Odonatologica 14 (2): 115-125 *June 1, 1985*

OBSERVATIONS ON THE CHROMOSOMES OF FOUR SOUTH AMERICAN LIBELLULIDAE (ANISOPTERA)

L.M. MOLA and S.S. AGOPIAN
Laboratorio de Genética, Facultad de Ciencias Exactas y Naturales,
Universidad de Buenos Aires, AR-1428 Buenos Aires, Argentina

Received January 15, 1984 / Accepted March 4, 1984

The meiotic sequence and the mitotic complements were examined in the males of *Tauriphila risi* Martin, *Perithemis icteroptera* (Sel.), *P. mooma* Kirby and *Miathyria marcella* (Sel.) (n=13, 2n=25). An *m*-pair is lacking in *P. icteroptera* only. The chromosome morphology at diakinesis (ring-shaped bivalents) and prophase II is discussed. Precocious disjunction is reported in *P. mooma* and *T. risi*. Variation in size of the *m*-bivalent in *P. mooma* and *M. marcella* could reflect a polymorphic and polytypic situation, respectively.

INTRODUCTION

According to SANTOS (1981) 314 species of Libellulidae are known in South America; about 90 of them were cytogenetically studied (CUMMING, 1964; KIAUTA & BOYES, 1972; FERREIRA et al., 1979; KIAUTA, 1979; GONI & ABENANTE, 1982; SOUZA BUENO, 1982).

This is the family with the greatest number of species in the Anisoptera suborder. The typical chromosome features of the order and of this family in particular are: haploid number of 13, presence of *m*-chromosomes and sex determining system of the XO/XX type, the male being the heterogametic sex.

Some cytological studies have attempted to describe odonate chromosome morphology and meiotic behaviour (e.g. OKSALA, 1943; FERREIRA et al., 1979; GAMA et al., 1981; SOUZA BUENO, 1982). Precocious disjunction of the *m*-bivalent has been reported in various species of Zygoptera and Anisoptera (KIAUTA, 1970, 1971, 1972; KIAUTA & BOYES, 1972; KIAUTA & VAN BRINK, 1978). Variation of the relative and absolute size of the *m*-chromosomes from species to species and within a single species in different geographical

populations, has been reported by KIAUTA (1968); intrapopulation variation has also been described (OGUMA & ASANA, 1932; in KIAUTA, 1968). Save for a note by AGOPIAN & MOLA (1984), this is the first chromosome study on Odonata from Argentina. The species dealt with in the present report are *Perithemis mooma, P. icteroptera, Miathyria marcella* and *Tauriphila risi.* The metaphase I description and the somatic chromosome number of *P. mooma* (CUMMING, 1964; FERREIRA et al., 1979; GONI & ABENANTE, 1982; SOUZA BUENO, 1982) and *M. marcella* (CUMMING, 1964; FERREIRA et al., 1979) were previously reported.

This paper describes the somatic chromosome number and the complete meiotic sequence of the four species, as well as the interpretation of some meiotic stages; inter- and intra-specific variation of the *m*-chromosomes is also discussed.

MATERIAL AND MEDHODS

All specimens were captured when in flight over the field. They were etherized, and the testes were subsequently removed and placed in 50% insect saline made according to DRETS & STOLL (1974) for 10 minutes, as hypotonic treatment; after this they were fixed in 3:1 absolute ethanol : glacial acetic acid. Some specimens were fixed in the field, without hypotonic treatment. No differences between both methods were observed.

Slides were made by the squash method. To facilitate cell spreading, a piece of testis was placed in 60% acetic acid for 2 or 3 minutes. Stain and squashes were performed in 2% propionic haematoxylin with 1% ferric citrate as mordant (SAEZ, 1960).

