SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Karyotype Characterization of *Anastrepha* Fruit Flies (Diptera: Tephritidae)

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Caracterização de Cariótipos de Moscas-das-Frutas do Gênero *Anastrepha* (Diptera: Tephritidae)

RESUMO - O estudo apresenta a descrição dos cromossomos mitóticos, através do bandamento-C, de cinco espécies de *Anastrepha*, *A. amita* Zucchi, *A. turpiniae* Stone, *A. zenildae* Zucchi, *A. grandis* (Macquart) and *A. leptozona* Hendel e uma reanálise dos carótipos de três outras espécies, *A. distincta* Greene, *A. obliqua* (Macquart) and *A. sororcula* Zucchi, segundo a mesma técnica. As espécies apresentam número diplóide igual a 12 cromossomos, exceto *A. leptozona* com 2n = 10. Os cromossomos sexuais são heteromórficos e com sistema XX/XY. Os autossomos são meta ou submetacêntricos em *A. grandis* and *A. leptozona* e acrocêntricos nas demais espécies. Os cromossomos sexuais apresentam comprimento e padrão de blocos de heterocromatina característicos para cada espécie.

PALAVRAS-CHAVE: Heterocromatina, cromossomo sexual, cromossomo mitótico, banda-C

ABSTRACT - The study comprises the description of the C-banded mitotic chromosomes of five species of *Anastrepha*, *A. amita* Zucchi, *A. turpiniae* Stone, *A. zenildae* Zucchi, *A. grandis* (Macquart) and *A. leptozona* Hendel , and a reanalysis of the chromosomes of three other species, *A. distincta* Greene, *A. obliqua* (Macquart) and *A. sororcula* Zucchi. The species have a diploid number of 12, while *A. leptozona* has 2n = 10. Heteromorphism of the sex chromosomes is present in all of the species, which have a XY/XX sex chromosome system. *A. grandis* and *A. leptozona* have meta and submetacentric autosomes while all of the chromosomes in the other species are acrocentric. The species can be characterized unequivocally by the length and the pattern of blocks of heterochromatin in the sex chromosomes.

KEY WORDS: Heterochromatin, mitotic chromosomes, sex chromosomes, C-band

The genus *Anastrepha* of frugivorous Tephritidae is endemic to tropical and subtropical regions of the American Continent occurring from southern United States to Northern Argentina, and in the Caribbean islands (Aluja 1994). It comprises about 200 species, distributed into 18 intrageneric groups (Norrbom *et al*. 1999). Of these, 94 species occur in Brazil, infesting native and introduced fruits of 31 different families (Norrbom *et al.* 1999, Zucchi 2000a, b). The taxonomy of fruit flies is usually based on the analysis of adult females, more specifically on the morphology of the aculeus tip (Zucchi 2000a). Structures of adult males, 3rd-instar larvae and eggshell morphology have been indicated as useful characters for species identification but these studies are incipient (Aluja 1994; Selivon & Perondini 1998, 1999; Norrbom *et al.* 1999).

Despite the studies so far done, there are some problems to ascertain the taxonomic status of some species. One example is the nominal species *A. fraterculus* (Wied.) that congregates a complex of cryptic species, as suggested by Baker *et al.* (1944) and Bush (1962), and evidenced by Morgante *et al.* (1980), Steck (1991), Steck & Sheppard (1993), McPheron *et al*. (1999) and Smith-Caldas *et al*. (2001). This complex of cryptic species is being characterized by Selivon (1996) Selivon & Perondini (1998) Selivon *et al.* (1999, 2004, 2005). The precise knowledge of the taxonomic status of these and other species is fundamental for the development of strategies oriented to control or supress these pests, since these methods are species-specific.

