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CHROMOSOMES OF THE CARIBBEAN FRUIT FLY (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Larval tissues of *Anastrepha suspensa* (Loew) (Diptera: Tephritidae) were examined to determine the optimal tissue and stage for chromosomal preparations and to determine the karyotype. Tissues were dissected in saline, stained in 2% aceto-orcein for 45 minutes, and squashed on a coverglass by thumb pressure. The compound eye imaginal discs from 6-day-old larvae yielded the best preparations of dividing cells. Mitotic figures also can be obtained from larval brain tissue, ventral nerve cord, and leg imaginal discs. In larvae 6 days old, many cells in the tissues examined were dividing. Cell division appears to be synchronized in the different tissues examined, with most cells in interphase or dividing at the same time during all instars. The male is heterogametic (XY) and the female is homogametic (XX). The chromosome number is 12 (10 autosomes + XX or XY). There are 3 pairs of subtelocentric and 2 pairs of submetacentric chromosomes. The X chromosome is subtelocentric and the Y chromosome is submetacentric. The two X chromosomes tend not to pair like the other chromosomes, and in males the Y chromosome often sticks to the short arm of the X chromosome.

Key Words: *Anastrepha suspensa*, karyotype, imaginal discs, mitotic figures.

RESUMEN

Los tejidos de larvas de *Anastrepha suspensa* (Diptera: Tephritidae) fueron examinados para determinar el tejido y la etapa óptima para las preparaciónes de cromosomas. Los tejidos fueron disectados en una solución salina, teñidos en 2% de aceto-orceino por 45 minutos, y aplastados debajo un cubre objeto por la presión del dedo pulgar. Los discos imaginales del ojo compuesto de larvas de 6 dias de edad resultaron ser las mejores preparaciones para dividir las cellulas. Figuras mitóticos también puede ser obtenidas del tejido del celebro de las larvas, la cuerda ventral de los nervios, y los discos imaginales de las patas. En larvas de 6 dias de edad, muchas de las celulas de los tejidos examinados estuvieron dividiendose. La división de celulas aparece ser sincronizada en los diferentes tejidos examinados, con la mayoria de las celulas en interfase o dividiendose al mismo tiempo durante todos los estadios. El macho es heterogamético (XY) y la hembra es homogamética (XX). El número de cromosomas es 12 (10 autosomas + XX o XY). Hay 3 pares de cromosomas subtelocentricas y 2 pares de cromosomas submetacentricas. La cromosoma X es subtelocentrica y la cromosoma Y es submetacentrica. Las dos cromosomas X suelen no aparearse como las otras cromosomas, y en los machos la cromosoma Y a menudo se pega al brazo corto de la cromosoma X.

The Caribbean fruit fly, *Anastrepha suspensa* (Loew), is an important immigrant in Florida, and has a significant economical impact on citrus (Greany & Riherd, 1993). No data are available on the cytogenetics of *A. suspensa*, but there is limited cytogenetic information on other species in the genus *Anastrepha.* Mendes (1958) published chromosomal studies on *Anastrepha fraterculus* (Wied.). Bush (1962) described the karyotypes of nine species of *Anastrepha,* including *A. ludens* (Loew), *A. fraterculus* (Wied), *A. distincta* Greene, *A. mombinpraeoptans* Sein, *A. zuelaniae* Stone, *A. spatulata* Stone, *A. striata* Schiner, *A. serpentina* (Wied), and *A. aphelocentema* Stone. All of the *Anastrepha* species studied by Bush (1962) have 12 chromosomes except males of *A. serpentina*, which has 11. The Mediterranean fruit fly, *Ceratitis capitata* (Weid.) also has 12 chromosomes (Radu et al. 1975). Our objectives were to determine the optimum stages and tissues for display of chromosomes, and to determine the karyotype of *A. suspensa*. Here we report 12 chromosomes as the karyotype of *A. suspensa*, comprising 10 autosomes and a pair of sex chromosomes, and that imaginal disk tissues in larvae are suitable for examining dividing cells and mitotic figures.

MATERIALS AND METHODS

Eggs of *A. suspensa* were obtained from the mass rearing facility at the Florida Department of Agriculture and Consumer Services, Gainesville, Florida. Larvae were reared at 28 ± 1°C and 80% relative humidity (RH) until the 5th or 6th day, and then larvae were moved to a cooler room at 22 ± 1°C and 80% RH. Under these conditions, development from hatching to pupation takes 8-9 days. Various imaginal disc tissues and brain tissue from 1-day-old to 7-day-old larvae were examined to determine optimal stages and tissues showing cell division and mitotic figures. Tissues observed were brain imaginal disc tissue, ventral nerve cord, compound eye imaginal discs, and imaginal discs attached to the nerve cord that develop into the first two pairs of legs.

