

Morphological Characterization of the Reproductive System of Irradiated *Anastrepha fraterculus*

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ABSTRACT: Field identification of released sterile insects is a major issue for eradication and suppression programs. Irradiated flies are normally identified by the presence of a fluorescent dye. When a fly lacks fluorescent dye, determination of gonadal state is necessary to identify between sterile or fertile flies. This is particularly relevant when population levels have decreased and identification is required to be as unequivocal as possible. Here we describe the reproductive system of irradiated *Anastrepha fraterculus* of different ages and we compare it with that of fertile flies in order to provide a diagnosis tool. Fertile and irradiated *A. fraterculus* were dissected from the day of emergence and until 15 days of age. Gross morphology was described and the gonads were measured. Germ cells were visualized in the testis. The reproductive systems of both males and females contained the same structures as other *Anastrepha* species. From day 1 to day 3, there were no detectable differences between irradiated and fertile males. The growing region encompassed half the testis total length and there was no free sperm in the seminal vesicle. On day 4 the presence of free sperm was seen in the seminal vesicle. At this stage irradiated males started differentiating from fertile ones: the growing region reduced in size and totally disappeared by day 11; sperm bundle zones occupied most of the testis; spermatids lost their triangular shape and sperm remained in the seminal vesicle without moving into the apical region. Testis length and width of irradiated males did not differ from fertile males. In females, the maturation of the ovaries involved a change in size that was more pronounced in the length of the ovary. This became noticeable at day 3. At this stage, the formation of yolk and the basal follicle began in fertile females and the oocyte had the same size as the trophocytes. From this point, the oocyte started growing. After day 8, the maturing oocyte reached its final length and the trophocytes disappeared. None of these occurred in sterile females and differences in ovary size were significantly different by day 4. We demonstrated the possibility to differentiate irradiated flies from non-irradiated ones after the latter begin the process of sexual maturation. Convenience and constraints in relation to its application as a tool for field monitoring in sterile insect release programs are discussed.

Key Words: South American fruitfly, sterile insect technique, field identification, testis, ovary

INTRODUCTION

The genus *Anastrepha* Schiner is the most diverse group of native tephritids in America with around 197 species described (Hernández-Ortiz 2003). Some of these species are of economic importance such as *A. ludens* (Loew), *A. obliqua* (Macquart), *A. striata* Schiner, *A. fraterculus* (Wiedemann), *A. suspensa* (Loew), and *A. serpentina* (Wiedemann) (Norrbon and Kim 1988).

The South American fruit fly, *A. fraterculus*, which is considered a species complex instead of a single species (Stone 1942, Morgante et al 1980, Solferini and Morgante 1987, Malavasi and Morgante 1982, Steck 1991, 1999, Selivon 1996, Goday et al. 2004, Hernández-Ortiz et al. 2004, Rocha and Selivon 2004, Selivo et al. 2005, Smith Caldas et al. 2001), is widely dis-

tributed (Salles 1995). In Argentina, is the only species of economic importance of the genus *Anastrepha*, and shares its distribution along with *Ceratitidis capitata* (Wiedemann). The sterile insect technique (SIT) is applied successfully for *Ceratitidis capitata*, and control actions are now extending to areas where *A. fraterculus* is present. This rises the need to develop control measures against *A. fraterculus* compatible with the use of SIT for *C. capitata*, being the SIT one alternative. Research efforts during the last years have been allocated to upscale the production and obtain a mass rearing protocol (Jaldo et al. 2001, Vera et al. 2007), to determine the adequate dose to induce sterility (Allinghi et al. 2007b), to assess sterile male competitiveness (Allinghi et al. 2007a), survival in the field (M. T. Vera unpublished), compatibility among populations (Petit Marty 2004ab, Vera et al. 2006), and to speed sexual maturation with the use of juvenile hormone analogues (Segura et al. 2005). The results from

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these studies encourage the implementation of SIT against this pest.