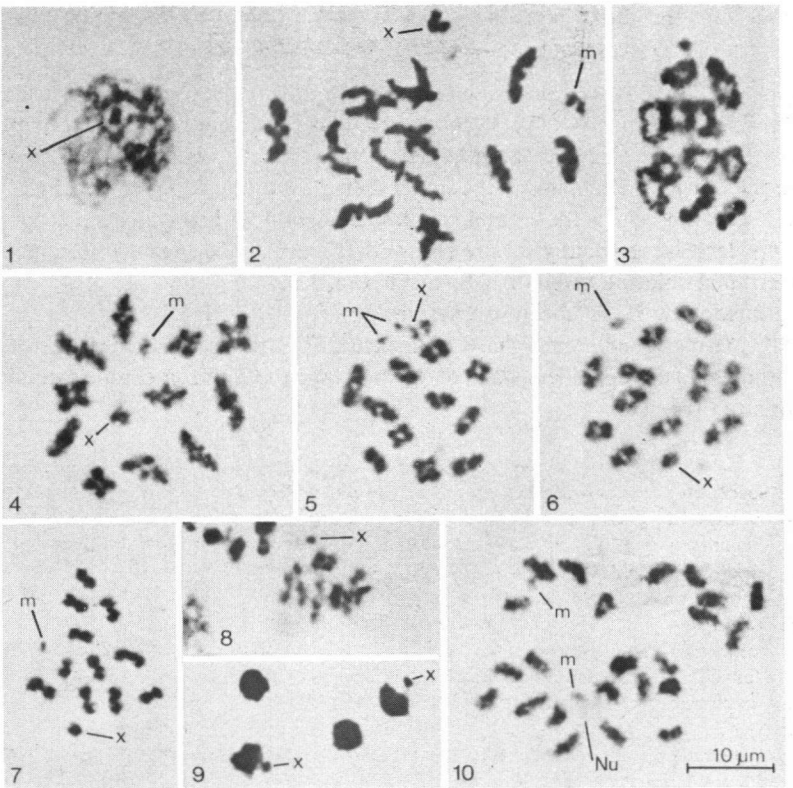
The specimens were identified by Dr A. Rodrigues Capítulo, Instituto de Limnología, Facultad de Ciencias Naturales y Museo, La Plata, Provincia de Buenos Aires, Argentina. They are kept in the said institute; their collection numbers are stated in brackets in the appropriate sections below.

TAURIPHILA RISI MARTIN

Figures 1-10

Material. — 6 ♂, Buenos Aires, Argentina, Jan., 1982 (1030, 1031, 1033-1035, 1037); — 1 ♂, Tigre, Prov. Buenos Aires, Argentina, Feb., 1982 (1036); — 2 ♂, Colonia, Uruguay, Feb., 1982 (1032, 1038). — [237 cells studied]

2n=25, n=13 (*m*). — The X chromosome is positively heteropycnotic until pachytene (Fig. 1) and in the remaining stages it is isopycnotic (Fig. 2). From diplotene (Fig. 2) onwards the *m*-bivalent is the smallest element, and the X chromosome is the second smallest one. The 12 bivalents always have a single subterminal chiasma (Fig. 2). A very interesting stage in which nearly all the bivalents are approximately "ring-shaped" follows (Fig. 3). At middle diakinesis (Fig. 4) the bivalents are more condensed than in the previous stage and again show their cross-shape. In two out of 69 diakinetic figures, referable to different specimens from the city of Buenos Aires, the *m*-chromosomes were unpaired (Fig. 5). The first prometaphase bivalents gradually decrease in size, except for the *m*-bivalent (Fig. 6).



Figs 1-10: Male germ cell chromosomes of *Tauriphila risi* Martin: (1) Pachytene; — (2) Late diplotene; — (3) "Ring-shaped" bivalents stage; — (4) Middle diakinesis; — (5) Late diakinesis, with precociously segregated *m*-bivalent; — (6) Prometaphase I; — (7) Prophase II; — (8) Early anaphase II; — (9) Telophase II; — (10) Spermatogonial prometaphase (Nu: nucleolus).

In second prophase the chromosomes show a charateristic ϵ-shape (Fig. 7) and both in prophase II and metaphase II, 13 chromosomes are seen; this indicates the postreductional mode of division of the X chromosome. At early anaphase II, the X chromosome migrates precociously to one pole (Fig. 8) and remains separated from the rest in telophase II (Fig. 9).

In spermatogonial prometaphase and metaphase there are 25 chromosomes, the *m*-chromosomes can be easily recognized (Fig. 10). On the other hand, the X chromosome is recognizable in only a few cells.

No differences in chromosome behaviour were found among the individuals from different localities.

