Establishing a timescale for plant evolution: palaeontological evidence and calibration consistency

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INTRODUCTION

Plants have utterly transformed the planet, from their influence on global biogeochemical cycles to the creation of environments habitable by animals. However, testing hypotheses of causality requires a timescale for land plant evolution that is as reliable as the geological timescale events that calibrates putative consequences such as perturbations in atmospheric chemistry. The molecular clock has long since usurped the fossil record in this endeavour, though fossil data remain integral in calibrating the clock. While clock methods have been extensively developed, for instance to accommodate rate variation, very little attention has been paid to the correct interpretation and appropriate implementation of fossil data. Fossil data cannot be used to directly calibrate the clock but, rather, can be interpreted to provide a minimum and maximum constraint on lineage divergences. Implemented as such, fossil data must be interpreted more rigorously than it has previously in establishing point calibrations. These principles have been implemented in analyses of animal evolution, but no such progress has been made in plant evolutionary studies.

RESEARCH OBJECTIVES

To address the lag between animal and plant molecular clock studies, we:

1. Researched 17 calibrations consisting of minimum constraints and soft maximum constraints for divergences between model representatives of major land plant lineages.
2. Utilising a dataset of seven plastid genes, we performed cross-validation techniques to these calibrations to explore their consistency under differing parameters
3. The same dataset was used to run a preliminary clock analyses to explore the effect of changing maximum constraints at nodes 1-3, and prior probability densities within calibrations, on divergence time estimates.

1. DERIVING CALIBRATION CONSTRAINTS

Because molecular clocks focus on divergences where sufficient sequence data is available and those which address interesting scientific questions, we calibrated divergences between 18 genome model organisms; taxa whose entire chloroplast or nuclear genomes have been sequenced (with the exception of *Ginkgo*).

Figure 1 is a representative phylogeny of the relationships between these taxa, based upon a consensus of the current literature. Each node has minimum and maximum fossil constraints.

- Minimum constraints are based upon the youngest age interpretation of an unequivocal fossil member of the clade delimited by that node.

- Soft maximum constraints may be inferred by absence of the clade from suitable deposits or older representatives of an outgroup