Previous cytological studies indicated that chromosome morphology may be useful for taxonomic purposes. However, the karyotype of only 10 percent of the species of *Anastrepha* has been described. These studies showed that similar acrocentric autosomes and a 2n = 12 are found in the karyotypes of the nominal *A. fraterculus* (Solferini & Morgante 1987, Basso & Manso 1998, Selivon *et al.* 2005), in *A. distincta* Greene, *A. ludens* (Loew), *A. obliqua* (Macquart), *A. sororcula* Zucchi, *A. zuelaniae* Stone (Bush 1962, Solferini & Morgante 1987, 1990), all of them belonging to the *fraterculus* group (Norrbom *et al.* 1999). On the contrary, large differences do exist in the karyotypes of *Anastrepha* species from other intrageneric groups, as to chromosome number and autosome morphology. The diploid number is 2n = 8 in *A. pickeli* Lima and *A. montei* Lima (Solferini & Morgante 1987, Morgante *et al.* 1996), while a 2n = 12 is found in *A. aphelocentema* Stone, *A. barnesi* Aldrich, *A. bistrigata* Bezzi, *A. pseudoparalella* (Loew), *A. serpentina* (Wied.), *A. spatulata* Stone and *A. striata* Schiner (Bush 1962, Solferini & Morgante 1987, 1990, Morgante *et al.* 1996). In all of these species most of the autosomes have a median or submedian centromere. Differences are also found in the sex chromosomes system, that is X_1X_2Y/ $X_1X_1X_2X_2$ in *A. serpentina* and *A. bistrigata* (Solferini & Morgante 1987, 1990), while all of the other studied species have a XY/XX system, therefore, heteromorphism of the sex chromosomes seems to be the rule.

In relation to the nominal *A. fraterculus*, Mendes (1958) found a karyotype having small Y chromosome in southern Brazil, while Bush (1962) described a large isomorphic pair of sex chromosome in Mexican populations. Later on, Solferini & Morgante (1987) described four karyotypes in Brazilian samples, one with a small Y in southern Brazil and the others with a large Y chromosome in northeastern region. However, it was shown later on that one of these karyotypes belong indeed to *A. sororcula* (Morgante *et al.* 1993). Polymorphism specially of the sex chromosomes was found by Basso & Manso (1998) and Basso *et al.* (2003), in nominal *A. fraterculus* collected in Argentina and southern Brazil. Further karyotypic analysis of nominal *A. fraterculus* samples from Brazilian populations, have shown the existence of three karyotypes, two of them with small Y and one with a large Y chromosome, as well as of a 4th type with a large Y chromosome occurring in a sample from Guayaquil, Ecuador (Selivon 1996; Selivon *et al.* 2004, 2005).

Further chromosome analyses of *Anastrepha* species are still necessary in order to help in taxonomic problems and to understand karyotypic evolution in this group of flies. The present report contains a more detailed analysis of the chromosomes of *A. distincta, A. obliqua* and *A. sororcula,* previously studied, respectively, by Bush (1962), Solferini & Morgante (1987) and Morgante *et al.* (1993). It includes measeruments of chromosome length and/ or heterochromatic blocks evidenced by C-banding, as well as a description of the mitotic chromosomes of five other *Anastrepha* species , *A. amita* Zucchi, *A. turpiniae* Stone, *A. zenildae* Zucchi, *A. grandis* (Macquart) and *A. leptozona* Hendel.

Material and Methods

Collection sites of host fruits infested with fruit flies are shown in Table 1. In the laboratory the species were maintained in population cages according to Selivon & Perondini (1998). Guavas or papayas were furnished as ovipositon sites for *A. obliqua, A. sororcula, A. turpiniae* and *A. zenildae,* and pumpkins for *A. grandis*. From these substrates, 3rd-instar larvae of the first generation were used for the chromosome analyses. For *A. amita, A. distincta* and *A. leptozona,* species not usually maintained in the laboratory, the chromosomes were analysed from larvae taken directly from the collected fruits. Although only adults of these three species emerged from the respective collected host fruit, to assure that the analysed larvae belonged to the same species, mitotic chromosomes where also analysed in samples prepared from adult gonads. Since the larval tissues furnished higher number of mitosis than the adult ones, samples of 16 to 20 larvae comprising males and females were analysed for each species.

For chromosome preparations neural ganglia and adjacent imaginal disks were employed as described by Selivon & Perondini (1997). After removal of the coverslip the preparations were air dried, and stored in a sealed box containing silica gel. Heterochromatic regions of the chromosomes were evidenced by the C-banding technique according to Selivon & Perondini (1997). The slides were examined in an Olympus BX60 microscope, and chromosome images were digitalized by a Leica DC100 CCD device coupled to the microscope. They were edited by the AdobePhotoshop 6.0 software, and chromosome

Table 1. Host fruits and collection sites of the species of *Anastrepha*.

