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THE CYTOTAXONOMY OF THE LARVAE OF SOME MEXICAN FRUIT FLIES IN THE GENUS *ANASTREPHA* (TEPHRITIDAE, DIPTERA)[1]

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INTRODUCTION

During a study of host relations of the Mexican fruit fly, *Anastrepha ludens* (Loew), difficulty was encountered in obtaining positive identification of tephritid larvae recovered from field infested fruit. Existing larval keys based on morphological characters (e.g. Phillips, 1946) were not adequate for differentiating between some closely related Mexican representatives of the family. Accurate identifications could only be obtained by rearing larvae to the adult stage. This proved time consuming and increased the chance of losing valuable host records when larvae failed to mature. For this reason, a preliminary cytotaxonomic study was made on some of the more common fruit infesting *Anastrepha* found in Mexico to see if chromosome morphology would be of any use in identifying larvae.

This method of species differentiation is not new. It has been used for many years by plant taxonomists to establish a more natural classification within certain groups of plants (Darlington, 1956). Its application to animal taxonomy has been somewhat restricted owing, in part, to the difficulties of handling some animal material. Many of these difficulties have now been eliminated through the use of new and improved techniques. A great deal is now known about the cytogenetics of animals and particularly of the insects. White (1954) has presented an excellent summary of our present knowledge of comparative cytology and its application to the study of animal evolution and taxonomy, while Patterson and Stone (1952) have

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discussed chromosome evolution in the genus *Drosophila* in detail.

Several investigations have been made concerning the cytology of the Tephritidae. Metz (1916), after investigating the chromosomes of *Euresta melanogaster* Loew [probably *Dyseuaresta mexicana* (Wied.)], concluded that flies of the family Tephritidae were not suitable for detailed chromosome studies. He did state, however, that this species appeared to have a haploid number of six, though he presented no figures. Keuneke (1924), on the other hand, obtained clear metaphase complements from *Tephritis arnicae* L., which had an interesting XO instead of the normal XY sex determining mechanism found in most Diptera. This configuration resulted in a diploid number of 11 in the male and 12 in the female. A reduced number of chromosomes has also been reported for *A. ludens* by Emmart (1935). A haploid number of 5 was found in spermatogenesis, though the findings of the present study do not support these observations for this species.

In more recent studies, Frizzi and Springhetti (1953) described the karyotype of the olive fruit fly, *Dacus oleae* Gmel., as having a haploid number of 6. This same modal number of 6, which seems to be common in most higher Diptera (White, 1954), has also been reported for six out of seven species of Queensland Dacinae by Davis (1955). One species had a haploid number of 7. Davis apparently encountered some technical difficulty, as he was unable to observe any details in the morphology of the chromosomes. Mendes (1958), however, was able to find distinct morphological differences in the chromosomes of two species of Brazilian tephritids, *Anastrepha fraterculus* (Wied.) and *Ceratitis capitata* (Wied.), both of which had the characteristic haploid number of 6. His description of the karyotype of *A. fraterculus* is of particular interest in that he found morphologically differentiated sex chromosomes. These distinguishable heterochromosomes were not found in the Mexican population of this species by the author. The importance of these differences will be discussed later.

Methods

The chromosomes of the following nine species of tephritids were analyzed during the course of this investigation: *Anastrepha ludens* (Loew); *A. zuelaniae* Stone; *A. fraterculus* (Wied.); *A. mombinpraeoptans* Seín; *A. distincta* Greene; *A. spatulata* Stone; *A. striata* Schiner; *A. serpentina* (Wied.); and *A. aphelocentema* Stone.

Larvae were reared from field collected fruit which was held in

racks over moist sand in well ventilated wooden boxes. A sample of each collection was reared to the adult stage to confirm preliminary identification. Some species, such as *A. ludens, A. mombinpraeoptans, A. fraterculus,* and *A. serpentina,* were also reared on a laboratory diet of ground carrots and yeast (Finney, 1956). Eggs of these species were collected from females which were induced to oviposit in wax impregnated cheese cloth shells, formed and pigmented to represent fruit (McPhail and Guiza, 1956). For most cytological investigations only larvae in the prepupal stage were used. Other larval stages had suitable but fewer metaphase plates.

