Letters

Methods for studying polyploid diversification and the dead end hypothesis: a reply to Soltis *et al.* (2014)

Introduction

The fate of polyploid lineages has been of long-standing interest to evolutionary biologists. In our previous work (Mayrose *et al.*, 2011a; reviewed in Arrigo & Barker, 2012), we used likelihood methods to estimate the effects of recent polyploidization events on diversification rates (speciation minus extinction). Our goal was to ask whether, across groups, there is a preponderance of evidence for or against the hypothesis that polyploid species diversify at different rates than diploid species. Our results demonstrated that polyploid lineages, when compared with their diploid relatives, tend to form new species more slowly and go extinct more quickly, for a combined effect of lower diversification rates. Soltis *et al.* (2014) recently called into question our conclusions and raised concerns about our analyses. Some of these concerns reflect open questions and limitations in the available data, but others are based on misinterpretations of the methods used and their implications. In the spirit of furthering the fascinating debate about the macroevolutionary consequences of polyploidization, we offer this response with the hope that it will clarify what has been shown, demonstrate the utility of the methods applied, and inspire future work.

Soltis *et al.* (2014) frame their concerns as a series of arguments, and we follow their framework in responding to the points raised. Before embarking, it is worth reemphasizing what we defined as a 'polyploid lineage'. Indeed, all angiosperms have one or more polyploidization events in their history if one looks back far enough (Jiao *et al.*, 2011), and thus polyploidy must be defined with respect to a reference time point. Because our focus was on the impacts on diversification of recent polyploidization events, we explicitly defined polyploids 'as those lineages that underwent a polyploidization event since divergence from their generic ancestor' (Mayrose *et al.*, 2011a). This definition enabled us to perform a large-scale comparative study using chromosome number changes to infer polyploid transitions in 63 clades of plants. Thus, when asking whether polyploidization impacts subsequent diversification, it must be borne in mind that our analyses were conducted on rather short evolutionary timescales, comparing those taxa that had undergone fairly recent polyploidization ('neopolyploids') with those that had not. As we concluded in Mayrose *et al.* (2011a), it remains an interesting open question whether earlier polyploidy

events had different impacts on speciation and extinction, as has been suggested, for example, in the context of mass extinction events (Fawcett *et al.*, 2009; Vanneste *et al.*, 2014).

### Addressing the concerns of Soltis *et al.* (2014)

Philosophical arguments

The first criticism leveled is that we overestimated the diversification rates of diploids because 'increased diversification rates are more likely to arise in large clades than in small ones' (Soltis *et al.*, 2014). Specifically, Soltis *et al.* (2014) noted that polyploid lineages, as we defined them, would have to be nested within a larger diploid group, hence the polyploid subclades must be more recent and would thus tend to be smaller.

We agree that the polyploid subclades will tend to be younger and, assuming equal diversification rates, polyploid species are expected to be fewer in number, but this should not bias the estimation of diversification rates. The likelihood method that we used ('binary-state speciation and extinction', BiSSE; Maddison *et al.*, 2007) estimates instantaneous 'birth' (speciation) and 'death' (extinction) rates per unit time, not the total number of speciation and extinction events. It thus simultaneously accounts for the amount of time spent in each state and for trait transitions. By analogy, the rate at which a jogger runs may be measured at 10 km h^{-1}, whether they have been running for a long time or a short time. The fact that the polyploid subclades have existed for a shorter amount of time does not bias their inferred diversification rates towards lower values.

At an intuitive level, the main phylogenetic information that allows BiSSE to infer trait effects on diversification are the internode distances (see also Ree, 2005). If a trait increases diversification, it should be associated with shorter distances between branching events, compared with the alternative character state, and this association should be seen regardless of the depth of the tree (power issues not withstanding – see the Statistical and analytical arguments section, later). Also at an intuitive level, the main information used to infer extinction rates is the rise near the present in the number of species (Nee *et al.*, 1994). If, compared with diploids, polyploids are found more often on the tips of the tree than expected, then this signal suggests that polyploids have a higher extinction rate: they arise but fail to persist.

