

1 **Title**

2 Successful asexual lineages of the Irish potato Famine pathogen are triploid

3

4 **Authors**

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7 **The oomycete *Phytophthora infestans* was the causal agent of the Irish Great Famine**
8 **and is a recurring threat to global food security¹. The pathogen can reproduce both**
9 **sexually and asexually and has a potential to adapt both abiotic and biotic environment².**
10 **Although in many regions the A1 and A2 mating types coexist, the far majority of**
11 **isolates belong to few clonal, asexual lineages³. As other oomycetes, *P. infestans* is**
12 **thought to be diploid during the vegetative phase of its life cycle³, but it was observed**
13 **that trisomy correlated with virulence and mating type locus⁴ and that polyploidy can**
14 **occur in some isolates^{5,6}. It remains unknown about the frequency of polyploidy**
15 **occurrence in nature and the relationship between ploidy level and sexuality. Here we**
16 **discovered that the sexuality of *P. infestans* isolates correlates with ploidy by comparison**
17 **of microsatellite fingerprinting, genome-wide polymorphism, DNA quantity, and**
18 **chromosome numbers. The sexual progeny of *P. infestans* in nature are diploid, whereas**
19 **the asexual lineages are mostly triploids, including successful clonal lineages US-1 and**
20 **13_A2. This study reveals polyploidization as an extra evolutionary risk to this notorious**
21 **plant destroyer.**

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24 Understanding the mechanisms of rapid adaption in devastating plant pathogens is of
25 critical importance for disease management. Plant oomycete pathogens employed a flexible
26 reproduction system to gain dual advantages in creating both genotype diversity via sexual
27 crosses and in achieving large population expansion via asexual cycles². Compared to other
28 *Phytophthora* sister species, *P. infestans* has a much larger genome that is enriched in
29 repetitive DNA (~74%). The genome can be largely divided into gene-dense and gene-sparse
30 compartments, with the latter containing fast-evolving effector repertoire that is required for
31 virulence and host adaptation^{7,8}.

32 An increasing number of reports showed that most *P. infestans* field strains from wide
33 geographical distribution were identified as belonging to a few asexual, clonal lineages⁹. The
34 first recorded clonal lineage HERB-1 was dominant in both Europe and North America
35 between 1845 and 1896, which triggered the Great Irish Potato Famine⁵. Its replacement, the
36 US-1 clonal lineage of the A1 mating type, firstly emerged in North America in the 20th
37 century and dominated the globe^{5,10}. Rather recently (1990's), US-1 was supplanted by more
38 aggressive lineages of the A2 mating type, including 13_A2 in Europe and Asia¹¹ as well as
39 US-22 in North America^{12,13}. As accumulation of deleterious mutations would lead to rapid
40 extinction of asexual lineages, a phenomenon coined as the Muller's ratchet¹⁴, it is puzzling
41 that a few clonal lineages could adapt to a wide geographical area for a considerably long
42 period. What is their common denominator? Although *P. infestans* was known as diploid for
43 decades³, several studies indicated trisomy and polyploidy^{5,6}. Polyploidy can enhance the
44 vigour in plants and buffer mutational load in asexual reproduction by masking deleterious
45 alleles as reported in both fungi, plants and animals^{9,15,16}. It remains unclear to what extent
46 polyploidy contributed to evolutionary advantage of clonal lineages and whether there is link
47 between sexuality and ploidy.

