Long-read transcriptome and other genomic resources for the angiosperm *Silene noctiflora*

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Abstract

The angiosperm genus *Silene* is a model system for several traits of ecological and evolutionary significance in plants, including breeding system and sex chromosome evolution, host-pathogen interactions, invasive species biology, heavy metal tolerance, and cytonuclear interactions. Despite its importance, genomic resources for this large genus of approximately 850 species are scarce, with only one published whole-genome sequence (from the dioecious species *S. latifolia*). Here, we provide genomic and transcriptomic resources for a hermaphroditic representative of this genus (*S. noctiflora*), including a PacBio Iso-Seq transcriptome, which uses long-read, single-molecule sequencing technology to analyze full-length mRNA transcripts. Using these data, we have assembled and annotated high-quality full-length cDNA sequences for approximately 14,126 *S. noctiflora* genes and 25,317 isoforms. We demonstrated the utility of these data to distinguish between recent and highly similar gene duplicates by identifying novel paralogous genes in an essential protease complex. Further, we provide a draft assembly for the approximately 2.7-Gb genome of this species, which is near the upper range of genome-size values reported for diploids in this genus and three-fold larger than the 0.9-Gb genome of *S. conica*, another species in the same subgenus. Karyotyping confirmed that *S. noctiflora* is a diploid, indicating that its large genome size is not due to polyploidization. These resources should facilitate further study and development of this genus as a model in plant ecology and evolution.


**Introduction**

*Silene* is the largest genus in the angiosperm family Caryophyllaceae and serves as a model system in many fields of ecology and evolutionary biology (Bernasconi *et al.* 2009; Jafari *et al.* 2020). For instance, *Silene* is used to study breeding system evolution, as the genus includes hermaphroditic, gynodioecious, gynonomonoecious, monoecious, and dioecious species (Desfeux *et al.* 1996; Charlesworth 2006). Despite the diversity of *Silene* sexual systems, there is only one available whole genome sequence for the entire genus—from the dioecious species *S. latifolia*, which has heteromorphic XY sex chromosomes (Papadopulos *et al.* 2015; Krasovec *et al.* 2018). Whole genome resources are not available for any of the hermaphroditic species, which has limited comparative genomic studies into the evolution of dioecy within this genus.

*Silene* is also used as a model system for investigating organelle genome evolution and the coevolution between nuclear and cytoplasmic genomes (i.e., cytonuclear interactions) (Olson and Mccauley 2002; Städler and Delph 2002; Klaas and Olson 2006; Garraud *et al.* 2011). *Silene conica* and *S. noctiflora* have two of the largest known plant mitochondrial genomes at 11 Mb and 7 Mb, respectively (Sloan *et al.* 2012a). In contrast, the mitochondrial genome of *S. latifolia* is only 0.25 Mb, about 45 times smaller than that of *S. conica* (Sloan *et al.* 2012a). Interestingly, the *Silene* species with expanded mitogenomes also display unusually high evolutionary rates and structural changes in mitochondrial and plastid DNA (Mower *et al.* 2007; Sloan *et al.* 2012a). The natural variation in organelle genome evolution found in this genus has been used to study how these differences affect cytonuclear interactions (Havird *et al.* 2015; Williams *et al.* 2019).

The ability to use *Silene* as a model for cytonuclear evolution is still limited by the lack of extensive nuclear genome resources. Previous work has characterized *Silene* nuclear genome size and chromosome number. Nuclear genome sizes in the genus vary considerably, although not as starkly as mitochondrial genome sizes, ranging roughly 4.5-fold among diploids (haploid sizes of 0.71 to 3.23 Gb) and 8-fold when the tetraploid *S. stellata* (5.77 Gb) is included (Kruckeberg 1960; Siroký *et al.* 2001; Bai *et al.* 2012; Dagher-Kharrat *et al.* 2013; Pellicer and Leitch 2020). Most of the available nuclear sequence data comes from short-read RNA sequencing, which has
been conducted on multiple *Silene* species (Blavet et al. 2011; Sloan et al. 2012b; Muyle et al. 2012; Casimiro-Soriguer et al. 2016; Havird et al. 2017; Bertrand et al. 2018; Balounova et al. 2019). These datasets have provided an important resource for molecular studies of *Silene*, but are limited because of the challenges associated with assembling short-read sequences, especially in distinguishing similar sequences arising from gene duplication, heterozygosity, and/or alternative splicing (Alkan et al. 2011; Schatz et al. 2012; Hahn et al. 2014; Lan et al. 2017).

We have generated genomic resources critical for investigations into *S. noctiflora*, a species of interest due to its extremely unusual organelle evolution and resultant use as a model for cytonuclear interactions, as well as its status as a hermaphrodite in a genus representing many types of breeding system. We include a high-quality transcriptome using long-read PacBio Iso-Seq technology, genome size estimates, and a draft nuclear genome assembly. These resources will expand opportunities for molecular and ecological studies within the genus.

**Materials and Methods**

**Study system**

*Silene noctiflora* (Figure 1) is largely hermaphroditic but can produce a mixture of hermaphroditic and male-sterile flowers on the same plant (gynonomoecy) (Davis and Delph 2005). Also known as the night-flowering catchfly, this annual species is native to Eurasia and introduced throughout much of the world (McNeill 1980; Davis and Delph 2005).

**Plant growth conditions, tissue sampling, and nucleic acid extractions**

Plants used for genome sequencing, Iso-Seq, and flow cytometry estimates of genome size were grown under standard greenhouse conditions with 16-hr light/8-hr dark at Colorado State University (Table 1). DNA for short-insert paired-end Illumina libraries was extracted from leaf tissue of a 7-week-old *S. noctiflora* individual from an Opole, Poland (OPL) population using a Qiagen Plant DNeasy kit. To obtain sufficient DNA quantity for construction of Illumina mate-pair libraries, additional DNA was extracted from the same individual 6 weeks later using a modified CTAB protocol (Doyle and Doyle 1987) for construction of Illumina mate-pair libraries. For Iso-Seq library construction, RNA was extracted from a single 12-week-old *S.
noctiflora OPL individual (grown from seed of the plant used for DNA extraction), using a
Qiagen Plant RNeasy kit. RNA extractions were performed for four different tissue samples: 1) a
large flower bud with calyx removed, 2) an entire smaller flower bud including calyx, 3) the
most recent (top-most) pair of cauline leaves, and 4) one leaf from the second most recent pair of
cauline leaves. The four RNA extractions were quantified with Qubit RNA BR kit (Thermo
Fisher Scientific). Purity and integrity were assessed with a NanoDrop 2000 (Thermo Fisher
Scientific) and TapeStation 2200 (Agilent Technologies). Different tissues and developmental
stages were sampled (and eventually pooled; see below) to capture a larger diversity of
transcripts and thereby increase the number of genes represented.