The correct identification of trapped insects represents a necessary tool in the implementation of the SIT against *A. fraterculus*. Most of field activities will be planned and directed based on these results (Guillen 1983). The identification of irradiated and wild flies implies a series of steps. Irradiated flies are dyed with a fluorescent powder before release. Population levels are monitored in the field with traps and flies captured in the traps are taken to the laboratory to determine their origin (i.e. wild or sterile) with the aid of UV lights. However, when a fly lacks fluorescent dye, it is necessary to use other tools to confirm the origin of the fly. When the population level is still high and the number of wild captures is numerous, all the undyed flies are considered wild flies in a conservative approach. When the population has been reduced, then the correct identification of the fly status is crucial since it may lead to the initiation of additional field procedures in the area where wild captures occurred. In this cases, the lack of fluorescent dye in itself is not sufficient to consider a fly as fertile and other techniques are used such as the dissection of the fly to observe the genitalia and verify the radiation damage produced in the gonads and hence confirm the origin of the fly. In the case of *C. capitata*, the variations produced by radiation in the maturation of the testes and ovaries are gradual and accentuate as flies age. This process has been well described and the development of irradiated and wild flies was compared (Guillén 1983). Once flies have reached their age of sexual maturity, it is possible to distinguish between fertile and irradiated flies representing nowadays a useful tool for field monitoring. However, when they are yet immature discrimination is not possible. Although this may represent a constrain, yet is a convenient practical tool given the fact that males are released almost at their age of sexual maturation and the damage is already noticeable. Sterile females can be misidentified as fertile immature females. In such cases, the spermathecae of the

female are also dissected to verify the presence of sperm as an indication of sexual maturity. If positive then the fly is classified as sterile. Other possible procedure is to perform a DNA analysis but this is not always possible. In cases where the genetic background of the mass-reared strain is from the target population or close to it, the identification is impossible. The use of genetic markers, such as the case of the *sergeant* mutation in some *C. capitata* strains has resulted a very interesting alternative (Niyazi et al. 2005). Yet the observation of the gonads is worldwide used for field identification.

In the genus *Anastrepha*, the study of the reproductive biology is poor and restricted to some few species. There is a limited number of contributions dealing with the anatomy of the reproductive apparatus and the development and sexual maturation of wild flies (Martínez et al. 1995, Ramírez et al. 1996, Martínez and Hernández-Ortiz 1997) and a detailed study of the spermathecae and ventral receptacle can be found for *A. suspensa* (Fritz and Turner 2002). Descriptions of the changes in the gonads along the process of sexual maturation either in fertile or irradiated flies are not available for *A. fraterculus*.

In this work we compare the development of the reproductive systems of irradiated *A. fraterculus* with fertile flies in order to provide a tool for field identification.

MATERIAL AND METHODS

Flies were obtained from the stock colony maintained at the EEAOC. This colony was established in 1997 from infested guavas collected in the vicinity of Tafí Viejo, Tucumán province (northwest Argentina). Rearing conditions were those proposed by Jaldo et al. (2001). Once pupae were obtained and aged until 5-7 days, they were sent by ground (15 hours) to Centro Atómico Ezeiza, National Atomic Energy Commission, in Buenos Aires province. Fruit fly pupae were irradiated with a Cobalt⁶⁰ source at 70 Gy (maximum dose

did not exceeded 75 Gy) two days prior to emergence, according to Allinghi et al. (2007b). Upon emergence, flies were sorted by sex and 10 flies/sex were placed in plastic containers with water and food (a mixture of corn protein, hydrolyzed yeast protein, sugar, vitamins and amino acids). The same procedure was performed on fertile, non-irradiated flies.

One container of each category (irradiated males and females and fertile males and females) was taken at random and flies preserved in 70% ethanol daily from the date of emergence until 15 days of age. Flies were dissected and the length and width of the gonads measured. Testes were transferred to a slide and stained with aceto-orcein to visualize germ cells (Guillén 1983).

In addition, 20 fertile females were mated to 20 sterile males and the percentage of egg hatch determined. The egg hatch obtained from fertile males and females were used as control and the presence of sperm in the spermathecae of females was determined.

