Sex chromosomes in mitotic and polytene tissues of *Anastrepha fraterculus* (Diptera, Tephritidae) from Argentina: a review

María Cecilia Giardini¹, Fabián H. Milla¹, Silvia Lanzavecchia¹, Mariela Nieves², Jorge L. Cladera¹

¹ Laboratorio de Genética de Insectos de Importancia Económica, Instituto de Genética ‘Ewald A. Favret’, CICVyA, Instituto Nacional de Tecnología Agropecuaria (INTA), Hurlingham, Buenos Aires, Argentina

² Grupo de Investigación en Biología Evolutiva, Departamento de Ecología, Genética y Evolución, IEGEBA-Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina

Corresponding author: María Cecilia Giardini (giardini.maría@inta.gob.ar)

Abstract

Cytogenetics, which is considered a fundamental tool to understand basic genetic and genomic issues of species, has greatly contributed to the description of polymorphisms both at inter- and intra-specific level. In fact, cytogenetics was one of the first approaches used to propose *Anastrepha fraterculus* (Diptera, Tephritidae) as a complex of cryptic species. Different morphological variants of sex chromosomes have been reported among Argentinean populations of *A. fraterculus*. However, since this high structural variability in sex chromosomes does not pose a reproductive barrier, their role in speciation is yet to be unveiled. This review provides an update on general aspects of cytogenetics in Argentinean *A. fraterculus* populations, focused on the prevalence of X-Y arrangements.

Keywords

Cytogenetics, karyotype variants, South American fruit fly, heterochromatin, centromeres, ribosomal genes
**General background**

Cytogenetic studies have provided significant information about intra- and inter-species genetic variation (Sumner 2003). Cytogenetic studies focus mainly on sex chromosomes, which present unusual features relative to autosomes (Traut et al. 2008). In organisms with the classical X-Y systems of sex determination, Y chromosomes lack genetic recombination, are male limited, and show an abundance of genetically inert heterochromatic DNA containing few functional genes, whereas X chromosomes also show sex-biased transmission, and are hemizygous in the heterogametic sex (Kaiser and Bachrog 2010). In particular, in some insect species, sex chromosomes show high structural variability (see Traut 1999, Kaiser and Bachrog 2010, Palacios-Gimenez et al. 2013). In the genus *Anastrepha*, Solferini and Morgante (1987), Selivon et al. (2005a), Goday et al. (2006) and García-Martínez et al. (2009) compared different species and reported specific sex-chromosome banding patterns. In addition, they described variability in the length, number, size, and position of heterochromatic blocks in ‘the South American fruit fly’, *Anastrepha fraterculus* Wiedemann (Diptera: Tephritidae). This species is distributed from southern United States to Argentina (Salles 1995, Steck 1999) and constitutes an economically important pest. Currently, it is considered a complex of cryptic species (for reviews and references, cf. Selivon et al. 2004, 2005a, Cáceres et al. 2009, Hernández-Ortiz et al. 2012, Cladera et al. 2014). Multivariate morphological studies including samples from different regions of the American continent have characterized seven distinct morphotypes (Hernández-Ortiz et al. 2012). Studies based on genetic differentiation, karyology, morphology, reproductive compatibilities combined with bionomic parameters, eggshell morphology and some aspects of early embryogenesis of samples from northern and southern Brazil have identified at least four entities of the *A. fraterculus* complex: *A*. sp.1 aff. *fraterculus*, *A*. sp. 2 aff. *fraterculus*, *A*. sp. 3 aff. *fraterculus*, and *A*. sp.4 aff. *fraterculus*. The first three entities have been reported in different regions of Brazil, whereas *A*. sp. 4 aff. *fraterculus* has been described in Guayaquil, Ecuador (Selivon and Perondini 1997, 1998, Selivon et al. 1997, 1999, 2004, 2005a, 2005b, Goday et al. 2006).