Indeed, the simulation study by Goldberg & Igic (2008) is highly relevant here: they simulated trees where the ancestor was in one state (diploid in the current case) and only forward transitions were allowed (say, to polyploidy). Although their focus was on the estimation of the transition rate, maximum-likelihood results kindly provided by E. E. Goldberg demonstrate that the extinction rates, speciation rates and diversification rates inferred by BiSSE had point estimates near their true values (Fig. 1). Thus, even
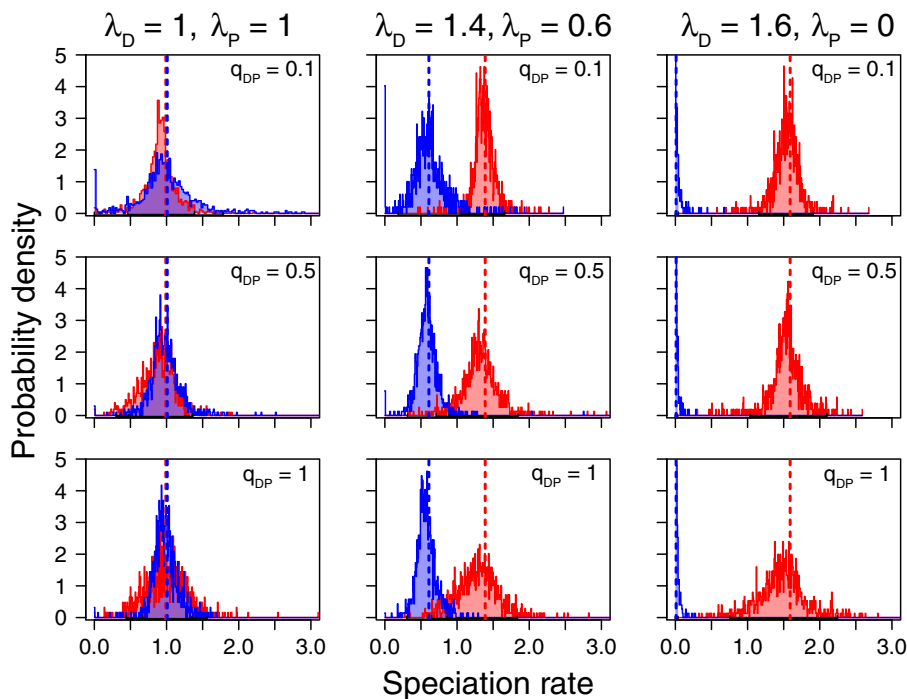
Fig. 1 The inferred speciation rates for diploids (D) and polyploids (P) based on 1000 simulated trees (data from Goldberg & Igic, 2008). For each simulated tree, one character state (here, diploidy) was assumed to be ancestral, with diploids giving rise to polyploids at rate q_{DP} and allowing no reverse transitions. Maximum-likelihood estimates for the five parameters (λ_D, λ_P, μ_D, μ_P, q_{DP}) were then inferred from each tree using binary-state speciation and extinction (BiSSE), assuming the root to be diploid. The inferred speciation rates are shown for the simulated parameters reported in Fig. 4 of Goldberg & Igic (2008) for diploids (red) and polyploids (blue). The distributions center around their true values, indicated by vertical dashed lines, despite the fact that the derived state (polyploidy) arose more recently. Similar results were observed for the extinction rate and the diversification rate (data not shown).

though polyploids had less time to diversify, the rate at which they did so is well estimated from simulated data (the age of the clade did not bias the rate estimates). It is worth clarifying a related misconception: Soltis *et al.* (2014) claim that 'older genera have more time to spawn polyploids than very young lineages; hence including 'young' genera while excluding 'older' genera will bias the outcome'. Because BiSSE accounts for the transition time between diploidy and polyploidy, younger clades will have fewer transitions and less time to accumulate species, but this is reflected in broader confidence intervals, not in lower estimated rates of diversification.

As suggested by Soltis *et al.* (2014), an alternative to the probabilistic framework is to restrict comparisons to clades of equal age (sister-clade comparisons). Doing so, however, drops a great deal of information from phylogenetic trees (i.e. dropping all branches not part of a contrast), reducing power. This is particularly true for traits, like polyploidy, which tend to appear on terminal branches of a phylogeny (Mayrose *et al.*, 2011a; Escudero *et al.*, 2014). Moreover, it is often not straightforward to identify sister clades with confidence and without subsequent transitions (Maddison, 2006). In addition, such sister-clade comparisons have been shown recently to be inherently biased in cases where one character state is more often the derived one. In these cases, there must be a transition from the ancestral to the derived state on the branch subtending the derived-state clade. The ancestral-state clade, however, gets a head start by already being in that state. So the time available for diversification of the derived state is less, causing it to artificially appear in clades with lower species richness (Kafer & Mousset, 2014).

The second philosophical criticism leveled is that reticulate evolution is common among polyploid lineages – indeed, it defines allopolyploids – and that this reticulation raises questions about the legitimacy of diversification analyses based on bifurcating trees. Notably, this criticism extends beyond our analyses; it applies to

most applications of comparative methods using phylogenies that contain taxa that have evolved via reticulation at some point in their histories. The effects of reticulation on comparative analyses in general, and on BiSSE-like analyses in particular, have not been well established. These biases may be particularly pronounced when polyploidy is involved as the effect on the topology and inferred branch lengths is still unexplored. To get some sense of the potential impacts of reticulation on our results, we subdivided our data into those genera whose phylogenies were based solely on chloroplast loci and those that were not. As the chloroplast is uniparentally inherited, the true phylogeny is expected to be bifurcating in the former groups, even in the face of hybridization. The results are shown in Fig. 2: the inferred diversification differences between diploids and polyploids are not significantly different between the cpDNA-based trees and the remaining trees ($P > 0.05$, two-tailed *t*-test). While further research is needed to explore the effect of reticulation on cpDNA-based phylogenies, this comparison suggests that hybridization did not strongly bias our results. Regardless, we agree with Soltis *et al.* (2014) that care should be taken when interpreting results based on models that ignore reticulation. We encourage future method development that accounts for hybridization and allows for its influence on diversification rate estimates to be considered.

