Current Status of Researches in Biosciences (2020) : 331-336
Editor : Prof. P.C. Joshi, N. Joshi, Reshman Yasmin, and D. K. Mansotra
Today & Tomorrow's Printers and Publishers, New Delhi - 110 002

MEIOTIC CHROMOSOMES OF GARDEN SLUG, *LAEVICAULIS ALTE* FROM PRACHI BELT OF RURAL ODISHA, INDIA

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ABSTRACT

Chromosomal studies in *Laevicaulis alte* was conducted in the laboratory on collection of garden slugs from Prachi River bed of rural Odisha. A standardized dose of colchicine was injected to the weighed species prior to dissection of ovotestis to obtain chromatin material. Standardized aceto-orcein squashing technique employed for the purpose recovered several meiotic stages of chromosomes with a diploid set determined as 2n=34. Haploid chromosome complement observed under high resolution microscope revealed the arrangement of 17 bivalents in different micro photographic plates of gonadal meiosis. Spermatogonial metaphase also confirmed the diploid number in the species consisting of metacentric & submetacentric configuration only as reported earlier

KEYWORDS: Chromosomes, Garden slug, Rural area, Prachi river, Gastropoda

INTRODUCTION

The tropical leatherleaf slug, *Laevicaulis alte* (Class: Gastropoda, Order: Systellommatophora, Family: Veronicellidae) is a serious agri-horticultural pest of India and neighboring countries (Raut & Mandal, 1984) where it is invasive (Herbert and Kilburn, 2004). The preliminary morphometric studies during laboratory observations of the eco-biology of the same species were first reported from coastal belt of Odisha (Das &

Parida, 2015). Chromosomal studies are an informative technique to establish phylogenetic relationship amongst species determining their course of evolution in the animal kingdom. The first ever account on the description of the karyotypic analysis of the slug *Laevicaulis alte* was reported from Philippines to confirm its taxonomic position within the family Veronicellidae (Cordova *et al.* 2010). The present investigation also aims at the chromosomal analysis of the exotic species to account for its taxonomic status as well as analyzing the impact of certain molluscicides in controlling the garden pests in a rural setup.

MATERIALS AND METHODS

The experimental animals were collected from the U.N. College garden located on the Prachi river bed of Odisha. Adult species of required length-weight were cultured in specialized rearing tanks constructed for the purpose as well as in laboratory terrarium properly maintained with an artificial environment. Each weighed specimen was injected with 0.02% colchicine (Fig.1) just 24 hours prior to removal of ovotestis meant for chromosomal studies. The dissected gonadal tissue was also undergone hypotonic treatment for 45 minutes using 0.075M KCl and later fixed in freshly prepared aceto-methanol mixture (1:3) with three changes in it. Now following the standardized aceto-orcein squashing technique (La Cour, 1941 and Stern,1975), temporary slides were prepared for examining under 2000x magnification of Microscan B-2 Universal Trinocular research grade microscope using high resolution digital camera with caliper pro software for photographic image analysis of meiotic stages in the species.

