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Cytological behaviour in relation to reproductive efficiency of some species of *Artemisia* L.

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ABSTRACT

Present study deals with four species of *Artemisia* L. namely *A. maritima*, *A. nilagirica*, *A. scoparia* and *A. tournefortiana* that dwell at variable altitudinal ranges (332-3305masl) of Jammu & Kashmir, India. Each species under study exhibits a variable sex-expression; these fall under three different sections of genus *Artemisia. Artemisia nilagirica* and *A. tournefortiana* belong to section Abrotanum, *A. maritima* to section Seriphidium while *A. scoparia* falls in section Dracunculus. The genus is cytologically very versatile. The analysis revealed four cytological races in *A. nilagirica* with chromosome numbers as 2n=18, 32, 34 and 54. Among these, *A. scoparia, A. maritima* and *A. tournefortiana* are cytologically stable*, A. scoparia* exhibits a diploid chromosome number of 2n=16, whereas *A. maritima* and *A. tournefortiana* have 2n=18. Reproductive output was maximum in *A. maritima* and minimum in *A. nilagirica*. Rootstocks provide an advantage, and help the perennial species of this genus to maintain a variable chromosome number, generated through a small percentage of normal seed- set.

Keywords : *Artemisia*, variability, sex-expression, reproductive output, rootstock

Artemisia L. (known as Wormwood) is a speciose genus of the family Asteraceae, and largest in the tribe Anthemideae comprising more than 500 taxa at the specific or sub-specific level. Taxonomically, it is considered as a complex genus since on one hand several species are reported to include different morphological forms, while on other extreme, there is a close resemblance among different species, which poses difficulties in proper identification. Many taxonomic rearrangements have thus been carried out for this genus which has led to several infrageneric classifications. According to Ling (1995 a, b), the genus has been divided into four groups treated as sections or sub-genera based on features mentioned against each section.

1. *Artemisia* (originally named as *Abrotanum* Besser.):— Heterogamous capitula with outer florets female and central florets hermaphrodite and fertile, glabrous receptacle.
2. *Absinthium* DC.:— Heterogamous capitula with outer florets female and central florets hermaphrodite and fertile, hairy receptacle.
3. *Dracunculus* Besser.:— Heterogamous capitula with outer female florets and central florets hermaphrodite, female sterile, glabrous receptacle.
4. *Seriphidium* Besser:— Homogamous capitula with all florets fertile and hermaphrodite, glabrous receptacle.

Genus *Artemisia* is distributed worldwide mainly across the temperate zones of Northern Hemisphere with some species reaching the Arctic, and a few species are found even in the Southern Hemisphere (Bremer 1994, Ling, 1994). The origin of *Artemisia* based on fossil data, is in semi arid steppes of temperate Asia at mid - coenozoic i.e. about 20 million years ago (Wang 2004). Central Asia is recognised as the main centre of speciation and diversification of the genus, from where it is believed to have expanded towards Indo-Turanian, Mediterranean and North - American regions (Pellicer *et al.* 2011). Holm *et al.*(1997) considered Europe as the centre of origin of the genus. A few species are also reported in Africa and Europe (Tutin *et al.* 1976, Ling 1994, Shultz 2006). The genus is interesting from cytological point of view with reports of several base numbers and polyploidy complexes.

Present work encompass details of morphology, cytology and sexual reproductive output of four species of *Artemisia* L. namely, *A. maritima* L., *A. nilagirica* (C.B. Clarke) Pamp., *A. scoparia* Waldst & Kit. and *A. tournefortiana* Reichb. inhabiting different altitudinal regimes of NW Himalayas. An attempt has been made to correlate these attributes to the distribution and dispersal capability of these species. *A. nilagirica* and *A. tournefortiana* belong to section *Abrotanum,* while *A. maritima* belongs to section *Seriphidium* and *A. scoparia* is from section *Dracunculus*

MATERIALS AND METHODS

Materials : Present work is based on sprawling populations of four species of *Artemisia* namely, *Artemisia maritima* L., *Artemisia nilagirica* (C. B. Clarke) Pamp., *Artemisia scoparia* Waldst & Kit. and *Artemisia tournefortiana* Reichb. (F: Asteraceae). Different populations of these species were tagged and surveyed in regions located at varied altitudes in Jammu and Leh region of Jammu & Kashmir (Table 1).

