Clonal architectures and diversity spatial pattern of different ploidies for Clintonia udensis in the Hualongshan Mountains

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Abstract

Clintonia udensis is a perennial herb possessing two cytotypes diploid (2n=14) and tetraploid (4n=28). In the Hualongshan Mountains, the diploid of C. udensis primarily grows in 2450 m areas on the south slopes, while the tetraploid grows mainly in 1900 m areas on the north slopes. So, this intra-polyploidy is regarded as an excellent material to study the origination, evolution and adaption of plant polyploidy. Through field investigations and molecular genotyping, we initially analyzed the bud bank spatial characteristics, clonal growth, and spatial genetic structure populations between the different ploidy of C. udensis. It found that the rhizome knot styles of C. udensis had zigzag, C, V, and Y models between the two cytotypes. There was no dominated clone present in the diploid or tetraploid. The clone architectures of two ploidies were both phalanxes. However, the number of rhizome knots, the number of buds of each rhizome knot, the ratio of rhizome branches, and average tetraploid clones were higher than that of the diploid. The diversity indices of the tetraploid, such as clone diversity index, genetic distribution uniformity, and genetic diversity index, were also slightly higher than that of the diploid. Thus, clonal reproduction differentiation and significant genetic variations occurred between the diploid and tetraploid of C. udensis. These two cytotypes, through seed reproduction and clonal growth, became a facultative clonal species and maintained its survival stability and reproduction. During the evolutionary process, the tetraploid of C. udensis with higher clonal diversity and genetic diversity responded and adapted to new surroundings that different from the ancestral diploid in the Hualongshan Mountains.

KEYWORDS

Clintonia udensis, intra-polyploidy, bud bank, clonal spatial structure, genetic diversity, adaption

1 INTRODUCTION

Angiosperms can reproduce not only through sexual reproduction, but also simultaneously with asexual reproduction. In asexual reproduction, the clonal growth of the organisms is the most important pathway (Geremew et al., 2018; Mandel et al., 2019; Sandén et al., 2020). It is estimated that ~80% of angiosperms can engage in clonal growth (Wang et al., 2010; Geremew et al., 2018; Wang et al., 2018a). Clonal plants general occupy dominant positions in forests, grasslands, wetlands, deserts, and other habitats, can expand their living space through clonal growth, and exhibit more morphological plasticity through clonal ramets. Meanwhile, clonal plants have a greater capacity to utilize resources in adapting to heterogeneous environments, and play a critical role in the maintenance of ecosystems (Kartzinel et al., 2015; Van Drunen et al., 2015; Wang et al., 2018a). Therefore, the growth characteristics and spatial structure of clonal plants have been a topic of intense interest in ecology and evolution research (Chen et al., 2016; Geremew et al., 2018; Wang et al., 2018a).

If the clonal plant has more buds, it would have relatively higher ratio of clonal growth, as which is the basis of the germination and development of ramets (Wang et al., 2016). Usually, clonal growth has significant impacts

on the spatial structures and level of diversity of plant populations. Some researchers showed the clonal plants can form spatially diverse genetic structures and different clonal diversity plaques, at even smaller spatial scales (Wang et al., 2018a). Meanwhile, asexual reproduction can decrease the level of genetic diversity of population (Zhang & Zhang, 2006) and influence and determine the adaptability of plants to specific environments during the evolutionary process (van Drunen et al., 2015; Pirog et al., 2017). Nevertheless, the spatial structures and diversity of clonal plants may be affected by biological and abiotic factors such as habitat heterogeneity, growth strategies, and seedling recruitment patterns (Kartzinel et al., 2015; Sandén et al., 2020). So, analyzing spatial genetic structures and clarifying the genetic patterns of different patches of clonal plants might be of great significance for exploring the formation, maintenance, and decline mechanisms of clonal plant populations (Kartzinel et al., 2015; Wang et al., 2018b; Mandel et al., 2019).

The perennial herb *Clintonia udensis* of the genus*Clintonia* Raf, which is widely distributed across Eastern Asia, has two cytotypes that include diploid (2n = 14) and tetraploid (4n = 28) (Wang et al., 2008; Wang et al., 2010; Wang et al., 2011; He et al., 2017). Although the diploid is continuously and extensively distributed from Yunnan Province of South China to Heilongjiang Province in Northern China, the tetraploid has only a limited presence in these areas, encompassing the Jade Dragon Snow Mountains in Yunnan Province, Shennongjia in Hubei Province, and the Hualongshan Mountains in Shaanxi Province, which overlap or are adjacent to the diploid distribution areas.