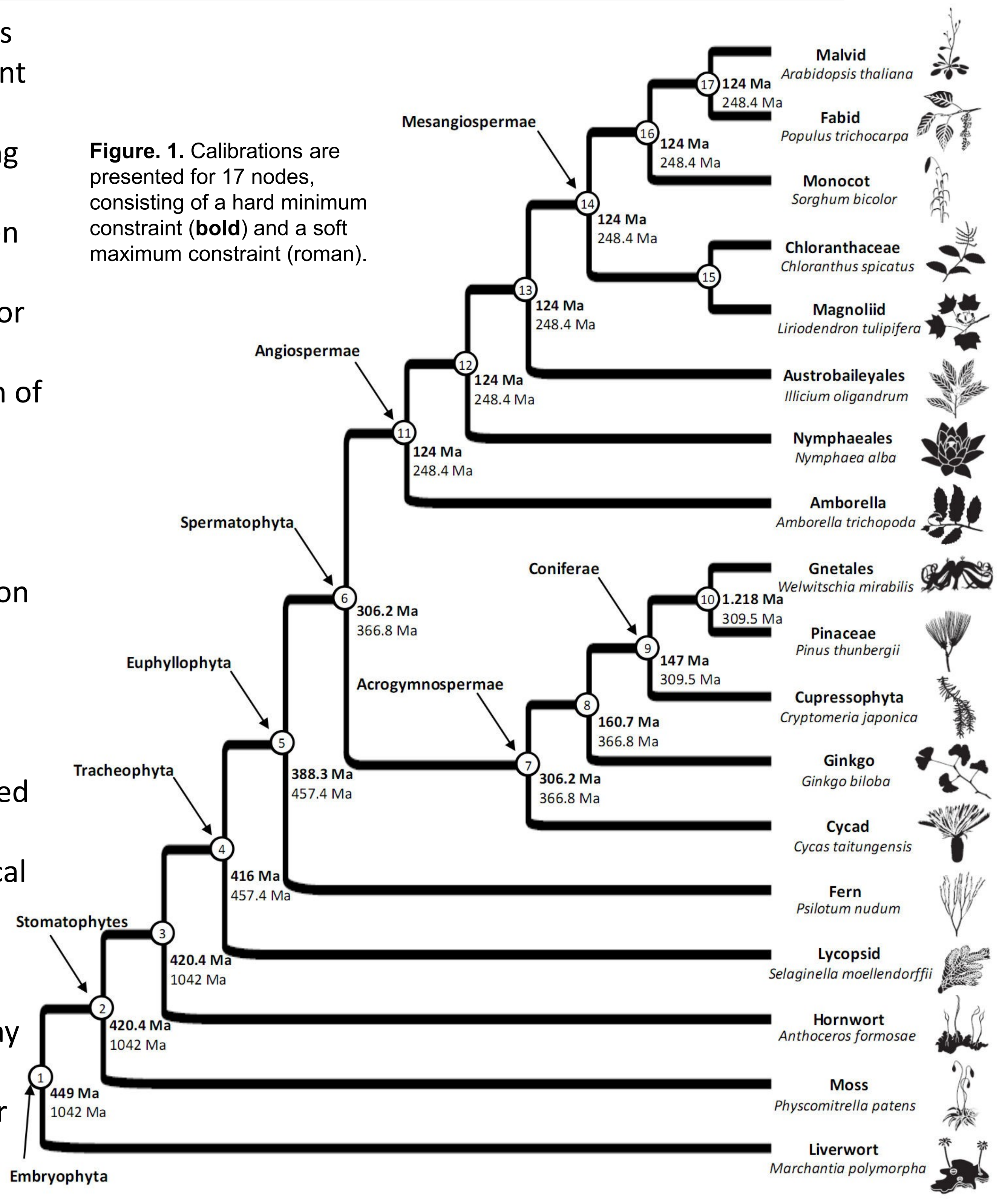


Figure. 1. Calibrations are presented for 17 nodes, consisting of a hard minimum constraint (**bold**) and a soft maximum constraint (roman).