Larvae were dissected in saline solution (9 g NaCl, 0.42 g KCl, and 0.25 g $CaC1_2$ in 1 liter of water) and the tissue to be examined was cleaned as much as possible from unwanted tissue. Dissected tissues were transferred immediately to a saturated solution of coumarin, as suggested by Bush (1962), but the time of exposure was reduced to 3 minutes. Following coumarin treatment, tissues were rinsed for 30 seconds in 1N HC1. The tissues were stained in 2% aceto-orcein for 45 minutes, and squashed on a coverglass by thumb pressure.

Cell suspension technique also was used in order to get more expanded chromosomes. Freshly dissected tissues were fixed in methanol:acetic acid (3:1) for 30 min, and then treated with 60% acetic acid for about 30 sec. The suspension of tissue was pulled into an eye dropper pipet, and drops were allowed to fall from several centimeters height onto a clean slide. The suspension on the slide was dried at 40-50°C on a hot plate. The cells and chromosomes were stained for 10-15 minutes by flooding the slide with Giemsa stain in 0.1M Sörensen buffer, pH 6.8.

Suitable preparations were photographed with a Zeiss III RS microscope and oil immersion with a 100x objective lens and a 10x ocular lens. Images of chromosomes were cut from the best photographs for the construction of the karyotype. The nomenclature for chromosome morphology and the centromeric index is that of Levan et al. (1964). The relative length of chromosomes was calculated by expressing the length of each chromosome as a percent of the summed length of all chromosomes. The centromeric index and the relative length of chromosomes were calculated from the mean of 19 measured metaphase preparations. The pairs of chromosomes were identified from their relative length and morphology.

Results and Discussion

Data on observations of dividing cells from the various ages of larvae and in different tissues examined are shown in Table 1. Cell division in the different tissues appears to be occurring at the same time, for example, brain and ventral nerve cord cells divide at the same time during the 1st day (instar 1), 4th day (instar 2) and the 6th day (instar 3). Eye imaginal discs begin cell division and growth earlier than the leg imaginal discs, but cell division in the leg imaginal discs occurred in synchrony with the brain and nerve cord cells. During the 6th day, when larvae are in the late 3rd instar, all tissues tested were dividing and this is the best stage of development in which to find workable chromosomes. Bush (1962) also reported good chromosomal preparations from mature 3rd instars.

Only a few dividing cells could be found in brain tissue of 1-day-old larvae. Brain cells in 2-day-old larvae tended to be in interphase and dividing cells were difficult to find. Three-day-old larval cells showed mostly interphase nuclei and a few prophase plates. Some cell division was observed in 4-day-old larvae, and metaphasic cells were found. Almost all cell nuclei from 5-day-old larvae were in interphase again, with few in methaphase. Tissue from 6-day-old larvae showed many dividing cells, and this was the best larval age class to observe the chromosomes. Dividing cells still could be observed in 7-day-old larvae that had not begun pupation. In these older larvae, however, dividing cells were few in number, cell nuclei were small, the chromosomes often remained bunched, and structural details were difficult to observe. By the 7th day, most of the larvae were in the wandering stage, had crawled out of the food, and were seeking a dry pupation site. The observations of distinct times of cell division in the brain are consistent with data showing that the brain does not grow in a linear manner (Nation et al. 1995), but is described by a sigmoid growth curve from day 1 (hatching) through day 8 (prepupa).

Table 1. Cell division in different tissues of *Anastrepha suspensa* larvae.

	Instar						
	I		II		III		
	Larval age (days)						
Tissues	1	2	3	4	5	6	7
Brain	+	—	—	+	—	++	+
Nerve cord	+	—	—	+	—	++	—
Eye discs			—	+	—	++	+
Leg discs						+	+

+Metaphase nuclei present; the number of plus signs indicates the relative number of metaphase nuclei found.
—Nuclei in interphase; mitotic figures not present.

Nerve cord cells in 1-day-old larvae were dividing, and some cells were observed in metaphase. With 2-and 3-day-old larvae, cells were in interphase. Cells in prophase and metaphase were found in 4-day-old larvae. Cell division stopped in 5-day-old larvae, and interphasic nuclei were observed, but at 6 days, cell division was evident and many metaphasic plates were observed. Cells in metaphase were difficult to find in the ventral nerve cord of 7-day-old larvae, and most of the cells had returned to interphase (Table 1).

