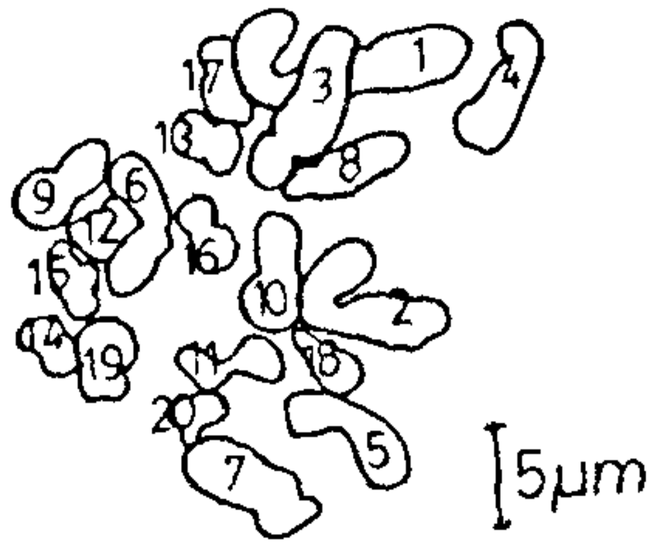

Figure 1. *Aphyllorchis montana* Reichb. *f*; Haploid number of chromosomes $n=20$ (×2000).

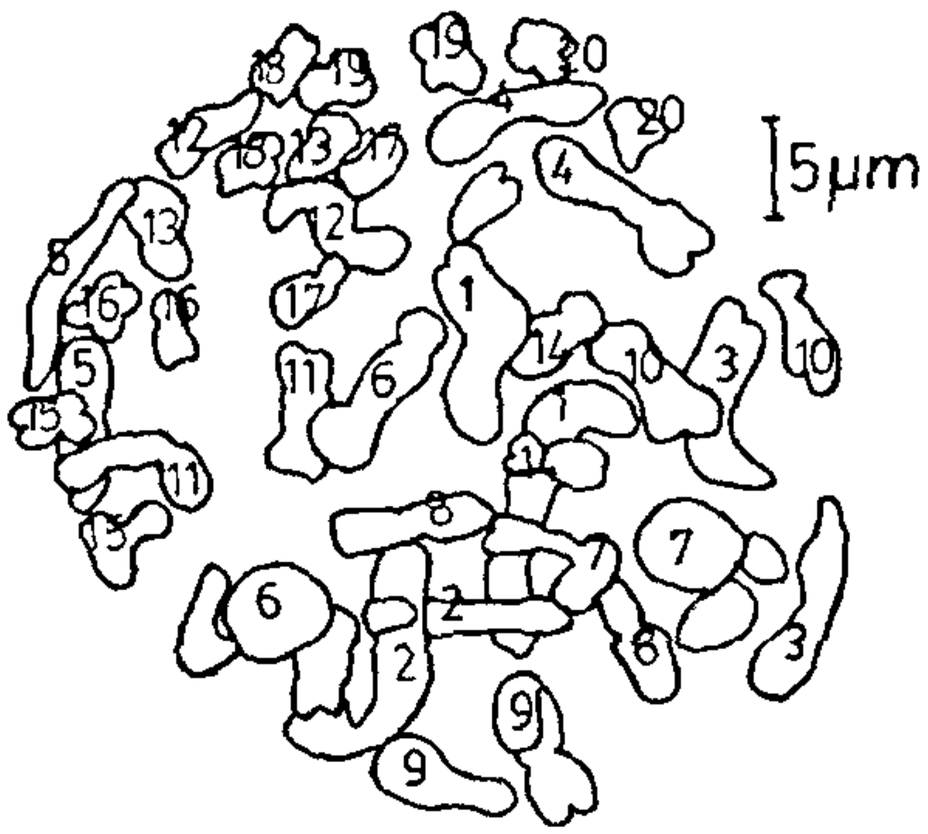

Figure 2. *Aphyllorchis montana* Reichb. *f*; Diploid number of chromosomes $2n=40$ (×2000).

numbered according to their length. Chromosome measurements were tabulated following the nomenclature of Abraham and Nagendra Prasad[2] (Table 1), Haploid chromosome number $n=20$ and diploid number $2n=40$. The same chromosome number is reported in some terrestrial orchids[3]. In *Aphyllorchis* the chromosomes are highly heterogeneous, because they vary considerably in their size and centromere position. Chromosome sizes are also astonishingly larger (3–15 μm). Usually in monandrous orchids the chromosome size ranges from 1.2–7 μm only and the diandrous species have been observed to possess large chromosomes[4].

