

Spermatogenesis in the aphid *Amphorophora tuberculata* (Homoptera, Aphididae)

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Abstract. Spermatogenesis was studied in *Amphorophora tuberculata* Brown & Blackman, a species of aphid with $n=2$. Spermatogonia have $2n=3$ (AA + XO). In early prophase I the autosomal homologues are united terminally to form a tandem bivalent. No evidence could be found of synapsis or of the formation and terminalisation of chiasmata. The terminal connection of the autosomes is retained until late in prophase II. Sister chromatids separate, and autosomal half-bivalents move apart at anaphase I, but the division is incomplete, the X chromosome forming a thin chromatin bridge between the two autosomal half-bivalents. In prophase II the autosomal half-bivalents double back on themselves, so that non-sister chromatids become aligned in parallel. The X chromosome then becomes associated with one of the autosomal half-bivalents. Anaphase II separates the non-sister chromatids, and meiosis is thus “post-reductional.”

Introduction

The holocentric chromosomes of Hemiptera generally behave in a characteristic way during the first division of meiosis. Pairing of homologues and crossing over are followed by an opening out of the bivalent, so that at metaphase I the homologues are joined end-to-end, in tandem fashion, by a single terminal chiasma. The orientation of these tandem bivalents on the first division spindle is then either axial, with the long axis of each bivalent lying along the spindle, or equatorial, with the long axis at right angles to the spindle. Equatorial orientation will lead to an “inverse” meiosis, in which the first meiotic division is equational for regions of the chromosome that have not crossed over, and the second division is the reductional one (see reviews by Battaglia and Boyes 1955; Sybenga 1981). “Post-reductional” meiosis was at one time thought to be the general rule in aphids and scale insects, and was referred to by Hughes-Schrader (1948) as the “aphid-coccid” type of meiosis, although in the case of aphids the generalisation was based on observations of a single species.

Meiosis in the Hemiptera in general thus seems to require obligate formation and terminalisation of chiasmata (White 1973). However, Blackman (1976) found no evidence of chiasmata or end-to-end alignment of homologues

in spermatogenesis of the aphid *Euceraphis betulae*. In this species, homologues remain closely aligned in parallel as they condense into metaphase I, and anaphase I separates the products of pairing in a strictly reductional manner. A similar type of achiasmate male meiosis has recently been reported in two families of Heteroptera, Saldidae and Nabidae (Nokkala and Nokkala 1983, 1984).

The discovery of a new species of aphid with $n=2$, *Amphorophora tuberculata*, from which males could easily be reared in the laboratory (Brown and Blackman 1985), prompted a detailed study of the behaviour of its chromosomes in spermatogenesis. The main aims were to find out whether or not chiasmata were formed and to establish whether meiosis was normal (pre-reductional) or inverted (post-reductional).

Materials and methods

Viviparous, parthenogenetic females of *A. tuberculata* were reared on excised leaves of their host plant, *Geranium macrorrhizum* L., in controlled environment rooms at 18° C with a 16-h photoperiod, and at 15° C with a 12-h photoperiod. Males were produced in both regimes, in the middle of the larviposition sequence of each mother, after an initial batch of entirely female offspring. Males produced at 18° C and 16 h were alate or brachypterous, whereas those produced at 15° C and 12 h were all apterous.

Testes were dissected from first, second, and third instar larvae in slightly hypotonic (0.75%) potassium chloride solution and fixed in cold methanol/acetic acid (3/1). They were then either hydrolysed for 5 min in 1 N hydrochloric acid at 65° C and stained with Feulgen before squashing, or squashed directly in a drop of 45% propionic acid for examination by phase contrast prior to freezing off the coverslip and staining with Giemsa. Some preparations were C-banded (Blackman 1976).

To study zonation in the testis and obtain information about the chronological sequence of events in meiosis, some of the Feulgen-stained testes were examined in distilled water under a coverslip without squashing. These preparations were then studied (using a Zeiss Planapo 63 oil-immersion objective) as the water was displaced by running 45% propionic acid under the coverslip to swell the cells and render their cytoplasm transparent. This technique was

useful to observe the three-dimensional disposition of chromosomes in the spermatocyte nuclei prior to squashing.