Table 1: Location of different populations of *Artemisia* spp. investigated

A. nilagirica	Altitude (masl)	*A. scoparia*	Altitude (masl)	*A. maritima*	Altitude (masl)	*A. tournefortiana*	Altitude (masl)
Jammu	332	Jammu	332	Kishtwar	1760	Leh	3305
Rajouri	915	Rajouri	915				
Poonch	1120	Kud	1705				
Bhaderwah	1680						
Kud	1705						

1. **Morphology:** The plants and flowers from these populations were studied for vegetative and floral morphology. Data were collected on various aspects like height of the plant, number of of fshoots arising from each root stock, shape, arrangement and size of leaves, length of inflorescence, number of disc and ray florets per inflorescence in the field. Floral structure with emphasis on essential organs was studied in the laboratory under a stereo - microscope (NIKON Type - 115).
2. **Fruit and Seed Set:** Fruit and seed set was observed in the plants growing open in the fields as well as those from bagged inflorescences. Number of flowers per capitulum and later number of fruits per capitulum were counted for these plants. Percentage fruit and seed set, both from open- pollination and bagging was calculated as:-

$$\text{Percentage fruit set per inflorescence} = \frac{\text{Fruit count per inflorescence}}{\text{Flower count of the same inflorescence}} \times 100$$

   For computing fruit set in *A. scoparia*, instead of floral count, ray florets were considered.
3. **Cytology :** For karyology, seeds of each species were germinated in petri-plates lined with moist filter paper. Seedlings with 4-5mm long root tips were washed with water and then pretreated with saturated aqueous solution of p- dichlorobenzene for 3-4 hours at 4°C. The pretreated seedlings were washed and fixed in Carnoy's fixative (three parts of ethyl alcohol and one part of acetic acid). After fixation for 24 h, these were washed in water and preserved in 70% alcohol. For preparation of root tip squashes, the seedlings were hydrolyzed in a mixture of 1% acetoorcein and 1N HCl (9:1) and placed in an oven maintained at 60° C for 13 min. Finally, the root tips from these seedlings were squashed in 1% propiocarmine. Battaglia's (1955) scheme was used for classifying the somatic chromosomes. Karyotypes were classified following Stebbin's (1971) chart of karyotype asymmetry.

Pollen mother cell meiosis was studied from immature flower buds fixed between 0800-0900h) in acetic- alcohol (1:3v/v). After fixation for 24h, the buds were washed in water and preserved in 70% ethyl alcohol at 4-6°C. Finally, the anthers were squashed in 1% propiocarmine. Chromosome preparations both (mitotic and meiotic) were made permanent by removing the paraffin ring and inverting the slide in a petridish containing 1:1 mixture of n- butyl alcohol and acetic acid. Both the slide and cover slip were then transferred to a petridish containing n- butyl alcohol. The slides were removed after 2-3 min and coverglass was restored using euparol.

All photomicrography of chromosomal preparations was done under a photomicroscope ECLIPSE E-400, Nikon attached with a digital color camera SAMSUNG SDS-312. Field photography was done with SONY DSC-H10 camera.

RESULTS AND DISCUSSION

Morphology and Reproductive cycle: Plants of all the species studied are shrubs that reach to a maximum height of 3.2m in *A .nilagrica*; *A. tournefortiana* includes the smallest plants with an average height of 0.51m (Figs. 1-4). All but *A. tournefortiana* undergo perennation by rootstocks that sprout in the months of March to April in different areas to produce aerial offshoots. *A. tournefortiana* propagates by seeds only that germinate after the snow melts in the area of occurrence normally during the month of March. The plants undergo a period of 4-5 months of vegetative growth before they bloom in Sept.-Oct. Seed set and seed dispersal is over by November. Thereafter, the plant dies. *A. tournefortiana* is therefore an annual.

Perennial shrubs predominate the genus *Artemisia*, and only 5% (approx. 10) are annual or biennial (Valles and Mc Arthu 2001). Annual life cycle is thus rare in the genus. Also while most of the *Artemisia* species enjoy wide distribution; few are reported to have restricted distribution, these include *A. bigelovii*, *A. rigida*, *A. tripartite* and *A. rothrockii* (Mc Arthur and Sanderson 1999). No correlation till date has been drawn between the life cycle and distribution in these species. Interestingly in the lot studied presently, *A. tournefortiana* occurring at higher altitudes 3305masl and having restricted distribution is an annual species. Out of the three perennials in the group *A. scoparia* and *A. nilagirica* are widely distributed, while *A. maritima* has a restricted range and sprawls at mid altitudes (1760masl) (Table 2).

