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Linkage disequilibrium and phylogenetic congruence between chloroplast and mitochondrial haplotypes in *Silene vulgaris*

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Both the chloroplast and mitochondrial genomes are used extensively in studies of plant population genetics and systematics. In the majority of angiosperms, the chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA) are each primarily transmitted maternally, but rare biparental transmission is possible. The extent to which the cpDNA and mtDNA are in linkage disequilibrium is argued to be dependent on the fidelity of co-transmission and the population structure. This study reports complete linkage disequilibrium between cpDNA and mtDNA haplotypes in 86 individuals from 17 populations of *Silene vulgaris*, a gynodioecious plant species. Phylogenetic analysis of cpDNA and mtDNA haplotypes within 14 individuals supports a hypothesis that the evolutionary histories of the chloroplasts and mitochondria are congruent within *S. vulgaris*, as might be expected if this association persists for long periods. This provides the first documentation of the evolutionary consequences of long-term associations between chloroplast and mitochondrial genomes within a species. Factors that contribute to the phylogenetic and linkage associations, as well as the potential for intergenomic hitchhiking resulting from selection on genes in one organellar genome are discussed.

Keywords: hitchhiking; cytoplasmic male sterility; mitochondrial DNA; chloroplast DNA; maternal transmission

1. INTRODUCTION

Plants have two major organellar genomes, which are thought to be of independent evolutionary origin: one associated with the chloroplasts and the other with the mitochondria. These genomes code for proteins integral to the photosynthetic and respiratory pathways, respectively, and thus their metabolic importance is inarguable. Sequence variation in chloroplast DNA (cpDNA) occurs at both inter- and intraspecific levels and has become the tool of choice for many plant systematic and population genetic studies. Coding regions of the chloroplast genome, such as *rbcL*, have been exploited for studies of relationships among distantly related plant taxa (Chase *et al.* 1993) while non-coding regions have been applied to analyses of population genetic structure (McCauley 1994). Although plant mitochondrial DNA (mtDNA) has not been used as widely for systematic and population studies, variation in this genome has been used to assess both systematic and population genetic questions (Latta & Mitton 1997; Caha *et al.* 1998; Dumolin-Lapègue *et al.* 1998; Wu *et al.* 1998; Tomaru *et al.* 1998; Desplanque *et al.* 2000; Bowe *et al.* 2000; Chaw *et al.* 2000). Despite the fact that the chloroplasts and mitochondria are responsible for different metabolic functions and replicate independently, the degree to which they evolve independently is unclear. Although these genomes are not physically linked, in most plant species both are maternally inherited and thus their evolutionary fates may be intertwined. If so, evolutionary factors such as selection on genes in one organellar genome may indirectly affect genes in the other organellar genome through 'hitchhiking' resulting from co-inheritance (Maynard Smith & Haigh 1974; Ballard & Kreitman 1995).

Once two taxa become fully reproductively isolated, their respective chloroplast and mitochondrial genomes should clearly share an evolutionary history. Because cpDNA and mtDNA often display maternal inheritance in angiosperms, co-inheritance of the two genomes should have effects equivalent to reproductive isolation at the intraspecific level. With strict co-inheritance, each pair of mitochondrial and chloroplast genomes is effectively a single clonal lineage and should share an evolutionary history even prior to speciation. At the population level, a shared evolutionary history should be expressed as a non-random association between specific chloroplast and mitochondrial haplotypes that is numerically equivalent to linkage disequilibrium within the nuclear genome (Schnabel & Asmussen 1989). The phylogenetic footprint of strict co-inheritance should be congruence of the chloroplast and mitochondrial phylogenies, i.e. the cpDNA and mtDNA haplotypes found in the same individual should be located on complementary positions in the two phylogenetic trees.

The potential for detecting linkage disequilibrium and reconstructing shared phylogenetic histories is dependent on the presence of molecular evolution occurring at roughly similar rates in the two genomes. Within species, some cpDNA introns and intragenic spacer regions evolve at a sufficiently high rate to allow detection of variation (McCauley 1994; Clegg *et al.* 1994). Although mtDNA sequences are largely conserved within plant species, intraspecific mtDNA structural variation often results from intragenomic recombination that generates gene rearrangements (Palmer 1992; Fragoso *et al.* 1989). Thus, it might be possible to match intraspecific cpDNA sequence variation and mtDNA rearrangements to study

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the joint evolution of these two genomes within plant species.

While most angiosperms inherit both the chloroplasts and the mitochondria largely from their mothers, infrequent paternal transmission of either mitochondria or chloroplasts has been observed for several species that otherwise transmit both matrilineally (see the review in Reboud & Zeyl 1994). In fact, observations consistent with rare paternal inheritance of the chloroplasts suggest that it may occur in over 15% of angiosperm species (Corriveau & Coleman 1988). As the level of biparental inheritance for either genome increases, opportunities for reassociation should increase and linkage disequilibrium between mitochondrial and chloroplast haplotypes should diminish (Schnabel & Asmussen 1989).

