Plant Cell Physiol. 48(4): 662–665 (2007)
doi:10.1093/pcp/pcm036, available online at www.pcp.oxfordjournals.org

Short Communication

Bi-Parental Cytoplasmic DNA Inheritance in *Wisteria* (Fabaceae): Evidence from a Natural Experiment

Jennifer L. Trusty [1,*], Kataren J. Johnson [2], B. Graeme Lockaby [1] and Leslie R. Goertzen [2]

[1] *Center for Forest Sustainability, School of Forestry and Wildlife Sciences, 602 Duncan Drive, Auburn University, Auburn, AL 36849, USA*
[2] *Department of Biological Sciences, 101 Life Sciences Bldg, Auburn University, Auburn, AL 36849, USA*

Cytoplasmic inheritance was investigated in interspecific hybrids of *Wisteria sinensis* and *W. floribunda*. Species-specific nuclear, mitochondrial and plastid DNA markers were identified from wild-collected plants of each species in its native range. These markers provide evidence for the bi-parental transmission of plastids in hybrid swarms of these two species in the southeastern USA. These population level molecular data corroborate previous cytological evidence of this phenomenon in *Wisteria*.

Keywords: Cytoplasmic inheritance — Fabaceae — Hybridization — Mitochondria — *Wisteria floribunda* — *Wisteria sinensis*.

In the majority of flowering plants, cytoplasmic inheritance is maternal. Although varying degrees of paternal 'leakage' of plastid DNA have been identified in a number of genera (Corriveau and Colman 1988, Sewell et al. 1993), exclusive paternal inheritance of plastids has been experimentally demonstrated in only five angiosperm species (Yang et al. 2000). In a recent issue of *Plant and Cell Physiology*, Hu et al. (2005) described fluorescent signals in *Wisteria sinensis* (Fabaceae) pollen that were consistent with the presence of plastid DNA, suggesting the potential for paternal inheritance of this organelle. Here, we present additional evidence for the paternal transmission of plastid DNA in hybrid swarms of invasive *Wisteria* in the Southeastern USA.

Cultivated for centuries in Asia, both Chinese and Japanese *Wisteria* (*W. sinensis* and *W. floribunda*, respectively) were introduced to North America as ornamental plants in the early 19th century (Remaley 1999). Their ease of propagation, spectacular floral display and vigorous growth has led to the widespread use of these species in urban and residential landscapes. Although they differ in the direction of twining and length of inflorescence, the two species are considered interchangeable in horticultural use (Valder 1995). Unfortunately *Wisteria* has become invasive, escaping its horticultural setting and becoming naturalized in several regions. To date, exotic *Wisteria* has been listed as an invasive pest species in 15 southeastern states (USDA, NRCS 2004).

Morphological and molecular data suggest that the overwhelming majority of naturalized invasive *Wisteria* in the southeastern USA are hybrids of *W. sinensis* and *W. floribunda* (Trusty et al. 2007). These extensive natural hybrid swarms, replicated independently in multiple locations, provide a means to examine the hypothesis of paternal plastid inheritance in *Wisteria* at the population level. Here we examine *Wisteria* species-specific nuclear, mitochondrial and plastid markers to identify the parental cytoplasmic DNA contribution in interspecific hybrids.

In total, 70 'wild' or naturalized *Wisteria* plants were scored for nuclear (two loci), plastid (one locus) and mitochondrial (two loci) genotype. All markers were clearly identifiable as either the *W. sinensis* or *W. floribunda* type, as found in the native range of either species (Fig. 1). No individual had more than two alleles for either nuclear region or displayed any polymorphism for either mitochondrial (both *nad4* and *cob* regions) or plastid DNA (*trn*L–F) markers.

Of all *Wisteria* individuals examined, 84% ($n = 59$) were identified as hybrids between *W. sinensis* and *W. floribunda*. These included individuals with hybrid nuclear genotypes, i.e. individuals heterozygous for species-specific alleles at one or both nuclear loci and individuals homozygous at each locus, but for different species-specific alleles (Table 1). Surprisingly, hybrids also included individuals with different, species-specific markers at plastid and mitochondrial loci. A key result of this study is that overall, 48% ($n = 28$) of all hybrid individuals possessed the plastid and mitochondrial markers of different species.

