BIOLOGY AND CYTOGENETICS OF GREENBUG, *SCHIZAPHIS GRAMINUM* (RONDANI), BIOTYPES

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PREFACE

The search for, and use of, pest resistant crop varieties should be an integral part of any pest management program. From 1967 to 1969, while studying under Dr. H. L. Chada, Professor of Entomology at Oklahoma State University, I screened sorghum varieties for resistance to Heliothis zea (Boddie) and Spodoptera frugiperda (J. E. Smith). During this time I became aware of some of the many problems involved in the development of resistant crop varieties as well as becoming acquainted with the development of greenbug, Schizaphis graminum (Rondani), biotypes. The development of biotypes with differential host plant resistance seriously complicates the problems involved in developing resistant varieties and creates a need for a more thorough understanding of the biotypes involved.

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INTRODUCTION

The greenbug, Schizaphis graminum (Rondani), has long been considered one of the most destructive pests of small grains in the United States. The appearance of a greenbug biotype in 1968 that was capable of damaging sorghums makes the greenbug a serious pest of the 2 major crops of the Great Plains, wheat and sorghum.

At present, chemical control is the only method available for applied control of the greenbug on wheat and sorghum. Due to possible deleterious environmental effects of toxic chemicals and the low cash value per acre of small grains and sorghums, the use of resistant crop varieties appears to be an ideal method of insect control. The development of greenbug biotypes with differential host plant resistance has hindered the development of resistant varieties.

This study was undertaken in hopes of providing additional information on greenbug biotypes which might be helpful in future greenbug studies, especially with respect to plant resistance studies. The greenbug was cytogenetically evaluated to determine if differences were apparent among biotypes. The sexual generation was evaluated to determine its possible significance to the species and its possible relationship to biotype development. Hopefully, information from this study will help in determining factors that influence biotype development.

REVIEW OF LITERATURE

Aphid Biology

The Aphididae are characterized by a type of development described as heterogamy or cyclic reproduction in which there is an alternation of parthenogenetic generations with a sexual generation (Johannsen and Butt 1941).

Beck (1968) described the generalized seasonal biology of aphids. He stated that polymorphism is the rule in this family and that it is closely associated with their complex seasonal biology. All combinations of alternation of generation and host plant alternation are found within the family.

Generalized Aphididae seasonal cycles were given by Johannsen and Butt (1941), Metcalf et al. (1962), Palmer (1952), Borror and DeLong (1971), Davidson and Peairs (1966), Beck (1968), and others. Figure 1, taken from Beck (1968), schematically shows the generalized seasonal biology of aphids. Many variations in this system occur in the Aphididae.

The Greenbug

History and Importance - The greenbug, Schizaphis graminum (Rondani), was originally described in Italy by Rondani (1852). It is virtually world wide in distribution, being reported from North and South America, Europe, Asia, and Africa (Wadley 1923). The greenbug was first reported in the United States in 1882 (Hunter 1909). Since

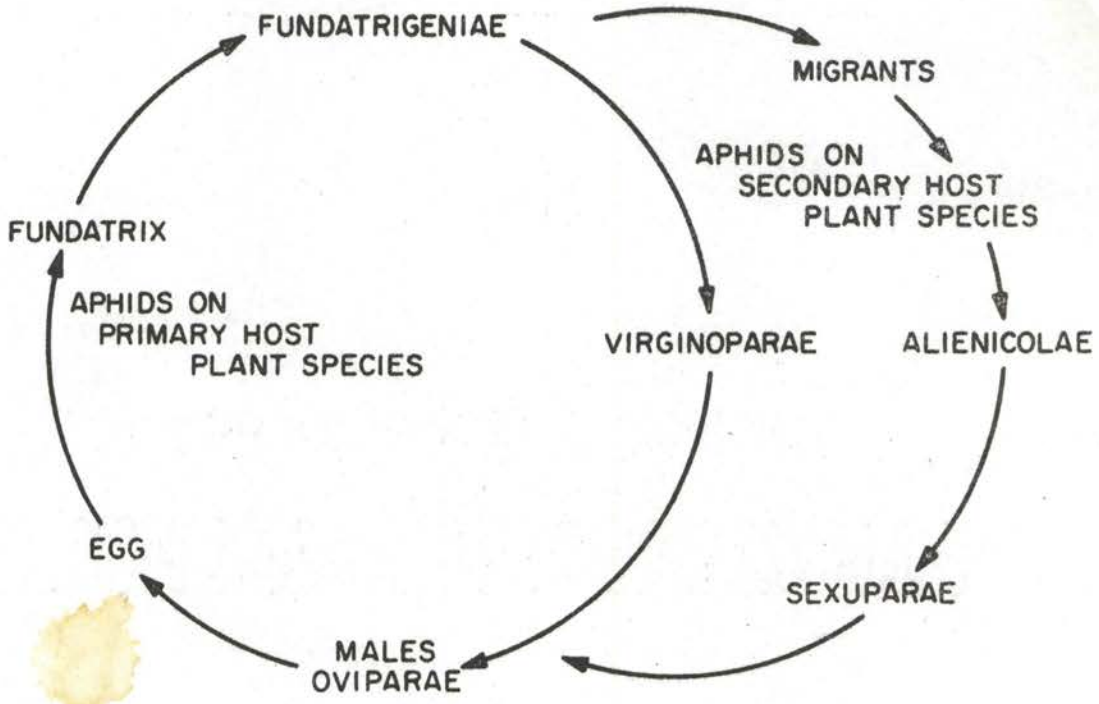

Fig. 1. Generalized seasonal biology of the Aphididae (Beck, 1968)

that time, it has become a very destructive pest of small grains in the Central and Southwestern States. Dahms et al. (1955) stated that the greenbug causes economic damage each year in the United States and estimated that severe outbreaks may cause losses of more than 50 million bu of grain.

Wadley (1931) reported the greenbug could feed on over 60 plant species, but found only wheat and oats to be severely damaged. Other studies reported greenbugs to attack at least 78 species of grasses and some species of other plant families (Patch 1938, Dahms et al. 1954, and Daniels 1960).

Seasonal Biology - The seasonal biology of the greenbug varies considerably with the climate. Generalized greenbug seasonal biology was reported by Metcalf et al. (1962) and Davidson and Peairs (1966). They stated that the greenbugs in the south remain active throughout the year. Further north the greenbugs may overwinter as nymphs or adults which enter a quiescent state during periods of cold extremes. In the more northern states, males and sexual females are produced with the approach of cold weather. They mate and the females oviposit on the plants. In the early spring the ova hatch, producing pale-green, apterous females. Washburn (1908a, b) reported finding eggs in the greenhouse in Minnesota but was unable to find them in the field. Later he reported finding some ova on Elymus canadensis and Beckmani sp. in the field. Webster and Phillips (1912) believed egg production to be largely restricted to north of the 35th parallel. However, sexuales have been reported in the field in Tennessee (Webster and Phillips 1912), and they have been reared in South Carolina (Luginbill and Beyer 1918), in Texas (Tucker 1918 and Daniels 1956), and in Oklahoma (Wood

1955 and Wood et al. 1969). Wadley (1931) concluded that the sexual stage appeared to be of little significance in the economic importance of the species.

Life Cycle - The greenbug passes through 4 instars. The development time is about a week under summer conditions but varies greatly under different temperatures. Alate and apterous females are the predominant forms. Fourth instar greenbugs already contain embryos in an advanced stage of development (Wadley 1931).

Greenbug Biotypes - Muntzing (1967) defined biotype as a group of individuals of a species having the same genotype. Biotypes have been reported for several species of aphids including the following: corn leaf aphid, Rhopalosiphum maidis (Fitch) (Cartier and Painter 1956 and Everly and Mulla 1962); spotted alfalfa aphid, Therioaphis maculata (Buckton) (Pesho et al. 1960); rubus aphid, Amphorophora rubi (Kalt) (Briggs 1965); pea aphid, Acyrthosiphon pisum (Harris) (Harrington 1943, 1945); and the greenbug, Schizaphis graminum (Rondani) (Dahms 1948, Ossiannilsson 1959, Orlob 1961, Wood 1961, 1971, Singh and Wood 1963, Dickson and Laird 1969, and Harvey and Hackerott 1969a, b). Hille Ris Lambers (1947) and Tambs Lyche (1959) reported Schizaphis species which were quite similar to the greenbug.

The criterion for determination of biotypes is usually differential host or host-plant injury by the aphids. The greenbug biotypes distinguished so far have been based largely on their differential plant reactions and differences in temperature requirements.

Dahms (1948) found greenbugs from Mississippi reacted differently from Oklahoma greenbugs when tested on 15 varieties of small grains. Wood (1961) reported a greenhouse biotype that was capable of destroying

wheat previously resistant to the "original" greenbug biotype in the Midwest. In 1968, a third biotype that was capable of damaging sorghum and withstanding higher temperatures was reported. This was reported from Arizona, Colorado, Kansas, Nebraska, Oklahoma, South Dakota, and Texas (Anonymous 1968). Three biotypes now designated as A, B, and C were described by Wood et al. (1969) in the following quotation:

> Biotype A - The "original" greenbug to which resistance has been developed in wheat, barley, and oats, but which does not occur in Southwestern small grain fields now. Dickinson Sel. 28A wheat and wheat hybrids containing this germ plasm are resistant to biotype A, but all wheats are susceptible to biotypes B and C. However, Will barley maintains resistance to biotype A. This greenbug is not morphologically or ecologically different from biotype B, but both differ in this respect from biotype C. Feeding is in the phloem sieve-tube of the leaf vascular bundle.
>
> Biotype B - This greenbug currently infests small grains in the Southwestern U.S. Dickinson Sel. 28A and all other wheats and wheat hybrids are susceptible, but Will barley maintains resistance to it. This greenbug is not morphologically or ecologically different from biotype A, but both A and B differ from biotype C in this respect. Feeding is in the parenchyma of the leaf in contrast to the phloem feeding by biotypes A and C.
>
> Biotype C - This "greenbug" infests and damages small grains and sorghums. All wheats and wheat hybrids are susceptible, but Will barley maintains resistance to it. It is morphologically and ecologically different from biotypes A and B. It is much lighter in color; the cornicles are yellowish-green with no blackening (1/3 of distal end black in A and B), tips not expanded, and wrinkles are present throughout their length (Wrinkles present on basal portions only for biotypes A and B); lateral abdominal tubercles are present on more abdominal segments than I and VII, as is the case for biotypes A and B; it has more sensoria on the third antennal segment than the normal 6 for biotypes A and B; feeding is in the phloem of the leaf vascualr bundle; development and reproduction rates on nearly mature sorghum plants are about 5 times greater than for biotypes A and B on similar plants; about 10% are males and eggs are deposited inside cages whereas, no males have been observed in colonies of biotypes A and B; and development takes place in the field at temperatures as high as 110 F, whereas biotypes A and B leave small grain plants when temperatures reach 80-85 F.

The development of biotype C makes the greenbug a pest of the 2 major crops of the Great Plains, wheat and sorghum (Harvey and Hackerott 1969a). Sorghum in America was considered to be only occasionally infested with greenbugs (Randolph and Garner 1961). Prior to the 1968 attack, the only report of damage to sorghum was made by Hayes (1922) in Western Kansas. Daniels and Jackson (1968) reported small infestations on Texas sorghum in 1967. Sorghum damage by greenbugs was reported from Europe (Barbulescu 1964) and Africa (Matthee 1962). Dickson and Laird (1969) suggested that the biotype was introduced from somewhere near the Mediterranean. Some authors feel that biotype C may prove to be another species or subspecies of Schizaphis (Saxena 1969 and Wood et al. 1969). However, taxonomists feel that the differences involved do not warrant description as a new species (Harvey and Hackerott 1969a and Wood et al. 1969).

Sexual Generation of Aphids

Low temperatures and short photoperiods are factors of major importance in the production of sexuales in the Aphididae (DeFluiter 1950, Kenten 1955, MacGillivray 1955, Lees 1959, 1963, MacGillivray and Anderson 1964, and others). Almost all authors agree that the seasonal biology is controlled by temperature and photoperiod. Short photoperiods and low temperatures are considered to stimulate production of the sexual generation.

Johnson and Birks (1960) stated that the sexual phase in aphid biology was the most primitive reproductive type. They also believed that work by Lees (1959) supported the theory that all embryos begin development as presumptive oviparae but can be switched to viviparous parthenogenetic types if exposed to long photoperiods and/or high

temperatures. Lees (1959) concluded that aphid polymorphism is regulated by the environment. The environmental influences acting upon a "switch" mechanism direct the oocytes and embryos into different developmental pathways. Lees (1961) further found that environment affected the parent aphid rather than directly influencing the embryos. He believed the site of stimulation to be located on the head or thorax. MacGillivray and Anderson (1964) suggested that the light and dark effects were accumulative, exerting more influence on second and following generations than on the first. They also believed that migratory forms were produced prior to production of oviparae.

Beck (1968) found the role of photoperiod to be uncertain in the production of male aphids. Lees (1959) found no definite effects of photoperiod on male production. He believed male production to be attributed to temperature and age of the reproducing female. Although photoperiod effects are not well understood, work by Kenten (1955), Lees (1959), Johnson and Birks (1960), and MacGillivray and Anderson (1964) indicated that males were produced under low temperature and short photoperiod conditions. However the effects were not as clear cut as for the oviparae.

Greenbug Sexual Generation

Males - Male greenbugs are winged and slightly smaller than winged females. Wing expanses were reported to be approximately 4.5 mm and body lengths to be about 1.3 mm (Washburn 1908a, Theobald 1927, and Palmer 1952). These authors also described the antennae as black except for the 2 basal segments and proximal half of the third. Their third antennal segments possessed about 20 circular sensoria, their fourth about 18, and their fifth about 9. Theobald (1927) further

described the male to have the apex of the tibiae and the tarsi dark.

Oviparae - Theobald (1927) and Washburn (1908a) reported the body length to be 2.2 to 2.5 mm in the oviparae and the 2 basal antennal segments and part of the third to be light colored, with no circular sensoria. They also reported the tibia of the hind legs to possess many sensoria and to be slightly swollen and dark. Palmer (1952) gave a slightly different description of the females. She stated the body length to be 1.35 to 2 mm and the hind tibia to possess about 23 large flat sensoria. Washburn (1908a) stated that oviparae can be easily identified by the swollen dark hind tibia and by the eggs which can be seen through the walls of the abdomen.

Ova - Ova have been described by Washburn (1908a, b), Metcalf et al. (1962), Daniels (1956), Davidson and Peairs (1966), Wood et al. (1969), and others. Washburn (1908a) described the eggs as oval in shape, averaging 0.65 mm long by 0.3 mm wide. The color was reported as blue-green when first laid and turning to a shiny black after a few days. Daniels (1956) found ova to average about 0.5 mm in length.

