

# Diversity of chromosome numbers and meiotic studies in genus *Anchusa* (Boraginaceae) from Iran

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## ABSTRACT

The present study reports the chromosome number and meiotic behaviour of 14 populations belonging to four taxa of *Anchusa* subgenus *Buglossum* Gusul. from Iran. All populations showed the chromosome number  $2n=4x=32$ . It is the first meiotic study for *A.* subg. *Buglossum*. We discuss some habit form and evolutionary aspect in the light of cytogenetic data. The origin of polyploidy (auto-allopolyploidy) were also surveyed. As the result of the present study and reviewing the chromosome numbers in *Anchusa* subg. *Buglossum* and *A.* subg. *Buglossoides* Gusul. in Iran, it can be concluded that polyploidy is the major force modeling the chromosome evolution within these subgenera. Almost all the studied taxa displayed regular bivalent pairing and chromosome segregation at meiosis. However, some meiotic abnormalities observed in different taxa are discussed here.

**Keywords:** *Anchusa* subg. *Buglossoides*, *Anchusa* subg. *Buglossum*, Cytomixis, laggard chromosomes, polyploidy.

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## Introduction

Tribe Boraginaceae Bercht. & J. Presl (including Anchuseae DC.) comprises approximately 170 species and 15 genera. The tribe is almost exclusively Eurasian and is represented in the New World. The genus *Anchusa* L. (Boraginaceae) is one of the largest genera centering in the Mediterranean and extending through Europe, Western Asia and Tropical Africa (1, 2). The genus is growing in different place of Iran, especially in west and south of country (3). It is morphologically characterized by bracteates cymes, hypocrateriform corollas with a long tube, spreading limb and faucal scales at the throat, and by strophiolate mericarps with ventral attachment to a planar gynobase. Fruits are important particularly at the subgenus level. They are substantially uniform within the four subgenera *Anchusa*, *Buglossum*, *Buglossoides* and *Buglosellum* Gusul. (4). *Anchusa* subg. *Buglossum* and *A.* subg. *Buglossoides* occur in Iran with eight taxa, two of which are endemic (3, 5).

The great diversity of form finding in the genus *Anchusa*, together with the clear-cut differences exhibited between the species suggested that the cytology of genus might be interesting. The first cytological observation on the genus was counting and documenting the chromosome numbers  $2n=16$ ,  $24$  and  $32$  in *A. barrelieri*, *A. ochroleuca* and *A. azurea* reported by Smith (6). Then, many more researchers have found at least eight different chromosome numbers from  $2n=12$  of *A. thessala* to  $2n=36$  of *A. barrelieri* (7, 8).

The objective of this paper is: presenting a comprehensive review of chromosome number in genus *Anchusa* from Iran; providing the first description of the meiotic stages and the chromosomal behavior of the genus, and also discussing about chromosome number, polyploidy and habit in subgenera occurring in Iran.

## Materials and Methods

For cytogenetic study 15 flower buds from at least five plants at an appropriate stage of development were fixed in 96% ethanol, chloroform and propionic acid (6:3:2) for 24h at room temperature and then stored in 70% alcohol at 4°C until used. Anthers were squashed and stained with 2% acetocarmine. All slides were made permanent using Venetian turpentine. Photographs of chromosomes were taken on an Olympus BX-51 microscope at initial magnification of  $\times 1000$ . Chromosome counts were made from well-spread metaphases in intact cells, by direct observation and from photomicrographs. Voucher specimens are kept at BASU (Bu-Ali Sina University), Hamedan, Iran (Table 1).

