

# Cytological Techniques in Auchenorrhyncha

S. DROSPOULOS

## Abstract

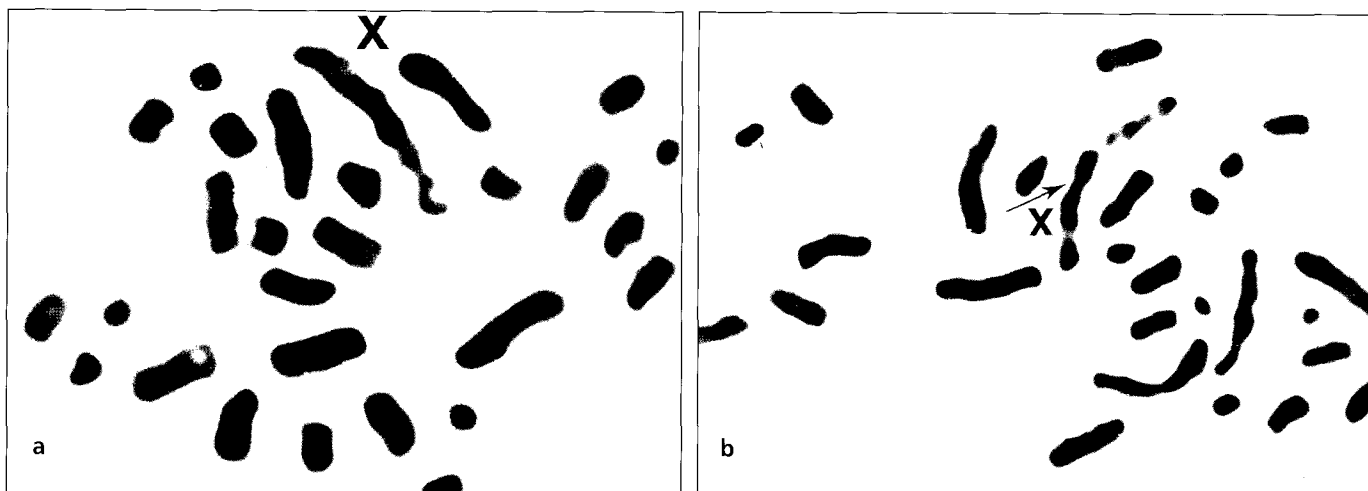
The use of cytological studies in Auchenorrhyncha is of great importance. By analysing the number of chromosomes and sex determination system in males it is very often possible to detect differences even in closely related species, while the same in females reveals cases of polyploidy associated with parthenogenesis or pseudogamy (clonal reproduction). Taxonomists should not hesitate to start applying these useful techniques. The author, although starting as a beginner, finally even developed a special technique for identifying the meiotic division in the egg-nucleus. In order to help and encourage the non-specialist to start and perform such investigations the technique mentioned above is presented here in addition to other published and unpublished data.

Denisia04,  
zugleich Kataloge des OÖ. Landesmuseums,  
Neue Folge Nr. 176 (2002), 483-490

## Introduction

The constitution and number of chromosomes of each species remain a field of research little investigated in Auchenorrhyncha. HALKKA (1959) noted that in the best investigated family of Cicadellidae only 1% of the total number of species, known at that time (10.000), had been studied cytologically. In

relations in Auchenorrhyncha is at the moment premature. In order to accomplish a reliable phylogenetic relation between various taxa of Auchenorrhyncha, investigations on chromosomes (number of chromosomes, sex determination systems, fusions and dissociations of chromosomes) must provide the relevant knowledge at least at the tribe level of each family.



other families (e.g. Derbidae, Achilidae, Meenoplidae, Kinnaridae, Issidae, Tettigometridae) cytological studies were even more scarce or lacking entirely. This is attributed to the fact that cytologists lack systematic knowledge and taxonomists cytological experience. HALKKA (1959), as a cytologist drawing on the systematic knowledge of many specialists in Finland 50-60 years ago, managed to contribute substantially to chromosome studies of many Auchenorrhyncha species. The same holds more recently for Russia, where these two disciplines have been integrated through the cooperation between the groups of A. Emelianov (taxonomist) and V. Kuznetsova (cytologist). Thus, by studying the chromosomes in some species of Auchenorrhyncha families that had been very little studied, (e.g. KUZNETSOVA 1985, KIRILLOVA 1986), further contributions have been made to this field. Also worth mentioning is the work of PARIDA & DALUA (1981) from India.

