SOME HOST RELATIONSHIPS OF APHIDS, INCLUDING DIFFERENTIATION BETWEEN GENERA AND SPECIES BASED ON CHROMOSOME STUDIES

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Rou-Yun Sun

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ABSTRACT

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Rou-Yun Sun

SOME HOST RELATIONSHIPS OF APHIDS, INCLUDING
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This study reports on certain host plant relationships of aphids in Manitoba, and on chromosome studies of aphids. When Rhopalosiphum padi (L.) was reared at constant temperatures on two varieties of barley, Hordeum vulgare L., the optimum was at 75° F, with reproduction ceasing at 45° and 100° F. In studies on host plants of two morphologically similar species, R. padi and R. fitchii (Sand.), it was shown that R. padi overwinters on Prunus virginiana L. and not on Pomeae. R. fitchii overwinters on species of Cotoneaster, Crataegus, Malus and Sorbus but not on Pruneae. Fundatrices and/or fundatrigeniae of R. fitchii could be successfully transferred between any of the four winter host plants.

In studies on chromosomes of aphids, two squash methods were used, a quick examination method stained by Orcein, and the Feulgen stain method. In fifty species of aphids studied, chromosome counts were 4, 6, 8, 10, 12, 14, 16, 18 or 20. More species (15) had 8 chromosomes than any other count. It is

postulated that the variations of chromosome numbers in aphids were caused by fragmentations of primitive chromosomes. This investigation indicates that studies of aphids chromosomes can be a useful means in solving difficult taxonomic problems and of suggesting possible evolutionary development in aphids.

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CHAPTER I

INTRODUCTION

Insect-host plant relationships and responses of plant varieties to insect attack have been observed dating back as early as 1792 when Havens described the Underhill variety of wheat as resistant to the Hessian fly, Phytophaga destructor (Say) in America. A well known example of a plant resistant to insects found in the middle of the nineteenth century, is that of the grape phylloxera, Phylloxera vitifoliae (Fitch) which was controlled by the use of phylloxera-resistant vines. Since then the inheritable resistant character of some plants to insects has been widely noticed, and also has been recognized as an effective method of controlling insects, especially those with high host specificity like aphids and scale insects. The first complete review of insect resistance in crop plants is that of Painter (1951). Some workers have looked for varieties resistant to insects. Others have dealt with the insect-host relationships. Resistance of plants to aphids has been described more frequently than for any other group of insects (Painter, 1958).

In studies on insect-host plant relationships various environmental factors may directly affect the insect or indirectly through effects on the plants. It is known that the environment may have a controlling effect on expression

of many genetic characters, hence it may be expected that a plant can exhibit a varying degree of resistance under different environmental conditions such as temperature, light intensity, humidity, soil nutrition or stage of plant growth. Among these factors temperature could be considered as the most important one affecting the behavior of insects and host plant resistance.

One of the problems encountered by those who study insect-host plant relationships is the correct identification of the insect species with which they are working. One method used to assist in solving these problems is to make transfers of plant-feeding insects from one host to another. Another method which is as yet not widely used, is the study of chromosomes of the insects. Host transfers and cytotaxonomic studies may be used in conjunction with studies on life history and morphology. The shape and size of chromosomes along with the details of their meiotic mechanisms may give fundamental information concerning evolution and taxonomy of higher categories.

Organization of the thesis

Although the various experiments performed for this thesis are part of the overall subject of insect-host plant relationships, they are more easily presented by introducing them separately in short chapters. The results of an experi-

ment to determine the effects of temperatures on reproduction are presented in Chapter IV. The results of some host transfers of two morphologically similar species of Rhopalosiphum padi (L.) and R. fitchii (Sand.) are presented in Chapter V. Chapter VI contains the observations of mitotic cell divisions and the chromosome counts of various aphid species. Pertinent literature review, and materials and methods used for the various studies, are given in Chapters II and III, rather than separately with each experiment performed. Chapter VII contains the summary and conclusions.

CHAPTER II

REVIEW OF LITERATURE

The first book on insect resistance in crop plants was published by Painter (1951). This book contains a comprehensive review of the world literature up to 1950 and covers over 1000 references. Painter (1951) defined the word resistance as "the amount of damage done by insects." He has divided the types of plant resistance to insects, as seen in the field, into three main categories: preference or non-preference, antibiosis and tolerance.

Preference or non-preference is referred to as the group of plant characters and insect responses that lead the insect to or away from a particular plant or variety. Plant characters such as color, texture, taste, might be involved in this type of resistance. Antibiosis involves the adverse effects on the life cycle of the insect feeding on the resistant plant or variety. Theseeffects might include decreased size and fecundity, abnormal length of life, and increased mortality. Tolerance is considered to be the ability of the resistant plant to grow favorably in spite of supporting an infestation comparable to that damaging a susceptible host.

Painter and Peters (1956), Daniels and Porter (1958) and Curtis et al (1960) have reported that resistance is a kind of genetic character, which should react in a certain way

to the various environmental conditions. These environmental factors may affect the insects, the plants and the insect-plant interaction in physiological and biotic aspect (Painter, 1954). Walton (1954) studied the seasonal fluctuation of the green peach aphid and turnip aphid in the field and stated that temperature is the most critical factor of many ecological factors that affect directly the aphid development or indirectly through the plants.

Temperature is known to affect the behavior of aphids (Isaak, Sorensen and Painter, 1965). Thorsteinson (1953) described a modification of host selection by temperature to a limited extent. It may increase the rate of feeding and of utilization of food and hence of growth. Headlee (1914) stated that humidity was less important than temperature on the feeding behavior of the greenbug, Toxoptera graminum. Moore (1914) noted the root-infesting habit of greenbug in hot weather in South Africa. Wadley (1931) observed that when the heat was over 100° F., some greenbugs left the plants and clustered on the soil under the pots. When pots were kept in a cage at 104° F., aphids left plants for soil till the soil became dry then returned to plants. The heat avoidance is a well-marked feature of their behavior.

Maxwell and Painter (1959) found that the rate of honeydew excretion increased with increased temperature. Lamb (1963) reported that increases in temperature may increase the

production of metabolic water and hence influence the weight loss.

Temperature may also affect the rate of development (Wadley, 1931; Barlow, 1962; Messenger, 1964). Harpaz (1955) and Dickson et al (1955) indicated that the development of spotted alfalfa aphid, Therioaphis maculata (Buckton) was reduced at lower temperatures. Messenger (1964) found that fluctuating temperatures could stimulate the development of spotted alfalfa aphid.

Wadley (1931) reported that the temperature may affect the longevity of the greenbug. Fluctuating temperatures caused longer longevity (Messenger, 1964). Blanchard and Dudley (1934) observed the pea aphid in the field in Wisconsin and found that the higher the average temperature, and especially the higher the maximum, the shorter the time aphids lived.

Headlee (1914) stated that the survival of greenbug at a low temperature (50° F) is more than at a high temperature (90° F). Pea aphid mortality on resistant alfalfa plants was higher at moderate than at low temperatures (Painter, 1954). Howe and Smith (1957) noted that spotted alfalfa aphids were unable to survive during hot summer months. Barlow (1962) also noted the effect of temperature on the survival of the potato aphid.

Polymorphism is controlled genetically, and is also greatly influenced by the environment (Lees, 1961). Wilson

(1931) stated that temperature is one of the important factors influencing the forms of Aphis chloris. MacGillivray and Anderson (1964) found that if other conditions were kept the same, temperature will influence the form of oviparae or viviparae of Macrosiphum euphorbiae (Thomas), with a greater percentage of oviparae at 51° F than at 61° F or 65° F.

The reproductive rate of aphids is higher at high temperatures than at low (Headlee, 1914; Wadley, 1931; Dickson et al, 1955; Harpaz, 1955; Barlow, 1962). Messenger (1964) found that increased reproduction under fluctuating conditions, at mean temperature of 14° C, is nearly 50% greater than that under constant conditions.

Since temperature is known to affect numerous physiological processes in plants and animals, it is possible that it should also affect the expression of resistance of plants to insects. Expression of host resistance under various environmental conditions may be determined by means of antibiosis. By comparing the number of live aphids on resistant plants with those on susceptible plants, the relative degree of resistance being expressed may be determined.

McMurtry and Stanford (1960) found that the physiology of alfalfa plants changed within a period as short as two days when the alfalfa plants were moved from one temperature to another. McMurtry (1962) stated that the effects of temperature caused the change in the host plant, which in turn

affected the aphids. Thorsteinson (1953) described that at 32°-36° C, the chemical constitution of leaf tissue may be changed by dissipation and evaporation of volatile repellent olfactory substances which are repellent to the fourth instar nymphs of Colorado potato beetle, hence increasing the consumption of less acceptable plants by them. Dahms and Painter (1940) found that at higher temperatures fecundity and survival of pea aphids, Macrosiphum pisi (Harris), were greatly retarded on resistant plants and suggested that available food in the resistant plant would be more limited at high temperature.

Hsu and Robinson (1962; 1963) found a greatly reduced fecundity of Rhopalosiphum padi (L.) reared on barley variety C.I. 3906-1 compared with that on Swan, at fluctuating temperatures, both in the field and laboratory. Studies on the same problem by Belvett (1965) at controlled temperatures showed no significant differences in fecundity of R. padi on the two varieties, and the resistance formerly found in C.I. 3906-1 could no longer be demonstrated. Albrecht and Chamberlain (1941) reported that low temperature may have resulted in a change in the apparent resistance of some strains of alfalfa. Painter (1954) stated that the differences between resistant and susceptible varieties in respect to preference were smaller at higher than at low temperatures. He further showed (1958) that temperature influences the expression of resistance differently. The reproduction of the pea aphid on

resistant alfalfa plants was sometimes greater at a lower temperature, while the degree of resistance of wheat to greenbug was less at high temperature than at low.

Resistance of alfalfa to spotted alfalfa aphids was decreased at low temperatures (Howe and Smith, 1957; Painter, 1958; Hackerott and Harvey, 1959; McMurtry, 1962; Isaak et al, 1963) and increased at high temperature (McMurtry, 1962). Within a certain range, pea aphid (Smith and Davis, 1962) and spotted alfalfa aphid (Dickson et al, 1955; Harpaz, 1955; Graham, 1959) populations on susceptible alfalfa increase directly with temperature. Dahms and Painter (1940), Howe and Smith (1957), Hackerott and Harvey (1959) found that the optimum temperature for the increase of both aphid species was lower on resistant than on susceptible alfalfa plants.

Painter (1951) stated that if two or more insects belong in the same family or genus this is a totally insufficient basis for assuming that the plant would react in the same way to the several insect species. Information on resistance to aphids is of questionable value unless the species involved have been correctly identified. Bruehl (1961) stated that Rhopalosiphum fitchii (Sand.) and R. padi (L.) are two important vectors of barley yellow dwarf virus and have been considered to be economically important. Smith (1963), Smith and Richards (1963) have worked on the transmission ability of the same two species. The resistance of barley varieties to

R. padi has been worked out by Hsu and Robinson (1962), Hsu (1963) and Belvett (1965).

The biology of R. padi and R. fitchii has been studied extensively by Orlob (1961), Smith and Richards (1963), Robinson and Hsu (1963), Hsu (1963) and Belvett (1965). They showed that the overwintering of R. padi on some Pruneae and of R. fitchii on various Pomeae is definitely established, and there is little difficulty in distinguishing the fundatrices or fundatrigeniae. Orlob (1961) has shown that R. padi was most common on Prunus virginiana L. and R. fitchii was found commonly on Malus sp. and Crateagus sp. There has been some uncertainty about the forms of R. fitchii from Cotoneaster and from Sorbus (Richards, 1960).

Because the summer viviparae of R. padi and R. fitchii are similar in morphology they are not easily separated taxonomically. The taxonomic problems involved in determinations of these two species have been discussed by many workers. Rogerson (1947) showed that two species were involved in R. prunifoliae (Fitch) of North America. One is R. crataegellum (Theo.), the other is R. padi (L.) of Europe. He further showed that only R. padi fed on the aerial portion of the grasses, while the North American R. fitchii is identical with the R. insertum (Walk.) which is subterranean in habit. A similar conclusion has been made by Hille Ris Lambers (1960). However this subterranean habit could not be confirmed by Orlob (1961).

Oswald and Houston (1953), Toko and Bruehl (1959) thought that the correct name for R. prunifoliae should be R. fitchii, the apple grain aphid, and it was considered to be a very efficient vector of barley yellow dwarf virus to barley, wheat, oats and grasses. But now it is believed to be R. padi by Smith and Richards (1963). It was noted by Orlob (1960), Bruehl (1961) that R. fitchii fed very poorly on cereals. Smith and Richards (1963) could only find very few on grass in Ontario. Robinson and Hsu (1963) could not find the summer forms of R. fitchii in the field at all in Manitoba. Smith (1963) and Smith and Richards (1963) showed that R. fitchii was not as efficient as R. padi as a vector of BYDV on cereals. Orlob (1961) referred to the name of "padi-fitchii complex." But Richards (1960) and Smith and Richards (1963) have recognized R. padi and R. fitchii as two distinct species. They stated that although the summer forms are easily confused, they are distinguishable by the slightly longer antennae and shorter, blunt antennal hairs in R. padi.