The morphological characteristics of *C. udensis* leaves, fruits, inflorescences, and pollens presented complex diversity between different populations; however, no significant differences were found between the different ploidies. The seed size of this perennial herb is significantly different between the diploid and tetraploid (Wang & Zhao, 2007). Field investigations revealed that the color of the scape in the diploid is brown, while that is green in the tetraploid (Bai et al., 2009), and both the diploid and tetraploid have a hard rhizome.*C. udensis* not only asexually reproduces through axillary buds but also generates seeds through sexual reproduction. In wild habitats, diploids and tetraploids can generally produce seedlings and grow in decayed fallen wood and moist mossy ground; thus, *C. udensis* is a facultative plant (Liu et al., 2016).

However, the trade-off between the sexual reproduction and asexual reproduction of plants is influenced by many factors. Changes in the ratios between sexual reproduction and asexual reproduction in different habitats are primarily determined by the modification of ecological factors such as light, water, and temperature (Huang et al., 2018; Wang et al., 2018; Alonso-Marcos et al., 2019; Wang et al., 2020). Under harsher environments, the ratio of asexual reproduction is increased, i.e. the occurrence and frequency of clonal plants might be higher under stressed conditions (Van Drunen et al., 2015; Wang et al., 2018a; Ma et al., 2019). In the Hualongshan Mountains, the diploid of *C. udensis* grows mainly in areas at 2450 m on the south slopes, while the tetraploid grows in areas at 1900 m on the north slopes which are different from the diploid surroundings. The vertical distribution pattern of different ploidies would be an ideal natural material for studying the clonal growth characteristics, clonal structures, and ecological adaptability of *C. udensis* .

According the previous study, it is an autotetraploid for the tetraploid of *C. udensis* (Wang & Zhao, 2007). The doubled genome of autoploid may imply higher potential and broader adaptability than its ancestral diploid (Wan et al., 2018). Within the autotetraploid with four same chromosomes, it can be expected to a certain extent having a larger effective population size. Thus, the gene diversity level of tetraploid should be higher than that of diploid (Brown et al., 2000; Li et al., 2008; Wang et al., 2016). However, these were mainly obtained from the inter-polyploidy of plants. For the intra-polyploidy, the similar pattern would be occurred between different polyploid still is uncertain.

In this study, we explored the clonal spatial structures of diploid and tetraploid *C. udensis* populations of the Hualongshan Mountains and verified the genetic diversity distribution pattern between intra-polyploid through field investigations and Simple Sequence Repeat (SSR) molecular markers. Our main aims were to: a) analyze the clonal diversity, genetic differentiation, and spatial clonal configurations of different ploidies; and b) reveal rationales for the differences in spatial structures between diploids and tetraploids. These results are likely to further enhance our understanding of the ecological adaptation characteristics of different intra-ploidies, provide insights toward enriching the spatial structures of clonal plants, and establish a foundation

for elucidating the adaptation mechanisms of clonal plants to their environments.

2 MATERIALS AND METHODS

2.1 Sample sites and materials

The sample sites are located at the Hualongshan Mountains National Nature Reserve in Shaanxi Province, with the geographic coordinates 109°16'41" - 109deg30'29" E, 31deg54'39" - 32deg08'13" N. The highest peak of the Hualongshan Mountains is 2917 m above sea level, which is the second highest peak of the Dabashan Mountains in China. Based on the vegetation regionalization of China, the Hualongshan Mountains belong to the subtropical evergreen broadleaved forest, north subtropical evergreen forest, and deciduous broadleaved mixed forest areas.

In the Hualongshan Mountains, the diploid grows within the long and narrow areas on the shady slopes of gullies, from west to east, whereas the tetraploid grows under the arbor forests at 40deg areas of the northern slopes. Ten 1 m x 1 m plots were set in the diploid and tetraploid sample areas, respectively. Within ten samples, a total of 93 and 107 diploid and tetraploid individuals were collected from all the visible individuals in the sample areas. And, the specific spatial positions of each sampled individual of the two ploidies were marked and recorded.

2.2 Clonal configuration analysis

Then the leaves of all the sampled individuals were cut off with scissors, dried using a color-changing silica gel, and stored at -80 in a laboratory refrigerator. Each sample was transferred to the laboratory, where the rhizome nodes (Fig. 1) were observed and measured, once the soil was removed from the roots. The number of *C. udensis* rhizome nodes could be added during each growing season per year. The number of buds (active and dormant buds) on each rhizome were counted. The ages and numbers of branches of the rhizome were also counted.

One-way ANOVA (analysis of variance) of all obtained data was analyzed using SPSS 20 software, and LSD (least-significant difference) was employed to compare the paired data for significance.

2.3 SSR analysis methods

Fresh leaf samples were used to extract the total genomic DNA following the modified CTAB method. The quality of extracted DNA was determined using electrophoresis in a 0.1 % agarose gel, and the concentration of the extracted DNA was detected using an UV nucleic acid analyzer.

