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ARTICLE

New chromosome number and unreduced pollen formation in *Achillea* species (Asteraceae)

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ABSTRACT Cytological studies were performed in 14 populations of 8 *Achillea* species growing in Iran. *A. eriophora*, *A. tenuifolia*, *A. oxyodonta*, *A. talagonica* and *A. biebersteinii* showed 2n = 2x = 18 chromosome number, *A. wilhelmsii* and *A. vermicularis* showed 2n = 4x = 36 and *A. millefolium* showed 2n = 6x = 54 chromosome number. The chromosome numbers of *A. eriophora* and *A. talagonica* are new to science and new polyploidy levels are reported for *A. tenuifolia* and *A. wilhelmsii*. Tetraploid and hexaploid species, they formed only bivalents in metaphase of meiosis-I showing diplontic behavior possibly due to allopolyploid nature of the species studied and the presence of control over pairing among homologous chromosomes. Multipolar cells were observed almost in all populations and species studied leading to the formation of abnormal tetrads and pollen grains as well as unreduced (2n) pollen formation.
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KEY WORDS

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The genus *Achillea* L. (Asteraceae) has approximately 130 perennial herb species (Saukel et al. 2004; Guo et al. 2004, 2005), mostly distributed in Eurasia, some in North Africa, while a few species can be found in North America and in the Southern Hemisphere (Bremer and Humphries 1993). A large number of species are endemic and restricted to certain regions, in contrast to other species from the genus growing over a wide geographical range.

Members of *Achillea* L. are usually herbaceous perennials, entomophilous and predominantly outbreeding, the basic chromosome number is x = 9 with most of the species being diploid. Polyploid taxa have originated in many clades including 4x, 6x and 8x species. The genus exhibits great ecological amplitude ranging from deserts to water-logged habitats and from sea coasts to the high mountains (Ehrendorfer and Guo 2006), as a result of which, several *Achillea* species show high morphological variability. For example, the unramnified forms of *A. millefolium* L. can easily be mistaken for *A. collina* Becker (Dabrovska 1977) under arid conditions; the leaf dissection of populations belonging to the *A. millefolium* complex differs dramatically along an altitudinal gradient in the Sierra Nevada due to both genetic and other components (Gurevitch 1988). Biste (1978) described considerable variations in height, leaf width, shoot number, branching and stomata length in populations of different origin of the same species.

The majority of the *Achillea* species are of medicinal values having therapeutic applications. The whole ground parts and mainly the inflorescences are effective as anti-inflammatory, spasmolytic, choleretic drugs. Essential oil and extracts of the plants are used for preparation of cosmetics, stomachic and digestive teas, creams, etc. Besides 1,8-cineole, compounds of bornane skeleton such as camphor and borneol are among the second and third most frequently characterized components of yarrow oil in *A. taygetea* and *A. fraasi* (Magiatis et al. 2002), *A. albicaulis* C. A. Mey. (Feizbakhs et al. 2003), *A. pseudoaleppica* Hub.-Mor. (Zen et al. 2003), *A. pachycephala* Rech.f. (Bamasian et al. 2002), *A. talagonica* Boiss. and *A. vermicularis* Trin (Rustaiyan et al. 1998).

Nineteen *Achillea* species are reported from Iran (Podlech 1986) growing in different regions of the country. Although *Achillea* species have been studies extensively in different regions of the world (Dabrovska 1977; Pireh and Tyrl 1980; Dabrowska 1989; Lambrou et al. 2004; Guo et al. 2004; Saukel et al. 2004; Guo et al. 2005; Ahmet 2006; Yasar et al. 2008), similar studies are almost completely lacking in Iran. Therefore the present study considers cytogenetic characteristics of 14 populations belonging to 8 *Achillea* species growing in Iran, reporting new chromosome number as well as the occurrence of B-chromosomes and unreduced gamete (2n) formation in some of the species for the first time.

Materials and Methods

Cytological studies were performed in 14 populations of 8 *Achillea* species growing in Iran namely 1- *Achillea biebersteinii* Afan., 2- *A. oxyodonta* Boiss. (two populations), from the sect. *Flipendulinae*, 3- *A. millefolium* L., from the sect.