The supraoesophageal and suboesophageal ganglion were used for the evaluation of all karyotypes with the exception of those of *A. spatulata* whose host and larva are not known, though the adult is collected in large numbers at certain times of the year. Adult spermatogonial metaphase plates were therefore used to establish the karyotype of this species. Attempts were made to obtain suitable oögonial metaphase plates, but these were unsuccessful. Larval and adult tissues were dissected out in normal saline (0.75 NaCl) and transferred immediately to a saturated solution of coumarin in distilled water for six to ten minutes following the technique of Sharma and Bal (1953) and Manna (1956). The majority of the species, including those treated statistically, were pretreated in coumarin for seven minutes. Care had to be taken not to exceed ten minutes as chromosomes tended to become condensed and unsuitable for study (Fig. 8). However, the shortening effect of coumarin, if used judiciously, makes it possible to obtain well flattened metaphase plates that show the structural features of the chromosomes distinctly. Without the use of coumarin, chromosomes remained bunched and no structural detail could be observed.

Tissue that had been pretreated in coumarin was then transferred either directly into aceto-orcein (2% orcein in 45% glacial acetic acid) for 30 minutes to one hour, or hydrolyzed in 1N HCl for 30 seconds to one minute at room temperature prior to staining. Hydrolysis improved the over-all qualities of the preparations. Squashes were then made in a drop of aceto-orcein on albuminized slides using coverslips treated with a silicon anti-wetting agent, such as Desicote[3], and made permanent following the simple and rapid quick-freeze method of Schultz *et al.* (1949), as modified by Conger and Fairchild (1953).

[3]Beckman Desicote 18772, Beckman Scientific Instruments Division, Fullerton, California.

Photomicrographs were taken on 35 mm. Adox KB-14 film with the aid of a Micro Ibso attachment using a Zeiss 90x apochromatic oil immersion objective of NA 1.3 and a Leitz 10x ocular. All films were developed with Neofin blau.[4] Prints were made on No. 5 Kodabromide paper. Final magnification of all prints used in statistical analysis was 3750x.

Measurements of chromosome lengths were made from photomicrographs after the method of Boyes and Wilkes (1953), as modified by Robertson (1957), on *A. fraterculus, A. mombinpraeoptans,* and *A. distincta* whose karyotypes could not be distinguished by visual inspection. All measurements were carried out to the nearest 0.5 mm. and the percent of the total complement length of each chromosome pair calculated. A sine transformation was then made on the resulting percentages to reduce any correlation between the means and their corresponding variances (Snedecor, 1956). An analysis of variance was made on both the longest and the shortest chromosome pairs which were the only chromosomes that could be consistently identified with certainty. There was not sufficient evidence to reject the null hypotheses that in these three species the mean lengths of the long chromosomes are the same or that the mean lengths of the short chromosomes are the same. (Short chromosome: $F=2.28<F_{.05(2,\ 69)}=3.13$. Long chromosome: $F=2.51<F_{.05(2,\ 69)}=3.13$.) The karyotypes of *A. fraterculus, A. mombinpraeoptans,* and *A. distincta* therefore could not be distinguished from one another on the basis of mensural observations.

Description of Karyotypes

The terminology used throughout the following descriptions and discussion of metaphase chromosomes is the same as that outlined by White (1957) except for the terms used to designate the position of the kinetochore or centromere. Major chromosome arms (MCA) were considered only when they were clearly visible in the metaphase plate as a point of flection or bend in the chromosome. This does not rule out the possibility of missing a short arm that would be visible only in anaphase configuration. Such chromosomes would be considered acrokinetic. A metakinetic chromosome has two major arms with the kinetochore located near the center. Acrokinetic chromosomes have the kinetochore located near the end of the chromosome giving the appearance at metaphase of being one-armed. Dot chromosomes are treated as though acrokinetic, although in future investigations

[4]Neofin blau, Tetenal-Photowerk, Hamburg, Germany.

these may prove to be metakinetic as has been shown in the IV chromosomes of *Drosophila melanogaster* Meigen (Kaufmann, 1934).

The locality and the host fruit from which the karyotype was described is also included in anticipation that future studies may uncover chromosomal polymorphism or sibling species within this genus. Whenever observable sex chromosomes were present, the heterogametic sex was always the male, as is normal for Diptera. This characteristic was checked by studying spermatogonial metaphase plates of adult males.