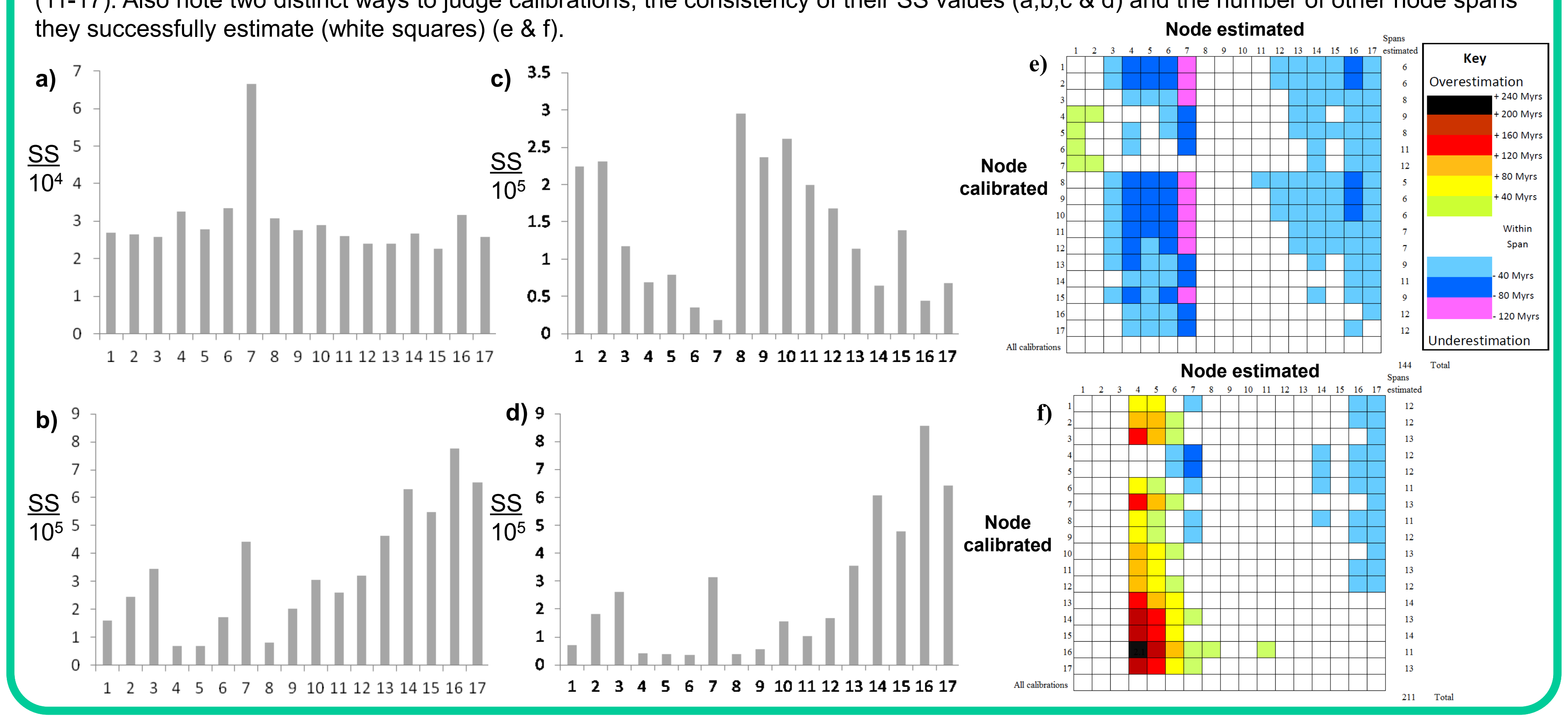
Table 1. Summary of the calibration constraints. Minimum constraints are based on the youngest age interpretation on the geological formation containing the relevant fossil. Maximum constraints are based on the oldest possible age of the sediments chosen.

Node No.	Clade	Lineage 1	Lineage 2	Minimum Providing Fossil	Youngest Date (Ma)	Minimum Age Constraint (Ma)	Evidence	Soft Maximum Providing Fossil/Sediments	Oldest Date (Ma)	Soft Maximum Age Constraint (Ma)	Evidence
1	Embryophyta	Hepaticae total group	Stomatophyte total group	Trilete spores	449	449.0	Biostratigraphy	Torridon group sediment devoid of plant-like spores	994 ± 48	1042.0	Direct date
2	Stomatophytes	Musci total group	Anthocerotae + Tracheophyta total group	*Cooksonia*	422.9 ± 2.5	420.4	Biostratigraphy				
3		Anthocerotae total group	Tracheophyta total group								
4	Tracheophyta	Lycopsida total group	Euphyllophyta total group	*Zosterophyllum sp.*	418.7 ± 2.7	416.0	Biostratigraphy	Trilete spores	454	454.0	Biostratigraphy
5	Euphyllophyta	Monilophyta total group	Spermatophyta total group	*Ibyka*	388.3	388.3	Biostratigraphy				
6	Spermatophyta	Angiospermae total group	Acrogymnospermae total group	*Cordaixylon iowensis*	307.2 ± 1.0	306.2	Biostratigraphy	Base of Vco zone which contains the first seeds.	366.8	366.8	Biostratigraphy
7	Acrogymnospermae	Cycadophyta total group	*Ginkgo* + Coniferae total group								
8		*Ginkgo* total group	Coniferae total group	*Ginkgo ginkgoidia*	164.7 ± 4.0	160.7	Biostratigraphy				
9		Gnetophyta + Pinaceae total group	Cupressophyta total group	*Araucaria mirabilis*	157 ± 10	147.0	Direct date	Sediments bearing Cordaixylon iowensis	309.5	309.5	Biostratigraphy
10		Gnetophyta total group	Pinaceae total group	*Liaoxia chenii* and Pityostrobus yixianensis	122.1 ± 0.3	121.8	Direct date				
11	Angiospermae	*Amborella* total group	Nymphaeales + Austrobaileyales + Mesangiospermae total group	Tricolpate pollen	125 ± 1.0	124.0	Magnetostratigraphy	Age of sediments below the oldest occurence of angiosperm-like pollen which are devoid of such pollen	248.12 ± 0.28	248.4	Direct date
12		Nymphaeales total group	Austrobaileyales + Mesangiospermae total group								
13		Austrobaileyales total group	Mesangiospermae total group								
14	Mesangiospermae	Chloranthaceae + Magnoliidae total group	Monocotyledoneae + Ceratophyllum + Eudicotyledoneae total group								
15		Magnoliidae total group	Chloranthaceae total group	*Endressinia brasiliana*	99.6 ± 0.9	98.7	Biostratigraphy				
16		Monocotyledoneae total group	Ceratophyllum + Eudicotyledoneae total group	Tricolpate pollen	125 ± 1.0	124.0	Magnetostratigraphy				
17		Malvidae total group	Fabidae total group	*Paleoclusia chevalieri and Dressiantha bicarpellata*	83.5 ± 0.7	82.8	Biostratigraphy	Oldest potential age of tricolpate pollen	127.2	127.2	Magnetostraigraphy