MIATHYRIA MARCELLA (SELYS)

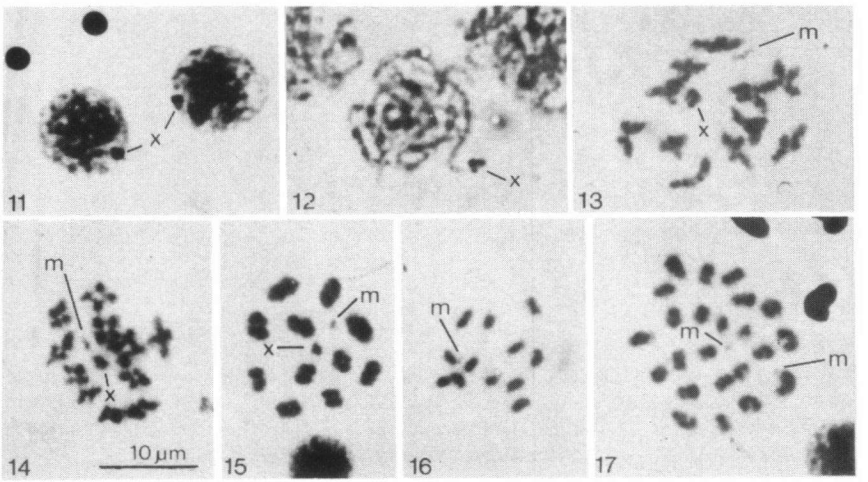
Figures 11-17

Material. — 9 ♂, Buenos Aires, Argentina, March, 1982 (1045-1053). — [209 cells studied]

2n=25, n=13 (*m*). — The X chromosome is positively heteropycnotic until pachytene (Figs 11-12). At late diplotene, the *m*-bivalent is always less stained than the other bivalents and is the smallest element of the complement (Fig. 13). The X chromosome is the second smallest element and the bivalents (except for the *m*) decrease gradually in size (Figs 14-15). As a rule, a single terminal or subterminal chiasma occurs per bivalent (Fig. 13).

In metaphase II, 13 chromosomes are observed (Fig. 16).

At spermatogonial metaphase there are 25 chromosomes, the pair of *m* chromosomes are again less stained. At this stage the X chromosome cannot be recognized (Fig. 17).



Figs 11-17: Male germ cell chromosomes of *Myathyria marcella* Selys: (11) Leptotene; — (12) Pachytene; — (13) Late diplotene; — (14) Diakinesis; — (15) Metaphase I; — (16) Metaphase II; — (17) Spermatogonial metaphase.

PERITHEMIS ICTEROPTERA (SELYS)

Figures 18-28

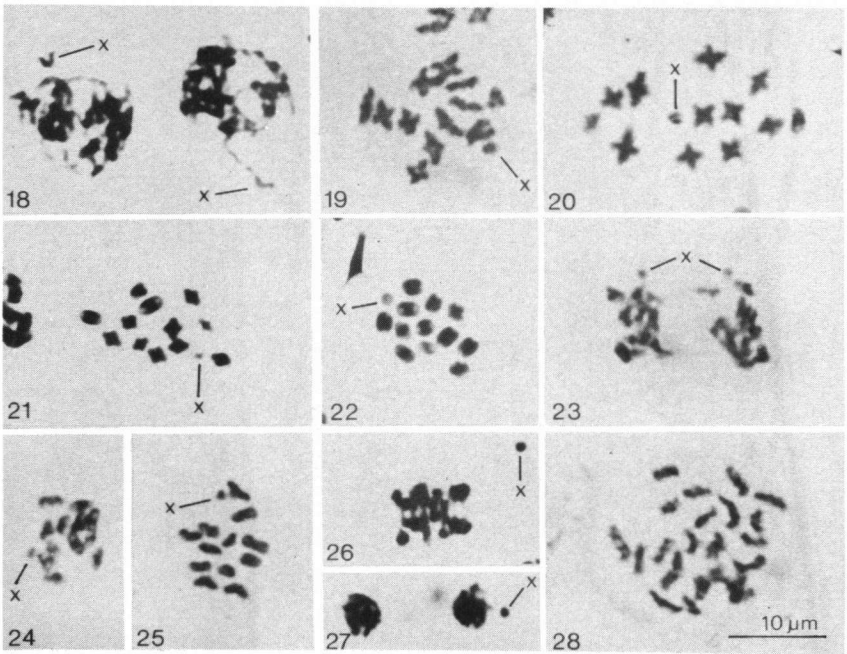
Material. — 6 ♂, Tigre, Prov. Buenos Aires, Argentina, Feb., 1982 (1039-1044). — [377 cells studied]

2n=25, n=13. — The X chromosome is isocyclic throughout the prophase I stages (Fig. 18, 19 and 20); it is the smallest of the complement, having approximately half the size of the smallest bivalent (Fig. 21). The bivalents are of

gradually decreasing size and show a single medial or subterminal chiasma (Fig. 19). In some cells, the X chromosome can be seen as negatively heteropycnotic during late diakinesis and metaphase I (Figs 21-22), while it is isopycnotic in the rest of the cells and also in the following stages. At anaphase I, the X chromosome divides equationally and simultaneously with the autosomes (Fig. 23).

