On the Cytology of *Umbonia crassicornis* (Amyot & Serville) (Homoptera: Membracidae: Hoplophorioninae)[1]

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ABSTRACT

Karyotype of the Membracid *Umbonia crassicornis* is 9 II + *XO*; 2n = 20 in female, 2n = 19 in male. It is presumably a fusion derivative from the basic Membracid 10 II + *XO* Accordingly, one of the autosomal pairs of *U. crassicornis* is notably large. All chromosomes are metacentric; and, except for the *X* chromosome, euchromatic. In the male, chiasma frequency is only 9.40 per nucleus. Chiasmata are confined to distal halves of the arms. Such a karyotype forms a conservative recombination apparatus that may have helped the species in its rapid adaptation to the Puerto Rican habitat, which presumably does not differ much from the habitat of the mother population.

INTRODUCTION

In 1973 Martorell and García-Tudurí (13) reported a successful invasion of Puerto Rico by the polyphagous pest insect, *Umbonia crassicornis*, a Membracid of wide distribution in American tropics and subtropics. If the original invasion took place in 1971 at San Juan, as data of these authors suggest, the speed by which the insect occupied the island is amazing. At Playa de Salinas in the south coast, the local people saw the first specimens in late 1972, feeding on *Pithecollobium dulce* (Roxb.) Benth. From 1969 to the mid year 1972, when intensive fortnightly insect collecting was done in this locality, not a single *Umbonia* specimen was encountered. It seems that this is one of those cases where the invader encountered a feebly defended, if not empty niche, and was capable of filling it very rapidly.

The basic cytogenetic apparatus of such a species is, of course, interesting. It appeared that the genus *Umbonia* is cytologically unknown, as is practically the whole subfamily Hoplophorioninae. Hernández and Peláez (12) have checked one species of this subfamily: *Metcalfiella monogramma* Germar, but their abstract does not contain details, not even the chromosome number.

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MATERIALS AND METHODS

Old larvae and adults of *Umbonia crassicornis* were collected on November 11, 1973, from *Pithecollobium dulce* at Playa de Salinas. Back in the laboratory, gonads of about 30 specimens, most of them male adults, were fixed for 1.5 min in Kahle-Smith fluid and individually stored at about 0° C in the "honeycombs" of a Disposable Microtiter "V" Plate 220-25 (Cooke Engineering Co., Alexandria, Va., U.S.A.)[3] containing Dyer's (6) stain. The "V"-plate was covered with a plain plastic plate, and the whole device was closed in a Snap-Off sandwich bag to avoid evaporation during the prolonged storage in a refrigerator. When needed, squash preparations were made from this material, using heat-albuminized slides. No permanent slides were made.

Observations and photography were made with a Zeiss Photo-microscope II provided with phase contrast optics. Kodak Plus-X Pan black and white film was used in photography.

RESULTS

GONIA

Gonia were more abundant in the larvae than in the adults. Spermatogonia are easy to localize. They show 19 chromosomes at metaphase. In the ovarioles both oogonial and other mitoses may occur. One of such mitoses, probably oogonial, is shown in figure 1,A. There are 20 chromosomes in the female. Chromatids can be distinguished in many of them, as well as some of the linear structure: light gaps, as often seen in monocentric chromosomes. These findings anticipate a karyotype 2n = 20; *XO*.

MEIOSIS

Only male meiosis was studied. Judging from the normal sex ratio, parthenogenesis should not occur.

Pairing of homologous chromosomes is an early event because haploid number of chromosomal loops can be counted as soon as they become visible. Autosomal telomeres and the entire *X* chromosome are polarized to form a bouquet (figs. 1,B and D). The *X* is rather compact in young bouquets but becomes more diffuse and even bipartite when it starts assembling the nucleolus (fig. 1,B). In addition to *X*, and some coarse chromomeric structure in autosomal loops, no conspicuous heteropycnosis was detected in the bouquet stages. There is a recession of contraction

[3] Trade names are used in this paper solely for the purpose of providing information. Mention of a trade name does not constitute a guarantee or warranty of the equipment by the Agricultural Experiment Station of the University of Puerto Rico or an endorsement over other equipment or materials not mentioned.

