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Cytogenetics of Two Species of *Euceraphis* (Homoptera, Aphididae)

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Abstract. Somatic cell divisions, spermatogenesis, and the prophase stages of primary oocytes, are described for two species of birch aphid, *Euceraphis betulae* (Koch) and *E. punctipennis* (Zetterstedt). Females of *E. betulae* have two autosome pairs, two pairs of X-chromosomes of different lengths, and two B-chromosomes. Females of *E. punctipennis* have the same number of X-chromosomes and B-chromosomes as *E. betulae*, but only a single pair of autosomes. The sex determination system is X_1X_20. *E. punctipennis* males sometimes have only one B-chromosome. In the spermatogenesis of *E. betulae*, pairing of homologous autosomes occurs in early prophase I, but no evidence was found of chiasmata or end-to-end alignment of homologues. Instead, homologues remain closely aligned in parallel as they condense into metaphase, and anaphase I separates the products of pairing in a strictly reductional manner. The two unpaired X-chromosomes and both B-chromosomes are stretched on the anaphase I spindle and all four pass into the larger secondary spermatocyte. The second division is equational. The B-chromosomes thus show accumulation in spermatogenesis, which must be compensated in some way by an elimination mechanism in oogenesis. Meiosis of *E. punctipennis* is highly anomalous. The two autosomes pair but separate again in early prophase I, then one homologue becomes heterochromatic and is apparently rejected from the late prophase nucleus. A single, equational maturation division follows. In female meiosis I, both species show highly characteristic diplotene figures with multiple chiasmata, the B-chromosomes remaining unpaired. These results are discussed in relation to previous work on aphid cytogenetics.

Introduction

Euceraphis are common aphids associated with birch trees (*Betula* species). Shinji (1927, 1931) was first to study a member of this genus cytologically, and reported a multiple sex chromosome system which appears to be unique

among the Aphididae, although found in related groups. Shinji's published observations relate only to the germ cells of a North American species, which he refers to as *E. betulae* Kalt. He reported that spermatogonia had four autosomes and four X-chromosomes, but his description of events in spermatocyte meiosis is difficult to follow, especially as he later lists the number of autosomal bivalents in primary spermatocytes also as four. Kuznetsova and Shaposhnikov (1973) identified Shinji's species as *E. punctipennis* (Zetterstedt) and deduced a diploid (female) chromosome complement of 2n=12 on the basis of Shinji's results.

In Europe, birch aphids which have been synonymised under the name *E. punctipennis* belong in fact to two closely similar species (Blackman in prep.), one with 2n♀=10 (*Euceraphis betulae* Koch) and the other with 2n♀=8 (the true *Euceraphis punctipennis*). *E. betulae* is primarily associated with *Betula pendula* Roth, and *E. punctipennis* with *B. pubescens* Ehrh. These species are probably also both present in North America. This paper reports on the cytogenetics of both species in an attempt to clarify the sex chromosome system and to resolve discrepancies in the literature. An examination of the critical stages of spermatogenesis and oogenesis in aphids using modern squash techniques seems in any case to be long overdue.

Materials and Methods

E. betulae and *E. punctipennis* were collected from birch trees in southern England at all times of year. Alate parthenogenetic ovoviviparous females are found from March to October, giving birth to oviparae (sexual females) and males from September. Mature oviparae mate and lay their eggs on birch twigs in late October and November.

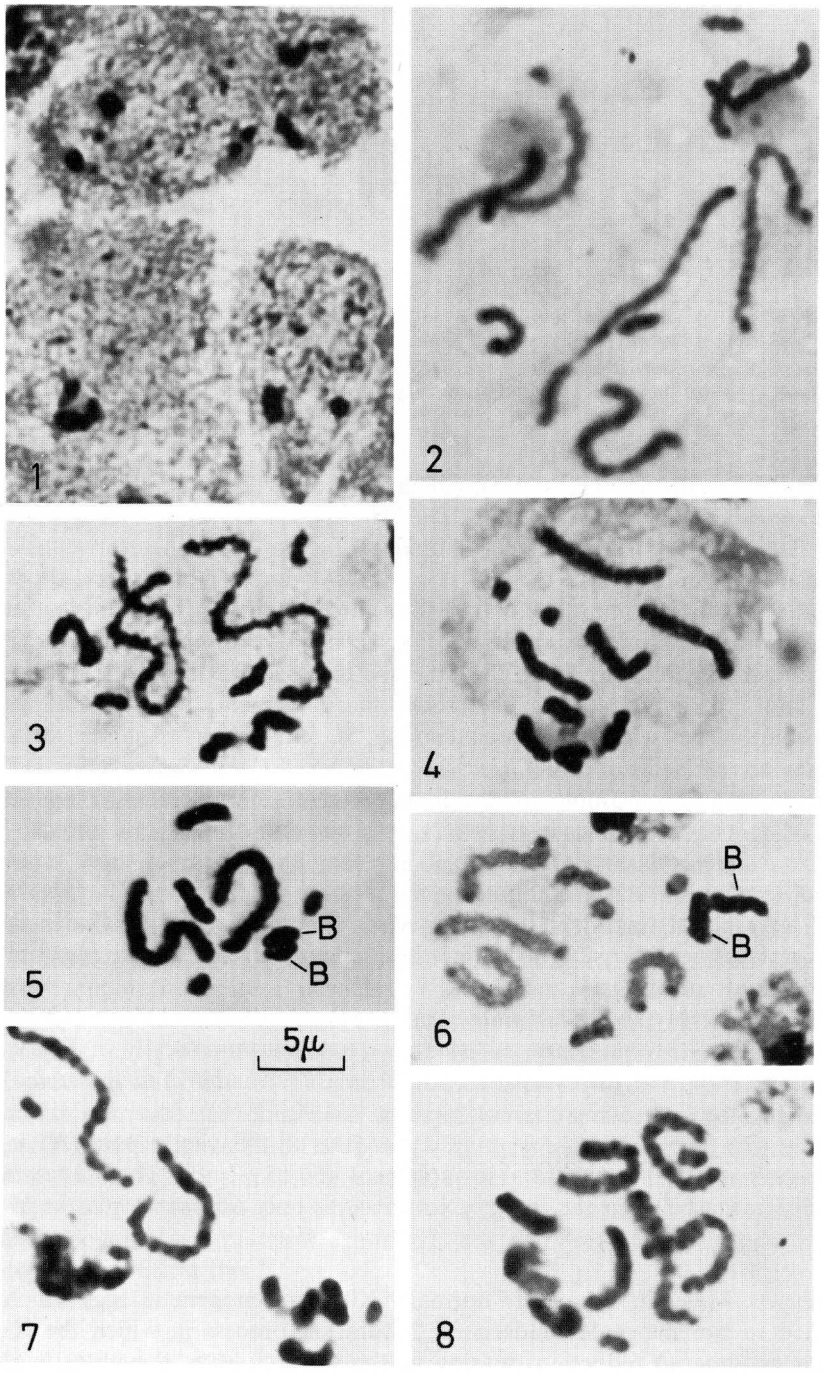
For studies of somatic cell divisions, embryos were dissected from immature or young adult parthenogenetic females in 0.75% potassium chloride solution, fixed in 3:1 methanol/acetic acid and squashed in a drop of 45% propionic acid on a clean microscope slide. After preliminary examination under phase contrast, a permanent preparation was made by freezing off the cover-slip and staining in 10% Giemsa in phosphate buffer at pH 6.8. In a few cases this method resulted in late prophase chromosomes having quite clear G-bands. Some preparations were C-banded using a modification of a technique suggested by Andrea K. Brown (personal communication); denaturation was for 2 min in 2×SSC at room temperature with pH adjusted to 12.0 with sodium hydroxide, and reassociation in 2×SSC at 65° for 2 h.