Results

Karyotype

Somatic cell divisions in female embryos show $2n=4$, with a single long pair of autosomes about twice as long as the X chromosome pair (Fig. 1a). C-banding techniques failed to demonstrate any significant blocks of C-heterochromatin. The genus *Amphorophora* shows a remarkable degree of karyotype instability; apart from *A. tuberculata*, chromosome numbers of other species range from $2n=12$ to $2n=72$ (Blackman 1980). The differences in all cases seem to be due to processes of autosome fusion or dissociation, and in all species except *A. tuberculata* the X chromosomes exceed the length of the longest autosomes.

Spermatogonial divisions

At prophase and metaphase, spermatogonial cells have $2n=3$ (Fig. 1b), comprising a pair of autosomes and an unpaired X chromosome (*A. tuberculata*, like other aphids, has XO sex determination). Condensation of the X is delayed in comparison with the autosomes and during spermatogonial prophase its length is 0.7–0.8 the length of one autosome, whereas at metaphase the ratio of X chromosome length to autosome length is closer to 0.6 (Fig. 1c). This contrasts with the situation in somatic cell prophases where the X chromosomes condense before the autosomes (Fig. 1a).

Meiosis

In the interphase preceding the maturation division the nuclei stain rather lightly and uniformly, with little sign of heterochromatin. The earliest stages of prophase I are, in comparison to other aphids studied by the author, relatively clear. As the autosomes become individually discernible it is evident that if synapsis occurred at all it is already over, and the homologues appear as unpaired, single threads (Fig. 2a). However, as condensation proceeds there is a tendency for the autosomes to appear united end-to-end (Fig. 2b, c). Autosomes showing the same degree of condensation in the same field are in some cells united terminally by a fine thread of chromatin, while in other cells their ends appear to be widely separated. Although this stage could be interpreted as the one at which terminal connections are formed, it seems more probable that the terminal connection is easily stretched or broken during preparation, and that in reality it is already present when the chromosomes enter prophase. As in spermatogonial prophase, the condensation of the single X chromosome lags behind that of the autosomes.

As prophase I proceeds, the terminal connection between the autosomal homologues becomes more consistent and the two chromatids of each homologue become discernible, especially in Giemsa-stained preparations (Fig. 3a). The unpaired X is less than half as long as the tandem autosomal bivalent and appears only about half as thick, its separate chromatids not being discernible at this stage (Fig. 3b). As condensation proceeds the point of union between the two autosomal homologues becomes obscure, and late in prophase I the separate chromatids of the X

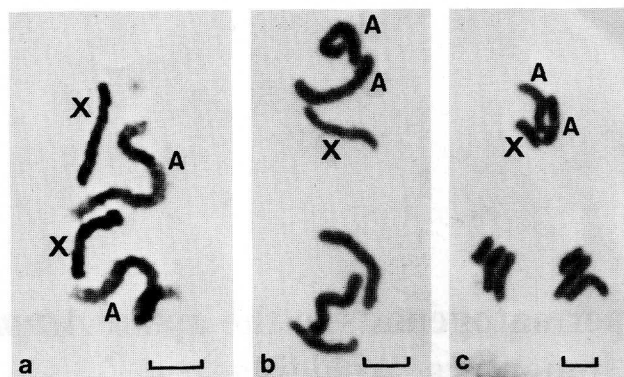


Fig. 1. a Somatic prophase from female embryo of *Amphorophora tuberculata*, $2n=4$. b Two spermatogonial nuclei in late prophase, $2n=3$. c Three nuclei in spermatogonial metaphase. Feulgen staining. A autosome, X X chromosome. Bars represent 5 μm

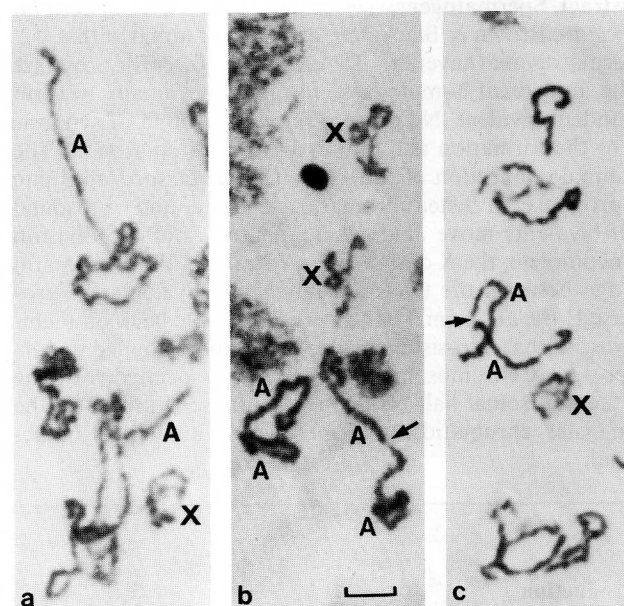


Fig. 2. a–c Successive stages of early prophase of male meiosis I in *A. tuberculata*. b Two spermatocyte nuclei are squashed together; c three nuclei are shown. Note apparent terminal connections between autosomes (arrowed) in b and in central cell in c. Feulgen staining. Bar represents 5 μm

