Chromosoma (Berl) (1988) 96:318–324

CHROMOSOMA


# Stability of a multiple X chromosome system and associated B chromosomes in birch aphids (*Euceraphis* spp.; Homoptera: Aphididae)

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**Abstract.** Autosomal dissociations are a common feature of aphid karyotype evolution, but multiple X chromosome systems are rare. Birch-feeding aphids of the genus *Euceraphis*, however, have $X_1X_2O$ males as a general rule, $X_1$ being always much larger than $X_2$. Only one species has XO males, and this condition appears to be secondary. Most *Euceraphis* karyotypes also have one or more, usually heterochromatic, elements that occur in the same numbers in both males and females, yet behave like X chromosomes at male and female meiosis I. They appear to be supernumerary, "non-functional" X chromosomes, although showing greater within-species stability in size and number than typical B chromosomes. *Euceraphis gillettei* forms a separate group within the genus and feeds on alders (*Alnus* species), yet has a similar system, and the two most closely related genera, *Symydobius* and *Clethrobius*, also have additional chromosomal elements possibly representing non-functional X chromosomes. Thus the multiple X chromosome system in these aphids seems to be a primitive condition.

## Introduction

In aphids, males are XO, and are usually produced in autumn. Autosomal dissociations are a common feature of aphid karyotype evolution (Blackman 1980), but multiple X chromosome systems are rare. *Euceraphis betulae* (Koch), a common aphid on the European silver birch, *Betula pendula* Roth, was originally reported to have $X_1X_2X_3X_4O$ sex determination (Shinji 1931), but Blackman (1976) showed that males of this species have only two chromosomes fewer than females and are therefore $X_1X_2O$ (Fig. 1). The other two elements, believed by Shinji to be X chromosomes, are heterochromatic and behave in most respects like B chromosomes. In female somatic cells of *E. betulae* these resemble the larger pair of X chromosomes in size and general appearance. Although they do not undergo reduction during the maturation of the male egg, they are both stretched on the anaphase I spindle at spermatogenesis in an identical manner to the X chromosomes, before passing with the Xs into one daughter spermatocyte. This strongly suggests that they are B chromosomes of X chromosomal origin, as is thought to be the case with the larger type of B chromosome found in certain grasshoppers (Hewitt 1973). However, unlike most B chromosomes they are consistently present, at least in British populations of *E. betulae*.

A second European species, *E. punctipennis* (Zetterstedt), feeds on downy birch, *B. pubescens* Ehrh., and has a different number of autosomes to *E. betulae*, but a similar X chromosome/B chromosome system (Fig. 1). In *E. punctipennis*, however, there is some variation in the size and number of B chromosomes, although at least one is always present (Blackman 1976).

Most other *Euceraphis* species occur in North America. This paper reports the karyotypes of the North American species and gives a preliminary account of their cytogenetics, paying particular attention to the system of sex determination.

## Material and methods

Karyotypes were analysed by examination of dividing cells of female embryos from parthenogenetic viviparous females in spring and summer populations and comparing these with (i) dividing somatic cells of male embryos from parthenogenetic viviparous females in autumn, and (ii) meiotic stages in immature males and sexual females (oviparae) in autumn. Aphids of all developmental stages were collected from the field and fixed directly in freshly mixed 3/1 methanol/acetic acid. For somatic cell preparations embryos were dissected from viviparous females in 75% methanol, transferred to 1 N hydrochloric acid (5 min), thence to distilled water, and then squashed under a coverslip in a drop of 45% propionic acid. Only the youngest embryos, from the upper parts of the ovarioles close to the germaria, were used. For germ cell preparations, testes and ovarioles were dissected from sexual morphs in their first or second instar.

Preparations were observed and photographed either by phase contrast without staining, or after staining with Giemsa (Gurr's R66).

## Results

### *Karyotypes*

Populations of *Euceraphis* on five native North American *Betula* species all have distinct karyotypes (Table 1, Fig. 1). Two are previously described species, *E. lineata* Baker on *B. populifolia* Marsh and *E. mucida* (Fitch) on *B. lenta* L. The other three are all very similar morphologically to the European species *E. betulae*, and share with it the same