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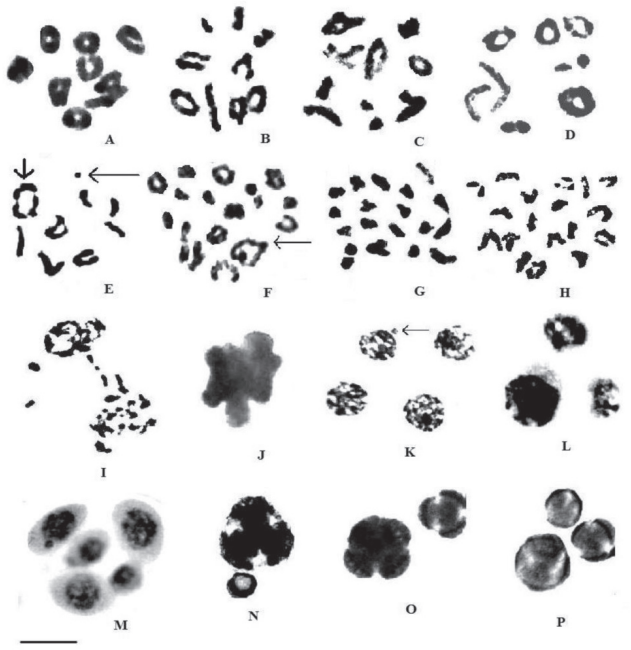

Figure 1. Representative meiotic cells in the *Achillea* species studied. A-D = Meiocyte in *A. eriophora, A. biebersteinii, A. oxyodonta* and *A. talagonica* showing 2n = 18 chromosome number. E = Meiocyte in *A. tenuifolia* showing 2n = 18 and heterozygote translocation (small arrow) as well as B-chromosome (big arrow). F = Meiocyte showing 2n = 38 and one quadrivalent in Polour population of *A. vermicularis*. G & H = Meiocytes showing 2n = 38 in Abali and Quem populations of *A. wilhelmsii*. I = Meiocyte showing chromosome stickiness in Touchal population of *A. oxyodonta*. J = Meiocyte showing chromosome clumping in *A. eriophora*. K = Meiocyte showing micronucleus in (arrow) in Quem population of *A. wilhelmsii*. L & M = Tripolar and multipolar cells in *A. wilhelmsii*. N – P = Bigger pollen (potential 2n) grains in Chitgar population of *A. wilhelmsii*, Polour population of *A. vermicularis* and Touchal population of *A. oxyodonta* respectively. Scale bar = 10 µm.

Millefolium, 4- *A. tenuifolia* Lam., 5- *A. vermicularis* Trin. (two populations), 6- *A. wilhelmsii* K. Koch. (five populations), 7- *A. eriophora* DC., Prodr., and 8- *A. talagonia* Boiss., from the sect. *Santolinoidea*. The voucher specimens are deposited in Tehran University Herbarium and Shahid Beheshti University Herbarium (SBUH).

Meiotic studies were performed on young flower buds collected using minimum 100 metaphase/ diakinesis pollen mother cells (PMCs) and 500 anaphase and telophase cells for data collection (Sheidai and Rashid 2007). Pollen satiability as a measure of fertility was determined by staining minimum 1000 pollen grains with 2% acetocarmine: 50% glycerin (1:1) for about ½ hr. Round. Complete pollens which were stained were taken as fertile, while incomplete, shrunken pollens with no stain were considered as infertile (Sheidai and Rashid 2007).

χ^2 test was performed to detect a significant difference in chiasma frequency and chromosome pairing as well as meiotic abnormalities (Sheidai and Rashid 2007). Similar test was performed among different species and populations having different chromosome numbers by using relative meiotic data. For this purpose the relative chiasma frequency was determined by dividing chiasma frequency and also the number of ring and rod bivalents by the haploid chromosome number (Sheidai and Bagheri-Shabestarei 2007).

In order to detect significant difference between potential unreduced pollen grains and the normal (reduced pollens), t-test was performed. Cytogenetic similarities and distinctness of the species was studied by using different clustering and ordination methods (Sheidai and Bagheri-Shabestarei 2007). Statistical analyses used SPSS ver. 9 (1998) and DARwin ver. 5.0.155 (2006) software.

Results and Discussion

Chromosome pairing and segregation

Data with regard to chiasma frequency and distribution as well as chromosome pairing are provided in Table 1, Figures 1-4. *A. eriophora*, *A. tenuifolia*, *A. oxyodonta*, *A. talagonica* and *A. biebersteinii* showed 2n = 2x = 18 chromosome number, *A. wilhelmsii* and *A. vermicularis* showed 2n = 4x = 36 and *A. millefolium* showed 2n = 6x = 54 chromosome number (Fig. 1A-H).