## Results

Chromosome numbers and meiotic behavior were determined in 75 individuals belonging to 14 populations of four taxa. A summary of their cytological features is given in Table 2, and the chromosomes are illustrated in Figures 1-3. A total of 2679 diakinesis/metaphase I (D/MI), 1700 anaphase I/telophase I (AI/TI), 231 metaphase II (MII) and 2530 anaphase II/telophase II (AII/MII) cells were analyzed. The meiotic irregularities observed in different populations included B-chromosomes, precocious division of centromeres, chromosome bridges resulting from stickiness, laggard chromosomes, formation of micronuclei in tetrad cells, and cytomixis, which have been discussed here. B chromosomes reported for populations of *A. azurea* var. *kurdica* 82 (kur82) and *A. strigosa* ssp. *tonsa* 00 (ton00). Most studied populations demonstrated ring and rod bivalents. Cytomixis has been detected in different phases of meiosis of *Anchusa* sp. Micronuclei were observed in *A. azurea* var. *kurdica* 56 (Figs 1-3).

**Table 1. Taxa studied of *Anchusa*. (n = Meiotic chromosome number)**

Taxon/ Abbreviation	n	Ploidy level	Locality	Alt. (m)	Voucher number
<i>A. azurea</i> Mill. var. <i>azurea</i> (AZU99)	16	4x	Fars, Noorabad	900	29999
<i>A. azurea</i> var. <i>azurea</i> (AZU47)	16	4x	Kermanshah, 5 km to Bayengan	1525	29447
<i>A. azurea</i> var. <i>azurea</i> (AZU33)	16	4x	Azerbaijan-e Gharbi, Mahabad	1368	25733
<i>A. azurea</i> var. <i>azurea</i> (AZU25)	16	4x	Kohgiluyeh va Boyer Ahmad, Yasuj	1135	23925
<i>A. azurea</i> var. <i>azurea</i> (AZU89)	16	4x	Azerbaijan-e Gharbi, Razhan village	1693	25489
<i>A. azurea</i> var. <i>azurea</i> (AZU825)	16	4x	Isfahan, Semirum	2250	30825
* <i>A. azurea</i> var. <i>kurdica</i> (Guşul.) Chamb. (KUR56)	16	4x	Azerbaijan-e Gharbi, 15 km to Ajab Shir.	1368	26456
<i>A. azurea</i> var. <i>kurdica</i> (KUR82)	16	4x	Hamedan, neck Zaghali	1905	30182
<i>A. azurea</i> var. <i>kurdica</i> (KUR57)	16	4x	Kermanshah, Tazeh Abad to Javan rud	715	32757
<i>A. azurea</i> var. <i>kurdica</i> (KUR98)	16	4x	Zanjan, 8 km before Solantanieh	2035	33998
<i>A. strigosa</i> Banks & Sol. ssp. <i>strigosa</i> (STR22)	16	4x	Kurdestan, 10 km after Sonqor	1745	27622
* <i>A. strigosa</i> ssp. <i>tonsa</i> Bornm. (TON00)	16	4x	Kermanshah, Kamyaran to Ravansar	1418	30000
<i>A. strigosa</i> ssp. <i>tonsa</i> (TON64)	16	4x	Kurdestan, 65 km after Divandarreh	1670	24964
<i>A. strigosa</i> ssp. <i>tonsa</i> (TON77)	16	4x	Kurdestan, After Khamush Abad village	1560	26477