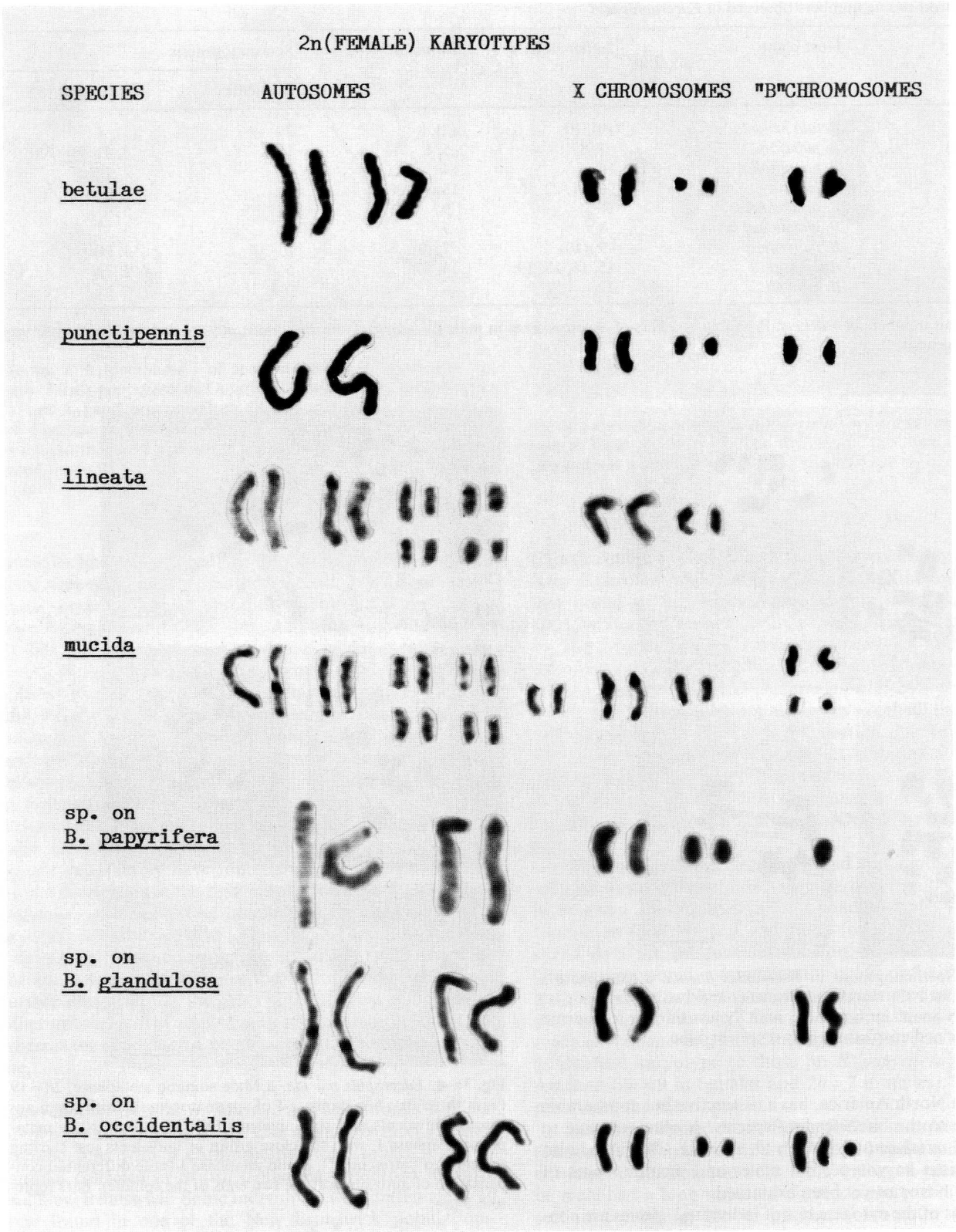


**Fig. 1.** The 2*n*(female) karyotypes of birch-feeding *Euceraphis* species

complement of two autosome pairs, differing only in the X chromosome/B chromosome system.

Aphids of the European *E. betulae* karyotype also occur in North America, but are restricted to European silver birch (*B. pendula*) planted as an ornamental in towns and gardens. Populations of *Euceraphis* on the dwarf birch of arctic Canada, *B. glandulosa*, have an identical karyotype to those from the closely related and possibly conspecific dwarf birch of northern Europe, *B. nana* L.