Oviposition was reported to be on the upper surface of dead leaves of grass by Washburn (1908a). Hunter (1909) reported finding eggs on breeding cages. Washburn (1908a) observed 10 nearly mature eggs in one female. Wadley (1931) reported an average of about 3 eggs per female and a maximum of 7. Although oviposition has been reported in the greenhouses in Texas and Oklahoma by Wood (1955), Daniels (1956), and Wood et al. (1969), no ova have been found in the field. Daniels (1956) did not observe hatching and further stated that the ova were possibly aborted embryos from old agamic females. However, a few males were found in field populations in 1968 (Wood 1968). Hatching of greenbug

eggs was reported by Washburn (1908b) and Webster and Phillips (1912).

Production of Sexuales - Wadley (1931) found that greenbugs produced sexuales under conditions of less than 12 hr day-length and temperatures averaging 21 C or less. He further stated that the day-length becomes favorable for production of the sexual generation the last week in September.

Insect Cytotaxonomy

Cytotaxonomy is concerned with cytological differences which exist among members of closely related species. These differences are often concerned with number, shape, and size of the chromosomes, but seldom with the genetic mechanisms of the insects. The differences among chromosome sets of closely related species are sometimes used to differentiate between sibling species that cannot be separated on the basis of external characters, or at best, can be distinguished only with great difficulty. These changes are the result of chromosome rearrangements which have become established in the phylogeny of the species (White 1957).

Chromosome rearrangements consist of centric fusions, dissociations, deletions, duplications, inversions, and translocations (White 1957). These rearrangements are accomplished according to several principles described mainly by Muller (1938, 1940a, b) and Muller and Herskowitz (1954). These include the following: (1) Chromosomes undergo spontaneous breakage periodically. (2) In order to be of any significance in future generations, such breaks must occur in the germ-line of an individual that happens to leave some descendents. (3) Freshly-broken chromosome ends seem to be "sticky" and capable of sticking to other freshly-broken ends. (4) Natural chromosome ends (telomeres) are

not "sticky" and will not fuse. (5) Consequently, telomeres cannot assume an interstitial position in a chromosome and freshly-broken chromosome ends cannot be converted to telomeres.

From these principles, White (1957) concluded that new chromosomes are produced by rearrangements involving at least 2 breaks and subsequent recombinations. Shinji (1931) stated that increases in aphid chromosome numbers appeared to be largely due to transverse divisions of one or more of the original chromosomes.

If the chromosome rearrangements do not lead to the loss of essential genes, the new chromosomes may persist in the population. Even if these new chromosomes are maintained for several generations, the rearrangements may be lost due to natural selection if no adaptive or superior features are involved in the heterozygous condition. Consequently, cytotaxonomic differences among species are usually preceded by a condition of cytological polymorphism. The study of chromosomes among related species may lead to consideration of problems of population genetics in the past as well as at the present time (White 1957).

The extent to which cytotaxonomy can be used in insect systematics varies considerably with the species involved. In some of the Diptera where giant chromosomes are present, most of the chromosome changes should be observable. In other insects with smaller chromosomes, only certain differences in gene sequence are detectable. The observable differences would entail chromosome number, shape, or size. It is probable that minute changes involving duplications or deletions are very significant in genetic evolution. In the salivary chromosomes of Drosophila spp., bands of identical appearance often exist and these probably represent duplications. However these would probably escape

detection in small chromosomes (White 1957).

The cytological study of differences between species has proved possible only in groups possessing small numbers of chromosomes (White 1957). Patterson and Stone (1952) analyzed the somatic metaphase chromosome configuration of approximately 200 species of Drosophila. Clayton and Ward (1954) later studied additional Drosophila species.

The use of cytotaxonomy to recognize sibling species has proved valuable in many insect species, especially in groups which possess giant chromosomes. Carson (1953) cytologically distinguished 3 sibling species of Drosophila, 2 of which were indistinguishable morphologically. Frizzi (1947, 1950) was able to distinguish sibling species of the Anopheles maculipennis group. White and Key (1957) reclassified previously described races of the Australian grasshopper, Austroicetis pusilla (Walker) into separate species based on 7 different cytological characters. Ohmachi and Matsuura (1951) determined that Gryllulus mitratus Burmeister actually consisted of 3 sibling species whose chromosome complements were different.

Sometimes it is possible to distinguish between species that have long, thin chromosomes at metaphase, and others that have much thicker and more contracted chromosomes. These chromosomes may be homologous from species to species even though they look quite different (White 1957).

Cytotaxonomy can be very useful in understanding the relationships of the intraspecific categories of races, microgeographic races, and local populations, but only in those that exhibit some degree of cytological polymorphism (White 1957). Scholl (1955) stated that cytotaxonomy may be used to analyze geographic variation in some

parthenogenetic species of Diptera.

Aphid Cytology and Cytotaxonomy

Boyes (1965) stated that cytological research is helpful in the solution of taxonomic problems. He further stated that the use of cytotaxonomy is more useful in those taxonomic groups in which karyotypic variations are the most abundant. In the study of 50 aphid species, Sun and Robinson (1966) found there were at least 24 karyotypes.

Shinji (1931) attempted to use aphid karyotypes to illustrate relationships and possible evolutionary development. He investigated 37 species of American aphids and found the chromosome number ranged from 6 to 38. Shinji also claimed the chromosome number and physical character of the species were so closely related that the evolutionary scale of any aphid could be judged from its chromosome number.

Shinji (1931) further stated that chromosome size, shape, and number were relatively constant within a genus and these characters could generally be used in determination of genera. He concluded that aphids with low chromosome numbers were the most primitive. Sun and Robinson (1966) concluded that possibly 8 was the primitive chromosome number and the very low frequencies of 4, 6, 18, and 20 represented the end products of evolutionary process.

Sun and Robinson (1966) further concluded that species with lower chromosome numbers were characterized by having large chromosomes. Those with higher numbers were generally characterized by having much smaller chromosomes.

Sun and Robinson (1966) found aphid species chromosome numbers to vary from 4 to 20. The most common number was 8 with 10 and 12 also being quite common. Colling (1955) stated that somatic chromosome

numbers were multiples of 4 in the species studied to that date.

In most multicellular organisms, each chromosome has a short region, called a centromere, which serves for spindle attachment. If chromosomes possess more than one centromere, they have a greatly increased possibility of chromosome disruption due to the migration of centromeres to opposite poles at meiosis. Because of the greater possibility of disruption of polycentric chromosomes most groups have evolved monocentric chromosomes (White 1957).

Schrader (1935) and Hughes-Schrader and Ris (1941) stated that the chromosomes of Homoptera and Hemiptera have either diffused centromere activity along their entire length or the chromosomes are polycentric with many centromeres distributed along the chromosome at intervals. Other groups included in this category are the Lepidoptera, an Anoplura, Haematopinus suis (L.), and a Mallophaga, Gyropus analis Burmeister (Bayreuther 1955, Scholl 1955, and Smith 1960).

Not all Homoptera and Hemiptera possess non-localized centromeres. A species of Pyrrhocoridae (Mendes 1949) and a species of Pentatomidae (Dutt 1955) were found to have localized centromeres. However, in the study of 50 aphid species by Sun and Robinson (1966), no species were found to possess localized centromeres. Toledo Piza (1956) is alone in stating that the chromosomes of Heteroptera are dicentric.

The large chromosomes of Ascaris spp. were found by Schrader (1935) to actually be composed of many small chromosomes grouped into a single unit. The spindle attachment of Ascaris thus resembles that of Hemipteran chromosomes. Schrader did not suggest that Hemipteran chromosomes were multiple. However this may be a possible explanation for the evolution of such a system in Hemiptera and Homoptera.

Choice of Tissue for Chromosome Staining - The proper choice of tissue for preparation of chromosome slides is very important. Smith (1943) stated that chromosome characters of taxonomic importance can only be determined from somatic tissue. He also stated that embryonic stages provided large numbers of rapidly dividing chromosomes but that they were frequently small and rarely desirable for study.

Despite objections raised by Smith (1943), embryonic tissue has proven to be the easiest and most useful tissue for aphid chromosome studies. Embryos were used for aphid chromosome studies by Shinji (1931), Dionne and Spicer (1957), MacDonald and Harper (1965), and Sun and Robinson (1966).

The advanced embryos removed from mature aphids already contain ova and embryos in early stages of segmentation. From these embryos, the largest chromosomes are found (Colling 1955). Sun and Robinson (1966) obtained the best results from apterous, viviparous females collected from early spring to late summer. During the spring to summer period when more rapid growth and development occur, it is easier to find cells in the metaphase stage. MacDonald and Harper (1965) found alate females to possess many fat bodies and the embryos to have few mitotic divisions. However, good chromosome preparations were obtained from nymphs with wing pads.

Staining Techniques - The inability to prepare quick, simple specimens is a major reason cytology and cytotaxonomy of aphids have been neglected for many years (MacDonald and Harper 1965). Stevens (1905), Morgan (1909), Shinji (1931), and Ris (1942) used sectioned material. Colling (1955), Dionne and Spicer (1957), and Cognetti (1961) described squash methods, but these methods were time consuming. Sun and Robinson

(1966) described a quick examination technique in which aceto-orcein squashes could be prepared in approximately 5 min. This greatly facilitated the ability to study aphid chromosomes.

The small size and close approximation of aphid chromosomes at metaphase mean that only the best chromosome configurations can be used for reliable counts and comparisons. Colling (1955) stated that the average metaphase chromosome size of aphids was approximately 1 micron and the diameter of the metaphase plate was about 4 microns. This creates a need for preparation of large numbers of slides in order to obtain adequate specimens for use in cytological studies.

Method of Slide Preparation - Smith (1943) stated that the squash technique provided the most valuable method for studying insect chromosomes. Dionne and Spicer (1957), Cognetti (1961), MacDonald and Harper (1965), and Sun and Robinson (1966) found the squash technique an expedient method for the study of aphid chromosomes.

Mitosis and Meiosis - Sun and Robinson (1966) described mitosis in the Aphididae. They found that the prophase chromosomes are not visible and that metaphase commences when the nuclear membrane disappears. The chromosomes become condensed and stain dark. Approaching late metaphase, they become condensed to such an extent that they appear as dots or short rods. These combine to form a rod-shaped mass at the metaphase plate. The mass separates in anaphase and part moves towards each pole. Then in telophase the chromosome masses swell, become spherical, and gradually fade upon entering interphase.

Meiosis was described as either pre-reductional or post-reductional by Battaglia and Boyes (1955). Pre-reductional was described as a reductional division followed by an equational division, post-reductional

being the opposite. Thomsen (1927), Ris (1942), and Helenius (1952) determined the meiotic divisions to be post-reductional in Hemiptera and the Sternorrhyncha Homoptera, but the order is reversed in the Auchenorryncha Homoptera.

Chromosome Stains

Smith (1943) stated that the most commonly used chromosome stains for insects were carmine, crystal violet, haematoxylin, and basic fuchsin. The discovery of the chromosome staining qualities of aceto-orcein by LaCour (1941) added an important additional tool for the study of chromosomes.

Haematoxylin has the disadvantage of creating difficulties in the interpretation of overlapping chromosomes due to the opaqueness of the stain. However, it has the advantage of staining the spindle and centromere which are not distinguished when crystal violet or basic fuchsin are used. Staining techniques often used are Delafield's, Heidinhain's, and Gomori's Haematoxylin (Smith 1943 and Dionne and Spicer 1957).

The main advantages of crystal violet are that it is simple to use, allows for maximum differentiation in the colorless cytoplasm, and is relatively transparent. One major disadvantage, especially with respect to aphid chromosomes, is the difficulty in removing the stain completely from the cytoplasm in specimens with small chromosomes (Smith 1943).

Feulgen's stain is one of the most specific of the nuclear stains. Many of the counterstains can be used in combination with Feulgen's, and it can be applied after other methods have proved unsuccessful on the same slide (Smith 1943). Feulgen's stain methods for aphids are

described by MacDonald and Harper (1965) and Sun and Robinson (1966). Both of these methods involved the use of aceto-carmine.

Aceto-carmine is a widely used stain in chromosome studies. Techniques have been described by Belling (1926), Painter (1934, 1939), Carlson (1942), Smith (1943), and Smith (1947). The main advantage of using aceto-carmine is the ability to prepare slides quickly and easily. However this stain has the disadvantage of not being particularly selective, especially for small chromosomes. It also stains the cytoplasm and often overstains the chromosomes (LaCour 1941 and Smith 1947).

Aceto-orcein for chromosome staining was first described by LaCour (1941). The stain has the advantages over aceto-carmine of being more selective for small chromosomes. It does not stain the cytoplasm and seldom overstains chromosomes (LaCour 1941). However, the main advantage is the speed with which slides can be prepared. The aceto-orcein technique described by Sun and Robinson (1966) for aphid chromosomes involves a minimum of time, in most cases less than 5 min.

Chromosome Measurements

Chromosome size, as determined from metaphase configurations, is relatively constant for a species. Changes in chromosome diameter and length are inversely proportional to each other (Swanson 1963). According to Burnham (1964), total lengths of individual metaphase chromosomes vary widely among species but the variance is much less within a species.

The major measuring techniques involve measurements taken from drawings or photographs. Techniques for chromosome drawings usually involve the use of a camera lucida. Darlington and LaCour (1969) described camera lucida use for chromosome studies. This method has been

widely used (Boyes 1953, Boyes and Wilkes 1953, Takenouchi 1965, and others). Hughes (1964) measured chromosomes from drawings made from projected negatives and from tracings of photographs. Measurements from photographs were used for aphid chromosome analysis by Dionne and Spicer (1957).

One of the most used measuring techniques is by the use of short arm to long arm ratios with respect to the centromere (Boyes 1953, Boyes and Wilkes 1953, and Burnham 1964). Becak (1968) used both total length and arm ratios to analyze species of Anura.

MATERIALS AND METHODS

Source of Aphids

The 3 biotypes of the greenbug, Schizaphis graminum (Rondani), used in the following studies were obtained from the U.S.D.A.-O.A.E.S., Entomology Research Division, at Stillwater, Oklahoma. The biotypes were cultured on Rogers barley, OK 612 sorghum, or RS 610 sorghum. The biotype cultures were periodically examined to make sure no contamination by the other 2 biotypes had occurred. Biotype C cultures were visually examined to verify if the cultures were pure. The only method to distinguish biotypes A and B from each other was their differential reactions to Dickinson Sel. 28A wheat.

The corn leaf aphid, Rhopalosiphum maidis (Fitch), oat bird-cherry aphid, Rhopalosiphum padi (L.), and the yellow sugarcane aphid, Sipha flava (Forbes), cultures used were also obtained from the U.S.D.A.-O.A.E.S., Entomology Research Division, at Stillwater, Oklahoma.

The cultures were maintained in the greenhouse as well as in bioclimatic chambers set at different temperatures.

Staining Techniques

The ability to cytologically and cytotaxonomically evaluate aphid species depends largely upon the ability to study large numbers of specimens. The speed with which slides may be prepared and evaluated is a prime consideration when selecting a staining technique.

Three staining techniques, Feulgen's leuco-basic fuchsin, aceto-carmine, and aceto-orcein, were compared to determine the fastest method that produced good preparations.

Feulgen's Stain - Feulgen's techniques for aphid studies have been described by MacDonald and Harper (1965) and Sun and Robinson (1966). The method used is listed below.