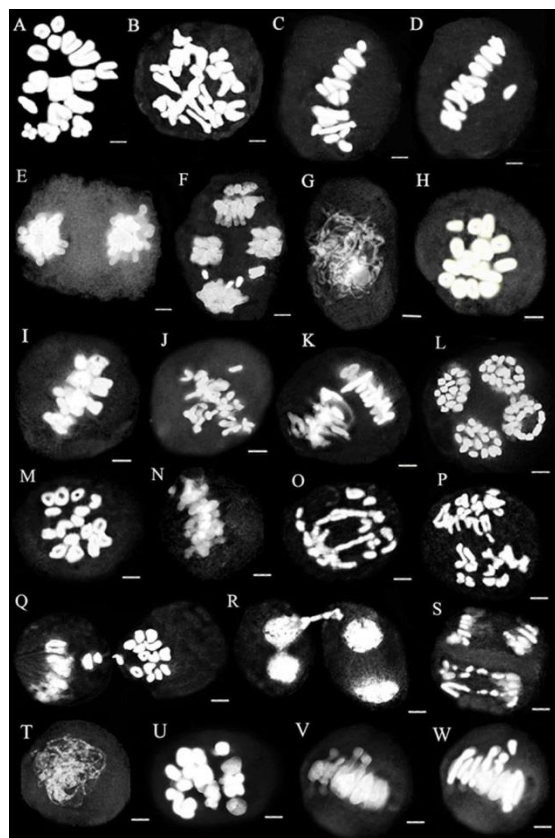
\*New records

**Table 2. Number of pollen mother cells (PMCs) analyzed and percentage of PMCs meiotic behavior in *Anchusa* subg. *Buglossum***

Meiotic characters/taxa	AZU25	AZU99	AZU47	AZU33	AZU89	AZU825	KUR98
Total cell number	185	176	741	606	289	435	249
% D/MI	42	12.5	66.8	51.3	38.2	75.4	50.2
% B-chromosome	0	0	0	0	0	0	0
% Cytomixis	0	0	2.1	1.6	1.7	0	0.8
% Precocious migration	6.4	4.5	0	0.6	1.7	3	12
% AI/TI	48.6	48.2	28.7	47.2	18.12	17	44.1
% Bridge	0	0	2.8	0	0	4	3.6
% Laggard chromosome	1.2	0	3.7	3.5	1.8	2.7	3.6
% Cytomixis	6.6	1.7	3.2	3.8	3.8	0	6.3
% Micronucleous	0	0	0	0	0	0	0
% MII	4.4	0.5	1.8	1	3.3	1.8	0
% AII/TII	7.9	38.6	2.5	0.66	43.6	5.7	5.6
% Laggard chromosome	1.2	0	10.2	0	0	0	14.2
% Cytomixis	0	0	42.1	0	0	0	0
N	16	16	16	16	16	16	16
Meiotic characters/taxa	KUR82	KUR56	KUR57	STR22	TON00	TON64	TON77

Total cell number	488	558	1375	157	772	585	339
% D/MI	50.6	34.4	1	88.5	46	48.5	34.5
% B-chromosome	1.6	0	0	0	2.1	0	0
% Cytomixis	0	0.5	0	0	0	3.8	0
% Precocious migration	5.2	2.08	0	4.3	4.1	12.3	4.2
% AI/TI	12.2	13.7	22	10.8	36.9	15.5	8.8
% Bridge	4.8	0	0	0	0	0	3.3
% Laggard chromosome	8	8.3	0.64	5	0.35	0	6.6
% Cytomixis	0	1.2	0	5.8	0.35	0	3.3
% Micronucleous	0	2.8	0	0	0	0	0
% MII	2.2	3	4	0	0.6	0	2.9
% AII/TII	34.4	48.5	72.8	1	15.5	35.8	58.2
% Laggard chromosome	0	1	0	0	0	0	0
% Cytomixis	2.9	0	1	0	0	0	0
N	16	16	16	16	16	16	16

Abbreviations: D/MI = diakinesis/metaphase I; AI/TI = anaphase I/telophase I; MII = metaphase II; AII/TII = anaphase II/telophase II.



**Figure 1.** Meiosis in AZU25 (A-F), AZU99 (G-L), AZU47 (M-S) and AZU33 (T-W) populations (n = 16) – A, B: diakinesis; C: metaphase I; D: precocious migration in metaphase I; E: telophase I; F: laggard chromosome in anaphase II; G: prophase; H: ring and rod bivalents in diakinesis; I: metaphase I; J: precocious migration in metaphase I; K: metaphase II; L: telophase II. M: ring and rod bivalents in diakinesis; N: metaphase I; O: laggard and bridge in anaphase I; P: anaphase I; Q: cytomixis in diakinesis; R: cytomixis in telophase I; S: laggard in anaphase II. T: prophase; U: ring and rod bivalents in diakinesis; V: metaphase I; W: precocious migration in metaphase I. Scale bars = 5  $\mu$ m.