Reproductive incompatibilities between *A*. sp.1 aff. *fraterculus* and *A*. sp. 2 aff. *fraterculus* living in sympathy were first described by Selivon et al. (1999, 2005a). Later, Vera et al. (2006) showed pre-mating isolation between flies from Peru and Argentina, Brazil and Colombia, as well as between flies from Piracicaba (São Paulo, Brazil) and Argentina. Cáceres et al. (2009) found that hybrids between strains from Peru and Argentina carried the expected mix of sex chromosome cytotypes, but presented sex ratio distortion and high rates of sterility or inviability. High levels of mating isolation have also been reported among Mexican, Peruvian and the Brazilian-1 morphotypes (Rull et al. 2013). Reproductive isolation between the four morphotypes of *A. fraterculus* complex and flies from “the Andean morphotype” were also found by Devescovi et al. (2014). These and other factors analyzed by the authors are indicative of incipient speciation, providing a strong evidence for a taxonomic revision of this species complex (Selivon et al. 2005a, Cáceres et al. 2009).
Polyploid chromosome analysis and the availability of polyploid maps of different genera of the family Tephritidae, as Ceratitis (Zacharopoulou 1990), Bactrocera (Mavragani-Tsipidou et al. 1992, Augustinos et al. 2014), Dacus (Drosopoulou et al. 2011), Rhagoletis (Kounatidis et al. 2008) and Anastrepha (Garcia-Martinez et al. 2009), have allowed identifying differences between closely related species. Moreover, several groups of cryptic species were initially identified using sequences of the polyploid chromosomes as genetic markers and later confirmed by molecular markers studies (Ramirez and Dessen 2000).

**Karyotype and sex chromosome configurations**

Karyological studies performed in wild populations of *A. fraterculus* from Argentina have shown structural variability in the sex chromosomes. Lifschitz et al. (1999) described an acrocentric X chromosome and a small submetacentric Y chromosome (see also Basso and Manso 1998, Basso et al. 2003). Lifschitz et al. (1999) and Manso and Basso (1999) also reported four morphological variants of the X chromosome (named $X_1$, $X_2$, $X_3$, and $X_4$) and six variants of the Y chromosome (named $Y_1$, $Y_2$, $Y_3$, $Y_4$, $Y_5$, and $Y_6$) at low frequency (Figure 1). Basso and Manso (1998) and Basso et al. (2003) also studied the viability and survival of individuals with different karyotype configurations under laboratory conditions, and showed that the cytogenetic differences found among these Argentinean populations do not represent evidence of reproductively separate species, but seem to be examples of intra-species chromosome polymorphisms.

In an experiment under field cage conditions Petit Marty et al. (2004a, 2004b) confronted *A. fraterculus* flies from extreme regions (NOA and NEA) inside Argentina and compared the frequency of homotypic and heterotypic crosses. No evidence of sexual incompatibility was found, either pre-zygotic (Petit Marty et al. 2004a) or post-zygotic (Petit Marty et al. 2004b). These studies confirmed the presence of a single *A. fraterculus* biological entity in Argentina.

After a revision of *A. fraterculus*’ chromosomes studies we concluded that the most frequent karyotype found in Argentina consists in five pairs of acrocentric autosomes, a submetacentric X chromosome (named $X_1$, Lifschitz et al. (1999) (CI average: 31.23) and a metacentric Y chromosome (named $Y_5$, Basso 2003) smaller than the X chromosome (Figure 2) (preliminary reported in Giardini et al. 2009b). It is important to highlight that the size and the patterns obtained with the different banding techniques for the X chromosome are the same as the one described by Lifschitz et al. (1999) as $X_1$. The only difference between them is the position assigned to the centromere and for that reason we kept on the same name. This difference in the centromere position could probably be explained by the lower resolution in the old pictures obtained by Lifschitz et al. (1999). This karyotype also corresponds to the one that was characterized by Selivon et al. (2005a) using samples from the southern Brazil (*A. sp.1 aff. fraterculus*) although without specifying the centromere position.
Figure 1. C-Band Ideogram. Sex chromosomes configurations of *A. fraterculus* found in Argentina (redrawn from Basso 2003). * Position of centromeres in each chromosome.

Figure 2. The most frequent karyotype of *A. fraterculus* found in Argentina. Mitotic chromosome preparations from third instar larvae of *A. fraterculus* male. A C-Bands B DAPI stain C CMA stain D Merged DAPI/CMA images.

