Acta Phytopathologica et Entomologica Hungarica,
25 /1-4/, pp. 273-282 /1990/

THE CHROMOSOMES OF LACHNIDAE

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ABSTRACT

Some features of the cytogenetics and cytotaxonomy of Lachnidae are discussed, with particular attention to differences in chromosome number between closely related species, the system of sex determination and the behaviour of the X chromosomes during spermatogenesis, and the occurrence and distribution of "B chromosomes" and constitutive heterochromatin.

INTRODUCTION

Karyotypes of more than 60 species of Lachnidae are known, of which more than 40 are Cinara species. Despite the generally large size of the aphids in this family, their chromosomes are often rather small and difficult to resolve. This is especially so in the case of Lachninae and Traminae, which mostly do not provide well-spread metaphase preparations. Nevertheless, lachnids show a number of features of cytogenetic and cytotaxonomic interest, which are described in this paper.

MATERIALS AND METHODS

Satisfactory squash preparations of somatic cell nuclei can generally be obtained from young embryos dissected out of aphids fixed directly in 3 parts methanol: 1 part glacial acetic acid, and kept for several months

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in this fixative, provided that the embryos are hydrolysed with 1N hydrochloric acid for 5 min at 65 C prior to squashing in 45% propionic acid (for details see Blackman, 1980a).

However, with Lachninae (notably Lachnus and Stomaphis) and Traminae, pretreatment of living tissue in a mildly hypotonic solution (e.g. 0.75% potassium chloride, or 1% sodium citrate) for 5-10 min prior to fixation is generally necessary in order to prevent late prophase and metaphase chromosomes from clumping together. Freshly-fixed material (left only 15-30 min in methanol/acetic acid fixative) can be squashed directly in propionic acid without prior treatment in hydrochloric acid, although acid hydrolysis will give a cleaner preparation with less cytoplasmic background.

Squashes made with freshly-fixed material can be "C-banded" to reveal constitutive heterochromatin (sections consisting mainly of highly repetitive DNA sequences). The method involves denaturation of DNA with strong alkali, followed by incubation in a saline solution that selectively renatures highly repetitive sequences (for details see Blackman, 1985).

Spermatogenesis occurs in the testes of 1st and 2nd instar males, and is virtually complete by the 3rd instar. Squash preparations of testis tissue can be made in the same way as for embryonic cells.

RESULTS AND DISCUSSION

Lachninae Somatic cell chromosomes of Stomaphis and Lachnus are particularly difficult to resolve, which is a pity because their karyotypes are potentially useful as taxonomic characters. Most European populations of Stomaphis quercus (L.) examined, collected from both Quercus and Betula,have a 2n(female)=10 karyotype like that of S. japonicus Takahashi (Fig. 1b). A sample from Quercus petraea in Czechoslovakia, however, had 2n=8 (Fig. 1a), lacking the the two smallest chromosomes. By analogy with

S. japonicus (see below), the missing elements are probably X chromosomes, although X chromosome numbers are usually very stable in aphids. S. cupressi (Pintera) is very different with 2n=14 (Fig. 1c), and S. yanonis Takahashi has 2n=?16 (but Honda, 1921, recorded a haploid number of 10 for yanonis).

Karyotype	Provenance of samples
2n= 7?	Q. robur, W. Germany (2 samples)
2n= 8 (7+1B)	Q. cerris, Czechoslovakia; Q. robur, W. Germany (2)
2n= 9 (7+2B)	Q. robur, Czechoslovakia, Denmark, Poland
2n=10	Castanea sativa, Portugal, ?UK
2n=11 (10+1B)	Q. robur, Sweden (1), UK (4)
2n=12?	Q. borealis, Portugal
2n=14	Castanea sativa, ?UK; Q. robur, UK
2n=15 (13+2B?)	Q. pyrenaica, Q. suber; both Portugal
2n=16	Q. ilex, Portugal (2)
2n=17?	Q. ilex, Portugal

Table 1 Karyotype variation in the Lachnus roboris group (23 samples) (uncertain karyotype determinations are indicated by "?")