Of the 42 *Wisteria* plants with plastids and mitochondria from the same species, 11 had uniform nuclear genotypes and were considered putatively 'pure' *W. sinensis* or *W. floribunda*. The remaining 31 individuals had nuclear

*Corresponding author: E-mail, jtrusty@auburn.edu; Fax, +1-334-844-1645.

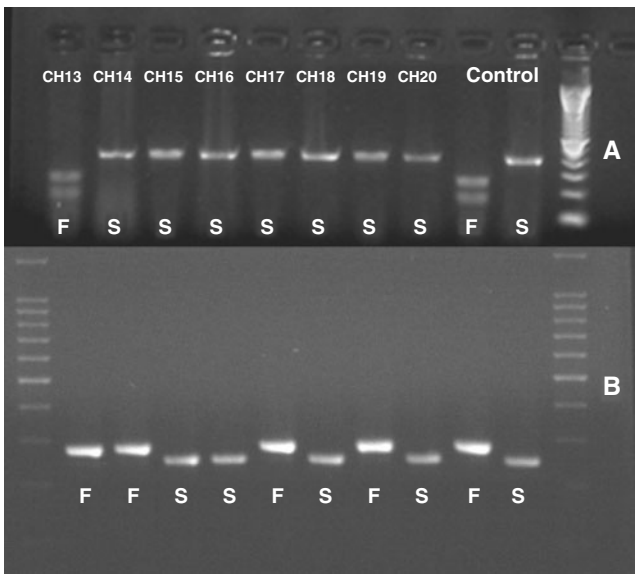

Fig. 1 (A) *Sal*I digestion patterns of the *Wisteria* mitochondrial *nad4* fragment and (B) *trn*L–F PCR product. *Wisteria* samples (Charleston individuals 13–20) are the same in A and B, including control *W. floribunda* and *W. sinensis* (control F and S). Each panel is the result of a single PCR, followed by a single restriction digest (A only). A 100 bp marker is shown in lanes 1 and 12.

Table 1 Plant sample location and nuclear, plastid and mitochondrial DNA designations

	Plastid	Mitochondrial		Nuclear	
ID	*trn*L–F	*cob*	*nad4*	824	997
CH1	F	S	S	F	F
CH2	**S**	**S**	**S**	**S**	**S**
CH3	**S**	**S**	**S**	**S**	**S**
CH4	**F**	**F**	**F**	**F**	**F**
CH5	F	S	S	F	F
CH6	F	S	S	F	H
CH7	F	S	S	F	H
CH8	F	F	F	F	H
CH9	S	S	S	H	S
CH10	S	S	S	H	S
CH11	S	S	S	H	S
CH12	S	S	S	F	S
CH13	F	F	F	H	F
CH14	F	S	S	F	F
CH15	S	S	S	H	S
CH16	S	S	S	H	S
CH17	F	S	S	F	F
CH18	S	S	S	H	S
CH19	F	S	S	F	F
CH20	S	S	S	H	S
CH21	S	S	S	F	H
CH22	S	S	S	H	S
CH23	F	S	S	F	H

(continued)

Table 1 Continued

	Plastid	Mitochondrial		Nuclear	
ID	*trn*L–F	*cob*	*nad4*	824	997
CH24	F	S	S	F	F
CH25	S	S	S	H	S
CH26	F	S	S	F	H
CH27	F	S	S	H	F
CH28	S	S	S	H	F
CH29	S	S	S	H	S
CH30	F	S	S	F	F
CH31	**S**	**S**	**S**	**S**	**S**
CH32	F	S	S	F	F
CH33	S	S	S	H	S
CH34	F	S	S	H	F
CH35	F	S	S	F	F
CH36	F	S	S	H	F
CH37	S	S	S	F	S
CH38	F	S	S	F	F
CH39	F	S	S	H	H
CH40	F	S	S	F	S
CH41	F	S	S	H	H
CH42	F	S	S	F	S
CH43	S	S	S	H	S
CH44	S	S	S	H	S
CH45	F	F	F	F	H
CH46	S	F	F	H	S
CH47	S	S	S	H	S
TA1	**S**	**S**	**S**	**S**	**S**
TA2	**S**	**S**	**S**	**S**	**S**
TA3	S	S	S	F	S
TA4	**S**	**S**	**S**	**S**	**S**
TA5	S	S	S	H	S
TA6	S	F	F	H	H
TA7	S	S	S	H	S
TA8	S	F	F	F	F
TA9	S	S	S	H	S
TA10	F	F	F	F	H
TA11	S	S	S	H	S
TA12	S	S	S	H	S
TA13	S	S	S	H	S
TA14	**S**	**S**	**S**	**S**	**S**
TA15	S	S	S	H	S
TA16	**S**	**S**	**S**	**S**	**S**
TA17	**S**	**S**	**S**	**S**	**S**
TA18	F	S	S	S	S
TA19	S	F	F	F	F
TA20	**S**	**S**	**S**	**S**	**S**
TA21	F	S	S	H	H
TA22	F	S	S	F	H
TA23	S	S	S	H	H