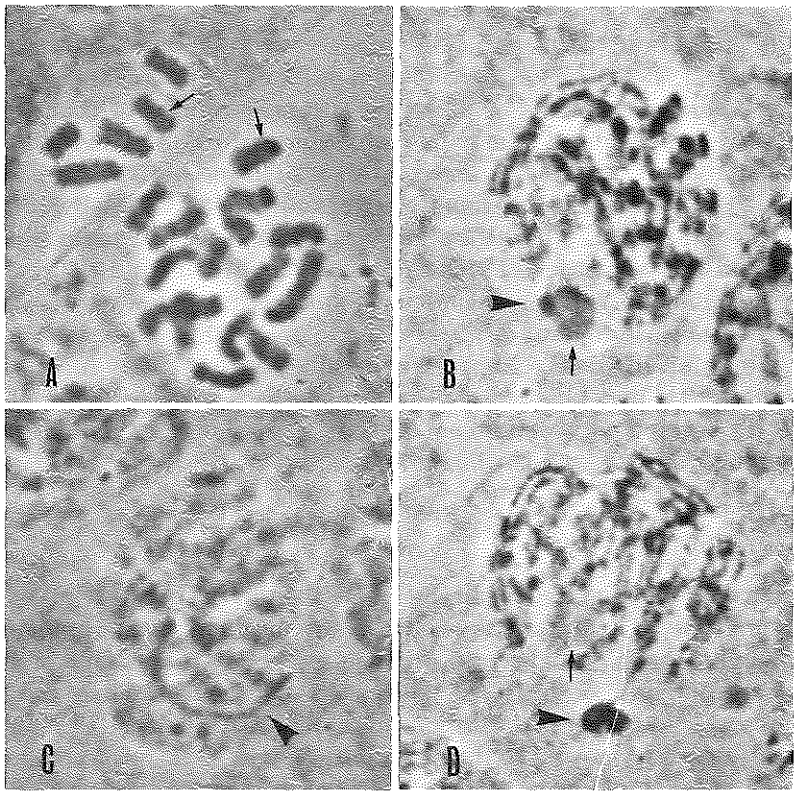

FIG. 1, A to D.—Meiosis in *Umbonia crassicornis*. 2700×, 5100×, 4900×, and 3700×, respectively. A, Ovariolar mitosis, 2n = 18 + *X* + *X*. Note the (centric?) gaps in each chromosome (marked by arrows in two cases). B to D, Pachytene bouquets in order of increasing age. C shows a recession in condensation. *X* chromosome marked with arrowhead; sex nucleolus, with arrow.

amid the pachytene, resembling a similar event found in the early prophase of several plants and animals (3,16,20). During this stage the autosomes become rather diffuse, the *X* appearing as a long rope or horseshoe (fig. 1,C). After recondensation the *X* is again compact, the sex nucleolus separated from it (fig. 1,D).

With diplotene the polarization ceases, and the bivalents approach the nuclear membrane. Nine autosomal bivalents can be counted with certainty from now on. Most of them have formed only one chiasma, and seldom is an unterminalized chiasma seen. The largest bivalent tends to be bibrachially bichiasmate. The ends of the *X* chromosome often touch, producing a ring-like structure (fig. 2,A).

Chiasma frequency per nucleus was determined in five males (table 1). Differences in the mean individual frequency are significant (P= .01),