For studies of spermatogenesis, testes were dissected from second and third instar male nymphs and squash preparations made as described for the embryos. To study oogenesis, oviparae were fixed at various stages of development from second instar to young adult, some in 3:1 methanol/acetic acid and some in Duboscq-Brasil Bouin. The germaria of the former were dissected out and treated with normal hydrochloric acid for 5 min at 65° before squashing in 45% propionic acid and staining in Giemsa. Longitudinal sections 6 μ thick of the oviparae fixed in Bouin were stained with Heidenhain or Feulgen.

Results

Somatic Cell Divisions

Female Embryos. Interphase nuclei of somatic cells from female embryos of both species of *Euceraphis* have either one or two conspicuous chromocentres



Figs. 1–8. Somatic cells from female embryos. **1** Interphase nuclei of *E. betulae*; **2** Prophase of *E. betulae*; **3** Prophase of *E. punctipennis*; **4** Pro-metaphase of *E. betulae*; **5** Pro-metaphase of *E. punctipennis*; **6** C-banded cell of *E. betulae*; **7** G-banded cell of *E. punctipennis*; **8** G-banded cell of *E. betulae*

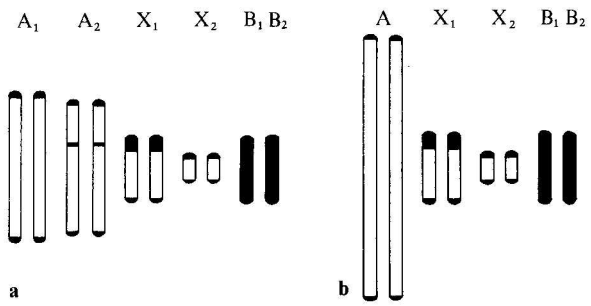


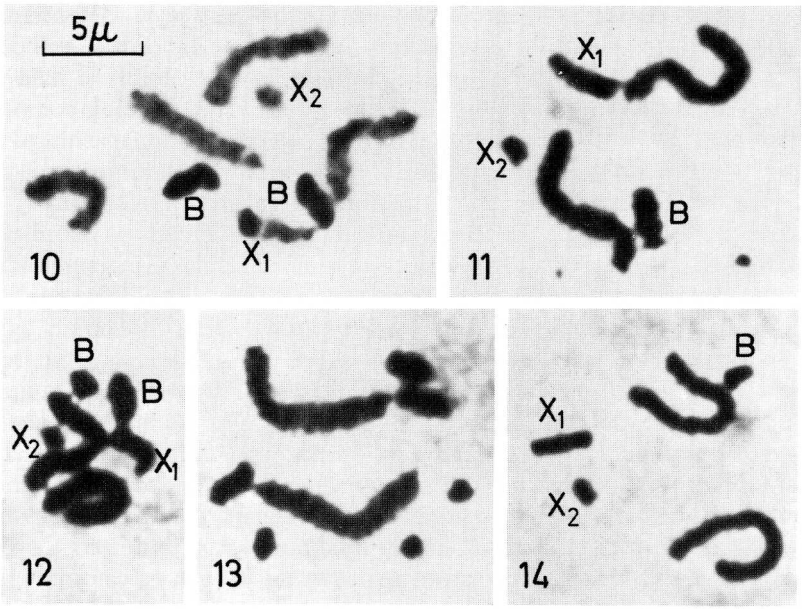
Fig. 9a and b. Karyotype diagram of **a** *E. betulae* and **b** *E. punctipennis*, with notation used for individual chromosomes. Relative lengths of chromosomes are represented to scale, based on measurements of 20 cells of each species. Distribution of constitutive heterochromatin, so far as it is known, is shown in black. The interstitial band on A_2 of *E. betulae* was only discernible at diplotene in oocytes (Figs. 41 and 42)

(Fig. 1). During prophase (Figs. 2–5), 10 chromosomes become apparent in *E. betulae*, and eight in *E. punctipennis*. The six shortest chromosomes appear to be similar in both species. They all tend to be positively heteropycnotic in prophase cells, and appear to consist of three pairs, one pair very short and the other two of medium length. The four chromosomes of medium length frequently show a nucleolar association (Fig. 4), but two of them stain more intensely and more uniformly than the others. For reasons which will become apparent later, these two strongly heterochromatic elements are regarded as B-chromosomes. They are often associated (Fig. 5), and even in C-banded cells (Fig. 6) they are stained uniformly.

The remaining chromosomes of the female somatic complement comprise two pairs of long chromosomes in *E. betulae*, and one pair of very long chromosomes in *E. punctipennis*. In C-banded cells the long chromosomes show small amounts of terminal heterochromatin (Fig. 6). In some preparations which were not given any special treatment they showed a complex pattern of G-bands, but it has not yet been possible to define these adequately for the comparison of the two karyotypes (Figs. 7 and 8).

