Methods of analysis of chloroplast genomes of C₃, Kranz type C₄ and Single Cell C₄ photosynthetic members of Chenopodiaceae

Richard M. Sharpe¹†, Bruce Williamson-Benavides¹,²†, Gerald E. Edwards²,³ and Amit Dhingra¹,²*

Abstract

Background: Chloroplast genome information is critical to understanding forms of photosynthesis in the plant kingdom. During the evolutionary process, plants have developed different photosynthetic strategies that are accompanied by complementary biochemical and anatomical features. Members of family Chenopodiaceae have species with C₃ photosynthesis, and variations of C₄ photosynthesis in which photorespiration is reduced by concentrating CO₂ around Rubisco through dual coordinated functioning of dimorphic chloroplasts. Among dicots, the family has the largest number of C₄ species, and greatest structural and biochemical diversity in forms of C₄ including the canonical dual-cell Kranz anatomy, and the recently identified single cell C₄ with the presence of dimorphic chloroplasts separated by a vacuole. This is the first comparative analysis of chloroplast genomes in species representative of photosynthetic types in the family.

Results: Methodology with high throughput sequencing complemented with Sanger sequencing of selected loci provided high quality and complete chloroplast genomes of seven species in the family and one species in the closely related Amaranthaceae family, representing C₃, Kranz type C₄ and single cell C₄ (SSC₄) photosynthesis six of the eight chloroplast genomes are new, while two are improved versions of previously published genomes. The depth of coverage obtained using high-throughput sequencing complemented with targeted resequencing of certain loci enabled superior resolution of the border junctions, directionality and repeat region sequences. Comparison of the chloroplast genomes with previously sequenced plastid genomes revealed similar genome organization, gene order and content with a few revisions. High-quality complete chloroplast genome sequences resulted in correcting the orientation the LSC region of the published Bienertia sinuspersici chloroplast genome, identification of stop codons in the rpl23 gene in B. sinuspersici and B. cycloptera, and identifying an instance of IR expansion in the Haloxylon ammodendron inverted repeat sequence. The rare observation of a mitochondria-to-chloroplast inter-organellar gene transfer event was identified in family Chenopodiaceae.

Conclusions: This study reports complete chloroplast genomes from seven Chenopodiaceae and one Amaranthaceae species. The depth of coverage obtained using high-throughput sequencing complemented with targeted resequencing of certain loci enabled superior resolution of the border junctions, directionality, and repeat region sequences.

*Correspondence: adhingra@wsu.edu
†Richard M. Sharpe and Bruce Williamson-Benavides contributed equally to this work
¹ Department of Horticulture, Washington State University, Pullman, WA 99164, USA
Full list of author information is available at the end of the article
sequences. Therefore, the use of high throughput and Sanger sequencing, in a hybrid method, reaffirms to be rapid, efficient, and reliable for chloroplast genome sequencing.

Introduction

Plastids convert light energy into chemical energy and are an essential site for the biosynthesis of pigments, lipids, several amino acids and vitamins [1, 2]. Comparative genomics studies have facilitated the understanding of chloroplast genome organization and phylogenetic relationships [3–5]. Additionally, availability of chloroplast genome sequences can be useful for constructing transformation vectors to enable chloroplast transformation via homologous recombination [6, 7].

Higher plant chloroplast genomes possess a characteristic organization comprising a Large Single Copy (LSC), a Small Single Copy (SSC) and two Inverted Repeat (IRa and IRb) regions, with only a few exceptions, e.g. in *Pisum sativum* and some other legumes [8–10]. Several methods have been used to sequence chloroplast genomes in plants, including primer walking [11–14] and high-throughput sequencing (HTS) [15]. HTS, both with isolated chloroplast DNA [16–18] and total cellular DNA [19–21], has been employed to generate physical maps of the chloroplast genome. However, the junctions of LSC/IRa, IRa/SSC, SSC/IRb and IRb/LSC need to be resolved using additional experimentation [22]. Genome sequencing and subsequent assembly of the chloroplast genome can be challenging due to variable IR borders; presence of chloroplast genome sequences in the nuclear genome; sequence homology between chloroplast and mitochondrial genes, such as the NAD(P)H and NADH dehydrogenase genes; as well as the NAD(P)H genes being distributed throughout the chloroplast genome [3, 23–28].

Chloroplasts, the green plastids in plants, are the site of photosynthesis where Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), captures CO₂ with synthesis of 3-phosphoglyceric acid (3PGA) in the Calvin-Benson cycle, leading to the synthesis of carbohydrates and cellular constituents. Three major types of oxygenic photosynthesis are known to date: C₃, C₄, and Crassulacean acid metabolism (CAM). In C₃ plants, Rubisco directly fixes atmospheric CO₂ introducing carbon into the Calvin-Benson cycle. In C₄ and CAM photosynthesis, CO₂ is first captured by phosphoenolpyruvate carboxylase (PEPC) with synthesis of 4-carbon organic acids which are sequestered in a spatial manner in C₄ plants and a temporal manner in CAM plants. Decarboxylation of the 4-carbon organic acid generates a CO₂-rich environment around Rubisco [29]. This mechanism suppresses the oxygenation reaction by Rubisco and the subsequent energetically-wasteful photorespiratory pathway. C₄ plants function with spatial separation of two types of chloroplasts, one type supports the fixation of atmospheric CO₂ by PEPC and synthesis of C₄ acids, while the other type utilizes the CO₂ generated from decarboxylation of C₄ acids in the Calvin Benson cycle. In Kranz type C₄ plants mesophyll chloroplasts support fixation of atmospheric CO₂ by PEPC, while bundle sheath chloroplasts utilize CO₂ generated by decarboxylation of C₄ acids. The unique single-cell C₄ (SCC₄) plants perform C₄ photosynthesis within individual chlorenchyma cells with spatial separation of two types of chloroplasts. One type supports capture of atmospheric CO₂ by PEPC and the other assimilates the CO₂ generated by decarboxylation of C₄ acids in the Benson-Calvin cycle [30–32].

Among dicot families, the Chenopodiaceae and Amaranthaceae families have by far the largest number (~800) of C₄ species, with up to 15 distinct lineages [33]. Although they are currently recognized as separate families in a clad, they are known to be closely related [34]. Chenopodiaceae species are acclimated to diverse ecosystems from xeric to more temperate salt marshes, including highly saline soils; while Amaranthus species predominantly occur in tropical and subtropical regions. The Chenopodiaceae family is very diverse, with six structural forms of Kranz anatomy present among its members [35]. Furthermore, it is the only family known to have SCC₄ species [34]. Phylogenetic analyses have identified independent origins of C₄ photosynthesis. In particular, the results allude to the unique independent origins of C₄ in subfamily Suaedoidae, including Kranz C₄ anatomy in *Suaeda* species and two independent origins of the SCC₄ system in *Bienertia* and *Suaeda* [33, 36–39]. In general the causation of these independent events is hypothesized to be a result of the harsh environments induced by global climate change and periodic reductions in CO₂ content over the past 35 million years [40, 41].

In this study, complete chloroplast genome sequences for seven Chenopodiaceae species and one Amaranthaceae species were generated using whole leaf tissue genomic DNA (gDNA) via HTS complemented with Sanger sequencing of targeted loci. The species analyzed were: *Bassia muricata* (C₄-Kochioioid anatomy, tribe Camphorosmoideae), *Haloxylon ammodendron* (C₄-Salsoloid anatomy, tribe Salsoleae), *Bienertia cyclop-tera* (C₃: SCC₄-tribe Suaedae), *Bienertia sinuspersici* (C₄: SCC₄-tribe Suaedae), *Suaeda aralocaspica* (SCC₄-tribe Suaedae), *Suaeda eltonica* (C₄-Schoberioid
type anatomy, tribe Suaedeae), and Suaeda maritima (C3-tribe Suaedeae). The chloroplast genome from Ama-
ranthus retroflexus (C4-Atriplicoid type anatomy, family Amaranthaceae, tribe Amarantheae), was also sequenced
and used for comparative analysis. These dicot species include representative species having C3-type photosyn-
tesis with monomorphic chloroplasts, and C4 species having dimorphic chloroplasts for C4 function includ-
ing its development in Kranz anatomy versus individual chlorenchyma cells. The purpose of the present study was
to determine among these representative dicot species whether the chloroplast genomes between C3 and C4 spe-
cies, and the chloroplast genomes between the various forms of C4, are highly conserved (in size and composi-
tion), and the degree of difference between the species.