The cytology and cytotaxonomy of aphids have been neglected in recent years due to the lack of a quick, simple technique for preparing specimens. Stevens (1905), Morgan (1909), Shinji (1931), Lawson (1936) and Ris (1942) used sectioned material in their investigations, while Colling

(1955), Dionne and Spicer (1957), Cognetti (1961) and MacDonald and Harper (1965) used squash techniques. Colling reported a squash method for the somatic chromosomes that apparently gives satisfactory results. Dionne and Spicer (1957) and MacDonald and Harper (1965) described a quite advanced squash method, but not a very satisfactory fixative was suggested.

Stevens (1905) was the first one who considered that the possible relation between chromosome numbers and the evolution of genera and species might lead to a more satisfactory system of classification of aphids. Her dissatisfaction with the system of classification of that time was because no less than three different forms or species of aphids infesting the rose were placed under the name of Aphis rosae L. White (1954) and Smith (1960) also stated that the comparative analysis of the chromosome number and chromosome shapes which frequently distinguish one species from its relatives throws new light on the problems of taxonomy. Lewis and John (1963) stated that the most useful chromosome characters at the lowest level of classification are those relating to their number, size, shape and detailed structure. This is especially true of those creatures with larger size and smaller numbers of chromosomes, or where conspicuous chromosome changes have played a major role in their evolution, for example, some species of the same genus do not have the same chromosome number. Smith (1943) mentioned that the character-

istics of taxonomic value such as relative size and the position of primary and secondary constrictions, are determinable only in somatic chromosomes. The number, however, can be determined during both mitosis and meiosis.

The pioneer work in this field was done by Stevens (1905), which covered over twenty different aphids. Unfortunately only Aphis oenotherae was well-identified as a known species (Fox, 1956). For the remainder she gives only host plant and sometimes a few brief words of description. Shinji (1931) investigated some 37 species of aphids, of which the somatic chromosome number and the physical characteristics of a species are so closely correlated that the evolutional scale of any aphid can safely be judged from its chromosome number. But Colling (1955) commented that in the view of the confused state of systematics and nomenclature of aphids at the time when Shinji wrote his paper, together with the complete lack of descriptions, no reliance can be placed on Shinji's identifications, even though his general conclusion may retain some validity.

Makino (1951) recorded 93 species of aphids in his book, a collection of references up to 1949. But all his compilations were to some extent incomplete and inaccurate, particularly as far as the taxonomy of the various species is concerned. Fox (1956) noted that in Makino's records for 93 species at least 31 counts are useless because of insuffi-

cient identification or they should not be included under the family Aphididae. Nine of the species now belong either in the Phylloxeridae or the Adelgidae. Some duplications in Makino's list have also been found, for instance, Amphorophora (Nectarosiphum) ribicola is quoted as Shinji's work in 1931; further on he was credited with a count for Nectarosiphum ribicola. According to the review of White (1954) the number of aphid chromosomes varied from 6 to 40.

Chromosomes of Homoptera have been regarded as having non-localized centromeres; they are considered to be polycentric or possess a dynamic activity diffused over their entire length (Hughes-Schrader and Ris, 1941; Schrader, 1958). In other words, each section of the chromosome is kinetically functionable. Shinji (1931) considered that the number six was the primitive one, from which all the others had been derived. The increase in number of chromosome seemed to have been brought about by transverse fragmentation of chromosomes. He noted the existence of species possessing the same number of chromosomes in each of the main tribes and he thought that this might indicate that the evolution of aphids proceeds, not at random, but along a definite path in each of the main tribes and in each chromosome of aphids there are certain points which may be places of fragmentations.

Stevens (1905), Morgan (1915), Doncaster (1924), Jeffrey (1933), Lawson (1936) and Shinji (1931) investigated

the meiosis of aphids to some extent. They agreed that all aphids are XO:XX type, there are only two sex chromosomes in females and one X in male cells of all species except Euceraphis betulae Koch, which has four X-chromosomes in male cells.

Schwartz (1932) described the life history or chromosome cycle of the aphid Tetraneura ulmi in considerable detail, referring to this aphid as a typical representative of the group. The eggs of fundatrices, and all parthenogenetic females, including migrants and alienicolae, only undergo a single mitotic division which does not reduce the chromosomes, so that all the descendants of a single fundatrix should be genetically identical (except for newly arisen mutations). The sexuparae differ from the other types of parthenogenetic females by production of two kinds of eggs, which will develop into males and oviparous females, respectively. Both kinds of eggs undergo a single mitotic division, but in those which will give rise to males, the two X-chromosomes pair to form a bivalent which remains in the middle of the cell after the autosomes have passed to either pole. One half of the XX bivalent passes into the polar body nucleus, the other half remaining in the egg. Thus the XO condition in the males arises through the X-chromosome alone undergoing reduction while the equational division of the autosomes are taking place. In the spermatogenesis, the bivalents of autosomes and univalent X shown at the first metaphase plate undergo a

reduction division. During anaphase the X univalent becomes stretched out between the two daughter nuclei, forming the so-called lagging chromosome, which goes to one of the daughter cells at cytoplasmic division. A similar phenomenon was observed by Stevens (1905), Morgan (1908), Shinji (1931), Lawson (1936), but denied by Jeffrey (1933), who thought that the lagging chromosomes only seem to indicate the existence of hybridism. The two kinds of secondary spermatocytes are thus formed, the one possessing X-chromosome receiving more cytoplasm than the one lacking X-chromosome. Only the larger ones undergo a secondary mitotic division and form sperms. The smaller ones called polar bodies will finally degenerate. Ris (1942) claimed that the unequal division of cytoplasm is caused by the X-chromosome, which is stretched in the axis of the spindle and prevents the cleavage furrow from cutting through the middle of the cell.

CHAPTER III

MATERIALS AND METHODS

Effects of different constant temperatures on reproduction of Rhopalosiphum padi (L.)

All the aphids in these trials belonged to the same parthenogenetic line of R. padi, and they were all apterous summer viviparae, seven days old. This was achieved by setting last instar apterous summer viviparae on uninfested seedlings, separated from the rearing cultures, and removing them 24 hours later. The difference in ages between the first born and the youngest aphid of any batch would be therefore less than 24 hours. The aphids were reared in a growth cabinet at a constant temperature of 70° F. Photoperiod was set at 16 hours light and 8 hours darkness.

Two seeds of each barley variety C.I. 3906-1 and Swan were grown in five-inch pots. At the end of six days after seeding the weaker of the two seedlings was removed. One adult aphid was then placed on each seedling and covered with a cage consisting of fine mesh organdy, 20 inches high and three inches in diameter which was supported by a rigid wire frame (Hsu, 1963, Figure 2). The aphids were transferred from the rearing cultures to the test plants with the aid of an aspirator developed and described by Robinson (1961).

The experiments were conducted at various constant temperatures from 45° F to 100° F in a growth cabinet. Individual apterous viviparous females of R. padi, in replicates of ten, were caged on seedlings of the two varieties of barley for five days. The number of adults remaining alive and the number of progeny produced per female were counted and recorded five days later. These results are shown in Chapter V.

Transfers of aphids between host plants

A series of transfers between plants were made in the field in the spring of 1964. Two species of aphids, Rhopalosiphum padi (L.) and R. fitchii (Sand.) (fundatrices and/or fundatrigeniae) used in this study were collected from Pruneae ---- Prunus virginiana L. (Choke-cherry) and Pomeae ---- Cotoneaster acutifolia Turcz., Malus spp. (Crabapple hybrids), Sorbus americana Marsh. and Crataegus spp. (Hawthorn hybrids) respectively. Since the fundatrices of R. fitchii on the four host plants were getting old and very few could be found after May 22, the aphids used before May 25 were adult fundatrices, while those after May 25 were last instar nymphs or adult fundatrigeniae. The aphids collected from their natural breeding hosts were immediately transferred to their new hosts to keep them fresh and free from starvation and to prevent too much disturbance.

Clip cages or Visking dialyzing tubing were used to confine the aphids after transferring. The clip cages

described by Robinson (1961) were used only on P. virginiana, since the leaves of this plant were big enough to fit this kind of cage and the feeding sites of the aphids on this plant were usually the under side of leaves rather than the terminal growth. Dialyzing tubing (25 mm in diameter, 90 mm in length) was used on Cotoneaster, Malus, Sorbus and Crataegus, because the aphids usually feed on the terminal growth and the leaves were not suitable for clip cages. The cages made by the dialyzing tubing enclosed the terminal growth of the plants, including three open leaves. The base of the cage was tightened with a twist-wire, and the top of cage was closed after transferring the aphids by folding over the top and clipping it with two paper clips.

The plants used in these experiments were on the campus of the University of Manitoba. Ten healthy leaves or terminal growths were used in each set of experiments. Every leaf or terminal growth picked was checked carefully to get rid of parasite eggs or parasites.

If the aphids on the new host were in a healthy condition and produced more than four nymphs they were considered to be successfully established colonies which were then recorded on the third and fifth day after the transfer. The results of this study are presented in Chapter IV.

Chromosome counts and studies

Fifty species were used in this study. All of them were collected around the campus of the University of Manitoba, Sandilands Forest Reserve, Manitoba, or Assiniboine Park of Winnipeg. Most of the work was done in the spring of 1965. It was necessary to examine the cells in their period of active division which was usually found only when the aphids were in active growth. The materials obtained after September of 1964 were not satisfactory for the chromosome study at all, because only very few metaphases could be observed in some of the aphids, and a reliable count could not be drawn from such metaphases, and furthermore, it was difficult to make the slides clean and clear from those aphids collected in the fall simply because of too many fat bodies mixed with the embryo. More than forty collections from September-October were discarded because of these reasons. The materials collected in the spring of 1965 provided the best information for chromosome counts and studies.

The embryos employed in the chromosome counts were from viviparous females because the diploid material was the most suitable. Though the testis material would provide theoretically large numbers of dividing nuclei in metaphase stage, in practice, it was very difficult to find metaphase in the adult male aphids due to the fact that spermatogenesis was completed in the embryo and nymphal stages, and as a result only

mature sperms were found in the adult male aphids. The use of males was further limited by the short period of time of year during which they could be collected. Viviparous females were readily identifiable and available in large numbers in spring and summer.

The two squash methods used through all the investigation are described as follows under the headings of The quick examination method and The Feulgen stain method.

1. The quick examination method:

   (a) Put a live aphid on a slide, and looking through a dissection microscope, break the end of the abdomen with a needle and then press out the embryos. Discard the adult body and the larger embryos (those in which the eyespots can be seen). Only three or four very small embryos or eggs are left on the slide.

   (b) Drop a little drop of Orcein on the embryos before the embryos become dry.

   (c) Put on a cover glass and place the edge of a razor blade between the slide and cover glass. Then hit the cover glass gently with an eraser pencil. In this way it will give a better spread of the cells and chromosomes.

   (d) Heat the slide a little bit on the top of a Bunsen flame.

   (e) Put the slide between two layers of blotting paper and press gently on the top to absorb the excess stain and

to make the chromosomes in the same plane of focus. The slides stained with Orcein cannot be used to make permanent mounts because the stain does not last very long. It will fade after about a month, or when in contact with acetic acid. The slides can be used only for a quick examination or photograph. When you want to take pictures from these slides, you should seal the cover glass with wax and wait for 2 or 3 days when the stain will get deeper.

2. Feulgen stain method:

(a) Remove embryos from aphids into Ringer's solution. Or simply just break the abdomens of the aphids and expose the embryos (in this way the very tiny embryos or eggs do not get lost and it is easier to handle them).

(b) Transfer the embryos with a pipette or forceps into a plastic holder (with small holes on the bottom and a piece of lens paper thimbled to the bottom of the holder) which is convenient for transferring the embryos from solution to solution.

(c) Absorb the excess Ringer's solution with blotting paper from the bottom of the plastic holder. Then put the holder into 1N warm HCl (60° F) to hydrolize for 7-8 minutes in 60° F.

(d) Transfer the plastic holder from HCl into Feulgen solution for 10-15 minutes and the embryos eventually become red.

(e) Pick out one or two small embryos (preferably those without eyespots) onto a slide with a needle. Drop a small drop of aceto-carmine on the slide and then squash it in the same way as described before.

The slide stained with Feulgen can be used to make a permanent mount and the stain is sharper than Orcein. The procedure of making a permanent mount is as follows:

(a) Soak the slide stained with Feulgen in Solution A till the cover glass releases from the slide.

(b) Transfer the slide and cover glass into Solution B and soak for 3 minutes.

(c) Transfer the slide and cover glass into Solution C and soak for 3 minutes.

(d) Put a drop of Canada Balsam on the slide and replace the cover glass over the stained specimens.