Anastrepha ludens (Loew)
Figure 1

The diploid number is 12. The MCA number is also 12 in both sexes as all chromosomes are acrokinetic. No secondary constrictions were noted. The male has a small dot Y chromosome about 1/4 to 1/3 the length of the rod-shaped X chromosome. Forty-seven metaphase plates were photographed from 16 larvae. A total of over 300 larval brain squashes from various localities were studied but not photographed.

Source of cytological material. Cuernavaca, Morelos, Mexico.

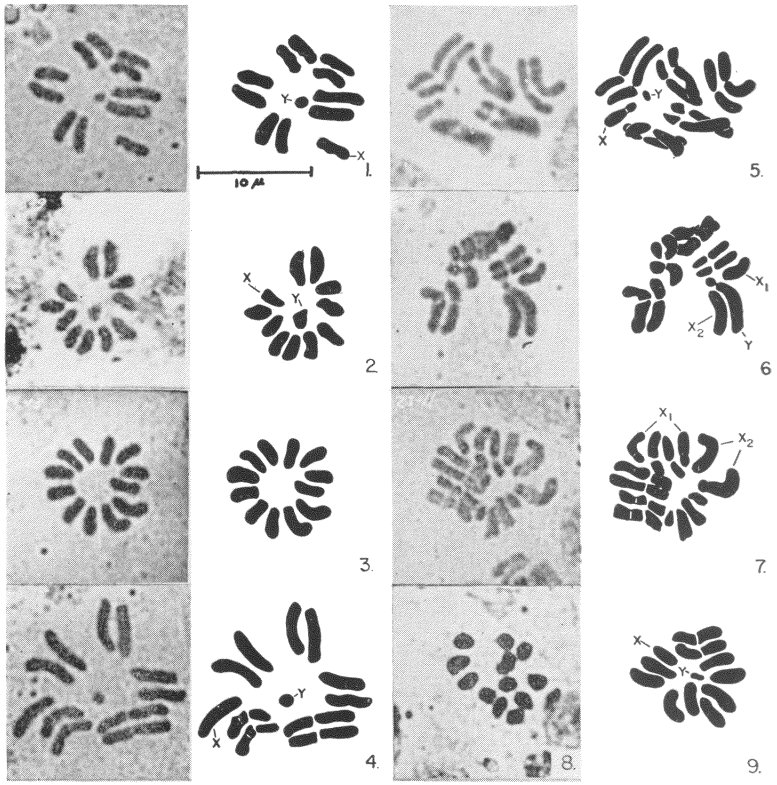
Host plant. Mango (*Mangifera indica* L.).

Collections of this species were made throughout the year from many host plants other than mango. These included sapote domingo (*Mammea americana* L.); avocado (*Persea americana* Mill.); yellow chapote (*Sargentia greggii* S. Wats.); white sapote (*Casimiroa edulis* Llave and Lex.); sweet orange (*Citrus sinensis* (L.) Osbeck); sour orange (*Citrus aurantium* L.); and grapefruit (*Citrus grandis* (L.) Osbeck).

Collections were also made in the states of Colima, Veracruz, Chiapas, Tamaulipas, Jalisco, Mexico, and Michoacan. No variation in the karyotype from these localities was noted.

Anastrepha zuelaniae Stone
Figure 2

The diploid number is 12 in both sexes. The MCA number is 12 with all chromosomes acrokinetic. No secondary constrictions were noted. The male has a rod-shaped Y chromosome about 2/3 the length of the X chromosome. Thirty-nine metaphase plates were photographed from nine larvae. Sixty-three larval brain squashes were studied but not photographed.



Figs. 1-9. Metaphase plates from the brain of: (1) *Anastrepha ludens;* (2) *A. zuelaniae;* (3) *A. fraterculus* (*A. mombinpraeoptans* and *A. distincta* are closely similar); (4) *A. striata;* (5) *A. aphelocentema;* (6) *A. serpentina* ♂ showing X_1X_2Y sex chromosomes; (7) *A. serpentina* ♀ showing $X_1X_1X_2X_2$ sex chromosomes. (8) Extreme contraction of chromosomes of *A. ludens* resulting from extended pre-treatment in coumarin. (9) Spermatogonial metaphase plate from testes of adult *A. spatulata.* (Magnification of all plates 1500×)

Source of cytological material. Tamazunchale, San Luis Potosi, Mexico.

Host plant. Volador (*Zuelania guidonia* Britt. and Millsp.).

Collections were made from late May to early July 1957. The larvae were found only in the fruit of the above host plant.

