Wulfenia **19** (2012): 97–106

Wulfenia
Mitteilungen des
Kärntner Botanikzentrums
Klagenfurt

On the meiosis in *Paeonia ludlowii* (Stern & Taylor) D. Y. Hong, an endangered species of SE Tibet, PR China

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Summary: The behavior of chromosomes during meiosis in pollen mother cells (PMCs) of *Paeonia ludlowii*, endemic to SE Tibet, PR China, was investigated in two natural populations represented by 32 individuals. The meiotic abnormalities, including bridges, fragments and univalents, occurred so frequently that they could be found in all individuals. At metaphase I, the mean chromosome configuration of each cell was 2n=10=0.34 I+4.83 II. Most of the chromosomes were ring bivalents, but some were rod bivalents or univalents. Pairing index ranged from 70% to 81.31%. Chromosome bridges, fragments, unequal separation and lagging chromosomes were observed both at anaphase I and at telophase I. All individuals produced bridges/fragments (9.81% of PMCs on average) at anaphase I. This shows that they were all heterozygotes of paracentric inversion. However, there were some variations among the individuals in frequency of bridge occurrence and in size of fragments, which indicates that many different inversions exist in this species. Further evidence is needed to answer why the structural heterozygosity exists so widely in the species, and whether there is a relationship between the heterozygosity and ecological adaptation.

Keywords: chromosome configuration, evolution, inversion, heterozygosity, meiosis, *Paeonia ludlowii* (Stern & Taylor) D. Y. Hong

The genus *Paeonia* (Paeoniaceae) comprises about 32 species, which are distributed in five areas in the northern hemisphere. According to Sang et al. (2004), there are three sections in *Paeonia*: sect. *Onaepia*, sect. *Moutan* and sect. *Paeonia*.

As a shrubbery, *Paeonia ludlowii* (Stern & Taylor) D. Y. Hong belongs to sect. *Moutan*. It is well known in Tibetan medicine and as ornamental flower. Because of the low rate of seed-setting and sprouting, overly excavation, catastrophes and human destructive activities to the environment, wild *P. ludlowii* is now becoming an endangered plant with a sharp decline in populations and individuals. Therefore it is classified as endangered (2nd level) in China.

Chromosome structure rearrangement plays an important role in evolution, and meiotic pairing configuration is used as primary proof to explain chromosome structure rearrangement (Singh 2003). Because the genus *Paeonia* has only few large sized chromosomes it has been extensively studied for chromosome structure variations (Wang et al. 2008). In spite of the variations, the karyotype in the whole genus *Paeonia* is the same (Koeva & Sarkova 1997). The basic chromosome number is x=5, in which three median chromosomes have been collectively named as 'M', one submedian as 'D' and one subterminal as 'E' (Tzanoudakis 1983; Hong et al. 1988).

Chromosome structure rearrangement has been recently investigated in two species (Pan et al. 2007; Wang et al. 2008). Meiotic abnormalities turned out to be chromosome structure mutations (Wang et al. 2008). However, the mutation reasons and the maintenance way in natural populations have remained unclear until now. This study is focused on observation and analysis of meiotic behavior of pollen mother cells (PMCs) of natural populations in order to

clarify whether the chromosome structure abnormalities existed or not in *P. ludlowii*, further to understand the genetic structure of populations and to deepen the understanding of the evolutionary pattern.

Materials and methods

Paeonia ludlowii is an endangered species with very few individuals per population, which limited material sampling. Material was collected from Wudaoban at 2937 m s.m., Nyingchi Prefecture, Tibet (Population 1, POP1) and from Hongwei Farm at 2945 m s.m., Nyingchi Prefecture, Tibet (Population 2, POP2).

Appropriate sized flower buds were collected and separately fixed in Carnoy's solution (absolute ethanol : glacial acetic acid = 3 : 1) and then transferred into 70% alcohol at -20 °C. By squashing method, slide preparations were made and stained with carbol-fuchsin (Darlington & La Cour 1975).

Meiotic chromosomes were observed by means of light microscope. Meiotic abnormalities including univalents, bridges, fragments, unequal separation and lagging chromosomes were recorded and analyzed. Pairing index was calculated as follows: Pairing index = (the number of ring bivalents × 2 + the number of rod bivalents) / the number of cells observed / 10 × 100%. Standard deviation was calculated by using Excel. The length of fragments was measured by Spot software (Diagnostic Instrument Inc.) in order to distinguish different inverted segments.