CH plants were collected near Charleston, SC and TA were collected near Tallahassee, FL. Taxa in bold possess markers from one species only. Samples with two *W. sinensis* alleles were scored as S, two *W. floribunda* alleles as F and hybrids as H.

genotypes consistent with F_2 or backcross generations—advanced hybrids in which cytoplasmic markers of the same species are easily reunited.

The recent and ongoing invasion of *Wisteria* species in the southeastern USA has provided an opportunity to test, at the population level, the hypothesis of paternal plastid transmission in this plant genus. Controlled crossing experiments are difficult with woody vines such as *Wisteria* that are space consuming and notoriously slow (~5 years) to flower and reproduce (Valder 1995). Instead, the widespread natural interspecific hybridizations driving the emergence of this invasive plant can be dissected with an array of species-specific markers for nuclear, plastid and mitochondrial genomes.

The data presented here reveal several novel aspects of the reproductive biology of *Wisteria*. First they confirm that the overwhelming majority of plants that have escaped from cultivation are hybrids between Japanese and Chinese *Wisteria* (Trusty et al. 2007). Hybridization is an important evolutionary mechanism in plants and has been implicated in the origin and success of numerous invasive plant species (Ellstrand and Schierenbeck 2000).

The data clearly show that in a large number (48%) of hybrid individuals, mitochondrial and plastid genomes are not inherited from the same parent. This pattern is repeatedly observed in hybrid swarms separated by approximately 500 km. It is important to note that the majority of hybrid individuals do not have simple F_1 genotypes; they are advanced generation hybrids and backcrosses in which pure cytoplasmic genotypes are expected to recur with high frequency. In addition, no plastid or mitochondrial heteroplasmy (presence of both *floribunda* and *sinensis* organelles) was observed in the 70 accessions sampled here or in the hundreds of other *Wisteria* individuals we have examined to date (J. L. Trusty and L. R. Goertzen, unpublished data).

Given that mitochondria and plastids are clearly inherited from different parents, the question arises of which parent provides which organelle. Mitochondrial inheritance in flowering plants is generally maternal, but exceptions have been noted (e.g. Havey 1997). In *W. sinensis*, Hu et al (2005) found that mitochondria in pollen were markedly degraded, while those in plastids remained intact. Given that plastid heteroplasmy has not been observed, these data provide strong evidence for the uniparental paternal transmission of plastid DNA in *Wisteria*.

Additional developmental cytology will be required to elucidate the exact mechanism of plastid inheritance in *Wisteria*, which appears to prevent the post-fertilization degradation of paternal plastid DNA and to preferentially exclude maternal plastids from the developing embryo. Continued research on these species may provide insight into the mode of uniparental paternal inheritance in angiosperms in general.

Materials and Methods

Wisteria populations were sampled in and around two urban areas: Tallahassee, Florida, and Charleston, South Carolina. Precise locations and descriptions of habitat are provided in Trusty et al. (2007).

Two nuclear sequence characterized amplified region (SCAR) loci '824' and '997', and the *trn*L–F region of the plastid genome were amplified and scored according to the protocol described in Trusty et al. (2007). The species-specific variation in these markers was identified in *W. floribunda* and *W. sinensis* individuals collected from their native range (where no hybridization occurs) and allows unambiguous identification of each species. All nuclear, plastid and mitochondrial markers were fully characterized through cloning and sequencing (with a minimum of two clones examined per marker) before survey methods using restriction enzymes were designed. Additionally, 2–4 clones of nuclear region 824 were sequenced for each individual plant for a total of >200 sequenced clones. PCR products of nuclear region 997 were cut with the restriction enzyme *Hpy*CH4IV, and the plastid *trn*L–F region was scored by size discrimination on agarose gels.