Anastrepha fraterculus (Wiedemann)
Figure 3

The karyotype of this species cannot be distinguished from those of *A. mombinpraeoptans* and *A. distincta* at metaphase. The diploid number in both sexes is 12. The MCA number is also 12, with all chromosomes acrokinetic. No morphologically differentiated heterochromosomes (XY) or secondary constrictions were observed. One hundred twenty-eight metaphase plates were photographed from 32 larvae. Over 250 larval brain squashes were studied but not photographed.

Source of cytological material. Monte Blanco, Veracruz, Mexico.

Host plant. Rose apple (*Eugenia jambos* L.).

Collections were made from early July to late August, 1957.

Anastrepha mombinpraeoptans Seín
cf. Figure 3 *(A. fraterculus)*

The karyotype of this species cannot be distinguished from those of *A. fraterculus* and *A. distincta.* The MCA number in both sexes is 12. No morphologically differentiated heterochromosomes (XY) or secondary constrictions were observed. Forty-six metaphase plates were photographed from 14 larvae. More than 150 larval brain squashes were studied but not photographed.

Source of cytological material. Cocoyoc, Morelos, Mexico.

Host plant. Hog plum (*Spondias mombin* L.).

Collections were made from September through October, 1957. Larvae which were reared from mangoes *(M. indica)* collected from Veracruz from June through July, 1957 were also studied.

Anastrepha distincta Greene
cf. Figure 3 *(A. fraterculus)*

This species cannot be distinguished from either *A. mombinpraeoptans* or *A. fraterculus.* It has an MCA number of 12 in both sexes. No morphologically differentiated heterochromosomes (XY) or sec-

ondary constrictions were observed. Forty metaphase plates were photographed from seven larvae. Over 80 larval brain squashes were studied but not photographed.

Source of cytological material. Cocoyoc, Morelos, Mexico.

Host plant. Inga inicuil Cham. & Schlecht.

Collections were made from August through October, 1957.

Anastrepha spatulata Stone
Figure 9

The diploid number is 12 in the male. The MCA number is 14, as one pair of chromosomes is metakinetic. The male has a small rod-shaped Y chromosome about 1/4-1/3 the length of the X chromosome. Only eight adult males were available for study; three of these gave suitable preparations for analysis. From these, four spermatogonial metaphase plates were obtained. Several photographs were taken of one particularly good metaphase plate that regrettably did not lie in one plane. A drawing made from a composite photograph is therefore presented in Figure 9.

Source of cytological material. Canyon de Lobos, Morelos, Mexico.

Host plant. Unknown.

Adults were collected from January to March 1957 in glass traps, using fermenting brown sugar as a lure (Baker *et al.,* 1944). Each adult was maintained for two weeks in the laboratory on a standard laboratory diet (Rhode, 1957) prior to examination.

Anastrepha striata Schiner
Figure 4

The diploid number is 12 in both sexes. The MCA number is 16. Two pairs of chromosomes are metakinetic. The shorter metakinetic chromosomes have secondary constrictions on their longest arms. The dot Y chromosome is present in the male and is about 1/4 to 1/3 the length of the X chromosome. Seventy-one plates were photographed from 20 larvae. Approximately 175 larval brain squashes were studied but not photographed.

Source of cytological material. Tequila, Jalisco, Mexico.

Host plant. Guava (*Psodium guajava* L.).

Collections were made from Tequila during the month of August 1957. Other collections were made from guava in the states of Veracruz, Chiapas, Morelos, and Michoacan in 1956 and 1957 with no variation in chromosome morphology noted.

Anastrepha aphelocentema Stone
Figure 5

The diploid number in both sexes is 12. The MCA number is 22 as there are five pairs of metakinetic autosomes. The dot Y chromosome is about 1/4 the length of the rod-shaped X chromosome which has a proximal secondary constriction. Twenty-five metaphase plates were photographed from five larvae. Approximately 35 larval brain squashes were studied but not photographed.

Source of cytological material. Tamazunchale, San Luis Potosi, Mexico.

Host plant. Socavite (*Lucuma standleyana* Pittier).

This species was studied only from the area surrounding Tamazunchale during the months May through July, 1957.

