A comparison between polytene chromosomes of two sibling species of *Drosophila*: *D. ananassae* and *D. pallidosa*.

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Abstract

Polytene chromosomes which occur in certain tissues of dipteran larvae have great utility in cytogenetic studies in *Drosophila.* A preliminary study on comparison between polytene chromosomes of two sibling species of *Drosophila*, *D. ananassae* and *D. pallidosa*, have revealed that they look similar. Interestingly, no asynapsis was observed in the polytene chromosomes of F_1 hybrids indicating that there is normal pairing between homologous chromosomes and homology between the banding patterns of the two species. However, the presence of heterozygous loops in F_1 hybrids in certain regions of autosomes demonstrates that these two sibling species differ in the order of gene arrangements. *D. ananassae* and *D. pallidosa* are sibling species. *D. pallidosa* is endemic to South Pacific regions where it coexists with *D. ananassae*, a cosmopolitan species, which is reproductively isolated by behavioral isolation. Presence of heterozygous loops in F_1 suggests that change in the order of genes in certain regions of chromosomes might have played role in speciation.

Introduction

Polytene chromosomes, which were discovered by Balbiani in *Chironomus*, have been extensively utilized for various kinds of studies in *Drosophila*, particularly preparation of chromosome maps, detection of chromosomal aberrations, population genetics of inversions, gene activity, deletion and cytological mapping, chromosome replication, molecular biology, mapping of any DNA segment to specific chromosome loci, and so forth (Zhimulev, 1996). Further, polytene chromosomes have also been used in hybridization studies to understand speciation genetics and divergence (Dobzhansky and Tan, 1936; Bock, 1971; Naveira and Fontdevila, 1986). In hybrids between *D. pseudoobscura* and *D. miranda*, pairing of homologous arms was found to be highly variable and generally poor. Gene arrangements were found to be very different in these two species, indicating phylogenetic divergence between them (Dobzhansky and Tan, 1936). Bock (1971) studied the polytene chromosomes of all the four species of the *Drosophila bipectinata* complex and their hybrids. All the species have similar polytene chromosome complements. In the interspecific hybrids, there was excellent synapsis of homologous chromosomes when *D. bipectinata*, *D. parabipectinata*, and *D. malerkotliana* were hybridized suggesting their close phylogenetic relationship. Heterozygous inversions were also observed in the species and their hybrids. Synapsis between homologous chromosomes in hybrids between *D. pseudoananassae* and each of the remaining three species of the complex is weak, which provides evidence that *D. pseudoananassae* may be remotely related with the other three species (Bock, 1971). This has also been confirmed by the studies on genetic interactions underlying hybrid male sterility in this complex (Mishra and Singh, 2006). Naveira and Fontdevila (1986) observed strong asynapsis in all the chromosomes of hybrids between two sibling species of *Drosophila*: *D. buzzatii* and *D. serido*.

D. ananassae Doleschall (1858) and *D. pallidosa* Bock and Wheeler (1972) are sibling species. *D. ananassae* is a cosmopolitan and domestic species whereas its sibling *D. pallidosa* is endemic to the Islands of the South Pacific Oceans. In spite of their sympatric distribution, these two species are reproductively isolated by strong sexual isolation, although post mating barriers such as hybrid inviabilty and sterility are absent (Futch, 1973; Doi *et al*., 2001; Vishalakshi and Singh, 2006). Further, the interspecific hybrids of *D. ananassae* and *D. pallidosa* are developmentally as stable as their parents (Vishalakshi and Singh, 2009).

Chromosomal polymorphism has been extensively studied in *D. ananassae* (Singh, 2010) following the chromosome map constructed by Ray-Chaudhuri and Jha (1966). Futch (1966) detected chromosomal heterozygous inversions in sympatric light and dark forms of *D. ananassae* and their hybrids. During the present investigation, we have compared the polytene chromosomes of *D. ananassae* and *D. pallidosa* and also analyzed the chromosomes in their hybrids to know pairing between homologous chromosomes and the presence of heterozygous loops. The results of these investigations are reported in this communication.