Lachnus roboris (L.) (Fig. 1d-f) shows great variation in chromosome number, some of which may be intraspecific and due to variable numbers of accessory heterochromosomes ("B chromosomes"). Table 1 summarises the available data and shows the difficulty of demonstrating any particular association between karyotype and host plant. A more intensive study, integrating karyotypic data with biological, morphometric and possibly enzyme/DNA studies, is needed to clarify the taxonomy of these aphids. L. tropicalis (van der Goot) in Japan and China shows similar variability (2n=12, 13 and 16 in 6 samples). One sample of L. iliciphilus (del Guercio) from West Germany had 2n=8.

In both S. quercus and L. roboris, males have two chromosomes less than females, so sex determination is $X_1X_1X_2X_2/X_1X_20$. In S. japonicus, X_2 is much smaller than X_1 (Fig. 1g), whereas in the L. roboris group X_1 and X_2 are more similar in size (Fig. 1h). "B chromosomes" behave like X chromosomes on the anaphase I spindle, although their number is unreduced in the male.

Maculolachnus submacula (Walker) yields much better preparations than other Lachninae studied. There are 10 chromosomes in female somatic cells; C-banding reveals interstitial blocks of constitutive heterochromatin on all the longer chromosomes (Fig. 1i). Male somatic cells have 9 chromosomes (Fig. 1j), so sex determination is XX/XO. The X chromosome is quite short, and there are two small "B chromosomes", one of which has a constriction (arrowed in Fig. 1i).

Tuberolachnus salignus (Gmelin) has "2n=20" in all populations so far examined (from India, Iran, Japan and the UK) (Fig. 1k). The X chromosomes, if they still exist in this permanently parthenogenetic aphid, are unidentifiable. Pterochloroides persicae (Cholodkovsky) also has 2n=20.

Spermatogonial divisions in the testes of 1st instar males show 9 chromosomes (Fig. 2a), the two "B chromosomes" appearing as dots. In prophase I and metaphase I of spermatogenesis (Figs 2b,c), the autosomes are paired to form bivalents, but the two "B"s are unpaired and situated close to the unpaired X chromosome. At anaphase I, the X and the two "B"s are stretched along the axis of the spindle as the autosomes move to the poles (Fig. 2d-j). Late anaphase (Fig. 2k) shows considerable attenuation of the X (and B) chromosomes, before they all pass into one of the daughter cells. At this stage the autosomes in the nucleus of the daughter cell that will eventually receive the X and the "B"s are already decondensed, whereas those in the other nucleus are still strongly condensed. Only the