chromosome become evident (Fig. 3c, d). C-banding at this stage reveals terminal or sub-terminal blocks of heterochromatin at each end of the X chromosome (Fig. 3e).

At metaphase I the autosomal bivalent comprises two short parallel rods, made up of the chromatids of each homologue aligned end-to-end. There is now no trace of the point of connection between the homologues, and no visible evidence remains of the fact that they are aligned in tandem (Fig. 4a). The thickness of the bivalent suggests that the autosomes are about twice as condensed as at spermatogonial metaphase (compare Fig. 1c). The X chromosome is also relatively more condensed than in spermatogonial metaphase, but its chromatids are not so clearly separated as are those of the autosomes. Just prior to anaphase the X chromosome orients with its long axis at right angles to the equator of the spindle.

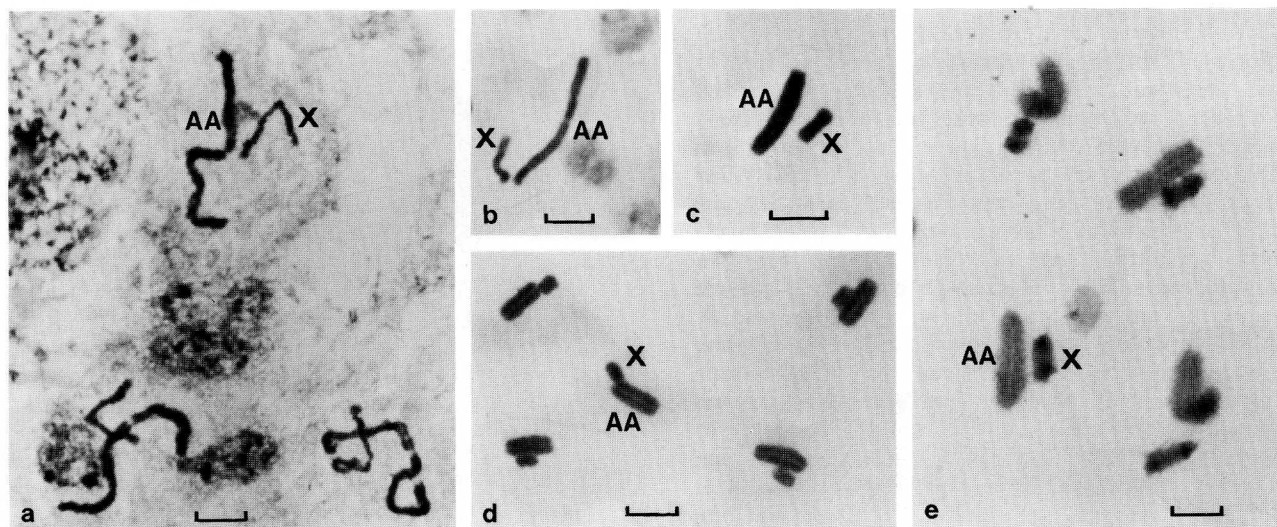


Fig. 3. a-e Later stages of prophase I in male meiosis of *A. tuberculata*. a, e Giemsa; b-d Feulgen staining. Bars represent 5 µm