The female karyotypes of *E. betulae* and *E. punctipennis*, and the notation used for individual chromosomes, are summarized diagrammatically in Fig. 9. The sum of the lengths of the two pairs of autosomes (A_1 und A_2) in *E. betulae*, relative to the total chromosome complement (64.90 ± 1.68), does not differ significantly ($P < 0.05$) from the summated lengths of the single pair (A) in *E. punctipennis* relative to the total complement (61.55 ± 1.68). This suggests that the difference between the two species involves only autosome rearrangement.

Male Embryos. Males in aphids are normally X0. Parthenogenetic eggs which are destined to become males undergo a maturation process in which the X-chromosomes undergo reduction division at the same time as the autosomes divide equationally (Schwartz, 1932). Male embryos were found in alate parthenogenetic females of both species of *Euceraphis* in September. Somatic cells from male *E. betulae* have eight chromosomes (Fig. 10). By comparison with the female karyotype (Fig. 4) it is evident that one of the medium length pairs



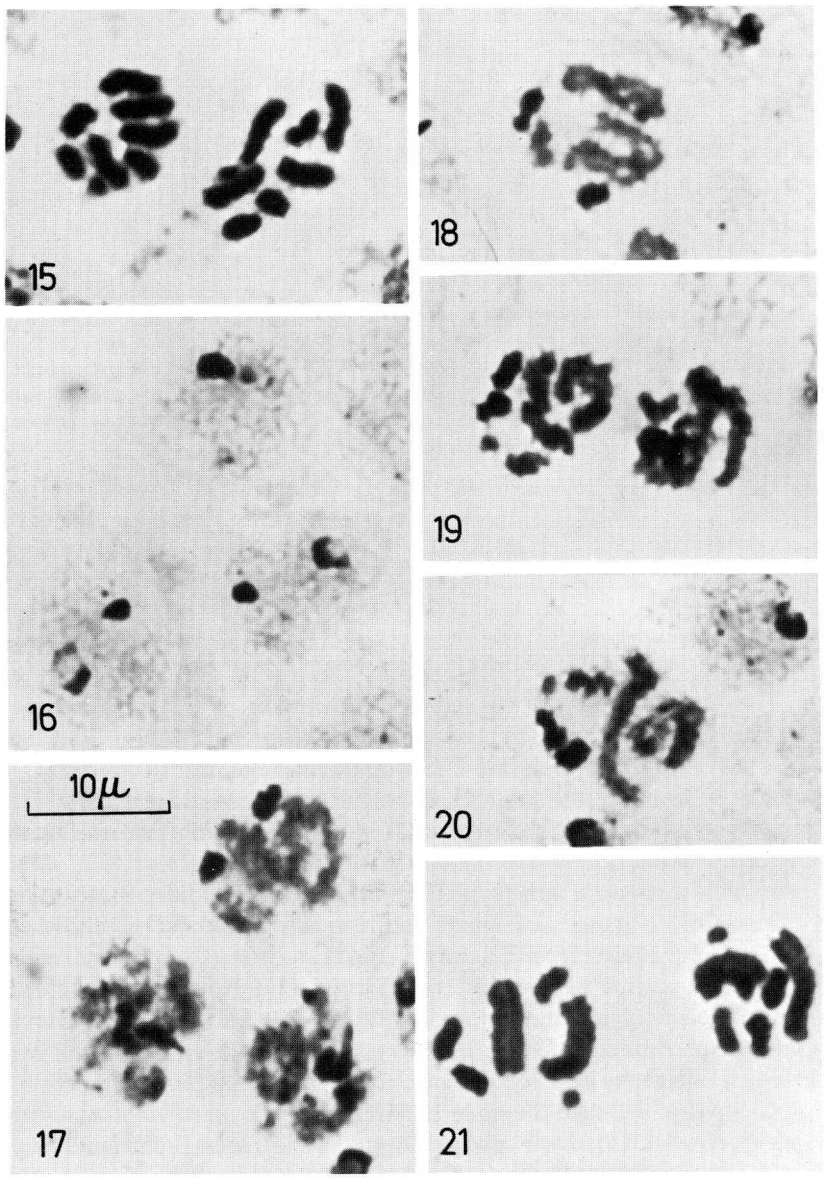
Figs. 10–14. Somatic pro-metaphase cells from male embryos. **10** *E. betulae* (2n=8); **11** *E. punctipennis* (2n=5); **12** *E. punctipennis* (2n=6), with short B-chromosome; **13** Female cell of *E. punctipennis* with a short B-chromosome; **14** Male *E. punctipennis* (2n=5) with a short B-chromosome

(X_1) and the short (X_2) have undergone reduction, whereas both the strongly heterochromatic B-chromosomes are still present in male somatic cells.

In *E. punctipennis,* however, male embryos were found with two different chromosome complements; they were either 2n=5 (Fig. 11) or 2n=6 (Fig. 12). By comparison with the female karyotype (Fig. 5), 2n=5 males have undergone reduction of X_1 and X_2 chromosome pairs, and have also lost a B-chromosome. The male with a 2n=6 constitution illustrated (Fig. 12) differs from the normal. It appears on first inspection to have undergone a reduction of the two medium length pairs, as two very short elements are still present. However, the parent of these males also contained female embryos with an abnormal karyotype (Fig. 13), in which one of the B-chromosomes has apparently suffered a deletion. Therefore in this particular case the male karyotype has B_1 and B_2 of unequal length. Male embryos with 2n=5, but with the single, shortened B-chromosome, were found in the same female (Fig. 14, cf. Fig. 11). The numbers of male embryos of *E. punctipennis* with one and two B-chromosomes appeared to be about equal in the material examined, but a more intensive study would be needed to confirm this.

Spermatogenesis in Euceraphis betulae

Spermatogonial stages mainly occur in late embryonic development, but can still be found in second instar male nymphs. Spermatogonial metaphase plates show the male karyotype of *E. betulae* very clearly (Fig. 15).