Results

Metaphase I

At metaphase I, five normal bivalents (Fig. 1A) were observed in most PMCs, but univalents occurred frequently in all individuals (for details see Appendix 1). Two or four univalents were found in 15.58% PMCs in POP1 and 17.53% in POP2. Ratios of three types of univalents (M, D and E) were 38.64% : 38.50% : 22.86% in POP1 and 40.33% : 34.43% : 25.25% in POP2. The univalents randomly moved to the same pole (Fig. 1B) or to the opposite pole (Fig. 1C).

Bivalents dominated this stage. In POP1, the average number of bivalents per PMC was 4.84 and 4.82 in POP2 (Appendix 1). Mean meiotic configurations were 2n=10=0.32 I+4.84 II in POP1, 2n=10=0.36 I+4.82 II in POP2 and 2n=10=0.34 I+4.83 II in POP1+POP2. The meiotic pairing index ranged from 70% to 81.31% in different individuals at metaphase I (Appendix 1).

Anaphase I and telophase I

Bridges and fragments: Although most of PMCs were normal at anaphase I (Fig. 1D), there were several sorts of abnormalities like: bridges without fragments (Fig. 1E), bridges with fragments (Fig. 1F), fragments without bridges (Fig. 1G), lagging chromosomes (Fig. 1H) and unequal separation (Fig. 1I) at anaphase I. At telophase I, abnormalities were found including bridges without fragments (Fig. 1J), bridges with fragments (Fig. 1K) and fragments without bridges (Fig. 1L). Every studied individual was found with bridges and fragments at anaphase I and telophase I. Frequency of abnormalities is shown in Appendices 2 and 3.

The frequency of cells with bridges and fragments varied between populations. PMCs with bridges and fragments were found in 11.55% (anaphase I) and 6.37% (telophase I) in POP1 and

