# Occurrence of monocentric chromosomes in *Pieris brassicae* L. (Lepidoptera, Pieridae)

S. Rishi and K.K. Rishi[1]

*Department of Zoology, Kurukshetra University, Kurukshetra 132119 (India), 26 August 1977*

*Summary.* Monocentric chromosomes with localized kinetochores were obtained in *Pieris brassicae* (Lepidoptera, Pieridae) by using colchicine treatment and air-drying method.

The application of new cytological techniques has shown that it is possible to study the lepidopteran chromosomes with greater precision than was done earlier[2-4]. By making use of colchicine and air-drying method, we obtained in *Pieris brassicae* elongated chromosomes which could be arranged in karyotypes on the basis of their length[4]. Earlier, Bigger published 2 papers in which detailed karyotypes of a number of lepidopteran species were prepared on length measurement basis[2,3]. On account of unavoidable limitations, these reports escaped our notice. We, therefore, regret our claim of having first obtained karyotypable chromosomes in Lepidoptera[4].

Bigger[2] described changes in the holokinetic organisation of the lepidopteran chromosomes studied after using colchicine and air-drying method. He is of the opinion that the chromosomes of the early mitotic stages are monocentric, but those of the later ones become holocentric. Bigger could see clear primary constrictions in the elements obtained from the early spermatogonial cells. Although we also noticed the hooked appearance of some elements during our earlier study, we were not able to confirm any localised kinetochores in *P. brassicae*[4]. In the light of Biggers' findings, we thought it fit to reinvestigate our material. In the present report, we confirm having obtained chromosomes with localized kinetochores in the spermatogonial cells of prepupal larvae of *P. brassicae* (figures 1 and 2). The technique used has already been described[4]. The chromosomes in figure 1 show quite clearly the position of the primary constrictions, but the chromatids of each element are not separated probably due to a milder effect of colchicine. However, in figure 2, obtained from another specimen, even the chromatids are much separated, revealing the primary constrictions more clearly, though the elements are very much condensed. Very good diakinetic (figure 3) and metaphase I (figure 4) stages were also available in these preparations from a dozen individuals. On the basis of the position of the primary constriction, 2 metacentric, 6 sub-metacentric and 7 acrocentric pairs of chromosomes in the diploid set of the present species (figures 1 and 2) can be seen.

The discovery of chromosomes with localized kinetochores in Lepidoptera is a rather unique finding in the light of the earlier belief of the holokinetic nature of the lepidopteran chromosomes[4-8]. White[9] stated that higher numbers of chiasmata per bivalent do not seem to occur in animal groups with diffuse centromeres. But this is also true of animals having monocentric and small-sized chromosomes (e.g. fishes), and may hold for Lepidoptera also. Thus a low