Solution A: 1 part 95% ethyl alcohol, 1 part glacial acetic acid, few drops of TBA (Tertiary Butyl Alcohol).

Solution B: 1 part 95% ethyl alcohol, 1 part TBA.

Solution C: TBA.

Both methods have given good results. The first method is recommended for its simplicity and convenience if the preparation of permanent slides is not necessary, and has the added advantage that it can be done in the field at the time of collecting, if so desired. At the time of writing, no fixative is known suitable for preservation of aphids so that chromosomes can be examined at a later date.

Microphotographs were usually taken from early and late metaphase in which the chromosomes were highly condensed and well-spread throughout the cell, and the chromosome number counts were more reliable. The photographs shown in all the plates were magnified at a power of 2400-2800X.

CHAPTER IV

EFFECTS OF DIFFERENT CONSTANT TEMPERATURES ON THE REPRODUCTION OF *Rhopalosiphum padi* (L.)

There is evidence from the literature that variations of temperature influence the fecundity of aphids feeding on the plants concerned. Most of the pertinent literature has already been discussed in Chapter II. The results of the effects of various constant temperatures on the reproduction rate of *R. padi* on two barley varieties, Swan and C.I. 3906-1, are shown in Figure 1. The optimum temperature for reproduction of *R. padi* is at 75° F. Reproduction nearly ceased at a minimum temperature of 45° F, and a maximum of 100° F. This probably resulted from the unfavorable influence of temperature on embryogenesis, or from the faster death rate of mothers, or from a combination of both factors. A similar pattern of reproduction curve was reported by Villanueva and Strong (1964). At 100° F all aphids on C.I. 3906-1 died before producing any young and only one adult was alive and one nymph was produced on Swan. At 45° F all adults, except one on Swan, were alive, and a total of 4 and 3 nymphs were produced on Swan and C.I. 3906-1 respectively. This indicates that the high temperature was adverse to the survival of aphids as well as on the development of embryos, but the low temperature has a greater influence on the development of embryos than on

the survival of aphids.

The size and color of aphids were also under the influence of ecological conditions, for example, aphids reared at 45° F were larger than those reared at 80° F, and the former was dark olive-green, and the latter lighter in color.

The build-up of an aphid colony on a plant host is apparently dependent on two major factors: (1) the reaction of the aphids' reproduction rate to variations of temperature in a certain range, (2) the reaction of the host resistance to variations of temperature in a certain range. The interactions between these two factors can be seen in Figure 1.

Hsu and Robinson (1962) reported that there was a greater reduction in fecundity of R. padi reared on barley variety C.I. 3906-1 than on Swan, at fluctuating temperatures, under both field and green house conditions. Belvett's study (1965) showed that there was no significant difference in fecundity of R. padi on the two barley varieties at a constant temperature. The variations of temperature really had an effect on the reproduction rate of aphids and resistance of plants to aphids, as it was repeatedly demonstrated by many workers listed in Chapter II.

Since Belvett's results coincided with that shown in Figure 1 but quite deviated from the report by Hsu and Robinson, if the temperature really acted as an important factor in the reproduction of aphids and resistance of plants

to aphids, the only possible explanation to account for the deviation between the two different results was that there were some differences between the effects of constant temperature and of fluctuating temperature. It was stated by Messenger (1964) that fecundity and longevity of spotted alfalfa aphid were different at fluctuating temperatures from those under constant temperatures.

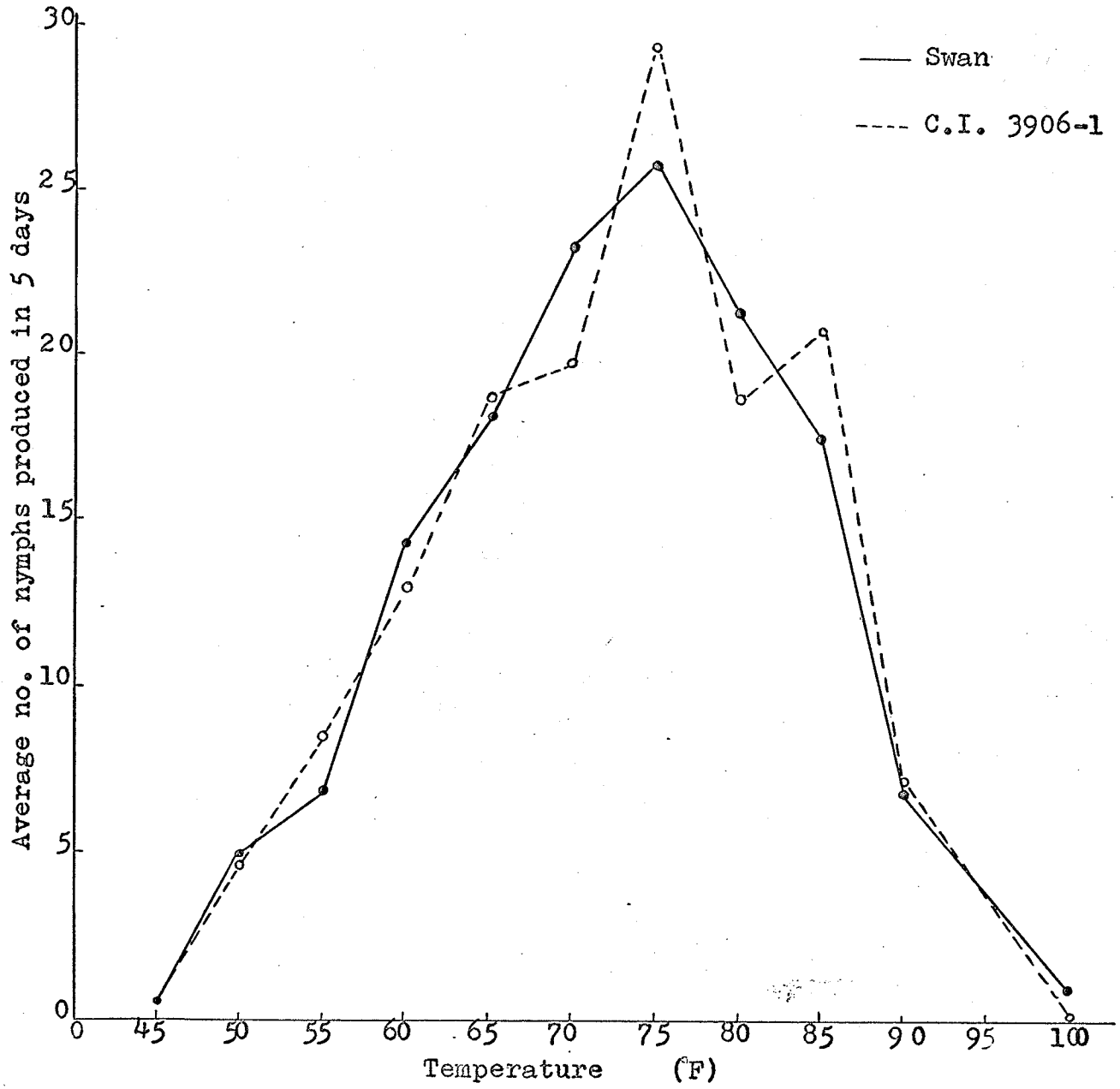


Fig. 1. Reproduction of apterous viviparae of Rhopalosiphum padi (L.) on two varieties of barley at different constant temperatures.

CHAPTER V

HOST TRANSFERS OF TWO MORPHOLOGICALLY SIMILAR SPECIES OF APHIDS

In studies of resistance of barley varieties to aphids in our laboratory, it was difficult to identify two morphologically similar species, Rhopalosiphum padi (L.) and R. fitchii (Sand.). The taxonomic problems associated with determinations of R. padi and R. fitchii have mostly been solved as cited in the literature review in Chapter II, but there is still no easy way to distinguish between the alienicolae of the two species. No R. fitchii were collected by Robinson and Hsu (1963) on cereal grains and grasses in Manitoba. It was necessary therefore to confirm the presence of the two species in Manitoba by transfer tests between the winter host plants.

There was also some uncertainty about the forms of R. fitchii from Cotoneaster and Sorbus (Richards, 1960). In Manitoba R. fitchii occurred in the spring of 1964 in much greater numbers on Cotoneaster, Crataegus and Malus than on Sorbus. To determine the relationships between R. padi, R. fitchii and their overwintering hosts, a series of transfers of fundatrices and/or fundatrigeniae were made in the field. The results are presented in Table I. It appears that R. padi overwinters on Prunus virginiana L. but is unable to

live and colonize on the winter hosts of R. fitchii. Similarly, R. fitchii overwinters on Cotoneaster, Malus, Crataegus and Sorbus in Manitoba and is incapable of living and colonizing on the winter host of R. padi. R. fitchii from any one species of the four winter hosts could colonize successfully on any other species of the winter hosts. Both aphids were found to be host specific within their host range.

TABLE I

EXPERIMENTAL TRANSFERS OF FUNDATRICES OR FUNDATRIGENIAE OF *Rhopalosiphum fitchii* AND *R. padi* BETWEEN VARIOUS HOST PLANTS*

Species	Morph	Host plant from	Transfer to	No. of aphids transferred	Number successfully established	Dates May 1964
R.fitchii	fundatrix	Cotoneaster	Cotoneaster	9	9	14-19
	fundatrigenia		Malus	10	9	25-30
	fundatrigenia		Sorbus	10	6	28-Jun.2
	fundatrix		Crataegus	10	8	20-24
	fundatrix		Prunus	10	0	16-21
	fundatrix	Malus	Cotoneaster	10	8	14-19
	fundatrix		Malus	10	9	22-27
	fundatrigenia		Sorbus	10	7	28-Jun.2
	fundatrix		Crataegus	10	7	20-24
	fundatrix		Prunus	10	0	19-24
	fundatrix	Sorbus	Cotoneaster	10	10	15-21
	fundatrigenia		Malus	10	7	25-30
	fundatrix		Crataegus	10	10	19-23
	fundatrix		Prunus	10	0	19-22

TABLE I (continued)

EXPERIMENTAL TRANSFERS OF FUNDATRICES OR FUNDATRIGENIAE OF *Rhopalosiphum fitchii* AND *R. padi* BETWEEN VARIOUS HOST PLANTS*

Species	Morph	Host plant from	Transfer to	No. of aphids transferred	Number successfully established	Dates May 1964
R. fitchii	fundatrix	Crataegus	Cotoneaster	9	9	15-21
	fundatrigenia		Malus	10	8	22-27
	fundatrigenia		Sorbus	10	6	28-Jun.2
	fundatrix		Crataegus	10	9	19-23
	fundatrix		Prunus	10	0	19-22
R. padi	fundatrix	Prunus	Cotoneaster	10	0	15-19
	fundatrix		Prunus	10	6	19-23

* *Cotoneaster acutifolia*, *Malus* sp. (crabapple hybrids), *Sorbus americana*, *Crataegus* spp. (hawthorn hybrids), *Prunus* spp. (*virginiana* and hybrids).

CHAPTER VI

OBSERVATIONS ON SOMATIC CELL DIVISIONS AND CHROMOSOME COUNTS ON FIFTY SPECIES OF APHIDS

Observations on somatic cell division of the pea aphid Acyrthosiphon pisum (Harris) (Plate I-II)

Mitotic cell division of aphids is basically similar to that of other organisms, though different in some ways at the metaphase stage. Detailed observations were made on the somatic cell division of the pea aphid, Acyrthosiphon pisum (Harris), as follows, and these observations were similar for all other aphid species studied.

In interphase the cells show little or no definable structure and prophase is initiated at the moment when the chromosomes emerge from the resting condition as irregularly twisted threads. Throughout prophase the chromatids are not visible. With the disappearance of the nuclear membrane metaphase is initiated and the chromosomes are condensed and dark-stained, in an entirely random distribution. Approaching late metaphase, the chromosomes condense to such an extent that they appear as dots or short rods (Plate I, Figure 5) which eventually unite together to form a rod-shaped mass (Plate I, Figure 6) at the metaphase plate. The rod-shaped mass soon divides into two chromatid masses directed towards either pole. In anaphase, the two chromatid masses separate

from each other gradually with both ends of the rods bent towards the pole (Plate II, Figure 9). In telophase, both halves reach the poles and swell to form spherical masses. Meanwhile cytokinesis is accomplished. In stained preparations the two spherical chromatid masses gradually fade until they finally enter the resting stage.

In embryos and ovarioles of the pea aphid, many large nuclei were found in which different levels of polyploidy were visible, for example from tetraploid to multiploid (Plate II, Figures 12-14). Chromosomes stained deeply to be countable, were present in these large nuclei and appeared as separated bodies when in a lower level of polyploid. They were similar in morphology to the chromosomes of diploid organisms. On the other hand, the chromosomes of multiploid were apparently small and irregular in morphology. These polyploid nuclei resulted from repeated duplication of the chromosomes without any following cytokinesis. This kind of duplication in chromosome numbers without any cytokinesis was mentioned by White (1954) as endomitosis. Painter (1940) called this kind of cell the nurse-cell and suggested that the nucleoprotein molecules derived from the thousands of nurse-cell chromosomes are used in the synthesis of the embryonic chromosomes during the cleavage division.