1. Dissect embryos from a live aphid into a drop of Ringer's solution and leave for approximately 5 min.
2. Transfer embryos to N HCl and hydrolyze at 60 C for 7-8 min.
3. Transfer into leuco-basic fuchsin for 10-15 min or until the embryos become red.
4. Place several of the smaller, stained embryos on a glass slide in a drop of aceto-carmine.
5. Place a cover glass on the embroys and tap gently with a blunt object.
6. Heat the slide gently.
7. Press the slide gently between 2 layers of blotting paper.

Aceto-orcein - Sun and Robinson (1966) described a quick examination technique involving aceto-orcein. The technique as listed below, involved a minimum of time, usually less than 5 min.

1. Dissect embryos from a live aphid onto a slide, discarding the larger embryos.
2. Place a drop of aceto-orcein on the embryos before they dry.
3. Place a cover glass on the embryos and tap with a blunt object.
4. Heat gently.
5. Place the slide between 2 layers of blotting paper and press

gently.

Aceto-carmine - Several aceto-carmine procedures have been described for insect chromosomes (Painter 1934, Carlson 1942, Smith 1943, and Smith 1947). None of the techniques so far described are specific for aphids, other than as a part of the Feulgen's stain method previously mentioned. The technique used in this study was the same as the aceto-orcein technique described earlier. This method is similar to that described by Smith (1947) for salivary gland preparations.

Embryonic Stage Best Suited for Staining

The embryonic stage of development greatly affects the quality of the slide. Aphid embryos can be roughly divided into 3 sizes as shown in Fig. 2. The largest size is a completely or almost completely developed embryo averaging about 0.58 mm in length. The large embryos appear green when freshly dissected from the aphid. The intermediate embryo stage appears as a clear jelly-like mass possessing 2 dark eye spots. After staining with aceto-carmine, appendages become apparent. This stage averages about 0.43 mm in length. The smallest embryo stage appears as a clear jelly-like mass that stains a bright red with aceto-carmine. Sometimes small appendages are visible after staining. This embryo stage averages about 0.25 mm in length.

Embryos were extruded from adult greenbugs. The embryos were separated into the 3 size groups by teasing them apart with a dissecting needle. They were then stained following the aceto-orcein technique already mentioned.

Thirty squashes were made for each embryo size. The squashes were observed under 1000 X magnification to determine if they possessed distinctly stained chromosomes capable of being counted.

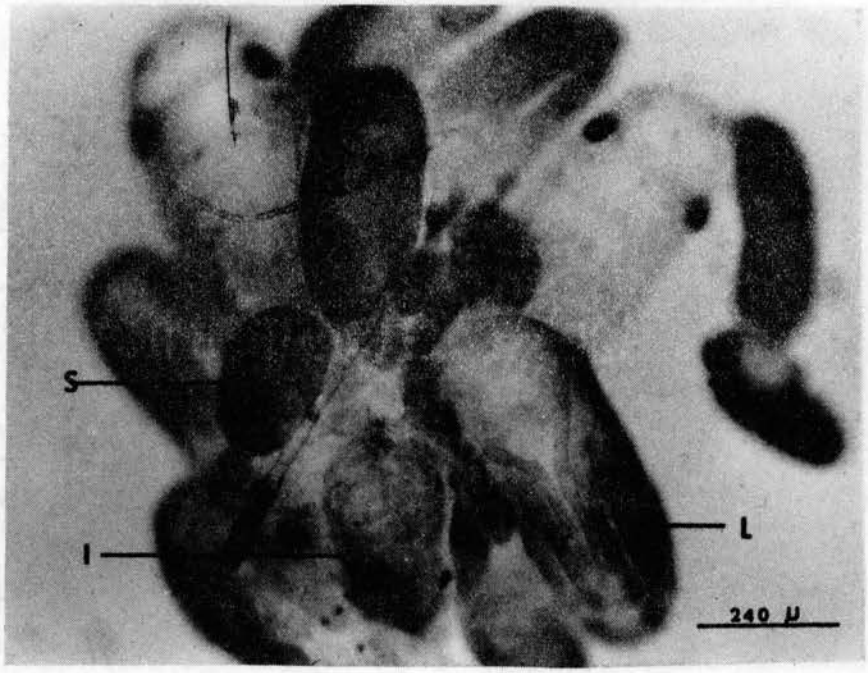

Fig. 2. Three embryo sizes of the greenbug. L, large embryo size; I, intermediate embryo size; S, small embryo size.

Determination of the Aphid Age Producing the Highest Number of Embryos in the Most Desirable Stages of Development

Attempts were undertaken to determine the developmental stage that affords the greatest possibility of finding large numbers of embryos in the most desirable stages of development. Embryos were extruded and counted from aphids that were 3, 5, 7 and 9 days old. These aphids were reared in a bioclimatic chamber at 24 C with a 14 hr photoperiod.

Embryo counts were accomplished by dissecting the embryos from each aphid onto a glass slide and placing a drop of aceto-carmine on the embryos. The embryos were then teased apart with needles and sorted into the 3 embryo size groups and counted.

Twenty-five 10 cm plastic pots were seeded with Rogers barley. The pots were fitted with clear plastic cages. The plants were thinned to 4 per pot when they were approximately 10 cm high. Fifteen of the pots were infested with 10 late instar greenbug nymphs (5 pots each with the 3 greenbug biotypes). The greenbugs were left on the plants for 4 days to insure that all of them were mature and reproducing. At the end of the 4 days, 2 pots were infested with a total of 30 mature greenbugs from the 5 pots of biotype A, 2 pots with 30 of biotype B, and 2 pots with 30 of biotype C. The pots were infested at 5 PM and the adults were removed the next morning at 9 AM. The embryos from these nymphs were checked 3, 5, 7, and 9 days after the adults were removed.

Chromosome Measuring Techniques

Two chromosome measuring techniques were compared. One technique involved direct measurements from the slide while the other involved measurements taken from enlarged photographs. All measurements were

made from aceto-orcein squashes of the 3 greenbug biotypes.

Chromosome measurements were made under 1000 X magnification. An ocular micrometer in the 10 X eyepiece was used for chromosome measurements. The micrometer was divided into 100 units with each unit equaling 0.952 micron. Chromosomes were measured by maneuvering the slide into a desirable position in the field of view while simultaneously turning the eyepiece to aline the micrometer and chromosome. Straight chromosomes were easily measured. Difficulties arose with bent or coiled chromosomes due to the inability to measure anything but straight lines. Bent chromosomes were visually divided into several short straight subsegments and measurements were taken and totaled to give the length of the chromosome. Drawings were quickly made of each chromosome set while it was being measured and the length recorded on the drawing. These drawings provided a means of identification for later reference and for comparisons with the photographs.

A photograph was taken of each chromosome set measured by the ocular micrometer. A 4 X enlargement was made of each negative and measurements were then taken from the photographic prints. Dionne and Spicer (1957) used 4 X enlargements for chromosome measurements. All photographs from which measurements were to be taken were printed at one time in order to avoid any fluctuations which might occur due to resetting the enlarger. All chromosomes that were distinct on the photographs were measured with a mm ruler.

Four chromosome sets were selected to calculate the exact enlargement of the photographs. The photographed chromosomes were measured and these values were compared to their corresponding lengths as taken directly from the slides.

These 2 measuring techniques were compared and analyzed as a paired experiment. All 3 greenbug biotypes were included in one test since only the measuring techniques were involved.

Chromosome Comparisons of Greenbug Biotypes

Confusion over the taxonomic status of greenbug biotypes as well as the inability to distinguish biotypes A and B from each other visually, led to attempts to cytotaxonomically evaluate the 3 biotypes. It was hoped that chromosome evaluations would detect differences in the chromosome configurations of the 3 greenbug biotypes. Biotypes were studied and evaluated on the basis of number, size, and general morphology of the chromosomes. Chromosome slides were prepared using the aceto-orcein squash techniques described earlier.

Biotypes B and C used in this study were cultured on Rogers barley at a temperature of 27 C. Biotype A was cultured on both Dickinson Sel. 28A wheat and Rogers barley. The temperature was about 24 C.

The embryos from 1 aphid were used in the preparation of each slide. Each slide was examined under 1000 X magnification to determine if distinct chromosome configurations were present. Only 1 chromosome set was measured from each slide prepared. The only criterion for selection of the chromosome sets was that all chromosomes be distinct enough to be separated from each other. The first such set found on each slide was measured and used in the analysis. Ten chromosome sets were measured for each of the 3 biotypes.

Total chromosome lengths were used for analysis. Due to differences in chromosome lengths at different mitotic and meiotic stages, the within the population variation in chromosome lengths was checked before comparisons of the biotypes could be made. A chi-square test

for homogeneity of data was used to check variation within the population of each biotype. A chi-square test for a 1:1 ratio was used to check variation in chromosome lengths between the 3 biotypes.

Comparisons of Chromosomes of Corn Leaf Aphids, Oat Bird-cherry Aphids, and Yellow Sugarcane Aphids With the Chromosomes of Greenbugs

Chromosome measurements were made of the corn leaf aphid, the oat bird-cherry aphid, and the yellow sugarcane aphid. The aphids were reared in the greenhouse on Rogers barley. The staining, measuring, analysis, and comparison techniques were the same as for the greenbug biotype comparisons. Measurements from the 3 aphid species were compared with each other and with the greenbug biotypes.

Sexual Generations

Greenbug sexual generations have occurred in the U.S.D.A.-O.A.E.S., Entomology greenhouse, Stillwater, Oklahoma, each year since the discovery of biotype C greenbug in 1968. Ova were first noticed in November 1968 and oviposition continued until April 1969. The fall sexual generation was not found in 1969. The second season sexual generation was first observed in late January 1970 and continued until mid April. The peak production period occurred during February and early March. The third season sexual generation was first noticed in November 1970 and continued through April 1971. Heavy oviposition was still occurring in late March.

All sexual generation studies described in this paper were conducted during the second and third seasons as described above. All collections of ova and sexual forms were made in the U.S.D.A.-O.A.E.S., Entomology greenhouse, Stillwater, Oklahoma.

Males - The collection of male greenbugs was accomplished by shaking aphids from infested plants onto a sheet of white paper. Male greenbugs were separated from other winged aphids and transferred to 29 ml medicine cups. The ratio of males to alate females was observed. The medicine cups provided a quick, easy method for collection and short term storage (less than 1 day).

The collected males were studied in the following manners:

1) Testes were dissected onto glass slides. Aceto-orcein squashes were prepared to determine sperm size and shape. Observations were made under 1000 X magnification.

2) The abdomens were pricked with a needle and the contents extruded into a drop of 0.85% saline solution. Five to 10 males were used in preparation of each slide. A total of 20 slides was examined. The slides were examined under 250 X magnification in an attempt to observe living sperm.

3) The males were mounted in Turtox[a] CMCP-9AF or 9AB mounting media to provide permanent slides. The slides were used for detailed examination. Measurements were made of the total body length and wing span and general taxonomic characters were evaluated.

Oviparae - Collection of oviparae was accomplished in the same manner as described for the males. Selection of oviparae was largely based on the yellowish tint of the abdomen which indicated possession of ova. The ratio of sexual to non-sexual apterous females was noted.

Oviparae were handled in the following manners:

1) Ova were dissected from the aphids onto a glass slide and

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[a]General Biological Supply House, Inc., 8200 South Hayne Ave., Chicago, Illinois 60620.

counted. Females were also placed on Rogers barley and the numbers of ova laid were counted. The site of oviposition was also checked to determine oviposition preferences.

2) The oviparae were mounted in Turtox CMCP-9AB or 9AF mounting media to provide permanent slides. The specimens were measured and observed for general taxonomic characters. Special observations and counts of the sensoria were made on the swollen hind tibia of the oviparae.

Ova - Ova were collected from cages and plants. The collection was accomplished by moistening the area around the base of an ovum and this loosened it from the surface. The ovum was then removed by an artist's small brush.

The following studies were conducted on the ova.

1) Aceto-orcein squashes were prepared from the ova. The squashes were prepared by removing the ova contents onto glass slides and discarding the shells. The aceto-orcein techniques, as described earlier, were followed. The slides were examined for chromosome sets.

2) Ova were observed to determine size, shape, and coloration. The effects of age on coloration were studied for 3 temperatures: 4, 21, and 27 C. Twenty freshly oviposited ova were placed in a regrigerator at a temperature of 4 C. The same numbers of ova each were placed in bioclimatic chambers set at 21 and 27 C, respectively. The bioclimatic chambers were set on a 14 hr photoperiod. No lighting was available for the 4 C test. No attempt was made to control humidity which remained low most of the time. The ova were checked every 12 hr for 2 days and then daily for 2 wk or until all ova turned black.

3) Incubation studies were performed to determine if embryonic

development occurred. In the spring of 1970, ova were incubated at temperatures of 2, 18, 21 and 27 C. A refrigerator was used for the 2 C temperature, the other 3 temperatures were maintained in bioclimatic chambers. Four Petri dishes containing 25 ova each were placed in each of the 4 chambers. The leaves containing the ova were clipped from the plants and placed on filter paper in the bottom of the Petri dishes. Observations were made once a week. Additional ova were placed in each chamber as they were collected throughout the season but weekly data were not taken on them. They were checked periodically to determine development.

Incubation studies, which began in December, were again conducted for the 1970-71 sexual generation. Ova were incubated at temperatures of 2, 4, 21, and 27 C. The ova were collected, removed from the leaf, and 25 each were placed on either glass slides or 1% agar plates. Four glass slides and 4 agar plates were placed under each of the 4 temperatures, and they were observed once a week. Additional ova were placed at the various temperatures as the season progressed but numbers of ova present and weekly data were not recorded. These ova were checked periodically to determine if development occurred.

Greenbug Type and Ova Production - Tests were conducted to determine the greenbug type that produces ova. Information on sexual population percentages was also collected.

Twenty-four 10 cm pots of Rogers barley were arranged in a completely randomized design, each pot containing 3 plants. The tests consisted of 3 treatments with 8 replications in each treatment. Rogers barley cultures containing biotype C, in which large numbers of ova were being produced, were selected to infest the treatments. The 3

cultures were selected by visual comparisons to determine cultures with high percentages of oviposition.

The 3 treatments consisted of dark green apterous females, light green apterous females, and alate females. The dark green females were very dark as compared to the "normal" coloration.

Each pot was infested with 1 aphid and observations were made at 4 or 5 day intervals. After oviposition or nymphal production began, the adult and nymphs, if produced, were removed and replaced by a corresponding aphid type from the cultures.

The first test was conducted in February and March 1970. The second study was conducted in December 1970 and January 1971. The same procedures were followed in both tests.

Oviposition by Oviparae When Exposed to Males as Compared to no Exposure to Males - Biotype C greenbugs were placed in a bioclimatic chamber having a temperature of 21 C with a 14 hr photoperiod for 1 mo. The test began December 10, 1969. On January 12, 1970, 30 Rogers barley pots each containing 4 plants were infested with 1 adult greenbug per plant. The following day the adults were removed and the nymphs, if produced, were left on the plants. On January 25, 30 Rogers barley cultures were infested with 1 greenbug nymph from the cultures. Nine of the infested nymphs developed into sexual forms. Males were placed in 4 of the 9 cultures. The cultures were observed to determine if oviposition occurred.