The approximately 3.8 kb intron 2 of subunit 4 of the mitochondrial NADH dehydrogenase complex 1 (*nad4*) gene contains a *Sal*I restriction enzyme recognition site (GTCGAC) at position 1527 in *W. floribunda*. A 385 bp fragment flanking this region was amplified using primers NAD4RSF1 (CTACTAG ACTACTAGAGGT) and NAD4RSR1 (GTTTGGCAACA AGCAAACG).

PCR amplification was carried out in 25 µl reactions containing 0.4 µM of each primer, 1 × *Taq* polymerase buffer, 0.2 µM each dNTP, 0.5 U of *Taq* polymerase (Eppendorf) and 1 µl of DNA template. Thermocycler conditions were 94°C for 1 min followed by 35 cycles of 94°C for 30 s, 52°C for 30 s, 72°C for 1 min; followed by 72°C for 10 min. A 5 µl aliquot of PCR product was digested with 0.5 µl of *Sal*I enzyme (NEB, Beverly, MA, USA), 2 µl of NEB buffer 3, 1 µl of 100 × bovine serum albumin (BSA) and 11.5 µl of distilled water for 1 h at 37°C. Restriction digests were visualized on a 1.5% agarose gel containing 0.5 µg ml^{-1} ethidium bromide in 1 × SB buffer on a UV transilluminator. Bands were scored by size as cut or uncut, indicating *floribunda* (F) or *sinensis* (S) mitochondria, respectively.

The approximately 1,500 bp intergenic spacer between mitochondrial cytochrome *b* (*cob*) and ribosomal protein S14 (*rps14*) contains an *Ase*I recognition site (ATTAAT) at position 677 in *W. sinensis*. A 398 bp fragment flanking this region was amplified using primers COBRSF1 (CATATTGACTTTCTCTCG CC) and COBRSR1 (GAATAGGATGACTCAGCGTC) according to the PCR conditions described above.

A 5 µl aliquot of PCR product was cut with 0.5 µl of *Ase*I enzyme, 2 µl of NEB buffer 3 and 12.5 µl of distilled water for 1 h at 37°C. Restriction digests were visualized as above and fragments were scored by size as cut or uncut, indicating *sinensis* (S) or *floribunda* (F), respectively.

Acknowledgments

We would like to thank John Kidd for laboratory assistance. We would also like to thank Javier Francisco Ortega,

Jeff Mower, and three anonymous reviewers for suggestions that greatly improved this manuscript. Funding was provided by the Center for Forest Sustainability at Auburn University.

References

Corriveau, J.L. and Coleman, A.W. (1988) Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperms. *Amer. J. Bot.* 75: 1443–1458.

Ellstrand, N.C. and Schierenbeck, K.A. (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? *Proc. Natl Acad. Sci. USA* 97: 7043–7050.

Havey, M.J. (1997) Predominant paternal transmission of the mitochondrial genome in cucumber. *J. Hered.* 88: 232–234.

Hu, Y., Zhang, Q. and Sodmergen (2005) Potential cytoplasmic inheritance in *Wisteria sinensis* and *Robinia pseudoacacia* (Leguminosae). *Plant Cell Physiol.* 46: 1029–1035.

Remaly, T. (1999) Exotic Wisterias (http://www.nps.gov/plants/alien/fact/wist1.htm)

Sewell, M.M., Qui, Y.-L., Parks, C.R. and Chase, M.W. (1993) Genetic evidence for trace paternal transmission of plastids in *Liriodendron* and *Magnolia* (Magnoliaceae). *Amer. J. Bot.* 80: 854–858.

Trusty, J.L., Zipperer, W.C., Lockaby, B.G. and Goertzen, L.R. (2007) Identity of naturalized exotic *Wisteria* in the Southeastern United States. *Weed Res.* in press.

USDA, NRCS (2004) The PLANTS Database, Version 3.5 (http://plants.usda.gov) National Plant Data Center, Baton Rouge, LA 70874, USA.

Valder, P. (1995) Wisterias: A Comprehensive Guide. Timber Press, Portland, OR.

Yang, T.W., Yang, Y.A. and Xiong, Z. (2000) Paternal inheritance of chloroplast DNA in interspecific hybrids in the genus *Larrea* (Zygophyllaceae). *Amer. J. Bot.* 87: 1452–1458.

(Received November 29, 2006; Accepted March 12, 2007)