Karyological Variation between Two Taxa of *Plantago major* L., ssp. *Major* and ssp. *Intermedia* (Gilib.) Lange

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Summary The two taxa *Plantago major* ssp. *major* and ssp. *intermedia* from different geographical regions were investigated karyologically. The separation of the two taxa depends mainly on seed number and size. The average karyotypic configuration for each population was used to contrast ideograms between populations from ssp. *major* and ssp. *intermedia*. The study revealed that the two taxa have distinct cytotypes which are somewhat correlated with seed number as an indication of taxonomic identity. The variation between two taxa is expressed by two main cytological groups based on the total form percentage (TF %) which measures the symmetry of the chromosomes over the whole karyotype. The study also revealed that the evolution of the *P. major* group may be in part due to chromosomal rearrangement. The study concluded that most *major* karyotypes are more symmetrical than those of *intermedia*, which may be an indication that *intermedia* is the derived type.

Key words Karyotypes, *Plantago*, Chromosome, Evolution, Recombination, Mitosis.

The first consistent chromosome counts in *Plantago* were reported by McCullagh (1934). Other studies were made in connection with taxonomic studies in order to explain the relationships in particular groups of related species (Böcher *et al.* 1955, Rahn 1957, Cartier 1971, 1973, Zemskova 1977). Studies also covered the cytology of *Plantago* species from different phytogeographic regions (Gregor 1939, Runemark 1969, Briggs 1973). Badr and El-Kholy (1987) studied *Plantago* chromosomes in the Egyptian flora. Of the 10 studied species, five were found to have a basic number of $x=6$: *P. arabica*, *P. lagopus*, *P. lanceolata*, *P. major* and *P. notata*. The study revealed that *P. major* was characterised by short chromosomes and a symmetric karyotype compared with other species. In general, the karyotype in *Plantago* consists of medium-sized chromosomes, mostly with meta- and submetacentric constrictions, with no marked size difference between individuals in the total length of the chromosome complement. One or two pairs of chromosomes possessed secondary constrictions. The chromosomes of *Plantago* could be classified as metacentric, submetacentric, or subterminal. Pramanik and Sen Raychaudhuri (1997) studied the karyograms of some species of *Plantago* obtained from different parts of the world and revealed that karyograms had standard features. Despite homogeneity in total features, each species differed from the other in minor karyotypic details indicating the role of structural changes of chromosomes in evolution. The basic chromosome number $x=6$ is the most common among the species *Plantago* and may be considered the ancestral number in the genus (Badr *et al.* 1987).

Material and methods

Seeds were sown from two taxa *Plantago major* L. ssp. *major* ('major') and ssp. *intermedia* (Gilib.) Lange ('intermedia') all of which were of known identity except for one location (Table 1).

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The definition of the two taxa depends mainly on seed number and seed size (Van Dijk *et al.* 1988, Morgan-Richards and Wolff 1999, El-Bakatoushi 2004). Seeds were germinated on moist filter paper in petri dishes; 4–5 mm long root tips were excised and pre-treated with aqueous solution of 0.002 M 8-hydroxyquinoline for 4 h at 4–5°C. The roots were fixed for 24 h in a mixture of ethanol: glacial acetic acid (3 : 1 v/v), and stored in the refrigerator. Roots were rinsed in distilled water, hydrolysed with 1 N HCL at 60°C for 8–9 min. The hydrolysed roots were stained with Feulgen's reagent. Deeply stained root tips were squashed on clean slides with a drop of 45% acetic acid. Some specimens were squashed in a drop of acetocarmine after Feulgen staining.

Cells with spread metaphase chromosomes were examined and photographed by photomicroscope, using 100× phase oil immersion lens and Kodak film. The chromosomes were measured from photomicrographs after scanning using Micromasure version 3.3 computer program. Magnification taking place during photography and scanning was calculated and was defined for the Micromasure program. All measurements are given by Micromasure program V.3.3.

