

SUPPORTING INFORMATION

Footprints of past intensive diversification and structuring in the genus *Zelkova* (Ulmaceae) in south-western Eurasia

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Appendix S1 The institutions and number of *ex situ* individuals of *Zelkova schneideriana*, *Z. serrata* and *Z. sinica* used in this study.

Abbr.	Institution	Country	<i>Z. schneideriana</i>	<i>Z. serrata</i>	<i>Z. sinica</i>
HILL	Sir Harold Hillier Gardens	UK	1	3	1
MOAR	Morris Arboretum	USA	1	2	1
BONN	Bonn Botanical Garden	Germany	1	1	1
KUT	Kutaisi Botanical Garden	Georgia	3		
AD	Botanic Gardens of Adelaide	Australia	1		
KOR	Kornik Arboretum	Poland		1	
POZ	Botanical Garden of the University of Poznan	Poland		1	
STR	Jardin Botanique de Strasbourg	France	1		
		Total	8	8	3

Appendix S2 The name, sequence, annealing conditions and source of primers used in this study. PCRs were performed in a 20- μ L total volume with 0.12 μ L of 5 U μ L⁻¹ Taq (Roche, Basel, Switzerland), 25 μ M MgCl₂, 10 μ M of each primer, 0.2 mM of each dNTPs, and 0.85 μ L of template DNA. ITS1 and ITS2 were amplified under the same conditions with minor modifications (2 μ M MgCl₂ for both regions and the GC-rich buffer for ITS1). All the PCRs were programmed for an initial denaturing step at 95 °C for 6 min, 35 amplification cycles (30 s at 95 °C, 30 s at the appropriate annealing temperature, an elongation step of 45 s at 72 °C for *trnH-psbA* or 90 s at 72 °C for *trnL*, ITS1 and ITS2), and a final step of 4 min at 72 °C.

Genome	Region	Primer name	Sequence (5' to 3')	Source	Annealing condition
Chloroplast	<i>trnH-psbA</i>	<i>trnH</i>	CGC GCA TGG TGG ATT CAC AAT CC	Shaw <i>et al.</i> (2005)	30 s at 48 °C
		<i>psbA</i>	GTT ATG CAT GAA CGT AAT GCT C		
	<i>trnL</i>	<i>trnLc</i>	CGA AAT CGG TAG ACG CTA CG	Taberlet <i>et al.</i> (1991)	60 s at 64 °C
		<i>trnLf</i>	ATT TGA ACT GGT GAC ACG AG		
Nuclear	ITS1	A	GCG AGA ATT CCA CTG AAC CT	Denk <i>et al.</i> (2002)	30 s at 56 °C
		B	TTG CGT TCA AAG ACT CGA TG		
	ITS2	3P	GCA TCG ATG AAG AAC GTA GC	White <i>et al.</i> (1990)	30 s at 52 °C
		8P	CAC GCT TCT CCA GAC TAC A		