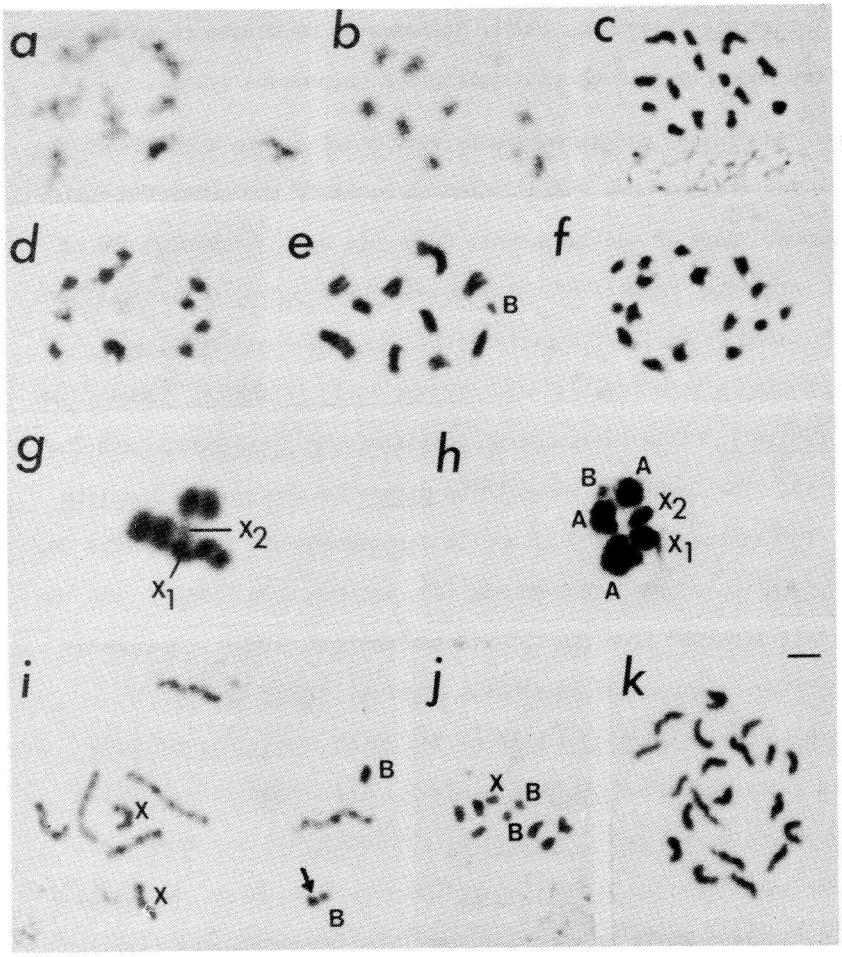

Figure 1.- a-b Somatic chromosomes of Stomaphis; **a**, S. quercus group (2n female=8); **b**, S. japonicus (2n=10); **c**, S. cupressi (2n=14). **d-f** Somatic chromosomes of Lachnus; **d**, L. roboris group from Castanea (2n=10); **e**, L. roboris group from Quercus robur (2n=10 + 1B); **f**, L. roboris group from Quercus ilex (2n=16). **g**, metaphase I of spermatogenesis in Stomaphis japonicus. **h**, metaphase I of spermatogenesis in L. roboris (2n=10 + 1B). **i**, C-banded somatic prophase of Maculolachnus submacula (2n female = 8 + 2B). **j**, male somatic metaphase of M. submacula (2n=7 + 2B). **k**, somatic metaphase of Tuberolachnus salignus (2n female = 20). Scale bar = 2μm.

daughter nucleus that receives the X and the "B"s is viable and divides again (Fig. 2l), and the resultant nuclei form spermatids (Fig. 2m).

This type of X chromosome/B chromosome system, with the "B"s being maintained in a constant number and inherited through the male, is like that found in Euceraphis (Blackman, 1988). It seems to be unique to Aphididae. The "B"s are possibly relicts of a multiple X chromosome system.

Cinarinae In Cinara, the karyotype is very stable; more than 70% of the species so far studied have 2n=10, which is probably the primitive number for the genus. Some of the deviations from this basic karyotype are of taxonomic interest. Members of the subgenus Cupressobium Börner all have karyotypes based on 2n=12, the permanently parthenogenetic C. fresai Blanchard having a structurally heterozygous 2n=13 (Blackman, 1980a). In subgenus Cinarella Hille Ris Lambers, aphids of the pinea group have 2n=8, 10, 11 or 14. The 2n=8 form seems to be pilosa Zetterstedt (= maculata Gmelin). Populations with 10, 11 and 14 chromosomes are found in the UK. The 2n=10 form is recorded from Canada (Sun and Robinson, 1966), and the 2n=14 form is recorded from the USSR (Rukavishnikov, 1979). Presumably these are hitherto unrecognised sibling species. Other species of Cinarella have 2n=14 (pergandei Wilson) and 2n=16 (maritimae Dufour). The pilicornis group (subgenus Cinaropsis Börner) also shows taxonomically useful karyotype variation; C. piceicola (Cholodkovsky) has 2n=8, and populations identified as C. pilicornis (Hartig) from UK and New Zealand have 2n=10, whereas 2n=14 is recorded from USSR (Rukavishnikov, 1972).

