Karyotype variation in the South American aphid genus *Neuquenaphis* (Hemiptera, Aphididae, Neuquenaphidinae)

ROGER L. BLACKMAN[1], PAUL A. BROWN[1], CLAUDIO C. RAMÍREZ[2] and HERMANN M. NIEMEYER[3]

[1] *Department of Entomology, The Natural History Museum, London, UK*
[2] *Centro de Investigación en Biotecnología Silvoagrícola, Talca, Chile*
[3] *Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Santiago, Chile*

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The endemic South American aphid genus *Neuquenaphis* (Hemiptera, Aphididae, Neuquenaphidinae) forms an important component of the phytophagous insect fauna associated with southern beeches, *Nothofagus* (Nothofagaceae), but has not previously been studied cytologically. As part of ongoing studies of the taxonomy, evolution and host relationships of this genus, the karyotypes of 12 species are described and illustrated. Species are mostly distinguishable by differences in number and/or relative lengths of chromosomes, with 2n (female) numbers ranging from 6 to 16. The taxonomic and evolutionary significance of the karyotype variation in this group are discussed.

Claudio C. Ramírez, Instituto de Biología Vegetal y Biotecnología, Universidad de Talca, Casilla 747, Talca, Chile. E-mail: clramirez@pehuenche.utalca.cl

Neuquenaphis Blanchard is an endemic South American aphid genus associated with southern beeches (*Nothofagus* spp.). It has taxonomic affinities with certain other southern hemisphere aphid genera, such as *Lizerius* in South America, *Paoliella* in southern Africa, and *Taiwanaphis* (including *Sensoriaphis*) in South East Asia and Australasia, but these groups have been placed in separate subfamilies (QUEDNAU and REMAUDIÈRE 1994). All retain certain primitive features in comparison with related groups (Drepanosiphinae, Chaitophorinae) in the northern hemisphere. *Neuquenaphis* species are an important element of the insect fauna associated with *Nothofagus* in temperate South America (MCQUILLAN 1993), and the biogeographical history of the *Nothofagus*-aphid-parasitoid association has received particular attention (SCHLINGER 1974).

Twelve species of *Neuquenaphis* have been described, and these have been placed in two subgenera, *Neuquenaphis* and *Spicaphis* (QUEDNAU and REMAUDIÈRE 1994). The taxonomy of the genus is complicated by the fact that the apterous and alate morphs differ considerably in morphology, and in some species only the alate morph is known, whereas in other species only the apterous morph is known (QUEDNAU and REMAUDIÈRE 1994).

To date, there have been no cytological studies of *Neuquenaphis*. Karyotype variation within aphid genera can sometimes be taxonomically useful in assigning morphologically divergent morphs to species, or as an indication of phylogenetic relationships (BLACKMAN 1980). Aphids are also a cytogenetically interesting group, because they have holocentric chromosomes (chromosomes that lack a localised centromere), and also because of peculiar features related to their cyclical parthenogenesis. One of these is their obligate XX/XO sex determination. For most of the year, aphid populations are comprised entirely of parthenogenetic (thelytokous) females. Therefore, no Y chromosome can exist, because it would have "nowhere to go" during this parthenogenetic phase. Once a year, a generation of XX parthenogenetic females produces eggs that develop as XO males, having lost half their X chromatin during the single maturation division (BLACKMAN 1987; BLACKMAN and HALES 1986). Here, as part of ongoing studies of the evolution and host relationships of *Neuquenaphis* (FUENTES-CONTRERAS et al. 1997; QUIROZ et al. 1999; RAMÍREZ et al., in preparation), we present information on karyotype variation in this genus, and assess its taxonomic and evolutionary significance.

MATERIAL AND METHODS

Samples of *Neuquenaphis* from various *Nothofagus* spp., and one species that feeds on *Gunnera* spp., were collected in central and southern Chile in 1998–2000 (Table 1). They were preserved in a cold, freshly-prepared mixture of 3 parts methanol:1 part glacial acetic acid. Embryos were dissected from specimens in 75 % methanol, hydrolysed in 1N hydrochloric acid at 65°C for 5 min and squashed under an

Table 1. *Chromosome numbers of Neuquenaphis species. The nomeclature of Nothofagus follows* VASQUEZ *and* RODRÍGUEZ *(1999)*