Anastrepha serpentina (Wiedemann)
Figures 6 and 7

The diploid number in the male is 11 and in the female 12. The male (Fig. 6) has an MCA number of 20 as there are four metakinetic pairs of autosomes, one of which in some metaphase complements has a secondary constriction on its longest arm (not visible in photomicrograph of the male metaphase plate). There are also three heteromorphic acrokinetic sex chromosomes designated X_1X_2Y. Both the X_1, which is the shortest, and the X_2 have small proximal secondary constrictions which are not always visible. The long Y chromosome is easily distinguished as it has a short arm separated from the rest of the chromosome by what has been interpreted as the kinetochore, though future studies may show this to be a secondary constriction. The female karyotype (Fig. 7) has an MCA number of 20 with four pairs of metakinetic autosomes and two acrokinetic pairs of sex chromosomes. Since the X_1 is much shorter than the X_2 (X_1 ca. 2/3 X_2), these two chromosomes are easily differentiated. Thirty-nine metaphase plates were photographed from 13 larvae. Approximately 75 larval brain squashes were studied but not photographed.

Source of cytological material. Monte Blanco, Veracruz, Mexico.

Host plant. Mamey (*Calocarpum mammosum* (L.) Pierre).

Collections were also made from the states of San Luis Potosi and Morelos in 1957. It should be noted here that one collection of this species was made from Tapachula, Chiapas in 1956 from mamey. Samples taken from this population did not demonstrate the com-

pound[5] sex determining mechanism. The diploid number was 12 and the MCA number was 24. No heteromorphic chromosomes were present. However, the collection was made at the beginning of the study when methods were not yet perfected for making temporary squashes permanent, and before photographic equipment was available. For this reason, only a few drawings were made. This difference in karyotype morphology may have resulted from inadequate technique, but more likely it represents a different species.

Discussion

To date at least 19 species representing 10 genera in the family Tephritidae have been investigated cytologically by several authors. It is apparent even from the few species thus far studied that a great deal of chromosomal variation exists within the family. Such variation not only includes characteristic positions of the kinetochore, secondary constrictions, and chromosome length, but also involves differences in chromosome number and sex determining mechanisms as well. These differences can be put to good use in the identification of immature forms and may possibly aid in establishing phylogenetic relationships. It must be stressed that cytotaxonomy is seldom if ever a "solve all" method of identification, and it is not surprising that three species of *Anastrepha* show no distinct chromosome differences. It is probable that as more species in this genus are investigated chromosome patterns will be found similar to the ones reported here as morphologically distinct. A combination of several criteria, including chromosomal variations, gross morphology of the larvae, and various ecological aspects of the species in question, may therefore be necessary before accurate identification can be made.

With such limitations in mind, the following key is presented as a tentative means of separating the larvae of six of the nine Mexican Tephritidae investigated cytologically so far. Due to the similarities of some female karyotypes, the key is based on the chromosome morphology of the male karyotype whenever it is known. This makes it advisable to study at least eight larvae (assuming a 1:1 sex ratio) in a given collection to be fairly certain that all are not of the same sex. No suitable means has yet been found to determine the sex of immature forms in this family without resorting to karyotype analysis.

[5]Schrader's (1928) terminology is followed here. A compound sex determining mechanism is one in which the X or the Y is represented by more than one element in contrast to a multiple sex determining mechanism in which there is an adherence of chromosomes belonging to different pairs.

Cytotaxonomic Key to the Metaphase Plates of the Common Species of Mexican Anastrepha (males only)

1. All chromosomes evidently acrokinetic (MCA=12) 2
— All chromosomes not all acrokinetic (MCA=13 or more) 4
2. No heteromorphic chromosome pairs present at metaphase *A. mombinpraeoptans, A. fraterculus, A. distincta*
— Heteromorphic chromosome pair present 3
3. Small dot Y chromosome present (Y<0.5 X) ..... *A. ludens*
— Rod-shaped Y chromosome present but shorter than X chromosome (Y>0.5 X) ..... *A. zuelaniae*
4. MCA=14 to 16 5
— MCA=17 to 22 6
5. MCA=14 ..... *A. spatulata*
— MCA=16 ..... *A. striata*
6. MCA=20, X_1X_2Y sex mechanism present (2n=11) ..... *A. serpentina*
— MCA=22, secondary constriction on X chromosome ..... *A. aphelocentema*