Spermatogenesis is relatively straightforward in Cinara (Fig. 2n-w). In C. pini (L.), sex determination is XX/XO, the X chromosomes being longer than any autosomes, and there are no "B"s (Fig. 2n). In anaphase I, the X chromosome is not stretched to the same extent as in Lachnini (Fig. 2p-s; cf. Fig 2d-k). During the short telophase between the meiosis I and II,

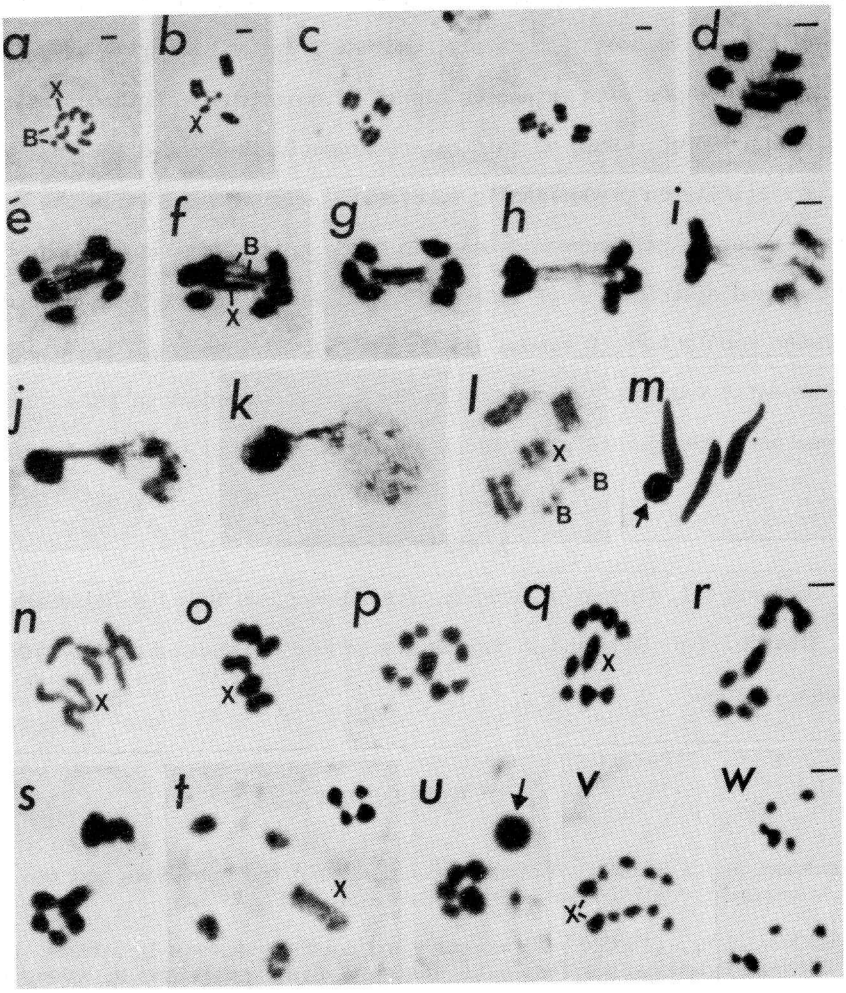

Figure 2.- a-m Spermatogenesis in Maculolachnus submacula; **a**, spermatogonial metaphase (2n=7 + 2B); **b**, prometaphase I; **c**, metaphase I; **d-k**, anaphase I (daughter nucleus destined to receive X and B chromosomes is to the right); **l**, metaphase II secondary spermatocyte, about to enter anaphase II: **m**, spermatids, start of elongate of sperm tails (round mass arrowed is a degenerating spermatocyte II without X or "B" chromosomes). **n-w** Spermatogenesis in Cinara pini; **n**, male somatic prophase, 2n=9; **o**, metaphase I; **p-s**, anaphase I; **t**, telophase (degenerating nucleus without X at top right); **u**, metaphase II (degenerating nucleus arrowed); **v-w**; anaphase II. Scale bars = 2 μm.

the chromosomes of the secondary spermatocyte with the X only partially decondense (Fig. 2t).

Traminae No functional sexual phase is known in Traminae, and there are no identifiable X chromosomes. Protrama have many small chromosomes that are difficult to count accurately, whereas Trama and Neotrama have fewer, large chromosomes with extensive blocks of constitutive heterochromatin (Blackman 1980a). Some of this heterochromatin is located terminally or interstitially on predominantly euchromatic chromosomes, while the rest forms separate heterochromosomes. In Trama troglodytes von Heyden, the extent and distribution of heterochromatin varies greatly both within and between populations (Blackman, 1980b and Fig. 3), but there are always 10 euchromatic sections (which contain the coding sequences of DNA). Two of these are often joined via a block of heterochromatin (Fig. 3c,d,g,h).