2. EXPLORING CALIBRATION CONSISTENCY

The principle of cross-validation is to use a single fossil calibration at a time to estimate all other nodes in the tree, until all available calibrations have been used. These estimates can then be compared to fossil estimates for their respective node, and the differences between these values compared. Some of our analyses are presented in **Figure 2.**

Figure. 2. Output from inconsistency analyses when comparing molecular estimates to fossil minima (a and b) and molecular estimates to calibration spans (c and d). e) and f) are visual representations of all the comparisons in c) and d), respectively. The top row had the maximum constraint from the three basal nodes at 509 Ma, and the bottom row had these maximums set to 1042 Ma. **Note** the inconsistency of calibration 7 in a), the inconsistency of nodes 1,2 and 8-10 in b), and the step-like increases in SS value through angiosperms in c) and d). Both e) and f) demonstrate two areas of the tree difficult to estimate, between nodes 4-7 and within angiosperms (11-17). Also note two distinct ways to judge calibrations, the consistency of their SS values (a,b,c & d) and the number of other node spans they successfully estimate (white squares) (e & f).



3. MOLECULAR CLOCK ANALYSES

- Using all 17 of the calibrations derived, we performed six molecular clock analyses.
- The first two explored the impact of the maximum placed on nodes 1-3 on divergence estimates, one where this maximum was 509 Ma, and the other where this maximum was 1042 Ma.
- The remaining four examined the effects of changing the peak in prior probability density on divergence estimates, generating 10%, 25%, 50% and 75% probability peaks within calibrations.