From January 25 until early March additional tests were conducted to determine if oviposition occurred both in the presence and absence of males. These tests were conducted under greenhouse conditions with temperatures ranging from 10-27 C. Large numbers of Rogers barley pots

were infested with 1 greenbug nymph each and observed to determine if oviparae were produced. Oviparae were found in 24 of the pots. Twelve of these pots were infested with males. Observations were made to determine if oviposition occurred in both treatments.

In the preceding tests, oviparae have been exposed to males but no copulation was observed. The following test was conducted to determine oviposition from oviparae observed copulating. Aphids were brushed onto white paper. When pairs were observed copulating, they were transferred to Rogers barley or RS 610 sorghum and caged. Fifteen pairs were checked in this manner. This test was conducted in 1970 and also in 1971.

Effect of Photoperiod and Temperature on the Production of the Greenbug Sexual Generation - Cultures of biotypes A, B, and C were colonized on Rogers barley for 1 mo in a bioclimatic chamber at 24 C with a 14 hr photoperiod. Each of the 3 cultures were the progeny of a single aphid. On September 1, 1970, 2 cultures of Rogers barley were each infested with 5 greenbugs from each biotype. One culture of each biotype was placed in a bioclimatic chamber set on a 14 hr photoperiod and the other cultures were given an 8 hr photoperiod. All cultures were maintained at 24 C. On September 17, 6 Rogers barley cultures for each biotype were infested with 2 aphids each for each of the 2 photoperiods. These cultures were examined September 30 to determine the effects of the photoperiods on oviposition and the occurrence of sexual forms.

On May 1, 1971, 4 cultures of biotype C and 2 cultures each of biotypes A and B were placed in a bioclimatic chamber set on daily temperature fluctuations of 13-21 C with a 9 hr photoperiod. The cultures

were checked May 31 to determine if sexual forms were produced or if oviposition had occurred.

RESULTS AND DISCUSSION

Staining Techniques

The Feulgen's staining technique provided good slide preparations with clear chromosome sets but the technique was slow. Another problem was in the handling of embryos. The transfer from one solution to the next often resulted in the loss or damage of the embryos.

The aceto-orcein technique proved to be a very easy method for obtaining good, quick slide preparations. The slides could be prepared in less than 5 min and were ready for immediate observation. Photographs could be taken immediately but the best pictures were obtained from slides that were at least 1 day old.

The aceto-carmine technique did not produce distinctly stained chromosomes. In most cases the cytoplasm was stained and this further created problems in distinguishing the chromosomes. However, aceto-carmine proved to be a useful stain for staining and counting embryos as they were dissected from the aphids.

The aceto-orcein technique was used in all of the chromosome studies in this manuscript.

Embryonic Stage Best Suited for Staining

The small embryonic stage was determined to be the most desirable stage for chromosome staining. Distinct chromosome sets were observed in about 50% of the slides prepared from the small embryos. Of the slides prepared from the intermediate embryo sizes, 37% were found to

possess distinctly stained chromosome sets capable of being counted. None of the slides prepared from the large embryos were found to possess such chromosome sets.

Many chromosomes were stained in the slides made from large embryos, but no complete sets were found in the same plane of focus. Usually, embryonic tissues masked part of the chromosome set. The large embryos were almost fully developed and their embryonic tissues and appendages apparently did not lend themselves readily to squash preparations.

The intermediate embryo size proved to be a fairly efficient size to use for squash preparations. Most of the slides had distinct chromosome sets, but many of them were not in a plane of focus that would enable the measuring and comparing techniques to be employed. Appendages were usually apparent in these slides which hindered the proper preparation of the slides in order to obtain chromosome sets in one plane of focus. In most instances, the slides did not possess areas of embryonic tissue that masked the chromosome sets below and within the tissue such as was the case for the slides made from the large embryos.

The slides prepared from the intermediate sized embryos also possessed some small embryos. It was not possible to remove all of the small embryos that stuck to the intermediate size embryos when they were being dissected and separated. These small embryos may have produced many of the distinct chromosome configurations observed on the slides. However, the possibility seems remote due to the small number of small embryos that remained on the larger embryos.

Few problems were encountered in preparing slides with distinct chromosome sets in one plane of focus when small embryos were used.

Determination of the Aphid Age Producing the Highest Number of Embryos in the Most Desirable Stages of Development

The counting of small embryos was difficult due to their sticking to the body of the aphid or to the larger embryos. Table 1 indicates that 5 day old aphids possessed the greatest number of small embryos. They also possessed very few intermediately sized embryos and none were found with fully developed embryos. The 1.4 mm average length is large enough to permit easy dissections. These data indicate that for aphids reared at 24 C, 5 day old aphids are the most desirable age to use in chromosome slide preparations.

Chromosome Measuring Techniques

The 4 chromosome sets chosen to calibrate the size of the photograph enlargements were selected because the chromosomes involved were fairly straight (Fig. 3). Thus the measurements of these straight chromosomes should more closely represent the true photographic enlargement size than other chromosome configurations.

One of the chromosome sets used in the measurement calibrations possessed what appeared to be a ninth chromosome. It was considerably shorter than the other 8 chromosomes and no homologue was apparent. It was believed that this probably did not represent a chromosome but was an artifact or some dark-stained cellular or extracellular substance. However, it was used in measurement calculations since only the measurements were important in calibrations.

If the 2 measuring techniques were comparable, the total measurements taken by each technique should be equal. Each ocular micrometer unit was calculated to equal 0.952 micron. Therefore, the total ocular measurements of 171 units would be equal to 162.8 microns (Table 2).

Table 1. Embryo counts from greenbugs that were 3, 5, 7, and 9 days old.

Aphid Age	Biotype	Avg. Length (mm)	Avg. No. of Embryos per Female		
			Small Embryo Size	Intermediate Embryo Size	Large Embryo Size
3 Days	A	0.9	6.3	0.0	0.0
	B	0.9	6.8	0.0	0.0
	C	1.0	7.5	0.0	0.0
5 Days	A	1.4	11.2	0.6	0.0
	B	1.4	11.0	0.1	0.0
	C	1.4	13.5	3.2	0.0
7 Days	A	1.5	7.8	2.9	1.7
	B	1.5	9.7	2.8	1.1
	C	1.6	10.5	3.8	2.4
9 Days	A	1.6	6.1	3.8	2.9
	B	1.5	8.3	3.0	2.9
	C	1.6	8.7	3.9	3.4

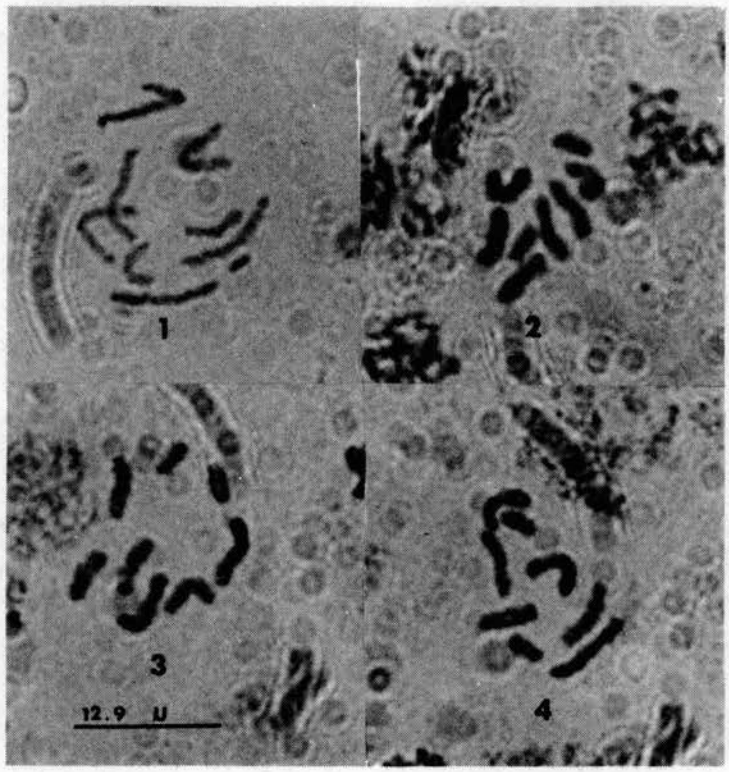

Fig. 3. Four chromosome sets used to calibrate the photograph enlargement size.

Table 2. Calculation of photograph enlargement scale for comparison of chromosome measuring techniques.

Chromosome Set No.	Chromosome No.	Ocular Micrometer Measurement[a]	Units Photograph Measurement[b]
1	1	13	16
	2	9	13
	3	9	13
	4	9	12
	5	8	12
	6	6	11
	7	6	7
	8	5	7
	9	2	3
2	1	5	8
	2	5	8
	3	5	7
	4	4	7
	5	4	7
	6	4	5
	7	3	4
	8	3	4
3	1	6	8
	2	6	9
	3	5	7
	4	5	8
	5	5	7
	6	4	7
	7	3	4
	8	3	4
4	1	6	11
	2	5	9
	3	5	8
	4	4	7
	5	4	7
	6	4	5
	7	3	4
	8	3	4
	Totals	171	253

[a] 1 ocular micrometer unit = 0.952 micron.

[b] 1 photograph measurement unit = 0.643 micron.

If 171 ocular units equals 162.8 microns, then the 253 photograph measurement units should also equal 162.8 microns. From this calculation, it was determined that each unit measured from the photographs equaled 0.643 micron. The values for the comparisons between the ocular and photographic measurements are shown in Table 3. Many chromosomes were unable to be measured from the photographs. In this test, 178 chromosomes were measured by the ocular micrometer method. Of this number, only 147 of the chromosomes were clear enough on the photographs to be measured. All chromosomes of each set were measured by this technique in only 8 of the 22 sets studied.

The ocular micrometer measuring technique enables the study of chromosome sets that are not in the same plane of focus. Each individual chromosome of the set can be focused upon and measurements made. Photographs taken of chromosome sets that are not in the same plane of focus may lead to the inability to measure some of the chromosomes from the photographs.

The 2 techniques were analyzed as a paired experiment. The t-calculated value was 1.034. This value was not large enough to consider significant differences between the 2 measuring techniques.

Exceedingly large numbers of slides must be prepared in order to obtain large numbers of chromosome configurations in the same plane of focus. The ocular micrometer measuring technique is very useful in studying aphid chromosomes since it enables the study and measurement of chromosome sets that may not photograph clearly. The ocular micrometer method works fairly well on short straight chromosomes but its value as a tool for the study of longer and less straight chromosomes is doubtful.

Table 3. Chromosome lengths as calculated using 2 different measuring techniques.

Chromosome Set No.	Chromosome No.	Chromosome Lengths in Microns	
		Ocular	Photograph
1	1	4.76	4.50
	2	3.81	----
	3	3.81	2.57
	4	3.81	3.22
	5	2.86	3.87
	6	2.86	----
	7	2.86	----
2	1	3.81	3.87
	2	3.81	----
	3	2.86	----
	4	2.86	2.57
	5	2.86	----
	6	2.86	----
	7	1.90	1.93
3	1	8.57	----
	2	7.62	----
	3	4.76	----
	4	4.76	4.50
	5	4.76	5.14
	6	4.76	5.14
	7	4.76	----
	8	4.76	4.50
	9	3.81	----
	10	3.81	----
	11	2.86	2.57
	12	2.86	2.57
	13	1.90	----
	14	1.90	1.93
4	1	5.71	5.14
	2	4.76	5.14
	3	3.81	----
	4	3.81	----
	5	1.90	----
	6	1.90	----
	7	1.90	----
	8	1.90	1.93

Table 3. (Continued)

Chromosome Set No.	Chromosome No.	Chromosome Lengths in Microns	
		Ocular	Photograph
5	1	7.62	8.36
	2	7.62	8.36
	3	6.66	7.72
	4	6.66	----
	5	6.66	----
	6	5.71	----
	7	3.81	3.87
	8	2.86	3.22
6	1	4.76	----
	2	4.76	----
	3	4.76	3.87
	4	4.76	4.50
	5	4.76	5.14
	6	2.86	3.22
	7	2.86	3.22
7	1	12.38	10.29
	2	8.57	8.36
	3	8.57	8.36
	4	8.57	7.72
	5	7.62	7.72
	6	5.71	7.07
	7	5.71	4.50
	8	4.76	4.50
	9	1.90	1.93
8	1	8.57	8.36
	2	7.62	----
	3	7.62	7.72
	4	6.66	7.72
	5	6.66	7.72
	6	6.66	6.43
	7	2.86	----
9	1	7.62	7.72
	2	8.57	7.72
	3	6.66	6.43
	4	6.66	5.79
	5	5.71	5.79
	6	4.76	5.79
	7	2.86	2.57
	8	1.90	2.57

Table 3. (Continued)

Chromosome Set No.	Chromosome No.	Chromosome Lengths in Microns	
		Ocular	Photograph
10	1	7.62	7.72
	2	6.66	6.43
	3	6.66	----
	4	5.71	5.79
	5	5.71	5.14
	6	4.76	----
	7	4.76	----
	8	3.81	3.87
11	1	6.66	6.43
	2	5.71	5.14
	3	3.81	4.50
	4	3.81	----
	5	3.81	----
	6	2.86	----
	7	1.90	2.57
	8	1.90	2.57
12	1	4.76	5.14
	2	4.76	5.14
	3	4.76	4.50
	4	3.81	4.50
	5	3.81	4.50
	6	3.81	3.22
	7	2.86	2.57
	8	2.86	2.57
13	1	5.71	5.14
	2	5.71	5.79
	3	4.76	4.50
	4	4.76	5.14
	5	4.76	4.50
	6	3.81	4.50
	7	2.86	2.57
	8	2.86	2.57
14	1	5.71	7.07
	2	4.76	5.79
	3	4.76	5.14
	4	3.81	4.50
	5	3.81	4.50
	6	3.81	3.22
	7	2.86	2.57
	8	2.86	2.57

Table 3. (Continued)

Chromosome Set No.	Chromosome No.	Chromosome Lengths in Microns	
		Ocular	Photograph
15	1	7.62	----
	2	3.81	3.22
	3	2.86	----
	4	2.86	3.22
	5	2.86	3.22
	6	1.90	----
	7	1.90	1.93
16	1	6.66	----
	2	5.71	5.79
	3	5.71	----
	4	4.76	3.87
	5	4.76	----
	6	2.86	----
	7	2.86	----
	8	2.86	----
17	1	6.66	5.79
	2	6.66	6.43
	3	5.71	5.14
	4	4.76	5.14
	5	4.76	4.50
	6	3.81	----
	7	2.86	2.57
	8	2.86	----
18	1	9.52	----
	2	8.57	9.65
	3	8.57	10.29
	4	8.57	7.72
	5	7.62	----
	6	7.62	8.36
	7	5.71	7.07
	8	5.71	5.79
19	1	6.66	9.00
	2	6.66	----
	3	6.66	5.14
	4	5.71	5.79
	5	5.71	5.79
	6	3.81	5.14
	7	2.86	3.22
	8	2.86	3.22

Table 3. (Continued)

Chromosome Set No.	Chromosome No.	Chromosome Lengths in Microns	
		Ocular	Photograph
20	1	6.66	5.79
	2	4.76	5.14
	3	4.76	5.14
	4	4.76	5.14
	5	3.81	3.87
	6	3.81	3.87
	7	2.86	2.57
	8	1.90	1.93
21	1	6.66	7.07
	2	5.71	6.43
	3	5.71	6.43
	4	5.71	5.79
	5	4.76	4.50
	6	4.76	4.50
	7	3.81	3.87
	8	3.81	2.57
22	1	6.66	6.43
	2	5.71	5.79
	3	5.71	5.79
	4	4.76	5.14
	5	4.76	5.14
	6	4.76	5.79
	7	3.81	3.87
	8	2.86	3.22

Chromosome Comparisons of Greenbug Biotypes

All 3 greenbug biotypes consistently possessed 8 chromosomes. A few chromosome sets were found with only 7 chromosomes. Shinji (1931) reported the male aphid to possess an XO type of sex determining mechanism. Possibly the chromosome sets of 7 represented male embryos. The loss of chromosomes from a set due to the slide preparation procedures is also possible. Some tetraploid chromosome sets were also observed. One slide was found that possessed several chromosome sets in a very small area. All of the sets were in approximately the same developmental stage (Fig. 4). Chromosome configurations of the 3 biotypes are shown in Fig. 5, 6, and 7. One pair of chromosomes was usually very small as compared to others in the set. In many of the sets studied, 1 large pair could also be distinguished from the other pairs. In general, the chromosomes could be classified as 1 large pair, 1 small pair, and 4 intermediate size chromosomes. Usually, the 4 intermediate size chromosomes could not be divided into their respective pairs.