**PacBio Iso-Seq transcriptome sequencing and analysis**

Iso-Seq is an application of Pacific Biosciences (PacBio) long-read sequencing technology that
uses cDNA templates to generate high quality reads for full-length transcripts. The high error
rate generally associated with PacBio sequencing is drastically reduced using circular consensus
sequencing (CCS), which uses hairpin adapters on each end of a double-stranded molecule to
create a circular, single-stranded topology (Au et al. 2012; Rhoads and Au 2015; Hestand et al.
2016; Wenger et al. 2019). This topology allows the polymerase to read the same full-length
molecule multiple times over, generating an accurate consensus sequence (Ono et al. 2013;
Wang et al. 2019). PacBio Iso-Seq has been used to study the transcriptomes of many organisms,
often in the context of identifying splice variants, or alternative transcripts (Xu et al. 2015;
Gordon et al. 2015; Rhoads and Au 2015; Guo et al. 2016; Abdel-Ghany et al. 2016; Wang et al.
2016; Weirather et al. 2017). Alternative transcripts can be identified using CCS because this
technology obtains consensus sequences for full-length single transcripts (Zhao et al. 2019). In
the same way, CCS can also be used to distinguish paralogs or gene duplicates.

To create an Iso-Seq library for *S. noctiflora*, the four RNA extractions (1.5 μg each) were
pooled into a single sample and sent to the Arizona Genomics Institute for PacBio Iso-Seq
library construction and sequencing. The library was constructed on the pooled RNA sample
using Poly(A) selection, following the standard PacBio Iso-Seq protocol ("Procedure &
Checklist – Iso-Seq Template Preparation for Sequel Systems,” Pacific Biosciences, PN-101-
and then was sequenced with a PacBio Sequel (first generation) platform on two SMRT Cells.

Raw movie files of long-read, single-molecule sequences (one per SMRT Cell) were processed using the PacBio Iso-Seq v3.1 pipeline (Anvar et al. 2018; Pacific Biosciences 2020). Circular consensus sequence calling was performed on each movie file separately using the command ccs with the recommended parameters --noPolish and --minPasses 1. Next, primer removal was performed on each dataset by running the command lima with parameters --isoseq and --no-pbi. Poly(A) tails were trimmed and concatemers were removed using the refine command with the parameter --require-polya. Data from the two cells were merged at this point using the commands dataset create --type TranscriptSet and dataset create --type SubreadSet. Finally, the merged data were run through the cluster and polish commands. We also ran the cluster and polish commands on each dataset individually after skipping the merge step.

Trinotate v3.2.0 (Bryant et al. 2017) was used to annotate the final polished sequences produced by the Iso-Seq pipeline after merging the datasets. To complete this process, we used Transdecoder v5.5.0 (https://github.com/TransDecoder/TransDecoder/wiki), SQLite v3 (Kreibich 2010), NCBI BLAST + v2.2.29 (Camacho et al. 2009), HMMER v3.2.1 (including RNAMMER) (Lagesen et al. 2007; Potter et al. 2018), signalP v4 (Petersen et al. 2011), and tmhmm v2 (Krogh et al. 2001). The Pfam (Bateman et al. 2004) and UniProt (“UniProt” 2015) databases were included in the Trinotate installation. The transcripts and Transdecoder-predicted peptides were searched against the respective databases, following the standard Trinotate pipeline. All of these results were loaded into a Trinotate SQLite database.

Cogent v4.0.0 (https://github.com/Magdoll/Cogent/wiki) and minimap2 v2.17 (Li 2018) were used to conduct family finding on the final sequences by the Iso-Seq pipeline by partitioning sequences into groups based on similarity. While the Iso-Seq pipeline collapses reads into individual transcripts, it does not collapse alternative transcripts originating from the same gene. Cogent further collapses alternative transcripts into groups, where each group is meant to represent a single gene. Next, coding genome reconstruction was performed on each group from the above step; thus, the Cogent output included both a file containing groups of alternative
transcripts (final.partition.txt at https://github.com/alissawilliams/Silene_noctiflora_IsoSeq) and a transcript-based genome. Finally, this transcript-based genome was used to determine total gene and isoform (alternative transcript) counts via cDNA_Cupcake scripts (https://github.com/Magdoll/cDNA_Cupcake/wiki; Wang et al. 2020; Jeffries et al. 2020). A modified form of the script make_file_for_sampling_from_collapsed.py was run with the parameter --include_single_exons in order to include all transcripts in the analysis. Gene and isoform counts were calculated using custom Python and R scripts on the resultant file. These Cogent, minimap2, and cDNA_Cupcake steps were performed on the merged dataset as well as individually on the datasets from each SMRT Cell.