Cytology : Out of four species studied presently**,** three are based on x=9, while *A. scoparia* exhibits x=8 as its base number. *A. tournefortiana* and *A. maritima* both with n=9 are

the species with restricted distribution. Both are strictly diploid (2n=18) with symmetric karyotypes consisting of metacentric and sub-metacentric chromosomes. 2n=18 is the only chromosome number reported for *A. tournefortiana* (Figs. 18-26).

A. nilagirica and *A. scoparia* are the widely distributed species of the lot, as these could be localised at altitudinal regimes with a range of 332 to 1705 masl. Out of these, *A. scoparia* is cytologically stable revealing 2n=16 in all the plants scanned (Figs. 24-26). In fact, it is among the few highly stable species in an otherwise cytologically flexible genus *Artemisia*. Meiosis in all the populations is normal. Species exhibit symmetric karyotype

Five populations of *A. nilagirica* studied for their cytological details revealed the presence of four different cytotypes. These are 2n=2x=18 (Bhaderwah population, Figs. 15-17), 2n=6x=54 (Rajouri, Poonch and Kud populations (Figs.33-35) and 2n=?=32,34 (Jammu population (Figs. 27-32). Cytotype with 2n=54 is the predominant one and most widely distributed. The pollen mother cells of this cytotype showed haploid chromosome number as n=27. Chromosomes pair during meiosis-I to form ring, rod and cross shaped bivalents, in addition to the presence of IIs, multivalent (trivalents, quadrivalents and hexavalents) were also observed (Figs.37-38). Segregation of chromosomes was normal at Anaphase-I (27:27) in most of the pollen mother cells observed. At the same time, some abnormalities in the form of laggards and bridges were also observed in different percentages in different populations. Going by n=9, this is a hexaploid.

Plants of Jammu population of *A. nilagirica* analysed for somatic chromosome complement and meiosis scored two haploid numbers i.e. n=16 and n=17, both of which are new numbers for the species. Out of 31 plants scanned, majority (n=25) had n=17 and few (n=6) had n=16. During meiosis-I chromosomes paired to form bivalents of different shapes. However univalents and multivalent forming different shapes were also observed in large number of cells (Tables 3 and 4, Figs. 30 and 36). 67.39% cells in plants with 2n=34 showed normal segregation of chromosomes. In rest (32.61%), either chromosome bridges or laggards were observed. In plants with 2n=32, 67.85% cells showed the presence of anomalies like laggards, bridges etc (Figs.39-40). Only small proportion (32.14%) of the cells were found to have regular 16:16 segregation of chromosomes. In some of the cells (n=8), chromosome fragments could be observed. The karyotype formulae for the plants of this cytotype (2n= 18 and 34) are 4M+14SM and 8M+26SM, respectively. Total chromatin length in the three hexaploid populations studied ranges between 109.43µm and 147.62µm.

Genus *Artemisia* is cytologically complex. It is among the few taxa that exhibit dysploidy(more than one base number). The two common base numbers in the genus are x=9 and 8, the later is considered as derived (Kawatani and Ohno 1964, Ehrendorfer 1964, Estes 1969, Persson 1974, Mc Arthur and Pope 1979, Oliva and Valles 1994, Mc Arthur and Sanderson 1999, Valles and Garnatje 2005, Pellicer *et al*. 2007 a and b). In addition few authors have reported new base numbers as n=7, 10, 13 and 17 (Weins and Richter 1966). In *A. maritima*, cytological polymorphism with intaspecific chromosomal races namely diploid (2n=18); tetraploid (2n=36) and hexaploid (2n=54) have been reported elsewhere in the world (Suzuka, 1950, Kawatani and Ohno 1964). From the Indian sub-continent, however only diploid cytotype is known (Khoshoo and Sobti 1958, 2005, Chehregani and Hajisadeghian 2009, Matoba and Uciyama 2009, Park, 2009).