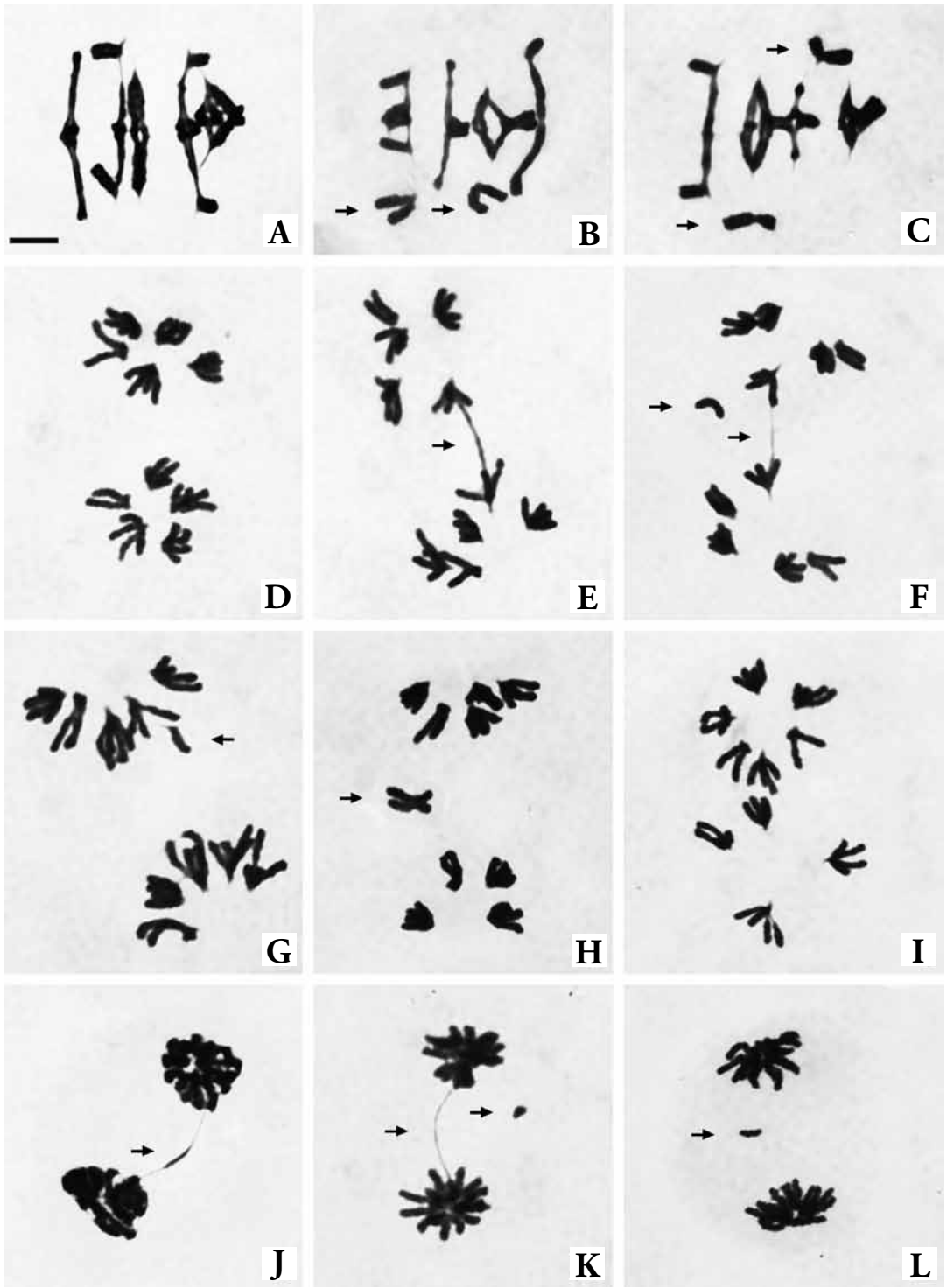

Figure 1. First meiotic division of pollen mother cells in *Paeonia ludlowii.* (A–C) Metaphase I. (A) Five normal bivalents. (B) Four pairs of normal bivalents and two univalents (arrow). (C) Four pairs of normal bivalents and two univalents (arrow). (D–I) Anaphase I. (D) Normal. (E) Bridge (arrow) without fragment. (F) Single bridge (arrow) with one fragment (arrow). (G) Fragment (arrow) without bridge. (H) Lagging chromosome (arrow). (I) unequal separation. (J–L) Telophase I. (J) Bridge (arrow) without fragment. (K) Single bridge (arrow) with one fragment (arrow). (L) Fragment (arrow) without bridge. Bar = 10 µm.

correspondingly in 7.83% (anaphase I) and 7.78% (telophase I) in POP2. The mean occurrence frequency of bridges and fragments of the species as a whole was 9.81% at anaphase I and 7.07% at telophase I (Appendices 2 and 3).

The frequency of cells with bridges and fragments also varied among individuals within a population at anaphase I. Cells with bridges and fragments ranged from 4.62% (1-7) to 34.66% (1-6) in POP1 and from 2.98% (2-9) to 14.17% (2-20) in POP2 (Appendix 2). Generally, percent of cells with all kinds of abnormal configurations together was higher at anaphase I than that at telophase I.

The fragment size varied considerably, ranging from an almost entire chromosome to hardly visible pieces. In order to know whether the variations in fragment size represent different inversions or not, fragment length at anaphase I and telophase I was measured (Appendix 4).

Lagging chromosomes: In a total of 137 anaphase I cells of the 30 individuals (1.51%) lagging chromosomes (Fig. 1H) could be observed. The frequency of such cells, however, was quite low in most of the individuals and only 4 cases exceeded 4%, with an extreme individual 1-1, where 5.06% of cells with lagging chromosomes were found (Appendix 2).

Unequal separation: The normal anaphase I chromosomal separation would be 5 : 5. However, unequal separation was observed, e.g., 6 : 4 segregation (Fig. 1I). Only 80 cells (0.91%) showed this phenomenon (Appendix 2).

Discussion

In *P. ludlowii*, the most obvious characteristics during meiosis are all kinds of abnormalities, particularly bridges and fragments. Comparing the frequency of the abnormalities among individuals, populations and species, leads to a better understanding of their evolutionary significance.

Univalents: Frequency of meiotic cells with univalents in *P. ludlowii* was considerably variable and ranges from 6.43% to 29.41%, that is 16.45% on average. Much more cells with univalents were found in *P. japonica* (32.4%) (Haga & Ogata 1956).