Figs. 15–21. Spermatogenesis in *E. betulae*. **15** Spermatogonial metaphase; **16** Three interphase nuclei; **17–21** Successive stages in prophase I of spermatocyte meiosis

The interphase prior to the first maturation division is characterized by an almost complete absence of stain in the greater part of the nucleus, except for one or sometimes two very sharply defined heterochromatic bodies which are evidently the associated or separate B-chromosomes (Fig. 16). The early stages of meiosis I are not very clear, but as condensation proceeds, separate elements become discernible, particularly the two B-chromosomes (Fig. 17), and

it becomes clear that homologous autosomes are paired (Fig. 18). Relational coiling is evident and there is sometimes a partial separation of homologues which is suggestive of diplotene, but as the autosomes condense further, the homologues become closely associated along their entire length (Figs. 19–22). No chiasmata were observed in an examination of over 1,000 cells in first meiotic prophase.

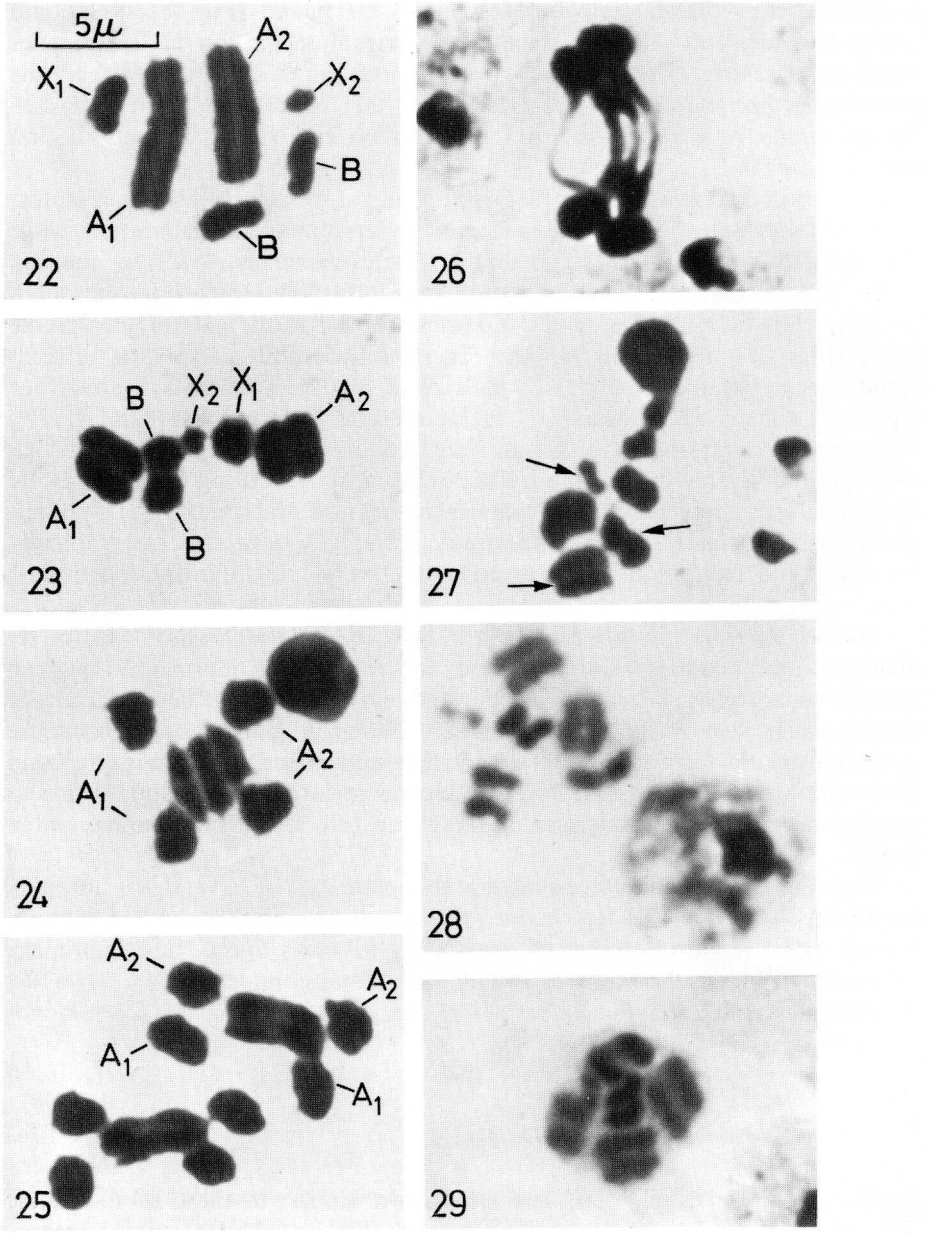
The autosomal bivalents contract greatly, and at metaphase I they are almost as broad as long (Fig. 23). The homologues undergo anaphase separation, while the unpaired X-chromosomes and the two B-chromosomes remain on the axis of the spindle (Fig. 24). Then, as homologous autosomes reach opposite poles, the characteristic stretching of the X-chromosomes on the axis of the spindle begins (Figs. 25 and 26). This has been described by various authors for different aphid species. In most anaphase I cells of *E. betulae* it is evident that four separate elements are stretched on the spindle, as observed by Shinji (1927). Thus the B-chromosomes, although they did not undergo reduction during maturation of the male egg, behave like X-chromosomes during the first meiotic division of spermatogenesis. The X-chromosomes and B-chromosomes eventually all pass into one secondary spermatocyte (Fig. 27). At this stage already the chromatid separation which precedes the second meiotic division can be seen.

The secondary spermatocyte which fails to receive the X-chromosomes remains as a rounded heterochromatic body for a short time and then disappears. There is an interphase in which relic coils and one or more large heterochromatic masses can be seen in the nucleus (Fig. 28). When the chromosomes condense again, chromatid separation is very evident in autosomes, X-chromosomes and B-chromosomes (Figs. 28 and 29). The second meiotic division thus seems to proceed in an equational manner, although no cells in second anaphase were found.