There are 13 chromosomes at prophase II and metaphase II (Figs 24-25). As in *T. risi,* the X chromosome is the first to migrate to one pole and remains separated from the rest in telophase II (Figs 26-27).

There are 25 chromosomes at spermatogonial prometaphase, but the X chromosome can not be distinguished (Fig. 28).



Figs 18-28: Male germ cell chromosomes of *Perithemis icteroptera* Selys: (18) Pachytene; — (19) Late diplotene; — (20) Diakinesis; — (21) Late diakinesis; — (22) Metaphase I; — (23) Late anaphase I; — (24) Prophase II; — (25) Metaphase II; — (26) Early anaphase II; — (27) Telophase II; — (28) Spematogonial prometaphase.

PERITHEMIS MOOMA KIRBY

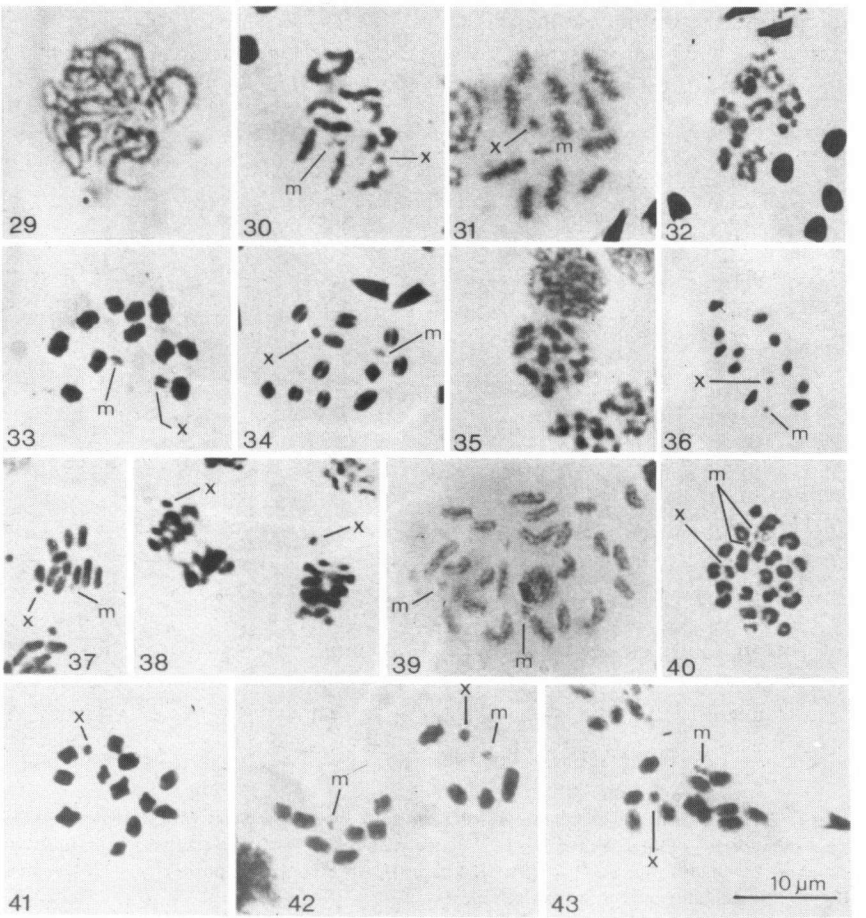
Figures 29-43

Material. — 8 ♂, Tigre, Prov. Buenos Aires, Argentina, Feb., 1982 (1056-1063); — 2 ♂, Buenos Aires, Argentina, March, 1982 (1054, 1055). — [556 cells studied]

2n=25, n=13 (*m*). — This observation is in agreement with reports of

CUMMING (1964), FERREIRA et al., (1979), GOÑI & ABENANTE (1982) and SOUZA BUENO (1982).