The mean karyotypes from each population were used to contrast ideograms between populations from *major* and *intermedia*. The nomenclature and classification of chromosomes were adopted from Singh and Singh (1976) as follows: The position of the centromere was taken as medium (M) and submedium (Sm) when the arm ratios ranges were 1.00–1.20 μm and 1.21–2.00 μm respectively. Chromosomes having secondary NOR constrictions were given the abbreviation "Sc". Chromosomes were classified arbitrarily into different types with respect to length as follows: Type A when it is >4 μm ; type B ranging between 3.10–4.00 μm ; type C when it is=2.10–3.00 μm and type D=1.10–2.00 μm .

The length of the two longest chromosome with satellites

The relative length of the longest pair of chromosomes (as identifiable markers) compared to the total karyotype was taken to compare populations from both taxa.

The two longest chromosomes with secondary constrictions for each karyotype for an individual were measured. Analysis of Variance ANOVA was applied to compare the relative lengths of these chromosomes to the remainder of the chromosomes in all populations studied. The relative length of each chromosome was adopted from Singh and Singh (1976) as follows:

$$\text{Relative length} = \frac{l}{\sum L} \times 100$$

where l is the long arm of the chromosome and $\sum L$ is the total length of the chromosome complement. The total form percent (TF%) which measures the average degree of symmetry over the whole karyotype was calculated as:

$$\text{Total form percent (TF\%)} = \frac{s}{\sum L} \times 100$$

Where s is the short arm of the chromosome and L is the total length.

Symmetry of the chromosomes over the whole karyotype in both taxa

The TF% value was taken for all karyotypic individuals considered. A t -test was applied to compare TF% values for all pairs of individuals. The lower the t -value, the smaller the difference between two populations. T -values obtained from t -test were placed in a Kulcinski table to match the degree of symmetry between each pair of the studied individuals (Table 2). The Kulcinski table was used before in comparisons between karyotypes of *Hordeum murinum* group with different chromosome numbers (Richards and Booth 1976) and also used to arrange 15 species of *Delphini-*

um and *Consolida* according to overall karyotype similarity (El-Kelidar and Richards 1981).

Results

Karyological characters of the P. major group

Karyological data of all populations are presented in Table 1. The somatic metaphases of individuals from all studied populations of both taxa *major* and *intermedia* revealed that $2n=12$. The chromosome morphology in the *P. major* group varied between metacentric and submetacentric. The majority of the chromosomes were of the short submetacentric type C and D (Table 1). Metacentric chromosomes were more frequent in *intermedia* than in *major*. The total complement length was the highest (40.49 μm) in *major* from Newcastle, England. The total chromosome length within the *P. major* group ranged from 18.96 μm to 40.49 μm . No marked difference in the total chromosome length in the two taxa *major* and *intermedia* was discovered. The most symmetrical karyotype with a TF value of 45.23% was of *major* in USA. The symmetry of the karyotype in *major* ranged from 31.24% to 45.23%, while the symmetry of chromosomes over the whole karyotype in *intermedia* ranged from 27.14% to 43.41%.

Most chromosomes of *P. major* group were short chromosomes. Satellites or secondary constrictions were generally observed in all populations studied. Two pairs of NOR satellites were observed in all populations studied (Figs. 2, 3). Satellite position varied within and between populations. However, it was noticed that in most populations, the satellite chromosomes were of comparable length, irrespective of their relative length compared with the rest of the chromosome complement. This suggests that the satellite pairs of chromosomes tend not to have been involved in major

Table 1. Locations, average seed number, total length of the chromosome complement (TL) and total form percent of the studies populations (TF%)