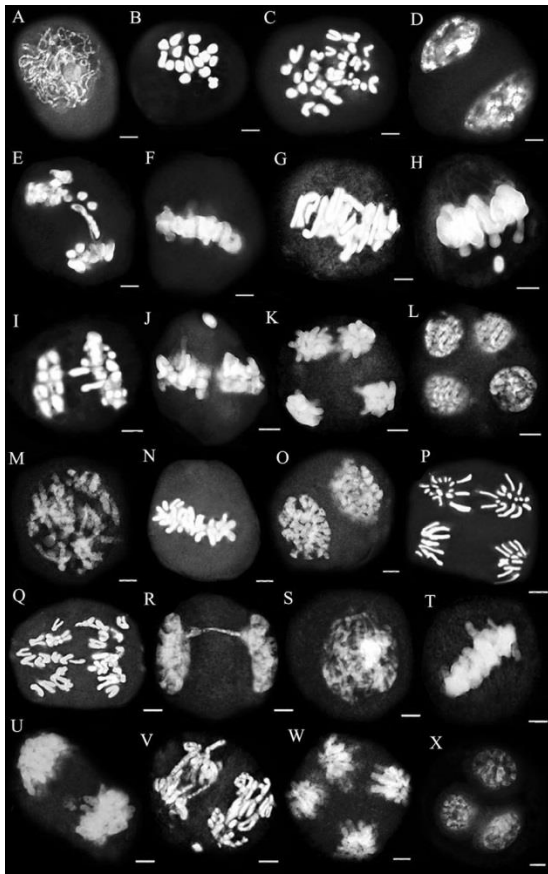


Figure 2. Meiosis in AZU825 (A-F), AZU89 (G-L), KUR98 (M-R) and KUR57 (S-X) populations ( $n = 16$ ) – A: prophase; B: diakinesis; C: anaphase I; D: telophase I; E: laggard and bridge in anaphase I; F, G: metaphase I; H: precocious migration in metaphase I; I: laggard chromosome in anaphase I; J: micronucleous; K: anaphase II; L: telophase II; M: prophase; N: metaphase I; O: telophase I; P: anaphase II; Q: anaphase I; R: bridge in telophase I; S: prophase; T: metaphase I; U: anaphase I; V: laggard in anaphase II; W: telophase II; X: microspore. Scale bars = 5  $\mu\text{m}$ .