Statistical and analytical arguments

Soltis *et al.* (2014) caution that extinction rates are difficult to estimate and claim that our conclusion that 'polyploids diversify at a lower rate is based on higher inferred extinction rates in polyploids'. We agree that extinction rate estimates should be interpreted cautiously. While simulation studies demonstrate that BiSSE-like methods can estimate extinction rates accurately when the model assumptions hold (Maddison *et al.*, 2007), extinction

(a) Diversification rates

(b) Speciation rates
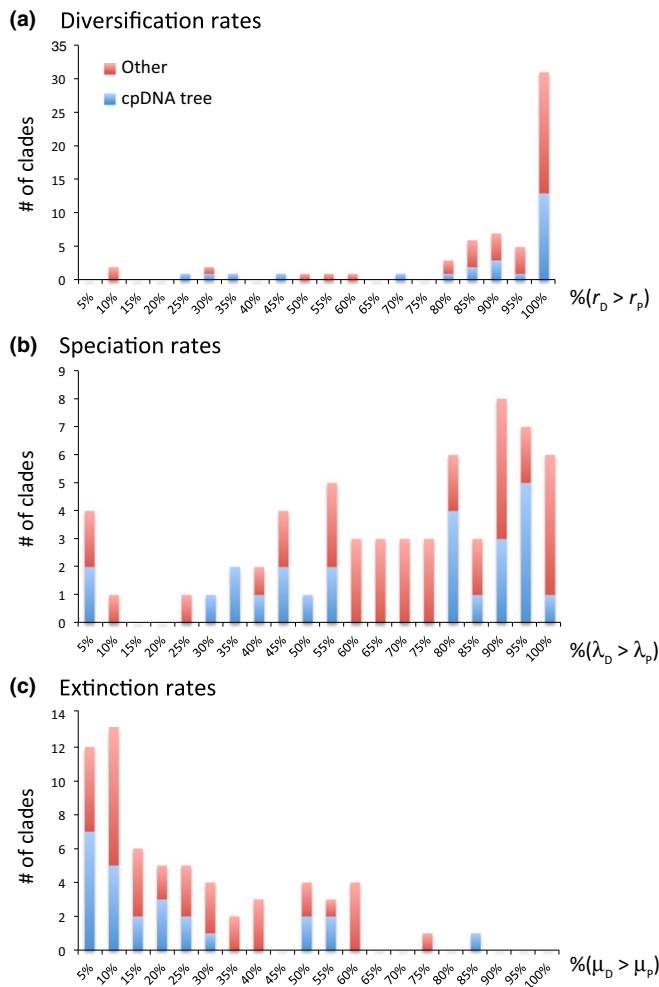
(c) Extinction rates

Fig. 2 Histograms illustrating the percentage of Markov chain Monte Carlo (MCMC) steps with diploids showing higher (a) diversification: $\%(r_D > r_P)$, (b) speciation: $\%(\lambda_D > \lambda_P)$, and (c) extinction rates $\%(\mu_D > \mu_P)$ than polyploids. The *y*-axis gives the number of clades (out of 63) for a given bin, with blue bars for the cpDNA-based phylogenies and red bars for the other clades.

rates are particularly sensitive to sampling biases and departures from the assumed model (Rabosky, 2010). That said, the primary focus of our work was not to estimate extinction rates, but rather to estimate the net diversification rate (r = speciation rate minus extinction rate) of diploids vs polyploids (r_D vs r_P). Typically, there are ridges on the likelihood surface, where the same diversification rate can be obtained by different combinations of speciation and extinction rates, all with similar likelihoods of explaining the data. Thus, comparative analyses have more power to measure the net diversification rate than to tease apart whether differences in diversification are due to speciation or extinction (Nee, 2006). Our main result that diploids diversify at a higher rate than polyploids (Fig. 1a of Mayrose *et al.*, 2011a) does not rely on a precise estimate of the extinction rate and accounts for correlations between speciation and extinction.

These results led us to conclude that polyploidy is 'most often an evolutionary dead end'. We realize, however, that some readers may interpret the term 'evolutionary dead end' to imply that polyploid

lineages will always go extinct before speciating. This was not our intention. Rather, we use the term to indicate that neopolyploids, on average, have lower – frequently negative – rates of diversification relative to congeneric diploids. Consequently, many polyploid lineages are ephemeral and contribute little to the diversity of plants, relative to their diploid relatives. That does not preclude the possibility that occasionally a polyploid lineage will radiate into a successful lineage, as stated in Mayrose *et al.* (2011a). We believe this nuanced view to be in keeping with that of G. L. Stebbins Jr (e.g. 'The long-continued evolution needed to differentiate genera, families, orders, and phyla appears to have taken place chiefly on the diploid level...Nevertheless, there is some evidence that many genera and even subfamilies or families of seed plants have had a polyploid origin'. Stebbins, 1950, p. 359).