Though biparental inheritance of either or both the cpDNA and the mtDNA would provide a mechanism for shuffling and breaking up otherwise long-term associations between the genomes, demographic processes could still cause new short-term statistical associations between haplotypes that do not share a long-term history of co-transmission. For instance, in species that undergo frequent population bottlenecks, such as are associated with high rates of extinction and colonization or range expansions, a limited number of chloroplast and mitochondrial haplotypes might be found within any particular population resulting from founder effects. In the most extreme case, all individuals in a local population might have identical chloroplast and mitochondrial genomes that can be traced back to a single colonist. Sampling across populations might then detect only a limited number of all possible mitochondrial and chloroplast haplotype pairs, resulting in linkage disequilibrium between the genomes. The signature of biparental inheritance combined with strong effects of demographic processes would be linkage disequilibrium between the genomes but no necessary phylogenetic congruence at the individual level.

Consider, then, three possible patterns of cpDNA–mtDNA population and phylogenetic association that could be generated by differing rates of biparental inheritance and different population structures. First, there might be little or no statistical association between cpDNA and mtDNA haplotypes within populations, and thus no phylogenetic congruence because of recent reshuffling of the genomes. Second, there might be a statistical association between cpDNA and mtDNA haplotypes but still no phylogenetic congruence. Finally, there could be linkage disequilibrium within and between populations, as well as congruent phylogenies evident when individuals are used as the taxonomic unit. Determination of such patterns in natural populations might help us to understand the degree to which the two genomes evolve independently.

Patterns of intraspecific associations between cpDNA and mtDNA, as well as their phylogenetic congruence, are not well studied. Two reports of associations between mitochondrial and chloroplast haplotypes, one in oaks (Dumolin-Lapègue *et al.* 1998) and the other in the sea beet (*Beta vulgaris* ssp. *maritima* (L.); Desplanque *et al.* 2000), showed high levels of statistical association (linkage disequilibrium) between haplotypes in the two organellar genomes. However, evidence for a shared evolutionary history was elusive. In the sea-beet study mtDNA and cpDNA phylogenies were not computed, whereas in the oak study too few mitochondrial haplotypes were detectable to assess phylogenetic congruence between the chloroplast and mitochondrial lineages within individuals (Dumolin-Lapègue *et al.* 1998).

In oaks and sea beets the chloroplast and mitochondrial genomes are both maternally inherited. In contrast, in conifers the mtDNA is maternally inherited and the cpDNA is paternally inherited. As might be expected, a study of cpDNA and mtDNA haplotypes in *Pinus flexis* showed that the genetic structure of the two genomes was very different, suggesting there are no long-term associations between the genomes (Latta & Mitton 1997).

In this study, we report complete linkage disequilibrium between the cpDNA and mtDNA haplotypes found in 86 individuals collected from 17 natural populations of *Silene vulgaris*, a weedy gynodioecious plant. In addition, we estimate the phylogenetic histories of chloroplast and mitochondrial lineages in 14 of these individuals using chloroplast sequence variation and mitochondrial restriction fragment length polymorphism variation. These data are used to test for congruence between the chloroplast and mitochondrial histories. Congruent phylogenetic patterns are consistent with long-term maintenance of linkage disequilibrium through co-transmission, while incongruent patterns suggest that opportunities for recombination exist and either assortative mating or selection maintain the disequilibrium in the shorter term.

2. METHODS

S. vulgaris is a weedy short-lived gynodioecious perennial native to Europe and naturalized in parts of the eastern USA. Leaf material was collected from 86 individuals distributed among 17 roadside populations found in Giles and Craig Counties, Virginia. These populations were all within *ca.* 25 km from each other and separated by at least 0.5 km. Some of these same populations have been studied for comparisons of nuclear and chloroplast population genetic structure (McCauley 1998) as well as the effects of population structure on the evolution of the sex ratio (McCauley *et al.* 2000*a*).

Our study was a two-stage process. First, to estimate linkage disequilibrium, we used polymerase chain reaction–restriction fragment length polymorphism (PC–RFLP) methods to screen chloroplast variation while concurrently using southern hybridization methods to screen mitochondrial RFLP variation. Second, to obtain detailed information for phylogenetic analyses, we chose 14 individuals, representing nine different mitochondrial RFLP haplotypes and five different chloroplast PCR–RFLP haplotypes, for more intensive study. For each of these individuals, the three chloroplast regions were sequenced and nine additional mitochondrial regions were screened for RFLP variation.