According to the SSR molecular markers from the EST (expressed sequence tag) information of Liliaceae (Yang et al. 2008), eight pairs of primers with clear amplification bands and good repeatability were screened out from 18 SSR pairs of primers (Table 1). Subsequently, all collected *C. udensis* individuals were amplified by PCR reactions using the selected primers.

The PCR reaction volume was 10 μL, including mix 5 μL, DNA template 1 μL, SSR primers 0.6 μL, and ddH_2O 3.4 μL. The reaction procedure was as follows: pre-denaturation at 94 for 5min, denaturation at 94 for 30 s, annealing at 45 - 60 for 30 s, extension at 72 for 2 min, 35 cycles in total, extension at 72 for 5 min, and the PCR products were finally stored at -4. The amplified products were separated using 10% denaturing polyacrylamide gel electrophoresis and visualized using silver staining.

2.4 Clonal diversity analysis

Clone diversity was estimated using the total genet number (G), average clone size (N /G), Simpson index (D), and Fager index (E). The calculation formulas of the Simpson and Fager indices were: D = 1-[?]{[N $_i$(N $_i$-1)] /[N (N -1)]}; E = (D-D $_{min}$) / (D $_{max}$ -D $_{min}$); D $_{min}$= (G -1) (2N -G) /N (N -1);D $_{max}$ = (G -1) N /G (N-1). Where, $N = \Sigma N$ $_i$ was the total number of sampled individuals (ramets), G was the total number of genotypes (genets), and N $_i$ was the number of individuals (ramets) with the i genotype.

2.5 Genetic diversity and structure analysis

A total of 51 loci were obtained. The linkage disequilibrium of each locus was detected using GENEPOP software (Raymond & Rousset, 1995), after which the loci that presented linkage disequilibrium were removed. Nei's gene diversity index (H), Shannon phenotype information index (I), population differentiation coefficient (G_{ST}), and the percentage of polymorphic bands (PPL) were calculated using the POPGENE software package (Francis et al., 1999).

The degree of molecular variation and the distribution pattern of genetic variation were analyzed using ARLEQUIN software (Expoffer & Lischer, 2010).

3 RESUILTS

3.1 Clonal characteristics and spatial architectures of diploid and tetraploid

The average number of buds on each diploid rhizome was 2.120, and the variance of buds was 0.277 (Table 2, 3). For the tetraploids, the average number and the variance of buds per rhizome was 2.315 and 0.408, respectively. For the proportion of active buds to total buds, it was 0.627 for the diploid and 0.473 for the tetraploid. The ratio of dormant buds to total buds for the diploid was 0.357, while it was 0.522 for the tetraploid. The extrema values of buds per rhizome for the diploids and tetraploids were same; both the minimum value = 1 and the maximum value = 4. These results revealed that the tetraploids possessed more rhizome buds; however, the number of active buds were lower (dormant buds were higher) than that in the diploids (Table 1). In addition, one-way ANOVA analysis showed that the above indexes, such as ratio of dormant buds to total buds, the ratio of active buds to total buds, and the ratio of dormant buds to active buds, were significantly different among diploid and tetraploid of *C. udensis* ($P<0.05$) (Table 3).

The rhizome styles of the diploids and tetraploids were primarily zigzagged, C, V, and Y (Fig. 2); the "one" style was very rare. The spatial positions between the buds of each rhizome were basically a symmetrical distribution when there were two buds, or formed 90° angles when having 3-4 buds.

The length of rhizome internode was about 1-2cm. Based on our observation, the rhizome bud usually occurred in the rhizome node, and was thought as the ramet produced by the genet. Moreover, the rhizome nodes of some individuals had a special branch architecture. The number of branches was typically from one to four, with the highest frequency of single branches. Additional branches were typically observed for older plants (Fig. 2).

Statistical analysis of the clone reproduction for *C. udensis* in that year revealed that the number of ramets produced by the diploid and tetraploid individuals was minimal, and the ratio of clone individuals between the diploid and the tetraploid were both relative lower (Table 3). Thus, the *C. udensis* clone growth pattern was the phalanx growth form (Fig. 3).

3.2 Clonal diversity and structure of diploid and tetraploid

Both the diploid and tetraploid of *C. udensis* were clonal populations. There were 89 and 46 genets in tetraploid and diploid, respectively. For this species, it also presented relatively higher clonal diversity (Table 4).

The average clone size (N/G) of the tetraploid was 1.146, the Simpson diversity index (D) and Fager evenness index (E) were 0.4695 and 0.6863, respectively. Correspondingly, these parameters for the diploid were 1.109, 0.3741 and 0.4612 respectively, indicating that the tetraploid had higher clonal diversity than the diploid.