Cytological studies may be useful in various fields of research in Auchenorrhyncha, as in animals and plants in general.

**a. Phylogenetics:** The use of chromosome numbers in constructing phylogenetic

**b. Taxonomy:** Cytogenetics as a tool in distinguishing even closely related species of Auchenorrhyncha may be very useful. Thus, species which do not display morphological differences can be distinguished by their number of chromosomes and sex determination mechanism. HALKKA (1959) and JOHN & CLARIDGE (1974) could detect differences between a complex of species of the leafhopper genus *Oncopsis* by analysing the male karyotype. Similarly, DROSOPoulos (1977) could separate the two closely related species of the planthopper *Muellerianella fairmairei* and *M. brevipennis*. However, cytogenetics was the only method to distinguish bisexual from triploid pseudogamic or parthenogenetic females in three genera of planthopper (*Muellerianella*: DROSOPoulos 1976, 1978; BOOIJ, 1982. *Ribautodelphax*: DEN BIEMAN 1988a, 1988b and *Delphacodes*: DEN BIEMAN & DE VRIJER 1987). These studies revealed the first cases of pseudogamy and the second of parthenogenesis in Auchenorrhyncha. Furthermore, three species of the spittlebug genus *Philaenus*, in addition to their morphological and allozyme differences (DROSOPoulos & ASCHE 1991; LOUKAS & DROSOPoulos 1993), can be separated now by their number of chromosomes and sex determination system (Drosopoulos, unpublished).

c. **Cytogenetics:** Several other interesting aspects of cytogenetics were based on studies of Auchenorrhyncha as for example chromosomal polymorphism in an Australian leafhopper (WHITTEN & TAYLOR 1968), unusual chromosome system (WHITTEN 1968) multivalents in *Eutettix apricus* (BHATTACHARYA 1975), orientation of the sex bivalent chromosome in *Muellerianella* (DROSOPoulos & SYBENGA 1977), the mode of meiosis in pseudogamic or parthenogenetic triploid female planthoppers (DROSOPoulos 1978; DEN BIEMAN & DE VRIJER 1987), as well as other chromosomal behaviour during meiosis (HALKKA 1959).

Finally various chromosomal abnormalities in the spermatocytes hereditary transmitted to males from radiated fathers and sterility caused by hybridization or other factors can be initially tested by cytological investigations (e.g. MAUDLIN 1976).

### Insect material to be studied

An entomologist working with Auchenorrhyncha should not hesitate to start cytological studies, since this insect group is excellently suited for this type of studies. Especially in males there is no difficulty whatsoever if spermatogenesis is at its early phase, since old males possess sperm only in their testes. The chromosomes of most Auchenorrhyncha species are fairly large and the average haploid number of chromosomes in Araeopidae and Cercopidae is about 15 while for Cicadellidae 9-10 (HALKKA 1959). When studying chromosomes in males care should be taken especially in univoltine species that hibernate in the adult stage. For example the leafhopper *Sulanicerus stali* (FIEBER 1868) infesting *Pistacea vera*, did not show any spermatogenesis before overwintering. In males and females, that emerge in May already, gonads developed early in spring of the next year, when copulation occurs (Drosopoulos, unpublished data). In species that hibernate in the egg or larval stage there is always spermatogenesis in young adults, but some times it is fast and the best material for studying meiotic divisions may be the late stage of fifth instar male larvae or, usually, freshly emerged males. However larvae should be used only when their identity is clear. The same of course implies to females, which in general are more difficult to identify than males.