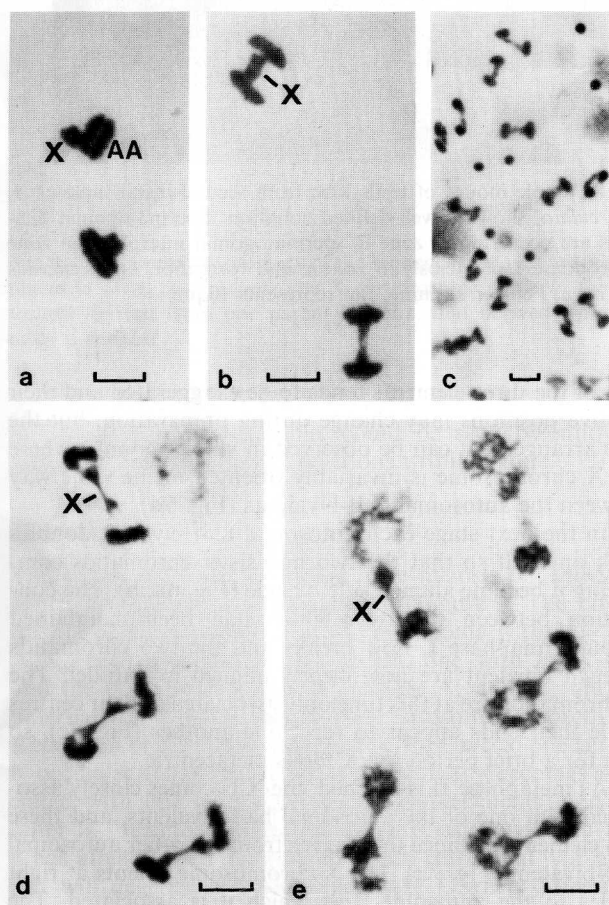


Fig. 4. a-e Metaphase I and anaphase I of male meiosis in *A. tuberculata*. a Two nuclei in metaphase I; b two nuclei in early anaphase I; c group of spermatocyte nuclei in anaphase I in an unsquashed preparation showing different aspects of the division figure; d, e later stages of anaphase I. Feulgen staining. Bars represent 5 µm

Anaphase I commences with the chromatids of the autosomal bivalent moving apart in parallel, their end-to-end alignment being maintained throughout the division (Fig. 4b, c). The X chromosome initially remains at the

equator of the spindle, maintaining the allocyclus evident from early prophase. Then, when the two halves of the autosomal bivalent have separated by a distance of about the length of the X chromosome, the latter starts to elongate along the line of the spindle. Initially it is situated to one side of the line of separation of the autosomal half-bivalents, as can be seen when the autosomes are viewed end-on (Fig. 4c). A constriction appears at about the mid-point of the X chromosome. As anaphase proceeds, the X becomes situated between the now widely spaced autosomal half-bivalents, and Feulgen-positive connections are visible between the autosomes and each end of the elongated X. The central part of the X chromosome becomes finely attenuated.

Late anaphase is marked by two events which destroy the symmetry of the arrangement. On one of the autosomal half-bivalents becomes partially decondensed and curls into a C-shape (Fig. 4d, e). At the same time there is an apparent shift in the position of the attenuated part of the X chromosome, so that the bulk of the X chromatin becomes associated with the decondensing half-bivalent, only a small part remaining at the other end of the spindle (Fig. 4). Eventually the other autosomal half-bivalent also becomes partially decondensed, but to a lesser extent (Figs. 4e, 5a).

This process can clearly be regarded as anaphase I of meiosis, but the nuclear division is not completed. The two clumps of chromatin, comprising in each case one autosomal half-bivalent and part of the X chromosome, remain linked by a thin bridge of X chromatin. All three elements then start to condense again, this time synchronously, entering prophase II of meiosis (Fig. 5b). During this condensation period the X chromosome at first comprises an irregularly shaped mass closely associated with, and sometimes connected by a chromatin bridge to, the autosomal half-bivalent that decondensed earlier and more fully during late anaphase I. A small trabant of X chromatin remains associated with the other autosomal half-bivalent, linked to the main mass of the X chromosome by a fine thread.

As condensation proceeds through prophase II the X separates from both autosomal half-bivalents and resolves itself into two chromatids, more closely associated at their centres than at their ends, with the trabant attached to the end of one chromatid (Fig. 5c). In squashes the separa-

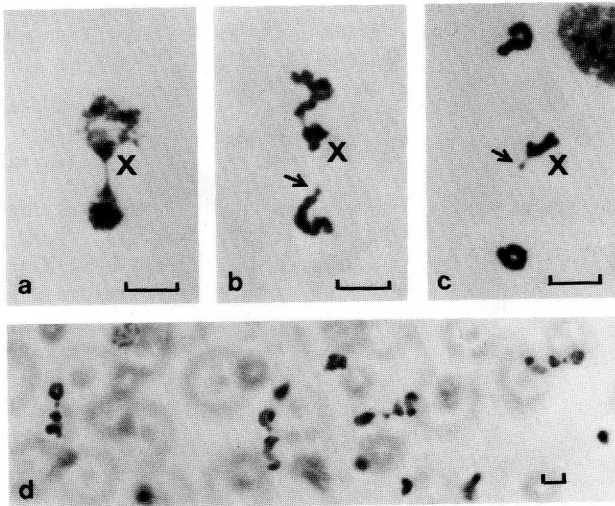


Fig. 5. "Telophase" (a) and early stages of prophase II (b-d) of male meiosis in *A. tuberculata*. Note the trivalent attached to the X chromosome (arrowed) in b and c. Feulgen staining. Bars represent 5 μ m