After C-band staining (Figure 2A), the X₁ chromosome shows two prominent and different-sized blocks of heterochromatin located at the terminal region, whereas the Y₅ chromosome also shows two heterochromatin blocks positioned in different arms, one on the proximal end and the other in the sub-median region (Figure 2B). Both chromosomes show DAPI-positive signals in the same position as the heterochromatic blocks (Figure 2B). No CMA-positive band is distinguishable on the X₁ chromosome (Figure 2C). However, on the Y₅ chromosome, CMA-positive bands are observed at the same position as C-bands and DAPI-positive bands (preliminary reported in Giardini et al. 2009b). These observations indicate that the sex chromosomes analyzed in these populations of *A. fraterculus* differ in the nucleotide composition of the heterochromatic regions: the heterochromatic regions on the X₁ chromosome are AT-rich, whereas those of the Y₅ chromosome are AT+CG-rich DNA sequences (preliminary reported in Giardini et al. 2009b). These findings are in agreement with those of Goday et al. (2006).

*Anastrepha fraterculus* has also been cytogenetically characterized by means of fluorescence in situ hybridization (FISH), to locate the ribosomal genes on the chromosome complement. The first studies carried out by Basso and Manso (1998) on cytological preparations of *A. fraterculus* from Argentina using a heterologous probe from *Drosoph-
ila hydei rDNA described two positive signals at the terminal positions in the X-chromosome. Later, Goday et al. (2006) carried out studies using a Drosophila melanogaster probe in a comparative analysis of the fraterculus complex using samples from Brazil. In A. sp.1 aff. fraterculus individuals, these authors found an rDNA positive signal that co-localized with a DAPI-positive band at a distal position in the X1 chromosome, and a second signal at the DAPI/CMA-positive regions of the Y5 chromosome. By using a specific probe designed from a region of A. fraterculus 18S rDNA (Figure 3), in our lab we have observed a pattern of signals equivalent to the one previously described by Goday et al. (2006) (preliminary reported in Giardini et al. 2009b). These two last studies confirmed the general tendency observed for the rDNA of reside on the heterochromatic regions of the sex chromosomes other than centromeres (Drosopoulou et al. 2012).

**Chromatin characteristics**

As a first attempt to study histone modifications in A. fraterculus chromosomes, we performed immunodetection assays with specific antibodies in mitotic preparations of Argentinean A. fraterculus to analyze the presence of histone H3 phosphorylated at positions 10 or 28 (preliminary reported in Giardini et al. 2011). Both variants of histone H3 serve as markers for chromosomal condensation and segregation during mitosis and meiosis (Goto et al. 1999). Using the H3S28ph antibody, we found positive signals in all centromeres (Figure 4), whereas using the H3S10ph antibody, we found characteristic positive signals of chromosome condensation in all the complement, showing the expected behavior of chromosomes during the mitosis (data not shown) (preliminary reported in Giardini et al. 2011). Considering that histone modification patterns are a particularly informative feature in relation to chromatin characterization, our results represent the first epigenetic characterization of A. fraterculus mitotic chromosomes. Although preliminary, these studies allow confirming the acrocentric nature of the autosomes.
Sex chromosomes in polytene tissues

The existence of polytene chromosomes in the salivary glands of *A. fraterculus* was first reported by Mendes (1958). Our group characterized these chromosomes and published the first polytene pictures of this species (Giardini et al. 2009a). These chromosomes show homogeneity in chromosome length, similar banding and puffing patterns between sexes, and the absence of a typical chromocentre, resulting in the observation of complete individual chromosomes. We have described each chromosome on the basis of constant morphological structures (landmarks) and specific features (e.g., puffing pattern) and performed an approximation to a linear map following a customary labeling system (see details in Giardini et al. 2009a). Currently, a detailed map of *A. fraterculus* is in progress (M. Cecilia Giardini, Antigone Zacharopoulou, in preparation).

We have also performed a simultaneous analysis of mitotic and polytene nuclei of Argentinean *A. fraterculus*, and observed that neither the number of polytene chromosomes nor their banding patterns differentiate males from females (Giardini et al. 2009a). This suggests that in *A. fraterculus*, as well as in other tephritid flies (Zacharopoulou 1987, Mavragani-Tsipidou et al. 1992, Zacharopoulou et al 2011a, 2011b, Garcia-Martinez et al. 2009), the sex chromosomes do not form polytene chromosomes. This finding was tested by FISH experiments using the specific 18S rDNA probe in polytene chromosomes, which revealed a hybridization signal in a region of granular and uncondensed heterochromatin (Figure 5) that corresponded to the non-polytene sex chromosomes (Giardini et al. 2012).

Conclusion and remarks for the future

This review summarizes the cytogenetic information available from Argentinean *A. fraterculus*, focused on sex chromosome variation. Figure 6 shows an ideogram illustrating the results of all the techniques applied so far in the cytogenetic characteriza-
Sex chromosomes in mitotic and polytene tissues of *Anastrepha fraterculus*...