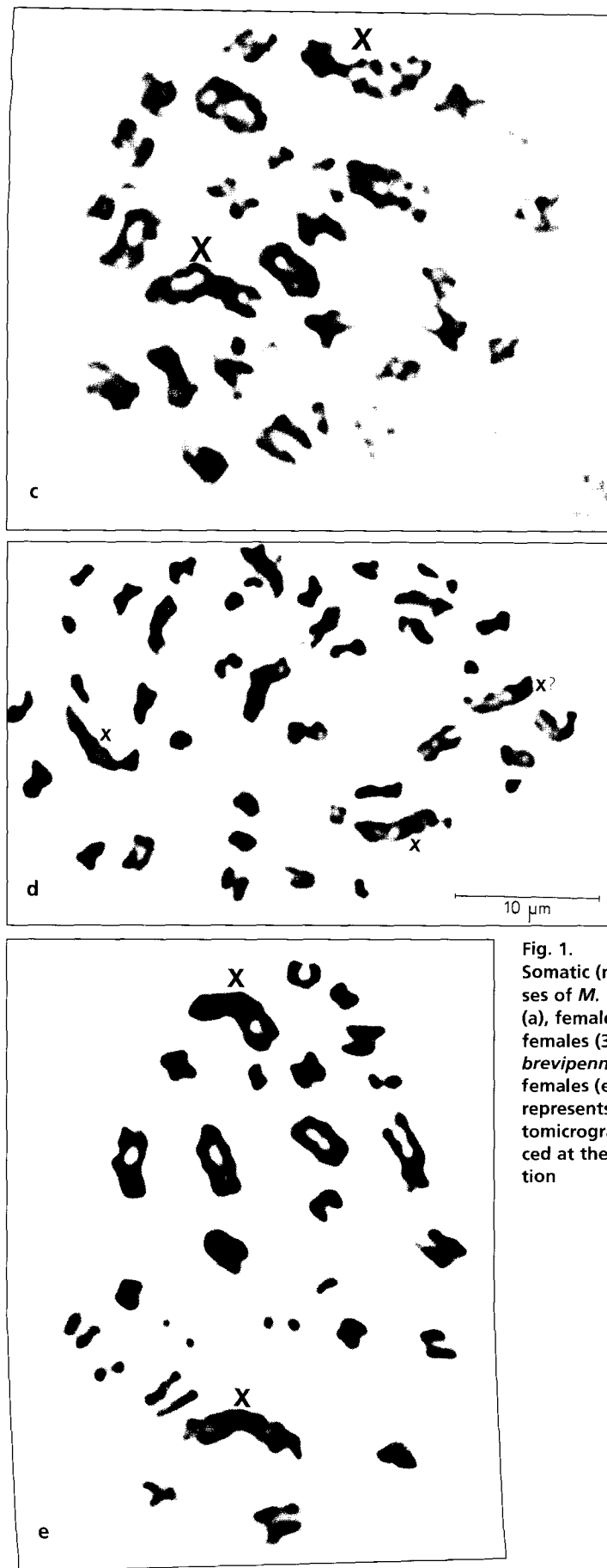