49 Previously we used multiplex microsatellites to fingerprint 520 *P. infestans* isolates
50 (Supplementary Table 1), including 397 isolates from nine major clonal lineages in four
51 continents and 123 isolates from three sexual populations from the Netherlands, Mexico and
52 Tunisia^{9,17,18}. In previous studies, the analysis was only based on the allele size, although
53 differences of allele dosage were observed by peak height. To survey the frequency of
54 polyploidy occurrence and to gain insights into the relationship of reproduction systems and
55 ploidy levels, we re-analyzed the fingerprinting dataset by including scoring of allele peak
56 height, besides molecular size of alleles. The ratio of allele peak heights allows assessing
57 allele dosage at each microsatellite locus (Supplementary Fig.). Surprisingly, we discovered
58 that eight of nine asexual lineages showed high ratio of triallelic loci (0.73-0.99), whereas the
59 value (0.14-0.35) in the three sexual populations is significantly lower ($p=0.008$). The only
60 exception is the clonal lineage in Northern China (CN-Northern), which is similar to sexual
61 populations in the percentage of triallelic loci. CN-Northern belongs to the A1 mating type
62 and it is a rather old population, as sub-populations evolved in the same lineage¹⁷. In China,
63 seed potatoes are produced in the North and shipped elsewhere. Strict quarantine is taken for
64 seed tubers to enter the area domestically and abroad. Therefore, the *P. infestans* population
65 there should be rather isolated and remains as diploid.

66

67 To further analyze the genome-wide ploidy level, we re-sequenced 11 representative
68 isolates for >50X genome depth from a meta-population from the Netherlands, a major source
69 of international seed potato trade. The meta-population includes three asexual lineages
70 (NL 13_A2, NL 06_A1, and NL 08_A1) and two sexual populations (NL pop 2 and NL pop3,
71 Table 1). Sequences were aligned to the T30-4 reference genome to determine single
72 nucleotide polymorphism (SNP). Relative read depth at each heterozygous SNP was used to
73 determine ploidy level. For diploid genome, the mean of read counts at heterozygous

74 positions should have a single mode at 0.5 (1/2) when the allele ratio is 1:1, while there
75 should be two modes, 0.33 and 0.67 (1/3 and 2/3) when the allele ratio is 1:2 and 2:1 for
76 triploid genomes (Supplementary Table 2). Consistent with the microsatellite fingerprinting
77 analyses, all the nine isolates from the sexual populations displayed diploid mode, whereas
78 the three isolates from the asexual lineages showed triploid mode (Table 2, Fig. 1 and
79 Supplementary Table 2). We also re-analyzed sequences of the seven isolates from a previous
80 report⁵ (Table 2 and Fig. 1), which confirmed that all the four isolates from asexual lineage
81 US-1 and EC-1 showed also triploid mode. These analyses indicate that sexuality and ploidy
82 are strongly associated.

83

84 In addition to deep re-sequencing, we used flow cytometry and microscope observation
85 to directly evaluate the DNA content and count chromosome number, respectively (Fig. 2).
86 The isolate NL07434 (A2) from the sexual population NL pop2 was used as control, since it
87 had been investigated previously⁵ as well as in this study. DNA content of the isolate
88 NL08797 (A2) from NL pop 2 is the same as NL07434. As expected, DNA content of the
89 three asexual isolate NL08080 (A1), NL08452 (A1), and NL07041 (A2) is about 1.5 times of
90 that of NL07434 and NL08797, confirming that they are triploid. The average of chromosome
91 number for isolates from sexual populations is 13.6, whereas 20.6 for isolates from asexual
92 lineages (Table 2, Supplementary Table 3). DNA content measurement and chromosome
93 counting further support that these asexual isolates are triploid.

94

95 In summary, *P. infestans* can no longer be considered to be only diploid, which should
96 be taken into account in future studies. The observed triploidy must play an imperative role in
97 the successful global epidemic of modern asexual lineages such as US-1, NL 13_A2, TU-1,
98 NL 06_A1 and NL 08_A1. Polyploidization in *P. infestans* is likely to enhance fitness, as

99 reported in yeast that tetraploid had a higher beneficial mutation rate than haploid and diploid
100 strains¹⁹. Ancient or old asexual lineages such as HERB1 and CN Northern are diploids,
101 therefore it is worth further investigation on when and how asexual lineages became triploid.
102 Was the transition of the ploidy level due to survival pressure from disease resistance genes
103 that were incorporated into potato breeding after the Great Famine or linked to the frequent
104 use of pesticide deployed in the last 50 years to control this disease? This study revealed a
105 new dimension of genomic feature of the great evolutionary risk of *P. infestans*, which should
106 also be considered in future agricultural management.
107

