

## TYROSINASE : A $\text{Fe}^{2+}$ CONTAINING ENZYME FROM TEA LEAVES

TYROSINASE of animal origin has been reported to be activated by  $\text{Fe}^{2+}$  with pteridine as co-factor<sup>1-3</sup>. *Fusarium vasinfectum* tyrosinase also requires  $\text{Fe}^{2+}$  for its activity<sup>4</sup>, while plant tyrosinases<sup>5-9</sup>, in general, are activated by  $\text{Cu}^{2+}$ . Tyrosinase from sugarcane<sup>9</sup> is strongly inhibited by  $\text{Fe}^{2+}$ .

In the present investigation, it is reported that tyrosinase from tea leaves is a  $\text{Fe}^{2+}$  containing enzyme.

Leaves of tea plant, *Camellia sinensis* L. (Küntz) collected from Dehra Dun, U.P., were homogenized in Waring blender containing sodium deoxycholate (0.04 M). The slurry was passed through two folds of muslin and made to 10% (w/v). The homogenate was raised to 30 to 70% saturation by stirring slowly with solid ammonium sulfate and centrifuged. The precipitate was suspended in water, dialysed against water and centrifuged at  $10,000 \times g$  for 30 minutes. The supernatant was tested for tyrosinase activity.

The determination of tyrosinase was based on the method of Alexander<sup>9</sup>. The assay system was comprised of L-tyrosine, 2.0 mg; enzyme preparation, 0.4 ml and acetate buffer (0.1 M, pH 5.5) to make up 1.0 ml. The tubes were incubated at 25° for 1 hour. The reaction was terminated by the addition of 1.0 ml of 10% TCA. In control tubes, L-tyrosine was added after deproteinization. The enzyme was assayed at 300 m $\mu$  by measuring O.D. increase. One unit of enzyme activity was defined as the amount leading to an increase of 1.0 O.D. under the assay conditions.

Proteins were estimated by a modified method of Khanna *et al.* for phenolic rich plant tissues<sup>10</sup>.

In 30-70% ammonium sulfate saturation, the activity of tyrosinase was recovered 70-80% and purity of enzyme was between 30-40 folds.

Dialysis of enzyme preparation against EDTA (1 mM, pH 7.0) brought about in almost complete loss of tyrosinase activity. Excess EDTA was removed by dialysis against water. This was further dialysed against  $\text{FeSO}_4$  solution (1 mM) which resulted in practically quantitative recovery of enzyme activity (Table I).

These results show that tyrosinase from tea leaves contains bound  $\text{Fe}^{2+}$ . This observation was further supported by dialysing enzyme preparation (3rd treatment of Table I) against 2, 2'-bipyridyl, a specific chelating agent for  $\text{Fe}^{2+}$ . This compound also resulted in complete loss of enzymic activity (Table I), confirming thereby that tyrosinase from tea leaves is a  $\text{Fe}^{2+}$  containing enzyme.

TABLE I

Effect of metal chelating agents and  $\text{Fe}^{2+}$  on tyrosinase activity from tea leaves

Treatment	Tyrosinase activity Units/mg protein
1. Before dialysis	30.0
2. Dialysis of enzyme preparation against: EDTA (1 mM, pH 7.0)	2.7
3. EDTA removal* followed by dialysis against: $\text{FeSO}_4$ (1 mM, pH 7.0)	28.9
4. Dialysis, of (3) against: 2, 2'-bipyridyl (1 mM, pH 7.0)	1.5

\* Excess of EDTA was removed by dialysis against water.

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Central Research Laboratory, D. V. SINGH.  
Antibiotics Plant, P. P. MUKHERJEE.  
Virbhadra, Rishikesh, U.P.,  
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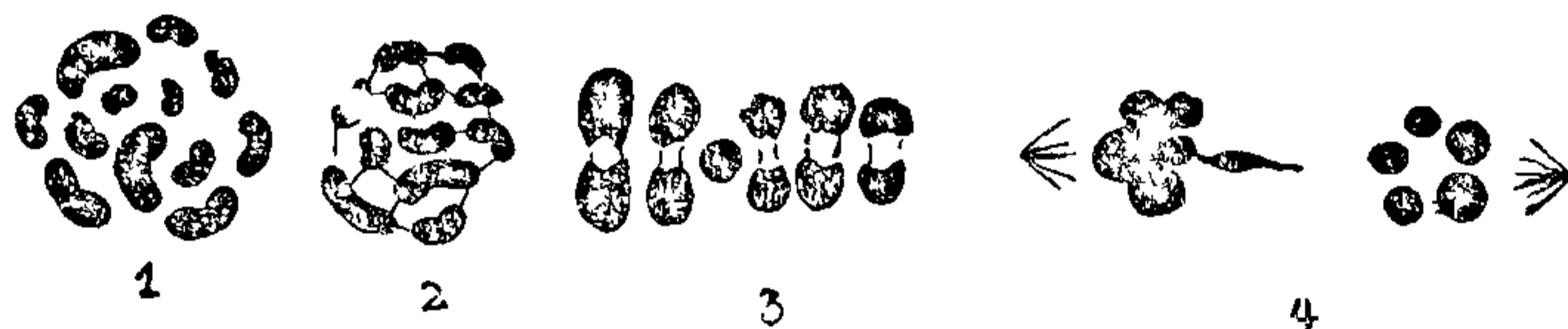
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## THE KARYOTYPE OF ZIZYPHOIDES PUNCTATUS, SP. NOV.