We used genes from the plastid caseinolytic protease (Clp) as a case study to assess the ability of Iso-Seq dataset to distinguish paralogs (gene duplicates) of various levels of divergence. To identify nuclear-encoded plastid Clp core genes in our dataset, we used blastn in conjunction with the Cogent family finding output. There are eight nuclear-encoded plastid Clp core genes in Arabidopsis thaliana: CLPP3-6 and CLPR1-4 (Nishimura and van Wijk 2015). Additionally, the genus Silene shares a duplication of CLPP5, denoted CLPP5A and CLPP5B (Rockenbach et al. 2016). We obtained the sequences of all nine of these genes from a previous study (Rockenbach et al. 2016) and used them as queries in blastn searches against the S. noctiflora Iso-Seq transcriptome. We then identified which groups of collapsed alternative transcripts (from the Cogent output) contained these BLAST hits. BLAST hits for eight of the nine nuclear-encoded Clp core subunits in Silene (including CLPP5A and CLPP5B) were found in a single Cogent group. The sequences within each group were confirmed to represent a single gene via alignment and manual inspection; thus, these eight core subunits are single copy in S. noctiflora. However, in the case of CLPR2, two different Cogent groups contained relevant transcripts, indicating a possible case of gene duplication. Sequence alignment and manual inspection of the transcripts within these two Cogent groups revealed that one group contained two unique sequences. These data, along with sequencing results from a separate project in which we cloned two versions of S. noctiflora CLPR2 using primers designed for S. latifolia CLPR2, suggested that there are actually three distinct CLPR2 sequences in S. noctiflora. In the subsequent phylogenetic analysis of CLPR2, we used the longest sequences from each of the three identified groups.
A phylogenetic tree was constructed using sequences from the three different *S. noctiflora* CLPR2 genes. In addition to the three *S. noctiflora* sequences, we also included *Agrostemma githago*, *S. conica*, *S. latifolia*, *S. paradoxa*, and *S. vulgaris* CLPR2 sequences from a previous study (Rockenbach et al. 2016), as well as three *S. undulata* CLPR2 sequences identified using blastn against the *S. undulata* TSA database (accession GEYX00000000). All 11 sequences were aligned using the *einsi* option in MAFFT v7.222 (Katoh and Standley 2013), and trimmed at the 5' end based on the trimming conducted in Rockenbach et al. (2016). The resultant sequence file was run through jModelTest v2.1.10 (Darriba et al. 2012) to choose a model of sequence evolution. We chose the top model based on the Bayesian Information Criterion (K80+I) and ran PhyML v3.3 (Guindon et al. 2010) with 1000 bootstrap replicates and 100 random starts.

**Genome size estimates by flow cytometry**

Leaf or seedling samples were collected from multiple individuals of varying age (between 2 and 14 weeks) for each of our target *Silene* species and shipped fresh to Plant Cytometry Services (Schijndel, Netherlands). Genome sizes were determined using the CyStain PI Absolute P reagent kit (05-5502). Samples were chopped with a razor blade in 500 μl of ice-cold Extraction Buffer in a plastic petri dish, along with *Pachysandra terminalis* tissue as an internal standard (3.5 pg/2C). After 30-60 sec of incubation, 2 ml of Staining Buffer was added. Each sample was then passed through a nylon filter of 50 μm mesh size, and then incubated for 30+ min at room temperature. The filtered solution was then sent through a CyFlow ML flow cytometer (Partec GmbH). The fluorescence of the stained nuclei, which passed through the focus of a light beam with a 50 mW, 532 nm green laser, was measured by a photomultiplier and converted into voltage pulses. The voltage pulses were processed using Flomax version 2.4d (Partec) to yield integral and peak signals. Genome sizes were reported in units of pg/2C. The conversion used to report each size (x) in units of Gb was (x/2)*0.978 (Gregory et al. 2007).

**Karyotyping**

*Silene noctiflora* OPL seeds were germinated on wet filter paper and grown for 5 days. Radicles were trimmed off and transferred to ice water for 24 hrs. The radicles were then fixed in a 3:1 solution of absolute ethanol and glacial acetic acid and stored at -20°C. Chromosomes were visualized using a squash preparation with Feulgen staining. Fixed radicles were rinsed in
distilled water for 5 min at 20°C. Radicles were then hydrolyzed in 5M HCl at 20°C for 60 min followed by three rinses in distilled water. The hydrolyzed radicles were transferred to Schiff’s reagent to stain the DNA for 120 min at 20°C and were then destained by rinsing in SO2 water at 20°C three times for 2 min, two times for 10 min, once for 20 min, and then transferred to distilled water. Squashes were prepared by placing a piece of tissue in 45% acetic acid for 10 min and then minced on glass. A coverslip was placed over the minced tissue and pressed with enough pressure to produce a monolayer of nuclei. Slides were placed on dry ice for 1 min, and the coverslip was removed. The slides were transferred to 96% ethanol for 2 min, air dried, and mounted with mounting medium. Chromosomes were observed using a compound light microscope at 100× magnification.

Genome sequencing and assembly
Extracted *S. noctiflora* OPL DNA samples were used for Illumina library construction and sequencing. A paired-end library with a target insert size of 275-bp was constructed at the Yale Center for Genome Analysis and sequenced on a 2×150-bp HiSeq 2500 run (three lanes). Two mate-pair libraries (with target insert sizes of 3-5 kb and 8-11 kb) were generated at GeneWiz and sequenced on a 2×150-bp HiSeq 2500 run (one lane each). Approximately 480M, 250M, and 230M read pairs were generated for the 275-bp, 3-5 kb, and 8-11 kb libraries, respectively. These reads are available via the NCBI SRA (accessions SRR9591157-SRR9591159). Reads were trimmed for quality and to remove 3′ adapters, using cutadapt v1.3 (Martin 2011) under the following parameters: -n 3 -O 6 -q 20 -m 30 -a AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC --paired-output. The trimmed reads were assembled with ALLPATHS-LG release 44837 (Gnerre et al. 2011). Estimates of mean insert size and standard deviation for each library were provided as input for the assembly by first mapping a sample of reads to the published *S. noctiflora* plastid genome (GenBank accession JF715056.1). These estimates were as follows: 274 bp (± 22 bp), 3752 bp (± 419 bp), and 9873 bp (± 1283 bp).

BUSCO analyses
Benchmarking Universal Single-Copy Orthologs (BUSCO) analysis (Seppey et al. 2019) compares an assembly (transcriptomic or genomic) to a set of highly conserved orthologs from a
particular clade in order to assess the completeness of the assembly. BUSCO (v4.1.4) analysis was performed on the Iso-Seq transcriptome and the genome assembly, as well as the output of the individual SMRT Cells. In each case, fasta files containing all genomic or transcriptomic sequences were run through BUSCO using the lineage eudicots_odb10 (2020-09-10) and default parameters. The graphical summary of results was produced using the script generate_plot.py included in the BUSCO installation.