Materials and Methods

During the present study, the following stocks of these two species were used:

(i) *D. ananassae* GL 10: it is a mass culture stock established from flies collected from Gwalior, M.P in 2010.

(ii) *D. pallidosa* NAN 57: Stock has been provided by Prof. M. Matsuda, Kyorin University, Tokyo, Japan (origin: Fiji, isofemale line).

These stocks were maintained on simple yeast-agar medium by transferring 15 pairs of flies to fresh culture bottles in every generation in the *Drosophila* culture laboratory maintained at approximately 24^{o}C. For getting interspecific hybrids, two species were hybridized by making reciprocal crosses.

Temporary squash preparations of salivary glands were made from third instar larvae of *D. ananassae*, *D. pallidosa*, and their hybrids using lacto-aceto-orcein stain. The slides were observed under the microscope, at different magnifications.

Results and Discussion

Both the species have the same chromosome karyotype and six arms in polytene chromosomes with identical tip morphology (XL, XR, 2L, 2R, 3L, and 3R; Figures 1, I and II) radiating from common chromocentre. In *D. ananassae*, two inversions, *i.e*., alpha (AL) in 2L (Figure 1, III) and delta (DE) in 3L (Figure 1, IV) were found to be present in heterozygous condition. Thirty larvae were analyzed, out of which 23 were found to have homozygous arrangement in 2L and 7 were found to be alpha heterozygotes. Sixteen larvae were found to have homozygous arrangement in 3L, and 14 were delta heterozygotes. The mean number of heterozygous inversions per individual was found to be 0.70. In *D. pallidosa*, one median inversion was detected in 2R (Figure 1, V). Out of 30 larvae examined, 26 were found to be inversion heterozygotes. Interestingly, no asynapsis was observed in the polytene chromosomes of F_1 hybrids indicating that there is normal pairing between homologous chromosomes and there is homology between the banding patterns of the two species (Figure 1, VI).