Chromosome counts of fifty aphid species

Fifty species from 32 different genera of aphids were employed in these studies of chromosome counts, which varied from 4 to 20 with all the intermediate classes between them, as shown in Table II. The frequency and distribution of the chromosome numbers among these fifty species are shown in Figure II, and all the photographs of each species are presented from Plate III, Figure 15 to Plate XII, Figure 69.

As far as the present investigation is concerned, no chromosomes associated with centromeres were found, which agrees with the observations of Hughes-Schrader and Ris (1941). They believed that the chromosomes of Homoptera are polycentric or with diffuse centromere activity. It was suggested that variation in number of entities may be due to fragmentation or duplication of their primitive chromosomes (Schrader, 1947; Schrader and Hughes-Schrader, 1956; Shinji, 1931). In this study of 50 species, those with the lower chromosome numbers 2n=4 and 6, are characterized by having large chromosomes, while those with higher chromosome numbers, 2n=18 and 20, always have smaller chromosome size (Plate III, Figure 19, 20; Plate III, Figure 18; Plate IV, Figure 21 and Plate XI, Figure 63, 64). On the basis of these facts, it could be assumed that evolution of aphids has involved fragmentation of chromosomes at different loci and subsequent behavior of the fragments as independent chromosomes. The diffuse nature of

the centromere facilitates survival of the fragments. This provides them with a method of increasing chromosome number other than polyploid. A similar assumption was made by Sharma and Sharma (1959) in the plant Lusula.

Shinji (1931) found that the lowest number in a female aphid was six, while in this study of 50 species the lowest number was four. Shinji concluded that the number six was the primitive one, from which all the others derived.

Figure 2 shows that the highest frequency of chromosome number is eight among these 50 species and the frequencies of 4, 6, 18 and 20 are very low, which suggests that the very high and very low numbers did not arise suddenly but represent the end products of evolutionary process.

The classification of aphids used was based on the system of Borner and Heinze (1957), except for Gypsoaphis oestlundi Hottes, which was not mentioned in their book. Hottes and Frison (1931) classified G. oestlundi under the Family Chaitophoridae. Periphyllus negundinis is, according to Borner and Heinze, a more primitive species, but it has a chromosome count of 2n=20. Shinji (1931) postulated that primitive species have a lower chromosome number. Therefore, either the taxonomists are in error in calling P. negundinis a primitive species, or there is no validity in the theory that primitive species always have a low chromosome number.

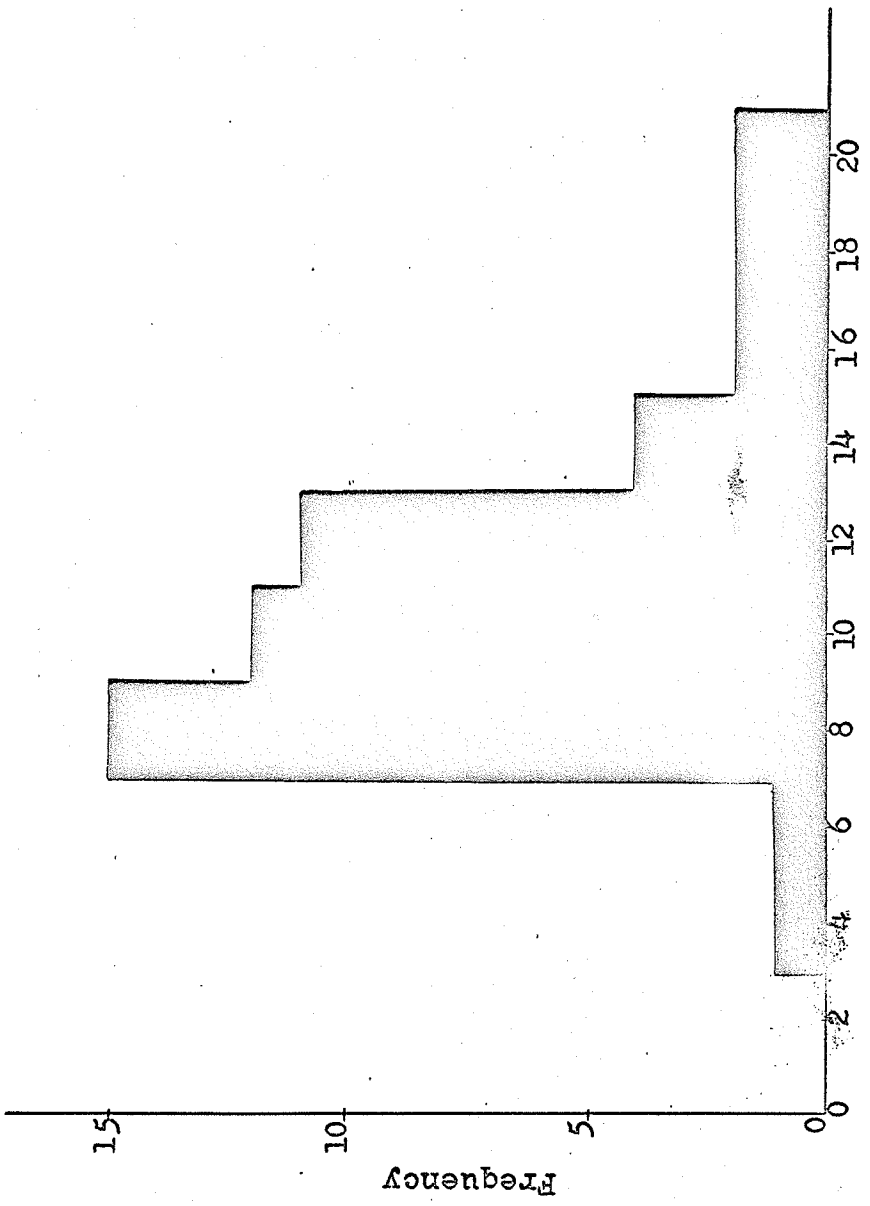


Fig. 2. The frequency of chromosome numbers of fifty species of Aphids

In the genus Aphis the chromosome number was 8 in all the species which were studied, while in the genus Macrosiphum the chromosome numbers varied from 10 to 18. It appears evident that changes in chromosome number have played a more important role in the evolution of Macrosiphum than of Aphis. From this point of view Aphis is more primitive than Macrosiphum.

Four kinds of chromosome karyotypes in the Family Aphididae were found to be quite common in more than one genus. The first type of chromosome is eight in number with three pairs of chromosomes of similar length and one pair of slightly shorter ones as found in Aphis spp., Rhopalosiphum maidis, R. padi and Schizaphis graminum (Plate IV, Figure 26, Plate V, Figure 27-32; Plate VI, Figure 33, 36-38; Plate VII, Figure 39). The second type of chromosome is also eight in number as found in Pterocomma smithiae and Acyrthosiphon pisum with three pairs of long chromosomes and one pair of very short ones (Plate IV, Figure 25; Plate IX, Figure 55). The third type of chromosome is ten in number as found in Rhopalosiphum fitchii, Kakimia essigi, Kakimia sp., Acyrthosiphon caraganae, Dactynotus cirsii, Macrosiphum euphorbiae, M. manitobensis and Masonaphis wahnaga, which show three pairs of long chromosomes and two pairs of very short ones (Plate VI, Figure 34-35; Plate VIII, Figure 47-48; Plate IX, Figure 54; Plate X, Figure 59; Plate XI, Figure 63-64; Plate XII, Figure 67). The fourth type of chromo-

some is twelve in number as found in Myzus persicae, Nasonovia lactucae, Rhopalomyzus lonicerae, Amphorophora laingi, A. rubicola, Dactynotus taraxaci, Macrosiphoniella absinthii and M. tanacetaria, which show four pairs of long chromosomes and two pairs of very short ones (Plate VIII, Figure 49; Plate IX, Figure 51, 53, 56; Plate X, Figure 57, 60-62). These different types of chromosome karyotypes might suggest the steps of an evolutionary process in aphids, which is by no means at random but along a definite path.

The Aphis spp. studied thus far have a common karyotype as mentioned above. Their morphology is also very similar, especially those species feeding on dogwood (Cornus spp.). The similarity of chromosome karyotypes as found in Aphis spp. is no assistance in species identifications. However, measurements and comparisons of the chromosomes of a large sample might help to separate morphologically similar species.

It is not necessary to have any occurrence of phenotypic changes associated with increases of chromosome numbers through certain kind of mechanisms, such as fragmentation, even though phenotype changes may occur beyond the level that can be detected. It was found, for instance in the genus Macrosiphum, that M. avenae has 2n=18, while M. euphorbiae and M. manitobensis have 2n=10, but the phenotypic differences between them are not great.

The taxonomic problem of the two morphologically similar species Rhopalosiphum padi and R. fitchii was discussed in

Chapters II and IV. There is no clear and easy way to distinguish the summer forms of these two species. The chromosome numbers of the two species provide the easiest way to tell one from the other. R. fitchii has ten chromosomes of the karyotype of the third form (Plate VI, Figure 34 and 35) and R. padi has eight chromosomes of the karyotype of the first form (Plate VI, Figure 38).

A very interesting chromosome figure is Aphthargelia symphoricarpi, shown in Plate VII, Figure 41, with four long chromosomes and ten very small chromosome dots of similar size. According to Shinji's (1931) hypothesis these small chromosomes might be the products of the fragmentations of large chromosomes. However, there is another possibility that A. symphoricarpi might be a hybrid between two different species with large and small chromosomes respectively. A similar figure of chromosomes was found in the hybrids of the cross between two plant species Lusula sudetica and L. campestris by Nordenskiold (1956). The F_1 hybrids showed six large chromosomes of L. campestris and twenty four small chromosomes of L. sudetica.

Shinji (1931) studied the spermatogenesis and chromosomes of Euceraphis punctipennis (= E. betulae) in considerable detail. He found four large autosomes and four small X-chromosomes in males of this species, and he described it as the first example of an animal with four X and no Y chromosomes in the male. Since it is known that aphids are XO:XX type,

then according to Shinji's observation of males, the female of this species should have 12 chromosomes. In the present study observations of chromosomes of viviparous females of E. punctipennis showed only four large chromosomes and four or five small ones as shown in Plate IV, Figure 22 and 24. In the four small chromosomes, one pair is larger than the other. Some cells of the same embryo showed five small chromosomes, two of them larger than the others. One of the three tiny chromosomes must be a supernumary chromosome because it does not appear in every somatic cell. The differences may be attributed to erroneous identification of E. punctipennis.

Amphorophora rubicola was also discussed by Shinji (1931). He stated that the univalent X-chromosome was larger than the other five bivalent autosomes in males. In the present study the largest pair of the 12 chromosomes stained more deeply than the others. This phenomenon has also been found in many other species. More observations in spermatogenesis, or the somatic chromosomes of males are required to prove that the largest and more deeply stained chromosomes in other species could also be X-chromosomes.

TABLE II

LIST OF SPECIES OF APHIDS, HOST PLANTS AND CHROMOSOME NUMBERS

Material no.	Name of species	Host plant	Date of collection	Diploid no. of chromosomes
	Family: Lachnidae			
	Subfamily: Cinarinae			
	Genus: *Cinara*			
1.	*C. braggii* (Gillette)	White Spruce (*Picea glauca* (Moench) Voss)	Jul.13/65	10
2.	C. pinea (Mordvilko)	Scots Pine (*Pinus sylvestris* L.)	Jun.10/65	10
	Family: Chaitophoridae			
	Subfamily: Chaitophorinae			
	Genus: *Periphyllus*			
3.	*P. negundinis* (Thomas)	Boxelder (*Acer negundo* L.)	Jun. 1/65	20
	Subfamily: Siphinae			
	Genus: *Sipha*			
4.	*S.* (*Rungsia*) *agropyrella* (H.R.L.) (Quack Grass Aphid)	Couch-Grass (*Agropyron repens* (L.) Beauv.)	Jul.15/65	6

TABLE II (continued)

LIST OF SPECIES OF APHIDS, HOST PLANTS AND CHROMOSOME NUMBERS

Material no.	Name of species	Host plant	Date of collection	Diploid no. of chromosomes
	Genus: *Gypsoaphis*			
5.	*G. oestlundi* Hottes	Honeysuckle (*Lonicera* spp.)	Jun.22/65	4
	Family: Callaphididae			
	Subfamily: Phyllaphidinae			
	Genus: *Calaphis*			
6.	*C. betulaecolens* (Fitch)	Paper-Birch (*Betula papyrifera* Marsh.)	Jul. 6/65	20
	Genus: *Euceraphis*			
7.	*E. punctipennis* Zetterstedt (=*E. betulae* (Koch))	Paper-Birch (*Betula papyrifera* Marsh.)	Jul.15/65	8
	Subfamily: Callaphidinae			
	Genus: *Myzocallis*			
8.	*M. punctata* (Monell) ?	Bur Oak (*Quercus macrocarpa* Michx.)	Jun.11/65	14