To estimate linkage disequilibrium, chloroplast variation was assessed using PCR–RFLP methods (McCauley 1994). DNA was extracted from leaf tissue using a QIAGEN DNeasy DNA extraction kit for plants (QIAGEN, Inc., Valencia, CA, USA). Three chloroplast regions were amplified using PCR: (i) L1–L2: a group I intron within the *trnL* gene (primers c and d; Taberlet *et al.* 1991); (ii) E–F: an intergenic spacer region separating the *trnL* and *trnF* genes (primers e and f; Taberlet *et al.* 1991); and (iii) H–A: a region that includes an intergenic spacer between

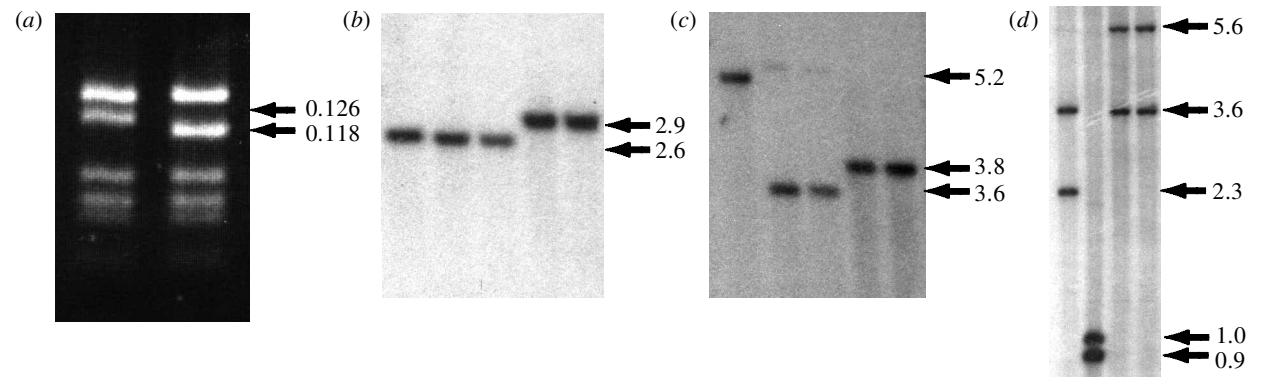

Figure 1. Screening methods for chloroplast and mitochondrial RFLP diversity. (*a*) Length variation in the *MseI*-digested L1–L2 cpDNA region. The difference in the two individuals results from an 8 bp deletion. (*b–d*) Mitochondrial variation was screened by assessing similarity of RFLP sites flanking known mtDNA genes. (*b*) A *coxI* probe hybridized to an *EcoRI* digest of five samples. Note two RFLP variants. (*c*) The same samples as figure 1*b*, digested with *HindIII* and probed with *coxI*. Note three RFLP variants. Both digests were used to distinguish among RFLP alleles associated with *coxI*. (*d*) Whole genomic DNA from four individuals digested with *HindIII* and probed with *cob*. *HindIII* cuts within the *cob* gene, thus the probe hybridizes to two regions of the DNA that flank the 5′- and 3′-ends of the *cob* gene. Note there are three restriction patterns, which were scored as three different alleles. RFLP band sizes are in kilobases.

the *trnH* and *psbA* genes (primers H and PSBA; Hamilton 1999). After amplification, each PCR fragment was digested with *MseI* restriction enzyme. The resulting products were electrophoresed on a 3.5% Metaphor agarose gel (FMC, Rockland, ME, USA) and variants were scored after staining with ethidium bromide (figure 1*a*).

Mitochondrial RFLP variation was assessed for restriction sites flanking the *coxI* mitochondrial gene (figure 1*b,c*). In two separate reactions, 0.5–1 μg of total genomic DNA was digested with either *HindIII* or *EcoRI*. Digested DNA was electrophoresed on a 0.7% agarose gel and transferred to a Hybond N^+ membrane (Amersham Pharmacia, Buckinghamshire, UK) via a capillary blot. Approximately 1.5 kb of the *coxI* gene was PCR-amplified from *S. vulgaris* using primers coxIf82 (Bowe *et al.* 2000) and coxIrl.6k (Cho *et al.* 1998). This PCR product was radioactively labelled and hybridized to the total genomic blots overnight. Hybridized blots were washed at moderate stringency (50 °C).

For the phylogenetic analyses, all three chloroplast regions (L1–L2, E–F, H–A) were sequenced. All sequences have been submitted to GenBank (accession numbers AF273273–AF273303). Each chloroplast region was directly sequenced from PCR products that were purified with QIA-quick PCR purification columns (QIAGEN, Inc.). Products were sequenced using standard protocols on an ABI 377 automated sequencer (Applied Biosystems, Inc., Foster City, CA, USA). Sequences were aligned manually and compared against GenBank to verify their identity.