Several structural polymorphisms have been described in sex chromosomes from wild and laboratory Argentinean populations. In contrast to that observed in Brazilian populations, these polymorphisms do not act as reproductive barriers between individuals of different populations. Deeper characterization of the *A. fraterculus* karyotype by FISH allowed the identification and location of ribosomal genes in terminal position on the

**Figure 5.** 18S rDNA FISH analysis in polytene and mitotic tissues. Polytenes and mitotic chromosome preparations obtained from third instar larvae of male (A) and female (B) of *A. fraterculus*. In each case: 1 Polytenes chromosomes DAPI stain 2 Polytenes chromosomes 18S rDNA hybridization signal (FISH) 3 Polytenes chromosomes merged image (DAPI/FISH) 4 Mitotic chromosomes DAPI stain 5 Mitotic chromosomes 18S rDNA hybridization signal (FISH) 6 Mitotic chromosomes merged image (DAPI/FISH).

**Figure 6.** Cytogenetic summary of sexual chromosome pair. Ideogram of sex chromosomes of *A. fraterculus* from Argentina (most frequent karyotype). Relative location of C-Bands, DAPI/CMA bands, 18S and anti-H3S28ph hybridization signals.
sex chromosomes. Chromatin characteristics were also explored, and allowed the specific detection of centromeric regions and chromosomal condensation status in mitotic chromosomes of this species. The first characterization of polytene chromosomes in this species provided the description of landmarks and specific features on this type of chromosomes, and the detection of sex chromosomes as granular and uncondensed heterochromatin in polytene tissues.

All the results described here represent valuable information to be further used in the identification of genetic entities in the *A. fraterculus* complex of cryptic species. Deeper characterization of the structural variation of the sex chromosomes and polytene chromosome needs to be addressed to have a complete genetic picture of this species, which represents one of the most destructive fruit flies of economic importance in Argentina and the South American region. A detailed taxonomic revision of *A. fraterculus* and the accurate elucidation of the complexity displayed by this species in South America are of uttermost importance to develop environment-friendly autocidal control methods as is the Sterile Insect Technique (SIT), ensuring its specificity and effectiveness.

**Acknowledgements**

The authors gratefully acknowledge the two anonymous reviewers for their comments and suggestions to improve the manuscript. This work was partially supported by the National Institute of Agriculture Technology (INTA) through the project AEBIO-242411 (module pests) to SBL, by Consejo Nacional de Investigaciones Científicas y Técnicas PIP0572 to MN, MAEC-AECID Fellowship for Researchers and Postgraduate Studies to MCG and by the Agencia Nacional de Promoción Científica y Tecnológica de Argentina (ANPCyT) through Fondo Nacional de Ciencia y Tecnología (FONCyT) (grants PICT 2012 0704) to JLC. We wish to thank Dr. Clara Goday (Departamento de Proliferación Celular y Desarrollo, Centro de Investigaciones Biológicas, CSIC, Madrid, España) who hosted a part of the research in her laboratory and for her valuable help in cytogenetic techniques. We are also grateful to Dr. Leonela Carabajal Paladino (Laboratory of Molecular Cytogenetics, Biology Centre ASCR, Ceske Budejovice, Czech Republic) and Dr. Alejandra Scannapieco (Laboratorio de Insectos de Importancia Económica, IGEAF, INTA) for their valuable contribution to improve this manuscript.

**References**

Augustinos AA, Drosopoulou E, Gariou-Papalexio A, Boutzis K, Mavragani-Tsipidou P, Zacharopoulou A (2014) The *Bactrocera dorsalis* species complex: comparative cytogenetic analysis in support of Sterile Insect Technique applications. BMC Genetics 15 (Suppl 2): 1–10. doi: 10.1186/1471-2156-15-s2-s16

Basso A (2003) Caracterización genética de los componentes del “complejo *Anastrepha fraterculus*” (*Anastrepha* spp. DIPTERA: Tephritinae, Trypetinae) (Wiedemann) mediante análisis
Sex chromosomes in mitotic and polytene tissues of Anastrepha fraterculus...

91

de la variabilidad cromosómica. PhD thesis, Universidad de Buenos Aires, Buenos Aires, Argentina.