Results and discussion

Genome sequencing and assembly

A summary of the sequencing data obtained from Illumina sequencing and assembly of A. retroflexus, B. muricata, B. cycloptera, B. sinuspersici, H. ammod-
endron, S. aralocaspica, S. eltonica, and S. maritima chloroplast genomes is presented in Table 1. Three
large contigs with overlapping 5′ and 3′ regions were generated during genome assembly for A. retroflexus,
B. muricata, B. cycloptera, B. sinuspersici, H. ammodendron, S. aralocaspica, and S. maritima. These three
contigs were identified as LSC, SSC, and IR via BLAST homology alignment [42], GE-Seq—Annotation of
Organellar Genomes [43] and DOGMA gene identity prediction [44]. The overlapping regions were present
at all four possible junctions when the IR region was reverse complemented (LSC-IR, IR-SSC, SSC-IR, and
IR-LSC). These overlapping areas ranged from 19 to 51 nt (illustrated in Additional file 1: Figure S1 with B.
cycloptera). The directionality of the LSC, SSC and IR, and all overlapping aligned junctions were validated
via Sanger sequencing of both strands of the ampli-
cons generated from these regions (Additional file 2: Table S1; Additional file 1: Figure S1). For S. eltonica,
the LSC-IRa and IRb-LSC overlapping regions were 23 nt long and were validated with Sanger sequencing
(Additional file 2: Table S1). The IRa-SSC and SSC-IRb sections were both missing a 1,475 nt section in the
IRa and IRb borders. The 300 nt sequence contiguous to the 1475 nt section had a low GC content of 19%.
A possible cause of the shortened contig flanking the IR-1475 area may be due to the low GC content value
which could impact the accuracy of the HTS genome assembly [45]. The 1,475 nt section was sequenced by
primer walking and Sanger sequencing (Additional file 2: Table S1). The GC content in the 1,475 nt region
and IR was 31.3 and 42.1%, respectively.

The average base depth of coverage for the eight assembled chloroplast genomes ranged from 1553-
to 5998-fold. For accurate assembly a minimum of 30–40× sequence coverage is recommended [46–48].
In this study, the only areas with less than 40× average coverage were identified in the last 1–3 nucleotides of
the IRb sequence for each of the eight genomes. This is expected due to the assembler algorithm parameters. The
end of the IRb and the beginning of the LSC were con-
catenated and these sections were remapped. Remapped
coverage results were reported to be above 40× for the
IRb ends and surrounding areas. The eight assembled
genomes (0.8/0.9 for the read length fraction/similarity
fraction mapping) were also compared with a more strin-
gent remapping of the reads to the contigs of 0.99/0.99
length fraction/similarity fraction. Analyses with both
levels of stringency show almost identical assembly min-
umum-coverage and average-coverage for the eight spe-
cies sequenced in this study (Additional file 3: Figure S2).

Table 1 Sequencing and assembly data when length fraction and similarity fraction parameters were set to 80 and 90 respectively during read mapping in the chloroplast genomes of eight Chenopod species

| Variable                          | A. retroflexus | B. cycloptera | B. muricata | B. sinuspersici | H. ammodendron | S. aralocaspica | S. eltonica | S. maritima |
|-----------------------------------|----------------|---------------|-------------|-----------------|----------------|----------------|-------------|-------------|
| Total number of reads             | 94,491,120     | 73,061,587    | 61,098,096  | 80,215,373      | 81,126,072     | 86,825,544     | 66,358,800  | 87,411,736  |
| Mean read length (nt)             | 78.98          | 79.04         | 79.44       | 79.66           | 78.5           | 78.83          | 78.35       | 79.88       |
| Minimum coverage (bases)          | 22             | 12            | 37          | 39              | 12             | 55             | 25          | 33          |
| Maximum coverage (bases)          | 29,721         | 6,135         | 23,533      | 15,558          | 15,284         | 23,266         | 14,652      | 6,991       |
| Average coverage (bases)          | 3,649.67       | 1,553.12      | 3,204.28    | 5,998.08        | 1,357.20       | 4,864.44       | 1,591.56    | 4,111.85    |
| Total (%) of reads assemble to genome | 7.37          | 4.12          | 10.01       | 14.39           | 3.44           | 10.42          | 4.55        | 8.95        |
Overall, the assembly and subsequent Sanger sequencing-based validation generated high quality and complete chloroplast genomes with all possessing a quadripartite structure as reported in other land plant species.

**Size, organization and gene content of the chloroplast genomes**

The size of the chloroplast genomes from the eight species ranged from 146,634 to 161,251 nt (Table 2). As expected, each chloroplast genome included a pair of inverted repeat regions, IRa and IRb, separated by an SSC and an LSC region (Table 2 and Additional file 4: Figure S3). With one exception, the size of the IRs ranged from 23,461 to 25,213 nt. (Table 2). The *H. ammodendron* inverted repeat sequence presented an instance of IR length expansion (29,061 nt) compared to the other seven species. The GC content was similar among the eight species and for all the plastomes, LSC, SSC and IRs it ranged from 36.4–36.6, 34.1–34.6, 29.1–30.2 and 42.1–43.0%, respectively (Table 2). All chloroplast genomes contained a similar number of protein coding, ribosomal, and tRNA genes. The number of genes and tRNAs ranged from 113 to 116 and 27 to 29, respectively in the eight genomes (Table 3 and Additional file 4: Figure S3). For seven of eight species, 60.1–61.9% of the chloroplast sequence consisted of coding region, which included 52.7–54.3% of protein coding genes and 7.4–7.9% of RNA genes. The *S. eltonica* chloroplast genome was composed of 56.8% coding region including 48.9% of protein coding genes and 7.9% of RNA genes. This difference between *S. eltonica* and the rest of chloroplast genomes is possibly due to the higher repeat content in intergenic sequences of the *S. eltonica* chloroplast genome (Table 4 and Fig. 1).

Gene order and content were largely conserved among the eight chloroplast genomes in this study. However, some structural rearrangements, gene losses and IR expansions were identified. The genes ycf15, ycf68, and rpl23 were identified as pseudogenes due to the presence of internal stop codons. The ycf15 and ycf68 genes are quite commonly classified as pseudogenes in angiosperms [23, 49]. The rpl23 is also classified as a pseudogene in some species such as the *Fagopyrum* spp., buckwheat, and spinach as well as *Suaeda* and *Haloxylon* species [22, 23, 50, 51]. In *S. eltonica*, rpl23 was not predicted to be in the chloroplast genome by GeSeq but it was identified as a pseudogene via the BLAST sequence analysis [42]. No stop codons were identified in the rpl23 of a previously published *B. sinuspersici* chloroplast genome [52]. In this study, 4 stop codons were identified at the same locations for *B. sinuspersici* and its close relative *B. cycloptera*.

At least one complete copy of the ycf1 gene was identified in the eight chloroplast genomes (total length of 5.3–5.6 Kb). In seven out of the eight chloroplast genomes, a duplicated ycf1 pseudogene (1,000–1,300 nt) was found at the IRa-SSC boundary. This is a common feature found in other species [23, 53]. In the case of *H. ammodendron*, there is a complete duplication of the ycf1 gene, therefore the *H. ammodendron* chloroplast genome has two full copies in the IR-SSC borders. The complete duplication of the ycf1 gene in *H. ammodendron* leads to the previously mentioned IR expansion (Additional file 4: Figure S3). This phenomenon has also been observed in *Amphilophium, Adenocalymma, Anemopaeogna*, and *Fagopyrum* species; these species possess an expanded IR region and two full-length copies of ycf1 gene [23, 54, 55]. The IRs for the other seven species are variable in length. In *A. retroflexus*, *B. muricata*, *B. cycloptera*, *B. sinuspersici*, *Fagopyrum*, *S. aralocaspica* and *S. maritima*, the IR includes the duplicated ycf1 pseudogene (1–1.3 kb) (Additional file 4: Figure S3). A small segment of the ycf1 gene is also duplicated in *V. vinifera*, *S. oleracea* and *B. vulgaris*. In *S. eltonica*, the IR has expanded to include the trnH-GTG and a fragment of the psbA gene (Additional file 4: Figure S3).