108 **Methods**

109 ***Definition of asexual and sexual populations***

110 This study only focuses on field isolates. We adopted the definition of clonality widely
111 accepted in papers dealing with the population structure of pathogens²⁰. The definition of
112 asexuality (clonal lineage) and sexuality (sexual population) in this study do not refer to the
113 cytological mechanism of reproduction, but rather to the population structure that results from
114 an absence or restriction of genetic recombination²⁰. The asexuality obtains wherever isolates
115 with the same mating type show multilocus genotypes (MLGs) that are identical or nearly
116 identical. In contrast sexual progeny shows extensive recombination of alleles.

117 ***Isolate collection***

118 In this study, isolates from China¹⁷, The Netherlands⁹, Ecuador¹⁸, Tunisia (manuscript
119 prepared), and Mexico were investigated (Table 2). The clonal lineages and sexual
120 populations have been defined by MLGs in those previous studies. The clonal lineages and
121 sexual populations used in this study have been defined by MLGs in the previous studies.

122 ***Microsatellite analysis***

123 Twelve microsatellite markers were used²¹⁻²⁴. Amplification of the SSR markers was
124 carried out as described by Li et. al²⁴. The amplicon was capillary electrophoresed on an
125 automated ABI 3730 according to the manufacturer's instructions. SSR allele sizing was
126 performed using GeneMapper v3.7 software (Applied Biosystems, USA).

127 ***Re-sequencing data and SNP calling***

128 The reference genome sequence of the artificial strain T30-4 and the re-sequencing data
129 used in this study were published in previous studies^{5,7,10,11}. The method of read mapping was
130 used as described⁵. The re-sequenced isolates selected from previous studies were analyzed
131 again here. Since the reference genome has 4921 supercontigs, we only analyzed the first 100
132 longest supercontigs.

133 The isolates were sequenced using the Illumina Hi-Seq 2000 sequencer. The sequencing
134 averagely generated 100 million 100-bp paired-end reads for each isolate. The sequencing
135 reads of each isolate were mapped to the T30-4 reference genome using BWA²⁵ and SNP
136 calling was conducted subsequently using Sequence Alignment/Maptools²⁶. Several criteria
137 was considered in SNP filtering: (1) a SNP should be bi-allelic between the isolate and T30-4
138 genomes; (2) at the SNP locus, the phred quality score of base sequencing and score of read
139 mapping should be both higher than 30; (3) each allele of a SNP should be supported by at
140 least 4 reads; (4) the non-reference allele frequency should be between 0.2-0.8 at a
141 heterozygous SNP locus.

142 ***Flow cytometry***

143 The nuclei were collected by simply chopping hyphae with a scalpel blade in cold
144 phosphate buffered saline (PBS)²⁷. Another method used was zoospore cultures. Zoospores
145 were induced at 4°C for two hours and harvested in excess of 10⁴ spores ml⁻¹. The nuclei were
146 stained by 10ug/ml propidium iodide (Sigma). The samples were delivered into a laser BD
147 FACSCalibur Flow Cytometer for data analysis.

148 ***Microscopy observation of chromosomes***

149 The young hyphae stained by 50ug/ml propidium iodide (Sigma) were transferred to a
150 microscope slide for observation. The observation was preformed with Zeiss LSM 780 Laser
151 Scanning Confocal Microscope.

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207 by flow cytometry in *Phytophthora infestans*. *Cytometry A***77**, 769-775 (2010).

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210 **Supplementary Information** includes one figure and three tables and is available in attached
211 files.

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221

222 **Author Contributions** Y. L. and S. H. designed the study and wrote the paper. Y. L.
223 performed SSR analysis and chromosome observation. Q. Z. analysed re-sequencing data.
224 Q.K. performed flow cytometry. T. L. provided the raw SSR data.