TABLE II (continued)

LIST OF SPECIES OF APHIDS, HOST PLANTS AND CHROMOSOME NUMBERS

Material no.	Name of species	Host plant	Date of collection	Diploid no. of chromosomes
	Family: Aphididae			
	Subfamily: Pterocommatinae			
	Genus: *Pterocomma*			
9.	*P. smithiae* (Monell)	Willow (*Salix* spp.)	Jun.24/65	8
	Subfamily: Aphidinae			
	Genus: *Aphis*			
10.	*A. armoraciae* Cowen (The Western Aster Root Aphid)	Dandelion (*Taraxacum officinale* Weber)	Jun.10/65	8
11.	*A. corniella* H.R.L. ?	Dogwood (*Cornus stolonifera* Michx.)	Jul. 1/65	8
12.	*A. helianthi* Monell ?	Dogwood (*Cornus stolonifera* Michx.)	May 31/65	8
13.	*A. nasturtii* Kaltenbach (=*A. abbreviata* (Patch))	Common Buckthorn (*Rhamnus cathartica* L.)	Jun.17/65	8
14.	*A. neogillettei* Palmer ?	Dogwood (*Cornus stolonifera* Michx.)	May 27/65	8

TABLE II (continued)

LIST OF SPECIES OF APHIDS, HOST PLANTS AND CHROMOSOME NUMBERS

Material no.	Name of species	Host plant	Date of collection	Diploid no. of chromosomes
15.	A. rubicola Oestlund ?	Wild raspberry (Rubus spp.)	Jun.15/65	8
16.	A. spiraecola Patch (The Spirea Aphid)	Spiraea spp.	Jun.14/65	8
17.	A. varians Patch (The Variable Currant Aphid)	Currant (Ribes alpinum L.)	Jul. 7/65	8
	Genus: Rhopalosiphum			
18.	R. fitchii (Sand.) (Apple Grain Aphid)	Cotoneaster acutifolia Turz.	May 31/65	10
19.	R. maidis (Fitch) (Corn Leaf Aphid)	Barley (Hordeum vulgare L.)	Jul. 6/65	8
20.	R. padi (L.) (The Oat Bird-cherry Aphid)	Choke-cherry (Prunus virginiana L.	Jun. 7/65	8
	Genus: Schizaphis			
21.	S. graminum (Rond.) (=Toxoptera graminum) (Greenbug)	Barley (Hordeum	Sept.5/65	8

TABLE II (continued)

LIST OF SPECIES OF APHIDS, HOST PLANTS AND CHROMOSOME NUMBERS

Material no.	Name of species	Host plant	Date of collection	Diploid no. of chromosomes
	Subfamily: Myzinae			
	Genus: *Aphthargelia*			
22.	*A. symphoricarpi* (Thomas) (*Brevicoryne symphoricarpi*)	Snowberry (*Symphoricarpos racemosus* Michx.)	Jun.15/65	14
	Genus: *Aspidaphis*			
23.	*A. adjuvans* (Walker) (The Armoured Knotweed Aphid)	Knotweed (*Polygonum aviculare* L.)	Jul. 4/65	12
	Genus: *Brevicoryne*			
24.	*B. brassicae* L. (The Cabbage Aphid)	Cabbage (*Brassica* spp.)	Feb.10/65	16
	Genus: *Capitophorus*			
25.	*C. hippophaes* (Walker)	Silverberry (*Elaeagnus argentea* Pursh)	Jun. 9/65	10
	Genus: *Cryptomyzus*			
26.	*C. ribis* (L.) (=*Capitophorus ribis* (L.) (The Currant Aphid)	Currant (*Ribes* spp.)	Jun.22/65	12

TABLE II (continued)

LIST OF SPECIES OF APHIDS, HOST PLANTS AND CHROMOSOME NUMBERS

Material no.	Name of species	Host plant	Date of collection	Diploid no. of chromosomes
	Genus: *Hayhurstia*			
27.	*H. atriplicis* (L.) (=*Hyalopterus atriplicis* (L.))	Lambs-quarters (*Chenopodium album* L.)	Jul.15/65	14
	Genus: *Kakimia*			
28.	*K. essigi* (Gillette and Palmer) (The Black-backed Columbine Aphid)	Columbine (*Aguilegia* spp.)	Jun.18/65	10
29.	*Kakimia* sp. (close to *K. thomasi* (Hottes and Frison))	Currant (*Ribes alpinum* L.)	Jun.10/65	10
	Genus: *Myzus*			
30.	*M. cerasi* (Fabricius) (The Black Cherry Aphid)	Pincherry (*Prunus pensylvanica* L.)	Jun.15/65	10
31.	*M. persicae* (Sulzer) (The Green Peach Aphid)	*Brassica* spp.	Jun. 7/65	12
	Genus: *Nasonovia*			
32.	*N. lactucae* (L.)	Sow Thistle (*Sonchus oleraceus* L.)	Sept.1/64	12

TABLE II (continued)

LIST OF SPECIES OF APHIDS, HOST PLANTS AND CHROMOSOME NUMBERS

Material no.	Name of species	Host plant	Date of collection	Diploid no. of chromosomes
	Genus: *Neoceruraphis*			
33.	*N. viburnicola* (Gillette) (*Aphis viburnicola* Gillette)	European Cranberrybush (*Viburnum opulus* L.)	Jun.17/65	14
	Genus: *Rhopalomyzus*			
34.	*R. lonicerae* (Siebold)	Honeysuckle (*Lonicera* sp.)	Jun. 1/65	12
	Subfamily: Dactynotinae			
	Genus: *Acyrthosiphon*			
35.	*A. caraganae* (Chol.) (The Caragana Aphid)	*Caragana arborescens*	Jun.15/65	10
36.	*A. pisum* (Harris) (The Pea Aphid)	Broad Bean (*Vicia faba* L.)	Sept.6/64	8
	Genus: *Amphorophora*			
37.	*A. laingi* Mason	Ostrich fern (*Matteuccia struthioperis* L.	Jul. 3/65	12
38.	*A. ribiella* (Davis) (The Ornamental Currant Aphid)	Golden Currant (*Ribes aureum* Pursh	Jun. 1/65	12

TABLE II (continued)

LIST OF SPECIES OF APHIDS, HOST PLANTS AND CHROMOSOME NUMBERS

Material no.	Name of species	Host plant	Date of collection	Diploid no. of chromosomes
	Genus: *Cryptaphis*			
39.	*C. poae* (Hardy)	Brome Grass (*Bromus inermis* Leyss.)	Jun. 4/65	16
	Genus: *Dactynotus*			
40.	*D. cirsii* (L.)	Canada Thistle (*Cirsium arvense* (L.))	Jun. 22/65	10
41.	*D. taraxaci* (Kaltenbach) (*Macrosiphum taraxaci* (Kaltenbach)) (The Dark Dandelion Aphid)	Dandelion (*Taraxacum officinale* Weber)	Jun. 16/65	12
	Genus: *Macrosiphoniella*			
42.	*M. absinthii* (L.)	Wormwood (*Artemisia absinthium* L.	July 4/65	12
43.	*M. tanacetaria* (Kaltenbach)	Common Tansy (*Tanacetum vulgare* L.)	June 14/65	12
	Genus: *Macrosiphum*			
44.	*M. avenae* (Fabr.) (English Grain Aphid)	Barley (*Hordeum vulgare* L.)	July 6/65	18

TABLE II (continued)

LIST OF SPECIES OF APHIDS, HOST PLANTS AND CHROMOSOME NUMBERS

Material no.	Name of species	Host plant	Date of collection	Diploid no. of chromosomes
45.	M. euphorbiae (Thomas) (=M. solanifolii Ashmead) (The Potato Aphid)	Rose and Spiraea (Rosa spp. and Spiraea spp.)	Jun.14/65	10
46.	M. (Sitobion) manitobensis Robinson	Dogwood (Cornus stolonifera Michx.)	Jun.14/65	10
	Genus: Metopolophium			
47.	M. dirhodum (Walker) (The Rose Grass Aphid)	Rose (Rosa spp.)	May 31/65	18
	Genus: Masonaphis			
48.	M. (Ericobium) wahnaga Hottes	Lily of the Valley (Convalaria majalis L.)	Jun.18/65	10
	Family: Thelaxidae			
	Subfamily: Anoeciinae			
	Genus: Anoecia			
49.	A. graminis Gillette and Palmer	Wild Barley (Hordeum jubatum L.)	Jun.16/65	8

TABLE II (continued)

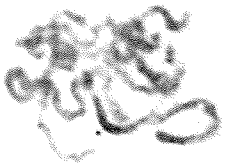
LIST OF SPECIES OF APHIDS, HOST PLANTS AND CHROMOSOME NUMBERS

Material no.	Name of species	Host plant	Date of collection	Diploid no. of chromosomes
	Family: Pemphigidae			
	Subfamily: Schizoneurinae			
	Genus: *Eriosoma*			
50.	*E. lanigerum* (Hausmann) (The Wooly Apple Aphid)	Elm (*Ulmus americana* L.)	Jun. 9/65	12

PLATE I

Explanation for Figures

3. Acyrthosiphon pisum; prophase; diploid complement.
4. Acyrthosiphon pisum; prometaphase; diploid complement.
5. Acyrthosiphon pisum; middle metaphase.
6. Acyrthosiphon pisum; late metaphase.
7. Acyrthosiphon pisum; anaphase.
8. Acyrthosiphon pisum; anaphase.



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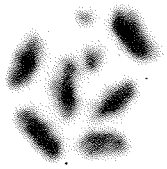

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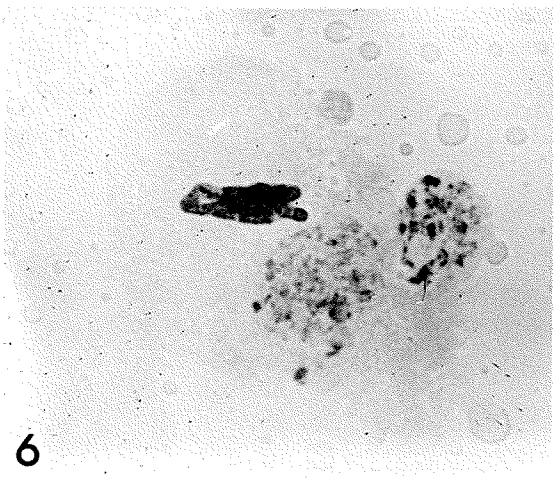

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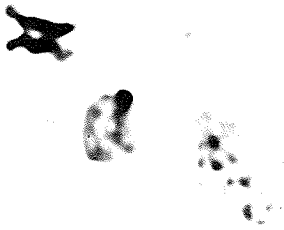

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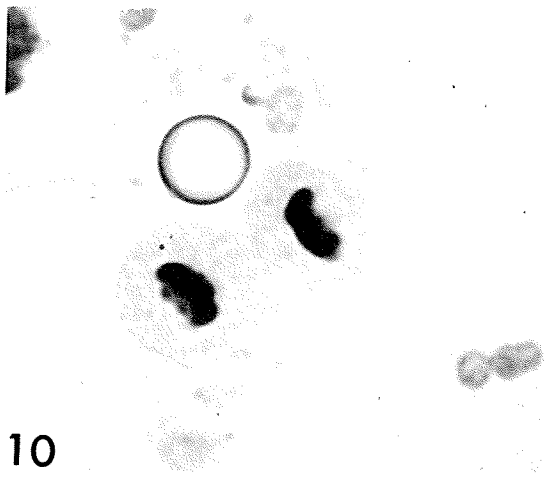
PLATE II

Explanation for Figures

9. Acyrthosiphum pisum; anaphase.
10. Acyrthosiphon pisum; telophase and cytokinesis.
11. Acyrthosiphum pisum; chromosomes from telophase enter the resting stage.
12. Acyrthosiphum pisum; a tetraploid cell, 4n=16.
13. Myzus persicae; a hexaploid cell, 6n=36.
14. Acyrthosiphum pisum; a multiploid cell at resting stage.