Basso A, Manso FC (1998) Are Anastrepha fraterculus chromosomal polymorphisms an isolation barrier? Cytobios 93: 103–111.

Basso A, Sonvico A, Quesada-Allue LA, Manso FC (2003) Karyotypic and molecular identification of laboratory stocks of the South American fruit fly Anastrepha fraterculus (Wied) (Diptera: Tephritidae). Journal of Economic Entomology 96:1237–1244. doi: 10.1093/jee/96.4.1237

Cáceres C, Segura DF, Vera MT, Wornoayporn V, Cladera JL, Teal PEA, Sapountzis P, Bourtzis K, Zacharopoulou A, Robinson AS (2009) Incipient speciation revealed in Anastrepha fraterculus (Diptera; Tephritidae) by studies on mating compatibility, sex pheromones, hybridization, and cytology. Biological Journal of the Linnean Society 97:152–165. doi: 10.1111/j.1095-8312.2008.01193.x

Cladera JL, Vilardi JC, Juri M, Paulin LE, Giardini MC, Gómez Cendra PV, Segura DF, Lanzavecchia SB (2014) Genetics and biology of Anastrepha fraterculus: research supporting the use of the sterile insect technique (SIT) to control this pest in Argentina. BMC Genetics 15 (Suppl 2): S2–S12. doi: 10.1186/1471-2156-15-s2-s12

Devescovi F, Abraham S, Roriz AKP, Nolazco N, Castañeda R, Tadeo E, Caceres C, Segura DF, Vera MT, Joachim-Bravo I, Canal N, Rull J (2014) Ongoing speciation within the Anastrepha fraterculus cryptic species complex: the case of the Andean morphotype. Entomologia Experimentalis et Applicata 152: 238–247.

Drosopoulou E, Nestel D, Nakou I, Kounatidis I, Papadopoulos NT, Bourtzis K, Mavragani-Tsipidou P (2011) Cytogenetic analysis of the Ethiopian fruit fly Dacus ciliatus (Diptera: Tephritidae). Genetica 139: 723–732. doi: 10.1007/s10709-011-9575-z

Drosopoulou E, Nakou I, Šíchová J, Kubíčková S, Marec F, Mavragani-Tsipidou P (2012) Sex chromosomes and associated rDNA form a heterochromatic network in the polytene nuclei of Bactrocera oleae (Diptera: Tephritidae). Genetica 140: 169–180. doi: 10.1007/s10709-012-9668-3

Garcia-Martinez V, Hernández-Ortiz E, Zepeta-Cisneros CS, Robinson AS, Zacharopoulou A, Franz G (2009) Mitotic and Polytene chromosome analysis in the Mexican fruit fly, Anastrepha ludens (Loew) (Diptera: Tephritidae). Genome 52: 20–30. doi: 10.1139/G08-099

Giardini MC, Milla FH, Manso FC (2009a) Structural map of the polytene chromosomes from the salivary glands of South American fruit fly Anastrepha fraterculus Wied. (Diptera, Tephritidae). Caryologia 62: 204–212.

Giardini MC, Milla F, Conte C, Lanzavecchia S, Cladera JL (2009b) Localización de los genes ribosomales en cromosomas mitóticos de Anastrepha fraterculus (WIED) mediante Hibridación in situ Fluorescente. JBAG. Actas XXXVIII Congreso Argentino de Genética.

Giardini MC, Milla F, Goday C, Cladera JL (2011) Explorando la epigenética en el díptero Anastrepha fraterculus (Wied). JBAG. Actas XL Congreso Argentino de Genética.

Giardini MC, Milla F, Conte C, Cladera JL, Lanzavecchia S, Nieves M (2012) Localización de los cromosomas sexuales de Anastrepha fraterculus (Wied.) en tejidos polítenicos. Journal of Basic Applied Genetics. Actas XV Congreso Latinoamericano de Genética, XLI Congreso Argentino de Genética, XLIV Congreso de la Sociedad de Genética de Chile, II Reunión Regional Sag-Litoral. Suppl. Vol. XXIII (1) 2012. ISSN: 1852–6233.
Goday C, Selivon D, Perondini ALP, Greciano PG, Ruiz MF (2006) Cytological characterization of sex chromosomes and ribosomal DNA location in *Anastrepha* species (Diptera, Tephritidae). Cyto genetic and Genome Research 114: 70–76. doi: 10.1159/000091931