---

**Table 2** A summary of the complete chloroplast genome, IR, LSC and SSC length (nt) and GC content from *A. retroflexus*, *B. muricata*, *B. cycloptera*, *B. sinuspersici*, *H. ammodendron*, *S. aralocaspica*, *S. eltonica* and *S. maritima*

| Species          | Complete chloroplast genome | IR | LSC | SSC |
|------------------|-----------------------------|----|-----|-----|
|                  | Size (bp) | GC content (%) | size (bp) | GC content (%) | size (bp) | GC content (%) |
| *A. retroflexus* | 150,786 | 36.67 | 24,353 | 42.64 | 83,963 | 34.51 | 18,117 | 30.20 |
| *B. muricata*    | 151,593 | 36.61 | 24,355 | 43.00 | 84,288 | 34.50 | 18,595 | 29.42 |
| *B. cycloptera*  | 153,341 | 36.50 | 24,942 | 42.92 | 84,541 | 34.42 | 18,916 | 29.69 |
| *B. sinuspersici*| 153,334 | 36.65 | 24,949 | 43.00 | 84,490 | 34.57 | 18,946 | 29.49 |
| *H. ammodendron* | 161,251 | 36.42 | 24,361 | 42.99 | 84,236 | 34.18 | 18,893 | 29.42 |
| *S. aralocaspica*| 146,634 | 36.53 | 24,942 | 42.94 | 81,878 | 34.42 | 21,834 | 29.30 |
| *S. eltonica*    | 148,729 | 36.44 | 25,213 | 42.72 | 83,482 | 34.11 | 18,103 | 29.17 |
| *S. maritima*    | 152,011 | 36.45 | 24,353 | 42.64 | 83,963 | 34.51 | 18,117 | 30.20 |
The biological significance of this duplication remains unknown.

Annotation of the ycf15 gene with the Dual Organellar Genome Annotator (DOGMA) [44] shows variability in terms of its physical location. In A. retroflexus, B. vulgaris and S. eltonica, the ycf15 is located between the rps12 and trnV-GAC. In B. cycloptera, B. muricata, B. sinuspersici, H. ammodendron, S. aralocaspica, and S. maritima the ycf15 is located between ycf2 and trnL-CAA. The ycf15 as well as other genes, such as the ycf2, psbA, clpP, and matK, have been reported to have variable physical location in different plants [56–59]. The genes ycf3, clpP, rpoc1, and rpl2 have been found to have a variable number of introns among and within some taxonomic groups [23]. The gain or loss of introns in these genes have occurred independently in several lineages of flowering plants [23, 60]. However, no differences were found in the number of introns among the eight species; the ycf3, clpP, rpoc1, and rpl2 contain 2, 2, 1, and 0 introns, respectively.

The orientation of the SSC region in A. retroflexus, and B. muricata differs from the orientation of the SSC in B. cycloptera, B. sinuspersici, H. ammodendron, S. aralocaspica, S. maritima and S. eltonica (Additional file 4: Figure S3). The SSC orientation has been shown to exist in the two different states within individual plants [61–64]. Therefore, SSC variation observed among taxa in this study is likely due to alternative states of the SSC region within individual plants. Although there was some variation in the SSC orientation, the number and content of

The biological significance of this duplication remains unknown.

Annotation of the ycf15 gene with the Dual Organellar Genome Annotator (DOGMA) [44] shows variability in terms of its physical location. In A. retroflexus, B. vulgaris and S. eltonica, the ycf15 is located between the rps12 and trnV-GAC. In B. cycloptera, B. muricata, B. sinuspersici, H. ammodendron, S. aralocaspica, and S. maritima the ycf15 is located between ycf2 and trnL-CAA. The ycf15 as well as other genes, such as the ycf2, psbA, clpP, and matK, have been reported to have variable physical location in different plants [56–59].

The genes ycf3, clpP, rpoc1, and rpl2 have been found to have a variable number of introns among and within some taxonomic groups [23]. The gain or loss of introns in these genes have occurred independently in several lineages of flowering plants [23, 60]. However, no differences were found in the number of introns among the eight species; the ycf3, clpP, rpoc1, and rpl2 contain 2, 2, 1, and 0 introns, respectively.

The orientation of the SSC region in A. retroflexus, and B. muricata differs from the orientation of the SSC in B. cycloptera, B. sinuspersici, H. ammodendron, S. aralocaspica, S. maritima and S. eltonica (Additional file 4: Figure S3). The SSC orientation has been shown to exist in the two different states within individual plants [61–64]. Therefore, SSC variation observed among taxa in this study is likely due to alternative states of the SSC region within individual plants. Although there was some variation in the SSC orientation, the number and content of
genes was the same among the eight species. The only exception is the presence of a trnU-TCA in the SSC of *H. ammodendron*.

**Repeat structures and microsatellites**

Seven out of the eight chloroplast genomes had 45–58 repeats, which ranged in length from 30 to 73 nt per repeat (Fig. 1). The majority of these repeats were shown to be between 30 and 40 nt in length. In the *S. eltonica* chloroplast genome, repeat analysis with REPuter [65] found a total of 174 repeats which ranged from 30 to 145 nt in length (Fig. 1). The number of repeats was similarly distributed among species for repeats found in intergenic regions and intron/exons (Table 4). An exception was *S. eltonica* in which a majority (80%) of repeats were located in the intergenic regions. Four species possessed reverse repeats; *S. maritima* and *S. aralocaspica* had one, *B. muricata* had two, and *S. eltonica* had four.

The presence of repeats varied for the genes ycf1, ycf2, ycf3, and psaA. Repeats were present in the gene ycf1 except for *A. retroflexus, S. aralocaspica* and *S. maritima*. All chloroplast genomes possessed repeats in the ycf2 gene except for *H. ammodendron*. Repeats in the introns of the ycf3 gene were only present in the *A. retroflexus*, *B. cycloptera*, and *B. sinuspersici*. All species presented at least one repeat in the psaA gene and *H. ammodendron* presented the highest number with six repeats.

Microsatellites, or simple sequence repeats (SSRs), were identified in the eight chloroplast genomes. The total number of microsatellites ranged from 41 to 72 of which the majority, 36–64, represent mononucleotide repeat microsatellites (Table 5). The complete list of microsatellites identified for each of the eight chloroplast genomes and their positions in the respective genomes is provided in Additional file 5: Table S2.

**Comparison of *Amaranthus retroflexus* chloroplast genome with previously sequenced *Amaranthus* spp. chloroplast genomes**

*Amaranthus retroflexus*, commonly known as pigweed, is used as a vegetable for human consumption as well as for fodder. It is the most widely distributed and damaging *Amaranthus* weed in the US and the world [66].

![Fig. 1](image-url) **Fig. 1** Histogram of number of repeated sequences (>30 nt) in length identified with REPuter for nine chloroplast genomes.

| Table 5 | Total number of microsatellites identified with MISA software for eight chloroplast genomes |
|---------|---------------------------------|
| **Species name** | **Mono** | **Di** | **Tri** | **Compound** | **Total** |
| *Amaranthus retroflexus* | 44 | 2 | 0 | 5 | 51 |
| *Bassia muricata* | 40 | 1 | 0 | 1 | 42 |
| *Bienertia cycloptera* | 41 | 1 | 0 | 2 | 44 |
| *Bienertia sinuspersici* | 48 | 0 | 0 | 3 | 51 |
| *Haloxylon ammodendron* | 46 | 0 | 0 | 2 | 48 |
| *Suaeda aralocaspica* | 47 | 3 | 0 | 1 | 51 |
| *Suaeda eltonica* | 64 | 2 | 1 | 5 | 72 |
| *Suaeda maritima* | 36 | 4 | 0 | 1 | 41 |
Availability of the *A. retroflexus* chloroplast genome provides an important tool for accurately monitoring the spread of this species and identifying possible hybridizations. Microsatellites were previously identified for *Amaranthus* spp. [67]. Six out of the nine polymorphic microsatellites were shown to be polymorphic between *A. hypochondriacus* and *A. retroflexus* (Table 6). Most of these microsatellites were located in the LSC regions and represented A or T mononucleotide repeats. SSRs can serve as molecular markers for future molecular breeding for *Amaranthus* spp. which are considered as emerging crops [67]. The chloroplast genomes of four *Amaranthus* spp; *A. hypochondriacus*, *A. cruentus*, *A. caudatus*, and *A. hybridus*, have been reported previously [67]. The *A. hypochondriacus* genome (GenBank accession KX279888.1) is 150,725 nt and the quadripartite regions of LSC, SSC and 2 IRs consist of 83,873, 17,941 and 24,352 nts, respectively. These sizes are very similar to the lengths of the *A. retroflexus* chloroplast genome reported in this study (Table 2). BLAST analysis showed a 99% sequence similarity between the chloroplast genomes of *A. hypochondriacus* and *A. retroflexus*.