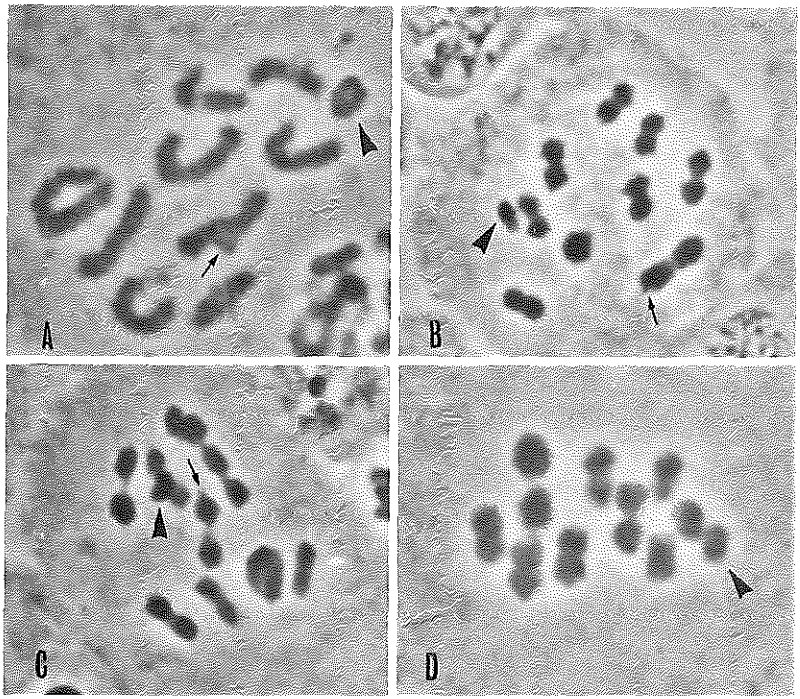

FIG. 2, A to D.—Meiosis in *Umbonia crassicornis*. 2600×, 2500×, 3700×, and 4900×, respectively. Arrowheads point to *X* chromosome. A, Late diplotene, arrow points to an unterminalized chiasma; B, Diakinesis. Arrow: One of the (centric?) "leaders" of the largest bivalent; C, Prometaphase I. Arrow shows one more "leader". D, Metaphase I.

TABLE 1.—*Number of chiasmata per nucleus in five males*

Individual	Chiasmata per nucleus			Total nuclei	Mean per nucleus
	9	10	11		
I	119	100	10	229	9.52
II	100	51	7	158	9.36
III	85	34	2	121	9.12
IV	107	79	4	190	9.47
V	105	17	1	123	9.15
Total	516	281	24	821	9.40

but this fact is hard to evaluate because intra-individual (inter-cyst) variation (7) and even environmental effects might be involved.

Diakinesis is beautiful, with far-progressed condensation of chromosomes. The *X* chromosome, being univalent, is smallest of all (fig. 2,B). In diakinesis, and even more in PM I, horn-like protuberances are sometimes seen extending from the extremes of the bivalents (figs. 2,B

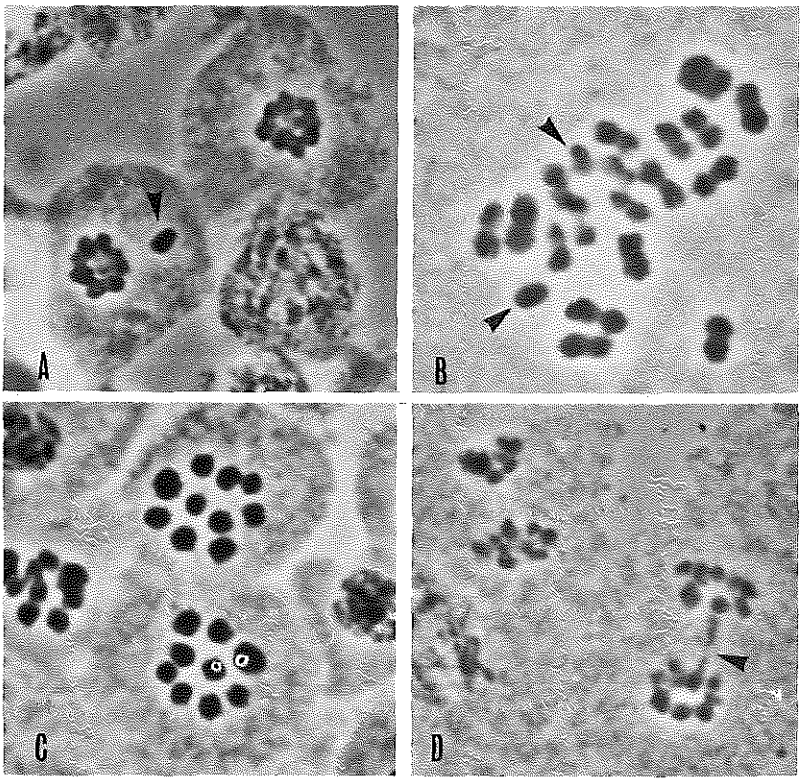

FIG. 3, A to D.—Meiosis in *Umbonia crassicornis*. A 2200×, B to D 2600×. Arrowheads point to *X* chromosome. A, Anaphase I. *X* goes undivided to one of the poles; B, Tetraploid metaphase I, 18 II + *X* + *X*; C, Metaphase II, plates with 9 + *X* (above) and with 9 chromosomes; D, Anaphase II. A pseudobridge in the cell containing the *X*.

and C). They resemble centromeric "leaders" as described in some organisms (see for instance 17, fig. 3).

At M I the unpaired *X* is sometimes seen neatly congressed with autosomal bivalents (fig. 2,D), sometimes advanced to one of the poles. This inconsistency could be due to repeated reorientations of the *X*, as discovered in live M I spindle of a grasshopper (15).