Locations	Code No.	Average seed number	TL	Average TF%	Karyotypic formula
<i>P. major</i> pop1 (9, 14) from Newcastle England	16	7	26.1	40.33	7SmC+2SmD
<i>P. major</i> pop3 (20) from Newcastle England	18	7.8	26.13	40.54	1MD+9SmC+2SmD
<i>P. major</i> pop4 (15) from Newcastle England	23	8.6	40.49	31.33	1MB+1SmB+8SmC+2SmD
<i>P. major</i> pop8 from Newcastle England	14	9.4	29.08	41.3	10SmC+2SmD
<i>P. major</i> pop7 (15) from Newcastle England	24	7.8	38.12	38.12	7SmC+5SmD
<i>P. major</i> pop7 (13) from Newcastle England	25	7.8	36.68	39.54	3MB+1SmB+7SmC+2SmC
<i>P. major</i> portpatrick England	12	7	24.31	39.59	5SmC+7SmD
<i>P. major</i> Labraye in Switzerland	7	6.6	21.86	43.27	5MD+2SmC+5SmD
<i>P. major</i> Belp, in Switzerland	5	10	23.19	41.47	5SmC+7SmD
<i>P. major</i> Venlo Norway	20	8.6	39.23	31.24	2SmB+8SmC+2SmD
<i>P. major</i> Villandry France	13	8.4	22.77	38.74	4SmC+8SmD
<i>P. major</i> Sarteano in Italy	2	7.5	20.68	44.31	2MC+2MD+8SmD
<i>P. major</i> from USA	3	8.1	29.66	45.23	4MC+1MD+6SmC+1SmD
<i>P. intermedia</i> pop1 (10) Newcastle England	15	12	23.28	42.41	2MC+2MD+3SmC+5SmD
<i>P. intermedia</i> pop3 (11) Newcastle England	17	20	23.12	38.04	1MD+9SmC+2SmD
<i>P. intermedia</i> Largoward in Scotland	11	16	19.22	40.7	1MD+1SmC+10SmD
<i>P. intermedia</i> (KO1) Denmark	6	16	23.03	42.52	1MC+3SmC+8SmD
<i>P. intermedia</i> Nybol Denmark	1	17.8	27.68	41.52	1MC+2MD+8SmC+1SmD
<i>P. intermedia</i> Venlo Netherlands	21	16	40.07	27.14	2MC+6SmC+4SmD
<i>P. intermedia</i> Noordpolderzijk Netherlands	22	14.2	39.02	29.49	1MB+10SmC+1SmD
<i>P. intermedia</i> Laudal Norway	19	18	37.13	31.9	3SmB+6SmC+3SmD
<i>P. intermedia</i> Yseel to Denver Netherland	10	—	21.04	41.41	4SmC+8SmD
<i>P. intermedia</i> Suamur France	8	20	20.65	41.49	1MD+2SmC+9SmD
<i>P. intermedia</i> Waldhut Germany	9	16	18.96	43.41	2MD+1SmC+9SmD
<i>P. intermedia</i> Benalis Athens (Greece)	4	17	21.87	42.19	4MD+5SmC+3SmD

Fig. 1. Relative lengths of the two longest chromosomes with secondary constrictions.

Fig. 2. Karyotype and ideograms representative of *P. major* (group 1).

Fig. 3. Karyotype and ideograms representative of *P. intermedia* (group 2).

had a wide range of relative lengths, probably due to the lowest number of individuals investigated (two only). These results showed congruence with data of the total chromosome length in both taxa, in which the total length of the chromosomes in *major* ranged from 20.68 μm to 40.49 μm , and the total length of the chromosomes in *intermedia* occupied the same range (18.96–40.07 μm).

Based on the *t*-value, the similarity between karyotypes of different populations were ranged from 0–8.2. The highest degree of similarity was considered at *t*-test value less than 1. Two main groups were separated in the Kulcinski table depending on the average degree of symmetry of chromosomes over whole karyotype. Each group contained individuals very similar to each other in the average degree of symmetry (Table 2). Most of individuals of group one were characterized by small average seed number, while the second group was characterized by a high average seed number (Table 2). Seed number is the best character to separate *major* into its two subspecies *major* and *intermedia* (El-Bakatoushi 2004). Group one included twelve individuals with an average seed number ranging from 7 to 9.4, except for three individuals with average seed numbers 16 and 16.4. The second group comprised individuals with high seed number with a maximum of 26, except for one with seed number of 7.5. One way Analysis of Variance was applied for the seed number of the two main karyological groups (gp 1 and gp 2, Table 3). The two taxa (based on the seed number) were significantly different in the symmetry of the chromosomes over the whole karyotype ($p=0.045$) (Table 3). Six individuals were ignored of large differences between their replicates (individuals) (some of them were in early metaphase).