9



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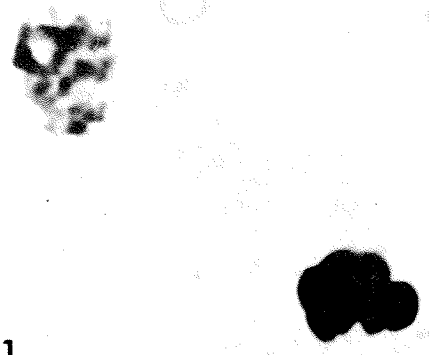

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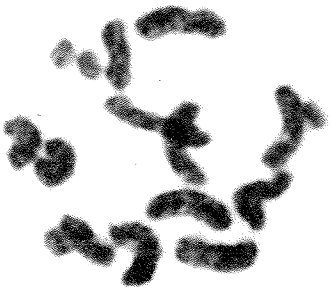

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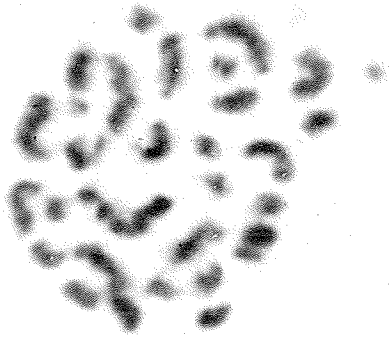

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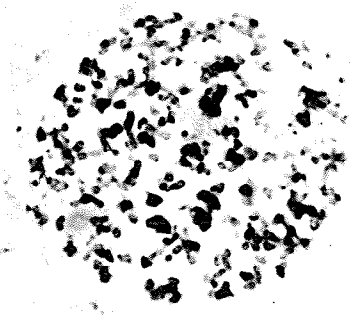

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PLATE III

Explanation for Figures

15. Cinara braggii; 2n=10.

16. Cinara braggii; middle metaphase.

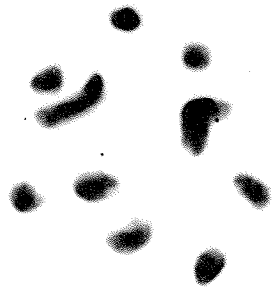
17. Cinara pinea; 2n=10.

18. Periphyllus negundinis; 2n=20.

19. Sipha agropyrella; 2n=6.

20. Gypsoaphis oestlundi; 2n=4.

15



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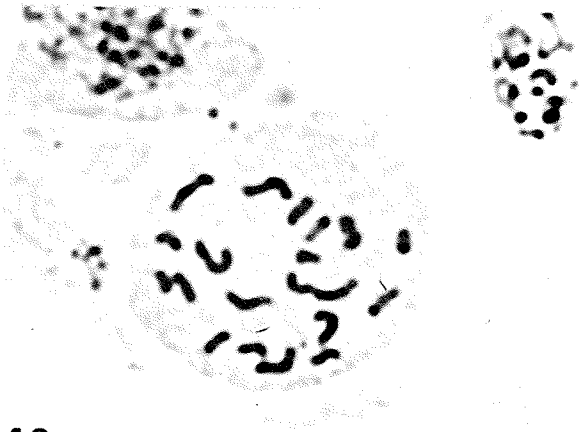

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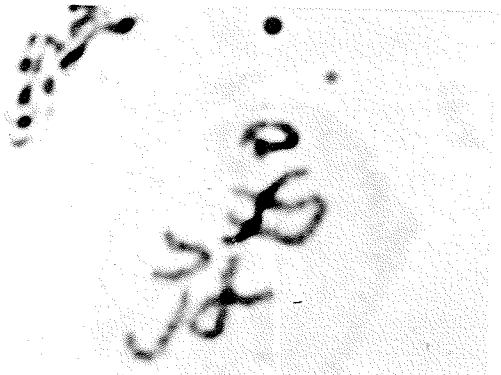

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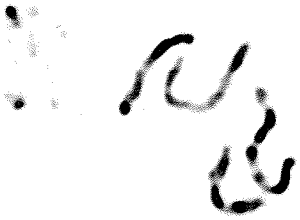

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PLATE IV

Explanation for Figures

21. Calaphis betulaecolens; 2n=20.

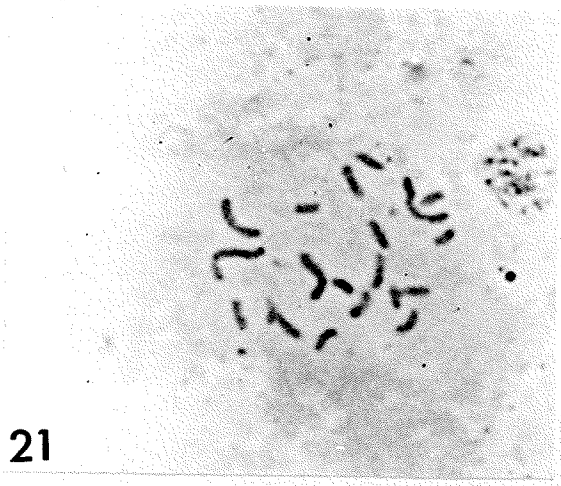
22. Euceraphis punctipennis; 2n=8.

23. Myzocallis punctata; 2n=14.

24. Euceraphis punctipennis; diploid complement with a super-numerary chromosome.

25. Pterocomma smithiae; 2n=8.

26. Aphis armoraciae; 2n=8.



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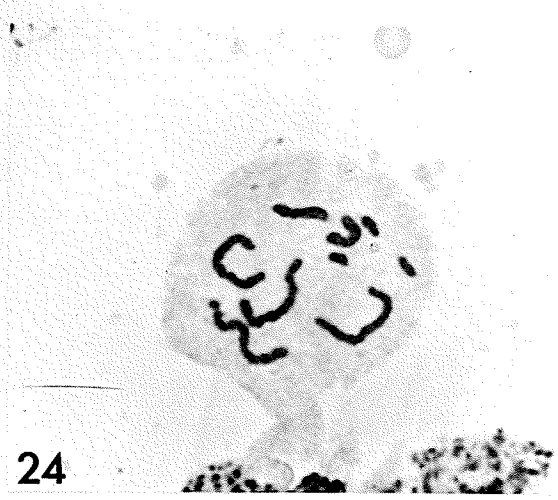

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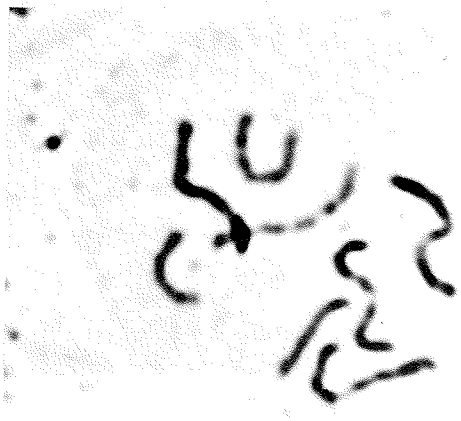

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PLATE V

Explanation for Figures

27. Aphis corniella; 2n=8.

28. Aphis helianthi; 2n=8.

29. Aphis nasturtii; 2n=8.

30. Aphis neogillettei; 2n=8.

31. Aphis rubicola; 2n=8.

32. Aphis spiraecola; 2n=8.

27

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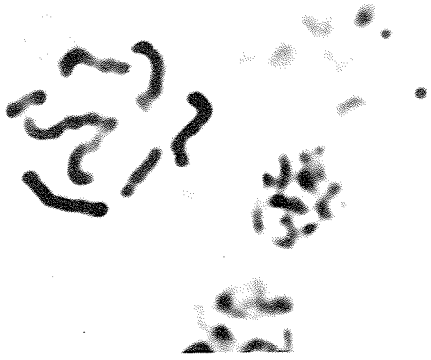

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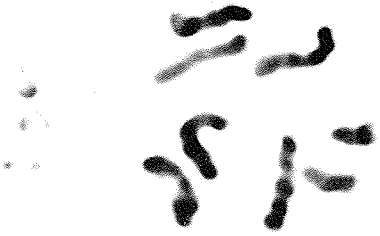

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32

PLATE VI

Explanation for Figures

33. Aphis varians; 2n=8.

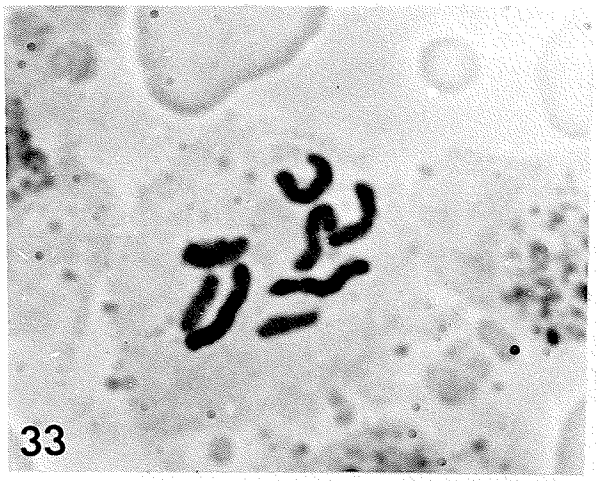
34. Rhopalosiphum fitchii; 2n=10.

35. Rhopalosiphum fitchii; 2n=10.

36. Rhopalosiphum maidis; 2n=8.

37. Rhopalosiphum maidis; middle metaphase.

38. Rhopalosiphum padi; 2n=8.



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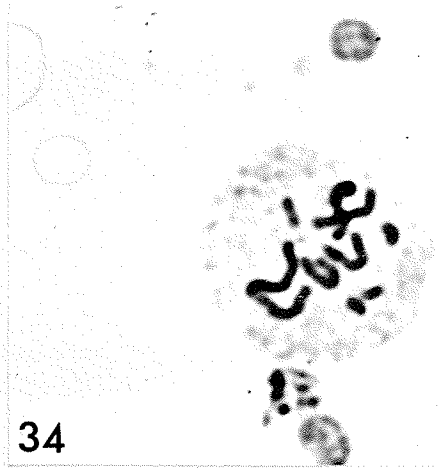

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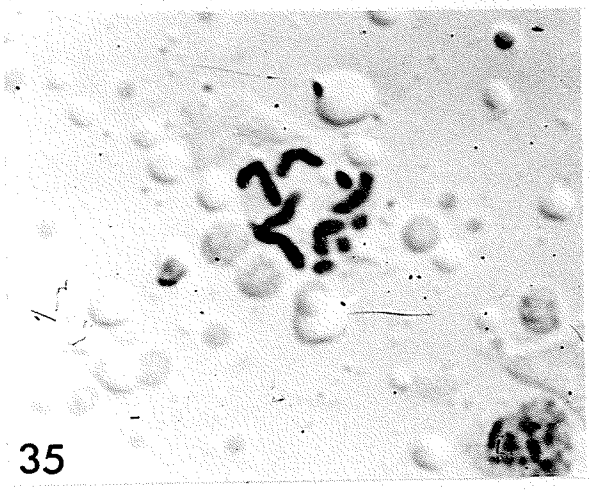

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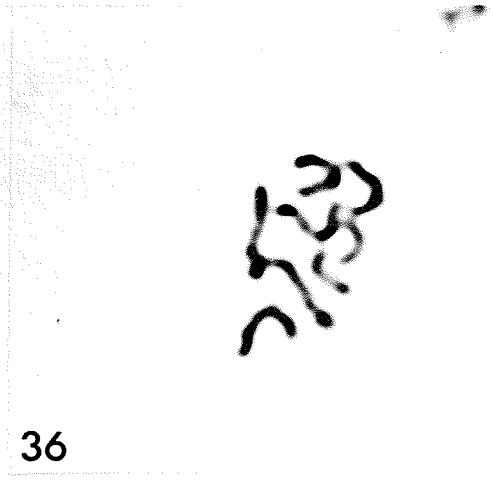

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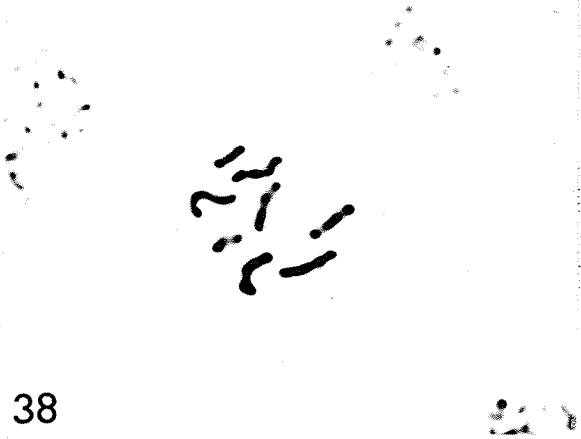

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PLATE VII

Explanation for Figures

39. *Schizaphis graminum*; 2n=8.

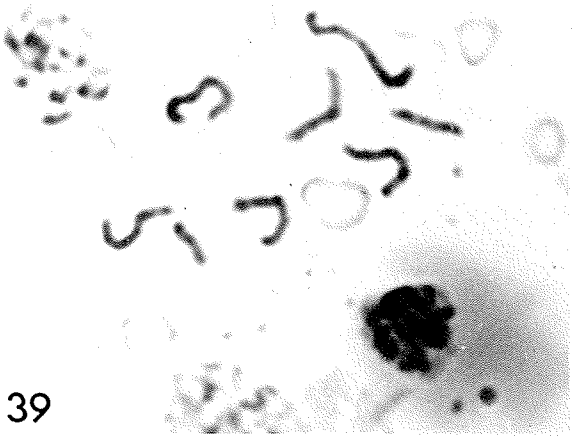
40. *Aspidaphis adjuvans*; 2n=12.

41. *Aphthargelia symphoricarpi*; 2n=14.

42. *Aspidaphis adjuvans*; middle metaphase.

43. *Brevicoryne brassicae*; 2n=16.

44. *Capitophorus hippophaes*; 2n=10.