Another statistical argument leveled in Soltis *et al.* (2014) revolves around the issue of power. In particular, they noted that the clades we examined were smaller than they should be to have sufficient power to infer diversification rate differences. Our approach was not, however, based on characterizing with confidence the diversification rates in individual clades. Instead, we performed a meta-analysis to assess the preponderance of evidence across dozens of clades. If polyploids and diploids diversified at equal rates, then we would expect to see higher polyploid diversification in roughly half of the clades. To assess this, we counted the fraction of Markov chain Monte Carlo (MCMC) steps in which $r_D > r_P$, treating this fraction for a clade as a single datum. As shown in Fig. 1(a) of Mayrose *et al.* (2011a), the majority of clades – 55 out of 63 – exhibited higher diversification for diploids, not polyploids ($P = 10^{-9}$ exact binomial test of obtaining an outcome as extreme as 55 or more out of 63 trials; $P = 10^{-12}$ one-sample *t*-test following a probit transformation testing whether the average fraction of MCMC steps differed from 0.5). By analogy, if one flips a coin once and gets a head, it would indeed be inappropriate to claim support for the coin being biased towards heads. However, observing 55 heads out of 63 coin flips is strong evidence that the coin is biased. Furthermore, there was no significant trend in the percent of MCMC steps showing $r_D > r_P$ and tree height (from Supporting Information Table S3 of Mayrose *et al.*, 2011a).

We argue that approaches like ours that consider the preponderance of evidence across multiple clades is the best way to assess whether a trait, like polyploidy, affects diversification in a repeatable way. This multiclade approach is particularly important given recent concerns raised by Maddison & FitzJohn (2015), who argue that analyzing single clades is susceptible to artifacts if there have been few transitions in the trait of interest, and by D. L. Rabosky & E. E. Goldberg (unpublished), who note that BiSSE can have a high false positive rate when other, unmodeled, processes affect diversification patterns. Thus, significant results from a single clade must be interpreted with caution. However, across many trait transitions, each false positive result, driven by chance associations with other processes, is equally likely to support increased or decreased diversification of the focal trait (here, polyploidy). Because we analyzed multiple clades in Mayrose *et al.* (2011a), we ensured that chance associations in any one clade did not drive our conclusions.

Although our recommended approach is meta-analytic as in Mayrose *et al.* (2011a), our result should be echoed in many of the analyses of individual clade datasets (albeit with reduced power), which Soltis *et al.* (2014) concluded was not the case. Unfortunately, the clade-specific statistics were incorrectly interpreted in Soltis *et al.* (2014) because they misinterpreted an error in Table S2 of Mayrose *et al.* (2011a). In that table we accidentally misordered the last three column headings, which should read $\%(\lambda_D > \lambda_P)$, $\%(\mu_D > \mu_P)$, and $\%(r_D > r_P)$, respectively, consistent with Fig. 1 of Mayrose *et al.* (2011a). Soltis *et al.* (2014) realized that there was a mistake, but assumed that it was only in the last column, and then interpreted this column as $\%(\mu_D < \mu_P)$ instead of $\%(\mu_D > \mu_P)$. (We were not asked about the error and have now posted a correction on the *Science* website.) Below, we summarize the results from the individual clades, using the corrected column headings.

The within-clade results are fully consistent with our meta-analysis. In short, 21 clades exhibited significantly higher rates of diversification among diploids than among polyploids (i.e. > 97.5% of the MCMC steps exhibited $r_D > r_P$; using a two-tailed test corresponding to an α-value of 0.05); none of the 63 clades showed a significantly higher rate of diversification for polyploids. While we caution against drawing conclusions about the impact of polyploidization in any one particular clade (as discussed above), the fact that the vast majority of MCMC steps (> 97.5%) supported $r_D > r_P$ in one third of the clades demonstrates that the signal for higher diploid diversification is both sufficiently strong and recurrent to be detected repeatedly. This trend was echoed in the extinction rate estimates, with nine clades exhibiting significantly higher extinction rates in polyploids, compared with none that exhibited significantly higher diploid extinction rates. Individual-clade speciation rates differed less by ploidy, with two clades showing significantly higher speciation rates in polyploids and two showing significantly higher speciation rates in diploids. Similar results were obtained using the less conservative cut-offs suggested by Soltis *et al.* (2014) of 5 and 95% (corresponding to an α-value of 0.1), with diploids diversifying significantly more in 31 clades (vs two for polyploids), speciating more in six clades (vs four for polyploids), and never exhibiting a significantly higher extinction rate (whereas polyploids went extinct significantly more often in 12 clades).

Sampling arguments

The next category of concerns raised in Soltis *et al.* (2014) relates to the datasets included in our analyses and our handling of them. The first concern is that recent polyploidization events may be underrepresented because many taxonomists use a 'conservative species concept' that ignores chromosomal variants within species. Defining species is, indeed, a thorny topic, particularly so for groups with ongoing polyploidization. In our Mayrose *et al.* (2011a) analyses, we ignored within-species variation in ploidy level, took the lowest reported chromosome number as the value for the species, and did not attempt to identify cryptic species. Doing otherwise, however, would have driven up the speciation rate of diploids (from which these new polyploids arise) and driven up the extinction rate of polyploids (because many more polyploid tips on

the tree would have arisen very recently and would appear to be short-lived). As pointed out in Soltis *et al.* (2014), 'most new polyploids go extinct early, probably at the population level before they are even detected'. By ignoring these highly ephemeral polyploids, our analysis was conservative; including them is expected to generate an even stronger signal that diploids diversify at higher rates than polyploids – contrary to what was implied in Soltis *et al.* (2014).