Univalents result from asynapsis or pairing failure. Because there are three M, one D and one E chromosomes per cell, the occurrence ratio of the three types of chromosomes should be 3 : 1 : 1 according to the univalent formation at random. In fact, the ratio between the three types of chromosomes, M : D : E, did not abide by the expected ratio 3 : 1 : 1. So M, D and E do not distribute by chance alone.

Bridges and fragments: The most peculiar feature of meiotic abnormalities observed in each individual in this species were the bridge/fragment at anaphase I and telophase I. Bridges and fragments of anaphase I and telophase I, resulting from crossing over in inversion regions, may be a symbol of paracentric inversion heterozygotes. By crossing over or gene conversion, chromosomal location of rDNA loci affected the tempo of concerted evolution (Zhang & Sang 1999). All individuals examined were obviously heterozygotes of paracentric inversions. Considerable variations in size of fragments, ranging from less than 10 µm to more than 1 µm long, were also found in *P. ludlowii* (Appendix 4).

Because of variations in both, frequency of bridge/fragment occurrence and size of fragments among individuals, there were different inversions in this species, i.e. inversion polymorphisms, and there were also few common inversions shared by the individuals.

The formation of imbalanced gametes is the most obvious genetic effect of inversions and causes microspore sterility (Grant 1975; Darlington 1956; Rees & Jones 1977). 5–90% sterile pollen grains were reported for *P. californica* (Walters 1942, 1952, 1956) and 15.82–54.97% for *P. jishanensis* (Pan et al. 1999).

We suggest that the paracentric inversion polymorphism might play an important role in adaptation to the varied ecological niches, as exemplified in previous studies, especially in *Drosophila* (Hoffmann et al. 2004). However, in *Paeonia*, further studies on the relationship between the chromosome structure heterozygosity and ecological adaptation are necessary.

Acknowledgements

This study was supported by the Natural Science Foundation of Hainan Province (310041), and the Key Discipline of Plant Ecology of Hainan Normal University (HS-1-2012-0713-2).

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Appendix 1. Chromosome pairing at metaphase I of PMCs in *P. ludlowii*.

			I (univalents)							II (bivalents)			
Population	Individual	No. of cells observed	No. of cells with univalents	Percent of cells with univalents	No. of M	No. of D	No. of E	Total	No. of univalents per cell	Total	No. of rod per cell	No. of ring per cell	Pairing index
One	1-1	213	42	19.72	56	18	10	84	0.39	4.80	2.21	2.60	73.99
	1-2	231	48	20.78	38	46	16	100	0.43	4.78	1.58	3.20	79.83
	1-3	306	36	11.76	20	24	28	72	0.24	4.88	2.65	2.23	71.11
	1-4	212	33	15.57	36	16	14	66	0.31	4.84	2.23	2.62	74.62
	1-5	275	39	14.18	44	26	16	86	0.31	4.84	2.42	2.42	72.65
	1-6	254	63	24.80	40	56	34	130	0.51	4.74	1.79	2.96	77.01
	1-7	274	28	10.22	32	20	12	64	0.23	4.88	2.23	2.65	75.33
	1-8	348	32	9.20	20	32	12	64	0.18	4.91	1.99	2.92	78.28
	1-9	298	42	14.09	40	24	20	84	0.28	4.86	2.09	2.77	76.31
	1-10	285	46	16.14	32	40	20	92	0.32	4.84	2.32	2.52	73.61
	1-11	231	42	18.18	26	28	34	88	0.38	4.81	1.79	3.02	78.27
	1-12	366	54	14.75	40	48	20	108	0.30	4.85	1.57	3.28	81.31
	1-13	304	50	16.45	22	50	32	104	0.34	4.83	1.66	3.16	79.93
	1-15	224	52	23.21	40	60	8	108	0.48	4.76	2.06	2.70	74.55
	1-19	233	32	13.73	30	18	18	66	0.28	4.86	2.25	2.61	74.68
	1-20	310	20	6.45	8	16	16	40	0.13	4.94	2.87	2.06	70.00
	Average	272.75	41.19	15.58	32.75	32.63	19.38	84.75	0.32	4.84	2.11	2.73	75.72
	S. D.	47.38	10.94	4.97	11.61	15.10	8.38	22.40	0.10	0.05	0.37	0.34	3.22
Two	2-3	240	44	18.33	40	32	16	88	0.37	4.82	2.15	2.67	74.83
	2-4	314	66	21.02	64	36	32	132	0.42	4.79	1.95	2.84	76.31
	2-6	204	60	29.41	40	56	28	124	0.61	4.70	2.11	2.59	72.84
	2-7	371	46	12.40	30	42	28	100	0.27	4.87	2.05	2.81	76.79
	2-8	234	20	8.55	14	14	12	40	0.17	4.91	2.13	2.78	76.97
	2-10	200	52	26.00	40	32	32	104	0.52	4.74	2.22	2.52	72.60
	2-11	328	58	17.68	40	36	40	116	0.35	4.82	2.04	2.79	76.10
	2-12	300	44	14.67	28	40	20	88	0.29	4.85	2.02	2.83	76.87
	2-13	288	81	28.13	90	48	36	174	0.60	4.70	2.29	2.41	71.04
	2-15	280	18	6.43	8	20	8	36	0.13	4.94	1.99	2.95	78.86
	2-18	204	37	18.14	34	16	24	74	0.36	4.82	2.03	2.78	76.03
	2-20	248	36	14.52	24	24	24	72	0.29	4.85	2.40	2.45	73.06
	2-22	254	32	12.60	40	24	8	72	0.28	4.86	2.03	2.83	76.85
	Average	266.54	45.69	17.53	37.85	32.31	23.69	93.85	0.36	4.82	2.11	2.71	75.32
	S. D.	52.61	17.90	7.13	20.92	12.51	10.39	37.73	0.15	0.07	0.13	0.17	2.26
Total	Average	269.97	43.21	16.45	35.03	32.48	21.31	88.83	0.34	4.83	2.11	2.72	75.54
	S. D.	48.98	14.38	6.00	16.32	13.76	9.42	30.00	0.12	0.06	0.28	0.27	2.79