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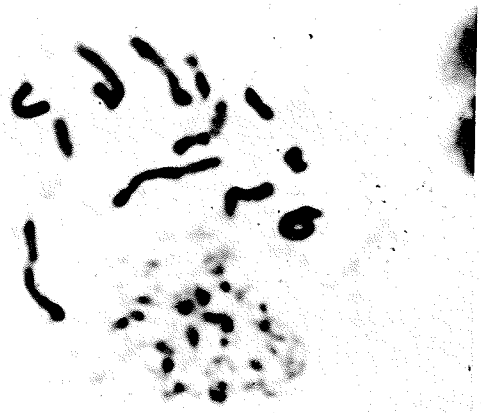

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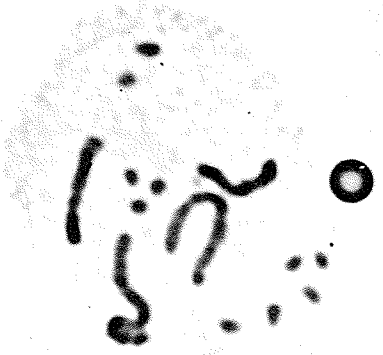

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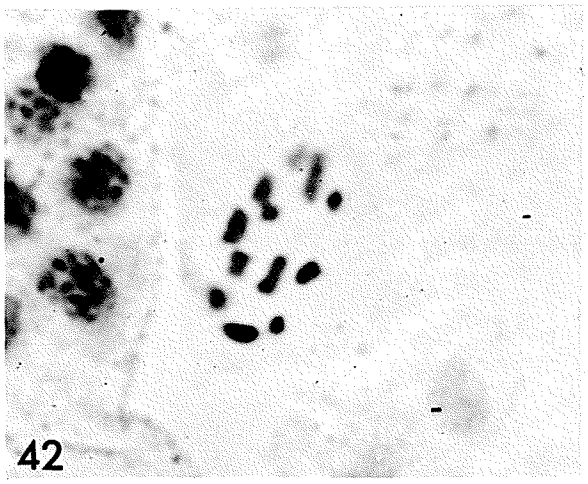

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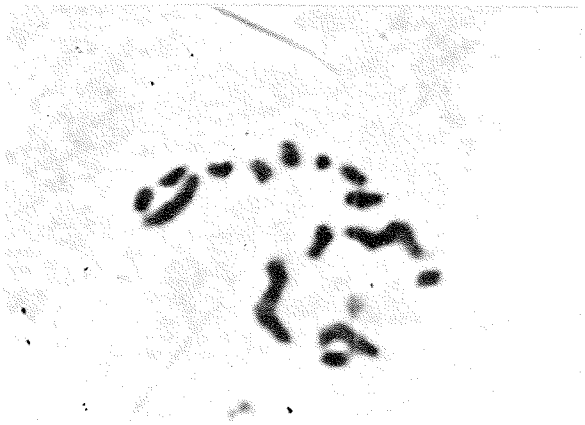

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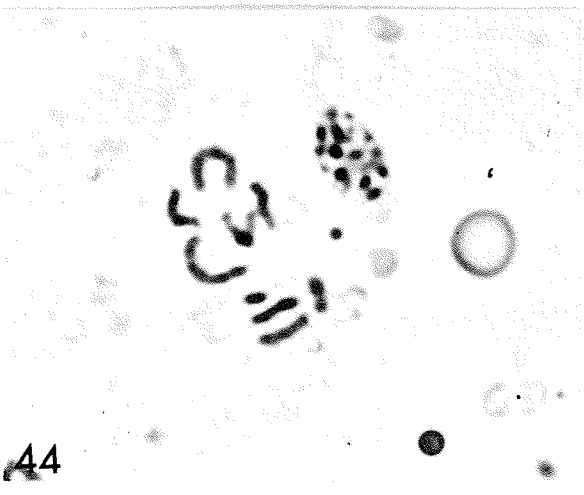


44

PLATE VIII

Explanation for Figures

45. Cryptomyzus ribis; 2n=12.

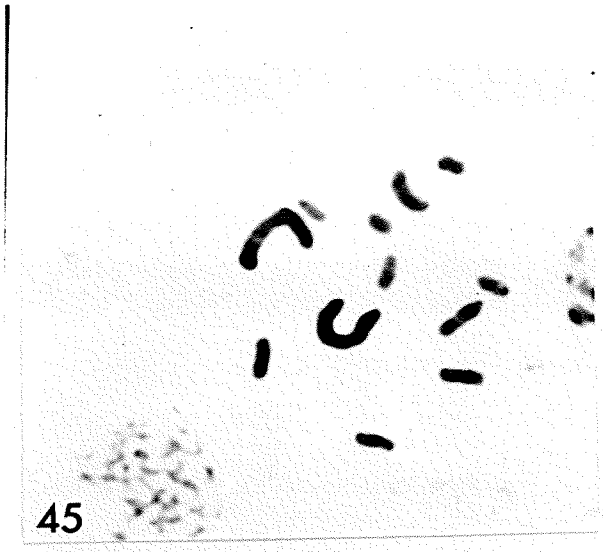
46. Hayhurstia atriplicis; 2n=14.

47. Kakimia essigi; 2n=10.

48. Kakimia sp., 2n=10.

49. Myzus cerasi; 2n=10.

50. Myzus persicae; 2n=12.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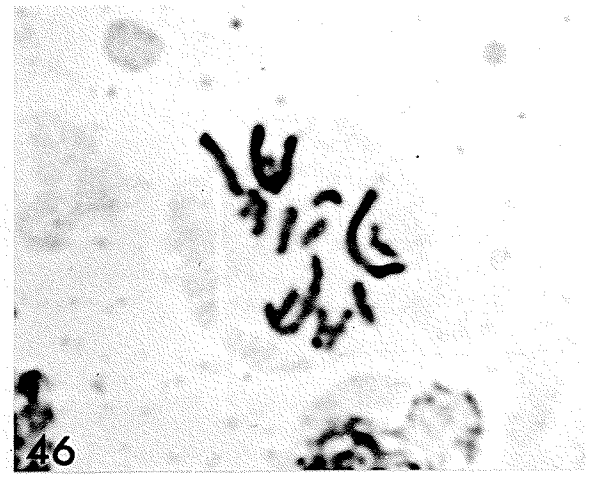
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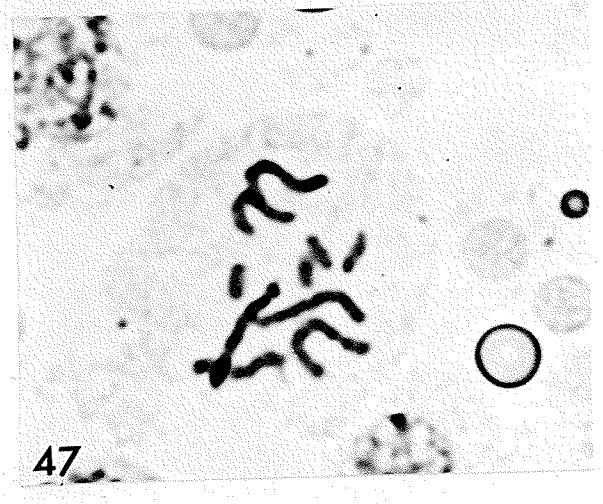
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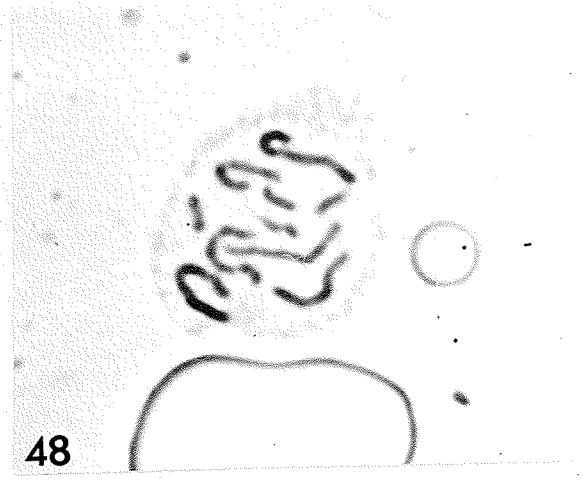
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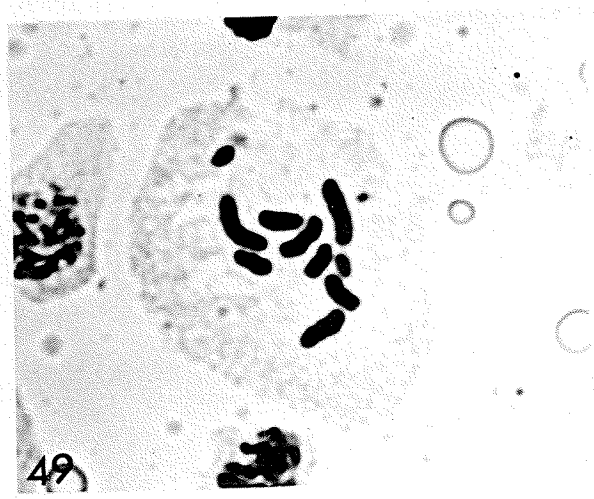
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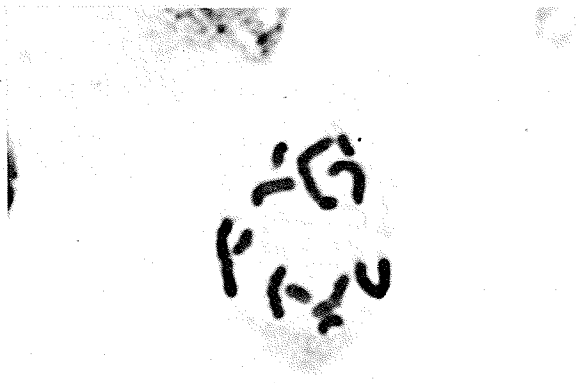
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PLATE IX

Explanation for Figures

51. *Nasonovia lactucae*; 2n=12.

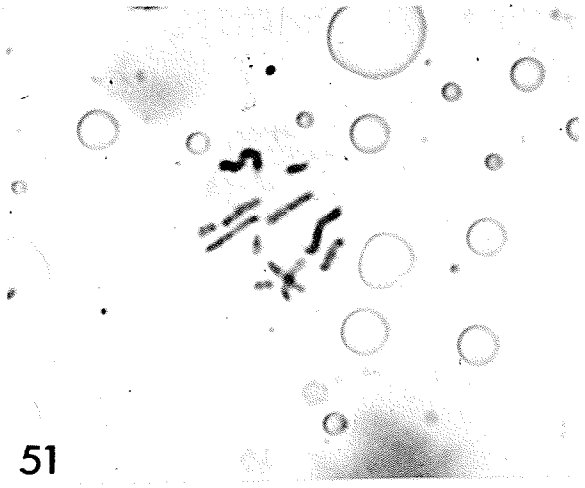
52. *Neoceruraphis viburnicola*; 2n=14.

53. *Rhopalomyzus lonicerae*; 2n=12.

54. *Acyrthosiphon caraganae*; 2n=10.

55. *Acyrthosiphon pisum*; 2n=8.

56. *Amphorophora laingi*; 2n=12.



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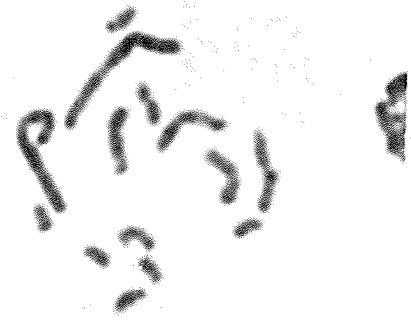

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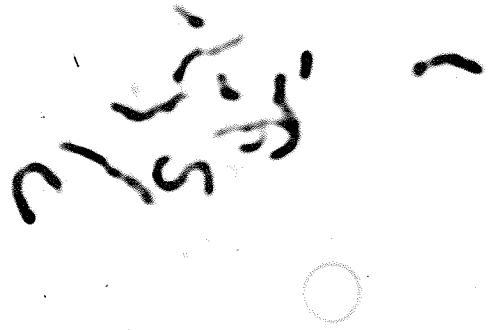

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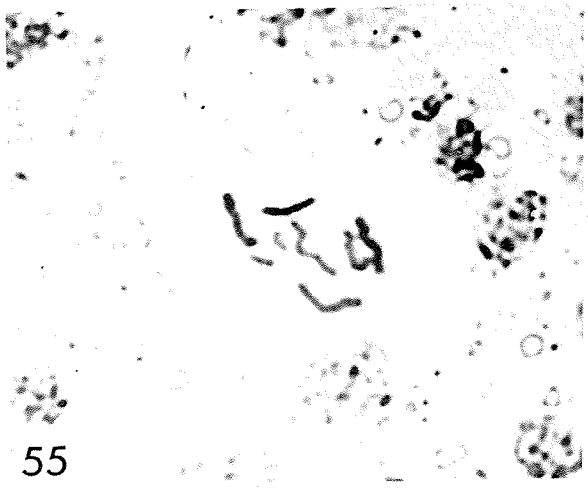

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PLATE X

Explanation for Figures

57. Amphorophora ribicola; 2n=12.

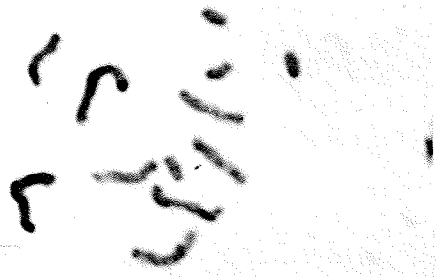
58. Cryptaphis poae; 2n=16.