Though cytological evidence *per se* is not always suitable for distinguishing some species of Tephritidae, it can support and elucidate certain phylogenetic relationships within the group. The cytogenetics of certain Diptera has been extensively studied in the past three or four decades so that many of the mechanisms of chromosome evolution in this group are now fairly well understood (Patterson and Stone, 1952; White, 1954; da Cunha, 1960). Since the number of species of Tephritidae so far investigated is extremely limited, it is as yet impossible to determine any conclusive generic or interspecific relationships, though some interesting possibilities do appear, particularly at the interspecific level in the genus *Anastrepha.*

It is possible that *A. mombinpraeoptans, A. distincta, A. ludens, A. zuelaniae,* and the Mexican and Brazilian forms of *A. fraterculus* may form part of a chromosome complex representing a subgenus or species group within the genus *Anastrepha.* This is supported by the similarity in the morphology of the adults of these species. The difference in karyotypes between the Mexican form of *A. fraterculus* reported here and the Brazilian population described by Mendes is interesting since this difference may represent a case of chromosomal polymorphism or, more likely, sibling species. Biological data support the latter (A. C. Baker *et al.,* 1944; E. W. Baker, 1945) in that slight but consistent morphological differences exist in the adults

from these widely separated areas. Such differences could be attributed to geographical variation; however, they also have distinctly different host preferences. The Brazilian population has a wide host range and is a destructive pest of citrus, while the Mexican population is of no economic importance, infesting the rose apple and only occasionally the guava.

The case of the compound sex determining mechanism encountered in *A. serpentina* is also interesting as this type of system appears to be rare in Diptera. Dobzhansky (1935) reported X_1X_2Y system in *Drosophila miranda* Dobzh. believing it to be an example of determinate disjunction. Cooper (1946), however, clearly showed that a X_1YX_2 trivalent was actually formed during meiosis. Boyes (1952) found the same type of trivalent formed in *Hylemya fugax* (Meig.). It is possible that *A. serpentina* may also produce a trivalent, but the preparations of gonadal tissue using the squash technique were not suitable for establishing the interaction of the three sex chromosomes.

The different chromosome number of $2n=10$ reported by Emmart (1935) for *A. ludens* probably resulted from an incorrect interpretation of chromosome morphology in her study of meiosis in pupal and adult testes. In the present study, larvae as well as adults were studied from Cuernavaca, Morelos, the same locality from which Emmart collected most of her material. A diploid number of 12 was always recorded. Meiotic figures in the testes without exception had a characteristic haploid number of 6.

Little can be said about the other *Anastrepha* species at this time. It is likely that a more thorough investigation of the karyotypes within this genus will uncover many interesting phylogenetic relationships which can now only be hinted at on the basis of the present study.

Spermatogonial metaphase plates can be put to good use in evaluating the chromosome morphology of those species of tephritids whose larvae are unknown, as in the case of *A. spatulata,* or whose larvae cannot be readily maintained in the laboratory. Such determinations can also be used to obtain tentative identification of larvae collected for the first time, and whose chromosome morphology is known only from previously captured adults.

From the cytological data thus far accumulated for the family Tephritidae, it appears that the variation between karyotypes is sufficient to warrant more attention from the taxonomists of this group. New methods of handling animal chromosomes, such as the many pre-treatments now available, followed by simplified squash tech-

niques, have eliminated many arguments against inclusion of cytological data in taxonomic studies. Cytological information in many cases offers the taxonomist who is interested in establishing better phylogenetic relationships a tool which can often supplement and strengthen his conclusions based on morphological data, as well as provide information not available by any other means. In the family Tephritidae this seems particularly true.

Summary

The karyotypes of nine species of *Anastrepha* (Tephritidae, Diptera) are described on the basis of mitotic metaphase morphology. The species include *A. ludens, A. fraterculus, A. distincta, A. mombinpraeoptans, A. zuelaniae, A. spatulata, A. striata, A. serpentina,* and *A. aphelocentema.* All species have a diploid number of 12, with the exception of the males of *A. serpentina* where an X_1X_2Y sex determining mechanism resulted in a diploid number of 11. Only six of the nine species investigated could be identified on the basis of chromosome morphology. It is suggested that *A. distincta, A. mombinpraeoptans,* and Mexican *A. fraterculus,* which have cytologically indistinguishable karyotypes, as well as *A. ludens, A. zuelaniae,* and the Brazilian form of *A. fraterculus* may represent part of a chromosome complex within the genus *Anastrepha.* The differences between the karyotypes of the Brazilian and Mexican populations of *A. fraterculus,* along with differences in external morphology and biology, suggest that these two forms may represent sibling species. In general, it is concluded that the metaphase chromosomes of the family Tephritidae can be used for critical cytotaxonomic and phylogenetic studies.