**Results and Discussion**

*Silene noctiflora* Iso-Seq transcriptome: Gene content and duplication

Sequencing of the Iso-Seq library on two Sequel SMRT Cells produced 711,625 and 686,576 reads for the first and second cells, respectively, where each read was derived from a single molecule. The two SMRT Cells differed substantially in data yield, with totals of 12,765,109 and 21,844,543 subreads, corresponding to subread counts of 17.9 and 31.8 per read, respectively. These reads were merged into 65,642 distinct high-quality transcripts according to the thresholds of the Iso-Seq 3.1 *merge* and *polish* commands. Of these transcripts, only 14 were found to be non-plant sequences, all of which were derived from *Frankliniella occidentalis* (the western flower thrip), a common greenhouse pest that likely contaminated our tissue samples. We annotated these transcripts using Trinotate (Bryant *et al.* 2017); our dataset contains 69,846 total entries for the 65,642 transcripts (transcripts with multiple predicted proteins are represented by multiple entries). Of the 69,846 entries, 48,742 (74.3%) have an annotated PFAM domain, 47,504 (68.0%) have a KEGG annotation, and 55,993 (80.2%) have at least one predicted Gene Ontology term.

Each high-quality transcript represents collapsed reads, meaning that identical or nearly identical sequences are represented by the same final sequence. However, the Iso-Seq pipeline does not collapse alternatively spliced transcripts, or isoforms; thus, this final dataset includes multiple transcripts derived from the same genes. In addition to separately representing isoforms, the transcriptome data could also contain alleles of the same gene and transcripts from paralogs (gene duplicates). Given sufficiently divergent alleles or paralogs, pairs of these types of
sequences will also be represented by separate final transcripts in this dataset. Due to the low levels of polymorphism and heterozygosity in *S. noctiflora* (Sloan *et al.* 2012a), we did not expect different alleles to comprise a major portion of this dataset.

Based on a BUSCO analysis (Seppey *et al.* 2019), the Iso-Seq transcriptome had a completeness of 74.9%. This estimate included a large number of duplicated BUSCOs (47.7%), but these do not necessarily represent true gene duplications for the reasons stated above (Figure 2). The merged dataset had a higher completeness percentage than either of the individual SMRT Cells, where the second SMRT Cell was more complete than the first, consistent with the differential data yield between the two cells (Figure 2). The estimated BUSCO completeness of the transcriptome was lower than that of the assembled nuclear genome (see below), which suggests that some genes with low or tissue-specific expression were not captured. Future efforts to generate deeper sequencing across a wider sample of tissues and environments may be beneficial in this respect.

We used the Cogent (https://github.com/Magdoll/Cogent/wiki) family finding algorithm to further collapse the transcripts into groups of isoforms (alternative transcripts) originating from the same gene. Notably, if paralogs (gene duplicates) have high enough sequence similarity, this binning could include them in the same group. We then used the Cogent data along with Cupcake (https://github.com/Magdoll/cDNA_Cupcake/wiki) to calculate the number of genes and isoforms represented in the transcriptome. Based on this analysis, the Iso-Seq transcriptome contains 14,126 *S. noctiflora* genes and 25,317 isoforms. Of the 14,126 genes, 7,027 had a single isoform (49.7%). We also calculated gene and isoform counts for each individual SMRT Cell; the first SMRT Cell produced 6,790 genes and 10,568 isoforms, while the second SMRT Cell produced 10,283 genes and 17,000 isoforms.

We wanted to test the ability of Iso-Seq to detect and distinguish known paralogs of varying levels of divergence using the Cogent family finding output. To this end, we used a sample gene family—the core subunit genes of the plastid Clp complex, as they have a rich history of paralogy. In *E. coli* and most other bacteria, the core of the Clp complex, which is responsible for proteolysis, contains 14 identical subunits (Yu and Houry 2007). In cyanobacteria, gene
duplication has led to four different core subunit-encoding genes (Stanne et al. 2007). Continued
gene duplication in the land plant lineage has further reshaped this complex in plastids; the 14
core subunits are encoded by nine different genes in A. thaliana, eight of which are nuclear
encoded (CLPP3-6, CLPR1-4), and one of which is plastid encoded (clpP1) (Nishimura and van
Wijk 2015). Further, we had previously identified a more recent duplication of CLPP5 in Silene,
as well as duplications of the plastid-encoded clpP1 in a small number of angiosperm species
(Erixon and Oxelman 2008; Rockenbach et al. 2016; Williams et al. 2019). The Clp complex is
one of the most highly expressed stromal proteases (Nishimura and van Wijk 2015). It is
expressed in most tissues throughout the life stages of the plant, including the tissues from which
we extracted RNA (Zheng et al. 2002). Thus, we would expect a transcriptome generated from
the tissues we used to yield sequences of the various components of the Clp complex.

We used the Cogent output to examine the nine nuclear-encoded Clp core genes in S. noctiflora.
The core genes CLPP3, CLPP4, CLPP5A, CLPP5B, CLPP6, CLPR1, CLPR3, and CLPR4 were
each represented by a single group in the Cogent output, whereas CLPR2 was represented by two
groups. Upon further examination, one of these groups actually represented two different genes,
yielding a total of three CLPR2 genes in S. noctiflora. Thus, CLPR2 was duplicated in this
lineage, and then one paralog underwent a second gene duplication. Based on a phylogenetic
analysis (Figure 3), these two duplications are shared with S. undulata but none of the other
sampled Silene species. Thus, these duplications likely occurred after Silene section Elisanthe
(including S. noctiflora, S. undulata, and S. turkestanica) diverged from the other members of
the genus (Jafari et al. 2020; Moiloa et al. 2021).

The Iso-Seq data allowed us to identify transcripts from every known nuclear-encoded Clp core
gene in S. noctiflora, including the closely related CLPP5A and CLPP5B subunits, as well as an
additional, previously unreported triplication of CLPR2. To corroborate the triplication of
CLPR2 in S. noctiflora that was identified using the Iso-Seq transcriptome, we used the CLPR2
sequence from Rockenbach et al (2016) as a query in a blastn search against the S. noctiflora
genome assembly. This search returned four scaffold hits. Upon examination, each CLPR2 gene
identified in the Iso-Seq transcriptome was represented by one scaffold. The fourth scaffold
represented all three gene copies in a short region of high sequence identity between them,
suggesting collapsing of similar sequence content within the genome assembly. Thus, each

**CLPR2** gene was fully represented by sequences on two scaffolds—there was one unique
scaffold per gene containing most of the sequence and one scaffold containing sequence shared
by all three genes.