In our material, from leptotene to pachytene the X chromosome is slightly positively heteropycnotic as can be seen in Figure 29, or isopycnotic as in the subsequent stages. This chromosome is the second smallest of the complement (Figs 30-31, 33-34). At diplotene and early diakinesis the bivalents have a single



Figs 29-43: Male germ cell chromosomes of *Perithemis mooma* Kirby: (29) Pachytene; — (30) Late diplotene; — (31) Early diakinesis; — (32) "Ring-shaped" bivalents stage; — (33) late diakinesis; — (34) Metaphase I; — (35) Prophase II; — (36) Metaphase II (polar view); — (37) Metaphase II (lateral view); — (38) Early and late anaphase II; — (39) Spermatogonial prometaphase; — (40) Spermatogonial metaphase; — (41) Diakinesis without *m*-bivalent; — (42) Diakinesis with precociously disjuncted *m*-bivalent; — (43) Diakinesis with deeply stretched *m*-bivalent.

terminal or subterminal chiasma (Figs 30-31). The stage with the "ring-shaped" bivalents (Fig. 32), as it occurs in *T. risi,* was also seen. At diakinesis and metaphase I, the *m*-bivalent is negatively heteropycnotic and the smallest of the complement (Figs 33-34).

Second division stages are shown in Figures 35 to 38. Thirteen chromosomes can be counted in all metaphase II cells. In early anaphase II the X chromosome migrates precociously to one pole (Fig. 38).

At spermatogonial prometaphase, both chromatids lay parallel in the 25 chromosomes (Fig. 39). At metaphase, this is only evident in some chromosomes, while in the others the chromatids seem to be attached by one end (Fig. 40).

In one specimen of the Tigre population there is no *m*-bivalent; all 12 bivalents seen are of gradually decreasing size and the smallest one is larger than the X chromosome, which remained constant in size (Fig. 41).

In another individual of the same population, 14 elements were observed in 50% of 70 late diakinesis cells studied. The two smallest elements look like univalents and are nearly equal in size, shape and chromatic features (negative heteropycnosis), each having approximately half the size of the *m*-bivalent (Fig. 42). In very few late diakinesis cells the *m*-bivalent is stretched (Fig. 43).

DISCUSSION

Table I shows the karyotypic features of the species studied in the three genera treated in this paper.

The males of *Tauriphila risi* and *Perithemis mooma* have a single chiasma per bivalent, as is common in Odonata. In spite of this, in these species at early diakinesis the bivalents seem to have two chiasmata (Figs 3, 32). A similar stage was depicted in *Orthemis ferruginea* and *Erythrodiplax umbrata* (FERREIRA et al., 1979), *Heteragrion* "b", *Dasythemis minki* and *Erythrodiplax famula lativittata* (SOUZA BUENO, 1982), among others. While in the first species it was claimed that "two chiasmata occur in at least eight bivalents", in the others, since the authors did not give information on numbers of chiasmata per bivalent, we assume that a single chiasma occurs, as is the rule.

Considering the size and degree of condensation of the bivalents, in comparison with the other prophase stages, we are of the opinion that, in our species, this stage could be early diakinesis with a single chiasma per bivalent. The cross shape of the bivalents at diplotene is lost and the chromatids remain terminally joined in such a manner that the bivalents acquire a ring shape at early diakinesis, returning again at late diakinesis to that characteristic cross shape. The meaning of this structural change during the meiotic process is still unknown and further studies are necessary to understand this strange behaviour.

Precocious disjunction of the *m*-bivalent generally occurs in only a small percentage of the diakinesis-metaphase plates (KIAUTA, 1970, 1971, 1972;

KIAUTA & BOYES, 1972). However, in a male of *Macrothemis declivata* (KIAUTA & BOYES, 1972) this percentage is approximately 33% and in *Perithemis tenera* KIAUTA & VAN BRINK (1978) observed that it "often segregates precociously".

The small percentage (3% approx.) of cells in *T. risi* (Fig. 5), which show precocious disjunction is in agreement with the cases reported and could be considered to have limited effects on fertility.

On the other hand, the high frequency of precocious disjunction (50%) found at diakinesis in one male of *P. mooma* (Figs 42-43) could be due to desynapsis, taking into account the cells with the *m*-bivalent far stretched (Fig. 43). But since it was not possible to obtain earlier stages, the possibility of asynapsis can not be dismissed. If the *m*-univalents migrate randomly, the fertility of this individual will be reduced by 25%.

In all the species studied, at prophase II the chromosomes have a characteristic ϵ-shape (Figs 7, 24, 35). This stage was also observed by OKSALA (1943) in the genus *Aeshna*. According to his analysis three "constrictions" can be seen, the central one being a "half chiasma" and the other two being centromers.

At present, however, there is some evidence of the polycentric condition of the chromosomes in *Enallagma cheliferum* (GAMA et al.,1981) and in our opinion the half chiasma of Oksala's drawings could represent a partial association of the two chromatids, that remain joined by one of their telomeric regions.