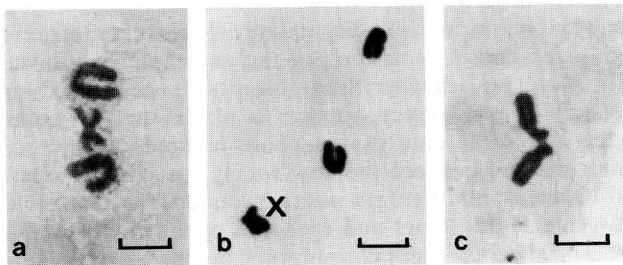


Fig. 6a-c. Later stages of prophase II showing realignment of non-sister chromatids. In b the X chromosome has been displaced during squashing from its position between the two half-bivalents. a Giemsa; b, c Feulgen staining. Bars represent 5 μ m

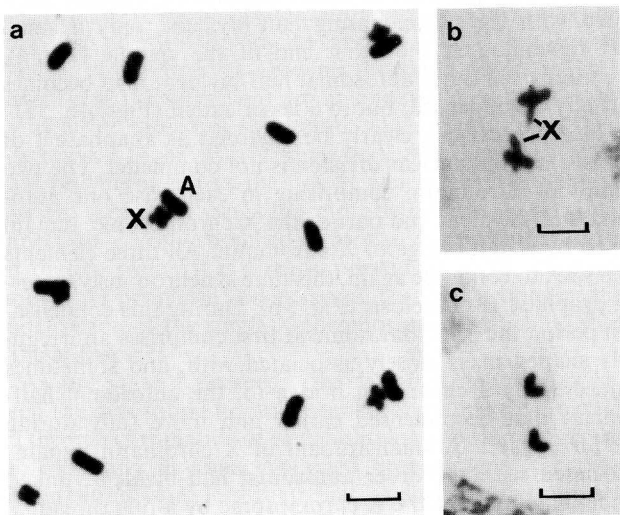


Fig. 7. a Metaphase II of male meiosis in *A. tuberculata*, showing associations between X chromosomes and autosomal half-bivalents. b and c Anaphase II separation respectively with (b) and without (c) an X chromosome. Feulgen staining. Bar represents 5 μ m

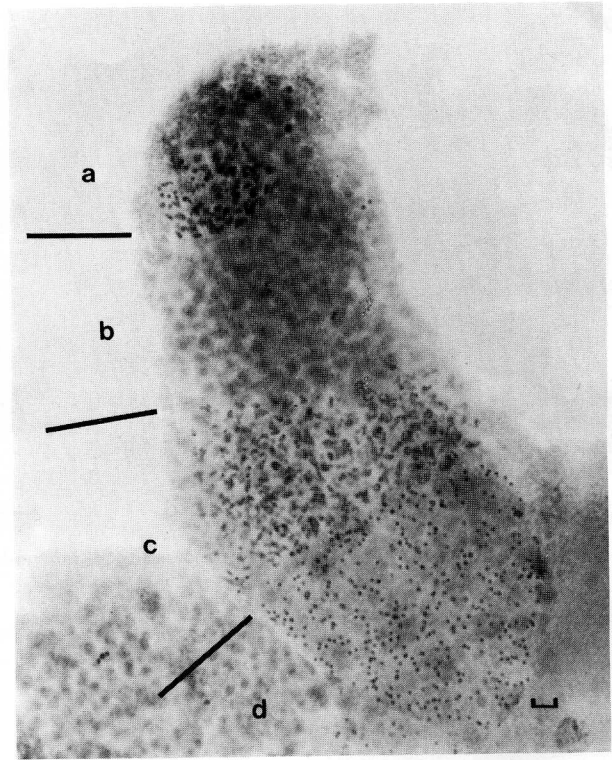


Fig. 8. Whole mount of testis lobe from second-instar male of *A. tuberculata*, showing well-defined zonation. Spermatogonial divisions are occurring in zone a, spermatogonial interphase in zone b, prophase I of meiosis in zone c and formation of spermatids in zone d. Feulgen staining. Bar represents 10 μ m

tion of the three elements tends to be exaggerated and their relative positions may change during preparation, but the true arrangement can be observed in whole mounts, where the X chromosome is invariably oriented in the same way between the autosomal half-bivalents (Fig. 5d).