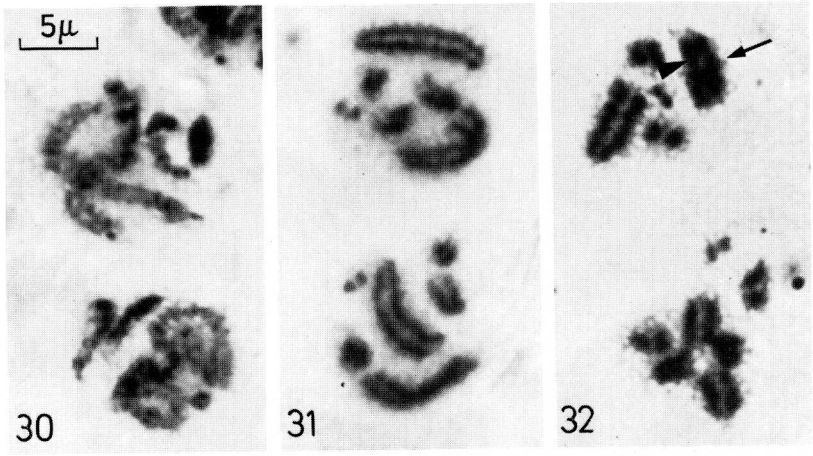
Thus the chromosome complement of the sperm cells of *E. betulae* is normally $n=6\,(=A_1A_2+X_1X_2+B_1B_2)$. In one preparation a group of secondary spermatocytes with five chromosomes ($A_1A_2+X_1X_2+B$) was found, but as primary spermatocytes from the same testis had the normal complement with two Bs, this appears to have been due to loss of a B-chromosome at an early spermatogonial division.

Spermatogenesis in Euceraphis punctipennis

Spermatogonial divisions of *E. punctipennis* are similar to those of *E. betulae*. The chromosome number is five or six, according to whether one or two B-chromosomes are present. The early prophase stages of meiosis are not clear, but when separate elements do become discernible a difference from *E. betulae* is immediately apparent; the two autosomes are unpaired (Fig. 30). In mid-prophase, chromatid separation is evident in all the chromosomes (Fig. 31). An examination of over 500 prophase cells revealed just one with a single, autosomal chiasma (Fig. 32). Although this was clearly an exceptional case, its occurrence nevertheless suggests strongly that the autosomes pair in early



Figs. 22–29. Spermatogenesis in *E. betulae*. **22** Late prophase I spermatocyte showing parallel association of homologues in the two autosomal bivalents; **23** Metaphase I; **24–25** Anaphase I showing separation of autosome homologues and stretching of X- and B-chromosomes; **26** Anaphase I with X_1, X_2 and the two B-chromosomes fully stretched between the secondary spermatocytes; **27** End of anaphase I with X- and B-chromosomes passing into the larger secondary spermatocyte. Note that chromatid separation is already occurring (arrows); **28 and 29** Interphase and prometaphase of the second meiotic division



Figs. 30–32. Spermatogenesis in *E. punctipennis*. Successive stages in meiotic prophase. **30** Two spermatocytes from 2n=5 male; **31** Two spermatocytes from 2n=6 male; **32** Two spermatocytes from 2n=5 male, the lower one with a single autosomal chiasma (an exceptional case). Note that one autosome (arrowed) is already becoming positively heteropycnotic

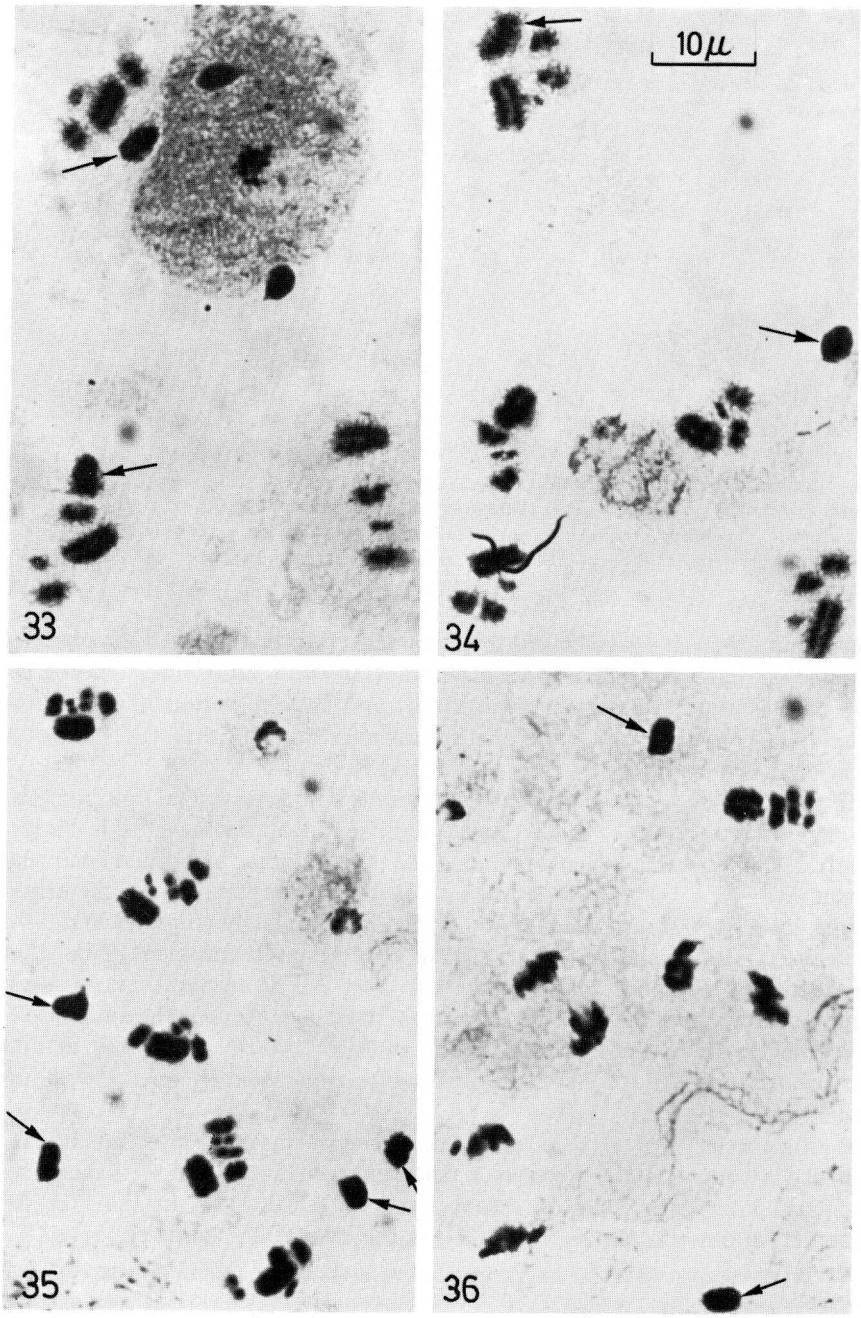
prophase, but separate again before their condensation has reached an observable stage. The complete separation of the homologous autosomes (except in the one case where a chiasma had occurred) and the separation of the sister chromatids of both autosomes and X-chromosomes during prophase I, are notably different from events at the same stage in *E. betulae*.