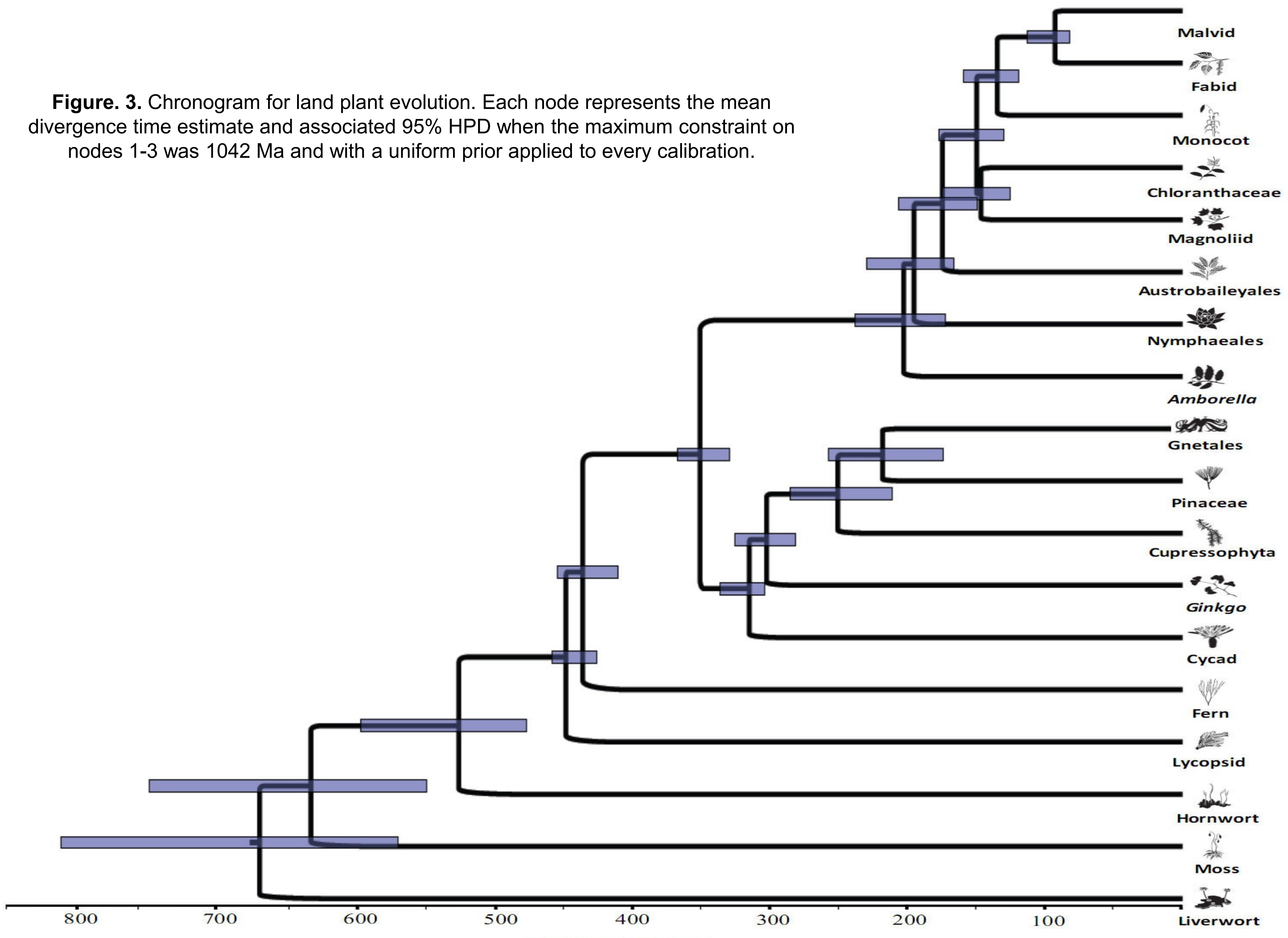
➡ An older maximum on nodes 1-3 generates older estimates for all nodes, although this effect is negligible for nodes 5 to 17.

➡ In all experiments, either those involving changes to maximum constrains or those changing prior probability densities, mean estimates and 95% HPD's fall outside the Cretaceous for crown-angiosperms, and outside the Ordovician for crown-land plants.

Table. 2. Divergence time estimates using all 17 calibrations with manipulations to the maximum constraints on nodes 1-3 and the prior probability densities within each calibration. Values in bold represent mean estimates, with 95% HPD's in parentheses. 'Raw prior' is the age which corresponds to the associated % between the minimum and maximum constraints for each node, in Ma.