The chromosome numbers obtained for *A. biebersteinii*, *A. millefolium*, *A. vermicularis* and *A. wilhelmsii* supports the earlier reports (Morton 1981; Dabrowska 1989; Khaniki 1995; Sulborska 2006), while the chromosome numbers of *A. eriophora* and *A. talagonica* are new to science. Khaniki (1995) reported 2n = 3x = 27 for *A. tenuifolia* and 2n = 18 for *A. wilhelmsii* from Iran while, we report 2n = 2x = 18 and 2n = 36 for these species respectively. Therefore two different polyploidy levels exist for these species. Similar situation is known to occur in other *Achillea* species like *A. millefolium* (2n= 36 and 2n= 45, Gervais 1977; Khaniki 1995) and *A. vermicularis* (2n = 18 and 2n= 36, Dabrowska 1989; Khaniki 1995), indicating the role played by polyploidy in the species diversification of *Achillea*.

Among three populations of *A. wilhelmsii* studied, the highest value of total and terminal chiasmata occurred in Karaj and Quem populations (24.90 & 242.81 respectively), while the lowest value of the same occurred in Damavand and Abali populations (20.70 & 13.00 respectively). The highest value of intercalary chiasmata occurred in Abali population (9.67) and the lowest value of the same occurred in Karaj population (2.16).

Chitgar-Park and Abali populations of *A. wilhelmsii* showed the highest mean value of ring bivalents (11.00), while Damavan population showed the highest mean value of rod bivalents (12.85).

Some of tetraploid and hexaploid *Achillea* species and populatins showed diplontic behavior and formed only bivalents in metaphse of meiosis-I (Fig. 1A-D, G & H), while

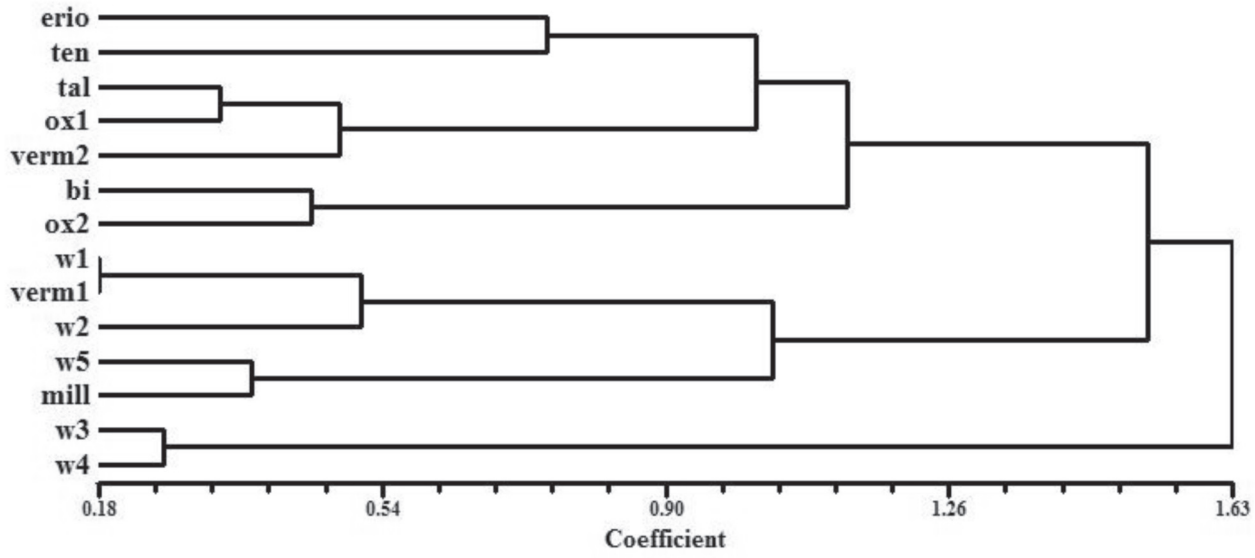

Figure 2. UPGMA dendrogram of *Achillea* species studied. Species abbreviations: erio = *A. eriophora*, ten = *A. tenuifolia*, tal = *A. talagonia*, ox1 & ox2 = Touchal and Darbandsar populations of *A. oxyodonta* respectively, verm1 & 2 = Polour and Darbandsar populations of *A. vermicularis* respectively, bi = *A. biebersteinii*, mill = *A. millefolium* and w1-5 = Quem, Karaj, Chitgar, Damavand and Abali populations of *A. wilhelmsii* respectively.

Damavan and Polour populations of *A. wilhelmsii* and *A. vermicularis* formed very low amount of quadrivalents (Fig. 1F). Such diplontic behavior may be due to allopolyploid nature of the species studied and the presence of control over pairing among homologous chromosomes also suggested in other *Achillea* species (Ehrendorfer 1959; Loidi et al. 1990).