**Comparative analysis of the *B. sinuspersici* chloroplast genomes**

Kim et al. [52], and Caburatan et al. [68] previously reported the chloroplast genome of *B. sinuspersici* (GenBank accession no. KU726550). Compared to our results with *B. sinuspersici* (Table 2), the size of their genome (153,472 nt) is 138 nt larger; the LSC and SSC in their study are 84,560 nt and 19,016 nt in size, respectively which is 70 nt larger than in our study (Table 2). The IR was reported to be 24,948 nt in length, versus 24,949 nt length in this study. The increase in length in the published *B. sinuspersici* chloroplast genome [52] is predominantly located at the LSC-IRa and SSC-IRb junctions, which has a repeat of 72 and 13 nts respectively. The two repeats are separated by spacer sequences of 1nt in the LSC-IRa junction and 48 nt in the SSC-IR junction. The 72 and 13 nt sequences were present just once in the *B. sinuspersici* chloroplast genome presented in the current study. The presence of a single occurrence of the 72 and 13 nt sequence in the genome was validated by Sanger sequencing of loci in question for both IRb-LSC and LSC-IRa loci (Additional file 2: Table S1). Further comparison of the two *B. sinuspersici* genomes identified 18 SNPs and 9 indels. In the published *B. sinuspersici* chloroplast genome, the LSC is inverted with respect to the rest of the sequence (IRa + SSC + IRb). In our study, the orientation of the LSC was validated using Sanger sequencing of PCR amplicons spanning the junctions IRb-LSC and LSC-IRa (Additional file 2: Table S1). As described above, there were also differences in the presence of stop codons in the rpl23 gene. In the previous study [68] a total of 110 unique genes were reported; a total of a total of 114 genes were identified in the current study (Additional file 4: Figure S3).

Differences between the previously reported chloroplast genome of *B. sinuspersici* compared to the current study likely stems from how the Celera assembler algorithm and the CLC algorithm process the read data. Each of these algorithms have their inherent pros and cons [69]. The assembly parameters for the previous *B. sinuspersici* chloroplast genome were not reported. Also, the chloroplast genome loci that were found to be different within the two previous versions [52, 68] were not resequenced. The chloroplast genome of *B. sinuspersici* presented in this study showed a minimum, maximum and

| SRR location in *A. hypochondriacus* chloroplast genome (nt) | Repeat unit | Number of repeats |
|-------------------------------------------------------------|-------------|------------------|
| 5,572–5,583                                                 | T           | 12               |
| 7,526–7,537                                                 | T           | 12               |
| 46,236–46,253                                               | TA          | 9                |
| 46,573–46,588                                               | AT          | 8                |
| 47,532–47,543                                               | A           | 12               |
| 52,543–52,557                                               | T           | 15               |
| 54,580–54,591                                               | A           | 12               |
| 65,482–65,496                                               | T           | 15               |
| 70,858–70,869                                               | A           | 12               |
| 79,076–79,087                                               | T           | 12               |
| 112,930–112,944                                             | T           | 15               |
| 116,360–116,371                                             | TATT        | 3                |

The table above shows the polymorphic simple sequence repeats (SSRs) in *Amaranthus hypochondriacus* and *A. retroflexus*.
average coverage of 37, 23,533, 3,204.28 nt. Furthermore, areas of ambiguity were validated via Sanger sequencing of PCR amplicons generated from selected loci. The combination of the assembly strategy utilized, and resequencing of loci, resulted in the generation of an improved version of the *B. sinuspersici* chloroplast genome.

Analysis of the two closest SCC4 related species, *B. cycloptera* and *B. sinuspersici*, chloroplast genomes showed a 99.70% sequence similarity between both sequences. *B. cycloptera* and *B. sinuspersici* chloroplast genomes differed in overall length by seven nt. *B. sinuspersici* IR, and SSC regions were larger than the *B. cycloptera* by 44 nt and *B. cycloptera's* LSC region was larger by 51 nt. The difference in size was due to changes in the intergenic region, length, and number of repeat regions. Number of genes with introns and repeats was the same between the two species. *B. cycloptera* had two larger repeats, one between 40–44 nt and the second greater than 45 nt. *B. sinuspersici* had one smaller repeat of 30–34 nt. Both species had the same number and identity of protein-coding, tRNA, and tRNA genes.

**Comparative analysis of *Haloxylon ammodendron***

chloroplast genomes: a case of transfer of mitochondrial DNA to the plastid genome

The chloroplast genome of *H. ammodendron* was published recently (GenBank accession no. KF534478) [70]. The size of the chloroplast genome was reported to be 151,570 nt, with a LSC of 84,214 nt, SSC of 19,014 nt and two IRs of 24,171 nt [70]. In our study, the genome assembled to a size of 161,251 nt, which is 9,681 nts larger. BLAST alignment of the two genomes indicated that the additional 9,681 nts were derived from the expansion of the IR, which is 4,868 nt in size. The IRs of *H. ammodendron* chloroplast genome in our study were 29,061 nt long. This represents an expansion of the IR that is also observed in *S. eltonica* (Table 2). Expansion and gene duplication are common phenomenon in the IR regions of chloroplast genomes [71, 72]. In grasses, the junctions between the IR and SSC regions are highly variable with the ends of genes ndhF, rps19, and ndhH repeatedly migrating into and out of the adjacent IR regions [73]. BLAST alignment between the two genomes revealed that the first 115 nt showed 78% homology with chloroplast sequences of *H. persicum*, and *H. ammodendron* present in the IRs of the published genomes [70]. The following region of 671 nt did not show any significant similarity and the last 4,028 nt showed homology to mitochondrial genome sequences. The highest significant hit (94%; E value = 0.0) for this 4,028 nt section resembled *Beta vulgaris* and *Spinacia oleraceae*. Interestingly, annotation identified the mitochondrial gene Cytochrome b (cob) in this 4,814 nt section, although the plastid copy had a nonsense mutation that resulted in a premature stop codon.

Evidence showing transfer of mitochondrial DNA (mtDNA) or nuclear DNA (nucDNA) to the plastid genome in plants had been lacking until recently. A few recent reports indicate that plastid genomes of carrot [74], milkweed [75], and bamboo [73] show evidence of gene transfer from mitochondria to the plastid. *Daucus carota* has a 1.5 kb region of mitochondrial origin located in the rps12-trnV intergenic space of the chloroplast genome. Only *Daucus* species and the close relative *Cuminum cyminum* (cumin) show the mitochondrial-to-chloroplast gene transfer [74]. It was concluded that a mitochondria-located DNA segment present in the ancestor of the Apiaceae subsequently moved to the plastid genome in the common ancestor of *Daucus* and cumin. *Asclepias syriaca*, the common milkweed, has a 2.4 kb mtDNA-like insert in the chloroplast genome. The mtDNA-like insert contains an intact exon of the mitochondrial ribosomal protein (rpl2) as well as a noncoding region [75]. There was a 92% sequence identity between the mitochondrial and plastid version of rpl2 in *A. syriaca* whereas the plastid copy had a nonsense mutation resulting in a premature stop codon. Similarly, the IR region in three herbaceous bamboo species of the *Pariana* genus had a 2.7 kb insertion [73]. The insertion was located in the trnL-trnF-CAU-GUU intergenic spacer region. Potential variations of this insertion in another *Pariana* species and species from the sister genus *Eremitis* were also reported. These studies suggest that the transferred sequence may have originated as a single event in a common ancestor; however, the inserted sequence evolved rapidly [73].