No shared genotype was found between diploid and tetraploid (Table 4). The analysis of variance between Simpson diversity index (D) and Fager evenness index (E) showed that there were significant differences in clonal diversity between diploid and tetraploid ($P<0.05$).

There were no dominant clones in the *C. udensis* diploids and tetraploids of the Hualongshan Mountains. The diploids consisted of 46 genotypes (genets), of which three genets had two clonal ramets, and one genet had three clonal ramets, while the other 42 genets had only a single clonal ramet. For the tetraploid, there was

89 genotypes (genets), of which three genets had two clonal ramets, and two genets had three clonal ramets, while the other 78 genets had only a single clonal ramet (Fig. 3). Therefore, both the diploids and tetraploids were polyclonal. Further, no identical individual genotype was found between the different ploidies, which verified that differentiation occurred between the diploids and tetraploids.

It is worth noting that there was differentiation in the clonal reproduction characteristics between the diploids and tetraploids. In the diploids, the numbers of clones (number of genets) were relatively lower, and the distribution of clones or genets was scattered. While for the tetraploids, the distribution of genets was relatively close and dense, which indicated higher clonal diversity.

All of the sample *C. udensis* diploid and tetraploid individuals were amplified by PCR using SSR markers (Table 1). Finally, 51 clear and stable DNA fragments were obtained for eight pairs of SSR primers. The sizes of the DNA fragments were from 350-750 bp. No linkage disequilibrium loci were found. Among the 51 bands, 40 bands were polymorphic. The percentage of polymorphic loci (*PPL*) was 78.4%, in addition, Nei's gene diversity index H was 0.2358, and the Shannon information diversity index I was 0.6337. All these results confirmed a high level of genetic diversity in *C. udensis* (Table 4).

Three genetic diversity indices of the tetraploid were $PPL = 68.5\%$, $H = 0.2332$, and $I = 0.3416$, respectively, which were slightly higher than that of the diploid ($PPL = 63.5\%$, $H = 0.2127$, and $I = 0.3012$) (Table 2). It showed that the genetic diversity of the tetraploid was slightly higher than that of the diploid.

The gene differentiation coefficient G_{ST} between the diploid and tetraploid was 0.6193, which indicated that 61.93% of the total genetic diversity existed between the different ploidies, and the genetic variation within different ploidies was 38.07%. This was consistent with the results of AMOVA analysis, namely, greatest genetic variation occurred between the diploid and the tetraploid (Table 5).

4 DISCUSSION

4.1 Clone architectures of *C. udensis* diploids and tetraploids in the Hualongshan Mountains

Different species have been found to possess different clonal architecture patterns. Furthermore, the clonal architectures of species might represent malleable changes under different habitat conditions (Kartzinel et al., 2015; Wang et al., 2018b; Huang et al., 2018). Within heterogeneous environments, clonal plants can adjust their strategies to secure resources through the pliable changes in their clonal architectures, which ultimately result in changes of the properties of its ramets (Kartzinel et al., 2015; Wang et al., 2018a). Our field investigations found that there was only a single active bud on many rhizomes of different *C. udensis* ploidies.

When *C. udensis* individuals normally grow and develop, they require only one active bud on each rhizome knot to develop aboveground parts every year. However, in some cases, there were 2-4 active buds on the rhizome knot, which began to grow through the year, all of which formed basal rosette seedlings. Each newly sprouted rhizome generated an indefinite number of buds on its rhizome, so that a former "one" style of belowground rhizome could generate new branching. Each new branching continues to sprout new buds in the later growth process (Fig. 2) and extend along a different direction from the original branching of the former rhizome. Thus, the active buds on each rhizome knot could form a symmetrical or 90° angle, which determined and affected the characteristics of the outward growth of new branches. Furthermore, the newly formed branching continued to grow and produce additional rhizome nodes to occupy more spatial positions, to gradually form a clonal plant comprised of multiple ramets (Liu et al., 2013; Ott et al., 2019).

The number and position of buds on the rhizome knot develop and form before the winter. When the temperature drops in the autumn and winter, the aboveground portions of *C. udensis* individuals die. The rhizome and formed rhizome buds continue to live underground through the winter, and during the ensuing spring, some of the active buds sprout aboveground to form caespitose clonal architectures (Qian et al., 2017). The number of active and dormant buds on many rhizome knots are approximately equivalent for the *C. udensis* diploids and tetraploids. Each spring, the active buds begin to germinate and form caespitose seedling sprouts, while the dormant buds remain in a dormant state continuously (Chen et al., 2016).