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Occurrence of B-chromosomes and karyotypic analysis in sedge genus *Eleocharis* R. Br.

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The presence of B-chromosomes in *Eleocharis* R. Br. is reported here. In *E. palustris* (L.) R. Br., $n=8$, the frequency of pollen mother cells with B-chromosomes is 35%. This species with localized centromere also shows variability in karyotype of different pollen when studied for pollen mitosis. The second species *E. atropurpurea* (Retz.) Kunth, $n=10$, shows polycentric chromosomes and highly symmetrical karyotypes. The study shows high amount of genetic diversity of sedges within species concept, indicating that evolution is operative at micro level.

SEDGES are fairly well represented in the extant flora and are cosmopolitan in distribution, growing mostly mingled up with grasses. In the sedge flora of Punjab state in North India, a wide variation for chromosome number[1–5] compared to earlier reports[6], was observed. Hence chromosomal analyses of sedge populations were initiated to find out the genetical basis of existing morphological variations within different species. The present paper reports the presence of B-chromosomes in *Eleocharis* R. Br. so far unrecorded for the genus, as well as karyomorphological variations within the species.

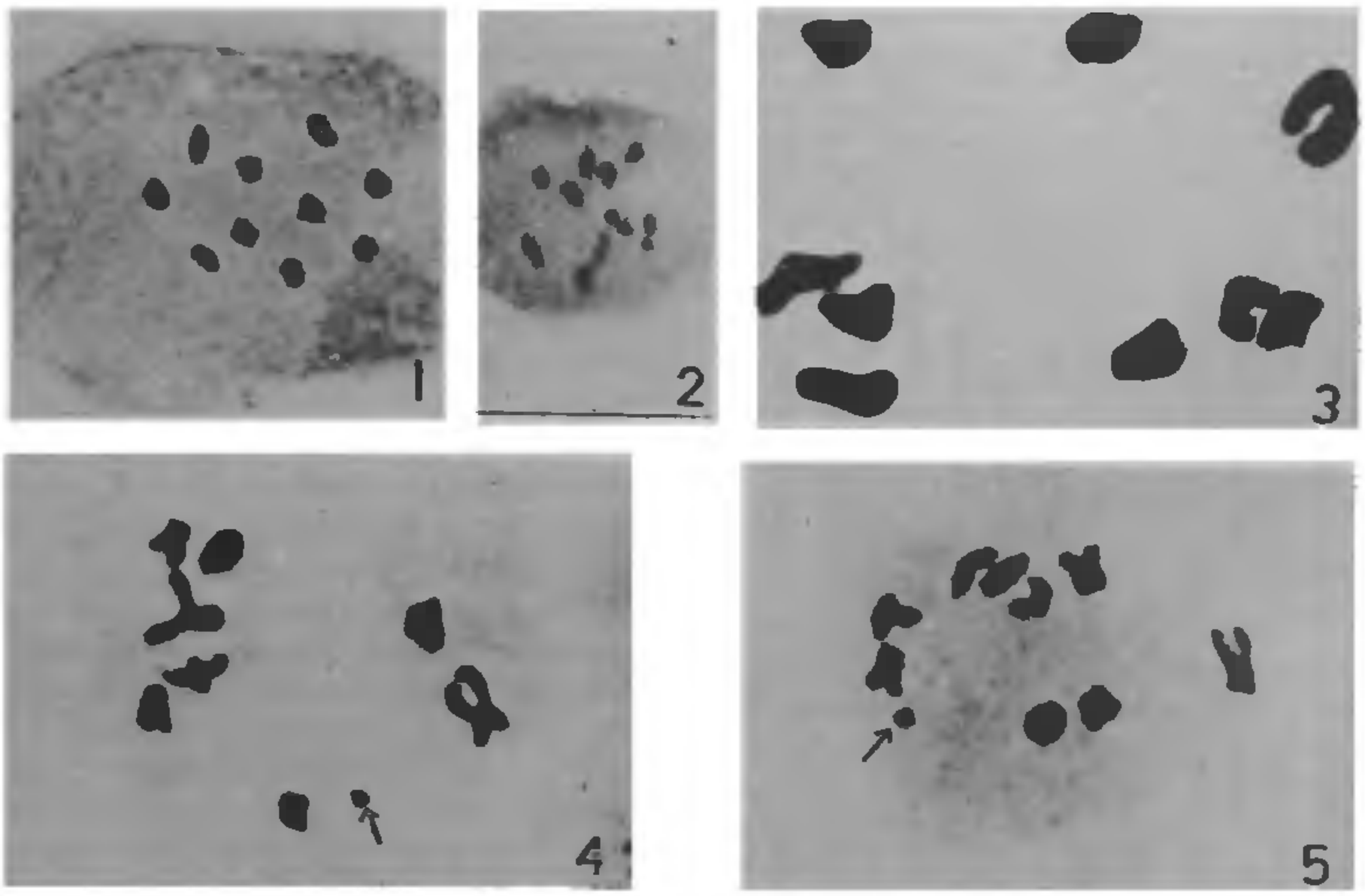
Two species of *Eleocharis*, viz. *E. atropurpurea* (Retz.) Kunth and *E. palustris* (L.) R. Br. were analysed for both meiotic and mitotic chromosomes. Young floral buds were fixed in standard Carnoy's fluid for 10–12 h. For the study of meiotic chromosomes young anthers were squashed in acetocarmine. Observations on division in the pollen nucleus were made for karyotypic analysis. This is a unique feature of Cyperaceae and Juncaceae, where pollen nucleus divides before the formation of exine[7]. On the basis of pollen mitosis, analysis of karyotypes of 13 sedge species was made and results for other genera as *Fimbristylis* Vahl, *Bulbostylis* Kunth and *Scirpus* Linn. are being published elsewhere.