Species group	Species	Host fruit		Locality
fraterculus	*A. amita*	"Pau-de-viola"	(*Citharexylum myrianthum*)	São Paulo, SP (23°33'S, 46°44'W)
	A. distincta	"Ingá"	(*Inga edulis*)	São Carlos, SP (21°55'S, 47°53'W)
	A. obliqua	Star fruit	(*Averrhoa carambolae*)	Indiatuba, SP (22°42'S, 47°37'W)
	A. sororcula	Guava	(*Psidium guajava*)	Hidrolândia, GO (17°02'S, 49°13'W)
	A. turpiniae	Guava	(*Psidium guajava*)	Hidrolândia, GO (17°02'S, 49°13'W)
	A. zenildae	Guava	(*Psidium guajava*)	Natal, RN (05°48'S, 35°13'W)
grandis	*A. grandis*	Pumpkin	(*Cucurbita pepo*)	Laranjal Paulista, SP (23°05'S, 47°45'W)
leptozona	*A. leptozona*	"Guapeba"	(*Pouteria* sp.)	Hidrolândia, GO (17°02'S, 49°13'W)

length measurements were done using the ProImage IPWIN32 software. The length of each chromosome was expressed as percentage of the total diploid complement length, according to Robertson (1957). Measurements were done in 10 mitosis of each sex from different individuals.

Results

The six analysed species of the *fraterculus* group (*A. amita, A. distincta, A. obliqua, A. sororcula, A. turpiniae, A. zenildae*), and *A. grandis* (*grandis* group), presented a diploid number of 12 chromosomes, while *A. leptozona* (*leptozona* group) had 2n = 10. The sex chromosomes were XY/XX in all of these species as shown in Figs. 1 to 8.

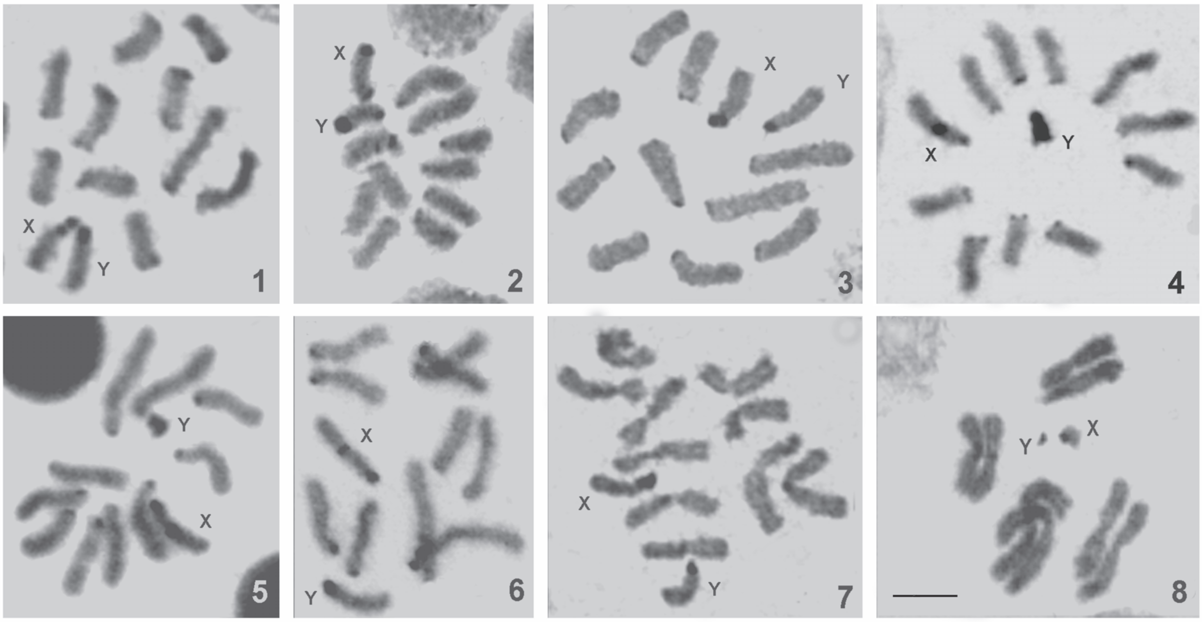
In previous publications on the mitotic chromosomes of *Anastrepha* species (Solferini & Morgante 1987, 1990) the sex chromosomes were considered the first pair (I), and the autosomes numbered according to an ascending order, the smallest being number II (or 2). However, in karyological studies of *Anastrepha* (Basso *et al.* 2003) and other genera of fruit flies [e.g. *Dacus cucurbitae* Coquillet (Gopalan 1972), *Ceratitis capitata* (Wiedemann) (Zacharopoulou 1986), *Rhagoletis pomonella* (Walsh) (Procunier & Smith 1993), *Bactrocera oleae* Gmelin (Canovai *et al.* 1996)], the autosomes are numbered in a descending order, the largest being number 2 (or II). The latter system will be used in the present report.