Interphase and prophase nuclei were observed in cells from the compound eye imaginal disc in 3-day-old larvae. In 4-day-old larvae, cells were actively dividing, but in 5-day-old larvae, most cells were in interphase again. At 6 days, many cells in the tissue were in division. By the 7th day, few cells were in metaphase and most were in interphase. The nuclei of cells in the compound eye imaginal

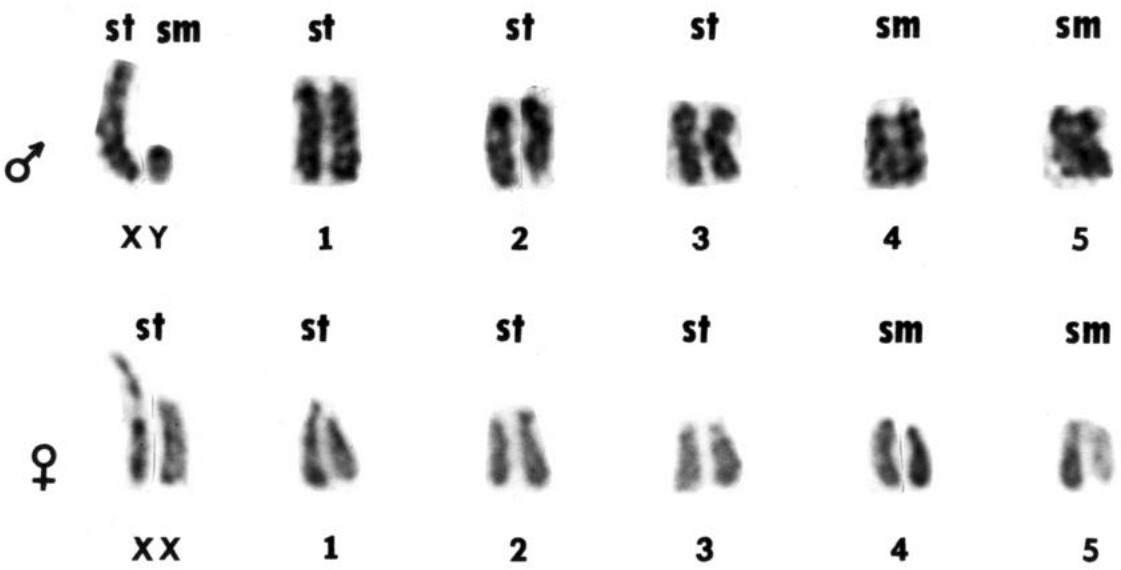

Fig. 1. Karyotype of *Anastrepha suspensa* (Loew); st, subtelocentric chromosome; sm, submetacentric chromosome.

discs are larger than nuclei of other tissues observed. The chromosomes from eye imaginal disc cells were expanded and nicely separated from each other (Table 1), and this is the best tissue for chromosome preparation in the Caribbean fruit fly larvae. In contrast, Radu et al. (1975) found that brain neuroblasts were the most favorable cells for chromosome studies in *Ceratitis capitata*.

Leg imaginal discs are small and poorly defined before the 5th day. In 5-day-old larvae, leg imaginal discs are growing in size, but still small and difficult to prepare for observation of cell division. Some metaphase plates were observed in leg discs from 6- and 7-day-old larvae.

The mitotic karyotype in both sexes of A. *suspensa* comprises 6 pairs of chromosomes (2n = 12), 5 autosomal pairs plus the pair of sex chromosomes (Fig. 1). As in most of the Diptera (White 1973), the male of *A. suspensa* is heterogametic (XY) and the female is homogametic (XX). Based on the centromeric index (Levan et al. 1964) the chromosomes of *A. suspensa* can be grouped into subtelocentric and submetacentric chromosomes. The largest pairs are subtelocentric (pairs 1, 2, 3 and XX), and the shortest pairs are submetacentric (pairs 4, 5, and Y) (Table 2). The relative length of the somatic chromosomes was about the same in all of the pairs (Table 2). One X chromo-

TABLE 2. CENTROMERIC INDEX AND RELATIVE LENGTH OF CHROMOSOMES OF *ANASTREPHA SUSPENSA* LARVAE.

Chromosome number	Centromeric Index* Mean ± SE	Relative length* Mean ± SE	Morphology*
1	25.9 ± 1.8	10.7 ± 0.6	Subtelocentric
2	25.8 ± 1.5	9.5 ± 0.3	Subtelocentric
3	25.7 ± 1.5	8.3 ± 0.3	Subtelocentric
4	23.3 ± 1.3	8.5 ± 0.3	Subtelocentric
5	22.6 ± 1.2	8.0 ± 0.2	Subtelocentric
6	23.8 ± 1.5	7.7 ± 0.1	Subtelocentric
7	27.1 ± 2.0	7.3 ± 0.1	Submetacentric
8	26.9 ± 1.9	7.4 ± 0.2	Submetacentric
9	26.4 ± 1.9	6.9 ± 0.1	Submetacentric
10	25.9 ± 1.7	6.8 ± 0.2	Submetacentric
X1	25.9 ± 2.1	11.3 ± 0.3	Subtelocentric
X2	22.1 ± 2.9	10.7 ± 0.9	Subtelocentric
Y	29.9 ± 3.8	3.5 ± 0.3	Submetacentric

*Calculations and morphology designation based upon 19 metaphase preparations. Relative length is calculated as the (average length of each chromosome divided by the summed length of all chromosomes) × 100. Morphology and centromeric index are based upon work of Levan et al. (1964).