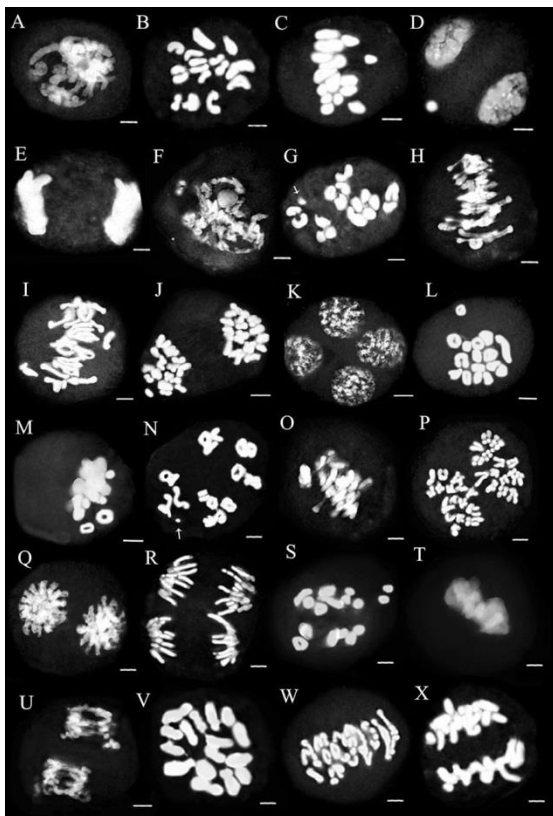


Figure 3. Meiosis in KUR56 (A-E), KUR82 (F-K), TON64 (L-M), TON00 (N-R), TON77 (S-U) and STR22 (V-X) populations ( $n = 16$ ) – A: prophase; B: diakinesis; C: precocious migration in metaphase I; D: micronucleous in telophase I; E: telophase I; F: prophase I; G: B chromosome in diakinesis; H: metaphase I; I: precocious migration in metaphase I; L, M: ring and rod bivalents in diakinesis; N: B chromosome in diakinesis; O: metaphase I; P: anaphase I; Q: metaphase I; R: anaphase II; S: ring and rod bivalents in diakinesis; T: metaphase I; U: anaphase II; V: diakinesis; W: metaphase I; X: metaphase II. Scale bars = 5  $\mu\text{m}$ .

## Discussion

### Ring and rod bivalents

During meiotic prophase, replicated homologs identify one another and become progressively more intimate juxtaposed until they are connected along their entire lengths. Then, they separate revealing a few remaining connections known as “chiasmata” (9). The rod configuration describes only one chiasma while the ring configuration, two chiasmata (9, 10, 11). In this study, ring and rod bivalents were observed in most studied populations.

### B chromosomes

B chromosomes are extra chromosomes to the standard complement that occur in many organisms. B-chromosome frequencies in populations result from a balance between their transmission rates and their effects on host fitness (12, 13). B chromosomes were reported for populations of *A. azurea* var. *kurdica* 82 (kur82) and *A. strigosa* ssp. *tonsa* 00 (ton 00).

### Cytomixis

Cytomixis is defined as the migration of chromatin between adjacent cells through cytoplasmic connection channels (14, 15). Cytomixis has been reported in many plant genus, such as *Onosma* and *Solenanthus* (16, 17). In this study, high percentage of cytomixis is concerned with *A. azurea*. The others *Anchusa* species demonstrated 0-3 percent of cytomixis. In recent years there is accumulating evidence that cytomixis is a normal, genetically controlled phenomenon influenced by physiological and environmental factors (15, 18, 19) rather than being due to fortuitous causes such as

fixation, mechanical injuries or pathological anomaly (20).

### Micronucleus

In general, univalents migrate precociously to the poles or behave as laggards in anaphase, but in both cases they can produce micronuclei in telophase I which normally remain until the tetrad stage (21, 22). This phenomenon were seen in *A. azurea* var. *kurdica* 56 (Fig. 2).

### Chromosome number and polyploidy

Johnston (32) and Riedl (33) regarded Boragineae as a natural group possibly originated from Lithospermeae, with Eritrichieae/ Cynoglosseae representing the ‘neighbouring’ lineage (2). Boragineae showed the broadest variation in base numbers, with  $x = 7$  (*Paraskevia*), 8 (*Anchusa*, *Phyllocara*, *Hormuzakia*), 9 (*Cynoglottis*), 10 (*Elizaldia*, *Symphytum*, *Nonea*) and 15 (*Elizaldia*, *Nonea*). Such broad series is likely to reflect a complex history of chromosomal evolution (34). The largest chromosomes in the family are found in *Anchusa*. All species studied here showed  $2n = 4x = 32$ . With exception of the new counts, chromosome numbers represented here are in agreement with those from earlier reports on the genus. The observations of the present study as well as the available data in Table 3 indicate that, the chromosome number of *A.* subg. *Buglossum* (*A. azurea* and *A. strigosa*) is  $2n = 4x = 32$ , and *A. aegyptiaca*, *A. milleri* and *A. iranica* of subg. *Buglossoides* is characterized by  $2n = 2x = 16$ . Also such variation in the ploidy levels can demonstrate a complex evolutionary pattern in subgenera.