Generally the *m*-bivalents do not show any differential meiotic behaviour and can only be distinguished because they are clearly smaller than the other bivalents of the complement (CUMMING, 1964) and because in many cases they are positively or negatively heteropycnotic at diakinesis and metaphase I (KIAUTA, 1968). So to give the name of *m*-bivalent to the smallest bivalent of the complement in each species, depends on the author's judgement.

As can be seen in Table I, all populations of *M. marcella* have the same haploid number and presence of *m*-chromosomes. Although differences were found regarding the size of the *m*-bivalent between the population treated in this paper and that reported by FERREIRA et al. (1979), in both populations the X chromosome is approximately of the same length. But, while in the two Brazilian males, the *m*-bivalent is isopycnotic and larger than the X chromosome, in the Argentinian sample the *m*-bivalent at metaphase I appears negatively heteropycnotic and rather smaller than the X (Fig. 15). We agree with FERREIRA et al. (1979) that the *m*-pair of their material is only "slightly smaller" than the other chromosomes and we believe that in this case these chromosomes should not be named *m*.

The differences between the populations could reflect a polytypic situation, though the possibility that we and FERREIRA et al. (1979) have dealt with two different subspecies can not be laid aside. This situation was seen in *Calopteryx virgo* (KIAUTA, 1968) where differences in the size of the *m*-bivalent were found

Table I
Cytological data of the genera *Miathyria, Tauriphila* and *Perithemis*

Taxa	n (♂)	Sex determination	*m*	Locality	References
MIATHYRIA					
marcella	13		+	Bolivia	CUMMING, 1964
	13	XO	+	Brazil	FERREIRA et al., 1979
	13	XO	+	Argentina	this paper
TAURIPHILA					
australis	13		+	Bolivia	CUMMING, 1964
azteca	13		+	Mexico	CRUDEN, 1968
risi	13	XO	+	Argentina	this paper
	13	XO	+	Uruguay	this paper
PERITHEMIS					
sp.	13	XO	—	Bolivia	CUMMING, 1964
cornelia	13	XO	—	Bolivia	CUMMING, 1964
domitia	13		+	Jamaica	CUMMING, 1964
electra	13	XO	—	Bolivia	CUMMING, 1964
icteroptera	13	XO	—	Argentina	this paper
lais	9	XO	—	Bolivia	CUMMING, 1964
	9	XO	—	Brazil	FERREIRA et al., 1979
	9	XO	+	Brazil	SOUZA BUENO, 1982
mooma	13	XO	+	Bolivia	CUMMING, 1964
	13	XO	+	Brazil	FERREIRA et al., 1979
	13	XO	+	Uruguay	GOÑI & ABENANTE, 1982
	13	XO	+	Brazil	SOUZA BUENO, 1982
	13	XO	+	Argentina	this paper
seminole	13		+	U.S.A.	CUMMING, 1964
tenera	13	XO	+	U.S.A.	KIAUTA & VAN BRINK, 1978

among *C. v. virgo, C. virgo japonica* and *C. virgo padana.*

Variation in the size of the *m*-chromosomes among specimens of the same population was reported by OGUMA & ASANA (1932) (in KIAUTA, 1968), and the same polymorphic situation was found in the Tigre population of *P. mooma.* This polymorphism plus the existence of precocious disjunction of the *m*-bivalent in another individual could indicate a sort of instability, at least for the *m*-chromosomes, in this population. In spite of this, all specimens studied from different populations are rather homogeneous in their chromosome characteristics at metaphase I.

The two studied species of *Perithemis* have similar chromosome features, but they can be distinguished because *P. icteroptera* has no *m*-bivalent.

As apparent from Table I, the presence or absence of *m*-chromosomes is characteristic of each species or, as in the case of *P. lais,* it is characteristic of geographical populations; it is not related to any given genus (KIAUTA, 1968).

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Dr J.H. HUNZIKER, Lic. C.A. NARANJO and Lic. C.J. BIDAU for having read the manuscript and for contributing many valuable suggestions. They are thankful to Dr A. RODRIGUES CAPITULO for the taxonomic identification of all specimens and to Ing. J.A. ROUSSILIAN for his collaboration in field collecting.

The research was supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), the Subsecretaría de Ciencia y Tecnología (SUBCYT), PNUD-UNESCO (RLA 78/024 No. 27) and the Universidad de Buenos Aires, to Professors Hunziker, Naranjo and L. POGGIO.

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