Analyses and comparisons were based on chromosome measurements taken from 10 aphids of each of the 3 biotypes. In order to obtain 10 measurements, 12 aphids were prepared from biotype B, 24 from biotype C, and more than 280 from biotype A. In all tests conducted, obtaining good slide preparations has been much easier for biotypes B and C than for A. All slides were prepared in August and September 1970. Possible differential growth patterns due to the season may account for the problems involved in obtaining good slide preparations from biotype A. Biotype A slides were made from aphids cultured on both Rogers barley and Dickinson Sel. 28A wheat and also under 2 temperatures, 24 and 27 C. At the present time, no explanation is available for the differential

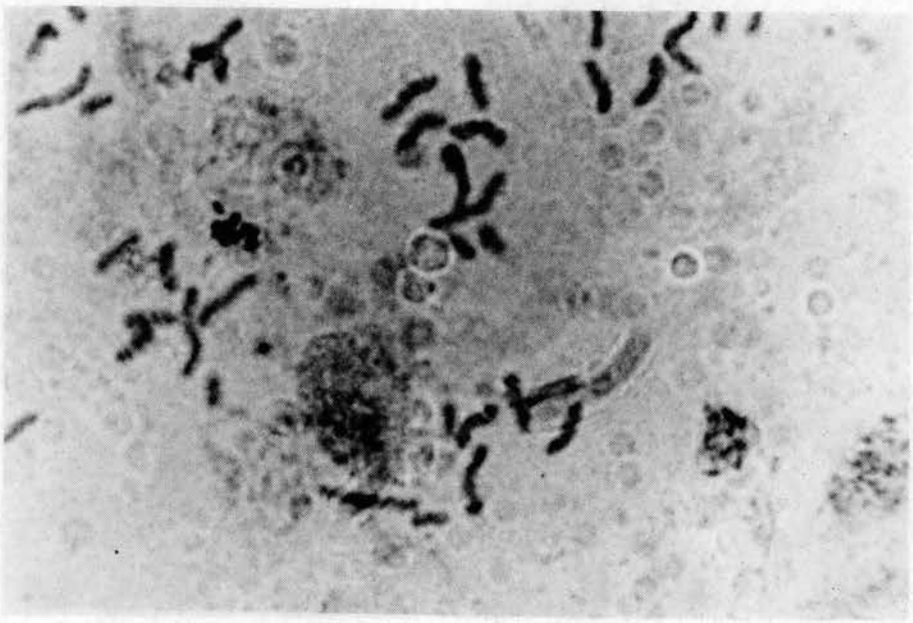

Fig. 4. Metaphase chromosomes of the greenbug.

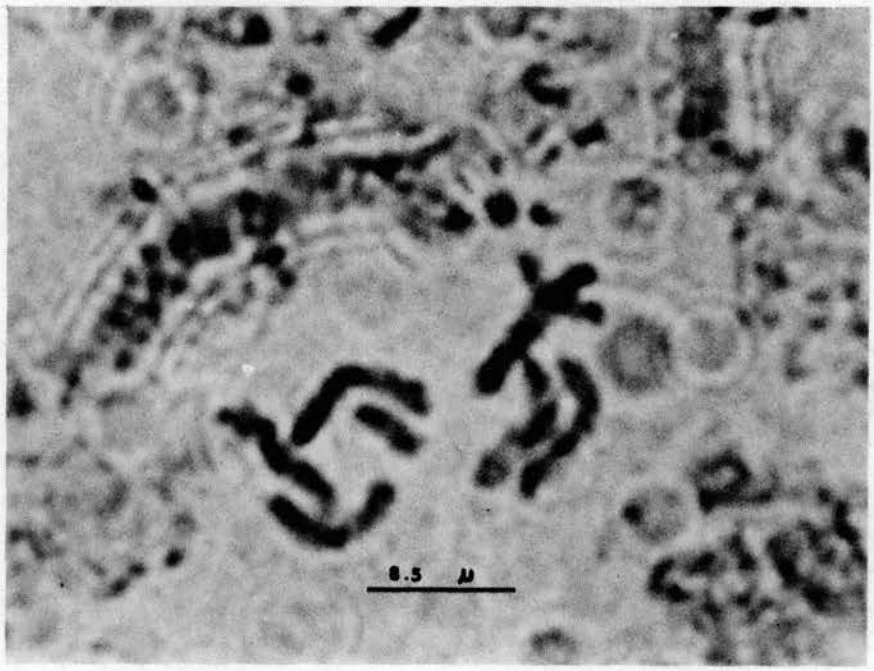

Fig. 5. Chromosomes of the greenbug, biotype A.

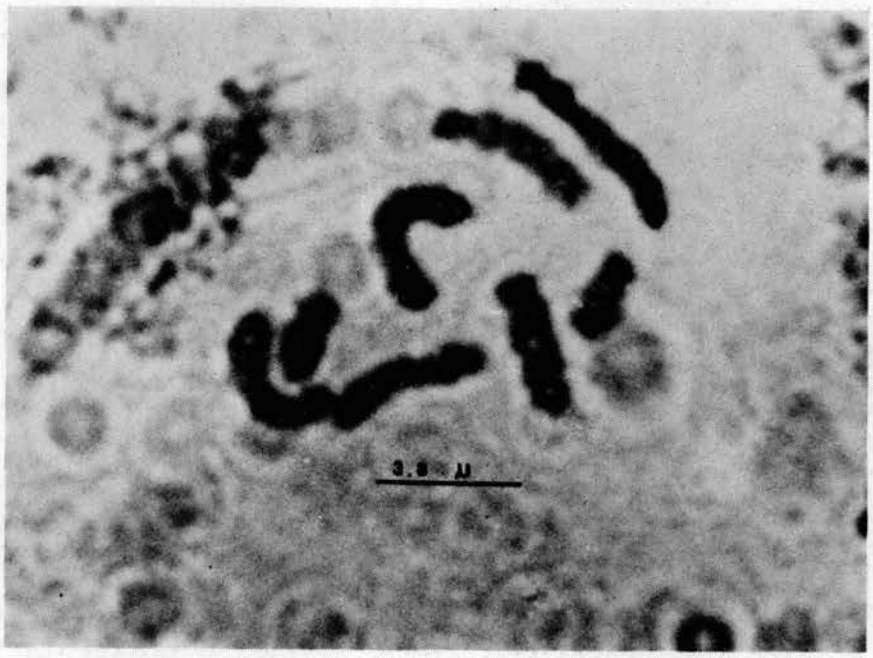

Fig. 6. Chromosomes of the greenbug, biotype B.

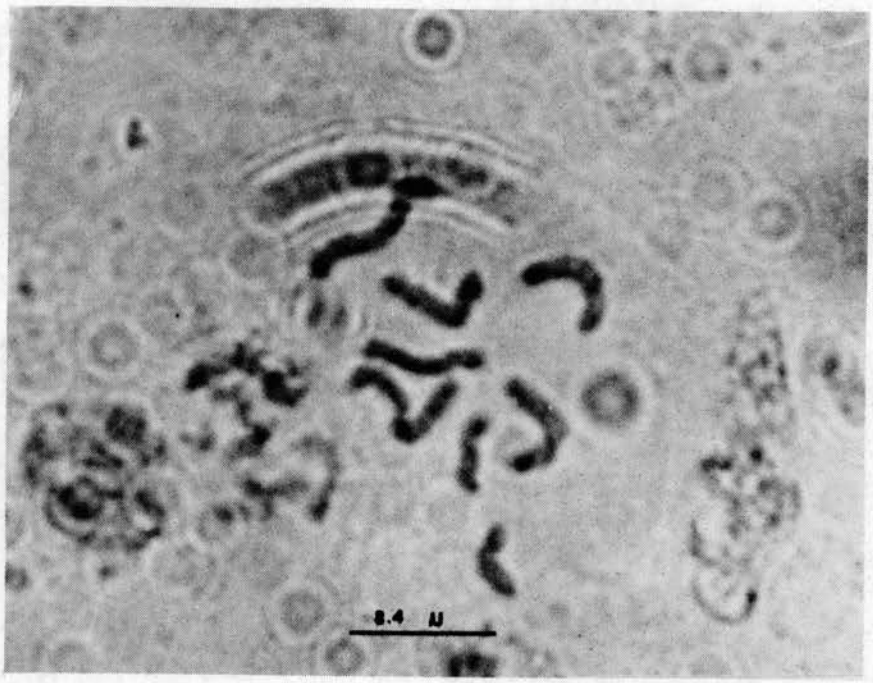

Fig. 7. Chromosomes of the greenbug, biotype C.

staining observed in the 3 biotypes.

The chromosome measurements of each of the 3 biotypes were analyzed for within the population variation (Table 4). The chi-square values for each of the 3 biotypes indicated no significant differences within any of the 3 biotypes.

Since no difference was indicated within the biotype measurements, the 3 biotypes could be compared to each other. The comparisons of the chromosome lengths for the 3 biotypes were accomplished by means of a chi-square to test goodness of fit to a 1:1 ratio (Table 5). The results indicated that biotype A was highly significantly different from either B or C. However, biotypes B and C were not shown to be significantly different from each other.

Comparisons of Chromosomes of Corn Leaf Aphids, Oat Bird-cherry Aphids, and Yellow Sugarcane Aphids, With Chromosomes of Greenbugs

The chromosome number for the corn leaf aphid and the oat bird-cherry aphid was found to be 8. The yellow sugarcane aphid had a chromosome number of 10. Sun and Robinson (1966) reported another species of Sipha, S. agropyrella Hille Ris Lambers, that possessed only 6 chromosomes. This represents a wide variation in the chromosome number of a genus. Figures 8, 9, and 10 show the chromosome configurations of each of the aphids. The corn leaf aphid and oat bird-cherry aphid possessed chromosomes that were approximately the same size as the other members in the set, but one chromosome pair was slightly smaller than the others. The yellow sugarcane aphid chromosomes could generally be grouped into pairs on the basis of lengths, each pair being slightly different in length from the others. The average length and chi-square values are given in Table 6. The chi-square values indicated that

Table 4. Average chromosome lengths and chi-square values of 3 greenbug biotypes.

Aphid No.	Biotype A		Biotype B		Biotype C	
	$\bar{x}$[a]	χ^2	$\bar{x}$	χ^2	$\bar{x}$	χ^2
1	3.63	.208	8.38	1.380	5.00	.298
2	3.13	.475	7.00	.350	8.13	.480
3	6.38	.679	5.63	.000	5.38	.151
4	5.25	.088	4.25	.325	5.50	.121
5	3.75	.160	3.88	.528	4.38	.626
6	3.38	.328	3.63	.693	5.38	.156
7	4.25	.028	4.75	.129	8.00	.411
8	5.75	.281	6.00	.028	9.00	1.075
9	6.25	.583	6.25	.075	5.13	.244
10	4.38	.011	6.25	.075	7.50	.196
Total	46.15	2.845	56.02	3.585	63.40	3.769
Avg.	4.62		5.60		6.34	

[a] = mean calculated by totaling all 8 values.

Tabulated χ^2 value

.05 = 16.92

.01 = 21.67

Table 5. Chi-square comparisons of chromosome lengths of 3 greenbug biotypes to each other and to the corn leaf aphid, oat bird-cherry aphid, and the yellow sugarcane aphid.

Comparisons of Biotypes and Species of Aphids		Chi-square Value[a]
biotype C with		
	: A	20.73**
	: B	3.64**
	: corn leaf aphid	98.78**
	: oat bird-cherry aphid	14.81**
	: yellow sugarcane aphid	8.22
biotype B with		
	: A	7.64**
	: corn leaf aphid	64.00**
	: oat bird-cherry aphid	3.38
	: yellow sugarcane aphid	0.73
biotype A with		
	: corn leaf aphid	28.26**
	: oat bird-cherry aphid	0.83
	: yellow sugarcane aphid	3.59
corn leaf aphid with		
	: oat bird-cherry aphid	38.56**
	: yellow sugarcane aphid	51.44**
oat bird-cherry aphid with		
	: yellow sugarcane aphid	0.97

[a]** = P 0.01% equal 6.63.

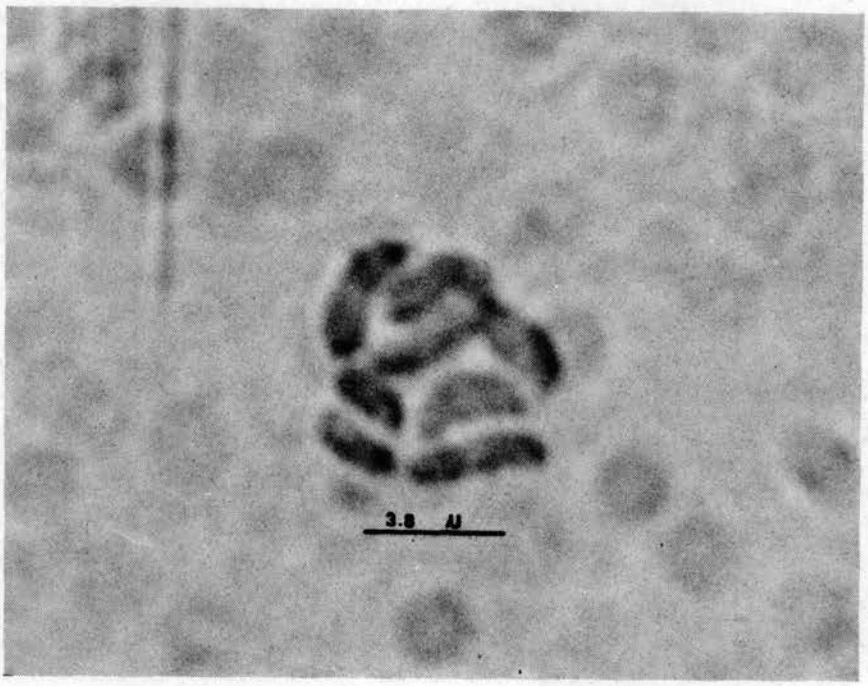

Fig. 8. Chromosomes of the corn leaf aphid.