Before condensation of chromatin is complete, it is noticeable that one member of the pair of autosomes in each cell is changing in appearance. It becomes positively heteropycnotic, the chromatids close together and the outline becomes more rounded (Fig. 33). Adjacent to nuclei with this apparently degenerating autosome are others which have apparently lost it completely (Figs. 33 and 34). At metaphase, all spermatocyte nuclei have only a single autosome (Fig. 35). In the vicinity of metaphase plates, however, there are heterochromatic masses of about the same size as the missing autosomes. In *E. punctipennis*, the anaphase I figures which seem to be so characteristic of aphids, were not found at all. Reduction of the autosome number from two to one evidently occurs without a cell division.

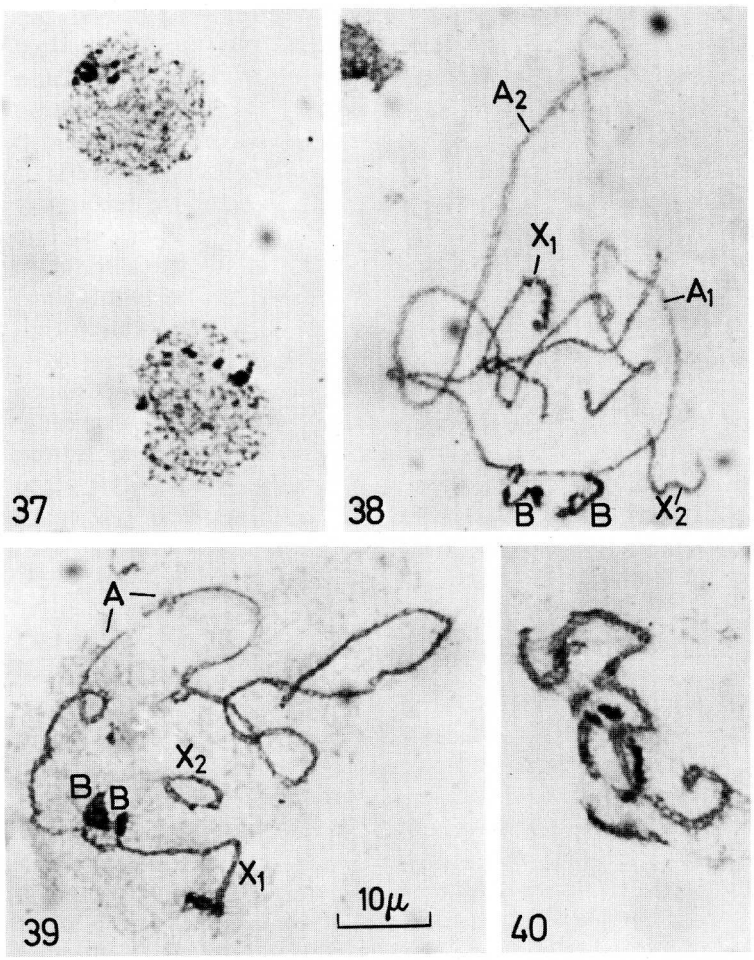
The single maturation division of *E. punctipennis* spermatocytes, which can be regarded a equivalent to the second maturation division of *E. betulae*, then occurs (Fig. 36). Sperm cells thus have n=4 ($A+X_1X_2+B$) in the case of 2n=5 males, and n=5 ($A+X_1X_2+B_1B_2$) in the case of 2n=6 males.

Oogenesis

Only the stages prior to vitellogenesis could be studied in squash preparations, as in spite of pretreatment the yolk seriously interfered with squashing. Oogonial divisions are mainly completed during embryonic development. The early stages