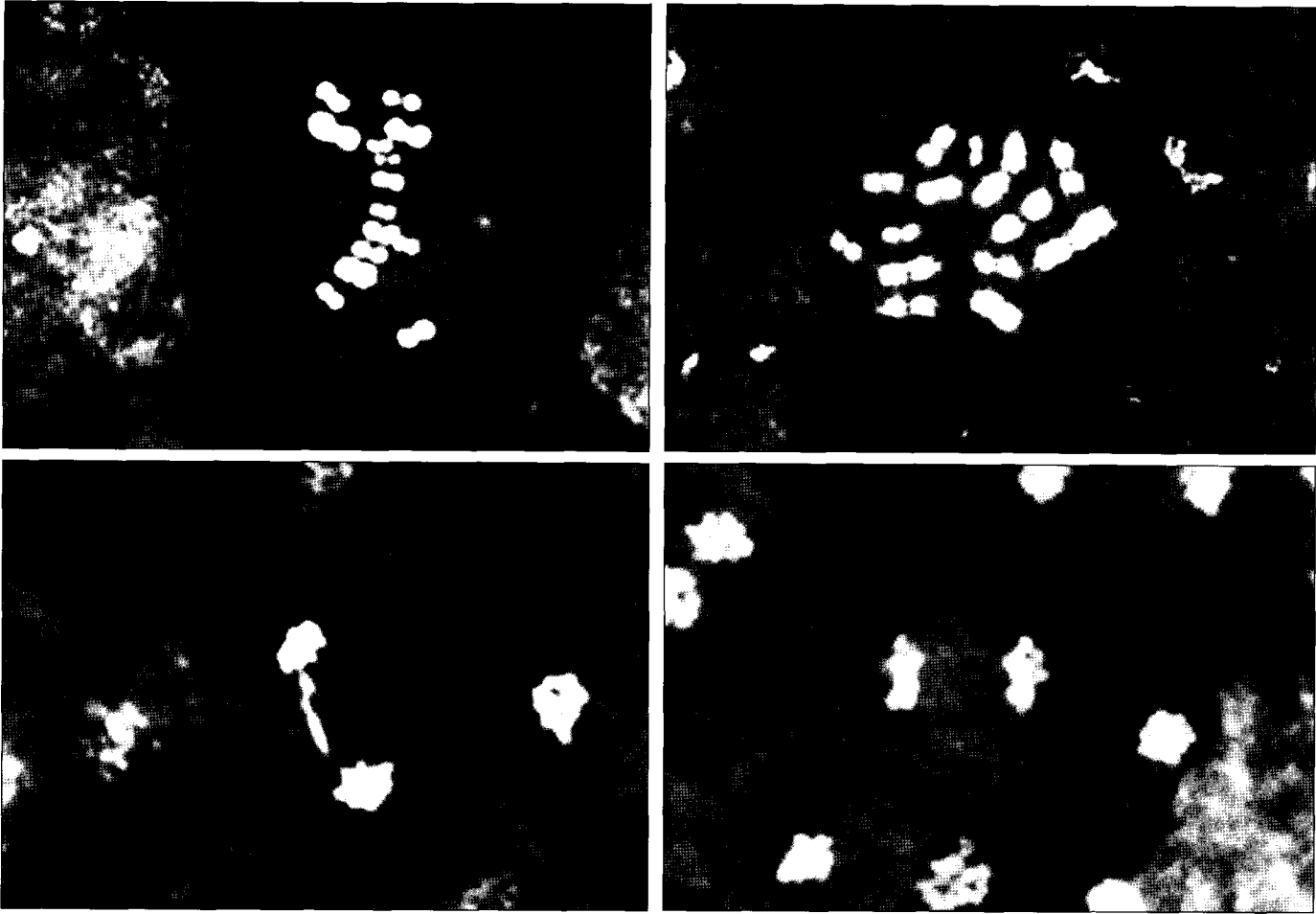


