Cytomorphological Studies in *Dugesia* Species of Iran

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Summary *Dugesia* species of Karaj river in Iran were investigated from cytological, and morpho-anatomical point of view. Two different populations were studied. Individuals from Gajereh region possessed $2n=25$ and those of Gachsar possessed $2n=26$. They varied in details of karyotypes, G-Banding pattern and morpho-anatomical features. Cluster analysis separated the two populations of *Dugesia* in different clusters. Two new species of *Dugesia* with new chromosome number having complete asexual reproduction are reported.

The fresh water planarians belong to the phylum Platyhelminthes, an old monophyletic group which branched from the rest of metazoans at an early stage and can be considered as the sister group of the other bilateral triploblastic organisms (Riutort *et al.* 1992, Addoutte and Philippe 1993). They possess a great morphological plasticity and regeneration power and are remarkable for the occurrence of local polyploid populations and the occasional presence of mixoploidy and mosaics having both diploid and triploid tissues (Hoshino *et al.* 1991, Benazzi and Lentati 1976, Oki *et al.* 1981).

Although there have been extensive cytological studies on planarian species in many countries (Hoshino *et al.* 1991, Benazzi and Lentati 1976, Oki *et al.* 1981, Tamura *et al.* 1988, Ribas *et al.* 1988), there has been no report from Iran. The present article is the first report on cytotaxonomy of *Dugesia* species from Iran.

Materials and methods

Dugesia species were collected from Gajereh and Gachsar regions of The Karaj river during 1995–1997. Specimens were brought to lab (temperature 4–8°C) and kept in the glass vials containing fresh well aerated water.

For karyotyping blastoma of the specimens were used. For this purpose; specimens were first cut from the head and tail regions and allowed to regenerate. It would take about one month for complete regeneration; specimens (blastomas) were collected after 5–8 days of regeneration and treated with 1% colchicin for 3–4 hr (4°C), then passed to hypotonic solution (double distilled water) for about 30–35 min (5–20°C). The specimens were kept in fixative (Carnoy's fluid) for 30 min (4°C). After dropping air dried slides were prepared by keeping them inside incubator (37°C) for 30 min. Staining was carried out by using 10% Gimsa solution in buffer phosphate (v : v) (pH=6.9) for 10 min. Chromosomes were identified according to Levans terminology (Levan *et al.* 1964). Karyotypic analysis was performed on 20 well prepared metaphase plates in each population.

For G-Banding all the steps involved in simple staining were followed plus using trypsin and 70% ethanol.

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Table 1. Characters and their coding

1. The body size: mm
2. Auricles: Short=1, long=2
3. Color pattern of the ventral side: Monotonous bright (unpigmented)=1, faint transversal regular strips=2
4. Color pattern of the dorsal side: Perpharyngeal area dark (pigmented), caudal area bright=1, cephalic area bright, post-pharyngeal area dark, caudal area bright=2
5. Pattern of pharynx area from ventral side: Distinguished bright margin=1, distinguished dark margin=2,
6. Pattern of pharynx area from dorsal side: Distinguished with humdrum marginal zone=1, distinguished with striped margin=2
7. 2n: 25=1, 26=2
8. No. SAT-Chromosomes: 6, 13=1, 13, 21=2
9. Mean of temperature change in environment °C

Statistical analysis

Karyotypic and morphological characters were coded (Table 1), standardized (mean=0, variance=1) (Chatfield and Collins 1995, Sheidai *et al.* 1996a, b) and used for multivariate statistical analysis. Cluster analysis using WARD and Single linkage methods (Sheidai and Inamdar 1997, Sheidai *et al.* 1996c, 1998) were performed. Fit of the clusters to the original data was checked by computing cophenetic correlation (Rohlf 1987, Stanton *et al.* 1994). For multivariate analysis SPSS (Norusis 1988) and NTSYS (Rohlf 1987) softwares were used.

Results and discussion

Specimens collected from Gajereh region possessed two prominent large eyes on the head; close to each other. The elongated and not very thin auricles formed a completely triangular head. The body color in the ventral side was bright color (yellow, brown or green) with a uniform pattern. The pharynx was without pattern and stain, dorsal side was also bright in color with no pattern. The body size ranged from 5–10 mm.

The mode of reproduction was asexual (fissiparity) throughout the year. They possessed 2n=25 chromosome number. All these characters indicate that Gajereh specimens belong to the genus *Dugesia*.

The specimens collected from Gachsar region possessed almost similar features as those of Gajereh with larger body size (5–15 mm), larger eyes and 2n=26. The members of these two populations however varied in some of the morphological characters such as type of auricle, color pattern of the ventral side, *etc.* (Table 1; code 1=Gajereh, code 2=Gachsar).

Karyotypic analysis

Details of karyotypes are presented in Table 2. The populations studied did not show any significant difference in their total chromatin length, but varied in type of chromosomes for example Gajereh possessed 3B type chromosomes while Gachsar possessed 4, *etc.* (Table 2). Both populations possessed two SAT-Chromosomes (Table 2). The secondary constrictions were present on the

Table 2. Karyotypic details

Character (size in μm)	Population	
	Gajereh	Gachsar
2n	25	26
TL	99.05	99.07
L	6.48	6.25
S	1.82	0.61
Type of chromosome:		
A: 6–6.5	1	1
B: 5.03–5.98	3	4
C: 4.07–4.65	5	3
D: 3.07–3.99	14	14
E: 2.78–2.93	1	2
F: 1.28–1.82	1	2
Karyotype formulae	4m+22sm	7m+18sm
No. Sat	2	2
SAT-CH	6, 13	13, 21

TL=Total chromatin length, L=Longest chromosome, S=Shortest chromosome, SAT-CH=SAT-Chromosome, m=Metacentric, sm=Submetacentric.

Fig. 1. Simple karyotype and G-Banding in *Dugesia* populations.

long arm of chromosome number 13 (in both populations) and on the short arm of chromosome number 6 (Gajereh) and 21 (Gachsar) (Figs. 1, 2).

G-Banding of the chromosomes also revealed differences in details of chromosomes in these two populations (Fig. 3). From karyotypic data it seems that numerical and structural changes of chromosomes have been occurred in genomic differentiation of these two populations.

The previous cytogenetical studies in *Dugesia* species have revealed the presence of $X=4, 7$ and 8 as the basic chromosome number (Benazzi and Lentati 1976, Oki *et al.* 1981), therefore $2n=25$ and 26 are new reports.

Cluster analysis of cyto-morphological data indicated presence of two main clusters (Fig. 4). The first main cluster is comprised of specimens collected from Gajereh (1, 2) and the second main cluster is formed by specimens collected from Gachsar (3 to 6). A little variation

Fig. 2. Ideograms of *Dugesia* Species.

Fig. 3. G-Banding patterns in *Dugesia* populations; top: Gajereh, down Gachsar.

Fig. 4. Cluster analysis (Single linkage method) of cytomorphological data.

observed among members of each cluster is only due to body size differences. High cophenetic value ($r > 0.80$) obtained for cluster analysis supports distinctness of the clusters obtained (Rohlf 1987).

According to the present findings we suggest specimens collected from Gajereh and Gachsar to be treated as two new *Dugesia* species reported from Iran namely *Dugesia Iranica* and *D. persica* respectively.

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