*E. gillettei* Davidson, which is widely distributed on *Al-*

**Table 1.** Chromosome numbers observed in *Euceraphis* spp

| Species | Host plant | 2*n*(female) | 2*n*(male) | Spermatogenesis | |
|---|---|---|---|---|---|
| | | | | Bivalents | Univalents |
| *betulae* | *Betula pendula* | (9), 10 | (7), 8 | 2 | (3), 4 |
| *punctipennis* | *B. pubescens* | 7, 8 | 5, 6 | 1 | 3, 4 |
| *lineata* | *B. populifolia* | 16 | 14 | 6 | 2 |
| *mucida* | *B. lenta* | 20, 21, 22 | 18, 19, 20 | 7 | 5, 6 |
| *sp. no.* 1 | *B. occidentalis* | 11 | 9? | 2? | 5? |
| *sp. no.* 2 | *B. glandulosa/nana* | 8 | 7 | 2 | 3 |
| *sp. no.* 3 | *B. papyrifera* | 9, (10) | 7, (8) | 2 | 3, (4) |
| *gillettei* | *Alnus* spp. | 15, 16, 18, 19 | 13, 17 | 5 | 3, 7 |
| *ontakensis* | *B. ermanii* | 22 | ? | ? | ? |

Less frequent numbers *in brackets*. Probable numbers of chromosomes in male *Euceraphis* from *B. occidentalis* are deduced from observations of oogenesis

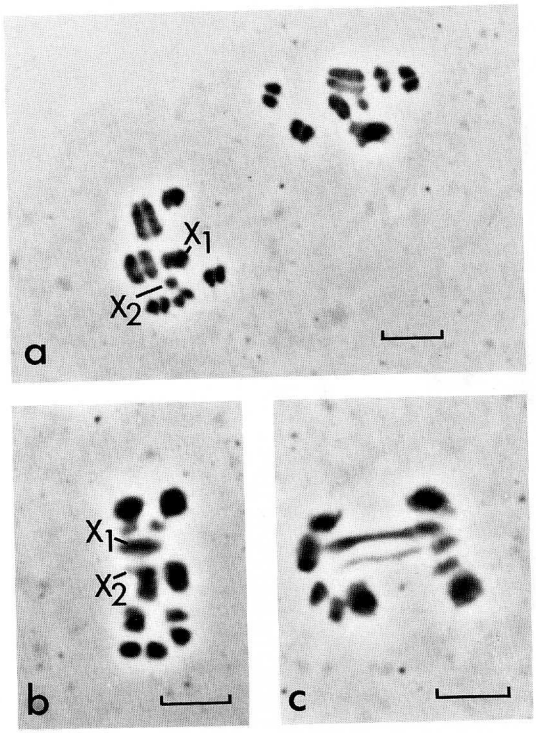


**Fig. 2a–c.** Spermatogenesis in *Euceraphis lineata*. **a** Metaphase I (two cells) with six autosomal bivalents and two univalents ($X_1$, $X_2$); **b** early anaphase I, with $X_1$ and $X_2$ just starting to undergo extension; **c** mid anaphase I. Bars represent 5 μm

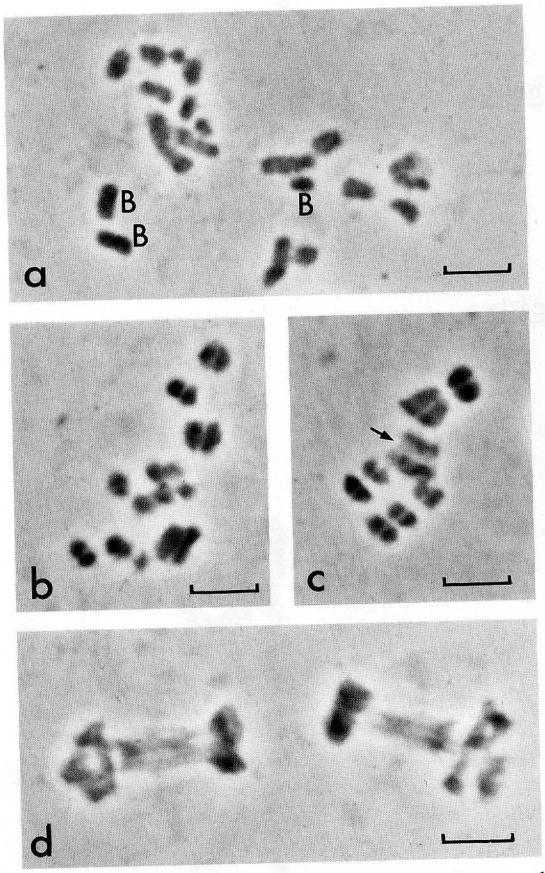


**Fig. 3a–d.** *Euceraphis mucida*. **a** Male somatic metaphase, $2n=19$ (with three Bs); **b** metaphase I of spermatogenesis, with seven autosomal bivalents and six univalents ($X_1$, $X_2$ + four Bs); **c** metaphase/anaphase I, with the close group of univalents just starting to undergo "stretching"; **d** mid anaphase I (note differential condensation of autosomes at the two ends of the spindle). Bars represent 5 μm

*nus* spp. in North America, has a distinctive but comparable karyotype to the birch-feeding species. A species native to Japan, *E. ontakensis* Sorin on *B. ermanii* Chamisso, also has a distinct karyotype, but males and meiotic stages of this aphid have not yet been examined.