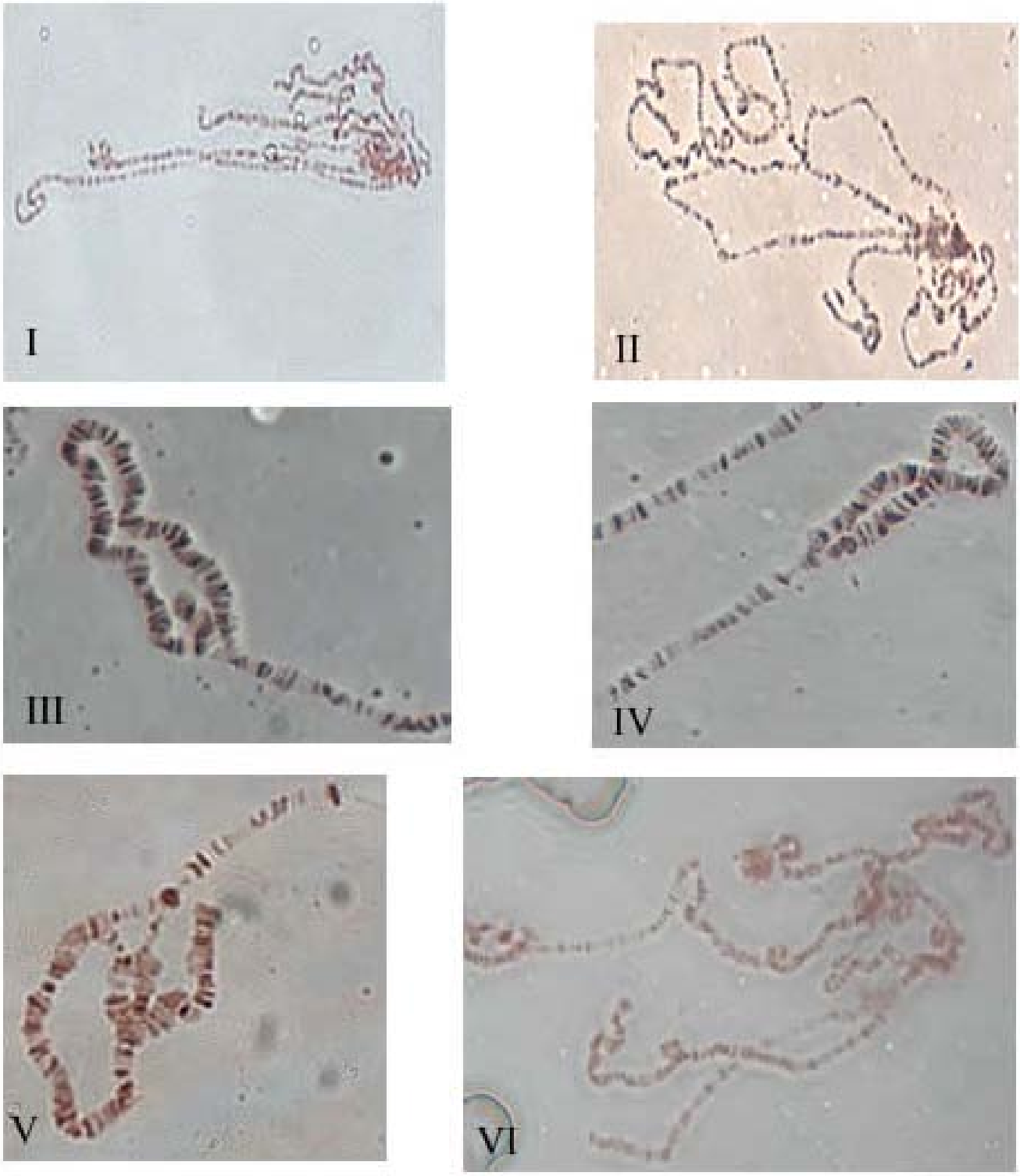

Figure 1, Part 1.