The autosomes of the species of the *fraterculus* group are acrocentric and no specific structural differences were found in any of them. Discrete heterochromatic blocks are usually seen at the centromeric ends of the autosomes except in *A. amita* (Fig. 1). In *A. grandis* and *A. leptozona*, of other intrageneric groups, the autosomes have a submedian centromere, as shown in Figs. 7 and 8 and in the ideograms of Fig. 9. Moreover, an ANOVA test revealed no significant differences in the length of corresponding autosomes of these species. *A. leptozona* was excluded since it was not possible to establish correspondence among its autosomes to those of other species (Table 2).

Significant differences among the species, including *A. leptozona*, were observed for the X and the Y chromosomes (Table 2). Details of the sex chromosomes revealed by C-banding will be described below.

In *A. amita* (Figs. 1 and 9), the X and Y chromosomes are large, the X chromosome presenting about the same length of the largest autosome (II), while the Y chromosome is similiar in size to autosome IV. The X to Y ratio is 1.28, and the X chromosome has a large block of heterochromatin at the centromeric end, and in which a constriction is frequently visible. The Y has also a heterochromatic block at the centromeric end.

The X chromosome of *A. distincta* (Figs. 2 and 9) presents the length of the smallest autosome, and has two blocks of heterochromatin, one at each tip, the block at the distal end being larger than the block at the proximal, centromeric tip. The Y chromosome, larger than the X (X/Y ratio = 0.83) has also two blocks of heterochromatin, similarly to the X chromosome. The distal block is larger than the proximal one, and also larger than the corresponding block of the X chromosome. Fig. 3 and Fig. 9, show that the X chromosome of *A. obliqua* has a large heterochromatic region at the centromeric end, usually divided into two blocks by a constriction. The Y chromosome also presents a block of heterochromatin at the proximal



Figs. 1-8. Metaphase plates of male individuals of *Anastrepha* species after C-banding. (1) *A. amita*; (2) *A. distincta*; (3) *A. obliqua*; (4) *A. sororcula*; (5) *A. turpiniae*; (6) A. *zenildae*; (7) *A. grandis*; (8) *A. leptozona.* Note the blocks of heterochromatin especially in the sex chromosomes. Bar = 10 µm

tip. The X has approximately the length of autosome III, while the Y is smaller than the smallest autosome. The X/Y ratio is 1.33.

A. sororcula (Figs. 4 and 9) has a small Y chromosome that seems to be entirely heterochromatic presenting a constriction at about 1/3 of its length. The X/Y ratio is 1.89, and the X chromosome has a length similar to autosome III. The X shows a conspicuous block of heterochromatin near its median region, and a smaller block at the centromeric end.

A median constriction is present in the small Y chromosome of *A. turpiniae*, one half being constituted by