Aspects of the cytogenetics of individual species are considered in more detail below.

*E. lineata*. Six populations of this species were examined, from *B. populifolia* in New York and Pennsylvania, USA. The karyotype was consistently 2*n*(female) = 16, and 2*n*(male) = 14, indicating $X_1X_1X_2X_2/X_1X_2O$ sex determination. At male metaphase I there were only two univalents (Fig. 2a, $X_1$ and $X_2$), positioned centrally on the equatorial plate. At anaphase I both these elements were stretched along the axis of the spindle (Fig. 2b, c) before passing into one of the daughter cells.

*E. mucida*. Ten populations of *E. mucida* were examined, from New York, Pennsylvania and Virginia, USA. Female

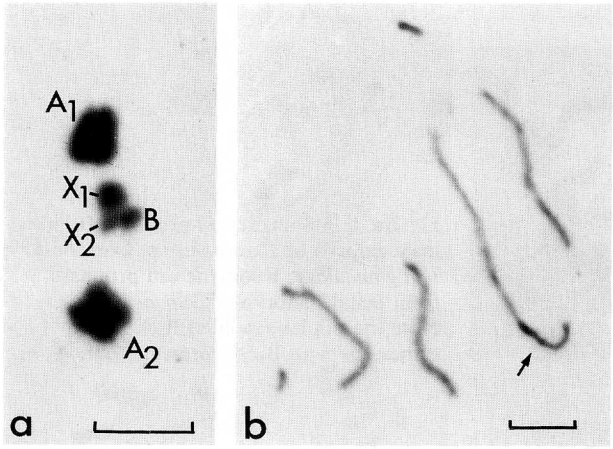


**Fig. 4a, b.** **a** Metaphase I of spermatogenesis of *Euceraphis* sp. from *Betula papyrifera* in Ontario, Canada, with two bivalents ($A_1$ and $A_2$) and three univalents ($X_1$, $X_2$ and a small B); **b** somatic cell prophase from male embryo of *Euceraphis* sp. from *B. papyrifera* in North West Territories, Canada. Note blocks of heterochromatin on long, unpaired chromosome (*arrowed*). Bar represents 5 μm

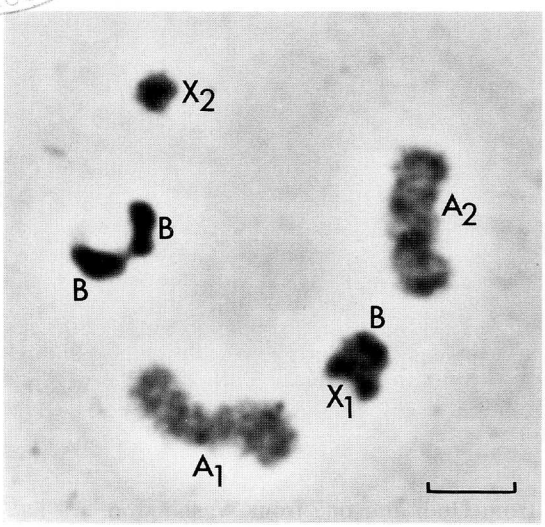


**Fig. 5.** Late diplotene oocyte of *Euceraphis* sp. from *Betula occidentalis* in Utah, USA, $n=7$. The smallest of the three condensed univalents is associated with the $X_1$ bivalent. Bar represents 5 μm