To infer phylogenetic associations among mitochondrial haplotypes, variation in restriction sites flanking ten mitochondrial genes was screened (table 1; see electronic Appendix A at The Royal Society Web site for molecular weights for all RFLP haplotypes). Homologous probes to each gene were created by PCR amplification (see table 1 and electronic Appendix B for primers) from *S. vulgaris* genomic DNA. After amplification, all PCR products were sequenced and compared to GenBank to verify their identities before they were used as probes. To assess RFLP variation associated with *cox3*, *atpA*, *atp9* and *coxI* (figure 1*b,c*), we digested two total genomic DNA samples (0.5–1 μg), one with *HindIII* and the other with *EcoRI*, as above. For hybridizations using the remainder of the genes, we digested the genomic DNA sample with a restriction enzyme that cleaved in the middle of the mitochondrial gene as well as at sites flanking the gene to reveal a two-band pattern when hybridized with the probe (figure 1*d*). Because mtDNA variation is thought to arise via rearrangement (Palmer 1992; Desplanque *et al.* 2000), we expect that as the number of restriction enzymes used to digest the mtDNA increases, the number of identifiable haplotypes will plateau.

For computational analyses of the data, sequences from all three chloroplast regions were combined into one data set consisting of 25 binary characters (electronic Appendix C). Chloroplast sequence variation among individuals included point mutations, insertions or deletions ranging from four to eight base pairs and variation in the number of adenine residues within repeats > 6 bp. Variations in the numbers of adenine residues were not included as characters because they might be prone to homoplasy (Desplanque *et al.* 2000). Exclusion of these characters did not change the general topological structure of the trees. The nature of the evolution of chloroplast sequences in non-coding regions is complex (Clegg *et al.* 1994), and we sometimes found two to four adjacent bases that appeared to have mutated in tandem. Therefore, we limited the effect of these regions on our phylogenetic estimation by coding them as single changes. We included all insertion or deletion events (indels) in our data set and coded them as either present or absent. We are aware that non-coding regions of the cpDNA are prone to repeated independent evolution of the same indels (Clegg *et al.* 1994). Independently derived indels probably had little effect on our topology because five out of the seven indels were autapomorphic and the topology of the cpDNA phylogeny when all indels were deleted was almost identical to the topology when all indels were included but it was less well resolved.

The mitochondrial data set consisted of ten characters: two characters were monomorphic and the remaining eight were multi-state (range four to 11 alleles per character; electronic Appendix C). For the mitochondrial data set, a character was defined as the DNA region surrounding and including a particular mtDNA gene. An allele was defined as a unique RFLP pattern associated with this region. For a particular character (mtDNA gene), individuals were assigned the same allele only if

Table 1. *Ten mtDNA probe and restriction enzyme combinations used to determine alleles for mtDNA phylogenetic analyses*

(When possible we screened with only one restriction enzyme, which cleaved in the centre of the mtDNA gene for which we were hybridizing. Otherwise, we scored two separate Southern blots, one with whole genomic DNA digested with *EcoRI* and the other digested with *HindIII*. Primers used to amplify probes can be found in the cited references or electronic appendix B. See §2 and electronic appendices for additional details.)

probe	restriction enzymes used	number of blots scored	primer reference
coxI	*HindIII*, *EcoRI*	2	Chaw *et al*. 2000; Cho *et al*. 1998
coxII	*StyI*	1	electronic Appendix B
coxIII	*HindIII*, *EcoRI*	2	Wu *et al*. 1998
cob	*HindIII*	1	Wu *et al*. 1998
atpA	*HindIII*, *EcoRI*	2	electronic Appendix B
atp6	*HindIII*	1	electronic Appendix B
atp9	*HindIII*, *EcoRI*	2	electronic Appendix B
18s	*StuI*	1	Chaw *et al*. 2000
nad3	*StyI*	1	Wu *et al*. 1998
nad5	*NdeI*	1	Wu *et al*. 1998

all restriction fragments matched. In cases where two different blots (genomic DNA digested with either *HindIII* or *EcoRI*) were hybridized with the same mtDNA gene (figure 1 and table 1), allelic similarity was assigned after comparing both digests.

Phylogenetic analyses were performed using PAUP v. 4.0b2 (Swofford 1998). Neighbour-joining (NJ) algorithms were used to construct phylogenetic trees (Saitou & Nei 1987). All bootstrap values are based on 10 000 bootstrap replicates.