### Silene genome size estimates and chromosome number

Genome sizes of *S. noctiflora*, *S. conica*, *S. vulgaris*, and *S. latifolia* were determined using flow
cytometry. Our estimates for *S. vulgaris* and *S. latifolia* (1.07 and 2.67 Gb, respectively; **Table**
1) were concordant with previously published estimates for these two species of 1.11 and 2.64
Gb (*Costich et al.* 1991; *Siroký et al.* 2001). Interestingly, despite their similar and extreme
patterns of organelle evolution (*Sloan et al.* 2012a, 2014), including large mitochondrial

genomes, *S. noctiflora* and *S. conica* have very different nuclear genome sizes. We found their
respective genome sizes to be approximately 2.74 and 0.93 Gb, respectively (**Table 1**), which are
on opposite ends of the spectrum for *Silene* diploids (*Pellicer and Leitch* 2020). The *S. noctiflora*
nuclear genome is almost three-fold larger than that of *S. conica* suggesting that mitochondrial
genome size is not necessarily correlated with nuclear genome size.

Most diploids in the genus, including *S. noctiflora*, have a chromosome number of 2n=24, which
is likely the ancestral number (*Bari* 1973; *McNeill* 1980; *Yildiz et al.* 2008; *Kemal et al.* 2009;
*Gholipour and Sheidai* 2010; *Ghasemi et al.* 2015; *Mirzadeh Vaghefi and Jalili* 2019). There are
also numerous polyploid *Silene* species, including tetraploid, hexaploid, and octaploid forms
(*Kruckeberg* 1960; *Popp and Oxelman* 2001, 2007; *Popp et al.* 2005; *Bai et al.* 2012). *Silene*
noctiflora has been previously reported as a diploid (*McNeill* 1980; *Yildiz et al.* 2008; *Ghasemi*
et al. 2015). Given its relatively large genome size, we sought to confirm this result in our
sampled population with a karyotype analysis (**Figure 4**), which indeed supported the conclusion
that *S. noctiflora* OPL is diploid.

### The *Silene noctiflora* nuclear genome

Illumina sequencing produced ~50× coverage of the *S. noctiflora* genome for a 275-bp paired-
end library and ~15-20× for each of two mate-pair libraries. By performing a *de novo* assembly
of these reads, we obtained a total assembly length (including estimated scaffold gaps) of 2.58 Gb, which is generally consistent with our estimate based on flow cytometry for *S. noctiflora* OPL (2.71 Gb). Given that we relied entirely on short-read sequencing technology, it was not surprising that the resulting assembly of this large genome was highly fragmented (79,768 scaffolds with a scaffold N50 of 59 kb; 222,040 contigs [minimum length of 1 kb for reporting contigs] with a contig N50 of 4.8 kb). Moreover, assembly gaps made up 73% of the total scaffold length, presumably representing the highly repetitive content that is typical of plant nuclear genomes. As such, the assembled gap-free sequences amount to only about a quarter of the genome (702 Mb). Given the expected low levels of polymorphism and heterozygosity in *S. noctiflora* (Sloan et al. 2012a), the assembly was interpreted as a single haplotype and no attempt was made to phase the two distinct haplotypes within the diploid.

BUSCO analysis (Seppey et al. 2019) provided an estimate of 89.5% completeness for the *S. noctiflora* genome assembly (Figure 2). Only 3.0% of BUSCOs were reported to be duplicated, in great contrast to the transcriptome, where 47.7% of BUSCOs were duplicated. Given that the final Iso-Seq dataset includes alternatively spliced transcripts as separate entries, it is not surprising that the transcriptome had a higher percentage of duplicated BUSCOs than the genome assembly.

As a complement to the Iso-Seq transcriptome, this *S. noctiflora* genome assembly should provide a useful resource to query for sequences of interest, especially in genic regions, and to compare against *S. latifolia* and other members of this genus. However, a more complete assembly that includes repetitive regions of the genome will require additional data from long-read technologies such as PacBio or nanopore sequencing. The Iso-Seq data generated in this study may be helpful in combination with improved genomic sequencing data in the future, as a means to improve scaffolding (Zhu et al. 2018), resolve paralogs (e.g., the collapsed regions of the CLPR2 paralogs in the genome assembly), and annotate gene models.

**Data Availability Statement**

The original subread bam files and final transcript sequences longer than 199 bp from the PacBio Iso-Seq transcriptome are available at NCBI Sequence Read Archive (SRA accession...
SRR11784995) and NCBI Transcriptome Shotgun Assembly Sequence Database (TSA accession GIOF01000000), respectively. The genome assembly has been deposited in GenBank (accession VHZZ00000000.1). Additional data have been provided at GitHub (https://github.com/alissawilliams/Silene_noctiflora_IsoSeq): 1) the full transcriptome as outputted by the PacBio Iso-Seq pipeline, 2) the annotation report for the transcriptome, 3) a custom script used to create a gene_trans_map file for our data in order to use Trinotate on non-Trinity-derived data (i.e. transcripts derived from sources other than a Trinity assembly, in this case Iso-Seq transcripts), 4) the Cogent output containing collapsed groups of transcripts, and 5) the set of trimmed, aligned sequences used in the CLPR2 phylogenetic analysis.

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Conflict of Interest Statement

The authors declare that there is no conflict of interest.

Funder Information

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Literature Cited

Abdel-Ghany, S. E., M. Hamilton, J. L. Jacobi, P. Ngam, N. Devitt et al., 2016 A survey of the sorghum transcriptome using single-molecule long reads. Nature Communications 7: 1–11.
Alkan, C., S. Sajjadian, and E. E. Eichler, 2011 Limitations of next-generation genome sequence assembly. Nat Methods 8: 61–65.

Anvar, S. Y., G. Allard, E. Tseng, G. M. Sheynkman, E. de Klerk et al., 2018 Full-length mRNA sequencing uncovers a widespread coupling between transcription initiation and mRNA processing. Genome Biology 19: 46.

Au, K. F., J. G. Underwood, L. Lee, and W. H. Wong, 2012 Improving PacBio Long Read Accuracy by Short Read Alignment. PLoS One 7:.

Bai, C., W. S. Alverson, A. Follansbee, and D. M. Waller, 2012 New reports of nuclear DNA content for 407 vascular plant taxa from the United States. Ann. Bot. 110: 1623–1629.

Balounova, V., R. Gogela, R. Cegan, P. Cangren, J. Zluvova et al., 2019 Evolution of sex determination and heterogamety changes in section Otites of the genus Silene. Scientific Reports 9: 1–13.