embryos had either 20, 21 or 22 chromosomes in somatic cells, depending on whether there were 2, 3 or 4 heterochromatic elements. The 21 chromosome form was most common, being found in 7 samples. At 2 sites, individuals with 21 and 22 chromosomes were found in the same populations. Somatic cells from male embryos had two chromosomes fewer than those of corresponding female karyotypes (e.g. Fig. 3a), indicating that the sex determination system was again $X_1X_2O$. Male metaphase I plates consistently had seven autosomal bivalents around a central group of univalents of varying number (Fig. 3b). Males containing metaphases with five and six univalents ($X_1X_2$ plus four B chromosomes) were found, presumably corresponding to male somatic karyotypes of $2n=19$ and $2n=20$. Males with $2n=18$ presumably have four univalents in meiosis I, but were not collected at the right stage to observe their spermatogenesis. At the start of anaphase I the univalents were grouped closely together (Fig. 3c). They all undergo "stretching" on the anaphase I spindle. At mid anaphase the autosomes at one pole of the spindle had decondensed further than those at the other (Fig. 3d). By analogy with other aphids, all the univalents probably pass to this pole, whereas the condensed autosomes at the other pole degenerate.

*Euceraphis species on B. papyrifera.* Populations on *B. papyrifera* were sampled in six Canadian provinces. Female somatic cells with 9 chromosomes were found in all 17 samples collected in British Columbia, Manitoba, Ontario and New Brunswick. Some individuals with $2n$(female)$=10$ were found in one of the New Brunswick populations. Males collected in Ontario had $2n=7$ in somatic cells, and were therefore $X_1X_2O$. Their karyotype was thus similar to that of European *E. betulae* on *B. pendula*, except that there was only one small B, similar in size to $X_2$. This B grouped with the two X univalents at metaphase I of the male meiosis (Fig. 4a), and passed into the same secondary spermatocyte as the X chromosomes. *E. betulae* populations in Europe and introduced into other parts of the world (North America, New Zealand) almost invariably had two large B chromosomes similar in size to $X_1$, the only exception found so far being in one sample from California, USA, which had some individuals with a single large B.

The $2n=10$ females from New Brunswick appeared to have two small B chromosomes, but no males were available to confirm this. However, males with $2n=8$ were obtained from a Manitoba population in which all the females karyotyped had $2n=9$. Testis preparations had four univalents (one large and three small) at metaphase I. Either these males had originated from undetected $2n=10$ females, or the males only had one chromosome fewer than the females, i.e. sex determination was XX/XO in this population.

Viviparous females with $2n=9$ and apparently of identical karyotype to those on *B. papyrifera* were collected from three other *Betula* species in Canada: *populifolia* (four times), *cordifolia* (once) and *lutea* (once). However, this aphid does not produce sexuales on *B. populifolia* (F.W. Quednau, personal communication), and probably its sexual generations in North America are restricted to *B. papyrifera*. In Japan, however, *Euceraphis* populations on *B. platyphylla* var. *japonica*, including sexuales, have an apparently identical karyotype to those on *B. papyrifera* in North America ($2n=9$ in females and $2n=7$ in males). They appear to be the same species.

Samples collected from *B. papyrifera* in arctic Canada (North West Territories and the Yukon) had a quite different karyotype. Somatic cells from embryos presumed to be male had a long unpaired chromosome with a terminal section containing blocks of heterochromatin (Fig. 4b). Possibly this karyotype could have arisen from the $2n$(male)$=7$ form by translocation of $X_1$ onto an autosome, but the female karyotype was not definitely identified, and examination of this and meiotic stages are needed for reliable interpretation.

*Euceraphis sp. on B. occidentalis.* Seven *Euceraphis* populations were sampled from this western North American

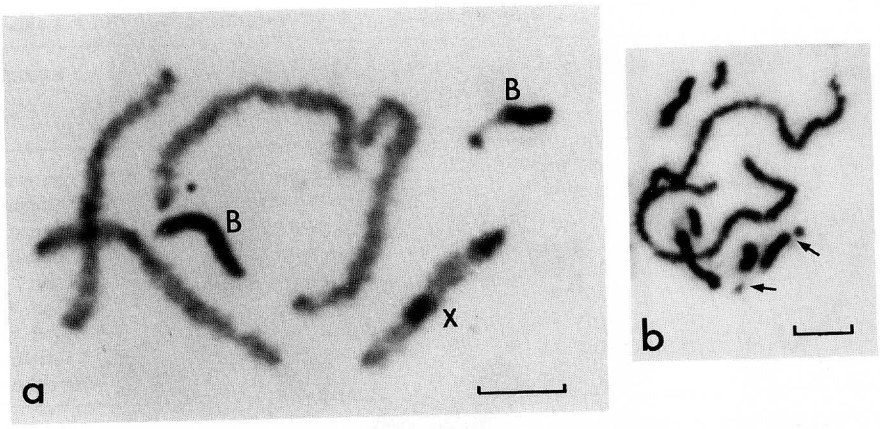


**Fig. 6a, b.** **a** Somatic cell prophase from male embryo of *Euceraphis* sp. from *Betula glandulosa;* **b** somatic cell prophase from female embryo of *E. punctipennis*. Note the similar satellited B chromosomes (*arrows*). Bars represent 5 μm