RESULTS

Male reproductive system. The reproductive system of *Anastrepha fraterculus* males is similar to that of other species of *Anastrepha* (Martínez & Hernández-Ortiz 1997). It is composed of paired testes, vas deferens and seminal vesicles, an ejaculatory duct, several pairs of accessory glands, a sperm pump, an eadeagal gland and an aedeagus. The testes exhibited oval shape and yellow color. In the sexually mature male, the testis can be divided into zones or regions which contain the different cells involved in spermatogenesis (Fig 1a). The apical region shows the growth zone formed by the apical spermatogonial cells followed by the primary and secondary spermatocytes. This zone is continued with a zone full of spermatids where the cells are grouped forming triangle like areas that can be visualized by a characteristic staining pattern. This is followed by the sperm

bundles zone formed by groups of sperm of filamentous form. The seminal vesicle is located in the basal region and will contain free sperm in sexually mature males. The free sperm, like in the bundles sperm zone, exhibits filamentous form which makes difficult to differentiate the head from the tail.

Maturation of testes in sterile males. The spermatogenesis in *A. fraterculus* males exposed to radiation in the pupal stage is halted. Aging and sexual maturation of males reveals changes in the organization of the testis and enables the differentiation between irradiated and fertile males.

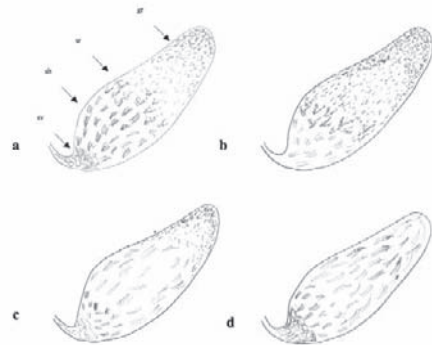


Figure 1: Line drawings of the testes of *A. fraterculus* indicating the variation on irradiated flies from different ages: **a** mature fertile male; **b** immature irradiated male (0 to 4 days); **c** irradiated male (5 to 7 days); **d** irradiated male (8 to 15 days). gr = growth region; sr = spermatid region; sb = sperm bundles; sv = seminal vesicle.

Day 0. The growth zone reaches half the length of the testis. The presence of spermatids and sperm bundles can be observed, but free sperm is not detected. No remarkable differences are detected between testes of fertile and irradiated adults (Fig. 1b, Fig 2a and b) and these observations remain until day 4.

Day 4. The seminal vesicle and the presence of free sperm became noticeable. At this moment remarkable differences are found between the growth zone of irradiated and fertile males. The staining of irradiated testis is weaker because it

does not have the positive reaction of the aceto-orcein within the chromatic material of the sperm nucleus (Guillen 1983). In fertile males, the different zones (growth region, spermatid region, and bundle sperm region) have a similar size; whereas in testes of irradiated males the proportion of each region is modified being the growing zone reduced in size.

Between day 5 and 6 the growth zone is reduced in size and the staining is not so pronounced. The spermatids are difficult to detect and the groups have lost their triangle like shape, becoming rounded. The sperm bundle region encompasses a greater proportion of the testis while the zone with free sperm is restricted to the seminal vesicle.

As of day 7 there is a great accumulation of sperm bundle lessening the growth zone to a small apical region (Fig 1c, Fig 2c and d).

At day 11 the growth zone has totally disappeared and the entire testicle is invaded by sperm bundles. The region with free sperm re-

mains restricted to the seminal vesicle, without advancing towards apical regions (Fig 1d, Fig 2e and f).

None of these changes are reflected in changes in size of the testicles (Table 1).