Indeed, recent analyses found exactly this pattern (N. Arrigo *et al.*, unpublished; see details in Supporting Information Notes S1). For each diploid species with a polyploid cytotype (an unsequenced and unnamed variant), the polyploid was elevated to species status by randomly 'injecting' a subbranch onto the terminal branch leading to that diploid for the genera *Draba*, *Gossypium*, *Ranunculus* and *Viola*. As expected, addition of these polyploid terminal taxa decreased polyploid diversification rates and increased diploid diversification rates (Table 1). Regardless of how branch lengths were assigned to the newly added terminal taxa (as exemplified in Fig. S1), net diversification rates for polyploids were much lower than diploids in each of these genera and were lowered still further by injecting the cytotypes into the phylogenies. This study confirms that excluding polyploid cytotypes is conservative with respect to the conclusions of Mayrose *et al.* (2011a).

The second concern raised was that we only analyzed a minuscule fraction of the angiosperm genera (estimated to be 14559; The Plant List, 2013). We agree; our analyses are only a start. As more phylogenies are constructed and coupled with chromosomal data, a fuller picture will emerge. That said, subsequent analyses with different groups have reached similar conclusions: polyploid lineages tend to diversify more slowly. In particular, Escudero *et al.* (2014) found that polyploid transitions generally occur towards the tips of the phylogeny and additionally that polyploid changes persisted over less time than changes in single chromosome numbers (dysploidy). A related criticism raised by Soltis *et al.* (2014) is that Mayrose *et al.* (2011a) did not include many of the most well-characterized genera of flowering plants. Recent analyses (N. Arrigo *et al.*, unpublished) have examined many of these classically studied genera and recover results consistent with Mayrose *et al.* (2011a). For example, analyses of *Draba*, *Gossypium*, *Ranunculus* and *Viola* – well-studied genera noted by Soltis *et al.* (2014) as absent from our previous analyses – again reveal lower net diversification rates for polyploids than diploids (Table 1).

The inference of lower polyploid diversification rates is consistent with two studies using entirely different methods (not phylogenetic). The first was a set of simulations conducted in Mayrose *et al.* (2011a) to ask whether the number of paleopolyploid events inferred from recent genomic analyses can be explained even if polyploidization does not increase diversification. These simulations were initiated with a single diploid ancestor and ran until the number of lineages reached 300 000, approximating the total number of angiosperm species. At each iteration of the simulation, a random species was selected to speciate or go extinct, with extinction happening with probability $\mu/(\lambda + \mu)$ and speciation otherwise. We assumed that extinction happened 60% as often as speciation ($\mu/\lambda = 0.6$, as estimated by Bokma, 2003; across a range of angiosperm taxa). At each speciation event, the

Table 1 Binary-state speciation and extinction (BiSSE) analyses of four plant clades (*Draba*, *Gossypium*, *Ranunculus* and *Viola*) with and without accounting for unnamed cytotypes (N. Arrigo *et al.*, unpublished)

Genus	SS[a]	SI[b]	SC[c]	$N_{2\times}$[d]	N_{pp}[e]	$N_{neo\text{-}pp}$[f]	%($r_P > r_D$)	%($r_P > r_D$) with neo-pp
Draba	233	350	43	27	12	4	0.08	0.07
Gossypium	44	50	39	28	8	3	8.83	0.41
Ranunculus	288	600	188	110	43	35	0.20	0.10
Viola (subsct. *Rostratae*)	31	50	23	11	7	5	0.42	0.07

BiSSE analyses were conducted using the R package diversitree (FitzJohn, 2012) across 50 Bayesian phylogenetic trees reconstructed using MrBayes v3.2 (Ronquist *et al.*, 2012) under a relaxed molecular clock model (Thorne *et al.*, 1998). The percentage of Markov chain Monte Carlo (MCMC) steps where the diversification rate of polyploids exceeded that of diploids was inferred as in Mayrose *et al.* (2011a), by considering only the named polyploid species (%($r_P > r_D$)). All unnamed polyploid cytotypes were then elevated to species status, and the analyses were repeated (%($r_P > r_D$) with neopolyploids variants; neo-pp). To do so, polyploid variants were inserted as sister lineages to the diploid species from which they arose, with the branching point chosen uniformly across the terminal branch for that diploid species (see details in Supporting Information Notes S1).
[a]Number of species sampled with sequence data. For all groups the internal transcribed spacer (ITS) marker was used for phylogeny reconstruction, except for *Viola* where *trnSG* was used.
[b]Number of species recognized in the ingroup.
[c]Number of sampled species with cytological data.
[d]Number of inferred diploid species.
[e]Number of described species inferred as polyploids.
[f]Number of tips assigned to unnamed polyploids (i.e. neopolyploid variants reported in otherwise diploid species).

daughter species was polyploidized with probability f_{het} (i.e. polyploidization evolved by 'cladogenesis' with shifts only at speciation), with f_{het} set to a range of values from 0.15 (Wood *et al.*, 2009) to 0.3 (Mayrose *et al.*, 2011a). According to the simulation results (Fig. S2 in Mayrose *et al.*, 2011a), if polyploids and diploids diversify at equal rates, the average number of paleopolyploidy events in the evolutionary history of a single angiosperm lineage is expected to be 4.6–8.9 for f_{het} ranging from 0.15 to 0.30. These estimates are higher than the observed number of 1–4 polyploidization events in the history of most angiosperms (Jiao *et al.*, 2011). Thus, not only is there no need to invoke higher diversification rates for polyploid taxa in order to account for the number of ancient polyploid events, but such higher rates would be incompatible with our current understanding of the frequency of paleopolyploidy. C++ source code that generated these simulations is available at http://www.tau.ac.il/~itaymay/cp/pp_diversification/.