In our study, the inserted section in *H. ammodendron* had an average coverage of 1,320X reported from the stringent 0.99–0.99 length fraction/similarity mapped to the assembly. The coverage corresponded well to the average coverage of 1,269X for other regions. Five kb regions flanking the 4.8 kb section had a similar coverage of 929 and 1,066 reads. The Illumina reads from *H. ammodendron* (0.99–0.99 99 length fraction/similarity fraction) were mapped to three randomly selected intronless mitochondrial genes identified from the *H. ammodendron* assembly [73]. The mitochondrial genes ccmFN, matR and rnr26 showed a much lower average coverage of 242, 211, and 447, respectively. Thus, the mapping results supported the result that the insertion in the *H. ammodendron* chloroplast genome was not an artifact of the assembly.

Since the *H. ammodendron* chloroplast genome reported in this study was assembled from reads obtained using total cellular DNA, the origin of 4.8 kb insert was confirmed using a complementary Sanger
sequencing approach. Amplified segments flanking the entire 4,814 nt insertion were 6,607, 7,172 and 8,132 nt long with the forward and the reverse primers flanking the ycf1 and ndhF genes, respectively (Fig. 2; Additional file 6: Table S3). Primers flanking both the ycf1 and ndhF genes coupled with a primer annealing to the middle section of the inserted region produced amplicons of predicted sizes of 3,810 and 4,458 nt (Fig. 2; Additional file 6: Table S3). The PCR results were the first line of confirmation since no PCR amplification should be expected from the published _H. ammodendron_ chloroplast genome due to primer mismatch. Interestingly, expected DNA amplicons were also obtained when PCR was performed on _Haloxylon persicum_, a close relative of _H. ammodendron_ (Fig. 2). A total section of 6.2 kb, including the 4,814nt inserted section, was sequenced and validated via primer walking (Additional file 6: Table S3). The sequenced amplicon results produced a 100% alignment match to the _H. ammodendron_ chloroplast genome assembly obtained in this study. Amplification and sequence homology validation of the 4,814 nt section confirmed the presence of the insertion in the _H. ammodendron_ chloroplast genome. The integration of intracellularly transferred DNA into the intergenic region of ycf1 and ndhF would be expected as insertion in the coding region would have disrupted gene function.

This is the first report to document mitochondria-to-chloroplast interorganellar gene transfer in the Chenopodiaceae family and the fourth example in angiosperms. However, the mechanisms underlying the transfer of genomic DNA fragments remains to be elucidated [73–75].

![Fig. 2](image-url) PCR amplicons flanking a 4.8 kb insertion in the chloroplast genome of _H. ammodendron_ (2–6) and _H. persicum_ (8–12). Agarose gel electrophoresis of PCR products (a); diagram representing location and length of each amplicon (b). Expected amplicon sizes are 6607 (2 and 8), 7172 (3 and 9), 8132 (4 and 6), 3810 (5 and 11), and 4458 (6 and 12). Primers for PCRs 2–4 and 8–10 flank the ycf1 and ndhF genes. Primers for PCRs 5 and 11 flank the middle section of the 4.8 kb insertion and the ndhF gene. Primers for PCRs 6 and 12 flank the ycf1 gene and the middle section of the 4.8 kb insertion. 1 and 7: exACTGene DNA Ladders 1 kb DNA Ladder.
Chloroplast genomes among different types of C4 species versus C3 species

The 8 chloroplast genomes studied, include the C3 species S. maritima and 7 forms of C4 species. The results indicate the chloroplast genomes are very similar in the number (82–84) and type of CDS genes encoding proteins. Despite some differences in gene content and organization among the chloroplast genomes, these differences do not coincide with the type of oxygenic photosynthesis (C3 or C4) that these 8 species represent. There is a general conservation of genes present in the C3 species B. muricata and the C4 species. This suggests nuclear genes encode most chloroplast-targeted proteins that are needed to support the C4 pathway. Both Kranz type and single-cell type C4 species have dimorphic chloroplasts (relative to function in carbon assimilation, starch synthesis, and in relative expression of photosystem I and photosystem II for balancing requirements for ATP and NADPH). In carbon assimilation one type of chloroplast supports fixation of atmospheric CO2 by PEPC with synthesis of C4 acids. They generate energy to support conversion of pyruvate to phosphoenolpyruvate utilizing pyruvate, P1 dikinase, adenylate kinase, and inorganic pyrophosphatase, and they support reduction of oxaloacetate to malate by NADP-malate dehydrogenase. The other type of chloroplast has the Calvin-Benson cycle with Rubisco fixing CO2 that is generated by decarboxylation of C4 acids (utilizing plastid-targeted NADP-malic enzyme in some C4 species). Currently all enzymes required in chloroplasts to support the C4 cycle and Calvin-Benson cycle are considered to be nuclear encoded except the gene for the large subunit of Rubisco which is in the chloroplast genome, while the small subunit gene is in the nucleus [39, 76–79]. In the dual-cell Kranz type C4 plants, cell specific control of transcription of nuclear genes may contribute to development of dimorphic chloroplasts. Other mechanisms must control development of dimorphic chloroplasts in SCC4 species (see hypotheses, selective protein import, selective mRNA targeting, selective protein degradation; [77]). Future studies are needed to determine how dimorphic chloroplasts develop to coordinate function of C4 in carbon assimilation, metabolite transport between chloroplasts, and requirements of energy from photochemistry.

Conclusions

This study reports high quality, and complete chloroplast genomes from seven Chenopodiaceae and one Amaranthaceae species. The procedures show the hybrid method of using high throughput and Sanger sequencing [80, 81] is rapid, efficient, and reliable for chloroplast genome sequencing. While genome organization, gene order, and content were largely conserved, there were a few structural differences, such as the variable location of the ycf15 gene; the high repeat content in the S. eltonica genome; the presence of two copies of ycf1 gene in H. ammodendron along with the IR expansion; and the IR expansion in S. eltonica that includes the trnH-GTG and psbA. The biological significance of these differences remains to be investigated.

The B. sinuspersici chloroplast genome presented in this study represents an improved version due to the high sequencing coverage and the validation of the junction regions through Sanger sequencing. The improvement in the B. sinuspersici chloroplast genome sequence allowed for the identification of a higher number of chloroplast genes. Interestingly, the H. ammodendron chloroplast genome presented in this study is 9,681 nt larger than the previously published genome [70]. This difference originated from a duplicated region of the IR, which is 4,868 nt in size and represented a rare instance of interorganellar DNA transfer from the mitochondria to the chloroplast genome.

The purpose of this study was to analyze chloroplast genomes in a few representative dicot species which have different forms of photosynthesis. Due to the high number of variable photosynthetic types present in Chenopodiaceae and almost 90% of the gene products in the chloroplast originating in the nucleus, there may be an expectation that the Chenopodiaceae may include chloroplast-encoded genes corresponding to each photosynthetic phenotype. However, to derive such phylogenetic conclusions requires extensive taxon sampling as exemplified in a recent analysis of 113 grass species [82]. Therefore, such an analysis was outside the purview of the current study.

C4 plants evolved independently from C3 species more than 60 times [33] leading to development of different forms of Kranz, along with single-cell C4 species, all of which have dimorphic chloroplasts coordinated in functions to support C4 photosynthesis. This includes differential expression of enzymes in carbon assimilation, selective expression of metabolite transporters to control flux of carbon between the two chloroplasts, and expression of photosystem I and II for production of ATP and NADPH. How these dimorphic chloroplasts develop through control of expression of nuclear and chloroplast genes remains unknown. Complete chloroplast genomic information on different forms of C4 species across dicot and monocot families should be useful in future studies on the control of its development, determining what is required for C4 photosynthesis, and determining the degree of conservation of the chloroplast genome in these photosynthetic types across phylogeny.
Materials and methods
Plant material and DNA extraction
Amaranthus retroflexus, Bassia muricata, Suaeda eltonica and Suaeda maritima plants were grown in a growth chamber with a 14/10 h photoperiod, light regime of 525 PPFD and day/night, and temperature of 28 °C/18 °C. The same photoperiod and light regime were used for Biennetia cycloptera, B. sinuspersici and Suaeda aralocaspica; however, the day/night temperatures were modified to 35 °C/18 °C. Haloxylon ammodendron plants were grown under natural annual environmental conditions in Pullman, WA. Total cellular DNA was isolated using fresh leaf tissue from each species with a Urea Lysis Buffer Method. Briefly, leaf tissue was flash frozen in liquid nitrogen and ground to a fine powder and approximately 100 mg tissue was placed in 600 μL buffer containing 42% w/v Urea, 250 mM NaCl, 50 mM Tris (pH 8.0), 1% sodium dodecyl sulfate (SDS) and 20 mM EDTA. Solution was briefly vortexed, extracted with equal volume of 1:1 phenol: chloroform and vortexed for 45 s. Samples were then centrifuged at 9,500 x g for 5 min and the supernatant was added to an equal volume of ice cold 2-propanol. The tube was rocked gently six times and centrifuged for 10 min at 9,500 x g. The pellet was washed in 1 mL ice cold 70% ethanol and centrifuged at 9,500 x g for 2 min and the supernatant was decanted. The pellet was dried and suspended in 500 μL TE buffer with 20 μg/mL RNAse A and incubated for 30 min at 37 °C prior to the addition of 1/10th volume 3 M sodium acetate (pH 5.3) and 2 volumes of 95% ethanol and rocked gently 6 times. The tube was centrifuged at 9,500 x g for 10 min, supernatant removed and the pellet was rinsed with 500 μL 70% ethanol, centrifuged for 2 min at 9,500 x g and the pellet was dried before being suspended in 50 μL TE buffer.