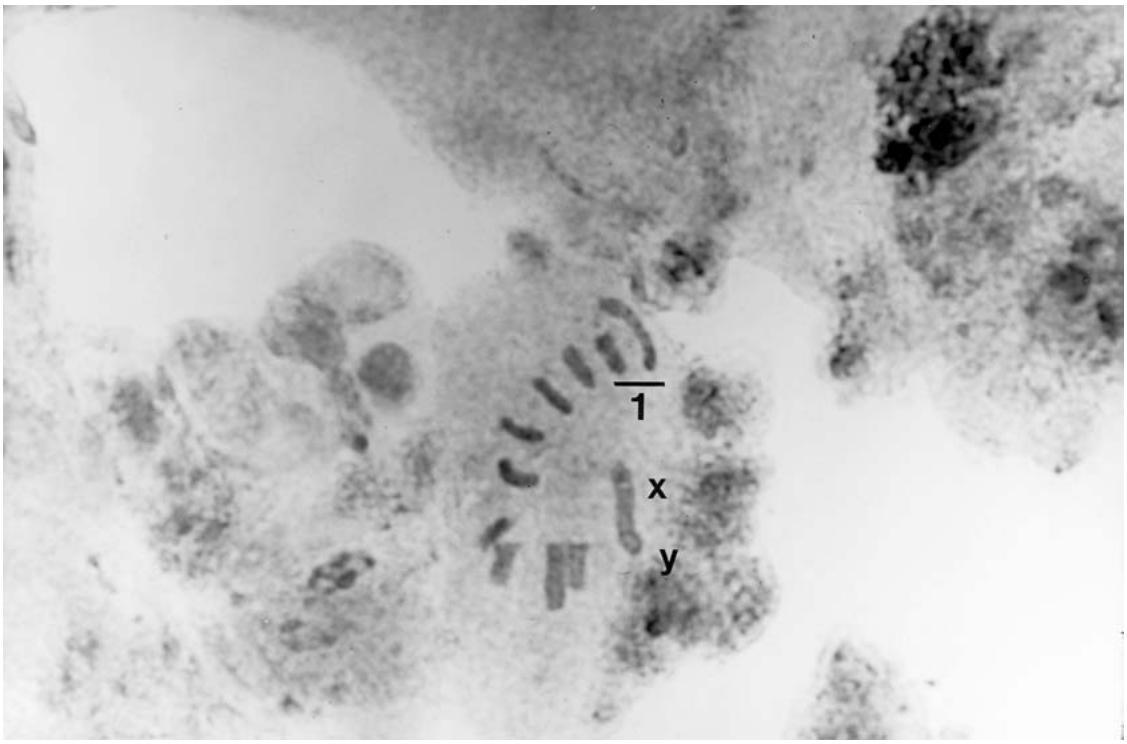

Fig. 2. Metaphase chromosomes from a male brain squash. The long, curved chromosome at the top is a member of pair 1, a pair that sometimes showed heteromorphology. The longer chromosome at the lower right shows the commonly observed short y chromosome associated with the longer x chromosome.

some was the longest, and the Y chromosome the shortest (Table 2). The somatic pairing reported in most dipterans (White 1973; Radu et al. 1975; Southern 1976) was also characteristic in *A. suspensa,* and homologous autosomes usually were associated as somatic bivalents. The Y chromosome was usually joined to the short arm of the X chromosome (Fig. 2). The sex chromosomes in females were often paired, but not joined end to end as the X and Y in the males. Another feature of the male karyotype was the heteromorphism sometimes observed in pairs 1 and 2, where one of the chromosomes appeared to be extended (Fig. 2).

Although all the larval tissues surveyed are suitable for studies of chromosomes, the best tissue for chromosome preparations was the compound eye imaginal discs because the size of the nuclei are larger than the nuclei from other tissues. The squash technique and the cell suspension procedure produced extended and separated chromosomes, but the squash technique was more useful because of the small size of the tissues. For multiple preparations, the squash technique was a relatively simple and faster procedure. Treating tissues with coumarin for 3 minutes helped to relax chromosomes, but prolonged exposure caused shrunken nuclei and very swollen chromosomes. The Caribbean fruit fly has 12 chromosomes, in agreement with 9 other species of *Anastrepha* (Bush 1962), and the Mediterranean fruit fly (Radu et al. 1975). Florida Agricultural Experiment Station Journal Series No. R-09467.

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