Congruence between the mitochondrial and chloroplast NJ trees was assessed with two statistics calculated by COMPONENT 2.0 (Page 1993): the nearest-neighbour interchange (d_{us}NNI) and partition metrics. The d_{us}NNI metric finds the smallest number of nearest-neighbour interchanges required to transform one binary tree into another, while the partition metric calculates the number of unshared clusters in two trees (Page 1993). To test whether the mitochondrial and chloroplast data sets were more similar than expected under a null hypothesis of random transmission of both genomes, we compared the distribution of each statistic (d_{us}NNI and partition metrics) computed for 1000 pairs of bootstrap-generated mitochondrial and chloroplast NJ trees (using our data sets) to the distribution of the same statistic for 1000 randomly generated pairs of trees, each with 14 terminal taxa. Random trees were generated from a uniform distribution of labelled binary trees (equiprobable model; Page 1993). The 95% confidence intervals were calculated as the 2.5% and 97.5% quantiles from the d_{us}NNI and partition metric distributions.

3. RESULTS

Eighty-six individuals from 17 populations were screened for both chloroplast and mitochondrial haplotypes. Among these individuals we identified seven unique cpDNA haplotypes by PCR–RFLP and 12 unique mtDNA *coxI*–RFLP haplotypes (figure 2). All but four *coxI* mtDNA haplotypes were observed in more than one population (figure 2). The cpDNA haplotypes and mtDNA haplotypes displayed a strict and highly statistically significant non-random association among individuals (Fisher's exact test $p < 2 \times 10^{-50}$; figure 2) presenting a pattern in which mtDNA haplotypes are nested within cpDNA haplotypes. That is, each mtDNA haplotype was associated with a single cpDNA haplotype while each cpDNA haplotype was associated with one or more mtDNA haplotypes.

Primarily maternal inheritance of the chloroplast PCR–RFLP haplotypes has been verified in a closely related species *Silene alba* (McCauley 1994). To verify that the mitochondrial variants were principally inherited matrilineally in *S. vulgaris*, we screened seven progeny from each maternal line from a reciprocal cross between

mitotype \ chlorotype	A	B	C	D	E	F	G
a	5 (2)						
b	14 (4)						
c	5 (3)						
d	5 (3)						
e	8 (6)						
f		26 (5)					
g			7 (3)				
h				1 (1)			
i				2 (1)			
j					9 (2)		
k						3 (1)	
l							1 (1)

Figure 2. Matrix of association between PCR–RFLP chloroplast (columns) and mitochondrial *coxI* RFLP (rows) variants within 86 individuals from 17 natural populations. Capital letters refer to PCR–RFLP chloroplast haplotypes. Lower case letters refer to different RFLP mtDNA haplotypes scored by hybridizing *coxI* probes to total genomic DNA. Numbers in cells refer to the number of individuals with each chloroplast–mitochondrial combination. Numbers in parentheses in cells refer to the number of populations in which each chloroplast–mitochondrial combination was found. Empty cells indicate that no individual was observed with this combination of PCR–RFLP chloroplast and mitochondrial *coxI* RFLP patterns.

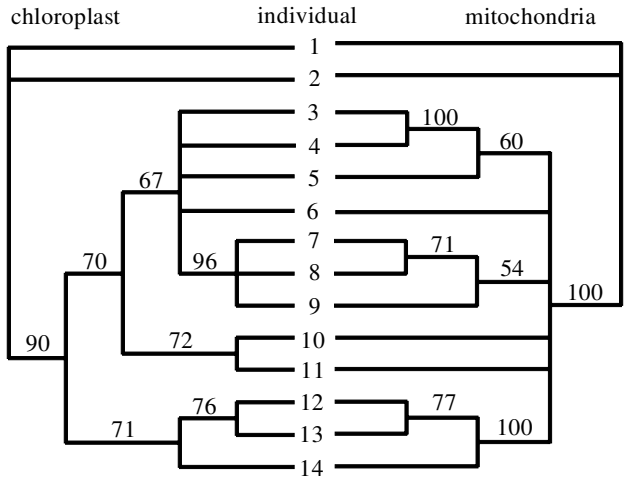

Figure 3. Chloroplast and mitochondrial bootstrap majority-rule consensus NJ trees. Different individuals are represented by numbers (1–14). Numbers on branches are bootstrap values for 10 000 bootstrap replicates.

two individuals with different *coxI*–RFLP genotypes (genotypes $g \times h$; figure 2). Inheritance of both the *coxI–EcoRI* and *coxI–HindIII* RFLPs was maternal for all seven progeny from both dams (14 progeny in total). The probability of this outcome occurring by chance under a hypothesis of equal biparental inheritance is $1/2^{14} \approx 6 \times 10^{-5}$. However, this sample size is only large enough to suggest with 95% confidence that biparental inheritance occurs less than 19.3% of the time (Milligan 1992).

The majority-rule consensus NJ trees for the chloroplast and mitochondrial lineages are presented in figure 3. Three clades, two with three taxa (7,8,9 and 12,13,14) and one with two taxa (1,2), were supported by $> 50\%$ of the bootstrapped NJ trees in both the cpDNA and mtDNA trees. The parsimony-based partition-homogeneity test (Farris *et al.* 1995) supported the null hypothesis of congruence, finding no partition of the data (out of 1000 replicates) that produced two trees with a combined sum greater than the original partition.