**Table 3. List of previous chromosome counts made for two subgenera of *Anchusa***

Species	Subgenus	Habit	2n	x	Ploidy level	Origin	Reference
<i>A. azurea</i> Mill.	<i>Buglossum</i>	Perennial	32	8	4x	France and Portugal	Britton 1951(23)
<i>A. azurea</i>	<i>Buglossum</i>	Perennial	32	8	4x	Albania	Strid 1971(24)
<i>A. azurea</i>	<i>Buglossum</i>	Perennial	32	8	4x	Bulgaria	Markova & Goranova 1995 (25)
<i>A. azurea</i>	<i>Buglossum</i>	Perennial	32	8	4x	Italy	Love & Love 1982 (26)
<i>A. azurea</i>	<i>Buglossum</i>	Perennial	32	8	4x	UK	Valdes et al. 1978 (27)
<i>A. azurea</i>	<i>Buglossum</i>	Perennial	32	8	4x	Sardegna	Valsecchi 1971 (28)
<i>A. azurea</i>	<i>Buglossum</i>	Perennial	32	8	4x	Italy	D'Amato & Trojani 1985 (29)
<i>A. azurea</i>	<i>Buglossum</i>	Perennial	32	8	4x	Iran	Ghaffari 1996 (30)
<i>A. strigosa</i> Banks & Sol.	<i>Buglossum</i>	Perennial	32	8	4x	Israel	Díaz Lifante et al. 1992 (31)
<i>A. aegyptica</i> (L.) DC.	<i>Buglossoides</i>	Annual	16	8	2x	Island of Dokos	Constantinidis & Kamari 1995 (32)
<i>A. aegyptica</i>	<i>Buglossoides</i>	Annual	16	8	2x	Israel	Díaz Lifante et al. 1992 (31)
<i>A. iranica</i> Rech. & Esfand.	<i>Buglossoides</i>	Annual	16	8	2x	Iran	Ghaffari 1996 (30)
<i>A. milleri</i> Willd.	<i>Buglossoides</i>	Annual	16	8	2x	Israel	Díaz Lifante et al. 1992 (31)

In autopolyploids, all genomes are identical and homologous chromosomes have equal opportunities to pair at meiosis. When pairing starts at different sites, multivalents are formed. However, the maintenance of the multivalence till metaphase I will depend on its frequency and chiasma localization. In segmental allopolyploids, the genomes are not identical. As they result from hybridization of closely related diploid species followed by the doubling of the chromosome numbers, many bivalents and a few multivalent is formed (35). Although, Sybenga (36) pointed out that this character is not necessarily a reliable indication of limited pairing affinity, and thus of homology, because even true autopolyploids may form quadrivalents with frequencies substantially lower than the theoretically possible.

Smith (6) showed that *A. azurea* is an allotetraploid species because there were "only two chromosomes of each

distinguishable shape" in species. Studying the meiotic behaviour in six populations of *A. azurea* var. *azurea*, four populations of *A. azurea* var. *kurdica*, three populations of *A. strigosa* ssp. *tonsa*, and one population of *A. strigosa* ssp. *strigosa* were presented no multivalent. From the observations of the present study and those of the previous reports, it can be concluded that *A. subg. Buglossum* is either a well-established autotetraploid or more likely an allotetraploid. Although it is necessary for adequate genome, characterization would certainly substantiate this process.

### Chromosome number and habit

Polyploidy is an important evolutionary process in plants and some animals (37, 38). The abundance of allopolyploid plant species in nature suggests a selective advantage conferred by allopolyploids over diploid

progenitors, and has implicated polyploidy as an important speciation process. Stebbins and Ledyard (39) presented the hypothesis that the vast majority of polyploid perennials arose from diploid perennials. This is in contrast to the suggestion made by Muntzing (40) that "a large number of perennial species must have originated from annual types with lower chromosome numbers". Britton (32) suggested that evolution in the Boraginaceae is proceeding from perennial species to biennial or annual species. The annual habit is typical of *A. subg. Buglossoides*, while the taxa of *A. subg. Buglossum* (*A. azurea* and *A. strigosa*) are always perennial. Moreover,

allopatry and specialized ecology are important systematic elements because they may contribute to reproductive isolation. Most species of *A. subg. Buglossoides* and *A. subg. Buglossum*, the more widely distributed and polymorphic ones, are usually synanthropic and almost ubiquitous occurring in a variety of secondary habitats on different soil types (3).

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