Both *E. atropurpurea* and *E. palustris* have leafless plants with simple, erect and densely tufted stems. In *E. atropurpurea* the rhizome is absent and the stems are

small, filiform, bearing a solitary terminal, ovoid, dark brown spike which is larger than the stems while in *E. palustris* the individuals are taller, with creeping rhizome, terete and longitudinally striated stem and a solitary terminal, cylindric, yellowish-brown spike again larger than the main axis. The former species appears annually in paddy fields during July to August and supports spikes when there is no standing water during ripening of rice. On the other hand, the latter species is perennial and grows in marshy places during December to April. Both the sedges frequently occur in Patiala district of Punjab state from where the materials for the present study were collected.

Meiosis. In the case of *E. atropurpurea*, for both the populations examined from villages Sheikhupura and Chhoti Daun, 10II are counted at M-I (Figures 1 and 2) but for *E. palustris* (village Bathli) normally 8II are countable at M-I (Figure 3). However, in some of *E. palustris* pollen mother cells (PMCs), an additional one B-chromosome (Figures 4 and 5) is countable. The frequency of PMCs with B-chromosome is 35%. Both the species are at diploid level based on $x=10$ and $x=8$ respectively.

Mitosis: In population ii (see Table 1) of *E. atropurpurea* we noticed 10 chromosomes, all polycentric or with diffuse centromere (Figure 6). Karyomorphological variability was noticed in population i of *E. palustris*. In population ia and ib categories of pollen, in addition to 8 chromosomes, one B-chromosome was noticeable (Figure 7) while in ic category, no B-chromosome was seen and only 8 chromosomes were countable (Figure 8). Karyotypic data of these two species are presented in Table 1 and the idiograms are shown in Figures 9–12.