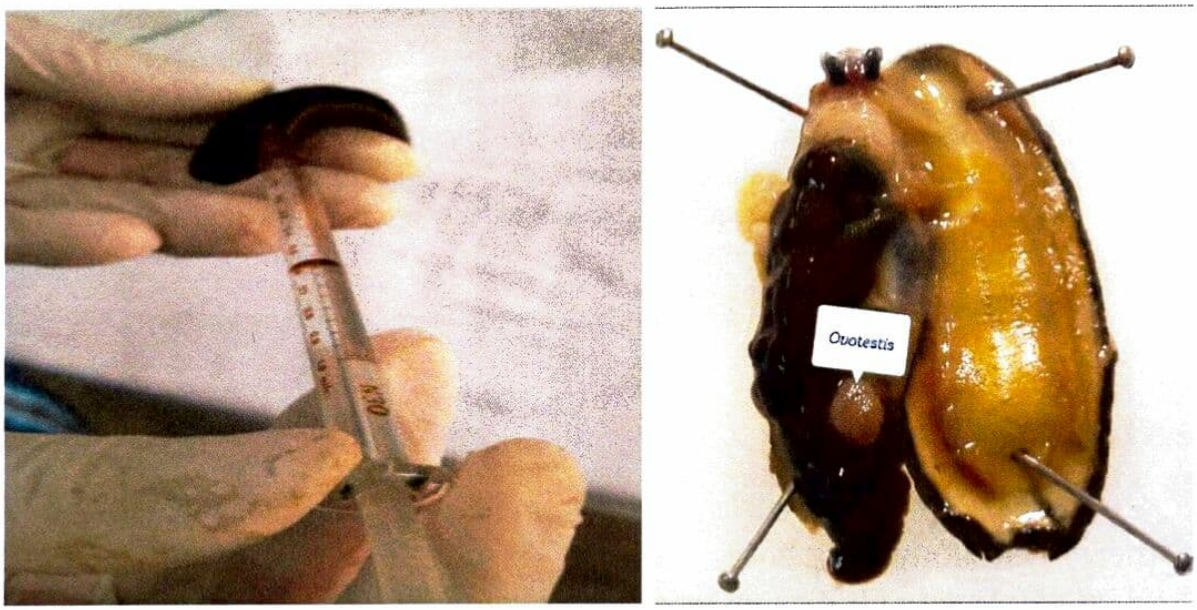

Fig.1. Colchicine pretreatment of *Laevicaulis alte* with ovotestis exposed for chromosome preparation