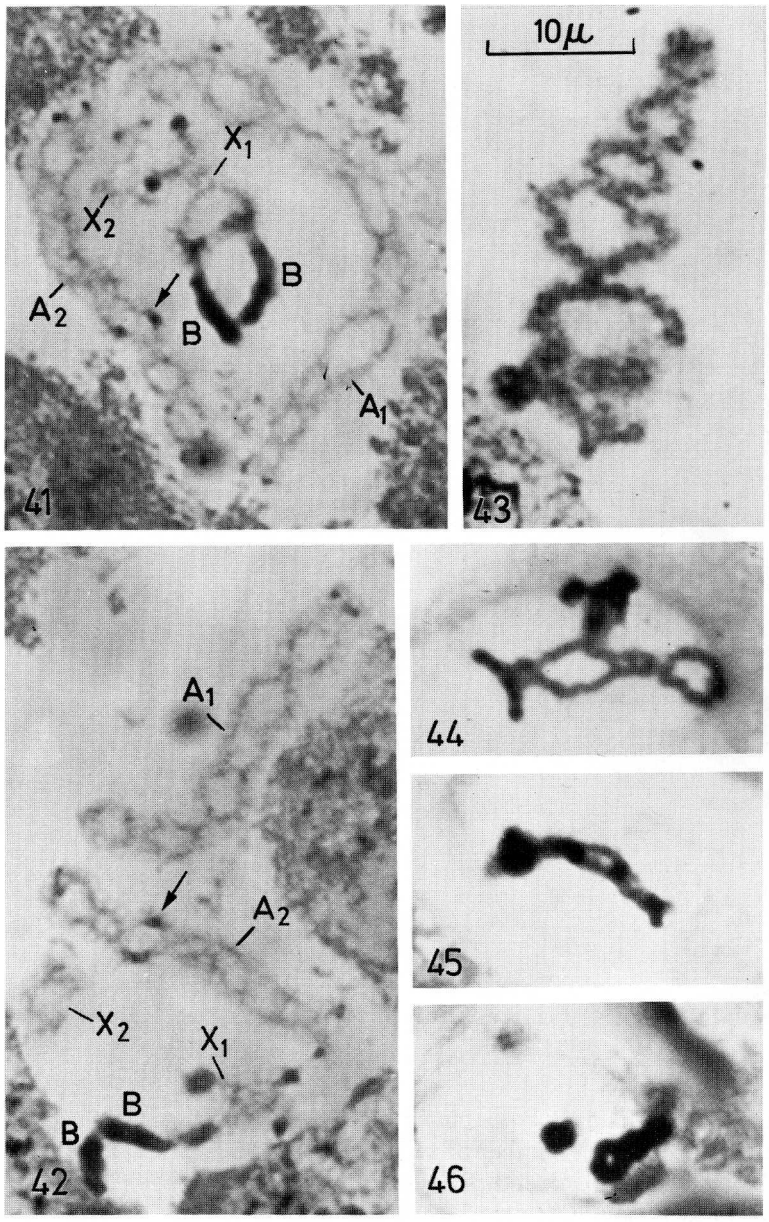


Figs. 33–36. Spermatogenesis in *E. punctipennis*. **33** Three spermatocytes in meiotic pro-metaphase from the testis of a 2n=5 male, two of which have heterochromatinized autosomes (arrowed); **34** Five spermatocytes in meiotic pro-metaphase from the testis of a 2n=5 male, only one of which still has a heterochromatinized autosome (arrowed); the other four have only one autosome (n=4); **35** Five spermatocytes in metaphase from a 2n=6 male. All have the reduced autosome number (n=5); **36** Metaphase and anaphase of meiosis in testes of a 2n=6 male. Note the heterochromatinized autosomes (arrowed)



Figs. 37–40. Prophase I of oogenesis in *Euceraphis*. **37** Leptotene nuclei of *E. betulae*; **38** Zygotene-pachytene in *E. betulae*; **39** Zygotene-pachytene in *E. punctipennis*; **40** Pachytene in *E. punctipennis*

of prophase I may be found in oviparae of the first to third larval instars. In leptotene nuclei (Fig. 37), a number of separate heterochromatic bodies may be visible, although they are sometimes associated. In zygotene-pachytene, the two B-chromosomes remain unpaired, while the paired X_1 has a positively heteropycnotic region in both species (Figs. 38–40). In diplotene, the chromosomes adopt a very characteristic configuration (Figs. 41 and 42). The paired autosomes have multiple chiasmata. Some of the apparent "crossovers" in early diplotene could be due to relational coiling, but nevertheless five or more definite chiasmata are common in the autosomal bivalents. The telomeres are heterochromatic, and in *E. betulae* there is also a short heterochromatic segment about a third of the way along each half-bivalent of A_2 (arrowed). The X_1 bivalent exhibits two or more chiasmata in its euchromatic part, but at one



Figs. 41–46. Oogenesis in *Euceraphis*. **41 and 42** Diplotene in *E. betulae* (squash preparations, stained with Giemsa); **43 and 44** Late diplotene in *E. punctipennis* (squash preparations, stained with Giemsa); **45 and 46** Autosomal bivalents in late diplotene and diakinesis (*E. punctipennis* – sections, stained with Heidenhain's haematoxylin)

end of each half-bivalent there is a extensive heterochromatic region. The X_2 bivalent seems to be entirely euchromatic at this stage. The two B-chromosomes, which have remained unpaired but close together throughout early prophase, usually associate with the heterochromatic region of the X_1 bivalent, or with each other.

Terminalisation of the chiasmata in the autosomal bivalents at first appears to proceed towards one end (Figs. 43 and 44). However, some oocytes in late diplotene had loops at both ends. The individual elements of the X-chromosome/B-chromosome complex clump together with increased condensation and become difficult to make out. At late diplotene-diakinesis the growth phase of the oocyte starts, and no later stages could be observed in squash preparations.

In sections of fourth instar oviparae, oocytes in late diplotene and diakinesis could be seen in the germaria (Figs. 45 and 46), along with the large, polyploid nurse-cells. As the growth phase starts, the bivalents enter a highly contracted, prometaphase state, in which they almost certainly remain until the fertilization of the fully-grown oocyte. The chromatin in the nucleus of the growing oocyte stains very densely with Heidenhain, and is usually clumped together in one part of the nucleus. The "clumping" is, however, likely to be an artefact.

Discussion

The species studied by Shinji (1927, 1931) was evidently *E. betulae* (Koch). Many of his figures agree closely with those in the present work. On the basis of observations of spermatogenesis he concluded, quite reasonably, that this species has a $X_1X_2X_3X_40$ system of sex determination. Although he refers to some observations on somatic cells, these were apparently not published, and he nowhere states the diploid female number of chromosomes. Had he done so, a discrepancy would have been apparent, as the female karyotype is $2n=10$, not the $2n=12$ expected if four X-chromosome pairs were present. In fact, only two chromosome pairs undergo reduction to produce the male karyotype, so the system is conventionally defined as X_1X_20. The other two heterochromatic elements, which Shinji believed were X-chromosomes, behave in most respects as B-chromosomes. Their size and general appearance in somatic cells, however, are very similar to that of the longer pair of X-chromosomes, and at anaphase I they are stretched on the spindle in an identical manner to the Xs. All the evidence strongly suggests that they are B-chromosomes of X-chromosomal origin, as is thought to be the case with the larger type of B-chromosomes found in certain grasshoppers (Hewitt, 1973).

The two B-chromosomes of *E. betulae* appear to be usually stable in mitotic divisions. The passage of both B-chromosomes into the sperm nucleus without reduction constitutes an accumulation mechanism, yet 37 populations of *E. betulae* sampled in southern England all had two B-chromosomes. The number must therefore be stabilized by some form of elimination mechanism. The B-chromosomes of the scale insect *Pseudococcus obscurus* accumulate in spermatogenesis in a similar way to those of *E. betulae*, but in oogenesis show some tendency to be eliminated by preferential segregation into polar body II

(Nur, 1962). In *P. obscurus*, the number of B-chromosomes is variable. It seems possible that the B-chromosome system in *Euceraphis* is relatively ancient, and that the accumulation-elimination mechanism has been perfected to give a stable number of B-chromosomes. Studies of the later stages of oogenesis will be necessary to clarify the nature of the elimination mechanism, but the most probable alternatives seem to involve segregation of both B-chromosomes into either polar body I or polar body II.