Figures 1–8. Photomicrographs of two species of *Eleocharis* (×1540) (except 3, 8 which are camera lucida druwings (×2740). **1–5.** PMCs. **1**, *E. atropurpurea* population i with 10 bivalents; **2**, *E. atropurpurea* population ii with 10 bivalents; **3**, *E. palustris* population ic with eight bivalents; **4**, *E. palustris* population ia with eight bivalents + 1B; **5**, *E. palustris* population ib with eight bivalents + 1B. **6–8**, Pollen cells. **6**, *E. atropurpurea* population ii with ten mitotic chromosomes; **7**, *E. palustris* population ia with 8+1B; **8**, *E. palustris* population ic with eight chromosomes. Chromosomes are marked by arrows.

Table 1. Data on karyotypes of *Eleocharis* R. Br of Cyperaceae from Punjab plains

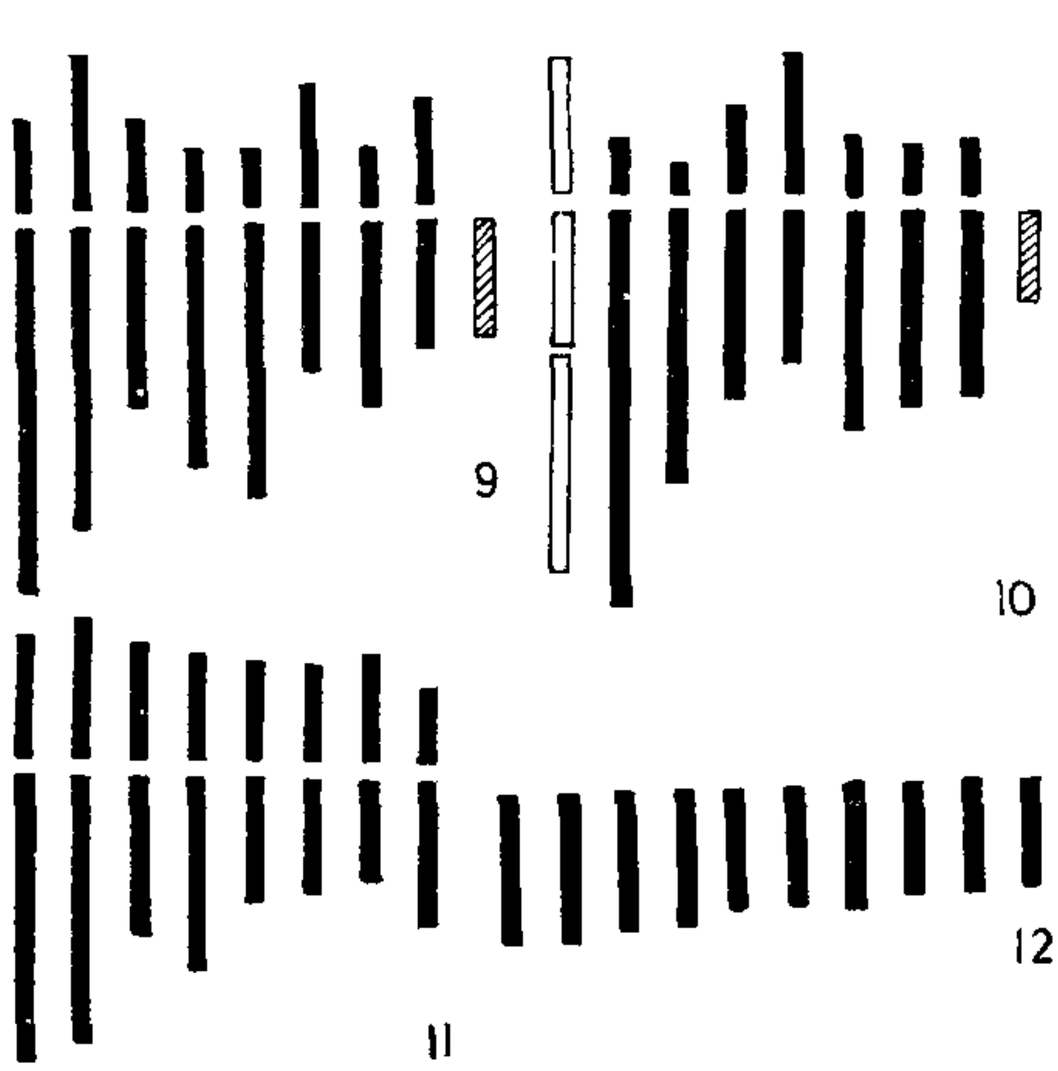
Species	Chromosome number*	Figure number	Genetic constitution	Karyotype formulae†	Karyotypic analysis†† on size basis	chromosome size range (μm)	Total chromatin length (μm)	Average chromosome size (μm)	Symmetry index (SI)	Gradient index (GI)	Stebbins's categorization (1958)
E. atropurpurea (Retz.) Kunth											
Population i	$n=10$	1	2x								
Population ii	$n=10$	2									
	'n'$=10$	6, 12	2x	P_{10}	L_{10}	1.28–1.82	15.35	1.53	—	70.33	—
E. palustris (L.) R. Br.											
Population ia**	$n=8+1B$	4									
	'n'$=8+1B$	7, 10	2x	L_6+J_2	$F_1+J_5+G_1+K_1$	2.91–5.47	35.43	3.93	41.57	53.19	2A
Population ib**	$n=8+1B$	5									
	'n'$=8+1B$	9	2x	$L_2+J_3+J_1^{s,c}$	$J_2+G_1+G_1I+K_4$	2.91–5.29	31.41	3.79	37.30	55.00	3A
Population ic	$n=8$	3									
	'n'$=8$	8, 11	2x	V_1+L_7	$I_1+F_2+J_5$	2.91–4.74	36.94	3.36	59.34	59.34	1A