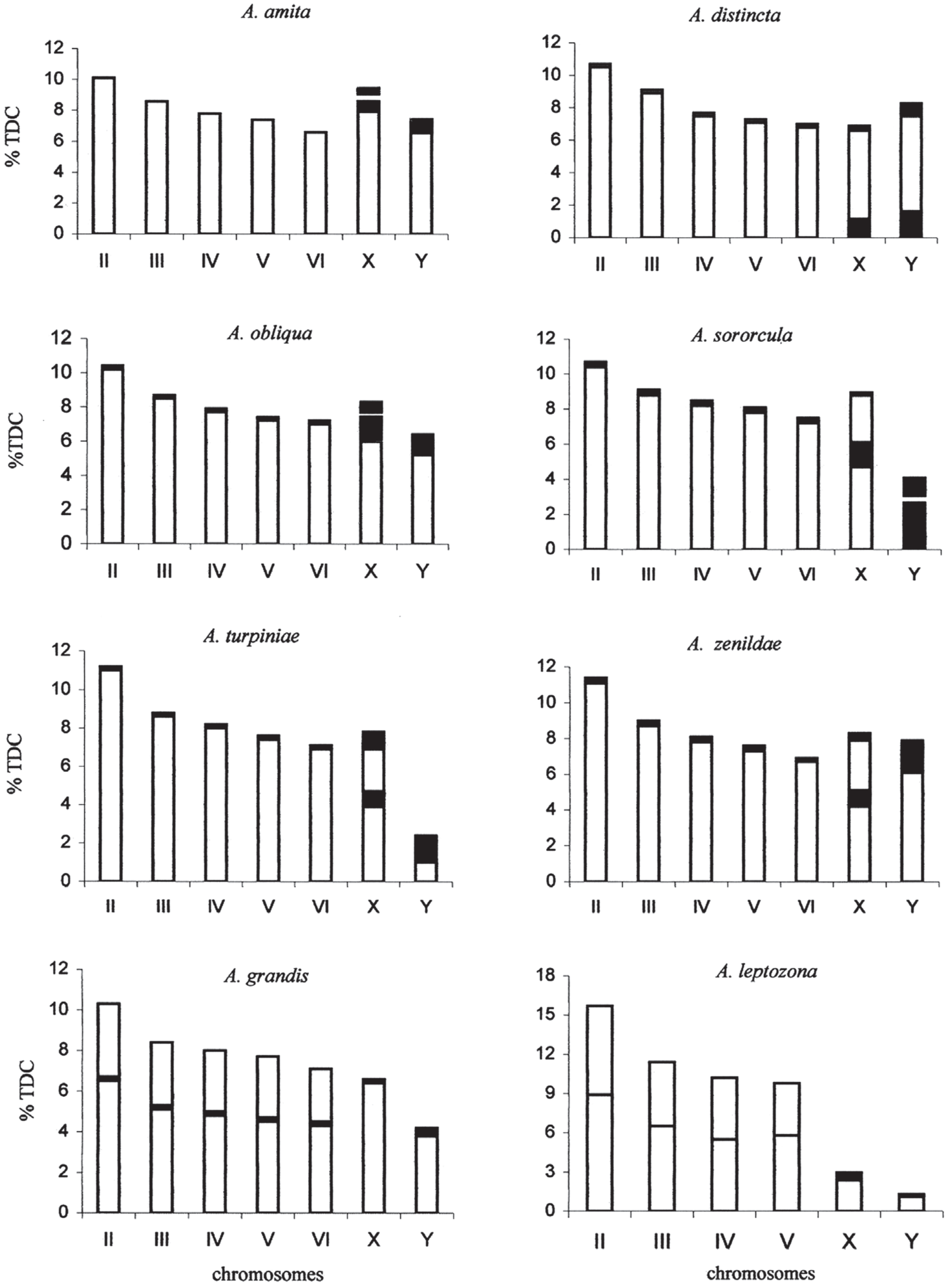


Fig. 9. Idiograms of the mitotic chromosomes of species of *Anastrepha.* Length of each chromosome as a percentage of the total diploid complement length (%TDC). Heterochromatic blocks marked in dark. The transversal line in the autosomes of *A. grandis* and *A. leptozona* indicate the position of the centromere. In the other species the centromeric end is at the top. Note the change of scale in the graph of *A. leptozona.*

Table 2. Comparison of the length of corresponding autosomes *leptozona* excluded) and the sex chromosomes (*A. leptozona* included) of the species studied in the present report.

Chromosomes	ANOVA			
	F	d.f.	P	Obs.
II	2.336	6, 34	>0.06	ns
III	1.865	6, 34	>0.11	ns
IV	0.986	6, 34	>0.50	ns
V	1.463	6, 34	>0.20	ns
VI	1.756	6, 34	>0,15	ns
X	52.773	7, 39	<0.001	***
Y	139.79	7, 39	<0.001	***

a block of heterochromatin (Figs. 5 and 9). The X chromosome has one block of heterochromatin at the proximal end and another one at a median region. The X has approximately the length of autosomes IV or V, and the X/Y ratio is 3.25 .

In *A. zenildae* (Figs. 6 and 9), the sex chromosomes are almost isomorphic, the X/Y ratio being 1.11, and exhibit a similar length to autosomes IV and V. The Y has a large block of heterochromatin at the centromeric end, and in the X two blocks are visible, one at the proximal tip, the other near the median region.

The sex chromosomes of *A. grandis* are the smallest in the karyotype, the X/Y ratio being 1.56 (Figs. 7 and 9). Both, the X and the Y chromosomes, exhibit a conspicuous block of heterochromatin at their proximal ends. The autosomes present unequal arms, the arm ratio (S/L) being 0.55, 0.62, 0.61, 0.62, 0.58 respectively for chromosomes II, III, IV, V and VI.