The distance between the rhizome internodes of the genet in *C. udensis* was relatively short (1-2 cm). The buds of the rhizome typically occurred at the rhizome knot. Generally, the ramets would grow adjacent to each other, whereas more collective clone patchiness in the microspace was formed. According to the annual statistical analysis for diploid and tetraploid clone reproduction, the ratio of individuals having clone reproduction was relatively low for both ploidies. The number of ramets generated by the diploid and tetraploid individuals was also limited, where the spatial distribution pattern of these ramets was quite repetitive (Fig. 2). Thus, the clonal architecture of *C. udensis* was a phalanx pattern (Fig. 3). The spatial pattern and architecture of the clonal population was thought to give rise to the capacity for utilizing environmental heterogenicity (e.g., various resources) for the clonal plants. Although this clonal structure of*C. udensis* had a limited expansion ability, it facilitated the easy utilization of environmental resources at localized sites (Wang & Zhao, 2007; Wang et al., 2008).

Clonal plants can improve the efficacy of clonal reproduction to optimize the survival rates of ramets through physiological integration and foraging behavior (Van Drunen et al., 2015). Due to the continuous expansion of rhizomes, the growth pattern of *C. udensis* gradually formed from a phalanx to a relatively scattered ramet in flocks. This change would meet the growing demands for space and nutrition for clonal growth, while enabling the diploid and tetraploid populations to more reasonably occupy and utilize environmental resources to maintain the sustainable development and survival of the *C. udensis* diploid and tetraploid population (Wang et al., 2008; Wang et al., 2010; Wang et al., 2011). However, *C. udensis* is not suitable for larger clonal populations, as there are no long-wandering spacers in its diploids or tetraploids.

The clonal growth of *C. udensis* belongs to the phalanx mode. If plants with phalanx clonal growth, the source of heterologous pollen is lower than that of plants with guerrilla clonal growth. The limitation of pollen resources would change the mating system of plants from outcrossing to inbreeding. Long-distance pollination by pollinators and the number of clonal ramets visited by pollinators in a flight round can maintain a high outcrossing rate for clonal plants (Bai et al., 2009).*C. udensis* is entomophilous pollination, and its breeding system is partial self-compatibility and outcrossing (Bai et al., 2009). This is a trade-off relationship between clonal growth and sexual reproduction and is a adaptability to the diverse environment (Zhang et al., 2006).

The relative proportion of clonal reproduction and sexual reproduction in plants are impacted by environmental conditions such as water and light. The balance between this two-breeding system primarily depends on environmental changes, resource demands, and interactions between organisms (Pirog et al., 2017; Alonso-Marcos et al., 2019). For *C. udensis* , the clone reproduction level of the tetraploid was higher than that of the diploid (Table 4). Compared with the sexual reproduction of plants, the growth probability and survival rate were much higher than those seedlings produced by seeds, although the resource input was higher when each ramet was produced through clonal reproduction (Van Drunen et al., 2015; Geremew et al., 2018; Smith et al., 2020).

In undisturbed environments, individual plants are consistently in a strong competitive state with each other. Newly generated clonal ramets more easily survive and grow in contrast to plants that are germinated by seeds. Therefore, plants are more likely to adopt clone reproduction when they grow under a long-term and stable environmental selection pressures. This might be related to the difficulty of establishing seedlings from seed reproduction (Chen et al., 2016). When the tetraploids of *C. udensis* form through genome duplication (Li et al., 1996), they explore new surroundings that are completely different from the ancestral diploids. The tetraploids might adapt to this new environment through higher level of clonal growth than its ancestral diploids and grow at relatively lower altitudes than that of the diploids in the Hualongshan Mountains.

To occupy and maintain spatial territories, *C. udensis* had to maintain sexual reproduction due to the instability of water, heat, climate, and other environmental factors in the subalpine environment. Thus, both the diploid and tetraploid populations preserved the sexual reproduction system through seeds as the main forms, and the ancestral diploids sustained a higher rate of sexual reproduction than did the tetraploids. Furthermore, *C. udensis* is a perennial rosette herbaceous species that grows under the shade of forests. Its population distribution was limited by changes in the canopy density of forest communities, environmental temperature, soil moisture, and the thickness of the humus layer. In conjunction with the lower altitudes of

the geographical distribution areas of *C. udensis* , the decomposition period of humus gradually shortened. The humus soil layer that the seeds of *C. udensis* depended on for dormancy and germination gradually became a limiting factor for sustaining the population. During the evolutionary process, the tetraploids might decrease the rate of sexual reproduction and increase clonal growth to stabilize the population.