ACKNOWLEDGEMENTS

I thank C.I. Carter, A. Czylok, V.F. Eastop, O. Heie, U. Heimbach, F. Ilharco, J.H. Sudd and H. Takada for providing samples of Lachnidae for these studies.

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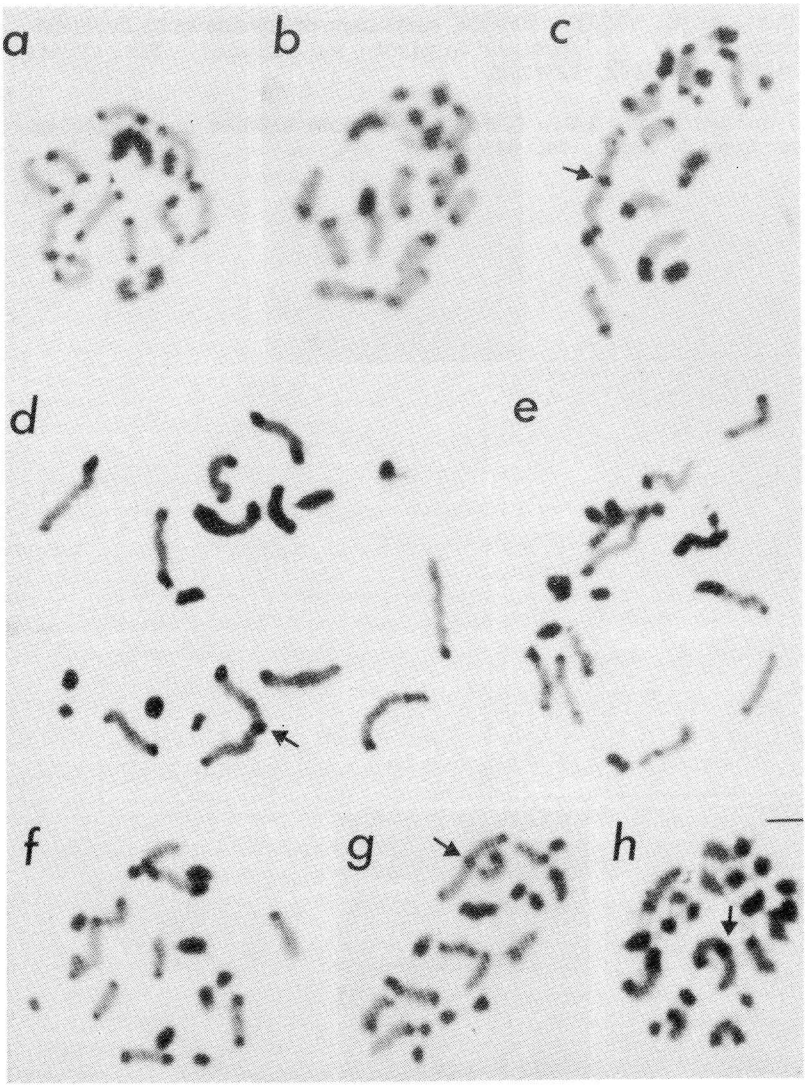

Figure 3. - Variable distribution of constitutive heterochromatin in Trama troglodytes in populations from S.E. England, revealed by C-banding. All karyotypes have 10 euchromatic sections, but in some two of these are joined by a block of heterochromatin (arrowed). **a**, 2n=14, from Artemisia vulgaris; **b**, 2n=16, from Sonchus oleraceus; **c**, 2n=16 (with fusion) from Cirsium arvense; **d**, 2n=18 (fusion), from C. arvense; **e**, 2n=17, from C. arvense; **f**, 2n=19, from Artemisia; **g**, 2n=20 (fusion), from C. arvense; **h**, 2n=22 (fusion) from Artemisia. Scale bar = 2 µm.

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