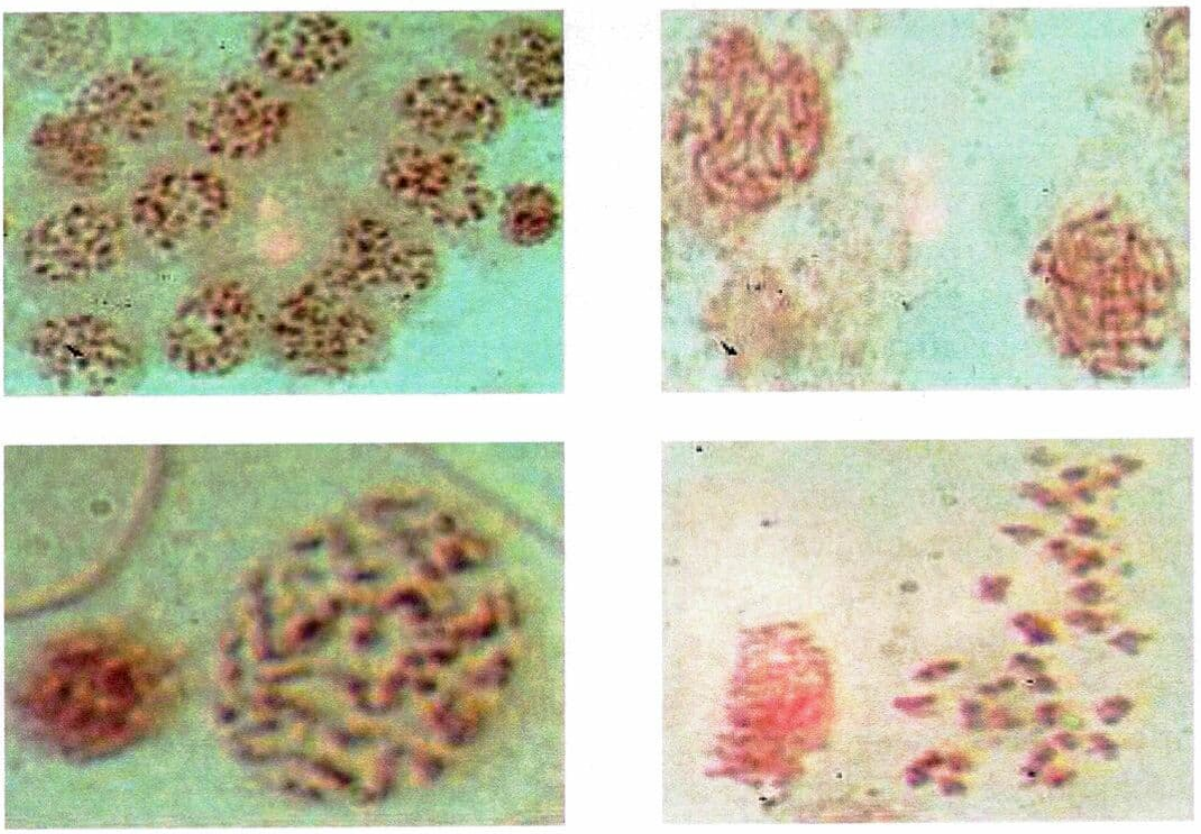

Fig.2. Photographic plates showing chromosomes of *Laevicaulis alte* in different stages of gonadal meiosis

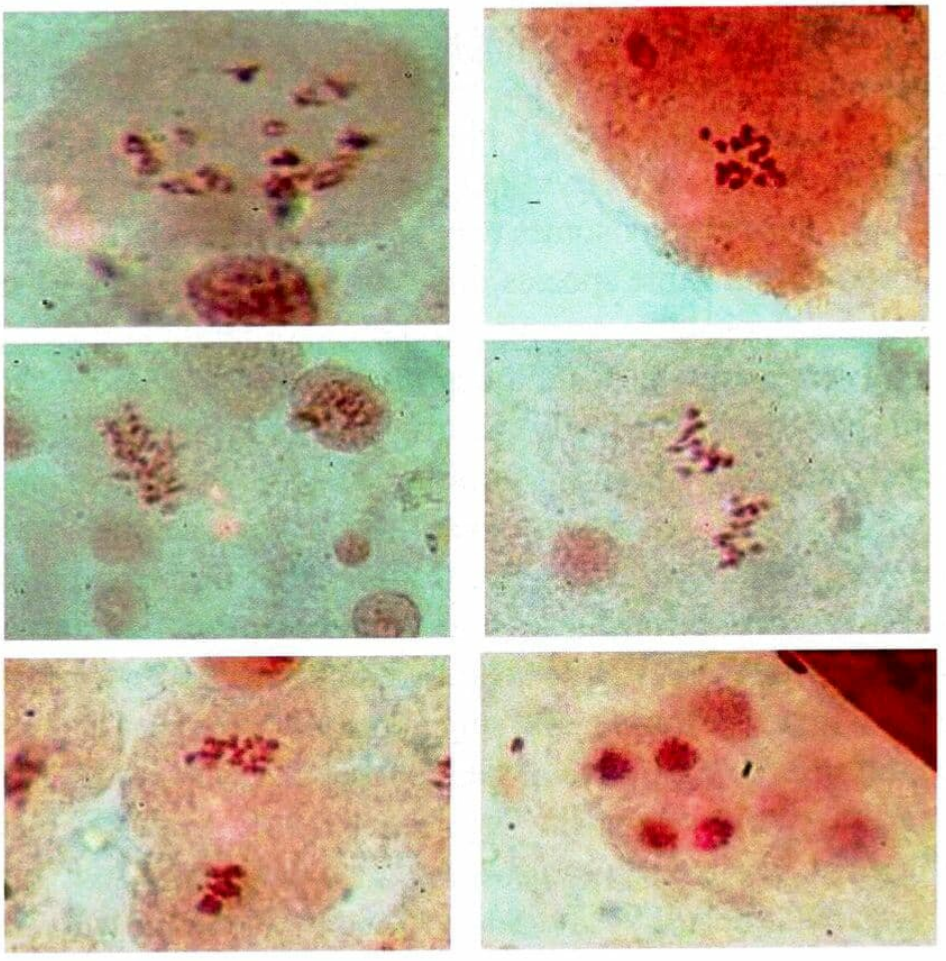

Fig.3. Photographic plates showing chromosomes of *Laevicaulis alte* in different stages of gonadal meiosis