THE genus *Zizyphoides* (Jassinae, Homoptera) was known by only two species from India<sup>1,2</sup>. *Z. punctatus*, sp. nov., was collected by the author from local *Zizyphus jujuba* plants. The new species has been described by Rao<sup>3</sup>. This paper gives an account of the mitotic chromosomes of both the sexes and meiosis in detail in males of *Z. punctatus*, sp. nov.

Testes from adult males were fixed in acetic alcohol and sanfelice. Squashed and sectioned tissues were stained with iron-alum haematoxylin and feulgen. Oogonial chromosomes were studied from temporary acetocarmine preparations.

The oogonial metaphase complement (Fig. 1) consists of 12 bean-shaped chromosomes with diffuse centromere. Members of the complement take up stain in equal intensity. Therefore the identification of the sex chromosome becomes obscure. There is a pair of large chromosomes while the rest are gradually seriated. The spermatogonial metaphase complement (Fig. 2) has 11 chromosomes similar to oogonial chromosomes in shape, size and behaviour. A careful homology reveals that the female has 2 X chromosomes and the male only one. These are the smallest members of the complement.



FIGS. 1-4. *Z. punctatus*. (Camera Lucida drawings,  $\times$  ca 1,500). Fig. 1. Oogonial metaphase. Fig. 2. Spermatogonial metaphase. Fig. 3. Spermatocyte metaphase I. Fig. 4. Anaphase I.

The beginning of the primary spermatocyte prophase is characterised by a contraction phase where a somewhat prominent, deeply stained chromatin mass is present. The contraction stage is followed by a diffuse stage where the large heteropycnotic mass appears to be more prominent. The number of elements present in the prophase stage of the primary spermatocyte division could be followed from the diplotene stage. There are six elements of which 5 are autosomal bivalents and the remaining one—the univalent X chromosome. Each bivalent has one chiasma, terminal or interstitial. At diakinesis much contraction takes place. Terminalization of the chiasmata is complete by the time metaphase I is reached. 1st division metaphase is characterized by dumbbell-shaped bivalents and the univalent X chromosome (Fig. 3). Bivalents at metaphase I show co-orientation. The 1st division anaphase is reductional and the X chromosome moves to one of the daughter nuclei (Fig. 4). There are two types of second division metaphases depending on the presence or absence of the X chromosome. The second division anaphases are equational. The relative percentage volumes of the haploid set of spermatocyte metaphase I chromosomes are—autosome No. 1 ( $A_1$ ) 23.84,  $A_2$

18.46,  $A_3$  16.92,  $A_4$  16.15,  $A_5$  13.46 and that of X 11.15.

Cytological information of a species is being utilized, now to determine its systematic position. Karyological studies on Jassids have been done by various workers<sup>4-7</sup> aiming at (1) cyto-taxonomy and (2) trend of evolution in the group. The subfamily Jassinae is divided into 9 divisions. The genus *Zizyphoides* has been put in Mukariaria. The diploid number ( $2n = 11$  male, 12 female) encountered in *Z. punctatus* sp. nov. is low compared to other divisions. The behaviour of spermatocyte chromosomes, however, has close similarity with the related divisions. The presence of the orthodox sex chromosome mechanism (XX:XO), the diffused nature of centromere, typical orientation of chromosomes at mitotic metaphase and spermatocyte metaphase I and the prereducational meiosis in males hold the identification cytologically valid.

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Department of Zoology, A. K. BHATTACHARYA,  
University of Kalyani,  
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#### NEW RECORDS OF PENTATOMOIDEA (HEMIPTERA) FROM INDIA

THE author had an opportunity to study a collection of unidentified Pentatomoidea from India in the National Pusa Collection, Division of Entomology, I.A.R.I., New Delhi. Besides this the author himself had collected many pentatomid bugs from Himachal Pradesh and Delhi, and examined them