Fig. 1. Somatic (mitotic) metaphases of *M. fairmairei* males (a), females (2n) (c) and females (3n) (d), and *M. brevipennis* males (b) and females (e). The bar in (d) represents 10  $\mu$ m. All photomicrographs are reproduced at the same magnification

For females HALKKA (1959) reported that they are difficult to work with, especially for studying oogenesis. Indeed eggs possess only one meiotically divided cell, which is surrounded by the yolk and difficult to stain since the chorion is not penetrated easily by most stains. In contrast to this, examination of testes reveals several meiotic cells, providing sometimes all phases of meiosis. However, an

Since the sex determination mechanism in Auchenorrhyncha is determined by males, it is obvious that males are sufficient for reporting the number and sex chromosomes of a species. In every species one sex determination system either the XO or the XY is present. Very rarely, some males are of the XO type and others XY, both types occurring within the same population, like in the planthopper



easy technique was developed about 25 years ago, which has been successfully used since, for studying the meiotic division of the egg nucleus (Fig. 2), both in planthoppers (DROSOPoulos 1976, 1978; BOOIJ 1982; DEN BIEMAN 1988a, 1988b) and leafhoppers (Drosopoulos, unpublished data). Finally, for mitotic divisions young embryos provide ideal material since somatic cells split more frequently (Fig. 1). Mitotic divisions can be found occasionally also in follicle cells of young ovaries and testes. In this material also polyploid cells may be found usually with small chromosomes, which may confuse a beginning cytologist.

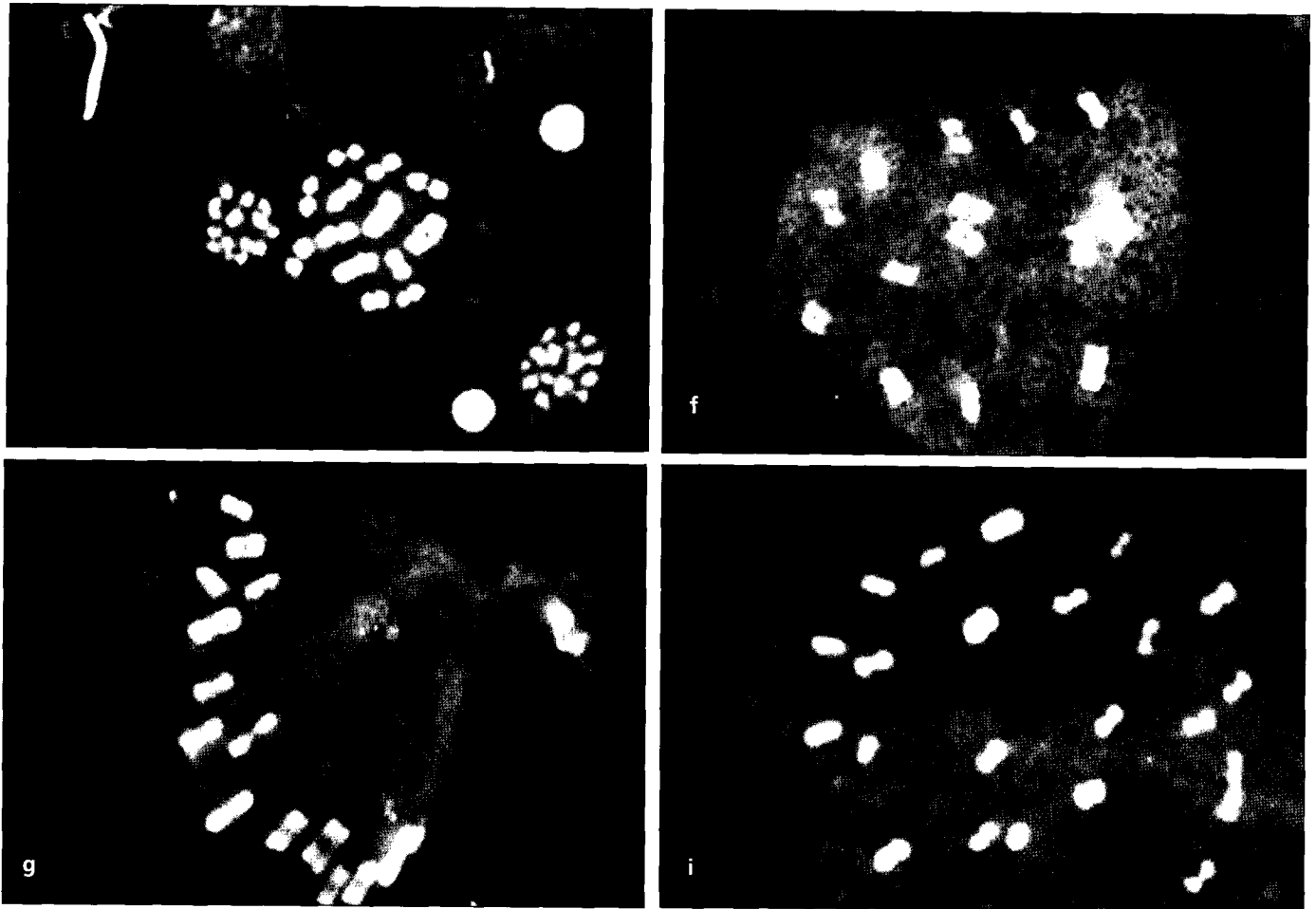
*Dicranotropis hamata* (HALKKA 1959). Several other formations of the sex chromosomes as for example a neo-XY system maybe found (see WHITTEN 1968; JOHN & CLARIDGE 1974). The definition of a sex determination system is basically made during meiotic divisions in the following ways: a) In metaphase I usually the sex chromosome is deeper stained (personal experience). This becomes more pronounced when the preparation remains stained for a few days. However in this phase the XO system is composed of one univalent in contrast to the other autosomes which form homomorphic bivalents. In the case of a XY or