birch, six from Utah and one from Washington. All had $2n$(female) = 11. No males were available for examination, but one immature ovipara was obtained that provided good preparations of late diplotene and diakinesis (Fig. 5). From these it was clear that there were four bivalents ($A_1$, $A_2$, $X_1$ and $X_2$), plus three condensed univalents of different sizes which were often associated either with each other or with the $X_1$ bivalent. Males are therefore presumably $2n = 9$, and $X_1X_2O$. Although the number of samples was small, aphids of this karyotype seem to be uniquely associated with *B. occidentalis*, and F.W. Quednau (personal communication) has found morphological discriminants from other aphids of the *E. betulae* group indicating that populations on *B. occidentalis* should be regarded as a separate species.

*Euceraphis sp. on B. glandulosa/nana.* Four populations on *B. glandulosa* from different parts of subarctic Canada (Yukon, North West Territories, northern Manitoba, northern Quebec) had an identical karyotype to a population on *B. nana* in Finland. Females had $2n = 8$ with two *betulae*-like autosome pairs. Males had $2n = 7$, indicating XX/XO sex determination. The X chromosome had an interstitial block of C-heterochromatin (Fig. 6a), and was larger than the $X_1$ of *E. betulae*, which had a terminal block of heterochromatin (Blackman 1976). There is thus a strong possibility that it has arisen by fusion between $X_1$ and $X_2$ of other members of the *betulae* group. One of the B chromosomes of this aphid, in both Canada and Finland, had a sub-terminal constriction separating off a small satellite. The B chromosomes of *E. punctipennis* in England sometimes have a similar satellite (Fig. 6b).

*E. gillettei.* Twenty-two populations of *Euceraphis* on *Alnus* species were sampled from different parts of North America. The number of chromosomes in somatic cells of female embryos was either 15, 16, 18 or 19 (Table 2). There was no clear association between karyotype and host plant, the $2n = 16$ form in particular being widely distributed throughout western USA and across Canada from British Columbia to New Brunswick, on several different *Alnus* species. The other karyotypes seem on present evidence to be more restricted in their distribution; the 18 and 19 chromosome forms occur in British Columbia on *Alnus rubra* (where the $2n = 16$ form also occurs on this host plant), and the 15 chromosome form is found on *A. rugosa* in Ontario.

**Table 2.** Karyotype variation in the *Euceraphis gillettei* group

| Host plant | Number of samples with $2n$(female) = | | | | |
|---|---|---|---|---|---|
| | 15 | 16 | 17 | 18 | 19 |
| *Alnus rhombifolia* Nutt. | – | 5 | – | – | – |
| *A rubra* Bong. | – | 7 | – | 4 | 2 |
| *A. rugosa* (Du Roi) Koch | 3 | 2 | – | – | – |
| *A. tenuifolia* Nutt. | – | 1(?) | – | – | – |

Stages of male meiosis were seen in a population of the 15 chromosome form from Ontario, and in populations of the 19 chromosome form from British Columbia. At prophase I in males from the Ontario population there were five bivalents and three univalents (Fig. 7a). Thus males must be $X_1X_2O$, with a single small B chromosome. At anaphase I, each secondary spermatocyte nucleus initially received five elements, while the three univalents were stretched along the axis of the spindle (Fig. 7b) before passing into the one functional daughter spermatocyte, which thus had eight chromosomes in its nucleus at metaphase II (Fig. 7c).

Males from the British Columbia population had five autosomal bivalents at prometaphase I, like those from Ontario, but in this case there were seven univalents, comprising $X_1$, $X_2$ and five B chromosomes (Fig. 7d). Some prophase I oocytes were also observed in preparations of ovarioles of second instar oviparae from this population; at early diplotene the five B chromosomes were condensed, separate bodies, but at late diplotene they were aggregated into a single dense mass.

Somatic cell prophases of the 16 chromosome form of *E. gillettei* had two small heterochromosomes, possibly both Bs, although examination of meiotic stages would be necessary to confirm this.

*Aphid species in related genera*

Species in the two genera most closely related to *Euceraphis*, which also feed on Betulaceae, show similar chromosomal anomalies.