In *E. punctipennis*, the X- and B-chromosomes appear to be essentially similar to those of *E. betulae*, except that reduction of the B-chromosomes sometimes occurs in male determination. Males of *E. punctipennis* and the sperm they produce can thus have either one or two B-chromosomes. From what is known of the behavior of B-chromosomes in other species, it is quite possible that they show a tendency to lag with the X-chromosomes during the maturation division of the male egg, and thus may be liable to reduction in number. Until this can be studied further it seems preferable to regard the sex determination mechanism of *E. punctipennis* as X_1X_20, in spite of the fact that males may have three chromosomes less than females. The B-chromosomes clearly cannot play any vital part in the sex determination system. It is difficult to understand how an elimination mechanism in oocyte meiosis can compensate for the variation in B-chromosome number of the sperm in such a way as to provide progeny invariably with two B-chromosomes. However, the only *E. punctipennis* so far examined have all been from one locality (Royal Botanical Gardens, Kew, Surrey), and one year (1975). Further sampling may reveal variations in B-chromosome number within this species.

The aberrant form of spermatogenesis found in *E. punctipennis* was unexpected, as all aphids previously studied have shown a highly characteristic form of anaphase I with stretching of the X-chromosome(s) on the spindle, as in *E. betulae*. In *E. punctipennis*, one homologue of the single autosome pair is eliminated from the germ line in a remarkable fashion, during the prophase of what then becomes a simple, equational division. The only situation which seems at all comparable is the Comstockiella system found in the scale insects, where heterochromatic chromosomes degenerate and are lost during spermatogenesis, which is then reduced to a single maturation division (Brown, 1963). In the Comstockiella system, however, one haploid chromosome set becomes heterochromatic during the early cleavage divisions of the male embryo, and the elimination of this whole set takes place in two different stages of meiosis: (1) in a "preprophase" stage, when some elements are apparently eliminated by intranuclear destruction (Kitchin, 1970, 1975), and (2) by a post-telophase ejection of the remaining heterochromosomes. In *E. punctipennis*, the elimination takes place during prophase and is clearly visible, and heterochromatinization is more-or-less coincident with loss or ejection of the autosome from the nucleus of the spermatocyte.

It is remarkable that two closely related aphids should have such different forms of spermatogenesis. However, in several coccid species forms of spermatogenesis with two (lecanoid) and one (Comstockiella) maturation divisions may occur within the same individual. The apparent uniformity of spermatogenesis in aphids which is suggested by all previous work could be due to the small

number of species which have been studied in any detail. In fact, spermatogenesis has been described for only 14 species of Aphidoidea, and several sub-families of the Aphididae (Anoeciinae, Hormaphidinae, Greenideinae, Chaitophorinae, Pterocommatinae) have not been examined at all.

The latest and most detailed account of spermatogenesis in an aphid is that of Ris (1942), who studied *Tamalia coweni*, which is in the same sub-family as *Euceraphis*. Working with sectioned material, Ris was unable to observe the details of the early prophase stages of meiosis I, but described and figured bivalents in diakinesis, some of which appear to show chiasmata. Basing his conclusions on what was known of spermatogenesis in other groups related to the aphids, Ris assumed that bivalents emerging from the "diffuse stage" of early prophase each consisted of two chromosomes joined end-to-end as the result of terminilization or opening out of one chiasma. As evidence for this interpretation he cites the changes in relative length which occur between the autosomal bivalents and the unpaired X-chromosome in the course of meiosis. The data he gives, however, do not indicate a large enough difference to be attributable to an end-to-end alignment of the autosomes.

In neither *Euceraphis* species could any evidence be found to support the hypothesis that the bivalents entering metaphase I of spermatocyte meiosis consist of homologues aligned end-to-end. Figure 22, for instance, clearly indicates that the homologues are closely aligned in parallel in the autosomal bivalents. Throughout late prophase I and metaphase I the length of each of the autosomal bivalents of *E. betulae* is about twice that of the unpaired X_1 and B-chromosomes, which is about the same length relationship as in spermatogonial cells (Fig. 15) and in somatic metaphase plates (Fig. 4). As X-chromosomes, B-chromosomes and autosomes are allocyclic with respect to one another through most of spermatogenesis, a very close agreement between relative lengths at various stages cannot be expected.

No clear indications of diplotene crossing-over or of the terminalisation of chiasmata could be found in the earliest prophase I cells in which individual chromosomes were discernible. In *E. betulae*, prophase I seems to follow a relatively simple course, similar to that found in male *Drosophila* and other Diptera with an achiasmate spermatogenesis. The homologues pair in early prophase and remain aligned in parallel as they condense into metaphase, anaphase I separating the products of pairing and being strictly reductional. This contrasts with the situation in coccids with an achiasmate meiosis, where pairing only occurs secondarily at a later stage. In *E. punctipennis*, early prophase pairing evidently occurs, but the attraction between homologues must cease at an early stage, and they have normally separated by the time the individual chromosomes become discernible. Only one chiasma was found in an examination of numerous primary spermatocytes. This chiasma was completely clear and unambiguous, supporting the conclusion that in both species male meiosis is virtually achiasmate.

Oogenesis in *Euceraphis* also conflicts with previous accounts in one significant respect. In contrast to the situation in spermatogenesis, there is a clear diplotene stage in which multiple chiasmata are evident. Bivalents with three or more chiasmata do not seem to have been previously observed in Homoptera

(Halkka, 1964). *Euceraphis* is rather unusual in only having one or two pairs of autosomes; the commonest number of autosome pairs in aphids is 5 or 6. Presumably if the recombination index is similar for other aphids the number of chiasmata in each bivalent would be much lower in most of them.

It remains to be seen how far these conclusions apply to other aphids. Clearly, the X-chromosome and B-chromosome systems of *Euceraphis* are unusual, as is the anomalous form of spermatogenesis found in *E. punctipennis*. There has, however, been a tendency to overgeneralize about the cytogenetics of aphids from observations of a very few species, and further work may reveal a far greater variety of cytogenetical mechanisms in this economically important group of insects.

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