A *t*-test was also applied to compare sites and populations using the values of the relative length of the two longest chromosomes with secondary constrictions. This did not reveal any regular pattern using the Kulcinski table.

Discussion

At the level of species, the evolution and divergence of species of higher plants are often associated with large variation in nuclear DNA amounts (Rees and Jones 1972). Most of these variations are due to amplification of DNA sequences in the chromosomes (Narayan 1982). The activity of chromosomal mutations such as deletions, insertions, inversions, translocations, and duplications can be enhanced by inbreeding or hybridisation (Levin 2002).

Completely stable karyotypes in sexually reproducing species are unlikely to occur. Little significant variation on a geographic scale has been reported in *Scilla vindobonensis*, *S. bifolia*, *S. mischtschenkoana* (Greilhuber and Speta 1977, 1978), *Adoxa* (Greilhuber 1979), several *Allium* taxa, *Brimeura fastigiata* (Vosa 1976a, b, 1979), or several *Hordeum* taxa (Linde-Laursen *et al.* 1980). In a previous study on *Plantago lanceolata* individuals collected from Kashmir Himalayas, although individuals were highly morphologically different, no specific differences in karyotypes of the different variants were detected (Jain 1978). The results were interpreted by Jain (1978) as morphological variants stemming from simple genetic differences.

In this study, in spite of the stability in chromosome number, there is significant karyotype variations between the two taxa studied. This variation is expressed by two main cytological groups based on the total form percent (TF %) which measures the symmetry of the chromosome over whole karyotype. Populations defined as *major* based on seed number which is the best character discriminating between two taxa (Van Dijk *et al.* 1988, Morgan-Richards and Wolff 1999, El-Bakatoushi 2004), tend to have TF % values higher than those defined as ssp. *intermedia* (they are more symmetrical). This suggests that *major* has the more symmetrical karyotype than *intermedia* which may be the derived type.

While there is no significant variation between the total chromosome lengths of both taxa, the two cytotypes are significantly correlated with morphology, as documented before in *P. albicans* (El-Bakatoushi 1997). Stebbins (1950, 1958) and Stebbins and Dawe (1987) reported that the sym-

metry of karyotype is decreased by pericentric inversion and unequal reciprocal translocations. Translocations seem to be the most common form of chromosomal rearrangement within plants. It was observed in a previous meiosis study of *P. albicans* that the percentages of translocations in populations were correlated with the symmetry of the karyotypes (El-Bakatoushi 1997). Whether all chromosomes are involved in translocations or not is unknown. If all chromosomes are involved in translocations, translocation heterozygotes will result. The presence of translocation heterogeneity also arises from a tendency for chromosome breakage that may be enhanced by inbreeding (Levin 2002). It was evident many years ago that chromosomal evolution via rearrangements, especially translocations, has accompanied morphological evolution in a multitude of plant genera (Darlington 1932, Lewis and John 1963).

These two subspecies of *P. major* seem to keep distinct with morphological and some genetical markers, although both taxa occur together in the same habitat in many of the studied populations. However, hybridization takes place between the two taxa and produces healthy looking individuals. Most of the character inheritance after crosses between the taxa shows matrilineal effects (El-Bakatoushi 2004). This could mean that parts of the chromosomes which influence characteristic features of both taxa can be exchanged between taxa. If these two taxa have become distinct due to the establishment of chromosome rearrangement leading to the formation of two karyotypes, nevertheless, it seems that these rearrangements do not involve the whole chromosome and have not resulted in reproductive isolation between the taxa.

Linked loci may be concerned with the fitness of individuals in the specific environmental conditions of the two taxa. *P. major* species is protogynous but this mechanism seems to be rather ineffective in preventing self-fertilization, because populations of this species are highly homozygous (Van Dijk and Van Delden 1981). Distinctness of the subspecies may result from the high selfing rate which is an important factor in the maintenance of gene complexes, consisting of such fitness-affecting genes.

The limited number of individuals used in this study for both taxa has shown karyological differences between these taxa. Further molecular karyosystematics and a meiosis study will give us more understanding about the evolution of these taxa, especially at the low level of difference that exists between them.

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