Appendix 2. Frequency of meiotic abnormalities at anaphase I of PMCs in *P. ludlowii*.

Population	Individual	No. of cells observed	No. of abnormal cells observed	Percent of abnormal cells	Percent of cells with laggards	Percent of cells with bridges		Percent of cells with fragments (without bridges)	Percent of cells with unequal separation
						With fragment	Without fragment		
One	1-1	237	93	39.24	5.06	5.06	10.13	16.46	2.53
	1-2	379	79	20.84	1.58	0.79	3.96	12.93	1.58
	1-3	315	36	11.43	0.32	2.54	2.22	5.71	0.63
	1-4	245	27	11.02	0.82	1.63	2.45	4.49	1.63
	1-5	313	34	10.86	0.64	2.56	2.24	5.11	0.32
	1-6	352	130	36.93	0.57	4.55	5.11	25.00	1.70
	1-7	260	21	8.08	0.38	0.77	1.54	2.31	3.08
	1-8	310	21	6.77	0.65	1.29	2.58	1.94	0.32
	1-9	261	23	8.81	0.38	1.15	1.15	4.60	1.53
	1-10	300	17	5.67	0.67	1.67	1.00	2.00	0.33
	1-11	264	22	8.33	0.38	2.65	2.27	2.27	0.76
	1-12	223	14	6.28	0.00	0.00	1.79	4.04	0.45
	1-13	317	24	7.57	0.63	1.58	1.89	2.84	0.63
	1-15	312	29	9.29	0.96	0.96	3.53	3.21	0.64
	1-19	249	41	16.47	0.80	3.21	4.02	7.23	1.20
	1-20	284	34	11.97	2.11	2.11	2.82	3.52	1.41
	Average	288.81	40.31	13.72	1.00	2.03	3.04	6.48	1.17
	S. D.	43.26	32.25	10.27	1.20	1.37	2.19	6.37	0.82
Two	2-3	386	48	12.44	3.11	0.00	1.04	6.74	1.55
	2-4	346	46	13.29	2.89	0.87	4.62	4.91	0.00
	2-6	345	45	13.04	3.19	3.19	1.74	4.35	0.58
	2-7	316	42	13.29	4.43	1.27	0.63	6.33	0.63
	2-8	325	22	6.77	0.62	2.15	2.15	1.85	0.00
	2-9	202	7	3.47	0.50	1.49	0.50	0.99	0.00
	2-10	244	18	7.38	0.82	1.23	0.00	5.33	0.00
	2-11	337	37	10.98	0.89	1.48	1.78	6.82	0.00
	2-13	312	26	8.33	1.28	0.32	1.60	4.17	0.96
	2-15	221	16	7.24	0.90	0.90	0.00	4.98	0.45
	2-18	306	44	14.38	4.90	1.31	1.31	4.90	1.96
	2-20	254	49	19.29	4.33	1.57	2.36	10.24	0.79
	2-21	226	10	4.42	0.44	0.44	0.00	3.10	0.44
	2-22	382	50	13.09	1.05	2.09	2.62	6.28	1.05
	Average	300.14	32.86	10.53	2.10	1.31	1.45	5.07	0.60
	S. D.	60.30	15.66	4.39	1.64	0.82	1.27	2.29	0.62
Total	Average	294.10	36.83	12.23	1.51	1.69	2.30	5.82	0.91
	S. D.	51.29	25.74	8.11	1.50	1.19	1.96	4.88	0.78