Gut (1976) recorded $2n$(female) = 16 for *Symydobius oblongus* (von Heyden) in the Netherlands. However, eight samples examined by the present author (six from England, one from Sweden and one from Czechoslovakia) all had

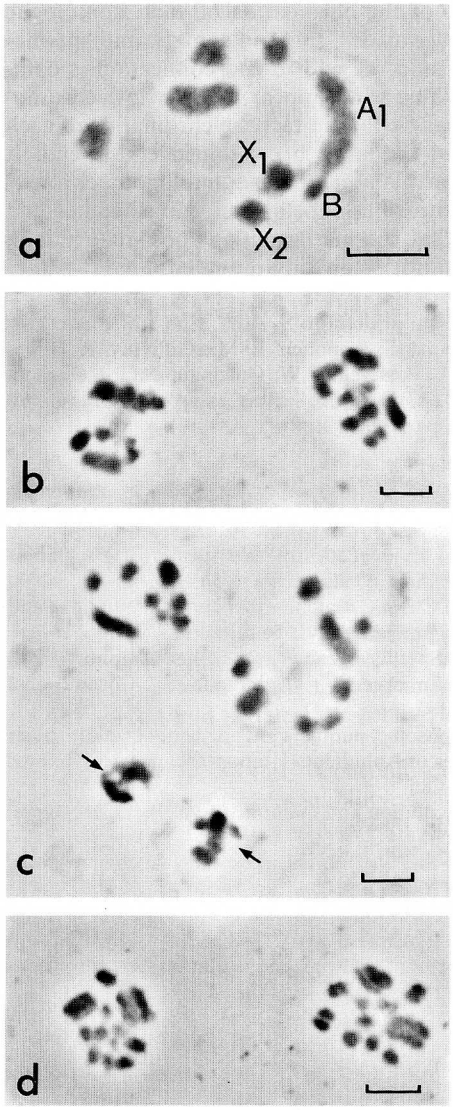


**Fig. 7a–d.** Spermatogenesis in *Euceraphis gillettei*. **a** Late prophase I of 15 chromosome form from Ontario, Canada; **b** metaphase I (2 cells); **c** 2 secondary spermatocytes at prometaphase II (the small *arrowed* nuclei lack Xs and Bs and are destined to degenerate); **d** metaphase I of 19 chromosome form from British Columbia, with 5 bivalents and 7 univalents. Bars represent 5 μm

$2n$(female) = 15. Males (2 samples) were XO with 14 chromosomes in spermatogonial metaphases (Fig. 8a), but metaphase I plates had 6 bivalents and 2 univalents (Fig. 8b), 1 of which must therefore have been a "non-functional" X or B. At anaphase I two elements, the X and the B, were stretched on the spindle (Fig. 8c).

Seven samples of *Clethrobius comes* (Walker) were examined, from *Betula* spp. in Japan (one sample), England (one sample) and Ireland (three samples), and from *A. incana* in Finland (two samples). In each case only somatic cells from female embryos were available, and these invariably had 11 chromosomes. Populations on *Alnus* are regarded by many authorities as a distinct species, *C. giganteus* (Cholodkovsky), yet their karyotype was indistinguishable from that of the *Betula*-feeding aphids. Interpretation of the karyotype in this genus must await study of the sexual generation.

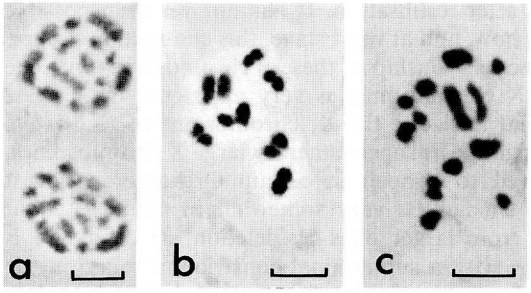


**Fig. 8a–c.** Spermatogenesis in *Symydobius oblongus*. **a** Two spermatogonial cells at metaphase, $2n = 14$; **b** metaphase I with 6 bivalents and 2 univalents (X, B); **c** anaphase I, with two lagging chromosomes. Bars represent 5 μm

## Discussion

Multiple X chromosome systems are rare in aphids, and occur only sporadically in other organisms, often being confined to single species within a genus. The mantids are one notable exception, with $X_1X_2Y$ sex determination in 24 genera, most of which probably form a monophyletic group (White 1973). The $X_1X_2Y$ condition of male mantids is believed to arise from the primitive XO system by translocation between an X and an autosome. Male aphids can never be $X_1X_2Y$ (or XY), because the Y chromosome would have "nowhere to go" in the unisexual spring and summer generations.