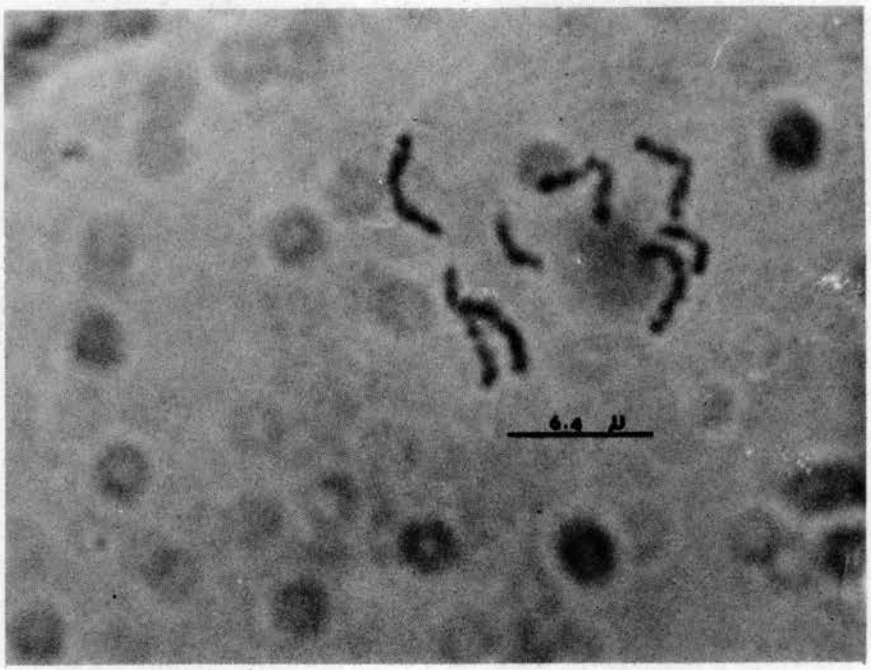

Fig. 9. Chromosomes of the oat bird-cherry aphid.

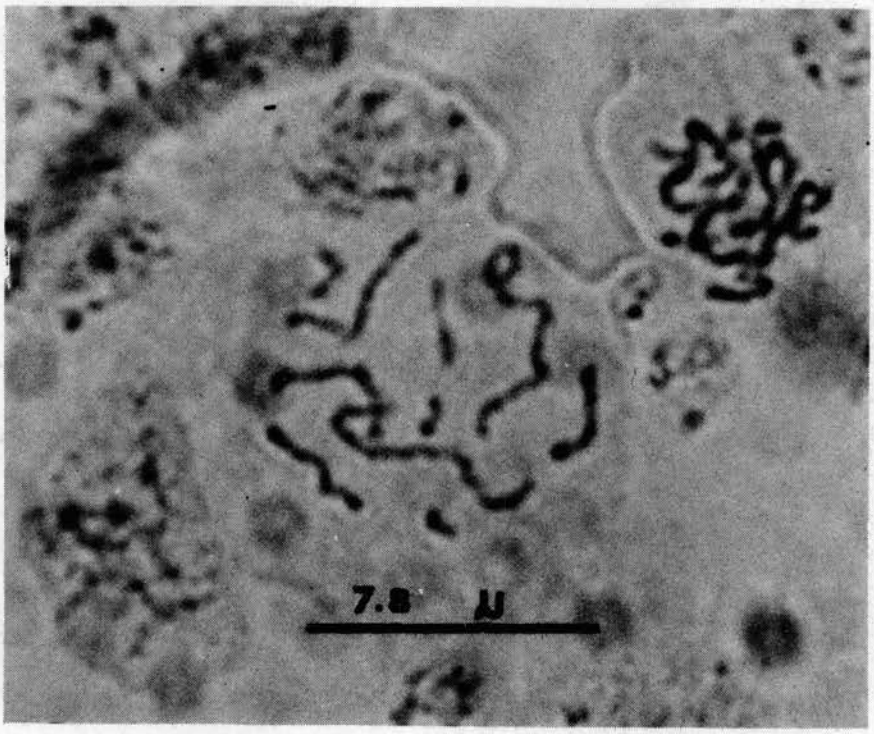

Fig. 10. Chromosomes of the yellow sugarcane aphid.

Table 6. Average chromosome lengths and chi-square values for the corn leaf aphid, oat bird-cherry aphid, and the yellow sugarcane aphid.

Aphid No.	Corn Leaf Aphid		Oat Bird-cherry Aphid		Yellow Sugar-cane Aphid	
	Mean	Chi-square[a]	Mean	Chi-square	Mean	Chi-square
1	2.62	.030	4.13	.109	4.6	.044
2	4.00	.399	4.50	.026	5.2	.254
3	3.50	.115	4.75	.002	3.8	.328
4	2.25	.153	4.25	.076	4.2	.000
5	2.25	.153	6.12	.326	3.4	.142
6	2.75	.009	5.37	.053	6.2	.988
7	3.62	.030	3.37	.456	3.6	.078
8	3.12	.013	6.25	.397	3.0	.328
9	3.75	.235	4.25	.076	4.4	.013
10	2.37	.012	5.62	.118	3.3	.181
Total	30.23	1.168	48.61	1.639	41.7	2.356
Avg.	3.02		4.86		4.17	

[a]Tabulated chi-square values

.05 = 16.92

.01 = 21.67.

variations within the population were not significant in any of the 3 aphids measured.

The chromosomal comparisons among species are shown in Table 5. As indicated in the table, the corn leaf aphid chromosome lengths were highly significantly different from all other aphids checked. The oat bird-cherry aphid was distinguished from biotype C greenbug and the corn leaf aphid. The chromosomes of the yellow sugarcane aphid were determined to be highly significantly different from biotype C greenbug and the corn leaf aphid.

The test results indicate that the chi-square analyses of total chromosome lengths for taxonomic comparisons among species are not reliable for species with completely different chromosome configurations. Visually, the yellow sugarcane aphid chromosomes were easily distinguished from the other aphid chromosome sets. However, the total chromosome lengths were not significantly different from the oat bird-cherry aphid and biotypes A and B greenbugs.

The analysis techniques described in this study can be used on species or biotypes which possess similar karyotypes. If the differences in chromosomes are large enough, this technique should detect them. The 2 species of Rhopalosiphum, in this study, were distinguished from each other. This technique will measure changes in sizes of chromosomes within a species but will not measure such things as inversions in which only the gene sequence may be changed. This is especially true in insects such as aphids which do not possess a centromere.

Based on chromosome lengths, a different karyotype was found for each of the 3 genera studied. Schizaphis was found to have 1 long pair of chromosomes, 1 short pair, and 2 intermediate size pairs. All 4

pair of chromosomes were about the same size in Rhopalosiphum. Sipha possessed 5 chromosome pairs, all of which were different in size.

Behavioral Observations of Greenbug Biotypes

The behavior of biotype A or B as compared to C is quite different. Biotypes A and B colonize and feed largely on the leaves and very small numbers are found on the plant stems. In contrast, biotype C is found in considerable numbers on plant stems as well as on the leaves. A and B biotypes have a tendency to adhere tightly to a plant. Biotype C has a tendency to move when disturbed and often falls from the plants. In biotype C, if the population is very dense, the disturbance of one greenbug usually results in the progressive disturbance of all aphids around it. The aphids will move around for several seconds before settling down.

Biotype C is a more vigorous animal than the other 2 biotypes. It moves quickly when disturbed or placed under unfavorable conditions. Dissection of biotype C was more difficult due to the tendency of the aphid to move from the field of view when placed under the microscope. Attempts to hold the aphid with a probe often resulted in the aphid clinging to or climbing up the probe.

Biotypes A and B were less vigorous and tended to remain relatively motionless, even when disturbed. Their movement was seldom as fast as that of C. Although they tended to move out of the field of view when placed under the microscope, their movement was usually slow. The behavior of the 3 biotypes indicated a definite behavioral difference between biotypes.

Sexual Generations

Although the sexual generation has occurred each year since 1968 in the U.S.D.A.-O.A.E.S., greenhouse at Stillwater, Oklahoma it has not been reported from fields in Oklahoma. Field surveys were conducted each of the 3 seasons. Several surveys were conducted in the spring of 1968, 3 in the spring of 1970, 1 in the fall of 1970, and 5 in the spring of 1971. Small grain fields and annual grasses were surveyed. The greenbug populations were very small in all surveys with the exception of a survey in mid-March 1971. In all surveys, no ovum was observed. However, males were observed in field populations in 1969.

The sexual generation was observed only in biotype C greenbugs and the following data were collected from studies on biotype C.

Males - The percentage of males varied greatly throughout the sexual generations. The percent males, as calculated from the total alate forms present, varied from 0 to 34%. When males were present they averaged 17% of the winged population. These percentages were figured from 10 collections. The numbers of males collected were exact counts but the total numbers of winged aphids were quick estimations of the numbers present (Table 7). These percentages may be high since the cultures chosen for examination were selected because they possessed large numbers of sexual forms with at least some males. There were many periods during which no males were found in the cultures even though large numbers of oviparae were present. The peak periods for male production occurred in January and again in early March for the 1970-71 season.

Spermatozoa were easily identified from aceto-orcein squashes of the testes. They were long thin rods averaging about 15 microns in length. Their general appearance was similar to that of a very small

Table 7. Percentage of males in winged populations of biotype C greenbugs during 1970-1971.

Sample No.	No. of Alates in Sample	No. of Males in Sample	Percent Males in Alates
1	35	0	0
2	50	17	34
3	40	9	20
4	37	0	0
5	25	0	0
6	13	2	15
7	40	4	10
8	30	8	27
9	38	3	8
10	40	2	5
Avg. of all samples			12%
Avg. of samples producing males			17%

nematode. The changes in form from the early to the late spermatozoa could usually be identified when different areas were examined along the testes (Fig. 11). None of the earlier stages of spermatogenesis were identified. Probably the study of testes from early instar males would produce the earlier stages of development. The fully developed testes of adult males contain, for the most part, only mature spermatozoa. The inability to identify immature males hinders the study of spermatogenesis in aphids.

Living spermatozoa were not observed in the saline solution in the slides prepared. More slides should be examined but the small size of the sperm and the difficulty in obtaining the males created problems in this test.

The male greenbugs were smaller than the alate females as Fig. 12 indicates. They averaged about 1.4 mm in length with a wing expanse of about 3.6 mm as compared to 2.1 mm and 5.4 mm, respectively for the alate females. The tarsi and the apex of the tibiae were dark. The first 2 1/3 antennal segments were light colored with the rest of the segments dark. This description of males corresponds closely with that given by Washburn 1908a, Hunter 1909, Theobald 1927, and Palmer 1952.

The numbers of circular sensoria on the antennal segments were not consistent from specimen to specimen. The average sensoria numbers were 25 for the third, 13 for the fourth, and 13 for the fifth antennal segments. This is slightly different from the 20, 18, and 9 sensoria reported by Washburn 1908a, Theobald 1927, and Palmer 1952, for the respective antennal segments of the males.

Oviparae - The percent oviparae in the samples ranged from 3 to 50% of the apterous population with an average of about 27% (Table 8).

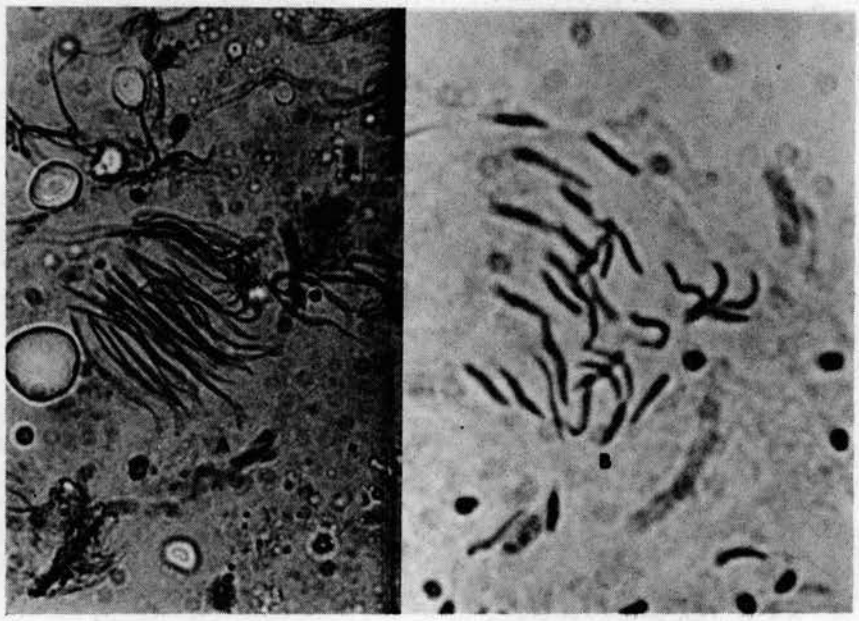

Fig. 11. Spermatozoa of the greenbug. A, mature spermatozoa; B, maturing spermatozoa.

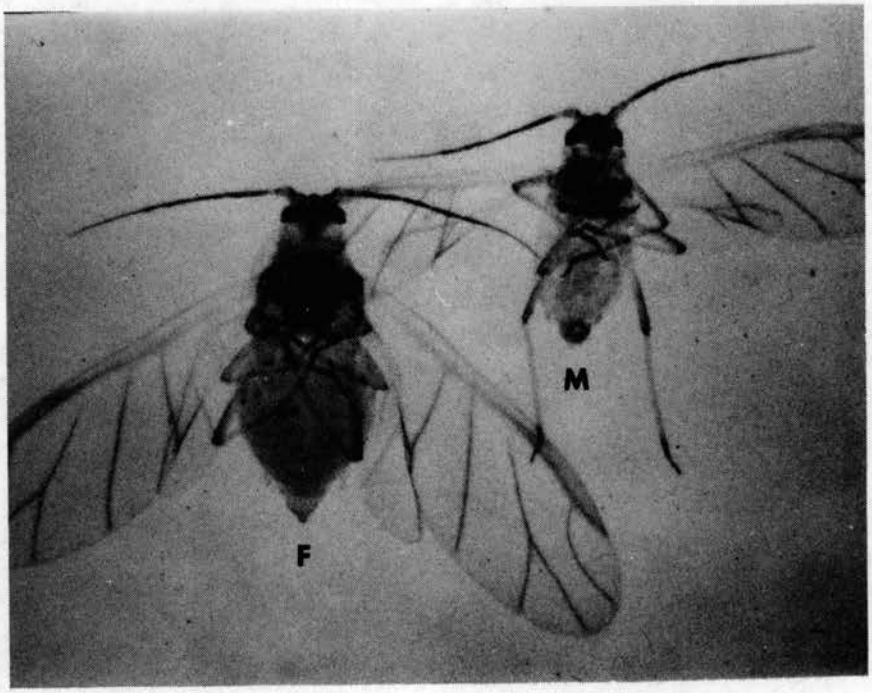

Fig. 12. Male and alate female greenbugs of biotype C. M, male; F, female.