DNA sequencing, validation and contig assembly
The paired-end DNA sample prep kit (PE-102–1001; Illumina, San Diego, CA) was used to generate a paired-end library according to manufacturer’s recommendations (Illumina, San Diego, CA) at the Research Technology Support Facility at Michigan State University (East Lansing, MI, USA). DNA samples were sequenced on the Illumina HiSeq 2000 utilizing the 100PE chemistry. Quality control on raw sequence data was performed using CLC Genomics Workbench ver. 6.0.1 (CLC), (QIAGEN, Redwood City, CA, USA). CLC was utilized for read trimming, merging reads and filtering out low quality sequences with a phred score below 40. Assembly and mapping of the reads to the contigs was accomplished with CLC software. Mapping of reads to contigs was conducted using the following mapping parameters: mismatch cost 2, insertion cost 3, deletion cost 3, length fraction 0.8 and similarity fraction 0.9. BLASTN searches on NCBI (https://www.ncbi.nlm.nih.gov/) were performed using the assembled contigs as query sequences to identify contigs with high homology to chloroplast large single copy (LSC), small single copy (SSC) and inverted repeat (IR) for each of the assembled libraries obtained from each of the eight plant species. Identified IR contigs were reverse complimented and overlapping borders of each of the identified contigs were aligned to assemble a complete chloroplast genome sequence in the following order of LSC+IR+SSC+IR. Chloroplast contig junctions from overlapping border regions were aligned and analyzed with MEGA6 version 6.0.6 (https://www.mega software.net/). Flanking primers for chloroplast junctions were designed utilizing Primer3 Software [83]. PCR amplification was performed using Platinum Taq High-Fidelity DNA polymerase (Invitrogen, CA) and PCR products were purified using the QIAquick PCR purification Kit (QIAGEN, MD). Amplicons, ranging in size from 0.2 to 0.5 kb, were Sanger sequenced to ensure sequence fidelity of the DNA assembly output (Eurofins Genomics, KY). A primer walking and Sanger sequencing method was utilized to identify non-overlapping regions in the LSC+IRa and IRb+LSC junctions of T. indica and the IRa+SSC and SSC+IRb junctions of S. eltonica. The primer walking and Sanger sequencing method was also employed to validate specific conflicting sequences in the H. ammodendron chloroplast genome when compared to the publicly available H. ammodendron sequence. A remapping of the Illumina sequenced reads was performed using the final predicted chloroplast genomes from the eight species utilizing CLC software. A length fraction and similarity fraction of 0.99 were chosen as remapping parameters to ensure high stringency alignment. Assemblies generated with 0.80–0.90 and 0.99–0.99 length fraction and similarity fraction were screened to identify regions with coverage below 40 ×. Sequence data have been deposited to GenBank database under accession numbers MT299584 (A. retroflexus), MT316306 (B. muricata), MT316305 (B. cycloptera), MT316307 (B. sinuspersici), MT316308 (H. ammodendron), MT316309 (S. aralocaspica), MT316310 (S. eltonica), and MT316311 (S. maritima).

Genome annotation and visualization
All the chloroplast genomes were annotated and visualized with GeSeq [43] which incorporates the Dual Organellar Genome Annotator (DOGMA) [44] and OrganellarGenomeDRAW (OGDRAW) [84].
Comparisons of gene content and gene order
Comparisons for both gene content and order were performed for the eight chloroplast sequences. This comparison included three chloroplast reference genomes: V. vinifera (NC_007957.1), S. oleracea (AJ400848.1) and B. vulgaris (EF534108.1). Gene order and content were parsed manually using pair-wise comparisons between sequences.

Examination of repeat structure and microsatellites
REPuter [65] was utilized to identify the number and location of forward, reverse, complementary, and palindromic repeats in the sequence of the eight species predicted chloroplast sequences. A minimum repeat size of 30 nt and a Hamming distance of 3 (>90% identity) was utilized. Shared and unique repeats were identified manually and with the use of BLASTN based on intergenomic comparisons.

Microsatellites were identified with MISA software [85] using standard thresholds. Specifically, a minimum stretch of 10 for mono-, six for di-, five for tri-, and three for tetra-, penta-, and hexa-nucleotide repeats, and a minimum distance of 100 nucleotides between compound microsatellites.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s13007-020-00662-w.

Additional file 1: Figure S1. Representative example of an overlap region amplicon sequenced with Sanger approach. The IRA-SSC junction showed a 100% match during nucleotide alignment.

Additional file 2: Table S1. Forward and reverse primers used to amplify and validate the overlapping regions present in all four possible junctions (LSC-IR, IR-SSC, SSC-IR, and IR-LSC) of eight chloroplast genomes.

Additional file 3: Figure S2. Stack column graphs of minimum coverage (MC) and average coverage (AC) for eight chloroplast genomes assembled with 80%-90% (blue) and 99%-99% (orange) length fraction-similarity fraction parameters.

Additional file 4: Figure S3. Representative maps of the chloroplast genome of A. Amaranthus retroflexus, B. Bassia muncata, C.Bienertia cycloptera, D. B. sinuspersicus, E. Haloxylon ammodendron, F. Suaeda aralocaspica, G. S. eltonica, and H. S. maritima. Genes shown outside the outer circle are transcribed clockwise whereas those represented inside are transcribed counterclockwise. Large single copy (LSC), small single copy (SSC), and inverted repeats (IRA, IRB) regions are indicated.

Additional file 5: Table S2. Forward and reverse primers used to amplify and validate the mitochondrial-to-plastidial DNA transfer in Haloxylon ammodendron and H. persicum.

Additional file 6: Forward and reverse primers used to amplify and validate the mitochondrial-to-plastidial DNA transfer in Haloxylon ammodendron and H. persicum.

Acknowledgements
The authors acknowledge Sequoia Dance for her support with PCR.

Authors’ contributions
RMS, GEE and AD designed the study. GEE and AD supervised the study. RMS and BWB performed the experiments and generated the data. RMS, BWB and AD analyzed the data. All authors read and approved the final manuscript.

Funding
Work in the Dhingra lab was supported in part by Washington State University Agriculture Center Research Hatch Grant WNP00011. BWB acknowledges graduate research assistantship support from Washington State University Graduate School.

Availability of data and materials
The sequence of the chloroplast genomes generated during the current study are accessible from GenBank accession numbers: MT299584 (A. retroflexus), MT316306 (B. muncata), MT316305 (B. cycloptera), MT316307 (B. sinuspersicus), MT316308 (H. ammodendron), MT316309 (S. aralocaspica), MT316310 (S. eltonica), and MT316311 (S. maritima), (https://www.ncbi.nlm.nih.gov/genbank/).

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1 Department of Horticulture, Washington State University, Pullman, WA 99164, USA. 2 Molecular Plants Sciences, Washington State University, Pullman, WA 99164, USA. 3 School of Biological Sciences, Washington State University, Pullman, WA 99164, USA.