*Somatic chromosome numbers studied from mitosis in pollen are denoted by 'n' and meiotic chromosomes studied from meiosis in PMCs are shown by n.

**Different 'karyotypic units' within the same population. Accessary chromosomes are expressed by 'B' and their length is counted only towards total haploid chromatin length of the complement and excluded for other parameters.

†V, L, J, I chromosome are categorized following White[14] whereas 'P' stands for chromosomes with apparently diffuse centromeres and hence no arms concept. Secondary constrictions are denoted by 's.c.'

†† In order to get an overall picture of evolution of karyotypes in sedge species[1,14] twelve basic types on size basis have been recognized as under: Long; A, B, C, D (double or more than double the length of shortest chromosome within total species of a genus worked out here); Medium; E, F, G, H (from one and a half times to double the length of the shortest chromosome); Short: I, J, K, L (less than one and a half times of the shortest chromosome). The position of centromere in relation to chromosome is A, E, I,: metacentric; B, F, J: submetacentric; C, G, K: acrocentric; D, H, L: telocentric in complements with normal chromosomes with localized centromeres but polycentric in complements having chromosomes with apparently diffuse centromeres. The symbol 'I' relates to chromosomes with secondary constriction on long arms.



Figures 9–12. Idiograms. **9**, *E. palustris* population ib with 8+1B; **10**, *E. palustris* population ia with 8+1B; **11**, *E. palustris* population ic with eight chromosomes; **12**, *E. atropurpurea* population ii with 10 chromosomes. B-chromosomes are shaded differently.

On the basis of relative analysis of karyotypic data, the following points emerge:

1. The two species differ in nature of centromere, i.e. *E. atropurpurea* possesses polycentric chromosomes while *E. palustris* has localized centromeres.
2. *E. atropurpurea* has short chromosomes ranging between 1.28 and 1.82 μm while *E. palustris* has short to medium chromosomes ranging between 2.91 and 5.47 μm.
3. Karyotypes of different pollen in *E. palustris* are also different for almost all the parameters (cf. Table 1).
4. Presence of B-chromosome results in the increase of total haploid chromatin length of complements in population ia and ib categories of *E. palustris*.
5. On the basis of gradient index value the karyotypes of *E. atropurpurea* are highly symmetrical..
6. In the case of ia and ib pollen categories of *E. palustris* the karyotypes are slightly symmetrical but for ic pollen category these are moderately symmetrical.
7. Based on symmetry index, the ia category of *E. palustris* is with slightly asymmetrical, ib with moderately asymmetrical and ic with slightly symmetrical karyotypes.
8. Stebbins' categorization[8] indicates that all the *E. palustris* populations, i.e. ia, ib and ic have highly symmetrical karyotypes falling under 1A, 2A and 3A categories respectively. It may be mentioned here that for *E. atropurpurea* this categorization is not possible because the chromosomes are polycentric.