In the next stage each autosomal half-bivalent doubles back on itself so that the two non-sister chromatids comprising it become aligned side by side (Fig. 6a, b). The connection between the two, which had been maintained through anaphase I, now breaks and the two chromatids of each bivalent are now clearly aligned in parallel. The X chromatids are at this time only associated at their centres while their ends appear to repel one another (Fig. 6c), so that for a brief period the X looks metacentric.

As metaphase II is reached, the X becomes closely associated with one of the autosomal half-bivalents, and these two elements distance themselves from the other autosomal half-bivalent (Fig. 7a). The X chromosome orients at right angles to the autosome with which it is associated. The anaphase II separation is peculiar; the two (non-sister) chromatids of the autosomal half-bivalent, and the two (sister) chromatids of the X chromosome, move apart in cross formation (Fig. 7b). The autosomal half-bivalent that is left on its own also undergoes anaphase separation of its constituent non-sister chromatids (Fig. 7c), but thereafter degenerates.

Zonation of the testis was clearly defined, with each stage occurring synchronously (Fig. 8). Lengthy stages such as spermatogonial interphase occupied broad bands extending right across a testis lobe, whereas the sequence of events

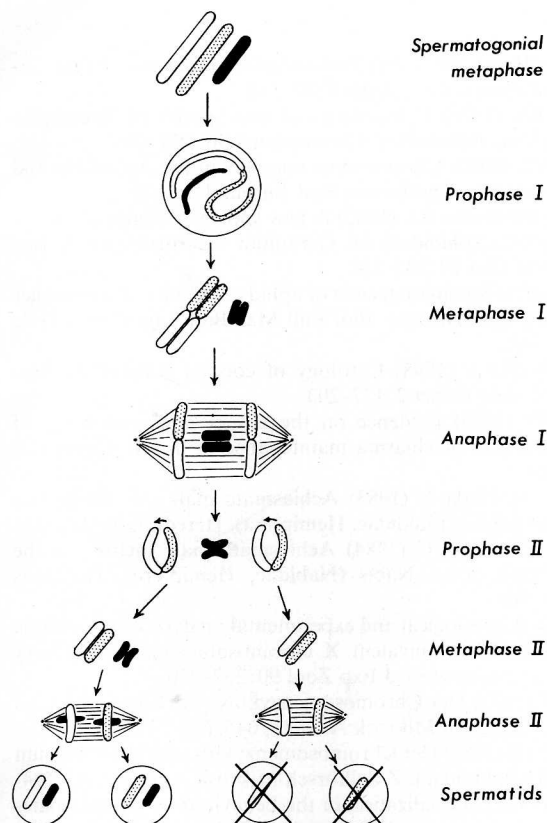


Fig. 9. Diagrammatic summary of the sequence of stages in spermatogenesis of *A. tuberculata*. The X chromosome is black; the autosome derived from one parent is white, that derived from the other is stippled

in prophase I, for example, could be seen by studying groups of cells in adjacent positions within a zone.

Discussion

The sequence of events during male meiosis of *Amphorophora tuberculata* is summarised in Figure 9. The consequence of the process is that spermatogonia with $2n = X + A_p A_m$ each give rise to two sperm, one with $n = X + A_p$ and the other with $n = X + A_m$, where A_p and A_m are the paternally and maternally derived autosomes respectively. Reduction of the autosome number from two to one actually occurs at metaphase II, when the X finally becomes associated with one of the autosomal half-bivalents, and the other is "rejected," although there is clear evidence that it has already been determined which autosomal half-bivalent will degenerate by late anaphase I, when differences in the timing and degree of condensation of the two half-bivalents, as well as in the behaviour of the X with respect to them, become apparent. As the two half-bivalents are believed to be genetically identical, their differential behaviour is perhaps due to asymmetry in the cytoplasmic architecture or other properties of the anaphase I spindle. It seems likely that the X chromosome associates with the same half-bivalent at metaphase II as it did at anaphase I, but there is as yet no proof of this.