Appendix 3. Frequency of meiotic abnormalities at telophase I of PMCs in *P. ludlowii*.

Population	Individual	No. of cells observed	No. of abnormal cells observed	Percent of abnormal cells	Percent of cells with bridges		Percent of cells with fragments (without bridges)
					With fragment	Without fragment	
One	1-1	296	18	6.08	0.68	1.35	4.05
	1-2	275	25	9.09	1.09	1.82	6.18
	1-3	312	2	0.64	0	0	0.64
	1-4	251	17	6.77	1.2	1.2	4.38
	1-5	252	3	1.19	0	0	1.19
	1-6	289	29	10.03	0.35	0.69	9
	1-7	421	14	3.33	0.24	0.24	2.85
	1-8	313	12	3.83	0.32	1.28	2.24
	1-9	314	13	4.14	0.32	0.32	3.5
	1-10	319	25	7.84	2.51	1.88	3.45
	1-11	205	15	7.32	0.98	1.46	4.88
	1-12	200	16	8	0.5	2	5.5
	1-13	212	13	6.13	0.47	2.83	2.83
	1-15	285	27	9.47	2.81	3.86	2.81
	1-19	306	28	9.15	1.96	2.94	4.25
	1-20	248	22	8.87	1.21	0.4	7.26
	Average	281.13	17.44	6.37	0.92	1.39	4.06
	S. D.	54.78	8.20	2.95	0.86	1.13	2.17
Two	2-3	329	26	7.9	0.91	1.22	5.78
	2-4	330	30	9.09	0.61	2.73	5.76
	2-6	323	23	7.12	2.48	0.62	4.02
	2-7	341	35	10.26	1.47	2.93	5.87
	2-8	332	22	6.63	0.6	0.6	5.42
	2-9	191	7	3.66	0.52	0.52	2.62
	2-10	304	24	7.89	0.99	0.99	5.92
	2-11	376	34	9.04	0.53	1.86	6.65
	2-12	330	29	8.79	1.21	3.03	4.55
	2-13	320	20	6.25	1.25	2.19	2.81
	2-15	316	15	4.75	0.63	0.63	3.48
	2-18	308	17	5.52	0	1.62	3.9
	2-19	214	28	13.08	0.93	3.74	8.41
	2-20	531	56	10.55	0.56	2.26	7.72
	2-21	322	9	2.8	0	0.62	2.17
	2-22	312	35	11.22	1.6	2.88	6.73
	Average	323.69	25.63	7.78	0.89	1.78	5.11
	S. D.	71.50	11.75	2.81	0.62	1.07	1.84
Total	Average	302.41	21.53	7.08	0.90	1.58	4.59
	S. D.	66.28	10.80	2.92	0.74	1.10	2.05

Appendix 4. Length (μm) range of fragments at meiotic anaphase I in *P. ludlowii*.

Meiotic stage	Length (μm)									Total fragments observed	No. of cells with fragments (%)
	1.1-2.0	2.1-3.0	3.1-4.0	4.1-5.0	5.1-6.0	6.1-7.0	7.1-8.0	8.1-9.0	9.1-10.0		
Anaphase I	26	54	120	141	176	91	47	32	15	702	681(7.52)
Telophase I	16	42	76	118	147	65	45	28	14	551	536(5.49)