Table 8. Percentage of oviparae in biotype C, apterous, adult, greenbug populations in the greenhouse during 1971.

Sample No.	Total Adults in the Sample	No. Oviparae	Percent Oviparae
1	70	25	36
2	120	60	50
3	90	22	24
4	130	45	35
5	200	75	37
6	150	5	3
7	125	16	13
8	70	16	23
9	80	21	26
10	160	41	25
Average			27%

The counts were conducted from January to the middle of March 1971. The numbers of oviparae were exact counts of the numbers collected. The total apterous counts were quick estimations of the adults present in the sample. Separation of late instar nymphs from adults was extremely difficult and exact counts were not possible for this reason. Although variations in percentages occurred, oviparae were present throughout the sexual season.

The oviparae ranged from 1.7 to 2.4 mm in length. This is slightly larger than the sizes reported by Theobald (1927) and Washburn (1908a). The first 2 1/3 basal antennal segments were light colored with the remaining segments dark. The hind tibiae were slightly swollen and dark and possessed many large flat circular sensoria. The number of sensoria per tibia ranged from 50 to 160 with most possessing between 80 and 110. This sensoria number is much higher than the 23 reported by Palmer (1952) or the 30 to 50 reported by Hunter (1909). The hind tibiae of oviparous and viviparous females are shown in Fig. 13.

The ova were often visible inside the abdomen of the oviparae and they served as a quick means of identification (Fig. 14). The ova sometimes imparted a yellowish tint to the aphid.

Oviparae dissections revealed that each female possessed from 3 to 10 ova in various stages of development. The average number per female was 6.6 (Table 9). Four of the 15 dissected aphids possessed embryos as well as ova. This appeared to represent a rather high percent when compared to other dissections made throughout the sexual season. However, this does show that at least some of the sexual females are capable of producing both ova and viviparous offspring.

Average oviposition, as indicated in Table 10, proved to be less

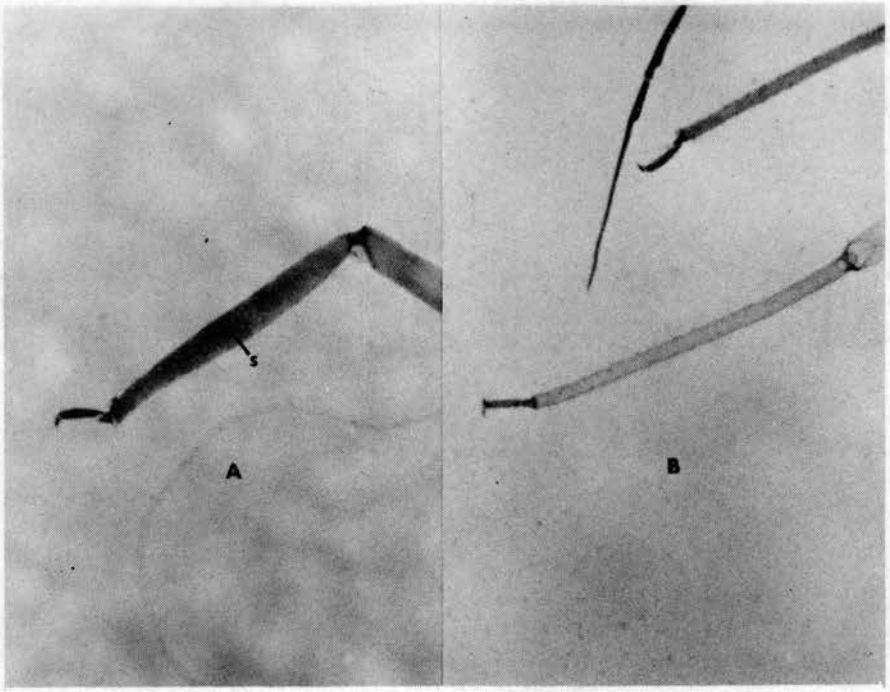

Fig. 13. Hind tibia of oviparous and viviparous females. A, oviparae tibia; B, viviparous female tibia; S, sensoria.

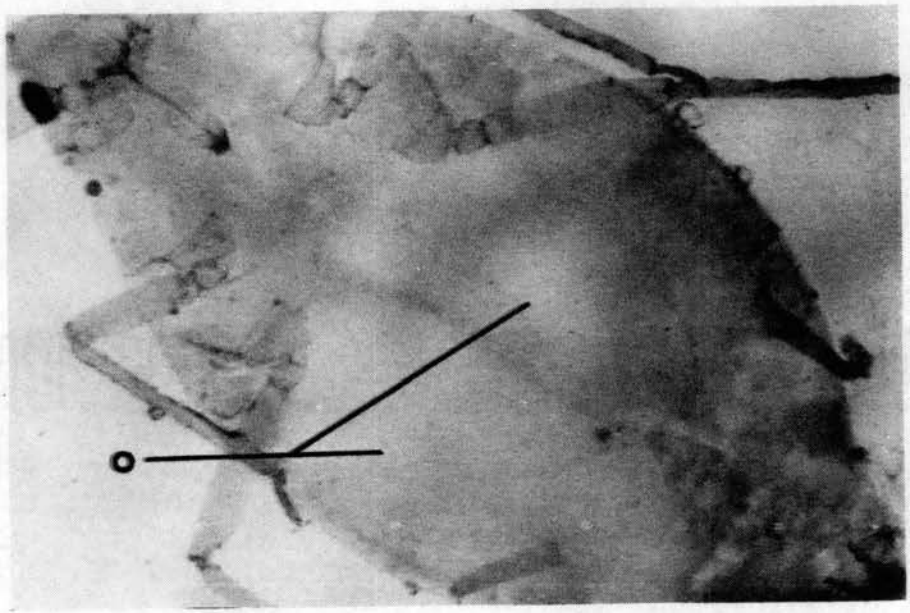

Fig. 14. Greenbug oviparae with ova visible within the abdomen. O, ova.

Table 9. Oviparae dissection to determine the number of ova and embryos present per female.

No. Aphids Dissected	Ova Present	Embryos Present
1	7	
2	8	
3	10	
4	6	
5	9	
6	5	2
7	7	
8	6	
9	9	
10	3	2
11	4	
12	5	3
13	6	
14	6	1
15	8	
Total	99	8
Average	6.6	0.5

Table 10. Average oviposition and oviposition surface preferences of the greenbug.

Cage No. (2 Oviparae/Cage)	Oviposition Site		Total Ova
	Cage	Plant	
1	8	0	8
2	6	2	8
3	4	5	9
4	0	7	7
5	2	6	8
6	0	7	7
7	5	0	5
8	3	0	3
9	5	3	8
10	6	4	10
11	0	0	0
12	6	1	7
13	2	4	6
14	0	1	1
15	0	1	1
Total	47	41	88
Avg./Female	1.6	1.4	2.9

than the number of ova found per female in the dissection studies. An average of 2.9 ova was oviposited per female as compared to the average of 6.6 ova per female from the dissection study. The ova were oviposited on the plant and on the cage in almost equal proportions. The ova found on plants were usually oviposited on the stem or upper leaf surfaces. Ova were usually oviposited singly but sometimes 2 or 3 were found together. The ova found on the cages were located on the cloth mesh where it came in contact with the plastic. Ova were rarely found on the plastic cage.

Greenhouse observations of cultures indicated exceptionally large numbers of ova on the cages. In some cages, the areas around the top of the cages appeared almost black due to the large number of ova. The caged plants also contained many ova but not to the same extent as on the cage.

Dissection of ova from the oviparae produced ova in various stages of development. Fig. 15 shows the progressive increase in size of the ova with the corresponding decrease in the size of the trophocytes. Oseto and Helms (1971) found the viviparous reproductive system of the greenbug to possess telotrophic ovarioles of the syncytial subtype. The oocytes of the sexual form, as indicated in this study, appear to possess attached trophocytes with very short nutritive cords.

Mating - No pre-mating behavior was observed among 10 pairs of mating greenbugs. The males approached the females and almost immediately climbed onto their abdomens. Some of the males used their antennae and front legs to probe the lateral parts of the abdomen a few times prior to climbing onto the abdomens. The male assumed an upright position on the posterior part of the female abdomen. The male then

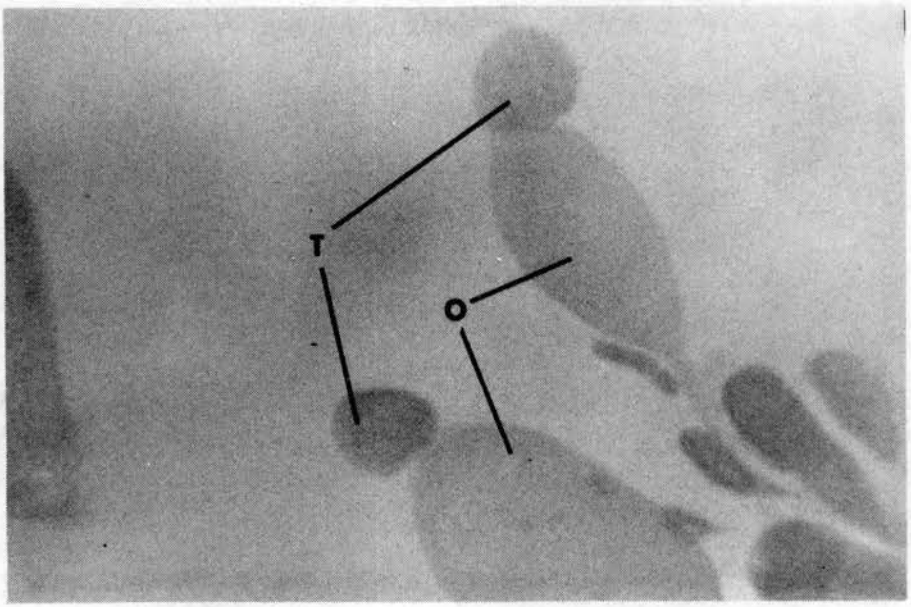

Fig. 15. Ova in various stages of development. O, ova; T, trophocytes.

lowered its abdomen until the male and female genetalia were linked together. Copulation continued for an average duration of 1 1/2 min with a range of 1/2 to 2 1/2 min. One pair was observed to copulate a second time after parting for about 15 sec. Figure 16 shows a greenbug pair copulating.

One female was observed with several males attempting to copulate with her simultaneously. None of the males were observed attempting to copulate with a non-receptive female even though several sexual and non-sexual females were present. The males moved rather quickly to a receptive female and were not observed probing with antennae or following non-sexual females.

These data, especially the multiple matings, indicate the probability of the release of pheromones by the females to attract the males. The possession of large numbers of antennal sensoria by the males is probably at least partially associated with the release of pheromones by the females.

Ova - Greenbug ova were oval in shape and averaged about 0.59 mm long by 0.21 mm wide. These measurements are slightly different from the 0.65 by 0.3 mm reported by Washburn (1908a) and the 0.5 mm length reported by Daniels (1956).

The freshly oviposited ova were yellowish-green in color and later changed to dark green and finally to black (Fig. 17). At a temperature of 27 C, 45% of the ova changed to a black color after 24 hr and 80% after 48 hr. Twenty percent of the ova in the 27 C test turned translucent brown and appeared desiccated. Ten percent of the ova were brown in the 21 C test. In the 21 C test, 40% of the ova became black after 24 hr and 90% after 48 hr. After 2 wk, only 25% of the ova were

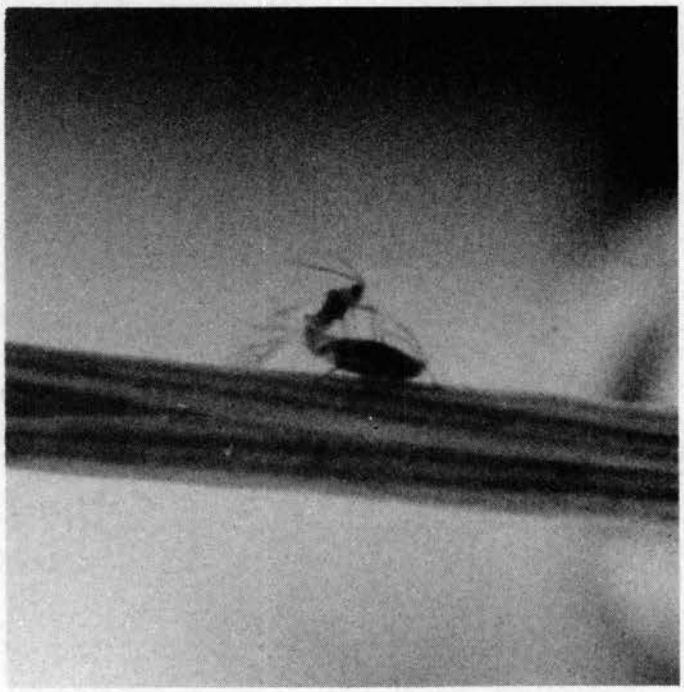

Fig. 16. Greenbug copulation.

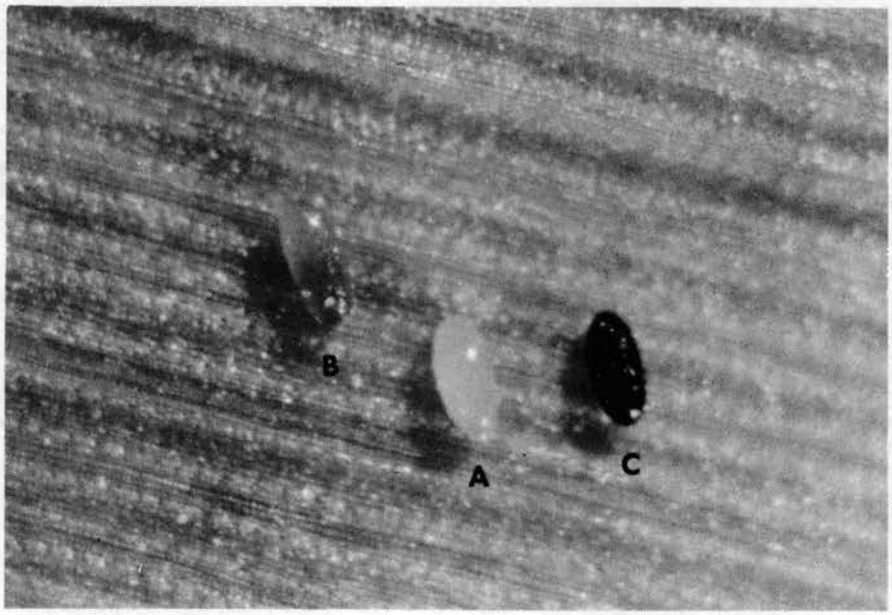

Fig. 17. Greenbug ova in 3 different stages. A, freshly oviposited; B, intermediate stage; C, final coloration.

black in the 4 C test and no brown ova were observed. With only one exception, all yellowish-green ova in the 21 and 27 C tests darkened within 24 hr. Most of the yellowish-green ova in the 4 C test also darkened within 24 hr but the time period ranged from 1/2 to 4 days.