As both the sexual reproduction and asexual reproduction of plants can contribute to risk sharing and resource acquisition capacities in plaque habitats, as well as the maintenance of genetic diversity, plants can adopt reproductive trade-off strategies under different environments (Zhang et al., 2015). Generally, habitats under forest shade are not helpful for seed germination and seedling growth. *C. udensis*would propagate offspring through clonal growth that could overcome the disadvantage of seedlings that are growing under the forest canopy. When fierce intraspecific competition is restricted by density, plants can avoid intraspecific competition by creating more resources to invest in seed reproduction through its own hormone regulation within a limited space. Therefore, to adapt to the changing subalpine environment, the diploids and tetraploids of *C. udensis* evolved into a facultative clone species, which would maintain population stability and reproduction through both seed reproduction and clone growth.

4.2 Diversity spatial structure of *C. udensis* diploids and tetraploids in the Hualongshan Mountains

Plants through clonal propagation can produce the descendant with the same genotype of parents and affect the genetic diversity and population spatial structure. For facultative clonal plants, it can supply the loss of genetic variation through sexual reproduction and increase the level of genetic diversity within population (Van Drunen & husband, 2018; Alonso Marcos et al., 2019). The genetic diversity of *C. udensis*and different ploidy populations in the Hualongshan Mountains was high (Table 4). This not only confirmed the facultative clonal characteristic of the species but also was consistent with previous studies (Wang et al., 2008; Wang et al., 2010; Wang et al., 2011; He et al., 2017).

The *C. udensis* breeding system is partially self-compatible and outcrossed through pollinators. Under ambient conditions, the rate of seed setting was 58%; however, by artificial xenogamy, the rate of seed setting was 82% (Bai et al., 2009). During the investigation and observation of the *C. udensis* breeding system, the fruit could be produced by no-clipping anther and bagging (including inflorescence and single flower) and there were seeds with in these fruits, which indicated that the self-pollination of *C. udensis* was fertile, although the seed setting rate was very low (Bai et al., 2009). Therefore, it was hypothesized that *C. udensis* facultative breeding system increased the survival fitness and improved the genetic diversity of the population.

Clonal growth typically leads to a decrease of genetic variation within populations (Ma et al., 2016; Jiang et al., 2018). However, increasing research has shown that the genetic diversity of clonal plant populations was not as low as anticipated (Pang et al., 2010; Pirog et al., 2017; Mandel et al., 2019). In this paper, both diploids and tetraploids had relatively high clonal diversity. Different clones had the capacity to occupy different microhabitats due to habitat heterogeneity under forest shade, clonal reproduction increased the survival of offspring and saved the resource consumption about sexual reproduction, impacted the fitness and the evolution of population (He et al., 2017). These might result in a high level of *C. udensis*clone diversity (Ma et al., 2019). Through morphological plasticity,*C. udensis* rhizomes could place their clonal ramets within favorable environments to achieve foraging behavior. Moreover, the rhizomes could renew their buds and preform photosynthetic products. These would provide material resources for seedling formation, clonal ramet growth, and the long-term survival of dormant buds, ensuring the success of clonal growth and improving the adaptability of *C. udensis* 's genet diploids and tetraploids (Wang et al., 2008).

The lifecycles of clonal plants are generally quite long, where a high degree of clonal diversity can be maintained even under very low levels of seedling regeneration (Wang et al., 2008; Liu et al., 2016). Nevertheless, the clonal diversity of different ploidies of *C. udensis* in the Hualongshan Mountains (Table 4) was lower than the average values of other clonal plants ($D = 0.62$) (Kartzinel et al., 2015), which was affected by the relative contribution ratio of sexual reproduction and asexual reproduction of diploids and tetraploids

in different habitats.

For the two different *C. udensis* ploidies, the diversity indices of the tetraploids were slightly higher than that of the diploids (Table 4). Being an autopolyploid, the tetraploids of *C. udensis* has a double genome relative to the diploids. The gene loss rate of tetraploids was half of the diploids. Thus, compared with the diploid, the genetic drift on the tetraploid was smaller. Consequently, the effective population size of tetraploid was relative larger than the diploid that resulting in the relative higher genetic diversity. Meanwhile, the genetic effective size within population of clonal plants mainly depends on the genet number not on the ramet number (Zhang & Zhang, 2006). In this study, the tetraploid has more genets than the diploid (Table 4), which might be another reason for the higher level of genetic diversity in the tetraploid of *C. udensis* .