A. leptozona has very small sex chromosomes which represent about 4.5% of the length of the diploid complement (Figs. 8 and 9). The X/Y ratio is 0.45, and both present a small block of heterochromatin at the proximal end. The S/L arm ratios of the autosomes are 0.76, 0.75, 0.85 and 0.69, respectively for chromosomes II, III, IV and V.

Discussion

The present results together with data obtained before for other species show a karyotypic constancy among species of the *fraterculus* group, in regard to the diploid number of 12, and to the size and morphology of the autosomes, which are acrocentric in the species so far studied. In other species groups, variation in the diploid number associated or not to differences in the morphology of the autosomes seems to be frequent. Since the karyotypes of *Anastrepha* species have a similar total length regardless the variation in chromosome number (data not shown), simple chromosome rearrangements could be invoked to explain the karyotypic modifications either of chromosome morphology (centromeric positions) or changes in the diploid number, as postulated by Solferini & Morgante (1987, 1990).

Contrary to the relative autosomes homogeneity, the acrocentric sex chromosomes of the *fraterculus* group, exhibit an expressive variation in length, as well as in size and location of heterochromatin blocks. Bush (1962), studying samples from Mexican populations, described the sex chromosomes of *A. distincta* and *A. obliqua* as being isomorphic. However, Solferini & Morgante (1987) showed that a heteromorphism does exist for the sex chromosomes in *A. obliqua* sample from southeastern Brazil, data that were confirmed by the present analysis. The present results on *A. distincta* collected in southeastern Brazil differ from those of Bush (1962) by showing sex chromosomes heteromorphism. Further analyses of *A. distincta* are required to interpret such variation since Steck (1991), through an isozyme analysis also recognized genetic differences in samples from Brazil and Venezuela.

Species of *Anastrepha* belonging to intrageneric groups other than the *fraterculus* group (Norrbom *et al*. 1999, Norrbom 2002), *A. aphelocentema* (*mucronota* group), *A. barnesi* and *A. leptozona* (*leptozona* group), *A. grandis* (*grandis* group), *A. pseudoparallela* (*pseudoparallela* group), *A. spatulata, A. pickeli* and *A. montei* (*spatulata* group), and *A. striata* (*serpentina* group), have small Y chromosomes, which are dot shaped in some or having from 1/4 to 1/3 of the length of the X chromosme (Bush 1962; Solferini & Morgante 1987, 1990; Morgante *et al.* 1993, 1996; present report). *A. serpentina* and *A. bistrigata* of group *serpentina*, have a X_1X_2Y sex chromosome system, the neo-Y being a large chromosome, possible resulting from a translocation of an original Y to an autosome. Considering this hypothesis the original Y must have been a small chromosome having about 1/4 to 1/3 of the original X, the actual X_1 chromosome (Solferini & Morgante 1990).

The available data on the karyotypes of the *fraterculus* group (Mendes 1958, Bush 1962, Solferini & Morgante 1987, Selivon 1996, Basso & Manso 1998, Selivon *et al.* 2004, 2005, present results), show that the species present sex chromosome heteromorphism and that, according to the size of the Y chromosomes, they can be arranged into two clusters: one exhibiting a small Y chromosome having 1/4 to 1/3 of the length of the X, as in *ludens, turpiniae, sororcula, fraterculus* complex (sp.1 and sp.3), and the other in which the Y is 2/3, equal or longer than the X chromosomes, as in *A. amita, A. distincta, A. fraterculus* complex (sp. 2 and sp. 4), *A. obliqua, A. zenildae, A. zuelaniae.* Heteromorphism of the sex chromosomes is thought to evolve through processes of chromosome degeneration that change the genetic content as well as the morphology of the chromosome that carries the sex determinant factor or genes related to other specific characteristics of the heterogametic sex, for example the Y chromosome (Bull 1983, Charlesworth 1990). If one assumes that the sex determinant for maleness in *Anastrepha* is located on the Y chromosome, as it occurs in another tephritid, *Ceratitis capitata* (Willhoeft & Franz 1996), it may be postulated that similar mechanisms of Y chromosome degenaration is involved in the evolution of the sex chromosomes in this genus, producing the small highly heterochromatic Y chromosome exhibited by some species.