neo XY system a heteromorphic bivalent (Fig. 2) or trivalent (when the X chromosome is fused rather recently with an autosome) is usually very clear. b) During the early anaphase I the sex chromosome X is placed peripherally and lying at a different level behaving thus as an "outsider" (HALKKA 1959). In the case of a XY system these chromosomes can be seen lagging, in contrast to the remaining

ta frequency is often doubtful because of the presence of pseudochiasmata (HALKKA 1959; BATTACHARYA 1980; KUZNETSOVA 1985).

### Methodology

There are many methods for obtaining the desired cytological preparations. However every scientist is using more or less different methods and techniques that sometimes are



autosomes which are concentrated in the plate just before the two daughter cells will be formed (Fig. 2) c) During the second metaphase in a XO system one plate will contain one more chromosome than the other, while in a XY system both plates will contain the same number of chromosomes. However in this phase it is very difficult to distinguish between each of the two sex chromosomes.

The chromosome type in Homoptera is holokinetic, which means that the centromere is diffuse. Consequently the frequency of chiasmata is very low (usually one or two per bivalent). Even the determination of chiasma-

difficult for a beginner to handle. The author has gained experience with several techniques which are time consuming and in some cases require expensive equipment and chemicals. Therefore methods used by some cytologists are cited (e.g. HALKKA 1959, BATTACHARYA 1975, WHITTEN & TAYLOR 1969, JOHN & CLARIDGE 1974, KUZNETSOVA 1985). The methods described by these authors are more or less suited for cytological studies of males. It is worthwhile to advise for the reader the chromosome preparations and photographic presentation in the paper of JOHN & CLARIDGE (1974).

**Fig. 2.** (from top left to the right, meiotic divisions) \*a. Metaphase I of *M. brevipennis* male \*b. Late diakinesis-early metaphase I of *M. fairmairei* males \*c. Early anaphase I, showing the delayed heteromorphic sex bivalent just before splitting into X-Y to complete the anaphase I of *M. brevipennis* males. \*d. Anaphase I of *M. brevipennis* males \*e. Two metaphases II, including an early metaphase I of *M. fairmairei* males. \*f. Meiotic metaphase I of *M. brevipennis* females. \*g. Meiotic metaphase I of *M. fairmairei* (2n) females. \*i Meiotic metaphase I in triploid (3n) *M. fairmairei* females. The asterisks indicate unpublished microphotographs.

Caryological investigations were made by the author equally successfully both in Fulgoromorpha and Cicadomorpha (males and females) by using the same method. For a beginner it is important to have cultures in the laboratory and to be able experimentally to use as many specimens as needed. Living or deep frozen specimens provide the best material for a caryological analysis and then do not need to be fixed as is done for material collected in the field or for material which is going to be analysed after some time.