Female reproductive system. Like the males of *A. fraterculus*, female reproductive system of this species resembles that of other species in this genus (Martínez and Hernández-Ortiz 1997, Cruz et al. 1996, Martínez et al. 1995). It is composed of paired lateral ovaries, oviducts and accessory glands, a common oviduct, three spermathecae, a vagina and the aculeus. The ovaries consist of polytrophic ovarioles formed by a terminal filament, a germarium, a vitellarium and the calyx. The calyx is a prolongation of the vitellarium connecting itself to the lateral oviducts. The vagina has two different regions: the bursa copulatrix, located in the anterior part, and the vaginal duct that is continued with the aculeus. The bursa copulatrix is formed by a cuticular extension

Table 1: Testis length and width of irradiated and fertile *A. fraterculus* males (mean \pm se).

Age (days)	Testis length (cm)		Testis width (cm)	
	Irradiated	Fertile	Irradiated	Fertile
0	0,75 \pm 0,04a	0,70 \pm 0,02a	0,29 \pm 0,01a	0,29 \pm 0,01a
1	0,76 \pm 0,03a	0,69 \pm 0,02a	0,30 \pm 0,01a	0,31 \pm 0,02a
2	0,79 \pm 0,03a	-	0,33 \pm 0,01a	-
3	0,73 \pm 0,03a	0,77 \pm 0,02a	0,31 \pm 0,01a	0,30 \pm 0,01a
4	0,80 \pm 0,02a	0,80 \pm 0,03a	0,30 \pm 0,01a	0,30 \pm 0,01a
5	-	0,81 \pm 0,03a	-	0,34 \pm 0,01a
6	0,77 \pm 0,03a	0,83 \pm 0,04a	0,28 \pm 0,01a	0,31 \pm 0,01a
7	0,81 \pm 0,02a	0,87 \pm 0,02a	0,31 \pm 0,01a	0,34 \pm 0,01a
8	0,82 \pm 0,03a	0,85 \pm 0,05a	0,28 \pm 0,01a	0,34 \pm 0,01a
9	0,77 \pm 0,03a	0,88 \pm 0,02a	0,29 \pm 0,01a	0,33 \pm 0,01a
10	0,76 \pm 0,05a	0,97 \pm 0,02a	0,28 \pm 0,01a	0,33 \pm 0,01a
11	0,80 \pm 0,03a	0,99 \pm 0,02a	0,25 \pm 0,01a	0,30 \pm 0,01a
12	0,81 \pm 0,02a	0,89 \pm 0,04a	0,25 \pm 0,01a	0,32 \pm 0,02a
13	0,84 \pm 0,03a	0,95 \pm 0,02a	0,24 \pm 0,01a	0,35 \pm 0,01a
14	0,80 \pm 0,03a	0,95 \pm 0,02a	0,24 \pm 0,01a	0,34 \pm 0,01a
15	0,78 \pm 0,04a	0,89 \pm 0,04a	0,28 \pm 0,02a	0,34 \pm 0,01a

For each variable, means followed by the same letter in the same row are not statistically different (*t* test, *p* < 0.05).

of the ventral wall called the ventral receptacle. In the third basal region of the vaginal duct, two cuticular pieces are located forming the signum (Fig 3). The spermathecae are sclerotized capsules surrounded by a glandular epithelium that continue in a spermathecal duct which opens into the dorsal side of the bursa copulatrix.

