être mis en évidence dans le cas de *ag*. C'est également pour cette raison que l'atrophie gonadique ne peut être directement reliée au phénomène de stérilité SF[11] ou à celui de la distorsion de ségrégation due à l'élément extrachromosomique delta[12], bien que l'on observe aussi dans ces cas des variations de l'expression du caractère liées à l'âge des mères ou à l'élévation de la température.
Dans l'état actuel du problème, il paraît donc préférable de rapprocher le phénomène observé pour l'atrophie gonadique d'une distorsion de ségrégation sans pouvoir préciser s'il s'agit d'un processus de méiose anormale[13], d'une différence de viabilité gamétique ou d'une utilisation différente de certains gamètes[14]. Cette transmission différentielle apparaît cependant très importante pour l'évolution de caractère *ag* dans les populations naturelles et expérimentales.

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Chromosome variations in *Xenopsylla astia* Rothschild, 1911 (Siphonaptera). A preliminary report

Cheriyan Thomas and R.S. Prasad[1]

University of Kerala, Department of Zoology, Kariyavattom, Trivandrum 695581, Kerala (India), 3 April 1978

Summary. 2 populations of rat fleas, *Xenopsylla astia*, collected from (A) Bombay and (B) Trivandrum, were found to differ in chromosome number as follows; (A) $2n=10$ in both sexes; (B) $2n=18$ (male) and 20 (female). Preliminary observations have further shown that the 2 'populations' differ in certain morphological characters and that their reciprocal hybrids are sterile in the male and partially sterile in the female sex.

Rat fleas are important as vectors of human and animal diseases. Though there are many reports of studies on ecology, general biology and vector potentialities of rat fleas, there are only 5 short accounts of observations made on the chromosome cytology of fleas in general[2-6]. However, the only recent information available is restricted to observations made on spermatogenesis and oogenesis in 3 different species of rat and mouse fleas, viz., *Nosopsyllus fasciatus*, *Xenopsylla cheopis* and *Leptopsylla segnis*[7-8].

Materials and methods. The fleas used in the present study consisted of *Xenopsylla astia* collected from Bombay (2061 km north of Trivandrum) and Trivandrum. Colonies of these were set up in the laboratory before using them for experiments.

'Sexing' of the larvae was carried out at the 3rd stage before pupation. The testes are larger than the ovaries and clearly discernible through the cuticle as 2 ellipsoidal translucent bodies occupying the lateral sides of the 6th abdominal segment. Meiotic preparations were made by dissecting the late larval and pupal testes in physiological saline solution, allowing them to stand in hypotonic solution (0.17% NaCl) for about 7 min, fixing in acetic-alcohol (1:1) for 1 min and staining in 2% acetic-orcein for about 5 min before squashing. Egg squashes were made by dechorionating the fixed (1:1 acetic-alcohol) eggs, 5 min after laying, staining in lactic-acetic-orcein for 3 days and squashing in 45% acetic acid[8]. Mitotic metaphase chromosomes were prepared from imaginal limb buds of the inactive bent larvae (after puparium was formed) by acetic-orcein squash method. In order to ensure an accumulation of mitotic plates, 0.1 µl of 1% chilled colchicine solution was injected into the thoracic region of the larvae through the intersegmental membrane. The injected larvae were kept at 15 °C for 1 h and the imaginal limb bud tissues were dissected out in a drop of physiological saline, thoroughly washed in it, and allowed to stand for 10–15 min in hypotonic solution (0.9%) of sodium citrate to effect swelling of the cytoplasm. The tissues were fixed in acetic-alcohol (1:1) for 1 min, stained for about 5 min in 2% acetic-orcein and squashed. Permanent slides were prepared by passing the slides through alcohol-xylol series and mounting in euparal.

Results and discussion. Though the fleas from Bombay and Trivandrum were morphologically similar with regard to the generally used criteria for the identification of fleas (confirmed by Prof. Robert E. Lewis), such as shapes of spermatheca in female (figure 1, a and b) and IX sternite in male (figure 1, c and d), the former has $2n=10$ chromosomes in both sexes (figure 2, a and c), and the latter has $2n=18$ (male) and 20 (female) (figure 2, e and h). The chromosomes of fleas from the Bombay population, mostly metacentrics, are relatively larger in size than those of the

Fig. 1. Spermatheca of female, and aedeagus and sternite IX (arrows) of male *Xenopsylla astia*. *a*, *c* Bombay population; *b*, *d* Trivandrum population. × 60.

Fig. 2. Chromosome complements of the Bombay *(a–d)* and Trivandrum *(e–i)* populations of *Xenopsylla astia. a, e* Diploid complement of male fleas. *b, f* Meiotic metaphase I of testis. *g* Anaphase of 1st meiotic division from spermatocyte. *c, h* Diploid complement of female fleas. *d, i* Meiotic metaphase I of egg. Magnification × 1610, except in *g* (× 1070).

Trivandrum population, which are acrocentrics except for a few metacentrics or submetacentrics.

Male metaphase I plates of fleas from Bombay showed a quadrivalent (2 metacentrics and 2 acrocentrics terminally associated with each other) apart from 2 metacentric bivalents and an acrocentric heteromorphic pair (figure 2, b 1-4). The female metaphase I preparation exhibited 3 metacentric bivalents and 2 acrocentric bivalents (figure 2, d). The quadrivalent present in the male metaphase I (the chromosomes marked 1-4 in figure 2b) represents the sex chromosomes as could be judged from the absence of this association in the female metaphase I plates. This indicates a sex-determining mechanism based on $X_1X_2Y_1Y_2$ (male) and $X_1X_1X_2X_2$ (female). Further work is needed to delineate the X and the Y chromosomes of the quadrivalent.

Metaphase I plates of male fleas collected from Trivandrum showed 7 bivalents and a complex involving 4 chromosomes, one of which was metacentric, the Y chromosome (figure 2, f), as is evident from the meiotic and mitotic metaphase preparations of both sexes (figure 2, e and f; h and i). We conclude that the sex-determining mechanism in this case is an $X_1X_2X_3Y$ type. Accordingly the diploid chromosome complement would be $2n=18, X_1X_2X_3Y$ (male) and $20, X_1X_1X_2X_2X_3X_3$ (female).

The wide cytological variation between the fleas collected from the 2 localities necessitated a careful examination of the morphological characters of the adult fleas.

A detailed study of the IX sternite of 30 males and the abdominal tergites and sternites of 30 each of males and females belonging to the 2 'populations' showed considerable variations in the number and arrangement of setae. For example, the total number of setae on the tergite and sternite of segments III–VII are more in the Bombay population than in the Trivandrum population. A detailed paper discussing these aspects is under preparation.

Reciprocal crosses between the 2 'populations' showed 100% sterility in males and partial sterility in females in the hybrid progeny.

Intraspecific variation in chromosome numbers has been reported for a number of animals[9–15]. Extensive studies on the biology, morphology and cytology of certain insects have resulted in the separation of what was thought to be a single species into several sibling species. Such is the case for example in mosquitoes[16,17].

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