Bari, E. A., 1973 Cytological Studies in the Genus Silene L. New Phytologist 72: 833–838.

Bateman, A., L. Coin, R. Durbin, R. D. Finn, V. Hollich et al., 2004 The Pfam protein families database. Nucleic Acids Res 32: D138–D141.

Bernasconi, G., J. Antonovics, A. Biere, D. Charlesworth, L. F. Delph et al., 2009 Silene as a model system in ecology and evolution. Heredity 103: 5–14.

Bertrand, Y. J. K., A. Petri, A.-C. Scheen, M. Töpel, and B. Oxelman, 2018 De novo transcriptome assembly, annotation, and identification of low-copy number genes in the flowering plant genus Silene (Caryophyllaceae). bioRxiv 290510.

Blavet, N., D. Charif, C. Oger-Desfeux, G. A. Marais, and A. Widmer, 2011 Comparative high-throughput transcriptome sequencing and development of SiESTa, the Silene EST annotation database. BMC Genomics 12: 376.
Bryant, D. M., K. Johnson, T. DiTommaso, T. Tickle, M. B. Couger et al., 2017 A Tissue-Mapped Axolotl De Novo Transcriptome Enables Identification of Limb Regeneration Factors. Cell Reports 18: 762–776.

Camacho, C., G. Coulouris, V. Avagyan, N. Ma, J. Papadopoulos et al., 2009 BLAST+: architecture and applications. BMC Bioinformatics 10: 421.

Casimiro-Soriguer, I., E. Narbona, M. L. Buide, J. C. del Valle, and J. B. Whittall, 2016 Transcriptome and Biochemical Analysis of a Flower Color Polymorphism in Silene littorea (Caryophyllaceae). Front. Plant Sci. 7:

Charlesworth, D., 2006 Evolution of Plant Breeding Systems. Current Biology 16: R726–R735.

Costich, D. E., T. R. Meagher, and E. J. Yurkow, 1991 A rapid means of sex identification in Silene latifolia by use of flow cytometry. Plant Mol Biol Rep 9: 359–370.

Dagher-Kharrat, M. B., N. Abdel-Samad, B. Douaihy, M. Bourge, A. Fridlender et al., 2013 Nuclear DNA C-values for biodiversity screening: Case of the Lebanese flora. Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology 147: 1228–1237.

Darriba, D., G. L. Taboada, R. Doallo, and D. Posada, 2012 jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772–772.

Davis, S. L., and L. F. Delph, 2005 Prior Selfing and Gynomonoecy in Silene noctiflora L. (Caryophyllaceae): Opportunities for Enhanced Outcrossing and Reproductive Assurance. International Journal of Plant Sciences 166: 475–480.

Desfeux, C., S. Maurice, J. P. Henry, B. Lejeune, and P. H. Gouyon, 1996 Evolution of reproductive systems in the genus Silene. Proc. Biol. Sci. 263: 409–414.
Doyle, J. J., and J. L. Doyle, 1987 A rapid DNA isolation procedure for small quantities of fresh leaf tissue. PHYTOCHEMICAL BULLETIN.

Erixon, P., and B. Oxelman, 2008 Whole-Gene Positive Selection, Elevated Synonymous Substitution Rates, Duplication, and Indel Evolution of the Chloroplast clpP1 Gene. PLOS ONE 3: e1386.

Garraud, C., B. Brachi, M. Dufay, P. Touzet, and J. A. Shykoff, 2011 Genetic determination of male sterility in gynodioecious Silene nutans. Heredity 106: 757–764.

Ghasemi, F. S., A. Jalili, and S. S. Mirzadeh Vaghefi, 2015 CHROMOSOME REPORT OF THREE SPECIES OF FLORA OF IRAN. 21: 165–168.

Gholipour, A., and M. Sheidai, 2010 Karyotype analysis and new chromosome number reports in Silene species (sect. Auriculatae, Caryophyllaceae). Biologia 65: 23–27.

Gnerre, S., I. Maccallum, D. Przybylski, F. J. Ribeiro, J. N. Burton et al., 2011 High-quality draft assemblies of mammalian genomes from massively parallel sequence data. Proc. Natl. Acad. Sci. U.S.A. 108: 1513–1518.

Gordon, S. P., E. Tseng, A. Salamov, J. Zhang, X. Meng et al., 2015 Widespread Polycistronic Transcripts in Fungi Revealed by Single-Molecule mRNA Sequencing. PLoS One 10:.

Gregory, T. R., J. A. Nicol, H. Tamm, B. Kullman, K. Kullman et al., 2007 Eukaryotic genome size databases. Nucleic Acids Res 35: D332–D338.

Guindon, S., J.-F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk et al., 2010 New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. Syst Biol 59: 307–321.
Guo, W., F. Grewe, W. Fan, G. J. Young, V. Knoop et al., 2016 Ginkgo and Welwitschia Mitogenomes Reveal Extreme Contrasts in Gymnosperm Mitochondrial Evolution. Mol Biol Evol 33: 1448–1460.

Hahn, M. W., S. V. Zhang, and L. C. Moyle, 2014 Sequencing, Assembling, and Correcting Draft Genomes Using Recombinant Populations. G3 (Bethesda) 4: 669–679.

Havird, J. C., P. Trapp, C. M. Miller, I. Bazos, and D. B. Sloan, 2017 Causes and Consequences of Rapidly Evolving mtDNA in a Plant Lineage. Genome Biol Evol 9: 323–336.

Havird, J. C., Whitehill Nicholas S., Snow Christopher D., and Sloan Daniel B., 2015 Conservative and compensatory evolution in oxidative phosphorylation complexes of angiosperms with highly divergent rates of mitochondrial genome evolution. Evolution 69: 3069–3081.

Hestand, M. S., J. V. Houdt, F. Cristofoli, and J. R. Vermeesch, 2016 Polymerase specific error rates and profiles identified by single molecule sequencing. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 784–785: 39–45.

Jafari, F., S. Zarre, A. Gholipour, F. Eggens, R. K. Rabeler et al., 2020 A new taxonomic backbone for the infrageneric classification of the species-rich genus Silene (Caryophyllaceae). TAXON 69: 337–368.

Jeffries, A. R., S. Leung, I. Castanho, K. Moore, J. P. Davies et al., 2020 Full-length transcript sequencing of human and mouse identifies widespread isoform diversity and alternative splicing in the cerebral cortex. bioRxiv 2020.10.14.339200.