As descendants of diploids, tetraploids might occupy myriad microhabitats of various areas with different genets coming from multiple genotypes during the initial establishment of populations (Table 4). Subsequently, this ploidy will propagate offspring through rhizome buds. The current individuals within tetraploid population are likely to be clone progeny, or the progeny of clone progeny from the original genet (Alix et al., 2017; Van Drunen & Husband, 2018; Wang et al., 2019). Compared with the high-altitude habitats of the diploids, the tetraploids occupied low altitude habitats with higher temperatures and increased precipitation. Other environment factors (e.g., light and nutrients) of tetraploid surroundings were also different from those of diploids. When in different habitats, the tetraploids of *C. udensis* presented higher clonal and genetic diversity to adapt to heterogeneous environments during the evolutionary process. These results supported the views proposed by Wang (Wang et al., 2008), but were different from the viewpoints of He et al. (2017). The clonal diversity, clonal spatial structures, and genetic diversity of clonal plants were influenced by sample scale, sample strategy, sample point layout, and individual sample selection (Van Drunen et al., 2015; Pirog et al., 2017; Wang et al., 2018a, b).

There was significant genetic differentiation between the diploids and tetraploids of *C. udensis* (Table 5), which might be closely related to its habitat distribution, breeding strategy, and life history. Through field investigations, we found that *C. udensis*was often distributed under the arbor and shrub forests of high-altitude areas, and highly dependent on the humid environment. In the Hualongshan Mountains, the diploids distribute across shady southern slopes at an altitude of 2400 meters (peak of 2917 meters), while the tetraploids distribute under the arbor forests of the 40° northern slopes at an altitude of from 1880-2000 meters.

The geographical distance between the two ploidy populations was 20 kilometers. Compared with the tetraploids, the growth cycles of the diploids were shorter due to the relatively higher altitude and lower temperature. The flowering time of the diploids was later than that of the tetraploids by about two weeks, whereas the fruits of the diploids matured in early August compared with those of the tetraploids, which matured in early September (Bai et al., 2009). The growth cycle of the diploids was about 45 days shorter than that of the tetraploids. The geographical isolation of the two ploidies and the asynchronous phenological period would effectively block gene exchange between the diploids and tetraploids, with limited wind or insect pollination to a certain extent, which enhanced the differentiation between the two ploidies.

Most of the *C. udensis* diploid and tetraploid genotypes were localized; thus, obvious clonal differentiation was observed between the two ploidies, which was consistent with the results of Wang et al. (2008) and He et al. (2017). The genotype distribution patterns of the different *C. udensis* ploidies in the Hualongshan Mountains resulted from the adaptation of the diploids and tetraploids to different habitats. Under the different selection pressures, various genotypes were fixed within the diploids and tetraploids, respectively, through the formation of different clones to adapt to diverse environments, whereafter the clone differentiation between the diploids and tetraploids were gradually manifest.

5 CONCLUSION

Clonal architectures and diversity spatial pattern of different *C. udensis* ploidies were the result of long-term adaptations to different habitats. Both the diploids and tetraploids adopted reproductive trade-offs to respond to different environments. As an autotetraploid, the tetraploids through high clonal reproductive rates and

genetic diversity adapted to completely different habitats from their diploid ancestors. The diversity model of two *C. udensis* ploidies were anticipant to be consistent with the hypothesis about the inter-polyploid. The results in this study will shed light on the evolution of the intra-polyploid and be helpful to better elucidating the adaptation mechanisms of facultative plant clones.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

YLW and GLS conceived the ideas; YG wrote the manuscript; YG, LL and WH analyzed the data; HZ and EZ collected the data; YLW and GLS reviewed and edited the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Morphological data and microsatellite genotypes will be archived on Dryad.

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Table 1 Characteristics of microsatellites for *Clintonia udensis*

Locus	Primer sequence (5'-3')	Anneal Temp Ta()	Number of alleles	Number of alleles
			2n	4n
ES07	F: GGCATCGAT-GATGAGGACTT R: ACAAGTAGGGCAACGGGTT	50.2	12	26
ES10	F: TGCTTGAGAT-GTGCGACGAG R: CAAGAGAATGCGACAAAGC	51.2	17	20
ES11	F: TGGCTCGAAC-CTTCTGAGTT R: CCTCGGATTGTTGATCCTGT	51.1	15	22
ES13	F:AGAGGTGGAACACGGACATC R: GCCCGGATCACTTTCTTCTC	56	10	8
ES16	F:GCTGGAGCAAGAGAAATTGG R: ACATTTGTTGGTCCCTCCTG	56	19	14
ES18	F: AACCTACCTC-CACCCCTACC R: AGAATCAGAGAGGCGGAAC	51.1	16	21
ES22	F:CACCCAGGCGTAAGAAGAAG R: GCACCTGTGTCAACACCCTA	46.8	16	25
ES23	F: GCAGGAATTCG-GCACGAG R: GCCACATTGGACCATAAACC	48	6	14

Table 2 Characteristic of rhizome buds of *Clintonia udensis* ' diploid and tetraploid