225

226 **Author Information** The authors declare no competing financial interests. Correspondence
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231 **Table 1. The allele dosage of microsatellite loci in asexual and sexual populations**

Lineage / Population	Area	MT ^a	mtDNA ^b	No. isolate	No. locus	3-allele loci ^c	2-allele loci ^c	1-allele loci ^c	3-allele ratio ^d
<i>Asexual lineages</i>									
CN Northern	Northern China	A1	IIa	67	10	106	213	346	0.33
CN 13_A2	Southwest China	A2	Ia	64	10	375	25	240	0.94
CN Fujian	Eastern China	A1	IIb	36	10	153	17	190	0.90
NL 13_A2	Netherlands	A2	Ia	73	12	591	5	280	0.99
NL 06_A1	Netherlands	A1	Ib	7	12	52	5	17	0.91
NL 08_A1	Netherlands	A1	Ia	16	12	91	15	47	0.86
EC-1	Ecuador	A1	IIa	73	12	422	124	330	0.77
TU-1	Tunisia	A1	Ia	57	12	312	114	251	0.73
US-1	US	A1	Ib	4	12	25	9	14	0.74
<i>Sexual population</i>									
NL pop2	Netherlands	A1, A2	Ia, IIa	37	10	45	83	241	0.35
Mex	Mexico	A1, A2	/	44	10	25	154	253	0.12
TU-2	Tunisia	A1, A2	Ia	42	10	56	105	238	0.35

232 ^aMating type

233 ^bMitochondria haplotype

234 ^c3-allele loci means triallelic loci, 2-allele loci means biallelic loci, and 1-allele loci means monoallelic loci

235 ^d=triallelic loci/(triallelic loci + biallelic loci)

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237

238 **Table 2. Ploidy analysis of isolates by genome re-sequencing, flow cytometry and**
 239 **microscope observation**

Isolate	MT ^a	Lineage / Population	Seq. Depth	DNA Content (pg)	No. chromosome	Ploidy Mode	Reference
NL00150	A1	NL pop3	53	nd ^b	13.5±1.0	Diploid	This study
NL04092	A1	NL pop3	54	nd	nd	Diploid	This study
NL04106	A2	NL pop3	39	nd	nd	Diploid	This study
NL05159	A1	NL pop3	51	nd	14.2±0.8	Diploid	This study
NL05385	A1	NL pop3	55	nd	nd	Diploid	This study
NL05387	A1	NL pop3	51	nd	13.2±0.9	Diploid	This study
NL05890	A2	NL pop3	40	nd	12.8±0.8	Diploid	This study
NL07434	A2	NL pop2	54	0.56 ±0.02	14.2±0.7	Diploid	Ref. 5
NL08797	A2	NL pop2	23	0.56 ±0.01	13.8±0.4	Diploid	This study
P17777	A1	nd	48	nd	nd	Diploid	Ref. 5
06_3928A ^c	A2	NL 13_A2	50	nd	nd	Triploid ^c	Ref. 5
DDR7602	A1	US-1	13	nd	nd	Triploid	Ref. 5
LBUS5	A1	US-1	9	nd	nd	Triploid	Ref. 5
NL07041	A2	NL 13_A2	106	0.81±0.03	20.7±0.4	Triploid	This study
NL08080	A1	NL 08_A1	43	0.82±0.03	20.3±0.9	Triploid	This study
NL08452	A1	NL 06_A1	41	0.83±0.02	20.8±0.4	Triploid	This study
P13527	A1	EC-1	20	nd	nd	Triploid	Ref. 5
P13626	A1	EC-1	34	nd	nd	Triploid	Ref. 5