Aceto-orcein squashes of ova contents produced chromosome sets in 33% of the slides prepared. Each slide consisted of the contents of 1 ovum. All of the ova examined were less than 2 days old. When chromosome sets were found, they appeared to be in a rapidly dividing state and many chromosome sets could be located in a small area. These data indicated that at least 1/3 of the ova are capable of initial development.

The ova incubated in 1970 failed to hatch or show any signs of embryo development. The ova placed in the 18 C temperature cabinet were transferred to the 21 C chamber after 4 wk due to mechanical problems. All of the ova incubated at 18, 21, and 27 C appeared desiccated within 4 wk. Dissections of the desiccated ova revealed their contents to be in a crystallized state. The ova stored in the 2 C chamber did not appear desiccated but no embryo development was determined from ova dissections. Approximately 300 additional ova were incubated at 2, 21, and 27 C but no development was found.

None of the ova in the 1970-71 incubation studies hatched. All of the ova stored at the 21 and 27 C temperatures were desiccated within 5 wk. The ova on the agar plates desiccated slightly slower than the ova on the glass slides. After 6 mo, approximately 70% of the ova stored at 2 and 4 C were dessicated. Many of the agar plates contained fungal growth. Two embryos were found when ova dissections were conducted. Both of the embryos were discovered from ova stored in the 4 C chamber.

One of the embryos, as shown in Fig. 18, was almost completely developed and appendages were apparent. Upon dissection from the shell a bright red eyespot was apparent. The other embryo was in an earlier stage of development with developing appendages (Fig. 19).

Greenbug Type and Ova Production - The results indicated oviparous females to be apterous. Oviposition was not demonstrated by alate aphids (Table 11).

Little difference was detected between the dark green and light green females. This indicated a color difference due to some factor other than a difference between oviparous and viviparous types. The difference was determined to be at least partially due to age.

The tests indicated that between 15 and 22% of the population was oviparous.

Oviposition by Oviparae When Exposed to Males as Compared to no Exposure to Males - Of the 30 cultures infested with 1 nymph each on January 25, 1970, 9 became oviparae, 18 viviparous females, and 3 did not reproduce. One of the 18 parthenogenics produced 5 offspring which all matured into males. The percent oviparae for this study was quite high when compared to later observations which usually ran in the area of 15 to 25% oviparae.

The results of both the 21 C test and the greenhouse test are shown in Table 12. In both tests, the oviparae not exposed to males produced fewer ova than when caged with males. However, the tests show that they are capable of oviposition in the absence of males. Two oviparae, one in the 21 C test and one in the greenhouse test, reproduced oviparously at first but later began reproducing viviparously. The data in these tests correspond closely with the average of 3 and maximum of 7 ova

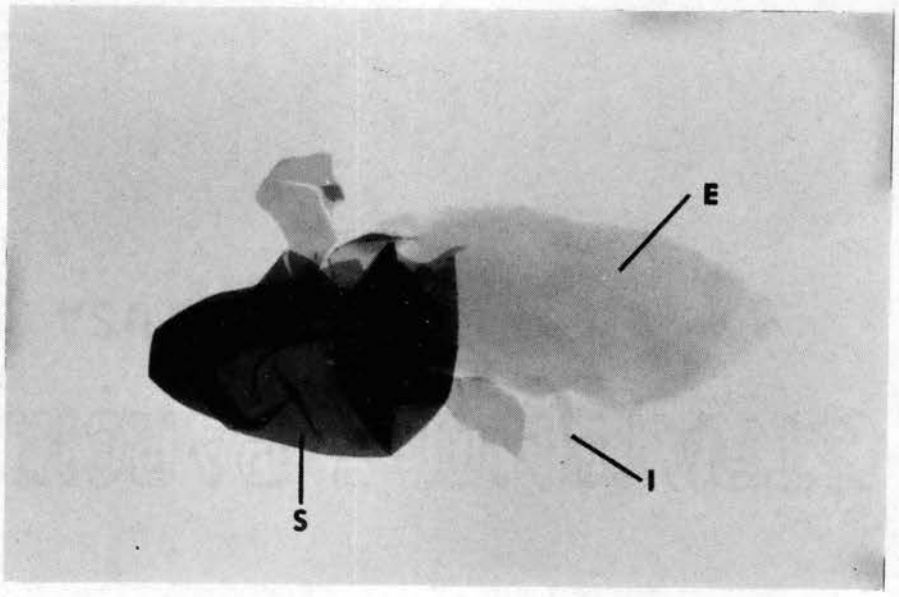

Fig. 18. Embryo from a biotype C greenbug ovum. S, ovum shell; E, embryo; 1, leg.

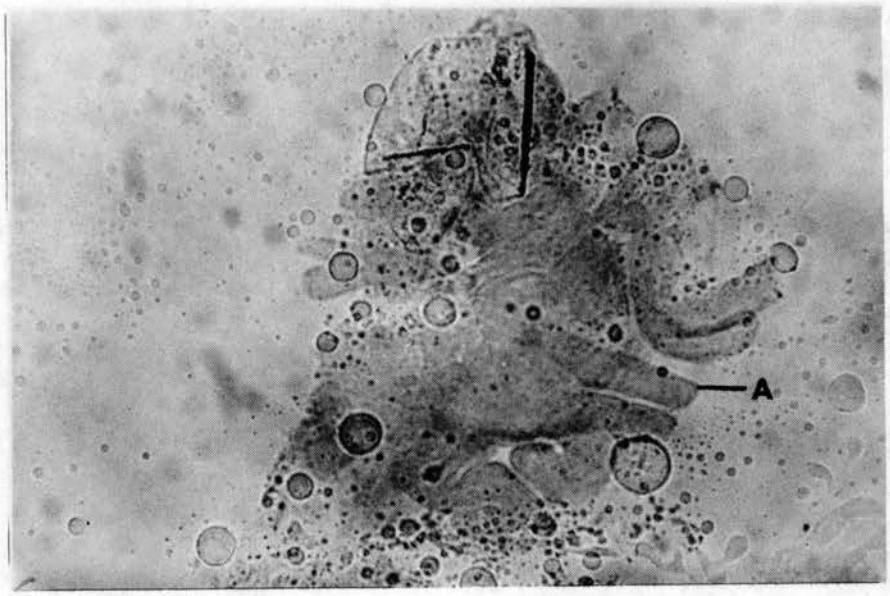

Fig. 19. Developing embryo from a biotype C greenbug ovum. A, appendages.

Table 11. Percentage oviparae in 3 phases of biotype C greenbugs.

Greenbug Form Studied	Test Dates	Total Aphids Studied	No. of Aphids Ovipositing	Percent Oviparae
Dark Green Females	1970	45	8	17.7
	1970-71	57	14	22.8
Light Green Females	1970	50	11	22.0
	1970-71	57	9	15.8
Alate Females	1970	44	0	0.0
	1970-71	60	0	0.0

Table 12. The effect of the presence of males on ova deposition by biotype C oviparous females.

Comparison No.	Number of Ova Produced			
	21 C Chamber Test		Greenhouse Test	
	Oviparae Only	Oviparae + Male	Oviparae Only	Oviparae + Male
1	3	7	1	5
2	5*	*	0	*
3	0	4	4	3
4	2	3	0	8
5	*		3	0
6			*	2*
7			6	4
8			5	0
9			2	5
10			0	1
11			2	2
12			1	5
Total	10	14	24	35
Avg.	2.0	3.5	2.0	2.9

*= Aphid produced living offspring either solely or in combination with ova.

reported by Wadley (1931) to be produced by greenbug oviparae.

The females observed copulating produced approximately the same number of ova as the oviparae caged with males in the previous tests. The results are shown in Table 13. Only 1 female failed to produce ova. These data suggest that the caging of oviparae and males together often leads to copulation. This would be the expected result if sex pheromones were emitted by the females as suggested in a previous section.

Effect of Photoperiod and Temperature on the Production of the Greenbug Sexual Generation - Sexuales were not produced under either of the photoperiod-temperature conditions studied. Both tests were conducted for only 1 mo and possibly this was not long enough to initiate the production of sexuales. However, Lees (1959) found sexual form determination to occur in the embryonic stages of Megoura viciae, and the change was expressed within 1 generation. Wadley (1931) stated that photoperiods of less than 12 hr at average temperatures of below 21 C produced greenbug sexuales. These conditions did not produce sexuales in this study.

Table 13. The number of ova produced by biotype C greenbugs known to have copulated.

No. of Pairs Observed Copulating	Caged Plant	Date Observed Copulating	No. Ova Produced
1	Barley	Apr. 2, 1970	6
2	Barley	Apr. 2, 1970	3
3	Barley	Apr. 2, 1970	3
4	Barley	Feb. 10, 1971	4
5	Barley	Feb. 10, 1971	5
6	Sorghum	Feb. 10, 1971	1
7	Sorghum	Feb. 11, 1971	2
8	Sorghum	Feb. 11, 1971	2
9	Sorghum	Feb. 11, 1971	8
10	Sorghum	Feb. 17, 1971	1
11	Sorghum	Feb. 17, 1971	7
12	Sorghum	Feb. 17, 1971	4
13	Sorghum	Feb. 17, 1971	3
14	Barley	Feb. 18, 1971	2
15	Barley	Feb. 18, 1971	0
		Total	51
		Avg.	3.4

SUMMARY AND CONCLUSIONS

Comparisons of Feulgen's, aceto-carmine, and aceto-orcein staining techniques indicated aceto-orcein to be the best technique for quick, clear chromosome preparations. The small greenbug embryos, averaging about 0.25 mm in length, were determined to be the best stage for slide preparation. At temperatures of 24 C, 5 day old aphids provided the greatest number of small embryos. The 5 day old aphids averaged 1.4 mm in length. The intermediate sized embryos, averaging about 0.43 mm in length, also provided several clear chromosome sets. The range of development within this embryo size group was considerable. Squashes of embryos just entering this size group probably resulted in the clear chromosome sets observed. More mature embryos from this size group possessed well developed tissue and appendages which prevented the easy preparation of clear chromosome sets.

Two chromosome measuring techniques were compared. One technique involved measurements taken from chromosome photographs. The other chromosome measurements were taken directly from the slide using an ocular micrometer. No significant difference was found between the 2 measuring techniques. The measuring of chromosomes directly from the slides was advantageous due to the ability to focus upon individual chromosomes. This resulted in the chromosome sets not having to be in one plane of focus as was necessary for production of clear photographs. Only 83% of the total numbers of chromosomes measured directly from the slides were clear enough on the photographs to be measured. The use of

an ocular micrometer for measurement of short, straight chromosomes appeared to be useful but its application for the study of long, curving chromosomes is doubtful.

The chromosome configurations of 3 greenbug biotypes, the corn leaf aphid, the oat bird-cherry aphid, and the yellow sugarcane aphid were compared. Total chromosome lengths of each set were used in a chi-square analysis for all of the species and biotypes studied. This study indicated biotype A chromosomes to be significantly different from biotypes B and C. Biotype B was not significantly different from biotype C. The corn leaf aphid chromosomes were determined to be significantly different from all aphid species studied. The oat bird-cherry aphid was significantly different from the corn leaf aphid and biotype C greenbug. The chromosome analysis of the yellow sugarcane aphid indicated significant differences from the corn leaf aphid and biotype C greenbug.

The 3 genera studied were found to possess different karyotypes. The greenbugs, corn leaf aphids, and oat bird-cherry aphids possessed 8 chromosomes per set, the yellow sugarcane aphid had 10.

Chi-square analysis for taxonomic purposes is not reliable for distinguishing species with different chromosome configurations. However, it can be used to detect differences between biotypes, sibling species, or other closely related species if the chromosome differences are large enough. This analysis technique will detect changes in chromosome size, but not changes that involve only the gene sequences.

The greenbug sexual generation was first observed in the Stillwater, Oklahoma area in the fall of 1968. The 1968-69 and 1970-71 sexual season occurred from November to late March. The fall sexual season

was not observed in 1969 but sexuales were found from late January to late March 1970. Ova were produced in the greenhouse at Stillwater, Oklahoma but were not observed in field surveys in the same area.

Greenbug males were found periodically throughout the sexual season. When they were present, they averaged about 17% of the winged population. The males were found to be slightly smaller than reported in the literature. They also possessed more antennal sensoria. Large numbers of spermatozoa were found from aceto-orcein squashes of the testes.

Oviparae were found to range from 3 to 50% of the apterous population with an average range of 15 to 25%. All of the oviparae observed were apterous and averaged 1.7 to 2.4 mm in length. Hind tibiae of oviparae possessed an average of 80-110 large flat circular sensoria. This is considerably above the numbers reported in the literature. Dissections of oviparae revealed an average of 6.6 ova per female, however, oviposition studies determined an average oviposition of 2.5 to 3.5 ova per female. Oviposition occurred on both the cages and the plants in about equal frequencies when populations were small. However, exceptionally large numbers of ova were observed on cages when greenbug populations were high. Some oviparae were found to produce both ova and living offspring.

Greenbug pairs were observed mating and there was an indication of the production of sex attractants by the females.

Biotype C greenbug ova were oval in shape and averaged 0.59 mm by 0.21 mm. They were yellowish-green when freshly oviposited and usually turned black within 48 hr. None of the ova hatched when incubated at temperatures of 2, 4, 18, 21, or 27 C. However, 2 developing embryos

were found by ova dissections. These embryos were found from ova stored at 4 C for 6 mo. Chromosome sets were also discovered from aceto-orcein squashes of ova contents. Squashes were made from ova less than 2 days old. The chromosome sets along with the embryo development indicate that at least some of the ova are capable of development if placed under the proper environmental conditions.

Photoperiods of less than 12 hr at average temperatures of 21 C or less did not initiate development of sexuales. Greenbugs were cultured for 1 mo under the conditions described. The light quality and intensity were not determined for the studies. Longer incubation may produce sexuales but more in depth studies must be conducted to determine factors responsible for the production of sexuales.

The morphological and physiological differences of the 3 greenbug biotypes described by previous authors, as well as the behavioral, chromosomal, and sexual generation differences reported in this study, indicate definite differences in the 3 greenbug populations. Whether these differences are large enough to warrant the description of the populations as subspecies rather than biotypes will depend on the interpretations of taxonomists.

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