Katoh, K., and D. M. Standley, 2013 MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Mol Biol Evol 30: 772–780.
Kemal, Y., E. Minareci, and A. Çirpici, 2009 Karyotypic study on Silene, section Lasiostemones species from Turkey. Caryologia 62: 134–141.

Klaas, A. L., and M. S. Olson, 2006 Spatial Distributions of Cytoplasmic Types and Sex Expression in Alaskan Populations of Silene acaulis. International Journal of Plant Sciences 167: 179–189.

Krasovec, M., M. Chester, K. Ridout, and D. A. Filatov, 2018 The Mutation Rate and the Age of the Sex Chromosomes in Silene latifolia. Curr. Biol. 28: 1832-1838.e4.

Kreibich, J. A., 2010 Using SQLite. O’Reilly Media, Inc.

Krogh, A., B. Larsson, G. von Heijne, and E. L. Sonnhammer, 2001 Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J. Mol. Biol. 305: 567–580.

Kruckeberg, A. R., 1960 CHROMOSOME NUMBERS IN SILENE (CARYOPHYLLACEAE). II. Madroño 15: 205–215.

Lagesen, K., P. Hallin, E. A. Rødland, H.-H. Stærfeldt, T. Rognes et al., 2007 RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 35: 3100–3108.

Lan, T., T. Renner, E. Ibarra-Laclette, K. M. Farr, T.-H. Chang et al., 2017 Long-read sequencing uncovers the adaptive topography of a carnivorous plant genome. Proc Natl Acad Sci U S A 114: E4435–E4441.

Li, H., 2018 Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics 34: 3094–3100.

Martin, M., 2011 Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal.
McNeill, J., 1980 THE BIOLOGY OF CANADIAN WEEDS.: 46. Silene noctiflora L. Can. J. Plant Sci. 60: 1243–1253.

Mirzadeh Vaghefi, S. S., and A. Jalili, 2019 CHROMOSOME NUMBERS OF SOME VASCULAR PLANT SPECIES FROM IRAN. The Iranian Journal of Botany 25: 140–144.

Moiloa, N. A., M. Mesbah, S. Nylinder, J. Manning, F. Forest et al., 2021 Biogeographic origins of southern African Silene (Caryophyllaceae). Molecular Phylogenetics and Evolution 107199.

Mower, J. P., P. Touzet, J. S. Gummow, L. F. Delph, and J. D. Palmer, 2007 Extensive variation in synonymous substitution rates in mitochondrial genes of seed plants. BMC Evol. Biol. 7: 135.

Muylde, A., N. Zemp, C. Deschamps, S. Mousset, A. Widmer et al., 2012 Rapid De Novo Evolution of X Chromosome Dosage Compensation in Silene latifolia, a Plant with Young Sex Chromosomes. PLOS Biology 10: e1001308.

Nishimura, K., and K. J. van Wijk, 2015 Organization, function and substrates of the essential Clp protease system in plastids. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1847: 915–930.

Olson, M. S., and D. E. Mccauley, 2002 Mitochondrial Dna Diversity, Population Structure, and Gender Association in the Gynodioecious Plant Silene Vulgaris. Evolution 56: 253–262.

Ono, Y., K. Asai, and M. Hamada, 2013 PBSIM: PacBio reads simulator—toward accurate genome assembly. Bioinformatics 29: 119–121.

PacificBiosciences, 2020 IsoSeq. Pacific Biosciences.
Papadopulos, A. S. T., M. Chester, K. Ridout, and D. A. Filatov, 2015 Rapid Y degeneration and dosage compensation in plant sex chromosomes. PNAS 112: 13021–13026.

Pellicer, J., and I. J. Leitch, 2020 The Plant DNA C-values database (release 7.1): an updated online repository of plant genome size data for comparative studies. New Phytologist 226: 301–305.

Petersen, T. N., S. Brunak, G. von Heijne, and H. Nielsen, 2011 SignalP 4.0: discriminating signal peptides from transmembrane regions. Nature Methods 8: 785–786.

Popp, M., P. Erixon, F. Eggens, and B. Oxelman, 2005 Origin and Evolution of a Circumpolar Polyploid Species Complex in Silene (Caryophyllaceae) Inferred from Low Copy Nuclear RNA Polymerase Introns, rDNA, and Chloroplast DNA. Systematic Botany 30: 302–313.

Popp, M., and B. Oxelman, 2001 Inferring the History of the Polyploid Silene aegaea (Caryophyllaceae) Using Plastid and Homoeologous Nuclear DNA Sequences. Molecular Phylogenetics and Evolution 20: 474–481.

Popp, M., and B. Oxelman, 2007 Origin and evolution of North American polyploid Silene (Caryophyllaceae). American Journal of Botany 94: 330–349.

Potter, S. C., A. Luciani, S. R. Eddy, Y. Park, R. Lopez et al., 2018 HMMER web server: 2018 update. Nucleic Acids Res 46: W200–W204.

Rhoads, A., and K. F. Au, 2015 PacBio Sequencing and Its Applications. Genomics, Proteomics & Bioinformatics 13: 278–289.

Rockenbach, K., J. C. Havird, J. G. Monroe, D. A. Triant, D. R. Taylor et al., 2016 Positive Selection in Rapidly Evolving Plastid–Nuclear Enzyme Complexes. Genetics 204: 1507–1522.
Schatz, M. C., J. Witkowski, and W. R. McCombie, 2012 Current challenges in de novo plant genome sequencing and assembly. Genome Biol 13: 243.

Seppey, M., M. Manni, and E. M. Zdobnov, 2019 BUSCO: Assessing Genome Assembly and Annotation Completeness, pp. 227–245 in Gene Prediction: Methods and Protocols, edited by M. Kollmar. Methods in Molecular Biology, Springer, New York, NY.

Siroký, J., M. A. Lysák, J. Dolezel, E. Kejnovský, and B. Vyskot, 2001 Heterogeneity of rDNA distribution and genome size in Silene spp. Chromosome Res. 9: 387–393.

Sloan, D. B., A. J. Alverson, J. P. Chuckalovcak, M. Wu, D. E. McCauley et al., 2012a Rapid Evolution of Enormous, Multichromosomal Genomes in Flowering Plant Mitochondria with Exceptionally High Mutation Rates. PLOS Biology 10: e1001241.