Wild populations of *A. scoparia* have been cytologically investigated by many authors. Most of these reveal the species to be diploid with chromosome number, 2n=2x=16 (Hindakova and Majovsky 1976, Kaul and Bakshi 1984, Kuzmanov *et al*. 1986, Volkova and Bokyo 1986; Mendelak and Schweizer 1986, Torrell *et al*. 2001, Valles *et al.* 2003, Saedi *et al*. 2006, Abdolkarim *et al*. 2010). Few authors (Yan *et al*. 1989, Abdolkarim *et al*. 2010, Qiao *et al*. 1990, Chehragani and Mehnfar 2008) have however reported 2n=18 as chromosome number for this species. Polyploid cytotype (2n=36) has been reported in *A. scoparia* by Kawatani and Ohno (1964) and Gupta *et al* (2010). From North West Himalayan region of J&K, Kaul and Bakshi (1984) have isolated it as a diploid species with 2n=16.

Khoshoo and Sobti (1958) have also reported polyploidy in some species of *Artemisia* from North West Himalayas with base numbers, x=8 and 9. The authors have worked out 11 species out of 27 reported by Hooker from the Indian sub–continent. The species have been sampled from the North–Western Himalayas; but materials from the Eastern–Himalayas and other areas have also been studied. Most of the species have been worked out for the first time. In addition to the 11 Indian species, the chromosomes number in *A. vulgaris* Linn. from Germany has been investigated cytologically. The chromosome counts given by them are *Artemisia scoparia* Waldst. and Kit.(n=8,2n=16), *A. absinthium* Linn. (n=9), *A. glauca* Pall.(n=18, 2n=36), *A. ricinata* Willd.(2n=18), *A. maritima* Linn.(Kashmir) (n=18), *A. maritima* Linn.(Japan) (n=27), *A. parviflora* Roxb. (n=9, 2n=18), *A. roxburghiana* Bess.(n=18, 2n=36), *A. tournefortiana* Besser.(n=9, 2n=18), *A. vestita* Wall. (n=9, 2n=18), *A. vulgaris* Linn. (2n=16), *A. vulgaris* Linn.(n=18) and *A. vulgaris* Linn. (India) (n=27, 2n=54). Hexaploid cytotypes (2n=54) in *A. nilagirica* has been reported by Gupta *et al*. 2014; whereas the diploid chromosome number of 2n=18+0-4B has been reported in the studies of Bala *et al*. (2012).

Reproductive output : Among the four species studied, *A. maritima* is the only species which shows seed set on selfing also; this is also the species which reveals maximum seed set on open pollination. In *A. maritima*, on open pollination, an average of 78.38% ± 2.2 seeds are formed out, of which a very high proportion is healthy (X = 75.2% ± 2.3), whereas on bagging, healthy seed set averages 20.8%.

Among other species, annual *A. tournefortiana* restricted in its distribution to high altitude (3305masl) revealed good seed set, it averaged 62.8%±2.7 (Healthy, 60.61%±1.55) on open pollination. On bagging, however, this species failed to set seeds. Of the two widely distributed species, cytologically stable *A. scoparia* has moderate to high seed set on open pollination, it ranged between 61.53-65.77% (Healthy, 52.5% to 60.46%) in three populations studied. On bagging this species also was unable to set healthy seeds (Table 2).

Widely distributed and cytologically flexible *A. nilagirica* displays significant differences in seed set in different populations. Here, the diploid cytotype displays maximum seed set (61.43%±1.6, healthy, 46.4%±1.8) followed by hexaploid cytotypes i.e. Rajouri (43.4%±2.0, healthy, 29.7%±2.1), Poonch (39.03%±1.9, healthy 25.7%±1.1) and Kud (37.7±2.0, healthy 22.5%±1.3); while Jammu population with a derived and new number has minimum value of seed set (15.4%±1.2, healthy, 7.5%±1.4). On bagging, shriveled seeds are formed in all the populations studied (Table 2).

Table 2: Comparative account of some morphological and reproductive features of four species of *Artemisia* L.