Cytogenetic studies in hybrid *Achillea* plants (Ehrendorfer 1959; Loidi et al. 1990) showed that chromosome pairing occurrs among homologous chromosomes only and not among homoeologous chromosomes of the parental genomes. For example in a pentaploid hybrid between *A. collina* (4x) and *A. millefolium* (6x), the five corresponding chromosomes of each set were assorted into a group of three (two synapsed plus one aligned) and a group of two (synapsed), reflecting different degrees of homology between the five genomes. The pentaploid hybrid received three homoeologues from its hexaploid parent (*A. millefoliurn*) and two homoeologues from its tetraploid parent (*A. collina*), pairing between the parental homoeologous groups seemed to be largely suppressed (Ehrendorfer 1959).

χ^2 test did not showed a significant difference for relative chiasma frequency and chromosome pairing among *Achillea* species and populatins studied indicating that no significant change has occurred in the number genes controlling chromosome pairing.

Although *A. tenuifolia* studied is diploid and is expected to form only bivalents in metaphse of meiosis-I, very low amount of quadrivalents were observed (Fig. 1E), indicating the occurrence of heterozygote translocations between two pairs of chromosomes. Such chromsomal structural changes may increase the amount of genetic variability in the gametes by forming new genetic linkage groups which may be used for adaptation to adverse environmental conditions.

Variation in chiasma frequency and localization is genetically controlled (Quicke 1993) and has been reported in populations of different species (Rees and Jones 1977). Such a variation in the species and populations with the same chromosome number is considered as a means for generating new forms of recombination influencing the variability within natural populations in an adaptive way (Rees and Jones 1977).

Several studies show the importance of hybridization along with polyploidy in the evolution of *Achillea* (Ehrendorfer 1959; Uotila 1979). For example in the group *Millefolium* having the most important medicinal species of the genus, in addition to diploid to the octoploid sppecies available, aneuploids also often occur presumably as a result of interspecific hybridization (Uotila 1979).

Grouping of the species based on UPGMA (Unweighted Paired group with Arithmetic Average) and NJ (Neighbor joining) clustering as well as ordinaton plots based on PCO (Principal Coordinate Analysis) and PCA (Principal Component Analysis) produced similar results (Figs. 2 & 3), almost separating the species of the three sections studied from each other and indicating their cytogenetic distinctness.

laggard chromosomes and chromosomes stickiness were observed during anaphase I and II as well as telophase-I and II (Fig. 1I) in most of the species and populations studied.