Sloan, D. B., S. R. Keller, A. E. Berardi, B. J. Sanderson, J. F. Karpovich et al., 2012b De novo transcriptome assembly and polymorphism detection in the flowering plant Silene vulgaris (Caryophyllaceae). Molecular Ecology Resources 12: 333–343.

Sloan, D. B., D. A. Triant, N. J. Forrester, L. M. Bergner, M. Wu et al., 2014 A recurring syndrome of accelerated plastid genome evolution in the angiosperm tribe Sileneae (Caryophyllaceae). Molecular Phylogenetics and Evolution 72: 82–89.

Städler, T., and L. F. Delph, 2002 Ancient mitochondrial haplotypes and evidence for intragenic recombination in a gynodioecious plant. PNAS 99: 11730–11735.

Stanne, T. M., E. Pojidaeva, F. I. Andersson, and A. K. Clarke, 2007 Distinctive Types of ATP-dependent Clp Proteases in Cyanobacteria. J. Biol. Chem. 282: 14394–14402.

UniProt: a hub for protein information, 2015 Nucleic Acids Res 43: D204–D212.
Wang, B., V. Kumar, A. Olson, and D. Ware, 2019 Reviving the Transcriptome Studies: An Insight Into the Emergence of Single-Molecule Transcriptome Sequencing. Front. Genet. 10:.

Wang, B., E. Tseng, P. Baybayan, K. Eng, M. Regulski et al., 2020 Variant phasing and haplotypic expression from long-read sequencing in maize. Communications Biology 3: 1–11.

Wang, B., E. Tseng, M. Regulski, T. A. Clark, T. Hon et al., 2016 Unveiling the complexity of the maize transcriptome by single-molecule long-read sequencing. Nature Communications 7: 1–13.

Weirather, J. L., M. de Cesare, Y. Wang, P. Piazza, V. Sebastiano et al., 2017 Comprehensive comparison of Pacific Biosciences and Oxford Nanopore Technologies and their applications to transcriptome analysis. F1000Res 6:.

Wenger, A. M., P. Peluso, W. J. Rowell, P.-C. Chang, R. J. Hall et al., 2019 Accurate circular consensus long-read sequencing improves variant detection and assembly of a human genome. Nature Biotechnology 37: 1155–1162.

Williams, A. M., G. Friso, K. J. van Wijk, and D. B. Sloan, 2019 Extreme variation in rates of evolution in the plastid Clp protease complex. The Plant Journal 98: 243–259.

Xu, Z., R. J. Peters, J. Weirather, H. Luo, B. Liao et al., 2015 Full-length transcriptome sequences and splice variants obtained by a combination of sequencing platforms applied to different root tissues of Salvia miltiorrhiza and tanshinone biosynthesis. The Plant Journal 82: 951–961.

Yildiz, K., E. Minareci, A. Çirpici, and M. Y. Dadandi, 2008 A karyotypic study on Silene, section Siphonomorpha species of Turkey. Nordic Journal of Botany 26: 368–374.
Yu, A. Y. H., and W. A. Houry, 2007 ClpP: A distinctive family of cylindrical energy-dependent serine proteases. FEBS Letters 581: 3749–3757.

Zhao, L., H. Zhang, M. V. Kohnen, K. V. S. K. Prasad, L. Gu et al., 2019 Analysis of Transcriptome and Epitranscriptome in Plants Using PacBio Iso-Seq and Nanopore-Based Direct RNA Sequencing. Front Genet 10: 253.

Zheng, B., T. Halperin, O. Hruskova-Heidingsfeldova, Z. Adam, and A. K. Clarke, 2002 Characterization of Chloroplast Clp proteins in Arabidopsis: Localization, tissue specificity and stress responses. Physiologia Plantarum 114: 92–101.

Zhu, B.-H., J. Xiao, W. Xue, G.-C. Xu, M.-Y. Sun et al., 2018 P_RNA_scaffolder: a fast and accurate genome scaffold using paired-end RNA-sequencing reads. BMC Genomics 19: 175.
Table 1: Genome sizes determined by flow cytometry

| Species          | Population | Location               | Samples, 2C (pg) | Mean Genome Size |
|------------------|------------|------------------------|------------------|------------------|
| *Silene noctiflora* | OPL*       | Opole, Poland          | 5.65, 5.61, 5.46, 5.44 | Mean Genome Size |
|                  | OSR        | Giles County, VA       | 5.75, 5.61       |                  |
|                  | BRP        | Nelson County, VA      | 5.63, 5.57       |                  |
| *Silene conica*  | ABR        | Abruzzo, Italy         | 1.92, 1.92, 1.88 |                  |
| *Silene vulgaris*| S9L        | Giles County, VA       | 2.19, 2.16       |                  |
| *Silene latifolia* | UK2600   | Bedford County, VA     | 5.46, 5.45       |                  |

*The *S. noctiflora* OPL population was used for Iso-Seq, genome assembly, and karyotyping.

Units: pg = picogram, Gb = gigabase, 1C = haploid amount, 2C = diploid amount

**Figure Legends**

**Figure 1**: *Silene noctiflora*, also known as the night-flowering catchfly.

**Figure 2**: BUSCO analysis of the *S. noctiflora* genome assembly, Iso-Seq transcriptome (full dataset), and the individual SMRT Cells that were merged to create the Iso-Seq transcriptome.

**Figure 3**: Phylogenetic analysis of *CLPR2* genes in *S. noctiflora* and related species. Branch lengths represent nucleotide sequence divergence. This tree was rooted on the *Agrostemma githago* sequence. The placement of *S. paradoxa* is in conflict with the species tree (Jafari et al. 2020), likely due to long branch attraction and the multiple independent evolutionary rate accelerations in this protein across *Silene* (Rockenbach et al. 2016).

**Figure 4**: Micrograph verifying the diploidy of *Silene noctiflora* at 100× magnification. Although an exact chromosome count is difficult to determine, this image suggests that *S. noctiflora* is a diploid with the typical number of 24 chromosomes previously documented in this species and the genus in general, rather than polyploid with 48 or more chromosomes (Bari 1973; McNeill 1980; Yildiz et al. 2008; Kemal et al. 2009; Gholipour and Sheidai 2010; Ghasemi et al. 2015; Mirzadeh Vaghefi and Jalili 2019).