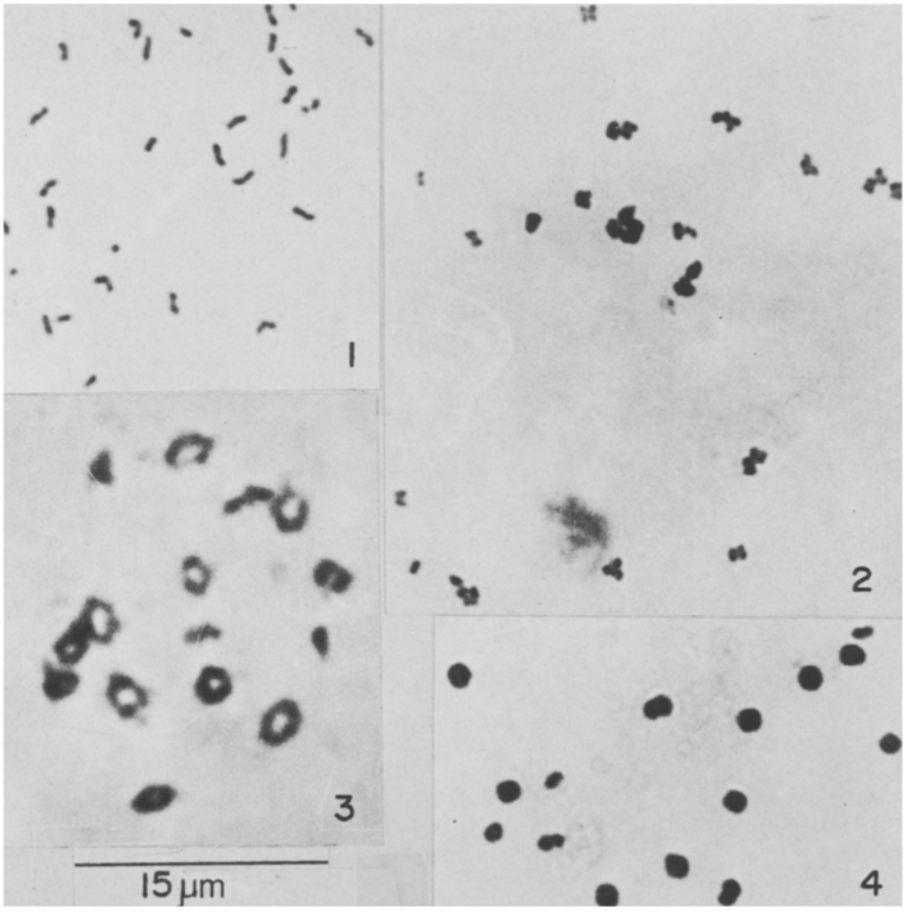


Fig. 1. Spermatogonial metaphase of *P. brassicae*.
Fig. 2. Another spermatogonial metaphase with more intense effect of colchicine.
Fig. 3. Diakinetic stage showing 1-2 chiasmata per bivalent.
Fig. 4. Metaphase I stage.

chiasma frequency (as can be seen in figure 3) does not necessarily indicate holocentric chromosomes, though the reverse may be true. On the other hand, the effect of ionizing radiations on the chromosomes of *P. brassicae* and other lepidopteran species has almost confirmed that the lepidopteran chromosomes are holokinetic[8]. Whether two types of centromeric organisations, as suggested by Bigger, exist in *P. brassicae,* was not clear. But the presence of monocentric organization, if detected in more lepidopteran forms, may invalidate our earlier ideas about the evolution of the karyotype in Lepidoptera. Suomalainen has already pointed out that in many groups of Lepidoptera the chromosome numbers are less variable than a diffuse centromere would theoretically allow. Thus, one would expect structural rather than merely numerical variation, as in the case of organisms having monocentric chromosomes. More work on recent lines on the lepidopteran forms is therefore well warranted.

1 We wish to express our thanks to Dr A.K. Datta-Gupta, Professor and Head of our Department for providing the research facilities and for encouragement.
2 T.R.L. Bigger, Cytologia *40,* 713 (1975).
3 T.R.L. Bigger, Cytologia *41,* 261 (1976).
4 S. Rishi and K.K. Rishi, Experientia *33,* 609 (1977).
5 E. Suomalainen, Hereditas *39,* 88 (1953).
6 E. Suomalainen, Chromosoma (Berl.) *16,* 166 (1965).
7 E. Suomalainen, Chromosomes today *2,* 131 (1969).
8 H. Bauer, Chromosoma (Berl.) *22,* 101 (1967).
9 M.J.D. White, in: Animal Cytology and Evolution, 2nd ed., p.231. Cambridge University Press, 1954.

## The chromosomes of *Bufo rubropunctatus* and *Bufo chilensis* (Anura, Bufonidae) and other species of the *spinolosus* group[1]

J. R. Formas[2,3]

*Instituto de Zoología, Universidad Austral de Chile, Casilla 567, Valdivia (Chile), 8 August 1977*

*Summary.* The chromosomes of *Bufo rubropunctatus* and *B. chilensis* (on basis of adults) are described for the first time. These chromosomic sets are compared with the karyotypes of *B. spinolosus* and *B. variegatus* and other species of *spinolosus* group. The importance of the secondary constrictions applied to the phylogeny of *Bufo* are discussed.