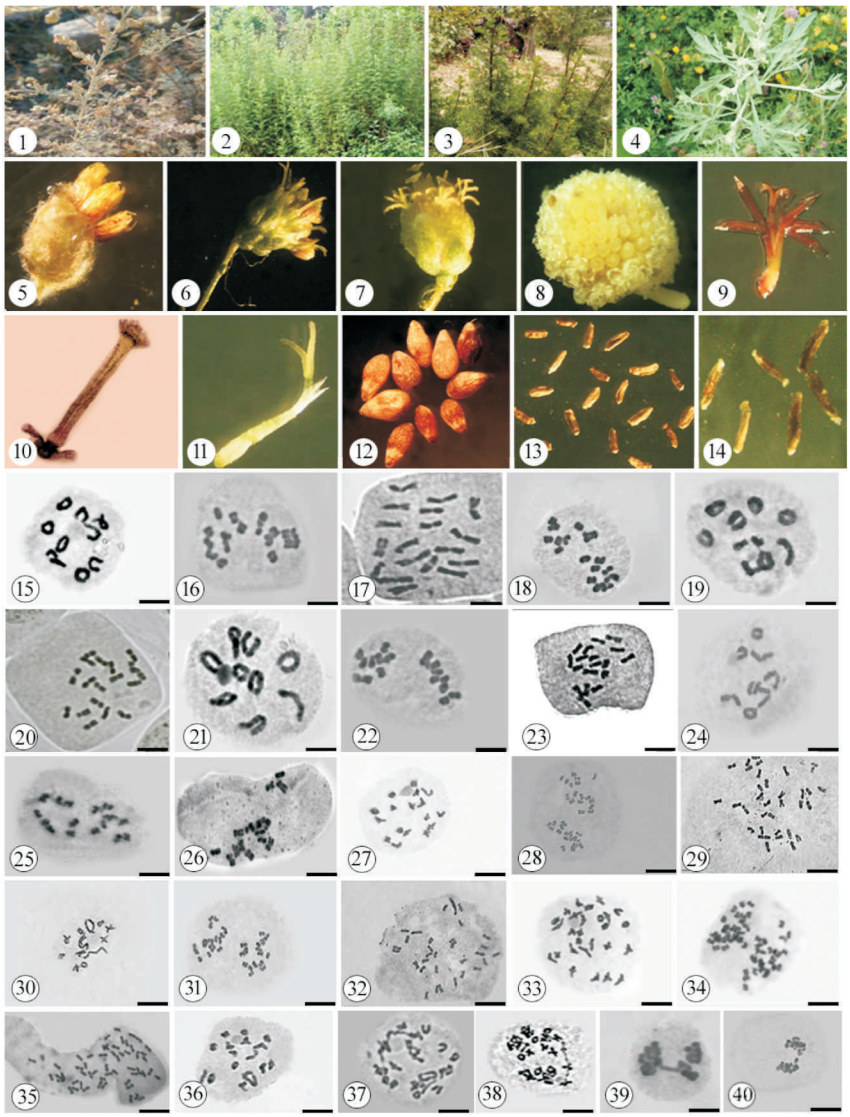
S.No.	Character		*A. nilagirica*	*A. scoparia*	*A. maritima*	*A. tournefortiana*
1	Habit		Perennial	Perennial	Perennial	Annual
2	Plant height (m)		1.2-2.9	1.2-2.4	0.72	0.51
3	No. of capitulum/plant		98,325	86,459	53,297	761
4	Size of capitulum (lxb) (mm)		4-5.1 x 2-4.8	1.2-2.3 x 1.0-2.5	2.0-2.5 x 1.6-2.3	3-5 x 3-7
5	No.of flowers/ capitulum		17-22	12-17	7-13	102-115
6	Size of individual flower (disc/ary)		1.8-2.8/1.5-2.6	0.8-1.6/0.6-1.3	2.0-2.6	2.1-3.0/2-2.8
7	Seed set %	Open Pollination	15.45- 61.43 7.57-46.4 (healthy)	61.5 – 65.7 52.5-60.4 (healthy)	78.3 75.2 (healthy)	62.8 60.6 (healthy)
		Selfing	9.3-18.5 All shrivelled	18.5-22.3 All shrivelled	18.5-36.220.8 (healthy)	1.0 All shrivelled

Table 3: Chromosomal associations observed in pollen mother cells at Metaphase-Iin *A. nilagirica*