OBSERVATIONS

The technique described above yields several stages of cells showing meiotic activity in gonadal tissue. The most frequent stages of meiotic chromosomes were observed as follows: Leptotene, Pachytene, Diakinesis, Metaphase-I, Anaphase-I, Anaphase-II, and Telophase-II (Fig.2). The chromosomes observed to form 17 bivalents in Diakinesis as well as in Metaphase-I confirming the diploid number to be 2n=34 as a characteristic feature of the species. Such bivalent configuration was supported by the presence of 17 thick and double structures with maximum condensation. The small unstained gaps present in the middle of each chromosome pair also proved their bivalent nature showing prominent chiasmatic regions. Spermatogonial metaphase plates were frequent in occurrence which revealed a diploid number of 34 small chromosomes exhibiting only metacentric and submetacentric configuration. Slug chromosomes at different meiotic stages exhibited a tendency of sticking together causing chromosomal overlapping as a result of which the cytogenetic study of gastropod of interest became more difficult.

DISCUSSION

Chromosome studies in gastropod molluscs have greatly increased since the reviews of Burch (1967), Burch & Natarajan (1967), Patterson (1969), Patterson & Burch (1978), Nagla *et al.* (1994), Nakamura (1986) and Thiriot-Quievreux (2002). As per the latest review the morphological features of gastropod chromosomes may be identical or very different within a family. Even within same species different karyotypes may be observed either due to polymorphism among different geographical populations or because differences in the techniques employed in order to obtain the chromosome preparations. Mostly Gastropod karyotypes include a majority of metacentric and submetacentric chromosomes following the nomenclature of Levan *et al.* (1964) with an exception in Veronicellid, *Sarasinula linguaeformis* (n=17) possessing equal number of different chromosome morphologies i.e.; four metacentric, four submetacentric, five subtelocentric and four telocentric (Majo A.D, 1996 and Majo A.D.et.al.1996). The haploid chromosome number in Veronicellid slug *Laevicaulis alte* (n=17) has already been reported previously by Natarajan (1960) and Majo A.D. (1996) but without any karyotypic detail. The first ever karyotypic analysis of Veronicellids like *Sarasinula plaebia* and *Laevicaulis alte* were reported with a haploid set to be n=17. The detailed karyotype of *Sarasinula plaebia* resembled to that of *Sarasinula linguaeformis* but with a minor difference in chromosome morphology as four metacentric, five submetacentric, four

acrocentric and four telocentric (Cordova *et al.* 2010). Another observation on meiotic chromosomes of Bean Slug, *Sarasinula plebeia* from Jammu & Kashmir also reported the haploid complement to be seventeen as specific in veronicellids but karyotypic differences from earlier consisting of three metacentric, six submetacentric, five subtelocentric and three telocentric configuration (Poonam *et al.* 2019). The present investigation on *Laevicaulis alte* also supported presence of 17 bivalents in metaphasic arrangements of gonadal meiosis. The first ever karyotypic formula in *Laevicaulis alte* has already been described as consisting of only four metacentric and thirteen submetacentric chromosomes in meiotic metaphase-II (Cordova *et al.* 2010). The present investigation emphasizing the impact of molluscicides on the karyotype of rural garden pest *Laevicaulis alte* is with a view to support the previous observation as similar considering the inclusion of the species under same family Veronicellidae with the haploid chromosome number falling within the established range as n=16-18 described in earlier chromosomal reviews.

ACKNOWLEDGEMENTS

The author is extremely thankful to the President GB and Principal of U.N. Autonomous College for provision of necessary infrastructural facilities required for research work with the financial support extended by UGC, New Delhi under the scheme of Minor Research Project. The author is highly indebted to the Director, Zoological Survey of India, Kolkata for taxonomic identification of the specimen.