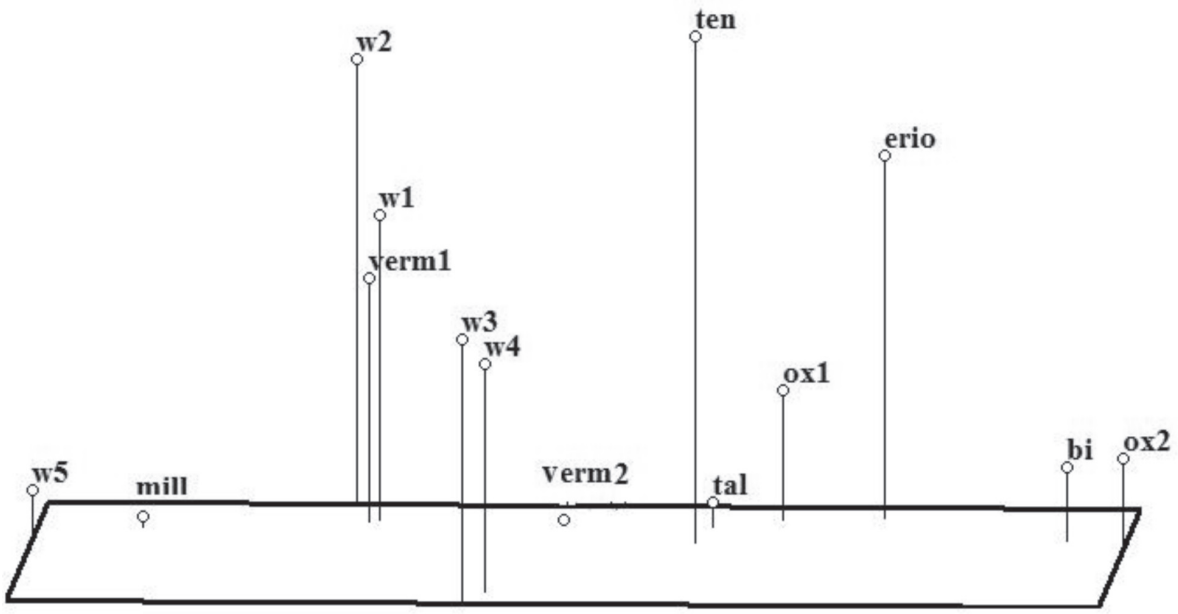

Figure 3. PCO plot of *Achillea* species studied. Species abbreviations: erio = *A. eriophora*, ten = *A. tenuifolia*, tal = *A. talagonia*, ox1 & ox2 = Touchal and Darbandsar populations of *A. oxyodonta* respectively, verm1 & 2 = Polour and Darbandsar populations of *A. vermicularis* respectively, bi = *A. biebersteinii*, mill = *A. millefolium* and w1-5 = Quem, Karaj, Chitgar, Damavand and Abali populations of *A. wilhelmsii* respectively.

The highest percentage of anaphase-I laggards occurred in Darbandsar population of *A. vermicularis* (3.20), while the highest value of the anaphase-II laggards occurred in Karaj population of *A. wilhelmsii* (1.80). The lowest value of cells showing telophase-I laggards occurred in Darbandsar population of *A. oxyodonta* (1.70%), while the highest value of the same occurred in Abali population *A. wilhelmsii* (Fig. 1I). Laggards observed may be the reason for micronuclus formation observed in some of the species studied (Fig. 1K).

The sticky chromosomes occurred from early stages of prophase to the final stages of meiosis. The number of chromosomes involved in stickiness varied from two to many forming a complete clumping of the chromosomes (Fig. 1J). χ^2 test showed a significant difference for the percentage of chromosome stickiness and laggards among the species and populations studied. Genetic and environmental factors as well as genomic-environmental interaction have been considered as the reason for chromosome stickiness in different plant species (Nirmala and Rao 1996; Baptista-Giacomelli et al. 2000).

Multipolar cells were observed (Fig. 1L & M) almost in all populations and species studied which may be due to spindle abnormalities. Such meiotic abnormalities may lead to the formation of abnormal tetrads and pollen grains, the occurrence of aneuploidy condition as well as unreduced (2n) pollen formation (Villeux 1985; Nirmala and Rao 1996). The *Achillea* species studied showed pollen fertility percentage of 77.00-99.00, meiotic abnormalities observed may be responsible for pollen sterility observed.

Unreduced pollen grain formation

The occurrence of large pollen grains (possibly 2n pollen grains) was observed along with smaller (normal) pollen grains almost in all species and populations studied (Fig. 1N-P). The large pollen grains comprised about 1.00-3.30% of pollen grains in these populations.

The mean diameter of normal (reduced) pollen grains ranged from 16.15 µm to 23.00 µm while, the mean diameter of unreduced pollen grains ranged from 28.10 µm to 38.90 µm. T-test analysis revealed a significant difference ($p < 0.001$) for the size between the larger sized pollen grains and smaller sized pollen grains. The presence of giant pollen grains has been used as an indication of the production of 2n pollen (Vorsa and Bingham 1979; Bertagnolle and Thomson 1995). Ramsey (2007) also reported the occurrence of unreduced pollen grains in *A. borealis* with the mean values of 19.00 µm for normal (reduced) pollen grains and 27.50 µm for unreduced pollen grains, well in the range we observed for other *Achillea* species.

Unreduced gametes are known to produce individuals with higher ploidy level through a process known as sexual polyploidization (Villeux 1985), which has been considered as the major route to the formation of naturally occurring polyploids. Different cytological mechanisms are responsible for the production of 2n gametes (Bertagnolle and Thomson 1995). The occurrence of multipolar cells and irregularities in anaphase segregation of the chromosomes might be considered as the possible mechanisms of unreduced pollen grain

formation in the *Achillea* species and populations studied. The occurrence of unreduced pollen grains was previously reported only in *A. borealis* (Ramsey 2007), therefore this is the first report on the occurrence of unreduced pollen grains in the other species of the *Achillea*.

B-chromosomes

B-chromosomes (Bs) of 0-1 were observed in *A. tenuifolia*. The Bs observed were much smaller than the A-chromosomes, round in shape and did not pair with the A-chromosomes. B-chromosomes are accessory chromosomes occurring in more than 1300 species of plants and almost 500 species of animals (Camacho et al. 2000). B-chromosomes have been reported in some other *Achillea*e species like *A. distans* (Dabrowska 1992), *A. glaberrima* (Dabrowska 1989), *A. lingulata* (Dabrowska 1989) and *A. nobilis* (Siljak-Yakovlev 1982). The B-chromosomes when present in high number affect negatively the growth and vigor of the plants, while in low number may benefit the plant possessing them.

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