Meiosis in *A. tuberculata* is therefore "post-reductional", in the sense that it is anaphase II, rather than anaphase I, which separates the products of pairing. In

this respect the situation in this species, which is a member of the subfamily Aphidinae, agrees with the findings of Ris (1942), working with the callaphidine aphid *Tamalia coweni*, and is contrary to that reported by Blackman (1976) in another callaphidine, *Euceraphis betulae*. However, Ris believed that in *Tamalia*, chiasmata formed and terminalised in a diffuse stage of early prophase, when the individual chromosomes were not discernible, and he depicted autosomal bivalents in a variety of configurations at diakinesis, some of them showing extensive chromatid separation. In *A. tuberculata* it is possible to resolve the autosomes very early in prophase I, when they invariably appear single-stranded, and no evidence could be found of the opening-out and terminalisation of chiasmata at this early stage of prophase. The chromatids in the autosomal bivalent remain closely aligned in parallel throughout prophase I of *A. tuberculata*; no chromatid separation was ever observed, and this clearly differs from the prophase I described for *Tamalia* by Ris. However it is still possible, although it seems unlikely, that synapsis, crossing over, and complete chiasma terminalisation could occur before the chromosomes can be resolved by the light microscope. This question can only be finally settled by electron microscope studies or use of genetic markers.

The simplest interpretation of the autosomal bivalent is that a terminal connection is formed between the two autosomes in interphase or early prophase I. This requires a specific and persistent bond to be established between non-sister telomeres, after replication but before sister chromatids can be distinguished. Possibly the ends of the homologues become associated on the nuclear membrane, and membrane material might be involved in the bonding mechanism. A more conventional explanation is that a highly localised, sub-terminal chiasma occurs, so that bonding is between sister rather than non-sister telomeres. However, the bonding cannot be due to generalised sister chromatid cohesiveness (Maguire 1985), because it is maintained when sister chromatids separate in anaphase I. It must therefore be of a special type, and the most parsimonious hypothesis is that it is between non-sister telomeres.

The "abortive" anaphase I, with the X chromosome maintaining a chromatin bridge between the autosomal half-bivalents, which do not finally separate until metaphase II, may be a feature peculiar to this species. Anaphase I separation seems to be complete in all other aphids studied except *Euceraphis punctipennis*, where it may be dispensed with entirely (Blackman 1976). However, Suomalainen (1933) reported that in *Acrythosiphon pisum* the X chromosome did not pass completely into one daughter spermatocyte at anaphase I, a small fragment (which he regarded as "eliminations chromatin") remaining behind in the other daughter spermatocyte. Possibly Suomalainen was observing a similar process to that described here for *A. tuberculata*, but was unable to observe what happened to this fragment in prophase II.

The remarkable process in anaphase II, whereby each autosomal half-bivalent doubles back on itself so that the two non-sister chromatids become aligned in parallel, can be clearly seen in *A. tuberculata*. Ris (1942) believed, by analogy with meiosis in primitive coccids, that the realignment prior to the second meiotic division in *Tamalia* must occur during a short interphase before prophase II, and must involve disjunction of the two chromatids followed by secondary pairing, so that the chromatids were already

aligned in parallel when they first became distinguishable in prophase II. In *A. tuberculata* this is clearly not the case. Realignment occurs quite late in prophase II, and disjunction does not occur until after the chromatids are aligned at least roughly in parallel. Whether *A. tuberculata* is peculiar in this respect or whether it has not been possible to observe the same detail in other aphids because they provide less favourable material is another question still to be resolved.

It is notable that during and after the realignment and loss of terminal connection of the autosomal half-bivalents in prophase II, the chromatids of the X chromosome appear to repel one another and are only held together centrally. They also adopt an orientation at right angles to the autosomal chromatids at anaphase II. It seems possible that the same forces that cause attraction between non-sister chromatids of the autosomal half-bivalent during late prophase II cause repulsion between the sister chromatids of the X chromosome, which therefore need to be held together until anaphase by some localised form of cohesion.

There seems to be no general rule in aphids about whether or not the autosome or autosomes that are left without an X chromosome go through a second meiotic division. In some species these autosomes degenerate before or during prophase II (Schwartz 1932; Ris 1942), in others there is a division before degeneration, and in others still small spermatids may be formed, which then regress (Honda 1921). In all cases, however, functional sperm without an X chromosome are never produced, presumably because the X chromosome carries factors essential for the completion of spermiogenesis.

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