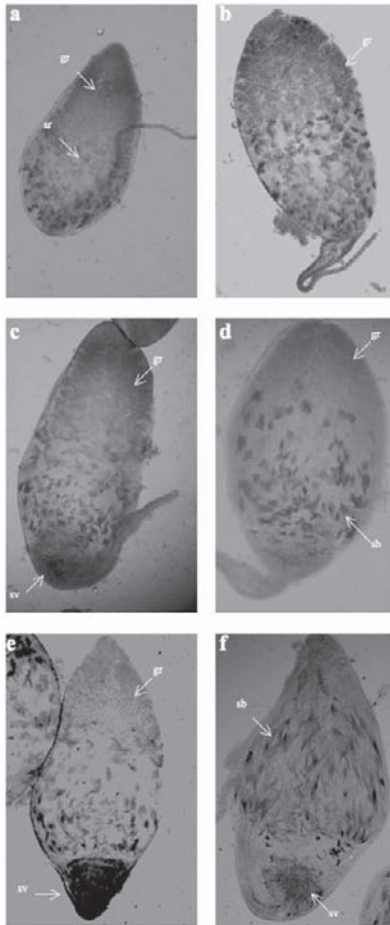


Figure 2: Testis of fertile and irradiated *A. fraterculus* males of different ages. **a** immature fertile male (0 days); **b** immature irradiated male (0 days); **c** fertile male of 6 days; **d** irradiated male of 5 to 7 days; **e** completely mature fertile male (more than 8 days); **f** irradiated male (8 to 15 days). gr = growth region; sr = spermatids region; sb = sperm bundles; sv = seminal vesicle.

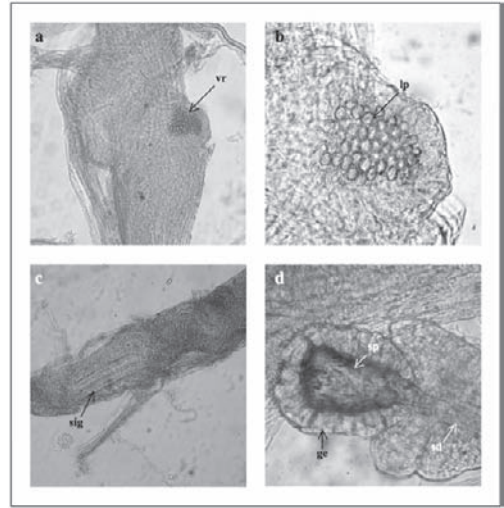


Figure 3: The reproductive system of *A. fraterculus* females. **a** bursa copulatrix with ventral receptacle; **b** ventral receptacle with lobular papillae; **c** signum; **d** spermathecae with spermathecal glandular epithelium and spermathecal duct. ge = glandular epithelium; sd = spermathecal duct; sig = signum; sp = spermatheca; vr = ventral receptacle.

Comparative studies of the genus show that the reproductive system varies mainly in the number of ovarioles, the morphology of the ventral receptacle, the signum, the spermathecae and the aculeus (Martinez & Hernandez-Ortiz 1997). In *A. fraterculus* the average number of ovarioles is between 20 and 24; the ventral receptacle is elongated with the presence of lobular papillae; the signum is short and more or less sclerotized; and the spermathecae presented an oval pear like form rather than rounded. The aculeus length is about 1.6 to 1.8 mm, with the presence of rounded teeth occupying the apical half and ends in a typical constriction.

Maturation of ovaries in sterile females compared to fertile females. Unlike the development of the testicles of the male, in the female the ovarian maturation implies a variation in size. This variation allows the characteristic differentiation between ovaries of irradiated and wild females. Since the radiation is applied at the pupal stage of the fly, and the oogenesis begins after the emergence of the

Table 2: Ovary length and width of irradiated and fertile *A. fraterculus* females (mean \pm se).

Age (days)	Ovary length (cm)		Ovary width (cm)	
	Irradiated	Fertile	Irradiated	Fertile
0	0.42 \pm 0.02a	0.40 \pm 0.01a	0.33 \pm 0.01a	0.33 \pm 0.01a
1	0.41 \pm 0.01a	0.45 \pm 0.01a	0.34 \pm 0.01a	0.35 \pm 0.02a
2	0.45 \pm 0.02a	-	0.33 \pm 0.01a	-
3	0.54 \pm 0.02a	0.51 \pm 0.03a	0.37 \pm 0.01a	0.37 \pm 0.02a
4	0.54 \pm 0.04a	0.76 \pm 0.05b	0.37 \pm 0.01a	0.48 \pm 0.02b
5	0.63 \pm 0.03a	0.94 \pm 0.09b	0.36 \pm 0.01a	0.57 \pm 0.04b
6	0.67 \pm 0.04a	1.37 \pm 0.15b	0.38 \pm 0.01a	0.85 \pm 0.11b
7	0.74 \pm 0.03a	1.78 \pm 0.10b	0.36 \pm 0.01a	1.11 \pm 0.11b
8	0.78 \pm 0.05a	1.96 \pm 0.13b	0.35 \pm 0.03a	1.14 \pm 0.12b

For each variable, means followed by a different letter in the same row are statistically different (*t* test, $p < 0.05$).

adult, the inhibition of the ovarian development is remarkable.