Goto H, Tomono Y, Ajiro K, Kosako H, Fujita M, Sakurai M, et al. (1999) Identification of a novel phosphorylation site on histone H3 coupled with mitotic chromosome condensation. Journal of Biological Chemistry 274: 25543–9. doi: 10.1074/jbc.274.36.25543

Hernández-Ortiz V, Bartolucci AF, Morales-Valles P, Frías D, Selivon D (2012) Cryptic Species of the *Anastrepha fraterculus* Complex (Diptera: Tephritidae): A Multivariate Approach for the Recognition of South American Morphotypes. Annals of the Entomological Society of America 105(2): 305. doi: 10.1603/an11123

Kaiser VB, Bachtrog D (2010) Evolution of sex chromosomes in insects. Annual Review of Genetics 44: 91–112. doi: 10.1146/annurev-genet-102209-163600

Kounatidis I, Papadopoulos N, Bourtzis K, Mavragani-Tsipidou P (2008) Genetic and cytogenetic analysis of the fruit fly *Rhagoletis cerasi* (Diptera: Tephritidae). Genome 51: 479–491. doi: 10.1139/G08-032

Lifschitz E, Manso FC, Basso A (1999) Karyotype study of the South American Fruit Fly, *Anastrepha fraterculus* (Wied.) in Argentina. In: IAEA (Ed.) The South American Fruit Fly, *Anastrepha fraterculus* (Wied.): Advances in Artificial Rearing, Taxonomic Status and Biological Studies. IAEA, Vienna. [IAEA-TECDOC-1064]

Manso FC, Basso A (1999) Notes on the present situation of *Anastrepha fraterculus* in Argentina. In: IAEA (Ed.) The South American Fruit Fly, *Anastrepha fraterculus* (Wied.): Advances in Artificial Rearing, Taxonomic Status and Biological Studies. IAEA, Vienna. [IAEA-TECDOC-1064]

Mavragani-Tsipidou P, Karamanlidou G, Zacharopoulou A, Koliasis S, Kastritisis C (1992) Mitotic and polytene chromosome analysis in *Dacus oleae* (Diptera: Tephritidae). Genome 35: 374–378. doi: 10.1139/g92-056

Mendes LOT (1958) Observacões citológicas em moscas das frutas. Bragantia 17: 29–39. doi: 10.1590/S0006-87051958000100034

Palacios-Gimenez OM, Castillo ER, Martí DA, Cabral-de-Mello DC (2013) Tracking the evolution of sex chromosome systems in Melanoplinae grasshoppers through chromosomal mapping of repetitive DNA sequences. BMC Evolutionary Biology 13: 167. doi: 10.1186/1471-2148-13-167

Petit-Martín N, Vera MT, Calcagno G, Cladera JL, Segura DF, Allinghi A, Rodriguero MS, Gómez-Cendra PV, Viscarret MM, Vilardi JC (2004a) Sexual behavior and mating compatibility among four populations of *Anastrepha fraterculus* (Diptera: Tephritidae) from Argentina. Annals of the Entomological Society of America 97: 1320–1327. doi: 10.1603/0013-8746(2004)097[1320:SBAMCA]2.0.CO;2

Petit-Martín N, Vera MT, Calcagno G, Cladera JL, Vilardi JC (2004b) Lack of postmating isolation between two populations of *Anastrepha fraterculus* from different ecological regions in Argentina. In: Barnes BN (Ed.) Proceedings of the 6th International Symposium on Fruit Flies of Economic Importance, 6–10 May 2002, Stellenbosch, South Africa. Isteg Scientific Publications, Irene.

Ramirez CCL, Dessen EMB (2000) Chromosomal evidence for sibling species of the malaria vector *Anopheles cruzii*. Genome 43: 143–151. doi: 10.1139/g99-103
Sex chromosomes in mitotic and polytene tissues of Anastrepha fraterculus...

Rull J, Abraham S, Kovaleski A, Segura DF, Mendoza M, Liendo MC, Vera MT (2013) Evolution of pre-zygotic and post-zygotic barriers to gene flow among three cryptic species within the *Anastrepha fraterculus* complex. Entomologia Experimentalis et Applicata 148: 213–222. doi: 10.1111/eea.12094

Salles LAB (1995) Bioecologia e Controle da Mosca das Frutas Sul-Americana. EMBRAPA-CPACT, Pelotas, R. S., Brazil, 58 pp.