In this scenario, the differences in the Y chromosome size may be the result of peculiarities occurred during the degenaration process or that the chromosomes are at different stages of this process. For example, in populations of *Megaselia scalaris* (Loew) (Willhoeft & Traut 1990) and *Drosophila miranda* Dobzhanski (Steinmann *et al.* 1993), Y chromosomes at different stages of degeneration process were found. Further studies encompassing a more expressive number of species is still required to provide new insights into the chromosome evolution in *Anastrepha* species.

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Literature Cited

Aluja, M. 1994. Bionomics and management of *Anastrepha*. Ann. Rev. Entomol. 39: 155-178.

Baker, A.C., W.E Stone, C.C. Plummer& H. MacPhail. 1944. A review of studies on the Mexican fruti fly and related Mexican species. United States Department Agric. Miscellaneous Publ. 531: 1-155.

Basso, A., A. Sonvico, L.A. Quesada-Allue & F. Manso. 2003. Karyotypic and molecular identification of laboratory stocks of the South American fruit fly *Anastrepha fraterculus* (Wied.) (Diptera: Tephritidae). J. Econ. Entomol. 96: 1237-1244.

Basso, A. & F. Manso. 1998. Are *Anastrepha fraterculus* chromosomal polymorphism an isolation barrier*?* Cytobios 93: 103-111.

Bull, J. 1983. Evolution of sex determining mechanisms. Benjamin/Cummings, Menlo Park, California, 298p.

Bush, G.L. 1962. The cytotaxonomy of the larvae of some mexican fruit flies in the genus *Anastrepha* (Tephritidae, Diptera). Psyche 69: 87-101.

Canovai, R., G. Macelloni & L. Galleni. 1996. Karyology of some Italian populations of the olive fly *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae). Caryologia 49: 247-255.

Charlesworth, B. 1990. The evolution of sex chromosomes. Science 251: 1030-1033.

Gopalan, H.N.B. 1972. The study of somatic, meiotic and salivary gland chromosomes of the melonfly, *Dacus cucurbitae*. Caryologia 25: 163-172.

McPheron, B.A., H-Y. Han, J.G. Silva & A.L. Norrbom. 1999. Phylogeny of genus *Anastrepha* and *Toxotrypana* (Trypetinae: Toxotrypanini) based upon 16S rDNA mitochondrial sequences, p. 343-361. In M. Aluja, & A.L. Norrbom (eds.), Fruit flies (Tephritidae): Phylogeny and evolution of behaviour. CRC Press, Boca Ratón, 944p.

Mendes, L.O.T. 1958. Observações citológicas em moscas-das-frutas. Bragantia 17: 29-39.

Morgante, J.S., A. Malavasi & G.L. Bush. 1980. Biochemical systematic and evolutionary relationships of neotropical *Anastrepha*. Ann. Entomol. Soc. Am. 73: 622-630.

Morgante, J.S., D. Selivon, V.N. Solferini & A.S. Nascimento. 1996. Genetic and morphological differentiation in the specialist species *Anastrepha pickeli* and *A. montei,* p.267-272. In B.A. McPheron & G.J. Steck (eds.), Fruit flies pests. St. Lucie Press, Delray Beach, 586p.

Morgante, J.S., D. Selivon, V.N. Solferini & S.R. Matioli. 1993. Evolutionary patterns in specialist and generalist species of *Anastrepha,* p.15-20. In M.Aluja & P. Liedo (eds.), Fruit flies: Biology and management. Springer, New York, 492p.

Norrbom, A.L. 2002. A revision of the *Anastrepha serpentina* species group (Diptera: Tephritidae). Proc. Entomol. Soc. Wash. 104: 390-436.

Norrbom, A.L., R.A. Zucchi, & V. Hernández-Ortiz. 1999. Phylogeny of the genera *Anastrepha* and *Toxotrypana* (Trypetinae: Toxotrypanini) based on morphology, p.299-342. In M.Aluja & A.L. Norrbom (eds.), Fruit flies (Tephritidae): Phylogeny and evolution of behaviour. CRC Press, Boca Raton, 944p.

Procunier, W.S. & J.J. Smith. 1993. Localization of ribosomal DNA in *Rhagoletis pomonella* (Diptera, Tephritidae) by *in situ* hybridization. Insect Mol. Biol. 2: 163-174.