To assess the statistical significance of the congruence between our cpDNA and mtDNA data sets we computed the similarity between bootstrap NJ trees from the chloroplast data and the mitochondrial data. We used two similarity indexes: the number of unshared clusters in the trees (the partition metric distance) and the number of nearest-neighbour interchanges (d_{us}NNI) required to transform one tree into the other (Page 1993). The null hypothesis of no similarity between cpDNA and mtDNA trees was tested by assessing the overlap of the distributions from cpDNA–mtDNA comparisons and comparisons between pairs of randomly generated trees.

The bootstrap cpDNA and mtDNA trees were significantly more similar than randomly generated 14-taxa trees. While random pairs of 14-taxa trees had an average of 23.6 unshared clusters, the bootstrap cpDNA and mtDNA trees had an average of only 14.9 unshared clusters (figure 4*a*) . There were also fewer nearest-neighbour interchanges (d_{us}NNI) separating the chloroplast and mitochondrial trees than there were separating pairs of random trees (figure 4*b*). Thus, for both the partition metric and the nearest-neighbour interchange metric

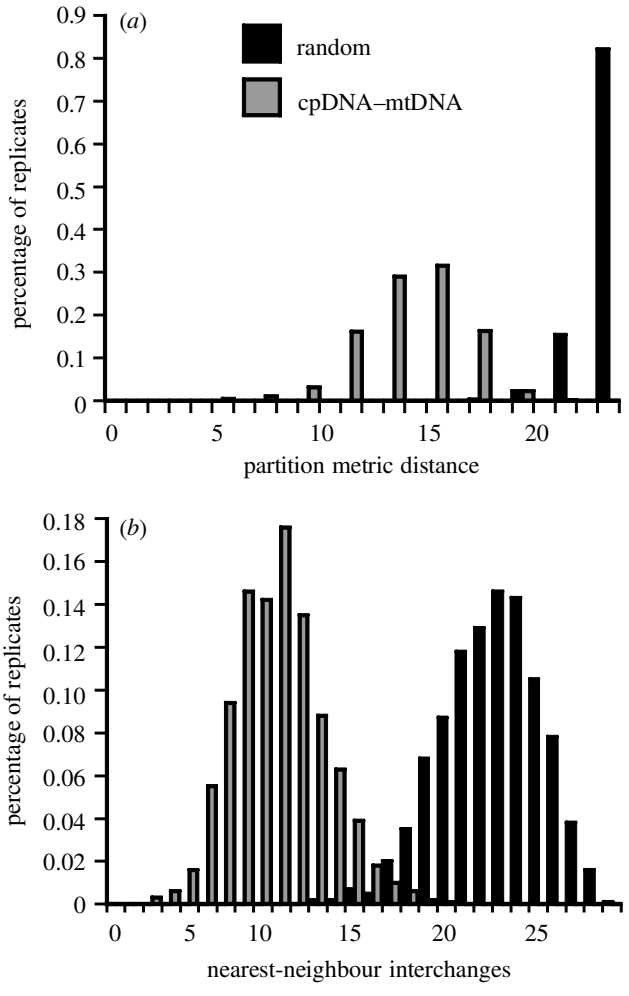

Figure 4. (*a*) Numbers of unshared clusters in bootstrapped cpDNA and mtDNA trees (grey bars; mean = 14.9; 95% confidence interval (C.I.) = 10–18) and randomly generated pairs of 14-taxa trees (black bars; mean = 23.6; 95% C.I. = 20–24). (*b*) Numbers of nearest-neighbour interchanges separating bootstrapped cpDNA and mtDNA trees (grey bars; mean = 9.8; 95% C.I. = 5–15) and randomly generated pairs of 14-taxa trees (black bars; mean = 21.5; 95% C.I. = 16–26).

there was a significantly higher consistency among the histories of chloroplast and mitochondrial haplotypes than is expected by chance.

4. DISCUSSION

We have shown that chloroplast and mitochondrial haplotypes in *S. vulgaris* exhibit complete linkage disequilibrium and have congruent phylogenetic histories. These patterns could not be produced without both genomes being largely co-transmitted. Additionally, in order to detect multiple haplotypes and generate phylogenetically useful information in both genomes, the molecular markers that were used must evolve at roughly similar rates. These results suggest a long-term shared evolutionary history of chloroplast and mitochondrial lineages in *S. vulgaris*, which might include episodes of inter-genomic hitchhiking selection.

The co-inheritance of the two genomes suggests that they may not respond to selection independently.