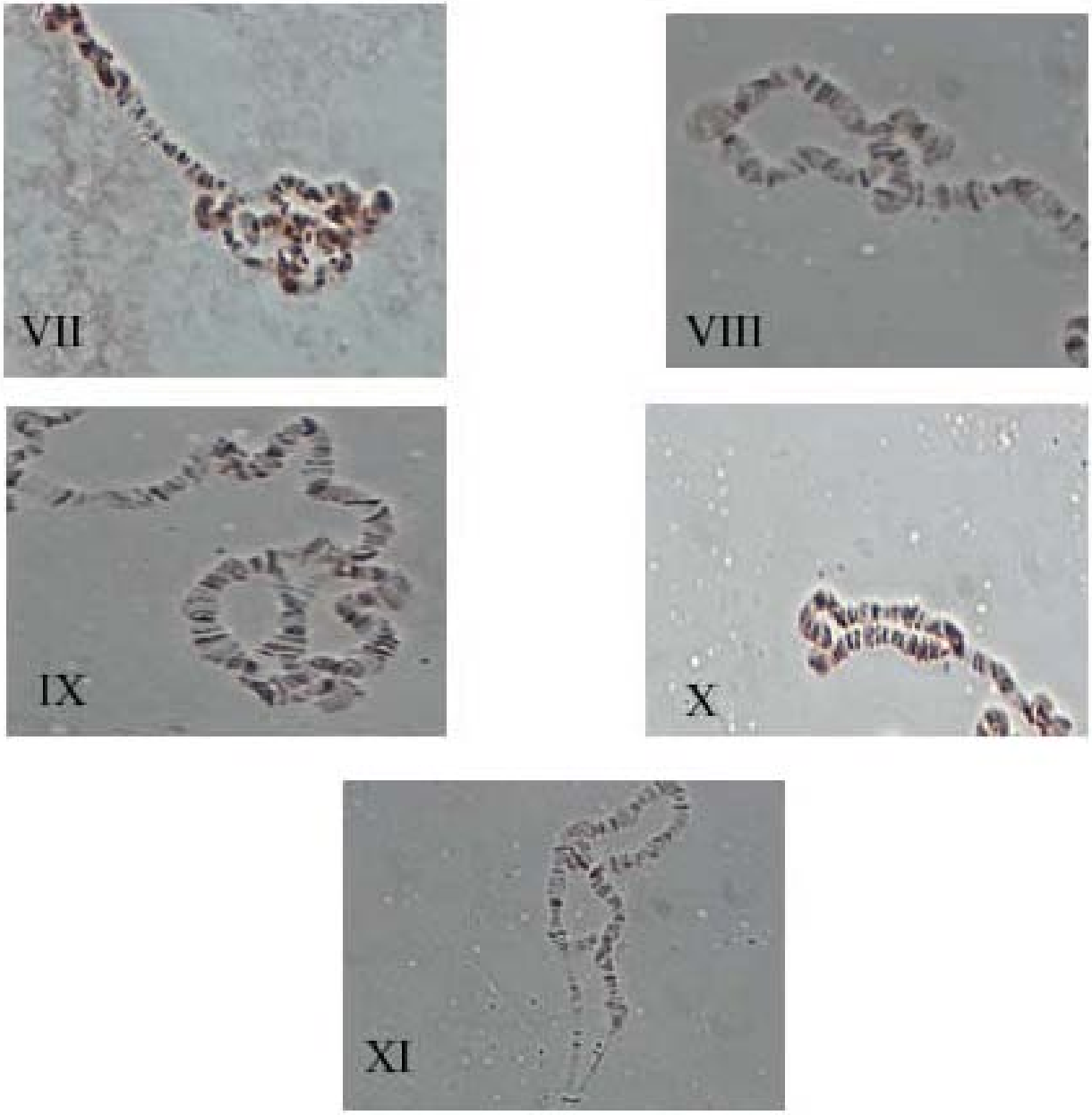

Figure 1. Microphotographs of polytene chromosomes of *Drosophila ananassae, D. pallidosa,* and their hybrids I, *D. ananassae* (25X); II, *D. pallidosa* (25X); III, AL (2L) inversion in *D. ananassae* (100X); IV, DE (3L) inversion in *D. ananassae* (100X); V, A median inversion in 2R in *D. pallidosa* (100X); VI, Hybrid polytene chromosomes showing no asynapsis (25X); VII, Complex configuration in 2L in hybrids (100X); VIII, A subterminal inversion in 2R in hybrids (100X); IX, Complex configuration in 2R in hybrids (100X); X, DE like loop in 3L in hybrids(100X); and XI, Complex configuration in 3R in hybrids (100X).

However, a number of paracentric inversion heterozygous loops were found in the autosomes of F1 hybrids. In 2L, complex configuration in sub-terminal region was detected (Figure 1, VII). In 2R two kinds of loops were detected, one was a simple sub- terminal loop (Figure 1, VIII) and the other a complex one (Figure 1, IX). In 3L, a loop similar to delta heterozygous loop could be detected (Figure 1, X). In 3R, a large sub-terminal complex configuration was found (Figure 1, XI). In XL and XR, no heterozygous loops were detected.

Based on these observations, it is concluded that: (i) these two sibling species, which belong to the *ananassae* subgroup of the *melanogaster* species group, have the same chromosome number, same number of arms in polytene chromosomes, homology and normal pairing between homologous chromosomes as there is lack of asynapsis in chromosomes of interspecific hybrids, (ii) *D. ananassae* stock has two heterozygous paracentric inversions (AL and DE) and *D. pallidosa* has one paracentric heterozygous inversion (a median inversion in 2R), (iii) Presence of heterozygous inversions and complex configurations in interspecific hybrids indicate that that these two sibling species differ in the order of gene arrangements in certain regions of autosomes.

These two sibling species show strong sexual isolation, which prevents gene flow between them in sympatric situations although post mating barriers such as hybrid inviabilty and sterility are absent (Futch, 1973; Doi *et al*., 2001; Vishalakshi and Singh 2006). Sawamura *et al.* (2008) have demonstrated that genes causing sexual isolation between the two species may be clustered in certain regions of chromosomes rather than being distributed randomly, and the presence of heterozygous loops and complex configurations in interspecific hybrids validate this. This indicates that certain segments in these regions differ in order of gene arrangements between the two species which might have played crucial role in speciation. Reversion in order has perhaps affected regulation. Indeed as it is known that it is not so much the divergence in sequences of genes as alterations in their regulation that can be attributed to speciation (De and Babu, 2010).

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