Days 0-2. The ovary of both the irradiated and fertile females has a minimum development at the time of the emergence and the vitellarium has not developed. The growth is very slow and differences between fertile or irradiated females are not observed (Fig 4a and e).

Day 3. As of the third day the vitellarium and basal follicle begins to grow in fertile females. As

a consequence there is an increase in the size of the ovary. These changes are not registered in irradiated females (Figure 4b and f).

Days 4-5. The growth of the ovary continues in fertile females. The oocytes have the same size than the trophocytes. These changes are not registered in the irradiated females and differences in ovary size became significant between the two types of females (Table 2, *t* test, $P < 0.05$).

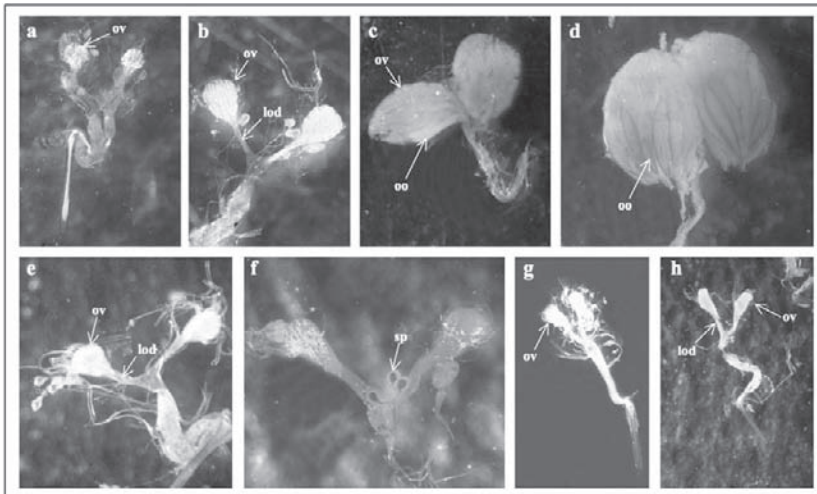


Figure 4: Ovary development of fertile and irradiated *A. fraterculus* females of different ages. **a** immature fertile female (0 days); **b** fertile female of 3 days; **c** fertile female of 6 days; **d** completely mature fertile female (more than 8 days); **e** immature irradiated female (0 days); irradiated male of 3 days; **f** irradiated female of 6 days; **g** irradiated female of 10 days. lod = lateral oviduct, oo = oocyte, ov = ovary; sp = spermatheca.

Days 6-7. In fertile females the basal follicle increases its length; the oocyte increases its size in comparison to trophocytes due to the vitellogenin uptake; the ovary becomes three times longer. These changes are not observed on the sterile females (Figure 4c and g).

Days 8-15. The growth of the ovary is accelerated as the oocyte uptakes the vitellogenin and the trophocytes are absorbed. At this stage the oocytes reach their maximum size being five times longer than on day 0. These changes are not observed on the irradiated females (Figure 4d and h).

The ovarian maturation is not uniform and it is possible to find females of the same age at different degrees of maturity.

Full sterility was confirmed in irradiated males as percent of egg hatch of fertile females mated to irradiated males was 0% while for the control it was 85%

CONCLUSION

We demonstrated the possibility to differentiate irradiated flies from non-irradiated ones after the latter begin the process of sexual maturation. Given that for other *Anastrepha* species, flies are released at 4 – 5 days of age, irradiated males could be easily differentiated from fertile yet immature males. Although the irradiated females could be misclassified as immature females, the search for sperm presence in the spermathecae can help in some situations. Hence, with the proper caution, the descriptions presented in our work can be considered a tool for field monitoring of undyed flies to confirm sterile or fertile fly status in areas with sterile insect release programs.