S. vulgaris was chosen as a model system for this study because there is evidence that cytoplasmic male sterility (CMS) factors in the mitochondrial genome affect individual fitness (Charlesworth & Laporte 1998; McCauley *et al.* 2000*a*). *S. vulgaris* is gynodioecious, with individuals expressing female or hermaphroditic phenotypes, and gender is determined by an interaction between maternally inherited CMS genes and nuclear male-fertility restorers (Charlesworth & Laporte 1998; Taylor *et al.* 2000). Both theoretical and empirical studies suggest that selection on CMS is strong. Theoretically, unrestorable CMS types may sweep to fixation (Frank 1989), though in natural populations there is negative frequency-dependent selection on females (McCauley *et al.* 2000*a*). Clearly, the co-ancestry of mitochondrial and chloroplast genomes in *S. vulgaris* indicates that the evolutionary fate of a new mutation in the chloroplast genome would be affected by selection on CMS genes in the mtDNA. In fact, we have documented elsewhere an association between certain cpDNA haplotype markers and female sex expression in natural populations (McCauley *et al.* 2000*b*). Given that the sex expression is determined by interactions between nuclear and mitochondrial genes, such an association between sex expression and cpDNA probably results from 'hitchhiking' of the entire chloroplast genome in response to selection on the CMS genes and the entire mitochondrial genome (Schnabel & Asmussen 1989; Ballard & Kreitman 1995).

The recognition that hitchhiking effects may result in non-neutral evolution of the mitochondrial genome in animals (Ballard & Kreitman 1995) should underscore the even greater potential for hitchhiking and selection to affect the independent evolution of plant organellar genomes, where two genomic compartments appear to have long-term histories of co-transmission. Similar hitchhiking effects have been studied in arthropods where intra-cytoplasmic micro-organisms (e.g. *Wolbachia*) feminize progeny leading to highly female-biased sex ratios (Marcadé *et al.* 1999; Rigaud *et al.* 1999). Selective sweeps resulting from colonization of new populations have been shown to result in high levels of population genetic structure similar to those caused by population bottlenecks (Johnstone & Hurst 1996).

The statistical congruence between the chloroplast and mitochondrial phylogenies supports the hypothesis that the two genomes have long-term associations through maternal inheritance. However, it is not clear whether paternal inheritance occurs at a low frequency, nor what frequency of paternal inheritance would be permitted while still yielding our results. Obviously, some cases of independent transmission might not be detectable through studies of linkage disequilibrium and haplotype-transmission histories. For reassortment to occur after an episode of biparental inheritance, the cpDNA and mtDNA genomes in the maternal and paternal sources must differ. Detecting reassortment of the cpDNA and mtDNA genomes in *S. vulgaris* might be limited by its breeding system and population structure. First, biparental transmission is undetectable in progeny resulting from self-fertilization. In *S. vulgaris*, the frequency of hermaphrodites, which are capable of self-fertilization, generally exceeds 50% (McCauley *et al.* 2000*a*). Second, the probability of detecting biparental transmission decreases as the population structure of the cytoplasmic genome increases, and both the mtDNA and the cpDNA haplotypes found in *S. vulgaris* populations used in this study exhibit a high degree of population structure (McCauley *et al.* 2000*a*; Taylor *et al.* 2000). Nevertheless, there was still ample opportunity for reassociation of the genomes if biparental inheritance were to occur in our study populations because in seven out of our 17 populations there were at least two chloroplast and two mitochondrial haplotypes present.

Our ability to detect linkage disequilibrium (or the lack of it) is contingent on similar rates of marker evolution in the mitochondrial and chloroplast lineages. We have shown that cpDNA–mtDNA reassociation via biparental inheritance is sufficiently rare to allow the build-up of phylogenetic signals in both the cpDNA and the mtDNA. Moreover, the mutation rates of our cpDNA sequence variants and mtDNA RFLPs must be roughly similar, allowing detection of phylogenetic congruence. As the difference in the mutation rates of the cpDNA and mtDNA increases, detection of congruent phylogenetic signals will be increasingly compromised. To obtain a more complete understanding of the mechanisms affecting our ability to detect the joint evolution of co-transmitted genomes, theoretical investigations will be necessary that focus on the effects of variation in the relative mutation rates of each genome, the potential for bi-parental transmission, the demographic context of the species under consideration and the potential for selective factors to homogenize associations.