Node	Clade	509Ma basalMax	--------1042Ma basalMax--------								
		uniform prior	uniform prior	10% prior peak		25% prior peak		50% prior peak		75% prior peak	
				Raw prior	Posterior	Raw prior	Posterior	Raw prior	Posterior	Raw prior	Posterior
1	Embryophyta	**505** (489-513)	**671** (570-817)	508	**589** (515-702)	597	**625** (553-736)	746	**729** (603-854)	894	**805** (610-997)
2		**499** (480-510)	**634** (549-753)	483	**555** (499-636)	576	**591** (536-668)	731	**691** (578-769)	887	**763** (584-909)
3		**459** (440-479)	**526** (477-599)	483	**477** (446-518)	576	**503** (460-563)	731	**553** (483-651)	887	**594** (494-782)
4	Tracheophyta	**428** (415-449)	**449** (426-459)	420	**421** (415-435)	426	**427** (417-443)	437	**439** (431-459)	447	**456** (443-617)
5	Euphyllophyta	**416** (395-439)	**436** (410-455)	395	**402** (390-420)	406	**409** (398-425)	423	**424** (415-436)	440	**447** (433-586)
6	Spermatophyta	**345** (324-366)	**351** (329-368)	312	**331** (315-351)	321	**325** (312-346)	337	**336** (322-348)	352	**357** (347-396)
7	Acrogymnospermae	**313** (302-332)	**315** (303-337)	312	**309** (303-317)	321	**294** (269-319)	337	**287** (266-313)	352	**328** (312-352)
8		**301** (281-323)	**303** (281-326)	181	**292** (258-309)	212	**276** (240-305)	264	**272** (256-298)	315	**315** (302-333)
9	Coniferae	**249** (208-284)	**251** (210-286)	163	**203** (160-260)	188	**203** (181-241)	228	**231** (217-248)	269	**272** (261-288)
10		**217** (170-257)	**218** (173-258)	141	**170** (133-231)	169	**174** (152-208)	216	**209** (185-224)	263	**252** (222-266)
11	Angiospermae	**195** (166-230)	**203** (172-238)	136	**167** (148-193)	155	**173** (157-195)	186	**202** (186-224)	217	**228** (216-256)
12		**188** (161-222)	**195** (166-230)	136	**161** (144-185)	155	**167** (153-188)	186	**196** (182-216)	217	**223** (211-248)
13		**169** (145-200)	**175** (149-207)	136	**147** (135-165)	155	**154** (142-170)	186	**183** (168-198)	217	**211** (192-231)
14	Mesangiospermae	**145** (128-172)	**150** (130-178)	136	**133** (124-144)	155	**139** (126-153)	186	**167** (148-182)	217	**192** (167-216)
15		**142** (123-169)	**147** (125-175)	114	**128** (114-141)	136	**136** (123-149)	174	**164** (144-179)	211	**189** (163-213)
16		**131** (117-154)	**135** (119-160)	136	**125** (117-134)	155	**127** (113-142)	186	**151** (131-170)	217	**173** (148-201)
17		**92** (81-111)	**93** (81-114)	87	**87** (82 - 95)	94	**92** (79-100)	105	**104** (89-112)	116	**116** (106-122)

Figure. 3. Chronogram for land plant evolution. Each node represents the mean divergence time estimate and associated 95% HPD when the maximum constraint on nodes 1-3 was 1042 Ma and with a uniform prior applied to every calibration.



CONCLUSIONS

➡ Improvements to calibrations and methods to better integrate palaeontological data with the molecular clock are essential to create more realistic timescales.

➡ Cross-validation highlights two distinct ways of judging calibrations: the consistency of their SS values and the number of calibration spans for other nodes they can estimate.

➡ Through creating plant calibrations that are 'fit for purpose' in the latest relaxed-clock techniques, we are able to reject a post-Jurassic origin for crown-angiosperms and also reject a post-Cambrian origin for crown-land plants.