Robertson, J.G. 1957. Somatic metaphase chromosomes in geographic isolates of the carrot rust fly, *Chamaepsila rosae* (F.) (Diptera, Psiliidae). Canadian J. Zool. 35: 453-458.

Selivon, D. 1996. Estudo sobre a diferenciação populacional em *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae). Ph.D. Thesis, Departamento de Biologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, 137p.

Selivon, D. & A.L.P. Perondini. 1997. Evaluation of techniques for C and ASG banding of the mitotic chromosomes of *Anastrepha fraterculus* (Diptera: Tephritidae). Brazil. J. Genet. 20: 651-654.

Selivon, D. & A.L.P. Perondini. 1998. Egg-shell morphology in two cryptic species of the *Anastrepha fraterculus* complex (Diptera, Tephritidae). Ann. Entomol. Soc. Am. 91: 473-478.

Selivon, D. & A.L.P. Perondini. 1999. Description of *Anastrepha sororcula* and *A. serpentina* (Diptera, Tephritidae) eggs. Fla. Entomol. 82: 347-353.

Selivon, D., A.L.P. Perondini & J.S. Morgante. 1999. Haldane´s rule and other aspects of reproductive isolation observed in the *Anastrepha fraterculus* complex (Diptera, Tephritidae). Genet. Mol. Biol. 22: 507-510.

Selivon, D., A.L.P. Perondini & J.S. Morgante. 2005. A genetic-morphological characterization of two cryptic species of *Anastrepha fraterculus* complex (Diptera, Tephritidae). Ann.Entomol. Soc. Am. (in press).

Selivon, D., C. Vretos, L. Fontes & A.L.P. Perondini. 2004. New variant forms in the *Anastrepha fraterculus* complex (Diptera, Tephritidae), p.253-258. In B. Barnes (ed.), Proc. 6th International Symposium on Fruit Flies of Economic Importance, Isteg Scientific Publications, Irene, South Africa, 510p.

Smith-Caldas, M.R.B., B.A. McPheron, J.G. Silva & R.A. Zucchi. 2001. Phylogenetic relationships among species of the *fraterculus* group (*Anastrepha*: Diptera: Tephritidae) inferred from DNA sequences of mitochondrial cytochrome oxidase I. Neotropical Entomol. 30: 565-573.

Solferini, V.N. & J.S. Morgante. 1987. Karyotype study of eight species of *Anastrepha* (Diptera: Tephritidae). Caryologia 40: 229-241.

Solferini, V.N. & J.S. Morgante. 1990. $X_1X_1X_2X_2$: X_1X_2Y mechanism of sex determination in *Anastrepha bistrigata* and *A. serpentina*. Rev. Bras. Genet. 13: 201-208.

Steck, G.J. 1991. Biochemical systematics and population genetic structure of *Anastrepha fraterculus* and related species (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 84: 10-28.

Steck, G.J. & W.S. Sheppard. 1993. Mitochondrial DNA variation in *Anastrepha fraterculus,* p.9-14. In M.Aluja & P. Liedo (eds.), Fruit flies: Biology and management, Springer, New York, 492p.

Steinemann, M., S. Steinemann & F. Lottespeich. 1993. How Y chromosomes become inert. Proc. Natl. Acad. Sci. USA 90: 5737-5741.

Willhoeft, U. & G. Franz. 1996. Identification of the sex-determining region of the *Ceratitis capitata* Y chromosome by deletion mapping. Genetics 144: 737-745.

Willhoeft, U. & W. Traut. 1990. Molecular differentiation of the homomorphic sex chromosomes in *Megaselia scalaris* (Diptera) detected by random DNA probes. Chromosoma 99: 237-242

Zacharopoulou, A. 1986. Cytogenetic analysis of mitotic and salivary gland chromosomes in the Medfly *Ceratitis capitata*. Genome 29: 67-71.

Zucchi, R.A. 2000a. Taxonomia, p.13-24. In A. Malavasi & R.A. Zucchi (eds.), Moscas-das-frutas de interesse econômico no Brasil: Conhecimento básico e aplicado, Holos, Ribeirão Preto, 327p.

Zucchi, R.A. 2000b. Espécies de *Anastrepha*, Sinonímias, Plantas Hospedeiras e Parasitóides, p.41-48. In A. Malavasi & R.A. Zucchi (eds.), Moscas-das-frutas de interesse econômico no Brasil: Conhecimento básico e aplicado, Holos, Ribeirão Preto, 327p.

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