	Buds number	Buds number	Buds mean	Buds mean	Active buds ratio	Active buds ratio	Dormant buds ration	Dormant buds ration	Ago
	2n	4n	2n	4n	2n	4n	2n	4n	2n
Number	25	54	39	39	39	39	39	39	39
Mean	2.1200	2.3148	2.1333	2.2827	0.6266	0.4733	0.3574	0.5222	7.9231
Variation	0.277	0.408	0.222	0.163	0.036	0.006	0.042	0.006	1.862
Range	3.000	3.000	1.700	1.200	0.550	0.238	0.550	0.238	4.000
Minimum	1.000	1.000	1.500	1.800	0.450	0.333	0.000	0.429	6.000
Maximum	4.000	4.000	3.200	3.000	1.000	0.571	0.550	0.667	10.00

Table 3 One-way ANOVA for *Clintonia udensis*

						SS	df	MS	F
Buds distribution on rhizome	Among groups	Among groups	Combination	Combination	Combination	0.400	5	0.080	0.098
			Linear term	Linear term	Weighted	0.009	1	0.009	0.011
					Deviation	0.391	4	0.098	0.120
	Within groups	Within groups				7.333	9	0.815	
	Total	Total	Total	Total	Total	7.733	14		
The ratio of dormancy buds	Among groups	Combination	Combination	Combination	Combination	0.061	5	0.012	9.546
		Linear term	Linear term	Weighted	Weighted	0.013	1	0.013	10.426
				Deviation	Deviation	0.048	4	0.012	9.326
	Within groups	Within groups	Within groups	Within groups	Within groups	0.009	7	0.001	
	Total	Total	Total	Total	Total	0.070	12		
Rates of active buds and dormancy buds	Among groups	Combination	Combination	Combination	Combination	0.764	5	0.153	6.147
		Linear term	Linear term	Weighted	Weighted	0.131	1	0.131	5.267
				Deviation	Deviation	0.633	4	0.158	6.366
	Within groups	Within groups	Within groups	Within groups	Within groups	0.174	7	0.025	
	Total	Total	Total	Total	Total	0.938	12		
Ratio of active buds	Among groups	Combination	Combination	Combination	Combination	0.072	5	0.014	11.063
		Linear term	Linear term	Weighted	Weighted	0.015	1	0.015	11.487
				Deviation	Deviation	0.057	4	0.014	10.956
	Within groups	Within groups	Within groups	Within groups	Within groups	0.009	7	0.001	
	Total	Total	Total	Total	Total	.081	12		

The average number of rhizome buds	Among groups	Combination	Combination	Combination	Combination	1.690	5	0.338	6.423
		Linear term	Linear term	Weighted	Weighted	0.747	1	0.747	14.201
				Deviation	Deviation	0.943	4	0.236	4.478
	Within groups	Within groups	Within groups	Within groups	Within groups	0.368	7	0.053	
	Total	Total	Total	Total	Total	2.058	12		

Table 4 Genetic variation of diploid and tetraploid populations of *Clintonia udensis*

Population	Total number of loci	Number of polymorphic loci	*PPL*	H	I	*N*	*G*	*N/G*	*D*
2n	51	32	63.5	0·2127	0·3012	93	46	1.1087	0.3741
4n	51	37	68.5	0·2332	0·3416	107	89	1.1460	0.4695
Total	51	40	78.4	0.2358	0.3552	76.5	67.5	1.1274	0.4218

PPL , The percentage of polymorphic loci. **H** , Nei's gene diversity. **I** , Shannon's information index. N , Sample size. G , Number of genotypes.N **/** $\boldsymbol{G}$, Average size of genotype. D , Simpson index. E , Fager index.

Table 5 Genetic variation of *Clintonia udensis* diploid and tetraploid populations

Source	df	SS	MS	Est. Var.	Variance percentage %	Fixation indices
Among groups	1	501.832	51.698	1.959	60.87	Φ_{CT} = 0.6089 (P = 0.00
Among populations within groups	17	378.867	39.803	0.923	28.66	Φ_{ST} = 0.5732 (P = 0.00
Within populations	311	78.371	17.472	0.337	10.47	Φ_{SC} = 0.3654 (P = 0.00

Φ_{CT}, genetic differentiation among groups.Φ_{SC}, genetic differentiation among populations within groups. Φ_{ST}, genetic differentiation among populations.

Fig. 1. *Rhizomes and rosette seedings of Clintonia udensis (**a**, rhizomes; **b**, rosette seedings).*

Fig. 2. *Clonal architecture of Clintonia udensis.*

Notes: first year. second year. . . . . and so on. ***F****, flowering.* ***a****, The diploid.* ***b****, The tetraploid.* ***c****, zigzag systyle.**d**, C systyle.* ***e****, V systyle.* ***f****, Y systyle.*

Fig. 3. *Clonal spatial structure of C. udensis**a**, The diploid.* ***b****, The tetraploid.*