Multiple X systems in aphids presumably arise from XO systems by dissociation. For *Euceraphis* this dissociation appears to be a primitive character. The species on *B. nana/glandulosa* with XO males is unlikely to show the primitive condition because it is closely related to *E. betulae* with $X_1X_2O$; the XO system in this species has probably arisen by a secondary fusion of $X_1$ with $X_2$.

The most notable feature of the *Euceraphis* sex determination system is the almost invariable presence of chromosomal elements which, although they are present in unreduced number in the male karyotype, nevertheless behave like X chromosomes at male meiosis. All species except *E. lineata* have at least one of these additional chromosomes, and similar elements are identifiable in related genera. Jones and Rees (1982) emphasise that it is the dispensable nature of B chromosomes that is their chief distinguishing characteristic. Whether the term "B chromosomes" should be used for elements that form such a stable component of the karyotype is therefore open to question. However, the dispensability of such chromosomes once they have arisen must be a matter of degree. B chromosomes are not necessarily neutral or deleterious in effect and may, under certain circumstances, confer advantages. Selection for stability of B chromosome number might then occur, for example, by the development of a balanced system of accumulation and elimination.

In *Euceraphis*, there appears to be just such a system, although the details are not yet understood. As all B chromosomes pass with the X chromosomes into one secondary spermatocyte, they have the potential to accumulate in the next generation. In order to provide a stable number of Bs in every generation, there must be a very efficient mechanism of elimination, either of the maternal set of B chromosomes during oogenesis, or possibly of the paternal set at

some stage after fertilisation. It has not yet been possible to establish how, and at what stage, this elimination occurs.

The close relationship of these B chromosomes with the functional X chromosomes is very apparent. The largest Bs are similar in size to the $X_1$ chromosome of *E. betulae*, and may originate as supernumerary large X chromosomes, as is believed to happen in certain Orthoptera (Hewitt 1973). The smaller Bs vary somewhat in size and could be derived from larger ones by deletion, as could the B chromosome with a subterminal constriction found in the species on *B. glandulosa/nana* and some populations of *E. punctipennis*. However, it is possible that the $X_2$ might not always undergo reduction in the male, i.e., it might cease to function as an X chromosome and become a small B. This appeared to be what had happened in males of a $2n$(female)$=9$ population of *Euceraphis* sp. on *B. papyrifera* in Manitoba. It was not possible to karyotype the actual mothers of these males, however, and an alternative explanation, that the mother had an extra small B chromosome as in New Brunswick populations, cannot be discounted.

There is still doubt about whether spermatogenesis in aphids is typically pre-reductional (i.e. normal) or post-reductional (i.e. inverse, as in many other Hemiptera, including their sister group, the scale insects). When spermatogenesis was examined in European *E. betulae*, no evidence could be found to support post-reductional meiosis: the autosomal homologues simply seemed to be aligned in parallel during late prophase I and metaphase I, and then to move apart at anaphase I (Blackman 1976). However, studies have since been made of spermatogenesis in an aphid in another subfamily, *Amphorophora tuberculata* Brown and Blackman, in which it is possible to observe events more clearly and at an earlier stage of prophase I (Blackman 1985). In this species it was shown that the single pair of autosomes undergo end-to-end alignment in early prophase, and remain connected end-to-end through anaphase I, separation of the homologues not occurring until anaphase II, i.e., meiosis *is* post-reductional. The appearance of the chromosomes at metaphase I is deceptive, because the DNA of the chromatids of each homologue is packed about twice as densely as in somatic and spermatogonial divisions, and the end-to-end connection is completely invisible. Thus, what appear to be whole chromosomes aligned in parallel are in fact the chromatids of each of the homologues, the latter being joined end-to-end. It seems likely that in *Euceraphis* the homologous chromosomes are also, contrary to appearances, joined end-to-end in meiosis I, and that spermatogenesis is post-reductional, although this could not be directly observed.

*Acknowledgements.* I am grateful to S. Aoki, Cho-Kai Chan, V.F. Eastop, O. Heikenheimo, S. Koponen, E. MacGillivray, R. O'Doherty, J.O. Pepper, A. Polaszek, F.W. Quednau, A.G. Robinson, A. Sutton and H. Takada for collecting samples of *Euceraphis* and other aphids for cytological study.

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Received December 10, 1987
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