Cytotype	Rajouri (chromosome association)	No. of cells	%age of cells	Poonch (chromosome association)	No. of cells	%age of cells	Kud (chromosome association)	No. of cells	%age of cells
2n=54	27IIs	136	69.84%	27IIs	164	84.97%	27IIs	150	84.74%
	2I + 23II + VI	11	5.82%	2I + 23II + VI	16	8.29%	22II + 1IV + 1VI	1	0.56%
	25II + 1 IV	26	13.75%	23II + 2IV	10	5.18%	24II + 1VI	19	10.73%
	24II + 1VI	17	8.99%	21II + 2IIIs + 1VI	2	1.03%	21II + 1IV + 1VIII	6	3.38%
	22II + 1IV + 1VI	03	1.58%	1I + 1III + 25II	1	0.51%	17II +2III+ 1IV + 1X	1	0.56%

Table 4: Chromosomal configuration in different populations of *Artemisia* L. at Metaphase-I of meiosis.

				Mean chromosome pairing per pmc					
S. No.	**Population**	**2n**	**I**	**II**	**III**	**IV**	**VI**	**VIII**	**X**
1.	Rajouri	54	0.18	25.92	-	0.24	0.16	-	-
2.	Poonch	54	0.2	26.15	0.05	0.29	-	-	-
3.	Kud	54	-	25.97	-	0.21	0.21	0.01	0.01
4.	Bhaderwah	18	-	8.86	-	0.06	-	-	-
5.	Jammu	34	0.16	16.13	0.02	0.18	0.06	-	-
6.	Jammu	32	-	15.33	-	0.33	0.1	-	-



Figs.1-4:. Plant morphology of *A. maritima, A. nilagirica, A. scoparia and A. tournefortiana.* Figs. 5-8:. their respective capitula, Fig. 9:. Opened disc floret of *A. nilagirica,* Fig. 10: Disc floret of *A. scoparia* with rudimentary ovary, Fig.11: A ray floret, Figs. 12-13: Healthy seeds of *A. maritima* and *A. nilagirica,* Fig. 14: Shrivelled seeds of *A. nilagirica,* Figs. 15-17:. pmcs and somatic metaphase spreads of *A. nilagirica* (Bhaderwah population with 2n-=18) (Scale Bar=10µm) Figs. 18-20: pmcs and somatic metaphase spreads of *A. maritima* with 2n=18; Figs. 21-23: pmcs and somatic metaphase spreads of *A. tournefortiana* with 2n=18; Figs. 24-26: pmcs and somatic metaphase spreads of *A. scoparia with* 2n=16: Figs. 27-29: pmcs and somatic metaphase spreads of *Artemisia nilagirica;* (Jammu population) with 2n=34, Figs 30-32: pmcs and somatic metaphase spreads of *Artemisia nilagirica* (Jammu population) with 2n=32; Figs. 33-35: pmcs and somatic metaphase spreads of *Artemisia nilagirica* (Rajouri population) with 2n=54, Figs. 36-38: Pmcs of plants of Jammu and Rajouri populations of *A. nilagirica* showing multivalent, Figs. 36:: 15II + 1IV (37). 17II +2III +1IV+ 1X , Fig. 38: 21II+1VIII+1IV, Fig.39:. pmc of *A. nilagirica* showing laggard, Fig. 40: pmc of *A. nilagirica* showing bridges

CONCLUSION

The present study lets one to comprehend how *Artemisia* uses its genetic system very efficiently at different altitudinal ranges. Out of the three species with stable meiotic system, annual *A. tournefortiana* and perennial *A. scoparia* use their breeding system to garner variation. *A. maritima* is the least variable, but is the highest seed setter of the lot having evolved the capacity to set some seed by selfing also (Table 2). Interestingly in *A. maritima*, a novel phenomenon of pollen germination on ovary wall has also been reported (Parihar *et al.*, 2009), suggesting the tilt towards self-fertilization in this species.

Widely distributed *A. nilagirica*, garners more variation through its meiotic system. Presence of distinct cytotypes at diverse altitudes points towards use of these variations for dispersal to new vistas. Although the sexual reproductive output in the new cytotypes (2n=32, 34) is low, they flourish well via a perennial rootstock. Combining an outcrossing breeding system with flexible meiotic system and the survival of the variation through perennial rootstock makes *Artemisia nilagirica*, a mighty species to rekon with.

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