As far as the incidence of B-chromosomes in sedges is concerned, the earlier data indicate +1B in *Carex bootiana*[9], +2B's in *Eriophorum schenchzeri*[10] and +1−3B's in *Scirpus pauciflora*[11]. Here, it will be interesting to note that in *E. palustris* so far only 1B is

noticed by us but from the same anther, pollen with no B's are also seen. The present note is the second report from India about the presence of B's in sedges. We have not noticed the presence of SAT-chromosomes in *E. atropurpurea* and *E. palustris* although their presence was reported earlier[12]. No secondary constrictions on metacentric chromosomes were seen by us for *E. palustris* but earlier studies show their presence[13].

The above observations clearly indicate the high amount of genetic diversity of sedges within species concept, indicating that the evolution is operative at micro level. As far as sedges are concerned, karyotypic variations are not only conserved but are also multiplied because of vegetative reproduction—a common feature of this group of plants.

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Gamma amylase activity—An alternate pathway of carbohydrate metabolism in animals

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An attempt has been made to establish the gamma amylase activity as an alternative pathway of glycogenolysis in starved condition of animal taking *Heteropneustes fossilis* as model. The enzyme activity was estimated in the liver, kidney, brain and muscle of diet supplemented (control), starved and refed fish groups. The maximum enzyme activity was noted at pH 4.8 and measured in the kidney followed by liver and brain in the control. But the highest activity of this enzyme was recorded in the liver of starvation-stressed fish. The reduction of the enzyme activity was noted in the tissues of refed group of fish suggests that the gamma amylase is responsible for the breakdown of glycogen to glucose other than phosphorylase pathway to meet the energy demand under certain stress.

THE gamma amylase activity other than phosphorylase[1] in mammalian and amphibian liver in diseased condition[2–5] have been reported to some extent. The gamma amylase can directly liberate glucose from glycogen by splitting both alpha 1, 4 and alpha 1, 6 linkages along with gamma dextrin[4] and appears as an important enzyme in the carbohydrate metabolism under certain stress. However, no reports are available on the gamma amylase activity in the starvation stress condition as it is in many ways similar to the diabetic condition[7]. We therefore studied the gamma amylase activity in different tissues under starvation stress as an alternate pathway of carbohydrate metabolism taking *Heteropneustes fossilis* as model.

The fish *H. fossils* was acclimatized in the laboratory and sorted into three groups. Group I, regularly fed on minced goat liver, earthworm and artificial diet, continued upto the 20th day was termed as control. Group II, kept without food upto the 20th day was termed as starved and group III, comprising of fishes starved upto the 10th day and thereafter supplemented with control diet, were named as refed group.

A batch of 6 fishes from each set were sacrificed and the liver, kidney, brain and muscle on the 5th, 10th, 15th and 20th days were taken out from the control and starved groups. Similarly, the aforesaid organs of the refed group were taken out on the 10th (just one hour of diet supplementation), 15th and 20th days. The enzyme gamma amylase was isolated[5] and the activity was determined following the procedure of Rosenfield[6]. The glucose[8] and the protein[9] were estimated for the calculation of the enzyme activity (EU) which is expressed as

$$EU = \frac{\text{mgm of glucose/100 ml}}{\text{mgm of protein/ml}}.$$

To evaluate the optimum pH of the medium (acetate buffer) for the maximum activity of the enzyme, a series of buffer solutions of pH 4.5, 4.6, 4.7, 4.8, 4.9, 5.0 and