Since this is the first study to compare the intraspecific phylogenetic histories of mitochondrial and chloroplast haplotypes that co-occur in the same individuals, it is not yet clear whether our results represent a condition common in angiosperm species. We are aware of few other studies that can be used to assess the intraspecific transmission histories of chloroplast and mitochondrial haplotypes. In one comparable study in oaks, it is unclear whether chloroplast and mitochondrial lineages, identified by PCR–RFLP and PCR–RFLP-single-strand conformational polymorphism (SSCP), respectively, have remained associated throughout the post-glacial colonization of France (Dumolin-Lapègue *et al.* 1998). Linkage disequilibrium between the oak chloroplast and mitochondrial haplotypes was very high, but not complete. The lack of complete linkage disequilibrium appeared to result from parallel independent evolution of the same mtDNA marker in two different geographical regions (Dumolin-Lapègue *et al.* 1998). Unfortunately, too few mitochondrial haplotypes were detectable to assess phylogenetic congruence between the chloroplast and mitochondrial lineages within individuals with confidence. In another study of the systematic relationships among *teosinte* races, mitochondrial and chloroplast RFLP variants were not in complete linkage disequilibrium across different races (Timothy *et al.* 1979). However, because this study focused only on taxonomic associations among and not within populations, we cannot infer whether new combinations of mitochondrial and chloroplast haplotypes resulted from rare biparental inheritance or some other process such as parallel evolution. Finally, in a study of gynodioecious *Beta vulgaris* spp. *maritima*, high (0.97) but not complete linkage disequilibrium

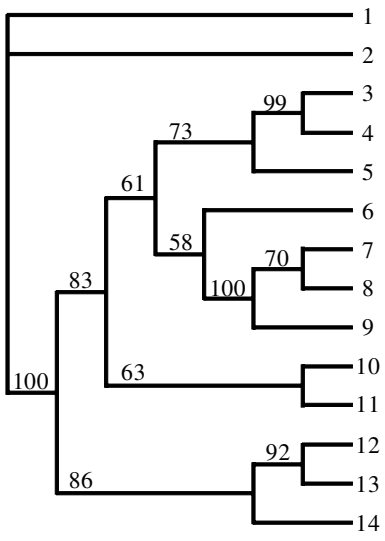

Figure 5. Majority-rule consensus NJ tree combining cpDNA and mtDNA data for the 14 haplotypes in this study. Different individuals are represented by numbers (1–14). Numbers on branches are bootstrap values for 10 000 bootstrap replicates.

between cpDNA and mtDNA haplotypes was found (Desplanque *et al.* 2000). The lack of complete linkage disequilibrium was ascribed in part to convergent evolution of strings of mononucleotide residues in the cpDNA (Desplanque *et al.* 2000).

It is interesting to contrast the intraspecific patterns expressed in these angiosperms with those in conifers where mtDNA is maternally transmitted and cpDNA is biparentally transmitted. Although Latta & Mitton (1997) did not calculate linkage disequilibrium between the genomes, they found much higher levels of population structure in the mtDNA than the cpDNA suggesting that linkage disequilibrium is minimal.

Our results showing some use of mtDNA RFLP data for assessing intraspecific phylogenetic and population genetic patterns support those of Desplanque *et al.* (2000). Mitochondrial RFLP variation sampled by hybridization of known mitochondrial genes has not been considered ideal for phylogenetic reconstruction at higher levels (Palmer 1992; Wu *et al.* 1998). In particular, it is thought that there may be 'hot spots' at which recombination is more common than at other sites, resulting in the generation of the same RFLP patterns in two evolutionarily independent lineages (Palmer 1992; Fragoso *et al.* 1989). There is evidence in *Arabidopsis* that specific rearrangements in the mtDNA depend on the nuclear background (Martinez-Zapater *et al.* 1992; Sakamoto *et al.* 1996). Although recombinational hot spots may be adding noise to our data, if the same RFLP patterns are evolving independently more than once they do not seem to be overwhelming all of the phylogenetic signal. If recombination occurred primarily at hot spots instead of randomly throughout the genome, we would expect to see inconsistencies in the chloroplast and mitochondrial phylogenies resulting from parallel evolution.

When the chloroplast and mitochondrial genomes share transmission histories, combining data from the two genomes may prove useful for phylogenetic reconstruction in plants. The observed congruence between the intraspecific phylogenetic signal in the non-coding cpDNA regions and the RFLP sites flanking mtDNA genes in *S. vulgaris* suggest that combining these two types of data may be useful for phylogeographical studies. In contrast to the pattern in a study of *B. vulgaris* spp. *maritima* (Desplanque *et al.* 2000), mtDNA haplotypes were nested within the cpDNA haplotypes in *S. vulgaris*. Thus, each genome might resolve relationships among individuals at a slightly different phylogenetic level. Figure 5 represents the phylogeny among the cytotypes of our 14 individuals when both the cpDNA and the mtDNA data sets are combined. This consensus phylogeny is fully resolved and has better overall bootstrap support than either of the phylogenies based on one organellar data set (see figure 3). Combining information from both genomes might allow a more complete understanding of intraspecific evolutionary patterns and potentially can be applied to studies ranging from detection of historical relationships among CMS factors in gynodioecious species to the invasive history of weedy plants.

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