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REFERENCES

- Allinghi, A., G. Calcagno, N. Petit-Marty, P. Gomez Cendra, D.F. Segura, M. T. Vera, J.L. Cladera, C. Gramajo, E. Willink, and J. Vilardi. 2007. Compatibility and competitiveness of a laboratory strain of *Anastrepha fraterculus* (Diptera: Tephritidae) after irradiation treatment. *The Florida Entomologist* 90: 27-32.
- Allinghi, A., M. C. Gramajo, E. Willink and J. C. Vilardi. 2007. Induction of sterility in *Anastrepha fraterculus* (Diptera: Tephritidae) by means of gamma radiation. *The Florida Entomologist* 90: 96-102.
- Fritz, A. H. and F. R. Turner. 2002. A light and electron microscopical study of the spermathecae and ventral receptacle of *Anastrepha suspensa* (Diptera: Tephritidae) and implications in female influence of sperm storage. *Arthropod Structure and Development* 30: 293-313.
- Goday, C., Selivon, D., Perondini, A. L. P., Greciano, P. G. & Ruiz, M. F. 2006 Cytological characterization and ribosomal DNA location in *Anastrepha* species. *Cytogenetics and Genome Research* 114: 70-76.
- Guillén Aguilar, J. C. 1983. Manual para la diferenciación de moscas del Mediterráneo *Ceratitis capitata* (Wied.). silvestres (fértiles) de moscas irradiadas (estériles). Programa Mosca del Mediterráneo, D.G.S.V., México.
- Jaldo, H. E., M. C. Gramajo, and E. Willink. 2001. Mass rearing of *Anastrepha fraterculus* (Diptera: Tephritidae): a preliminary strategy. *The Florida Entomologist* 84: 716-718.
- Hernández-Ortiz, V. 2003. Familia Tephritidae: Clasificación actual, relaciones filogenéticas y distribución de taxa americanos. *Memorias del XV Curso Internacional Sobre Moscas de la Fruta*. Metapa de Domínguez, Chiapas, México pp.11-23.
- Hernández-Ortiz, V., J. A. Gómez-Anaya, A. Sánchez, B. A. Mc Pheron and M. Aluja. 2004. Morphometric analysis of Mexican and South American populations of the *Anastrepha fraterculus* complex (Diptera: Tephritidae) and recognition of a distinct Mexican morphotype. *Bulletin of Entomological Research* 94: 487-499.
- Malavasi, A., and J. S. Morgante 1982. Genetic variation in natural populations of *Anastrepha* (Diptera: Tephritidae). *Revista Brasileira de Genética* 5: 253-278.
- Martínez, I., and V. Hernández-Ortiz 1995. Desarrollo y maduración sexual en *Anastrepha serpentina* (Wiederman) (Diptera: Tephritidae). *Acta Zoológica Mexicana* (n.s.) 65: 75-88.
- Martínez, I., and V. Hernández-Ortiz. 1997. Anatomy of the reproductive system in six *Anastrepha* species and comments regarding their terminology in Te-