59. Dactynotus cirsii; 2n=10.

60. Dactynotus taraxaci; 2n=12.

61. Macrosiphoniella absinthii; 2n=12.

62. Macrosiphoniella tanacetaria; 2n=12.



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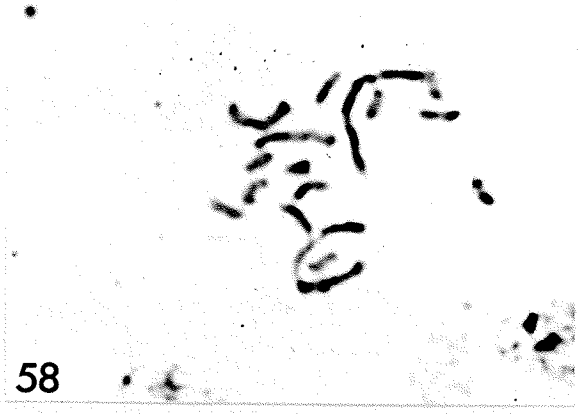

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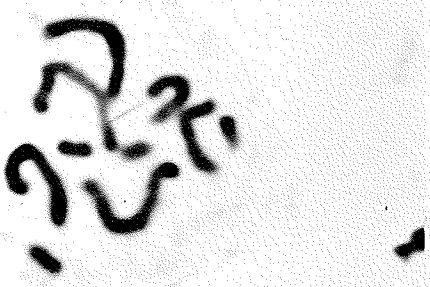

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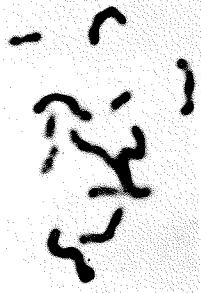

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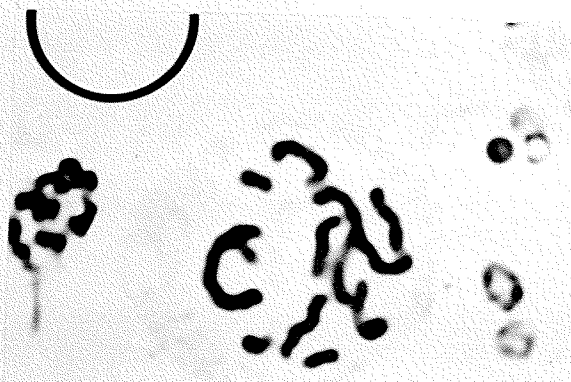

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PLATE XI

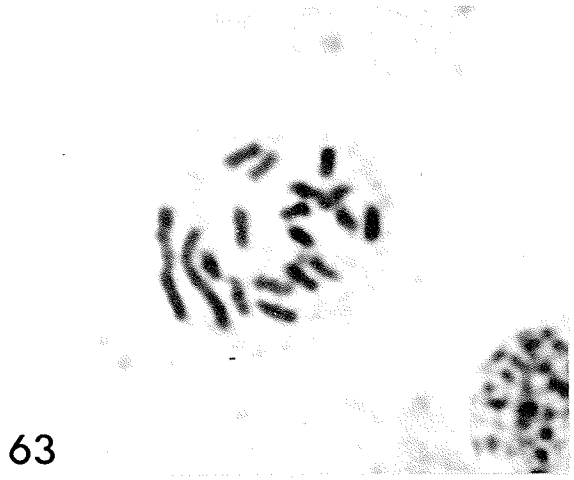
Explanation for Figures

63. Macrosiphum avenae; 2n=18.

64. Metopolophium dirhodum; 2n=18.

65. Macrosiphum euphorbiae; 2n=10.

66. Macrosiphum manitobensis; 2n=10.



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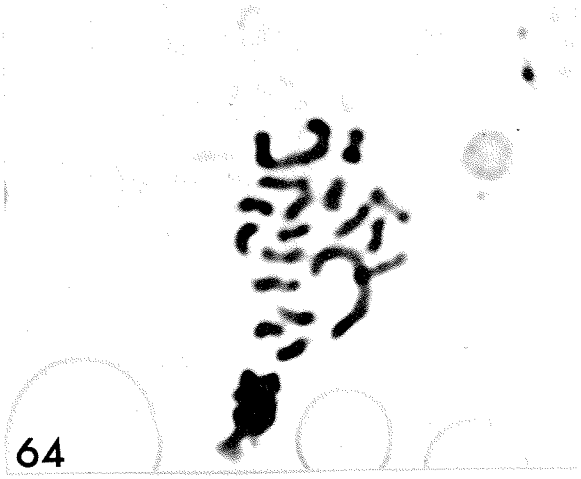

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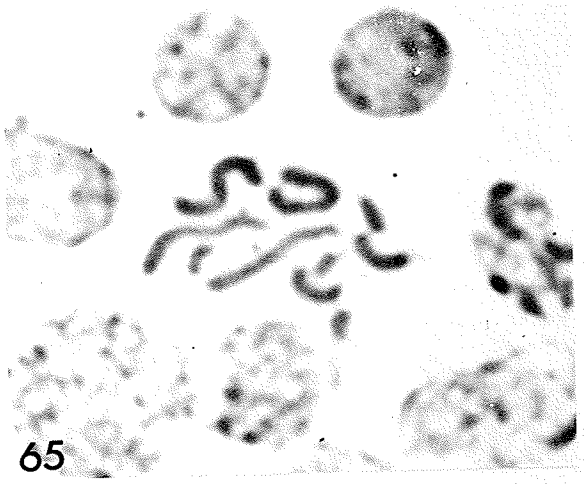

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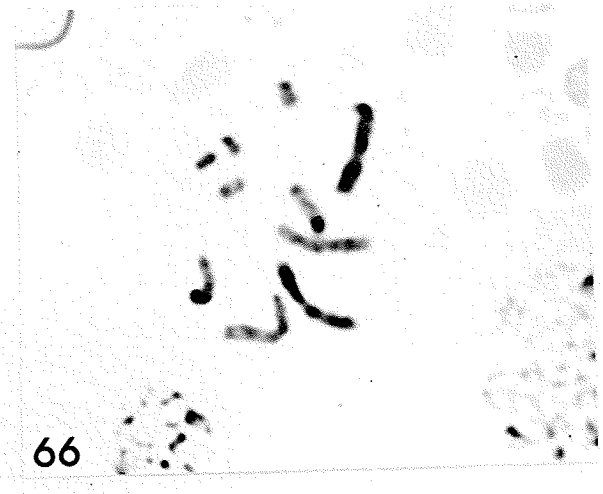

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PLATE XII

Explanation for Figures

67. *Masonaphis wahnaga*; 2n=10.

68. *Anoecia graminis*; 2n=8.

69. *Eriosoma lanigerum*; 2n=12.

67

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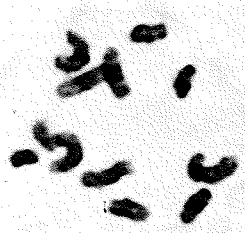

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CHAPTER VII

SUMMARY AND CONCLUSIONS

In Chapter I the subjects of insect-host plant relationships and differentiation between genera and species based on chromosome studies are introduced. In studies on insect-host plant relationships various environmental factors may directly affect the insect or indirectly through effects on the plants. Among these factors, temperature could be considered as the most important one. One problem encountered by those studying insect-host relationships is the correct identification of the insect species with which they are working. One method used for solving these problems is to make transfers of insects between different host plants. Another method is to study the chromosomes of insects. The present study reports research on the effect of various constant temperatures on reproduction of the aphid Rhopalosiphum padi on two barley varieties C.I. 3906-1 and Swan; transfers of two morphologically similar species of aphids R. padi and R. fitchii between their host plants; and studies of the chromosomes of 50 species of aphids.

Chapter II reviews the important literature of recent years on attempts by other workers to understand insect-host relationships of aphids, especially the effects of various environmental factors on both host plants and insects; references on the taxonomic problems of R. padi and R. fitchii

encountered by those who study insect-host relationships and by taxonomists; and the research studies on the cytology and cytotaxonomy of aphids.

In Chapter III the materials and methods are described. In the experiments on effects of different constant temperatures on R. padi apterous female aphids, 7-8 days old, descendants of one female were used under cages in a growth cabinet. The constant temperatures varied from 45° F to 100° F. The reproduction of R. padi on C.I. 3906-1 and Swan was recorded after 5 days.

In the experiment of transfers of aphids between host plants, R. padi and R. fitchii (fundatrices and/or fundatrigeniae) collected from their winter hosts, Pruneae or Pomeae, were transferred to new hosts and confined by clip cages or cages made of Visking dialyzing tubing on the underside of leaves or on the terminal growth of new host plants. If the aphids on the new hosts were in a healthy condition and produced more than four nymphs they were considered to be successfully established colonies and were then recorded on the third and fifth day after the transfer.

The chromosome studies were mostly conducted in the spring of 1965. The embryos employed in the chromosome counts were from viviparous females and the materials collected in the spring provided the best material for these studies. Two squash methods, the quick examination method stained by Orcein

and the Feulgen stain method were used through all the investigation. The first method is recommended for its simplicity and convenience if the preparation of permanent slides is not necessary.

Chapter IV presents the results of effects of different constant temperatures on the reproduction of R. padi. It was found that the optimum temperature for reproduction of R. padi on both barley varieties, Swan and C.I. 3906-1, was at 75° F. Reproduction nearly ceased at a minimum temperature of 45° F, and maximum of 100° F. The high temperature was adverse to the survival of aphids as well as to the development of embryos, while the low temperature had a greater influence on the embryogenesis than on the survival of aphids. The size and the body color of aphids were also influenced by temperatures. Aphids reared at 45° F were larger and deeper in body color than those reared at 80° F. The resistance of two barley varieties, Swan and C.I. 3906-1, was determined by means of antibiosis -- the fecundity of R. padi on them. There was no significant difference of antibiosis between the two barley varieties to R. padi at various constant temperatures.

In Chapter V the presence of the two species of aphids, R. padi and R. fitchii, in Manitoba was confirmed by transfer tests between the winter host plants. R. padi overwinters on Prunus virginiana L. but is unable to live or colonize on the winter hosts of R. fitchii. Similarly, R. fitchii overwinters

on Cotoneaster, Malus, Crataegus and Sorbus in Manitoba and is incapable of living and colonizing on P. virginiana L. R. fitchii from any one species of the four winter hosts could transfer and colonize successfully on any of the other winter hosts. Both aphids were found to be host specific within their host range.

Observations on somatic cell divisions and chromosome counts of fifty species of aphids are given in Chapter VI. The mitotic cell division of aphids is basically similar to that of other organisms, though different in some ways at metaphase stage. At metaphase, chromosomes condensed to such an extent that they appeared as dots or rods. At the metaphase plate chromosomes united together to form a rod mass and then divided longitudinally through the middle of the whole mass. No separated chromosomes could be seen until the prophase of the next division. Numerous products of endomitosis were found from tetraploid up to multiploid in embryos and ovarioles.

The chromosome numbers of the 50 species in 32 genera vary from 4 to 20. The highest frequency of chromosome numbers is eight. Those species with the lowest chromosome numbers are characterized by having large chromosomes, while those with higher chromosome numbers always had smaller chromosome size. These facts might be an evidence of fragmentation. Since the highest frequency of chromosome number is eight among these 50 species and the frequencies of 4, 6, 18 and 20 are very low,

it is suggested that the very high and very low numbers did not arise suddenly but represent the end products of evolutionary process.

Four kinds of chromosome karyotypes in the Family Aphididae were found to be quite common in more than one genus. The chromosome numbers of these four karyotypes are 8, 8, 10 and 12. These different types of chromosome karyotypes might suggest the steps of an evolutionary process in aphids, which is by no means at random but along a definite path.

The chromosome numbers and karyotypes of the two morphologically similar species R. padi and R. fitchii are different. R. padi has eight chromosomes and R. fitchii has ten chromosomes, which provides an easy way to tell one from the other.

The very peculiar chromosome karyotype of Aphthargelia symphoricarpi shows four large chromosomes and ten very small chromosome dots of similar size. The ten small chromosomes might be the products of the fragmentations of large chromosomes, or this species might be a hybrid between two species with large and small chromosomes respectively.

Evidence obtained in the present study on aphid chromosomes suggests that this could be a useful additional means of solving difficult taxonomic problems, and of elucidating evolutionary development in aphids.

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