Hap.	540ID	548ID	550ID	553ID	561ID	563ID	565ID	572ID	584D	585DTV	586TV	591ID	594TV
A	-8	-2	-3	-8	-2	T	T	-12	G	A	A	T	T
B	-8	-2	-3	-8	-2	T	T	-12	G	A	A	T	T
C	-8	-2	-3	-8	-2	-2	-7	-12	G	A	A	T	T
D	T	-2	T	T	T	-2	T	G	G	A	A	T	T
E	-8	-2	-3	-8	-2	-2	T	-12	G	A	A	T	T
F	T	T	T	T	-2	-2	T	-12	G	A	A	T	T
G	T	T	T	T	-2	-2	T	-12	G	A	A	T	T
H	-8	-2	-3	-8	-2	-2	-7	-12	G	G	A	T	T
I	-8	-2	-3	-8	-2	-2	-7	-12	G	A	A	T	T
J	-8	-2	-3	-8	-2	-2	-7	-12	G	A	A	T	T
J3	-8	-2	-3	-8	-2	-2	-7	-12	G	A	A	T	T
K	T	-2	T	T	T	-2	T	G	G	A	A	T	T
L	T	-2	T	T	T	-2	T	G	G	A	A	T	T
M	T	-2	T	T	T	-2	T	G	G	A	A	T	T
O	T	-2	T	T	T	-2	T	G	G	A	A	T	T
Q	T	-2	T	T	T	-2	T	G	G	A	A	T	T
R	T	-2	T	T	T	-2	T	G	G	A	A	T	T
S	T	-2	T	T	T	-2	T	G	G	A	A	T	T
T	T	-2	T	T	T	-2	T	G	G	A	A	T	T
U	T	T	T	T	T	-2	T	G	G	A	A	T	T
V	T	-2	T	T	T	-2	T	G	G	A	A	T	T
W	T	-2	T	T	T	-2	T	G	G	A	A	T	A
X	T	-2	T	T	T	-2	T	G	G	A	A	T	T
Y	-8	-2	-3	T	T	T	T	-12	G	A	A	T	T
Z	-8	-2	-3	T	-2	-2	T	-12	-5	?	?	T	T
Z5	-8	-2	-3	T	-2	-2	T	-12	-5	?	?	T	T
Z2	-8	-2	-3	T	-2	-2	T	-12	-5	?	?	T	T
Z3	-8	-2	-3	T	-2	-2	T	-12	-5	?	?	T	T
SH4	-8	-2	-3	-8	-2	T	T	-12	T	A	A	-	T
Total													

Hap., haplotype; freq., frequency; Zab, *Z. abelicea*; Zca, *Z. carpinifolia*; Zsc, *Z. schneideriana*; Zse, *Z. serrata*; Zsic, *Z. sicula*; Zsin, *Z. sinica*. The mutation numbering starts at the end of the *trnH* primer at position 537 of the complete tobacco chloroplast genome (Shinozaki *et al.*, 1986), with the following notations: ID, indel; IV, inversion; D, duplication; TS, transition; TV, transversion. Question marks '?' correspond to indels for which the mutation state could not be defined.

(b) The substitutions and indels in the chloroplast haplotypes at the locus *trnL* in six species of *Zelkova* from across Eurasia. The number of individuals and the species identity are given for each haplotype.