REFERENCES

1. Alderete-D.M, A.M., Mercado, L. A.C. & Usandivaras, E. M. (1996). The chromosomes of *Sarasinula linguaeformis* (Semper, 1885) (Gastropoda, Veronicellidae). *Iberus*, 14: 155–160.

2. Alderete-De Majo, A.M. (1996). The Veronicellidae (Mollusca, Gastropoda) in the light of new techniques for the karyotype and gametogenesis analysis. *Iberus*, 14: 147–154.

3. Burch, J.B. and Natarajan, R. (1967). Chromosomes of some Opisthobranchiate mollusks from Eniwetok Atoll, Western Pacific. *Pacific Science*, Vol.XXI (2), 252-259.

4. Burch, J.B. (1967). Cytological relationships of some pacific gastropods. *Venus*: 25,118-135

5. Cordova K. A. T., Batomalaque G. A. and Fontanilla I. K. C. (2010). Karyotypic Analysis of Two Veronicellid Slugs, *Laevicaulis alte* (Ferussac, 1821) and *Sarasinula plebeia* (Fischer, 1868) Tropical Natural History, Supplement 3, 17th International Congress of Unitas Malacologica, World Congress of Malacology

6. Das,B. and Parida, L. (2015). Morphometric studies of the tropical leatherleaf slug *Laevicaulis alte* from prachi belt of Odisha. *Journal of Entomology and Zoology Studies* 3: 132-134

7. Herbert D and Kilburn D. (2004). Field guide to the land snails and slugs of eastern South Africa. Pietermaritzburg: *Natal Museum*; Pp 336.

8. La Cour. L, (1941). Aceto-orcein: a new stain-fixative for chromosomes. *Stain Tech.* Vol.16.169 -174

9. Levan A., Fredga K. and Sandberg A.A., (1964). Nomenclature for centromeric position on chromosomes. Hereditas, 52: 201-220.

10. Nagla, Z. El-Alfy, K.A.Al-Ali and A.H. Abdel-Rehim, (1994). Karyotype, meiosis and sperm formation in the land snail, *Macrochlamys indica*. *Qatar Univ. Sci. J.* 14(1),122-128

11. Nakamura, H.K. (1986). Chromosomes of Archaeogastropoda (Mollusca Prosobranchia) with some remarks on their cytotaxonomy and phylogeny. Publications of the Seto Marine Biological Laboratory, 31: 191–268.

12. Natarajan R., (1960). Further cytological studies in Pulmonata (Mollusca-Gastropoda). *J. Zool. Soc. India.*, 12: 69-79.

13. Patterson, C.M. and Burch, J.B. (1978). Chromosomes of pulmonate molluscs. In: Pulmonates: Systematics, evolution and ecology, 2A. (V. Fretter & J. Peake, eds), 171–217. Academic Press, New York.

14. Patterson, C.M. (1969). Chromosomes of molluscs. In: Proceedings of the 2nd Symposium of Mollusca, Ernakulam, Cochin, India, 2: 635–689. Marine Biological Association of India.

15. Poonam, Tripathi, N.K. and Kour, P. (2019). First report of meiotic chromosome number and Karyotype analysis of Bean slug, *Sarasinula plebeia* from Jammu & Kashmir. Proceedings of 106th Indian Science Congress, Jalandhar, p-369

16. Raut, S.K. and Mandal, R.N. (1984). Natural history of the garden slug *Laevicaulis alte*. *Journal of the Bengal Natural History Society*; 3: 104-105.

17. Stern, E.M. (1975). A technique for the preparation of gastropod chromosomes. *Veliger*. 17.296-298

18. Thiriot-Quiévreux, C. (2003). Advances in chromosomal studies of gastropod molluscs. *Journal of Molluscan studies*, 69: 187-201