The second study was based on the ratchet model of Meyers & Levin (2006). These authors pointed out that the high abundance of polyploidy can be explained even when polyploidy is disadvantageous, due to the high frequency of polyploid formation coupled with slow reversal to the diploid state. Recently, Scarpino *et al.* (2014) extended the ratchet model to allow for diversification and polyploidization rates to depend on the ploidy level of a lineage. They then used approximate Bayesian computation to estimate the model parameters for 60 angiosperm genera (only five are shared with the Mayrose *et al.*, 2011a dataset), chosen based on high coverage of chromosome number data. Using the number of species at each ploidy level within a genus as data, the study reached a similar conclusion: diploids diversify at higher rates than polyploid congeners, due primarily to a higher speciation rate from the diploid state via polyploidization. The consistency of the conclusions, using different data and methods, confirms the view that polyploidization does not increase species diversification, at least over genus-level timescales.

A related concern raised by Soltis *et al.* (2014), which we fully share, is that sampling biases may mislead inferences about

diversification rates. In particular, if systematists avoid sampling polyploid species when inferring trees or avoid clades with speciose polyploid groups, then diversification rates could be biased. In addition, if higher level taxa, such as genera, are biased with respect to polyploidy (e.g. if taxonomists commonly break diverse polyploid clades into multiple genera) estimated diversification rates of polyploids could be affected. Such biases can be corrected, if known, but the extent of these biases is generally unknown. We endeavored to make the best use of the data available at the time and welcome efforts to revisit these conclusions with new data.

Data-mining arguments

Of great concern are the issues raised in Soltis *et al.* (2014) about inconsistency between the data analyzed in Mayrose *et al.* (2011a) and the original sources. As described later, the data that we used are consistent with the literature cited and follow the methods described in Mayrose *et al.* (2011a) (with two exceptions).

The first concern raised about data consistency involved chromosome counts in *Physalis*. Soltis *et al.* (2014) wrote that we had failed to list three polyploid taxa based on their IPCN chromosome counts: *P. angulata* ($2n = 48$), *P. hederaefolia* ($2n = 24$, 48; correcting the typo stating '12, 24' in Soltis *et al.*, 2014; D. E. Soltis, pers. comm.), and *P. peruviana* ($2n = 48$, 72). Our compilation of chromosome numbers included additional sources besides IPCN (see citations in Mayrose *et al.*, 2011a), and these indicated additional diploid counts of $2n = 24$ for both *P. angulata* and *P. peruviana* (Fedorov, 1969), as confirmed by more recent chromosome counts (Rodríguez & Bueno, 2006; Wahua & Sam, 2013). Because all three species have diploid chromosome counts of $2n = 24$, we did not treat any of these species as polyploid to avoid ephemeral within-species variation (see earlier).

Soltis *et al.* (2014) then presented their Table 1 listing 21 groups in which they claimed that taxa were seemingly omitted from

Mayrose *et al.* (2011a). We traced the causes of these inconsistencies (see additional details in Notes S2), as follows.

Two errors misreported in Soltis *et al.* (2014) In the first two rows of their Table 1, Soltis *et al.* (2014) report that we only used five out of 14 *Sium* species and 17 out of 32 of the species of *Antirrhinum*. However, the analyses reported in Mayrose *et al.* (2011a) were based on the full number of species (see data deposited in Dryad, Mayrose *et al.*, 2011b). These two inconsistencies resulted from a bug in the software used by Soltis *et al.* (2014) to view the tree files (D. E. Soltis, pers. comm.)

Nine cases where Soltis *et al.* (2014) mistakenly included intraspecific variants Because we analyzed diversification patterns at the species level, the inclusion of intraspecific variants would bias the results towards a higher diversification rate for those species with denser intraspecific sampling. Thus, we excluded subspecies and variants as detailed in the methods of Mayrose *et al.* (2011a) (see also details in Supporting Information), while Soltis *et al.* (2014) did not (affected: *Phacelia*, *Achillea*, *Pelargonium*, *Houstonia*, *Digitalis/Isoplexis*, *Mimulis*, *Cerastium*, *Aichryson*, *Arisaema*).

Four cases where Soltis *et al.* (2014) inappropriately included outgroup taxa As stated in the supporting information of Mayrose *et al.* (2011a), the outgroup taxa were used only to root the tree and were removed before our chromEvol and BiSSE analyses. As discussed in FitzJohn *et al.* (2009), outgroup taxa must be excluded in order to apply BiSSE, because they are typically not well sampled and their diversification pattern would not necessarily reflect that of the analyzed ingroup, biasing the estimated diversification rates of the ingroup. We thus removed outgroups, as stated in Mayrose *et al.* (2011a) (affected: *Mimulis*, *Graptopetalum*, *Aristolochia*, *Trillium*).