Living specimens were pinned and the abdomen was cut out into a physiological solution of 1% Na-citrate. Then in males the genital segment was torn and together with the genital segment also the testes were removed out of the abdomen. In females it was necessary to dissect the abdomen caudally and to remove the ovaries together with accessory glands and spermatheca. These preparations were moved to a glass-slide where with the tip of a piece of filter paper most of the physiological solution was removed. Shortly one small drop of propionic or acetic acid 50% was left on the material which was going to be squashed. Hard tissues have to be removed before squashing. After 5-6 minutes a coverslip was left on the slide and the preparation was examined under a phase contrast microscope. Suitable preparations were preserved permanently by the following method. The slide with the coverslip was transferred to a flat surface and on top of the coverslip a filter paper was placed and on top of that a cork. The squashing was done by using a monocular or a small pressing apparatus and for 5-10 minutes. For fixation the slide with the coverslip were transferred to a petri-dish and submerged in CARNOY (6 parts ethanol or methanol, 3 parts chloroform and 1 part glacial acetic acid). After 10-20 minutes the coverslip could be seen removed by waving the CARNOY. When the coverslip was not removed one of its sides was fixed properly while under the other a fine needle was added. Both the coverslip and the slide were transferred to absolute alcohol for more than 10 minutes. Staining of the almost dried preparation was made in aqueous crystal violet 0,1%. In order to see the chromosomes which usually were fixed on the coverslip and the slide, the coverslip was removed properly from its origi-

nal position during squashing. Permanent preparations were mounted in Curr's Neutral Medium.

For female chromosome analysis, ovaries with semi-mature eggs were placed in propionic acid 50% for 2-6 minutes. During this period the eggs swell considerably and become free of their follicle cells and chorion. Before the eggs are diluted in the propionic acid they have to be transferred to a slide by using a capillary glass-tube. By removing the proper quantity of the propionic acid squashing can be done as mentioned previously for males. Mature eggs of fixed or unfixed ovaries should also be transferred into propionic acid and the chorion has to be removed after 10-15 minutes by penetrating it carefully and without damaging the yolk. The egg starts getting swollen but it never gets diluted in this concentration of propionic acid. Then it is transferred to a slide for squashing. In order to save time more than one egg may be transferred to the same slide. In *Muellerianella* the egg nucleus used to be located at the centre of the longitudinal egg axis, opposite of the micropyle.

Field and laboratory material of males and females with swollen abdomens may be fixed in CARNOY which can be composed of methanol-acetic acid solution (3:1). This material can be kept in a refrigerator for more than a year. When working out this material it is better to place it in 20-50% acetic acid before dissecting the ovaries or the testes and then to transfer the material into a drop of lacto-acetic orcein. Before the orcein starts spreading on the slide this has to be covered with another special slide having a cavity. Eggs should be kept overnight but testes may be squashed after 1-3 hours.

### Zusammenfassung

Karyologische Untersuchungen an Auchenorrhyncha haben eine erhebliche Bedeutung erlangt. Die Analyse der Chromosomenzahl und des Geschlechtsbestimmungssystems der Männchen ergibt häufig Unterschiede zwischen nahe verwandten Arten, während sie bei den Weibchen Hinweise auf mögliche Polyploidie aufgrund von Parthenogenese oder Pseudogamie (klonale Reproduktion) gibt. Taxonomen sollen hiermit ermutigt werden, diese nützlichen Techniken einzusetzen.

zen. Dem Autor ist es selbst als Anfänger bereits gelungen, eine spezielle Technik für die Darstellung der Meiose im Kern der Eizelle zu entwickeln. Der vorliegende Beitrag möchte den Nicht-Spezialisten zum Einsatz und zur Erprobung derartiger Techniken bei seinen Untersuchungen ermutigen.

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Address of the author

Prof. Dr. Sakis DROSPOULOS  
Agricultural University of Athens,  
75 Iera Odos  
GR-11855 Votanicos, Athens,  
Greece