Hap.	Species	Freq.	71TS	135TS	151ID	176TV	254TV	262ID	289IV	304IV	307TV	310ID	341IV	359TS	360TV	375TS	378TS	384TS	401TV	560TS	586TS	603TS	610TS	628ID	745IV	752IV	888TS
A	Zab	5	G	G	-10	A	T	A	T	A	A	-7	G	A	T	A	C	T	A	T	C	T	G	-13	A	G	C
B	Zab	6	G	G	-10	C	T	A	T	A	A	-7	G	A	T	A	C	T	A	C	C	T	G	-13	A	G	C
C	Zab	5	G	G	-10	A	G	A	T	A	A	-7	G	A	A	A	C	T	A	T	C	T	G	-13	A	G	C
D	Zsin, Zse, Zsc	7	G	G	-10	A	T	-6	G	C	A	-7	C	G	A	A	C	C	A	T	C	T	G	-13	A	T	C
E	Zsin, Zsc, Zse	12	G	G	-10	A	T	-6	G	C	A	-7	C	G	A	A	T	C	A	T	C	T	G	-13	C	T	C
F	Zab	16	G	G	-10	A	T	A	T	A	A	-7	G	A	A	A	C	T	A	T	C	T	G	-13	A	G	C
G	Zab	1	G	G	-10	A	T	A	T	A	A	-7	C	A	T	A	C	T	A	T	C	T	G	-13	A	G	C
H	Zab	18	G	G	-10	A	T	A	T	A	A	-7	G	A	T	A	C	T	A	C	C	T	G	-13	A	G	C
J	Zab	10	G	G	-10	A	T	A	T	A	A	-7	G	A	A	A	C	T	A	C	C	T	G	-13	A	G	C
K	Zca	3	A	G	-10	A	T	-6	T	A	A	-7	C	G	A	A	C	C	C	T	T	T	G	T	A	G	T
L	Zab	2	G	G	-10	A	T	A	T	A	A	-7	G	A	T	A	C	T	A	T	C	T	G	-13	A	G	C
O	Zab	4	G	G	-10	C	T	A	T	A	A	-7	G	A	T	A	C	T	A	T	C	T	G	-13	A	G	C
P	Zca	2	A	G	-10	A	T	-6	T	A	A	-7	C	G	A	A	C	C	C	T	T	C	G	T	A	G	T
R	Zca	10	G	G	-10	A	T	-6	T	A	A	T	C	G	A	A	C	C	C	T	T	T	G	T	A	G	C
S	Zca	9	G	G	T	A	T	-6	T	A	A	-7	C	G	A	A	C	C	C	T	T	T	G	T	A	G	C
T	Zca	15	G	G	-10	A	T	-6	T	A	A	-7	C	G	A	A	C	C	C	T	T	T	G	T	A	G	C
U	Zca	3	G	A	-10	C	T	-6	T	A	A	-7	C	A	A	C	C	T	A	T	C	T	G	-13	A	G	C
V	Zsic	5	G	G	-10	A	T	A	T	A	A	-7	C	A	A	A	C	T	A	T	C	T	G	-13	A	G	C
W	Zsic	5	G	G	-10	A	T	-6	T	A	T	-7	C	A	A	A	C	T	A	T	C	T	G	-13	A	G	C
X	Zca	5	G	G	-10	A	T	-6	T	A	A	-7	C	A	A	A	C	T	A	T	C	T	A	-13	A	G	C
Y	Zca	11	G	A	-10	A	T	-6	T	A	A	-7	C	A	A	A	C	T	A	T	C	T	G	-13	A	G	C
Z	Zca	19	G	G	-10	A	T	-6	T	A	A	-7	C	A	A	A	C	T	A	T	C	T	G	-13	A	G	C
Total		173																									

Hap., haplotype; freq., frequency; Zab, *Z. abelicea*; Zca, *Z. carpinifolia*; Zsc, *Z. schneideriana*; Zse, *Z. serrata*; Zsic, *Z. sicula*; Zsin, *Z. sinica*. The mutation numbering starts at position 49,376 of the complete tobacco chloroplast genome (Shinozaki *et al.*, 1986) with the following notations: ID, indel; IV, inversion; D, duplication; TS, transition; TV, transversion.

(c) The substitutions for the nuclear ribotypes at the locus ITS1 for *Zelkova abelicea* (Zab), *Z. sicula* (Zsic) and *Z. carpinifolia* (Zca). The frequency and the species identity are given for each ribotype. Mutation positions are numbered from the ninth position after the end of primer A of Denk *et al.* (2002).

[illegible]

(d) The substitutions and indels for the nuclear ribotypes at the locus ITS2 for *Zelkova abelicea* (Zab), *Z. sicula* (Zsic) and *Z. carpinifolia* (Zca). The frequency and the species identity are given for each ribotype. Mutation positions are numbered from the end of the forward primer 3 of White *et al.* (1990).

Ribotypes	Sequence reference	Species	Allele frequency	150	222	249	308	380	387
B	Zab-OMA22	Zab, Zsic	8	-	A	G	G	A	C
C	Zsic-A1-2	Zsic	1	-	G	G	G	G	C
D	Zab-KED1	Zab, Zsin	126	G	G	G	G	A	C
E	Zca-GUN2	Zca, Zsic, Zsc	153	G	G	G	G	G	C
F	Zca-NER1	Zca	3	G	G	A	G	G	C
H	Zsic-A1-3	Zsic	1	-	A	G	G	G	C
I	Zsic-A1-4	Zsic	1	-	A	G	G	A	T
K	Zsic-A1-5	Zsic	1	G	G	G	A	A	C
Total			294						

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