- phritidae (Diptera). Proceedings of the Entomological Society of Washington 99: 272-743.
- Morgante, J. S., A. Malvasi, and G. L. Bush. 1980. Biochemical systematics and evolutionary relationships of Neotropical *Anastrepha*. Annals of the Entomological Society of America 73: 622-630.
- Niyazi, N., C. Cáceres, A. Delprat, V. Wornoyaporn, E. Ramirez santos, G. Franz, and A. S. Robinson. 2005. Genetics and Mating Competitiveness of *Ceratitis capitata* (Diptera: Tephritidae) Strains Carrying the Marker *Sergeant*, *Sr2*. Annals of the Entomological Society of America 98: 119-125.
- Norrbom A. L., and K. C. Kim. 1988. A list of the reported host plant of the species of *Anastrepha* (Diptera: Tephritidae). U.S. Dep. Agric., Animal and Plant Health Inspection Service, Plant Protection and Quarantine, Washington, DC. 114 pp.
- Petit-Marty, N., M. T. Vera, G. Calcagno, J. L. Cladera, D. F. Segura, A. Allinghi, M. Rodriguez, P. Gómez Cendra, M. M. Viscarret, and J. C. Vilardi. 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.
- Petit-Marty, N., M. T. Vera, G. Calcagno, J. L. Cladera, and J. C. Vilardi. 2004b. Lack of post-mating isolation between two populations of *Anastrepha fraterculus* from different ecological regions in Argentina, pp. 79-82. In Proceedings of the 6th International Fruit Fly Symposium, 6-10 May 2002, Stellenbosch, South Africa.
- Ramírez Cruz, A., V. Hernández-Ortiz and I. Martínez. 1996. Maduración ovárica en la "Mosca de la Guayaba" *Anastrepha striata* Shiner (Diptera: Tephritidae). Acta Zoológica Mexicana (n.s.) 69: 105-116.
- Rocha, L. S. and Selivon, D. 2004. Studies on highly repetitive DNA in cryptic species of the *Anastrepha fraterculus* complex. Proceedings of the 6th International Symposium on Fruit Flies of Economic Importance. B. N. Barnes [ed.], Isteg Scientific Publications, Irene, South Africa. 415-418.
- Segura, D. F., M. T. Vera, M. F. Rodriguez, M. E. Utgés and J. L. Cladera. 2005. Maduración sexual en machos de *Anastrepha fraterculus*. Proceedings of the VI Congreso Argentino de Entomología. 271.
- Segura, D. F., N. Petit-Marty, R.B. Sciarano, M.T. Vera, G. Calcagno, A. Allinghi, P. Gómez Cendra, J.L. Cladera y J.C. Vilardi. Lekking behavior of *Anastrepha fraterculus* (Diptera: Tephritidae). The Florida Entomologist 90: 154-162.
- Selivon, D. 1996. Estudio sobre diferenciação populacional em *Anastrepha fraterculus* (Wiederman) (Diptera: Tephritidae). Ph D. dissertation, Instituto de Biociencias, Universidade de Sao Paulo, Sao Paulo, Brazil.
- Selivon, D., Perondini, A. L. P. & Morgante, J. S. 2005. 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.
- Smith-Caldas, M. R. B., McPherson, B. A., Silva, J. G. & Zucchi, R. A. 2001. Phylogenetic relationships among species of the *fraterculus* group (*Anastrepha*: Diptera: Tephritidae) inferred from DNA sequences of mitochondrial *cytochrome oxidase 1*. Neotropical Entomology 30: 565-573.
- Solferini, V. N. and J. S. Morgante. 1987. Karyotype study of eight species of *Anastrepha* (Diptera: Tephritidae). Caryologia. 40: 229-241.
- Steck, G. J. 1991. Biochemical systematic and population genetic structure of *Anastrepha fraterculus* and related species (Diptera: Tephritidae). Annals of the Entomological Society of America 84: 10-28.
- Steck, G. J. 1999. Taxonomic status of *Anastrepha fraterculus*, pp 13-20. In 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 pp.
- Stone, A. 1942. The fruit flies of the genus *Anastrepha*. U.S. Dep. Agric. Misc. Publ. No. 439.
- Vera, M. T., C. Cáceres, V. Wornoyaporn, A. Islam, A. S. Robinson, M. H. de la Vega, J. Hendrichs, y J-P Cayol. 2006. Mating Incompatibility Among Populations of the South American Fruit Fly *Anastrepha fraterculus* (Wied.) (Diptera: Tephritidae). Annals of the Entomological Society of America 99: 387-397.
- Vera, M. T., S. Abraham, A. Oviedo and E. Willink. 2007. Demographic and quality control parameters of *Anastrepha fraterculus* (Diptera: Tephritidae) artificial rearing. The Florida Entomologist 90: 53-57.