Three errors traced back to the original studies Specifically, the GenBank entries for *Lathyrus* and *Vaccinium* did not match the accessions reported in the original studies, and accession numbers were missing for two species of *Gunnera*. These accessions were thus not used in Mayrose *et al.* (2011a).

Seven cases where we filtered species with little and/or poor sequence data As described in Mayrose *et al.* (2011a), we chose to exclude species that lacked data for one or more of the genes sequenced in a study, rather than including all species with any amount of sequence data. We further excluded sequences stated to be of poor quality. In cases where requiring all loci resulted in a loss of too many ingroup species (> 20%), we narrowed our analysis to subsets of loci that maximized the number of species analyzed. These procedures were detailed in the Supporting Information of Mayrose *et al.* (2011a). While we agree that alternative inclusion criterion could have been made, for example, to use all loci regardless of coverage, we made this decision *a priori* without regarding any specific clade. Thus, these inclusion criteria should not bias the relative diversification rates of diploids and polyploids

(affected: *Gaura/Stenosiphon*, *Achillea*, *Penstemon*, *Mimulis*, *Cerastium*, *Coreopsis*, *Graptopetalum*).

Two genuine errors in Mayrose *et al.* (2011a) In *Cuphea*, we mischaracterized *C. sessiliflora* and *C. sessilifolia* as duplicate entries. In *Cerastium*, we accidentally omitted the count for *C. lithospermifolium* (see later).

Soltis *et al.* (2014) then reexamined three clades. In the Mayrose *et al.* (2011a) analysis of *Tiquilia*, several taxa reported by Moore *et al.* (2006) were dropped because they were not sequenced for the full set of genes (*matK*, *ndhF*, *rps16*, ITS and *waxy*). As the dropped taxa contained a disproportionate number of polyploids (six out of nine; Soltis *et al.*, 2014), their exclusion could have biased our results. Indeed, based on the Soltis *et al.* (2014) reanalysis, the difference in diversification rate was substantial (% of MCMC steps with $r_D > r_P$: 82% in Mayrose *et al.*, 2011a; using the corrected column headings, vs 24% in Soltis *et al.*, 2014), largely due to a difference in speciation rate; the difference in extinction rates was fairly minor (% of MCMC steps with $\mu_D > \mu_P$: 8% in Mayrose *et al.*, 2011a; vs 23% in Soltis *et al.*, 2014). (Soltis *et al.*, 2014 reported this as the % of MCMC steps with $\mu_D < \mu_P$, but this is inconsistent with their Fig. 2c.; verified by D. E. Soltis, pers. comm.) Thus, *Tiquilia* represents a potential example where a bias against genotyping polyploids led to their underrepresentation in our dataset. However, because the genes used to generate the Soltis *et al.* (2014) phylogeny (*rps16* and ITS) were said by Moore *et al.* (2006) to be incongruent and, for ITS, difficult to align, reliable conclusions about *Tiquilia* must await a more complete and robust genetic dataset.

In the second example, we had accidentally omitted the chromosome count for *Cerastium lithospermifolium*. As indicated by Soltis *et al.* (2014), the ancestral chromosome count is affected by properly including *C. lithospermifolium*. Doing so, Soltis *et al.* (2014) found that support for higher diploid diversification in this clade dropped from 100 to 76% (% of MCMC steps with $r_D > r_P$).

In their last example, Soltis *et al.* (2014) revised the basal chromosome number for the GAMA clade of *Greenovia/Aeonium/Monanthes/Aichryson* by including *Sedum* ($n = 8$) as an outgroup not only to reconstruct the phylogeny (based on Mort *et al.*, 2002), but also to infer ploidy shifts using ChromEvol (Mayrose *et al.*, 2010). Doing so caused Soltis *et al.* (2014) to infer that a group of *Aichryson* (for which there were no chromosome counts in the dataset) were similar in chromosome number to the outgroup ($n = 8$) and to label these as diploids in Fig. 4 in Soltis *et al.* (2014). Both Fedorov (1969) and Uhl (1961), however, report several $n = 15$ chromosome counts for *Aichryson*, and the latter study concludes that this is the basal chromosome number for the genus (with subsequent polyploidization in *A. pachycaulon* and *A. villosum*). These data are thus inconsistent with *Aichryson* having the same chromosome count as the *Sedum* outgroup. Accordingly, the ancestral chromosome count for the base of the group once the outgroup is removed would be c. 15–18, as in our original analysis. Note also that in the diversification analysis of Soltis *et al.* (2014), the tree used included multiple accessions of two polyploid species (*Aeonium pseudourbicum*, *Aeonium percameum*), which also

artificially increased the inferred diversification rates of polyploid lineages reported in Soltis *et al.* (2014).

Nevertheless, even if we use the three datasets as reanalyzed by Soltis *et al.* (2014), the preponderance of evidence across the 63 datasets continues to support higher diversification rates for diploids (53 out of 63 clades; $P = 10^{-8}$ exact binomial test).