Received: 7 May 2020 Accepted: 20 August 2020
Published online: 31 August 2020

Bibliography
1. Neuhaus HE, Emes MJ. Nonphotosynthetic metabolism in plastids. Annu Rev Plant Physiol Plant Mol Biol. 2000;51:111–40. https://www.annualreviews.org/doi/abs/10.1146/annurev.arplant.51.1.111.
2. Namitha KK, Negi PS. Chemistry and biotechnology of carotenoids. Crit Rev Food Sci Nutr. 2010;50:728–60. https://tandfonline.com/doi/abs/10.1080/10408398.2010.499811.
3. Jansen RK, Cai Z, Raubeson LA, Daniell H, Delpampfils CW, Leebens-Mack J, et al. Analysis of 81 genes from 64 plastid genomes resolves relationships in angiosperms and identifies genome-scale evolutionary patterns. Proc Natl Acad Sci. 2007;104:19169–74.
4. Shaw J, Lickey EB, Schilling EE, Small RL. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. Am J Bot Wiley Online Library. 2007;94:275–88.
5. Green BR. Chloroplast genomes of photosynthetic eukaryotes. Plant J. 2011;66:34–44. https://doi.org/10.1111/j.1365-313X.2011.04541.x.
6. Suv Z, Hajdukevicz P, Maliga P. Stable transformation of plastids in higher plants. Proc Natl Acad Sci. 1990;87:8526–30.
7. Dhingra A, Daniell H. Chloroplast genetic engineering via organogenesis or somatic embryogenesis. Arab Protoc. 2006;323:245–62. https://doi.org/10.1111/j.1365-313X.2006.04133.x.
8. Palmer JD, Osorio B, Aldrich J, Thompson WF. Chloroplast DNA evolution among legumes: Loss of a large inverted repeat occurred prior to other sequence rearrangements. Curr Genet. 1987;11:275–86. https://doi.org/10.1007/BF00355401.
9. Wu C-S, Chaw S-M. Large-scale comparative analysis reveals the mechanisms driving plastomic compaction, reduction, and inversions in conifers II (Cupressophytes). Genome Biol Evol. 2016;8:3740. https://academic.oup.com/gbe/article-lookup/doi/10.1093/gbe/evw1278.
10. Braukmann TWA, Broe MB, Stefanovic S, Freudentstein JV. On the brink: the highly reduced plastomes of nonphotosynthetic Ericaceae. New Phytol. 2017;216:254–66. https://doi.org/10.1111/nph.14681.
11. Dhingra A, Folta KM. ASAP: amplification, sequencing & annotation of plastomes. BMC Genom. 2005;6:176.
12. Cahoon AB, Sharpe RM, Myasiphon C, Thompson EJ, Ward AD, Lin A. The complete plastome grammar of tall fescue (Lolium arundinaceum, Poaceae) and comparison of whole plastomes from the family Poaceae. Am J Bot. 2010;97:49–58. https://www.amjbot.org/cgi/content/abstract/97/1/49
13. Zhang Y-J, Ma P-F, Li L-Z. High-throughput sequencing of six bamboo plastome genomes: phylogenetic implications for temperate woody bamboos (Poaceae: Bambusidoideae). PLoS ONE. 2011;6:e20596. https://doi.org/10.1371/journal.pone.0020596
14. Wu J, Liu B, Cheng F, Ramchary N, Choi SR, Lim YP, et al. Sequencing of plastome genome using whole cellular DNA and solasea sequencing technology. Front Plant Sci. 2012;3:243. https://www.frontiersin.org/Plant_Genetics_and_Genomics/10.3389/fpls.2012.00243/abstract
15. Moore MJ, Dhingra A, Solits PS, Shaw R, Farmerie WG, et al. Rapid and accurate pyrosequencing of angiosperm plastid genomes. BMC Plant Biol. 2006;6:17.
16. Nagy E, Hegedűs G, Taller J, Kutasy B, Virág E. Illumina sequencing of the chloroplast genome of common ragweed (Ambrosia artemisiifolia L.). Data Br. 2017;15:606–11.
17. Sakaguchi S, Ueno S, Tsumura Y, Setoguchi I, Hito M, Hattori C, et al. Application of a simplified method of chloroplast enrichment to small amounts of DNA for chloroplast genome sequencing. Appl Plant Sci. 2017;5:1700022.
18. Sakulsathaporn A, Wannapinyo P, Vuttipongchaikij S, Apisitwanich S. The complete plastome genome sequence of Asian Palmyra palm (Borassus flabellifer). BMC Res Notes. 2017;10:740.
19. Keller J, Rousseau-Gueutin M, Martin GE, Moret B, Courtois E, et al. The evolutionary rate of the chloroplast and nuclear rps16 genes as revealed through the sequencing and comparative analyses of four novel legume plastome genomes from Lupinus. DNA Res. 2017;24:343–58.
20. Schuster TM, Setaro SD, McCready JD, Termizi AAA, Playford J, et al. Wild origins of macadamia domestication identified through intraspecific plastome genome sequencing. Front Plant Sci. 2019;10:334.
21. Dong W, Xu C, Cheng T, Lin K, Zhou S. Sequencing angiosperm plastid genomes made easy: a complete set of universal primers and a case study on the phylogeny of saxifragales. Genome Biol Evol. 2013;5:989–97. https://academic.oup.com/gbe/article-lookup/doi/10.1093/gbe/evt063
22. Logacheva MD, Samigullin TH, Dhingra A, Penin AA. Comparative plastid genomics and phylogenetics of Fagopyrum esculentum ssp. ancestrale—a wild ancestor of cultivated buckwheat. BMC Plant Biol. 2008;8:59.
23. Weng M-L, Ruhlman TA, Jansen RK. Expansion of inverted repeat does not increase substitution rates in Petunia genome plastid genomes. New Phytol. 2013;201:482–51. https://doi.org/10.1111/nph.14375
24. Zuo L-H, Wang A-Q, Zhang S, Yu X-Y, Ren Y-C, Yang M-S, et al. The first plastome sequencing of Ulmus species by de novo assembly and annotation. Front Plant Sci. 2017;8:1246. https://doi.org/10.3389/fpls.2017.01246
25. Peng L, Yamamoto H, Shikanai T. Structure and biogenesis of the plastid genomes of Poaceae and comparison of whole plastomes from the family Poaceae. BMC Genomics. 2011;12:465. https://doi.org/10.1186/1471-2164-12-465
26. Gu C, Tembrock LR, L Y, Lu X, Wu Z. The complete plastome of queen's crape myrtle (Lagerstroemia indica) and cDNA assembly from 454 DNA sequencing. J Proteome Res. 2015;14:2490–108. https://doi.org/10.1021/pr5011907
27. Edwards GE, Franchessi VR, Voznesenskaya EV. Single-cell C4 photosynthesis versus the dual-cell (Kranz) paradigm. Annu Rev Plant Biol. 2004;55:173–96. https://doi.org/10.1146/annurev.arplant.55.031900.314172
28. Voznesenskaya EV, Francheschi VR, Kirats O, Artysusheva EG, Freitag H, Edwards GE. Proof of C₄ photosynthesis without Kranz anatomy in Bienertia cycloptera (Chenopodiaceae). Plant J. 2002;21:649–62. https://doi.org/10.1046/j.1365-313X.2002.01385.x
29. Sharpe RM, Christin P-A, Edwards EI. The C₄ plant lineages of planet Earth. J Exp Bot. 2011;62:3155–69. https://jxb.oxfordjournals.org/content/62/9/3155.abstract
30. Hernández-Ledesma P, Berendsohn WG, Borsch T, Von Mering S, Akhani H, Arias S, et al. A taxonomic backbone for the global synthesis of species diversity in the angiosperm order Caryophyllales Willdenowia. JSTOR. 2015;45:281–383.
31. Edwards GE, Voznesenskaya EV. Chapter 4 C₄ photosynthesis: Kranz forms and single-cell C₄ in terrestrial plants. Dordrecht: Springer; 2010. p. 29–61.
32. Kaderere G, Borsch T, Wessing K, Freitag H. Phylogeny of Amaranthaceae and Chenopodiaceae and the evolution of C₄ photosynthesis. Int J Plant Sci. 2003;164:959–86. https://doi.org/10.1086/378649
33. Schütze P, Scheunemann K, Wessing K, Borsch T, Freitag H. Phylogenetic relationships in the Chenopodiaceae and Salsolaceae clade and a clarification of the phylogenetic position of Bienertia and alexandra using multiple DNA sequence datasets. Syst Biol. 2006;55:1–85. https://www.bioone.org/doi/abs/10.1043/06–01
34. Sharpe RM, Offermann S. One decade after the discovery of single-cell C₄ species in terrestrial plants: what did we learn about the minimal requirements of C₄ photosynthesis? Photosyn Res. 2014;119:169–80. https://doi.org/10.1007/s11120-013-9810-9
35. Sharpe RM. The evolution of C₄ photosynthesis. New Phytol. 2004;161:341–70. https://doi.org/10.1111/j.1469-8137.2004.00974.x
36. Christin P-A, Scale TL, Edwards EJ, Ogburn RM, Khoshravesh R, Sharpe R. Complex evolutionary transitions and the significance of C₃-C₄ intermediate forms of photosynthesis in Molluginaceae. Evolution. 2011;65:643–60. https://doi.org/10.1111/j.1558-5646.2010.01168.x
37. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol Elsevier. 1990;215:403–10.
38. Schütze P, Freitag H, Weising K. An integrated molecular and morphological study of the subfamily Suedaeoideae Ull. (Chenopodiaceae). Plant Syst Evol. 2003;239:257–86. https://doi.org/10.1007/s10036-003-132-3
39. Kapralov MV, Akhani H, Voznesenskaya EV, Edwards G, Francheschi V, Roalson EH. Phylogenetic relationships in the Salsolinae / Suauideoe / Salsolideoe s.l. (Chenopodiaceae) clade and a clarification of the phylogenetic position of bienertia and alexandra using multiple DNA sequence datasets. Syst Biol. 2006;55:1–85. https://www.bioone.org/doi/abs/10.1043/06–01
40. Sharpe RM, Offermann S. One decade after the discovery of single-cell C₄ species in terrestrial plants: what did we learn about the minimal requirements of C₄ photosynthesis? Photosyn Res. 2014;119:169–80. https://doi.org/10.1007/s11120-013-9810-9
41. Christin P-A, Scale TL, Edwards EJ, Ogburn RM, Khoshravesh R, Sharpe R. Complex evolutionary transitions and the significance of C₃-C₄ intermediate forms of photosynthesis in Molluginaceae. Evolution. 2011;65:643–60. https://doi.org/10.1111/j.1558-5646.2010.01168.x
42. Tillich M, Lehwark P, Pellizer T, Ulbricht-Jones ES, Fischer A, Bock R, et al. GeSeq–versatile and accurate annotation of organelle genomes. Nucleic Acids Res. 2017;45:W6–11.
43. Wyman SK, Jansen RK, Boore JL. Automatic annotation of organellar genomes with DOGMA. Bioinformatics. 2004;20:3252–5. https://bioinformatics.oxfordjournals.org/content/20/17/3252.abstrac
51. Kim Y, Park J, Chung Y. The complete chloroplast genome of Suaeda japonica Makino (Amaranthaceae). Mitochondrial DNA Part B. 2019;4:1505–7.