One tetraploid spermatocyte was encountered at M I; it has 18 II + + *X* (fig. 3,B). The low chiasma frequency has prevented formation of multivalents.

In the following anaphase (AI) the *X* chromosome passes undivided to one of the poles (fig. 3,A). Consequently, there are two kinds of M II plates: 50% have 9+*X*; the other 50% have only 9 autosomes (fig. 3,C). At A II the *X* tends to divide slightly belatedly, which helps to identify the two kinds of spermatocytes II still at this late stage (fig. 3,D).

Thus, two kinds of spermatozoa are produced in 1:1 proportion, those with *X* and those without it. As usual in insects, spermatozoa derived from the first definitive (encysted) spermatogonium keep together throughout the entire spermatogenesis, finally forming a sperm bundle. The bundle is relatively short in *U. crassicornis* and lacks the cyst cell, at least in bundles nearing maturity. The sperm heads, although in a parallel order, are thus scattered widely along the length of the bundle (fig. 4). Attempts at counting the number of spermatozoa per bundle (spz/b) have produced counts around 2000 (up to 2238), which suggests that $2^{11} = 2048$ might be the exact number. This means a cell cluster produced by nine synchronous mitoses of definitive gonia plus the two meiotic divisions. This is a relatively high count, suggesting primitiveness (18,19).

DISCUSSION

KARYOTYPE AND CHROMOSOMAL STRUCTURE

Karyotype formulae of Membracidae are quite monotonous, the prevailing status being 10 autosomal pairs plus *XO* system of sex

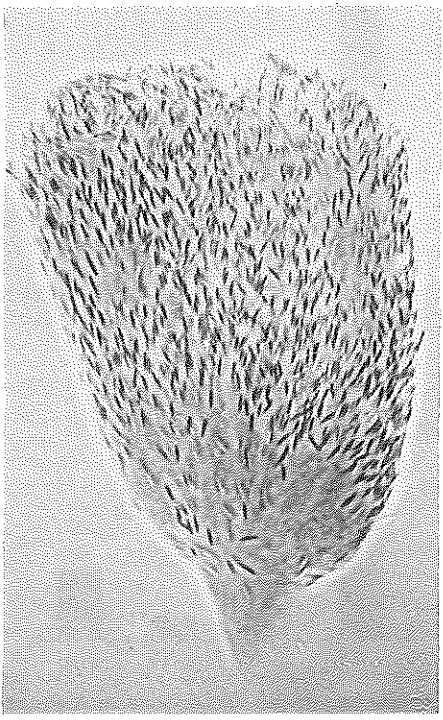

FIG. 4.—Sperm bundle of *Umbonia crassicornis*. 1400×.

determination. Next in frequency is 9 II + *XO* (fig. 5). These two formulae occur in all Membracid subfamilies where more than one karyotype is known. Several authors depict or mention presence of a large autosomal pair in species with 9 II + *XO*. *U. crassicornis* is one of such species although length of the oversized chromosome is not very notable here. Halkka (9) and Bhattacharya and Manna (2) plausibly suggest that the large chromosome is a fusion product of two smaller autosomes. Thus 9 II + *XO* is a derivative of 10 II + *XO* which, in turn, seems to be basic for Membracidae. Provided that the division of Membracidae into subfamilies is phylogenetically sound, one must conclude that there is a rather generalized trend in Membracidae to 1-2 autosomal pairs to fuse, two-by-two, and that this trend is realized polyphyletically in different subfamilies.

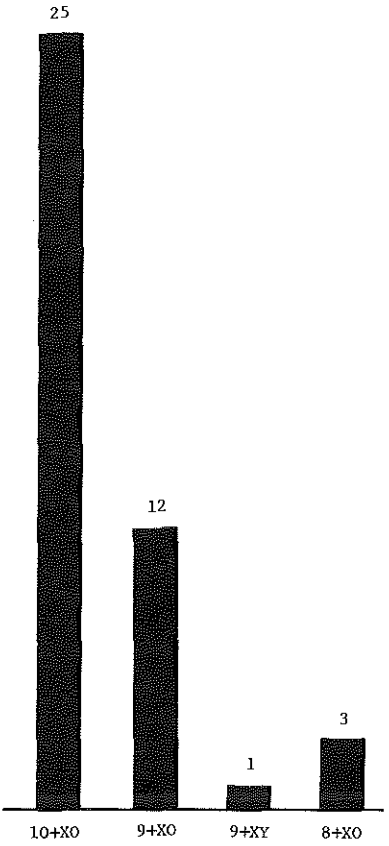

FIG. 5.—Frequency of the karyotypes encountered in Membracidae. Compiled from chromosome lists (1,9,11,14,21), one later paper (2), and the present paper.