Selivon D, Perondini ALP (1997) Evaluation of techniques for C and ASG banding of the mitotic chromosomes of *Anastrepha fraterculus* (Diptera: Tephritidae). Brazilian Journal of Genetics 20: 651–654. doi: 10.1093/ajg/91.4.473

Selivon D, Perondini ALP (1998) Egg-shell morphology in two cryptic species of the *Anastrepha fraterculus* complex (Diptera, Tephritidae). Annals of the Entomological Society of America 91: 473–478.

Selivon D, Morgante JS, Perondini ALP (1997) Egg size, yolk mass extrusion and hatching behaviour in two cryptic species of *Anastrepha fraterculus* (Wied.) (Diptera, Tephritidae). Brazilian Journal of Genetics 20: 587–594.

Selivon D, Morgante JS, Perondini ALP (1999) Haldane’s rule and other aspects of reproductive isolation observed in the *Anastrepha fraterculus* complex (Diptera, Tephritidae). Genetics and Molecular Biology 22: 507–510. doi: 10.1590/S1415-47571999000400007

Selivon D, Vretos C, Fontes L, Perondini ALP (2004) New variant forms in the *Anastrepha fraterculus* complex (Diptera, Tephritidae). In: Barnes BN (Ed.) Proceedings of the 6th International Symposium on Fruit Flies of Economic Importance, 6–10 May 2002, Stellenbosch, South Africa. Isteg Scientific Publications, Irene, 253–258.

Selivon D, Perondini ALP, Morgante JS (2005a) A genetic morphological characterization of two cryptic species of the *Anastrepha fraterculus* complex. (Diptera: Tephritidae). Annals of the Entomological Society of America 98: 367–381. doi: 10.1603/0013-8746(2005)098[0367:AGCMCT]2.0.CO;2

Selivon D, Perondini ALP, Rocha LS (2005b) The mitotic chromosomes of eight species of *Anastrepha* (Diptera: Tephritidae). Neotropical Entomology 34: 273–279. doi: 10.1590/S1519-566X2005000200015

Solferini VN, Morgante JS (1987) Karyotype study of eight species of *Anastrepha* (Diptera: Tephritidae). Caryologia 40: 229–241. doi: 10.1080/00087114.1987.10797826

Steck GJ (1999) Taxonomic status of *Anastrepha fraterculus*. The South American Fruit Fly, *Anastrepha fraterculus* (Wied.): Advances in Artificial Rearing, Taxonomic Status and Biological Studies. IAEA-TECDOC-1064. International Atomic Energy Agency, Vienna, 202, 13–20.

Sumner AT (2003) Chromosomes: organization and function. Blackwell Publishing, 287 pp.

Traut W (1999) The evolution of sex chromosomes in insects: Differentiation of sex chromosomes in flies and moths. European Journal of Entomology 96: 227–235.

Traut W, Sahara K, Marc F (2008) Sex Chromosomes and Sex Determination in Lepidoptera. Sexual Development 1:332–346. doi: 10.1159/000111765

Vera MT, Cáceres C, Wornooyporn V, Islam A, Robinson AS, De La Vega MH, Hendrichs J, Cayol J-P (2006) Mating incompatibility among populations of the South American fruit fly *Anastrepha fraterculus* (Diptera: Tephritidae). Annals of the Entomological Society of America 99: 387–397. doi: 10.1603/0013-8746(2006)099[0387:MIAPOT]2.0.CO;2
Zacharopoulou A (1987) Cytogenetic analysis of mitotic and salivary gland chromosomes in the Medfly *Ceratitis capitata*. Genome 29: 67–71. doi: 10.1139/g87-011

Zacharopoulou A (1990) Polytene chromosome maps in the medfly *Ceratitis capitata*. Genome 33: 184–197.

Zacharopoulou A, Augustinos AA, Waheed A, Sayed A, Robinson AS, Franz G (2011a) Mitotic and polytene analysis of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). Genetica 139: 79–90. doi: 10.1007/s10709-010-9495-3

Zacharopoulou A, Sayed WAA, Augustinos AA, Yesmin F, Robinson AS, Franz G (2011b) Analysis of Mitotic and Polytene Chromosomes and Photographic Polytene Chromosome Maps in *Bactrocera cucurbitae* (Diptera: Tephritidae). Annals of the Entomological Society of America 104(2): 306–318. doi: 10.1603/AN10113