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LITERATURE CITED

BAKER, A. C., W. E. STONE, C. C. PLUMMER, and M. MCPHAIL.
1944. A review of studies on the Mexican fruitfly and related Mexican species. U. S. Dept. Agr. Misc. Publ. 531:1-155.

BAKER, E. W.
1945. Studies on the Mexican fruitfly known as *Anastrepha fraterculus*. Jour. Econ. Ent. 38:95-100.

BOYES, J. W.
1952. A multiple sex-chromosome mechanism in a root maggot. Jour. Hered. 43:194-199.

BOYES, J. W., and A. WILKES.
1953. Somatic chromosomes of higher Diptera. I. Differentiation of tachinid parasites. Can. Jour. Zool. 31:125-165.

CONGER, A. D., and L. M. FAIRCHILD.
1953. A quick-freeze method for making smear slides permanent. Stain Technol. 28:281-283.

COOPER, K. W.
1946. The mechanism of non-random segregation of sex chromosomes in male *Drosophila miranda*. Genetics 31:181-194.

DA CUNHA, A. B.
1960. Chromosomal variation and adaptation in insects. Ann. Rev. Ent. 5:65-110.

DARLINGTON, C. D.
1956. Chromosome Botany. G. Allen and Unwin Ltd. 186 pp.

DAVIS, J. J.
1955. A note on chromosomes of some Queensland *Dacinae* (Trypetidae, Diptera). Queensland Jour. Agr. Sci. 12:161-173.

DOBZHANSKY, TH.
1935. *Drosophila miranda,* a new species. Genetics 20:377-391.

EMMART, E. W.
1935. Studies of the chromosomes of *Anastrepha* (Diptera: Trypetidae). I. The chromosomes of the fruit-fly, *Anastrepha ludens* Loew. Proc. Ent. Soc. Wash. 37:119-135.

FINNEY, G. L.
1956. A fortified carrot medium for mass-culture of the Oriental fruit fly and certain other tephritids. Jour. Econ. Ent. 49:134.

FRIZZI, G. and A. SPRINGHETTI.
1953. Prime ricerche citogenetiche sul "*Dacus oleae* Gmel.". Ricerca Sci. 23:1612-1620.

KAUFMANN, B. P.
1934. Somatic mitoses of *Drosophila melanogaster*. Jour. Morph. 56: 125-155.

KEUNEKE, W.
1924. Über die Spermatogenese einiger Dipteren. Z. Wissenschaftliche Biol. 1:357-412.

MANNA, G. K.
1956. Handling human chromosomes by a coumarin technic. Stain Technol. 31:45-50.

MCPHAIL, M., and F. E. GUIZA.
1956. An oviposition medium for the Mexican fruit fly. Jour. Econ. Ent. 49:570.

MENDES, L. O. T.
1958. Observaçoes citológicas em "moscas das frutas". Bragantia 17: 29-39.

METZ, C. W.
1916. Chromosome studies on the Diptera. II. The paired association

of chromosomes in the Diptera, and its significance. Jour. Exptl. Zool. 21:213-279.

PATTERSON, J. T., and W. S. STONE.
1952. Evolution in the Genus *Drosophila*. The MacMillan Co. 610 pp.

PHILLIPS, V. T.
1946. The biology and identification of trypetid larvae (Diptera: Trypetidae). Mem. Amer. Ent. Soc. No. 12.

RHODE, R. H.
1957. A diet for Mexican fruit flies. Jour. Econ. Ent. 50:215.

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

SCHRADER, F.
1928. Die Geschlechtschromosomen. Berlin: Borntraeger.

SCHULTZ, J., R. C. MACDUFFEE, and T. F. ANDERSON.
1949. Smear preparations for the electron microscopy of animal chromosomes. Science 110: 5-7.

SHARMA, A. K., and A. K. BAL.
1953. Coumarin in chromosome analysis. Stain Technol. 28:255-257.

SNEDECOR, G. W.
1956. Statistical Methods. 5th ed. Iowa State College Press. 534 pp.

WHITE, M. J. D.
1954. Animal Cytology and Evolution. 2nd ed. Cambridge Univ. Press. 454 pp.
1957. Cytogenetics and systematic entomology. Ann. Rev. Ent. 2:71-90.