*Bufo rubropunctatus* and *Bufo chilensis* belong to the *Bufo spinolosus* group species which is restricted to the South-Western part of South America (from Ecuador to South Chile and Argentina) specially on the Andes[4]. Other species of this group are *B. atacamensis, B. limensis, B. trifolium* and *B. flavolineatus. B. variegatus* has been included in this group, however this possibility is discussed[5,6]. *Bufo rubropunctatus* is an uncommon toad of the humid and cool forests of Southern Chile which is characterized by its ventral pattern (white spots on a black belly). *Bufo chilensis* is a typical inhabitant of the Central Chilean arid steppe (*Acacia caven* association). Bogart[7] analyzed the chromosomes of several species of genus *Bufo.* He described, among others, the karyotypes of *B. spinolosus, B. variegatus* and *B. chilensis.* The chromosomes of this last species were studied on basis of hybrid tadpoles which were produced in combination of *B. chilensis* (from Zapallar, Central Chile) with 5 species of *Bufo* from North America, *B. viridis* from Eurasia and *B. arenarum* and *B. flavolineatus* from South America.

In this paper, the chromosomes of *B. rubropunctatus* are described for the first time, and new karyological information of *B. chilensis* is presented on basis of adult toads from 2 different populations. Also the karyotypes of *B. spinolosus* and *B. variegatus* are redescribed because our chromosomic results, specially on *B. spinolosus,* are not in agreement with Bogart's data[7]. All the karyological information obtained is here employed in the analysis of karyological relationships among the species of the *Bufo spinolosus* complex.

The karyological materials here studied were obtained from: *B. rubropunctatus,* 16 juveniles from Riñinahue (Valdivia province), *B. chilensis,* 5 males and 2 females from Los Angeles (Bio Bio province) and 3 males from Santiago (Santiago province), *B. spinolosus,* 3 males from Malargue (Mendoza, Argentina) and *B. variegatus,* 5 females from Antillanca (Osorno province). All specimens were deposited in the amphibian collection of Instituto de Zoología, Universidad Austral de Chile, Valdivia (IZUA). Methodology and nomenclature are used as described in previous paper[8]. Secondary constructions were named according to Bogart[7]. The karyotypes for each species are shown in the figure and the results of the chromosomes measurements are included in the table.

According to our results, only the karyotype of *B. variegatus* is more in agreement with Bogart's data; however, minute differences were observed. In relation to *B. spinolosus* and *B. chilensis* karyotypes, important karyological differences were found. The karyotype of *B. spinolosus* has been described with 2 secondary constrictions on pair 3[7]; however in 32 mitotic plates of this species we have found only 1 secondary constriction on pair 10. In *B. chilensis* karyotype constrictions E (pair 2), F (pair 3), K (pair 7) and L (pair 11) were seen by Bogart[7]. The specimens of 2 different populations (Santiago and Los Angeles) here examined have only 1 secondary constriction on pair 7 (K). 4 possibilities are advanced to explain the remarkable differences here found.

1. A sibling species could exist into the morphological complex of *B. chilensis,* this cryptic species could be located in Zapallar (Coastal Range, Central Chile) where Bogart's toads were collected.
2. *B. chilensis* from Zapallar population could show a chromosomic difference in relation to the other populations of this species.
3. Another possibility might be that the karyotypes of the tadpoles are different to those of the adults, Beçak[9] demonstrated this fact in the South American tadpoles of *Odontophrynus americanus,* which have 2 more secondary constrictions than the adults; these have only one.
4. The last possibility might be that the hibrid condition of the tadpoles could involve a modification in number of secondary constriction.

For many years, *B. chilensis* was considered as a subspecies of *Bufo spinolosus (B. s. chilensis)*[10,11]. From the serological point of view, Cei[12] proved that *B. chilensis* is a full species,