Soltis *et al.* (2014) also highlighted several differences between the topologies of our trees and those in the original papers, although they noted that most of these differences were minor. The original papers typically reported nonultrametric likelihood- or parsimony-based trees. Because a requirement of BiSSE is that branch lengths be proportional to time (otherwise rates cannot be estimated), ultrametric trees had to be generated for each dataset from the original sequence data. To do so, we developed a pipeline that applied likelihood methods to infer trees with branch lengths proportional to time for each of the groups analyzed. This pipeline further standardized our data analysis across the dozens of plant groups studied, which we believe is a necessary step when conducting such large-scale analyses. Importantly, in our analyses we also accounted for phylogenetic uncertainty (in both topology and relative branch lengths) by conducting the diversification analysis across a sample of Bayesian trees rather than relying on estimates from a single inferred tree. We make no claim of superiority for these trees, only that they can be used for analyses that require time-proportionality for branch lengths.

An additional concern was that examining slightly more inclusive trees by including the next closest sister group could have dramatically changed the ploidy level assignments. Certainly, if one were to study older trees, then earlier polyploid events would be included, and these might well change ploidy assignments (e.g. incorporating all of *Sedum* as an ingroup alongside GAMA would lead to the inference that the entire GAMA clade is polyploid, albeit with a mixture of tetraploids and higher ploidy levels). If one were to conduct BiSSE using these older trees, one would be assessing the impact on diversification of earlier polyploid events. We welcome such analyses, but they would not change our result: that polyploidy events which have happened in the recent past (within the genus for most of our clades) tend to lower diversification rates. It is tantalizing to contemplate the possibility that earlier polyploidization events may tell a different story. For example, additional gene loci may have provided substantial 'genetic degrees of freedom' and have been highly beneficial during earlier rounds of polyploidization in angiosperms, but no longer. But evaluating this speculation awaits analyses of deeper trees.

A final concern raised in Soltis *et al.* (2014) is that we assumed that all genera are equal in age. This is not, however, an assumption of our method. If one wanted to compare the diversification rate within one group (e.g. r_D of *Actinidia*) to the diversification rate within another group (e.g. r_D of *Tiquilia*), then it would indeed be critical that both diversification rates were measured in the same units of time. In our analysis, however, we compared the relative diversification rates of polyploids and diploids within a clade (e.g. r_D to r_P within *Actinidia*). No units of time are needed and the groups can be of any age when asking, for example, the percentage of MCMC steps where $r_D > r_P$.

The R script that was used for the diversification analysis presented in Mayrose *et al.* (2011a) is available at http://www.tau.ac.il/~itaymay/cp/pp_diversification/.

Conclusions

The majority of the philosophical, statistical and analytical arguments made in Soltis *et al.* (2014) stem from misinterpretations of our methodology, an incorrect assumption regarding our mislabeled column headings, and differences of opinion about how to define polyploids in a comparative analysis. However, we entirely agree with the broader point that our paper should not be considered the end of the story, that more data are needed from a wider variety of plant groups, that having more completely sampled clades would improve accuracy of the estimates, and that conducting analyses at deeper phylogenetic levels might reveal greater evolutionary success for polyploidization events earlier in plant evolution (just as deeper phylogenetic analyses carried out in fish did not find lower polyploid diversification; Zhan *et al.*, 2014). We look forward to learning, as more data are gathered and analyzed, whether the patterns we inferred from the 63 genera included in Mayrose *et al.* (2011a) are or are not representative of the fate of polyploid plants more broadly.

Nevertheless, we believe that model-based methodology applied over many character state transitions (either a meta-analysis as in Mayrose *et al.*, 2011a, or on a single clade with many transitions) is the best currently available approach for studying the effects on diversification of traits, like polyploidy, both in terms of its power and its relative insensitivity to biases. Having reexamined the data and results, we stand by our original conclusion based on the 63 clades examined (Mayrose *et al.*, 2011a): 'our results indicate that polyploidy is most often an evolutionary dead end, but the possibility remains that the expanded genomic potential of those polyploids that do persist drives longer-term evolutionary success'.

But we emphasize the wording. There is a critical difference between *polyploids are always evolutionary dead ends* and *polyploids are most often evolutionary dead ends*. Because polyploidization occurs so often (Meyers & Levin, 2006) and because occasional polyploids are endowed with phenotypic attributes that improve their success (Levin, 1983), we should not wonder that plant evolution has been repeatedly shaped by polyploidization. Like most mutations, our evaluation of the evidence to date is that polyploidization is typically deleterious, both at the within-species level (Ramsey & Schemske, 2002) and the between-species level (Mayrose *et al.*, 2011a; Arrigo & Barker, 2012). That most mutations are harmful does not preclude the occasional adaptive mutation, whether caused by a change in nucleotide or a change in ploidy level; indeed, evolution depends on such changes.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Elevating polyploid variants to species status.

Notes S1 Diversification analysis of four plant clades (Table 1).

Notes S2 Clarification of taxon count discrepancies in Table 1 of Soltis *et al.* (2014).

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