It is generally agreed by the cytologists of Homoptera that the chromosomes of this order are holocentric. Our preparations produced some hints favoring monocentry: unstained gaps in gonial chromosomes, and protruding "leaders" in some late diakinectic and PM I bivalents. This is insufficient evidence to challenge the prevailing opinion but deserves a closer study, especially since it is now known that the width of a diffuse centromere may vary in different taxa (8).

RECOMBINATION APPARATUS AND SUCCESS IN INVASION

A completely free assortment of genes is impossible because they are packed in chromosomes. A chromosome transports all the genes it contains as a unit, or linkage group. Generally, the bivalents orient independently from one another at M I, assuring a free recombination of maternal and paternal chromosomes in the gametes. The higher the number of chromosomes into which the genotype is stuffed, the more liberal is the recombination at the gene level. Thus, fusion of chromosomes reduces the chances of recombination, and fission increases them. *U. crassicornis*, with one fusion, has a less liberal recombination apparatus than the basic 10 II + *XO* of Membracidae.

Most bivalents are maintained by chiasmata. Each chiasma (result of cross-over) breaks the chromosome, producing an additional linkage group. Thus the effective number of freely recombining gene blocks for each A I pole is n (haploid chromosome number) + the number of chiasmata per nucleus. The latter factor must be estimated for a given specimen as a mean of a meiocyte I sample counted. Thus the factors of Darlington's (5) *recombination index* are determined. Obviously, the minimum *r.i.* for an individual with all bivalents arranged by one chiasma equals to $n + n$. The sex chromosomes often have achiasmate modes of pairing, or lack a partner, as in just male *U. crassicornis*. Thus the minimum *r.i.* is one less: $n + (n\text{-}1)$; for male *U. crassicornis*, $10 + 9 = 19$. The empirical *r.i.*'s for the five specimens of table 1 vary from 19.15 to 19.58, e.g., they are close to the minimum. The figures for female *U. crassicornis* remain unknown, but Homopterans have little sexual difference in frequency and distribution of chiasmata (10).

The extent of recombination is further limited by preferred location of chiasmata in distal halves of the chromosomes in *U. crassicornis*. Since the autosomes are largely euchromatic, long sequences of significant major genes are thus protected against crossing over. Smith and Virkki (16), following Cox (4), have recently suggested that there might be a mutability gradient in such chromosomes, correlated with frequency of chiasmata. Following this line of thought, one may suggest that the median regions of *Umbonia* chromosomes contain: 1) archaic genes controlling very basic events of cell life; and 2) more recent genes in

selected sequences with very beneficial position effects, whereas 3) the distal regions produce most of the mutations, to be tried in varying recombinations. The archaic genes apparently should not mutate and recombine frequently, the selected sequences should be kept as such in a given, static niche, whereas the distal regions tolerate mutations and recombination in a great variety of conditions.

According to this model, a successful invasion of a new niche differing much from the old one would require restructuring of the selected sequences, by means of a controlled (liberated or restricted) chiasma distribution and/or chromosomal rearrangements. The authors did not see hints of these in the Salinas sample of *Umbonia*, where the patterns of chiasma formation were conservatively Homopteran (10). Actually, the Puerto Rican habitat does not differ drastically from the habitat of the continental (or Antillean) mother population. In such a case a conservative recombination apparatus only helps in occupying the new niche efficiently.

RESUMEN

El cariotipo del membrácido *Umbonia crassicornis* es 9 II + *XO*; 2n = 20 en la hembra y 2n = 19 en el macho. Supuestamente se trata de un derivativo del cariotipo básico 10 II + *XO* de los membrácidos por una fusión. Un par de autosomas notablemente largos en *U. crassicornis* sugiere esto. Los cromosomas son metacéntricos y, excluyendo al *X*, eucromáticos. La frecuencia de los quiasmas es solamente 9.40 por núcleo (macho). Los quiasmas están confinados a las mitades distales de los brazos. Un cariotipo así forma un aparato conservativo de recombinación, que puede haber ayudado al insecto en su invasión rápida del habitat puertorriqueño, supuestamente no muy diferente del de la población original.

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