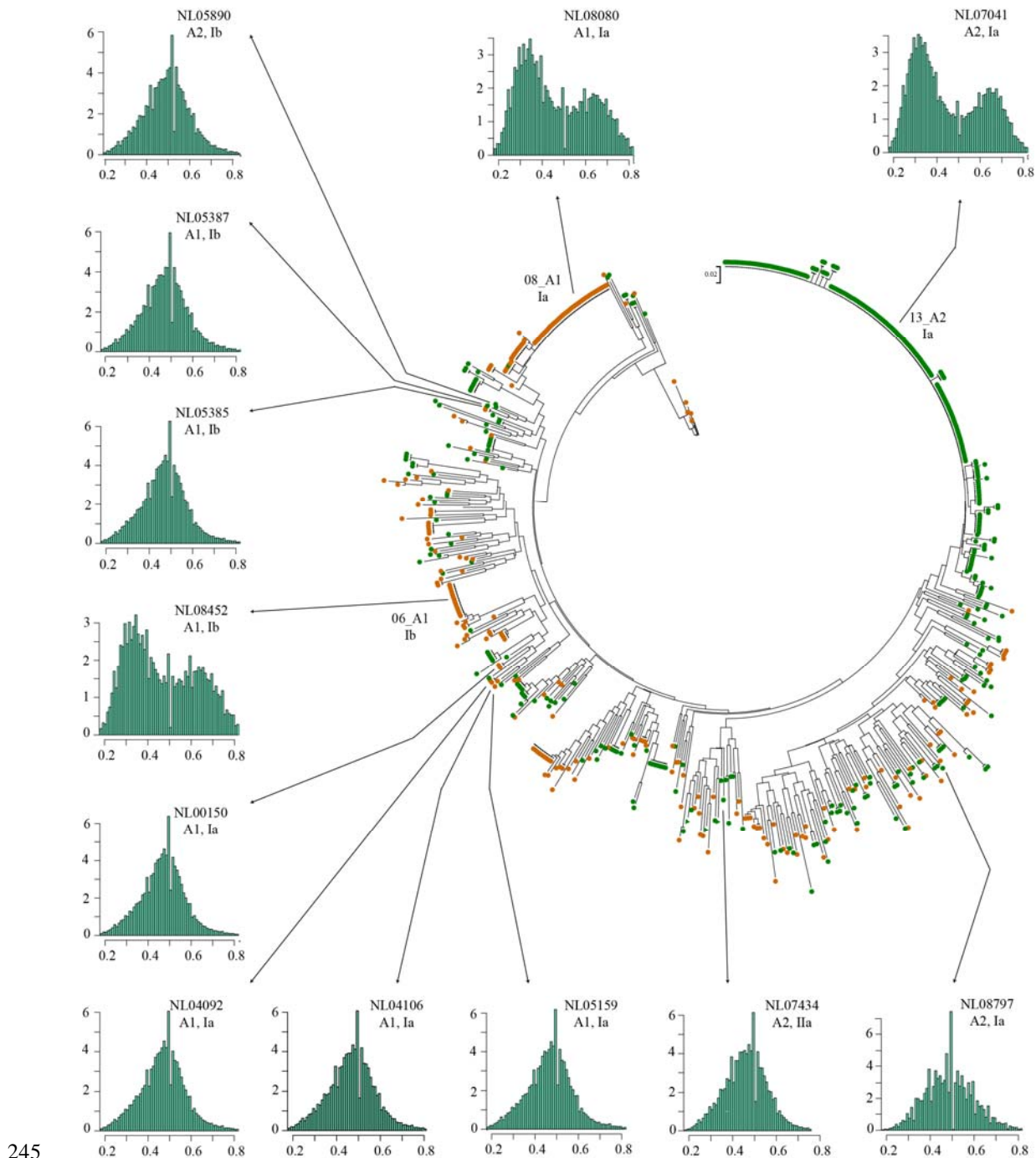
240 ^aMating type

241 ^bNot determined

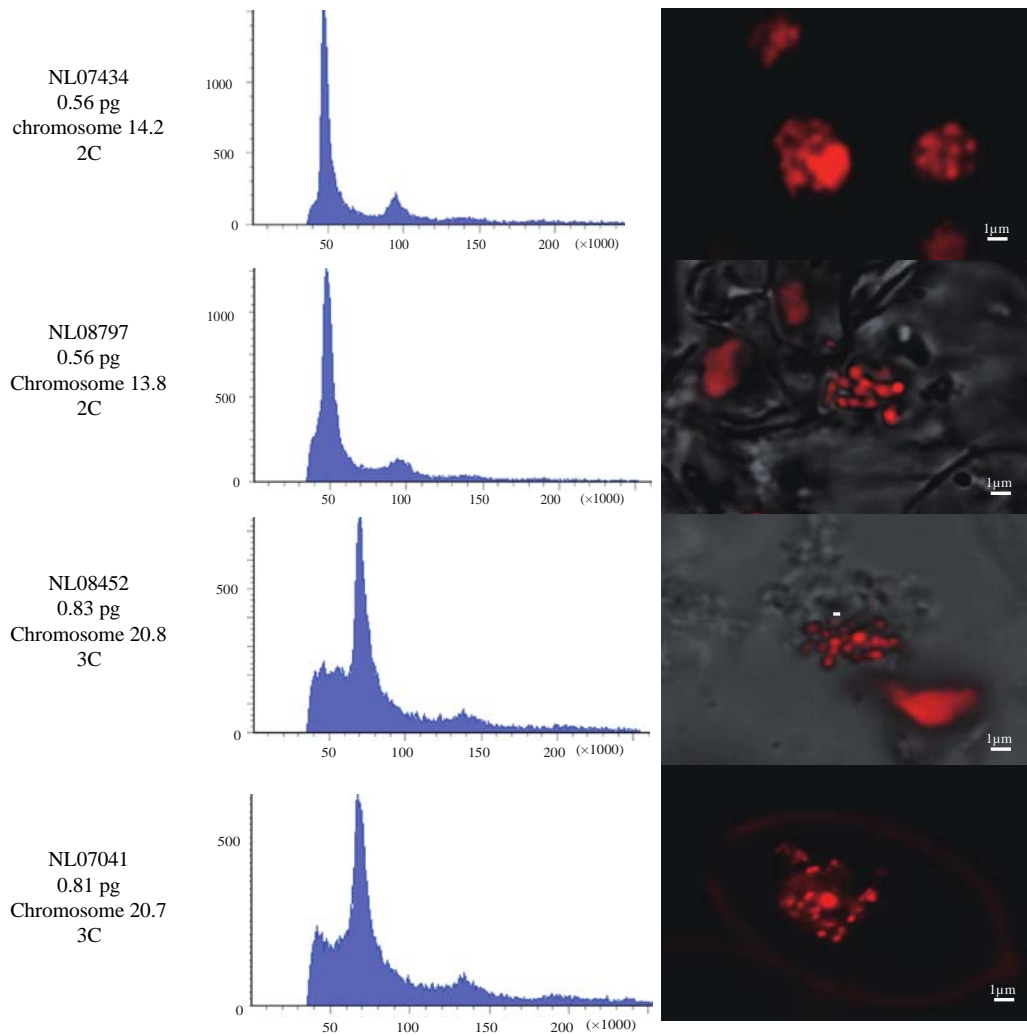
242 ^c06_3928A appeared to be tetraploid in previous research⁵. However, our analysis showed it is triploid.

243

244



251 frequency of read counts at heterozygous positions, a single and diploid mode at 0.5, while
252 two modes, 0.33 and 0.67 were for triploid genomes.



253

254 **Figure 2. DNA content and chromosome number.** Two isolates from asexual lineage
255 (NL08452 and NL07041) and two isolates from sexual populations (NL07434 and NL08797)
256 were investigated. DNA content was determined by flow cytometry. The chromosome number
257 was counted by adjusting the focal levels on Laser Scanning Confocal Microscope (LSM780,
258 Zeiss).

259