52. Kim B, Kim J, Park H, Park J. The complete chloroplast genome sequence of Bienertia sinuspersici. Mitochondrial DNA Part B. 2016;1:388–9.

53. Yang M, Zhang X, Liu G, Yin Y, Chen K, Yun Q, et al. The complete chloroplast genome sequence of date palm (Phoenix dactylifera L.). PLoS ONE. 2010;5:e12762.

54. Firetti F, Zuntini AR, Gaiarsa JW, Oliveira RS, Lohmann LG, Van Sluys M. Plastome rearrangements in the ‘Adenocaulon’ complex (Solanaceae) and the phylogenetic implications. Front Plant Sci. 2017;8:1875.

55. Fonseca LHM, Lohmann LG. Plastome rearrangements in the “Adenocaulon” complex (Solanaceae) and the phylogenetic implications. Front Plant Sci. 2017;8:1875.

56. Qian J, Song J, Gao H, Zhu Y, Xu J, Pang X, et al. The complete chloroplast genome of Suaeda japonica Makino (Amaranthaceae). Mitochondrial DNA Part B. 2016;1:388–9.

57. Downie SR, Olmstead RG, Zurawski G, Soltis DE, Soltis PS, Watson JC, et al. Six independent losses of the chloroplast DNA rpl2 intron in plastid monocotyledon evolution. PLoS ONE. 2013;8:e67350.

58. Kim K-J, Lee H-L. Complete chloroplast genome sequences from Korean ginseng (Panax schinseng Nees) and comparative analysis of sequence evolution among 17 vascular plants. DNA Res. 2004;11:247–61.

59. Dong W, Liu J, Yu J, Wang L, Zhou S. Highly variable chloroplast markers for evaluating plant phylogeny at low taxonomic levels and for DNA barcoding. PLoS ONE. 2012;7:e35071.

60. Jiao J, Song J, Gao H, Zhu Y, Xu J, Pang X, et al. The complete chloroplast genome sequence of the medicinal plant Salvia miltiorrhiza. PLoS ONE. 2013;8:e57607.

61. Ma P-F, Zhang Y-X, Guo Z-H, Li D-Z. Evidence for horizontal transfer of chloroplast DNA to the plastid genome in a bamboo genus. Sci Rep. 2015;5:11608.

62. Oldenburg DJ, Bendich AJ. Most chloroplast DNA of maize seedlings for horizontal transfer of chloroplast DNA to the plastid genome in a bamboo genus. Sci Rep. 2015;5:11608.

63. Iorizzo M, Grzebelus D, Senalki D, Szklarczyk M, Spooner D, Simon P. Against the traffic: the first evidence for mitochondrial DNA transfer into the plastid genome. Mob Genet Elements. 2012;2:261–6.

64. Firetti F, Zuntini AR, Gaiarsa JW, Oliveira RS, Lohmann LG, Van Sluys M. Plastome rearrangements in the ‘Adenocaulon’ complex (Solanaceae) and the phylogenetic implications. Front Plant Sci. 2017;8:1875.

65. Kurtz S, Choudhuri JV, Ohlebusch E, Schleiermacher C, Stoye J, Giegerich R. RePuter: the manifold applications of repeat analysis on a genomic scale. Nucleic Acids Res. 2001;29:4633–42.

66. Tranel PJ, Trucco F. 21st-century weed science: a call for Amaranthus barcoding. PLoS ONE. 2012;7:e35071.

67. Chaney L, Mangelson R, Ramaraj T, Jellen EN, Maughan PJ. The complete chloroplast genome sequence and inversion variation in Lasthenia burkei (Madieae, Asteraceae) . Am J Bot. 2015;102:1008.

68. Yang M, Zhang X, Liu G, Yin Y, Chen K, Yun Q, et al. The complete chloroplast genome sequence of prickly morning glory (Ipomoea carnea Jacq.). Nucleic Acids Res. 2019;47:W59–64. https://doi.org/10.1093/nar/gkz238.

69. Li Z, Chen Y, Mu D, Yuan J, Shi Y, Zhang H, et al. Comparative analysis of the complete chloroplast genome sequences in psammophytic Haloxylon species (Amaranthaceae). PeerJ. 2016;4:e2699. https://peerj.com/articles/2699.

70. Dong W, Xu C, Li D, Jin X, Li R, Lu Q, et al. Comparative analysis of the complete chloroplast genome sequences in psammophytic Haloxylon species (Amaranthaceae). PeerJ. 2016;4:e2699. https://peerj.com/articles/2699.

71. Goulding SE, Wolfe KH, Olmstead RG, Morden CW. Ebb and flow of the chloroplast inverted repeat. Mol Gen Genet. 1996;252:195–206.

72. Lee S-B, Kaittanis C, Jansen RK, Hostetler JB, Tallon LJ, Town CD, et al. The complete chloroplast genome sequence of Gossypium hirsutum: organization and phylogenetic relationships to other angiosperms. BMC Genomics. 2006;7:61.

73. Kim B, Kim J, Park H, Park J. The complete chloroplast genome sequence of Suaeda japonica Makino (Amaranthaceae). Mitochondrial DNA Part B. 2016;1:388–9.

74. Iorizzo M, Grzebelus D, Senalki D, Szklarczyk M, Spooner D, Simon P. Against the traffic: the first evidence for mitochondrial DNA transfer into the plastid genome. Mob Genet Elements. 2012;2:261–6.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.