SPECIES	No. of samples	Host plant (No. of collections in brackets)	Karyotype (2n female)
N. bulbicauda Hille Ris Lambers	3	*Nothofagus dombeyi*	14
N. edwardsi (Laing)	5	*Nothofagus obliqua* s. lat. (3), *N. obliqua valdiviana* (2)	12
N. palliceps Hille Ris Lambers	6	*Nothofagus dombeyi* (3), *N. nitida* (1), *N. obliqua* s. lat. (2)	6
N. schlingeri Hille Ris Lambers	5	*Nothofagus alessandrii* (1), *N. glauca* (3), *N. obliqua* s. lat. (1)	12
N. sensoriata Hille Ris Lambers	10	*Nothofagus glauca* (1), *N. obliqua* s. lat. (7), *N. macrocarpa* (2)	16
N. similis Hille Ris Lambers	2	*Nothofagus pumilio*	14
N. staryi Quednau & Remaudière	3	*Nothofagus alessandrii*	14
N. valdiviana Carrillo	2	*Gunnera tinctoria*	6
Neuquenaphis "sp. A"	2	*Nothofagus dombeyi* (1), *N. nitida* (1)	12
Neuquenaphis "sp. B"	3	*Nothofagus antarctica* (1), *N. pumilio* (2)	16
N. (*Spicaphis*) *chilensis* Essig	1	*Nothofagus obliqua valdiviana*	10
N. (*Spicaphis*) *essigi* Hille Ris Lambers	2	*Nothofagus obliqua* s. lat.	12

18 mm square cover-slip in a drop of 45 % propionic acid (BLACKMAN 1980). Whole mounts of the specimens from which embryos had been dissected were prepared for species identification.

Chromosome preparations were examined with a Zeiss Axiophot microscope, and good spreads of prometaphase and metaphase chromosomes of somatic cell nuclei were located and photographed on Kodak TMX-100 film. Chromosome lengths were measured from digitised photographs using a Kontron Videoplan image. Only a small number of spreads (see legend to Fig. 2) could be measured with sufficient accuracy in most species. Relative chromosome lengths were calculated as percentages of the total length of all chromosomes in the diploid set.

Chromosomes were ranked in descending order of relative length, and presumed homologs were paired to give data for the haploid set of each species. Chromosomes of the same length ranking in species with the same chromosome number, and therefore presumed most likely to be homologous between species, were compared using one–way nonparametric ANOVA, and individual comparisons were tested for significance using the Mann-Whitney U test.

RESULTS

Karyotypes were recorded for 10 described species of *Neuquenaphis* and two undescribed species (Table 1). The genus shows much karyotype variation, with diploid female chromosome numbers ranging from 6 to 16. Of the 12 species, two had $2n = 6$, one had $2n = 10$, four had $2n = 12$, three had $2n = 14$ and two had $2n = 16$ (Fig. 1). However, when relative lengths of chromosomes were compared in species with the same chromosome number, there were significant interspecific differences except between two of the species with $2n = 12$; *edwardsi* and *schlingeri* (Fig. 2B). Some differences were apparent without measurement, for example the different lengths of the shortest chromosome pair in the two species with $2n = 6$, *palliceps* and *valdiviana* (Fig. 1A,B). Numbers of chromosome spreads of *similis*, *staryi* and *bulbicauda* were too small for statistical comparison, but these species also seemed to have relative length differences (Fig. 2C).

The identity of the X chromosomes could only be determined with certainty in two species, *essigi*, in which male embryos with $2n = 11$ were found in an autumn-collected sample (Fig. 1G), and *similis*, in which some oocytes were found at late prophase of the maturation division (Fig. 1J), at which stage the X chromosomes of aphids are associated (BLACKMAN and HALES 1986). However, the X chromosome pair can usually be identified even in female somatic cells of aphids at prophase because they condense early and typically each has a nucleolar organising region (NOR) at one end, with an associated "cloud" of nucleolar material. In most species the X chromosomes could be tentatively identified as the longest pair (*edwardsi*, *schlingeri*, *staryi*, *similis*, *sensoriata* and sp. A) or the second longest (*essigi*, *chilensis*), but they were the shortest pair in the two species with $2n = 6$ (*palliceps* and *valdiviana*). The absence of homology between the longest chromosomes of *essigi* and the longest chromosomes of *schlingeri* and *edwardsi* is also indicated by the relative length differ-

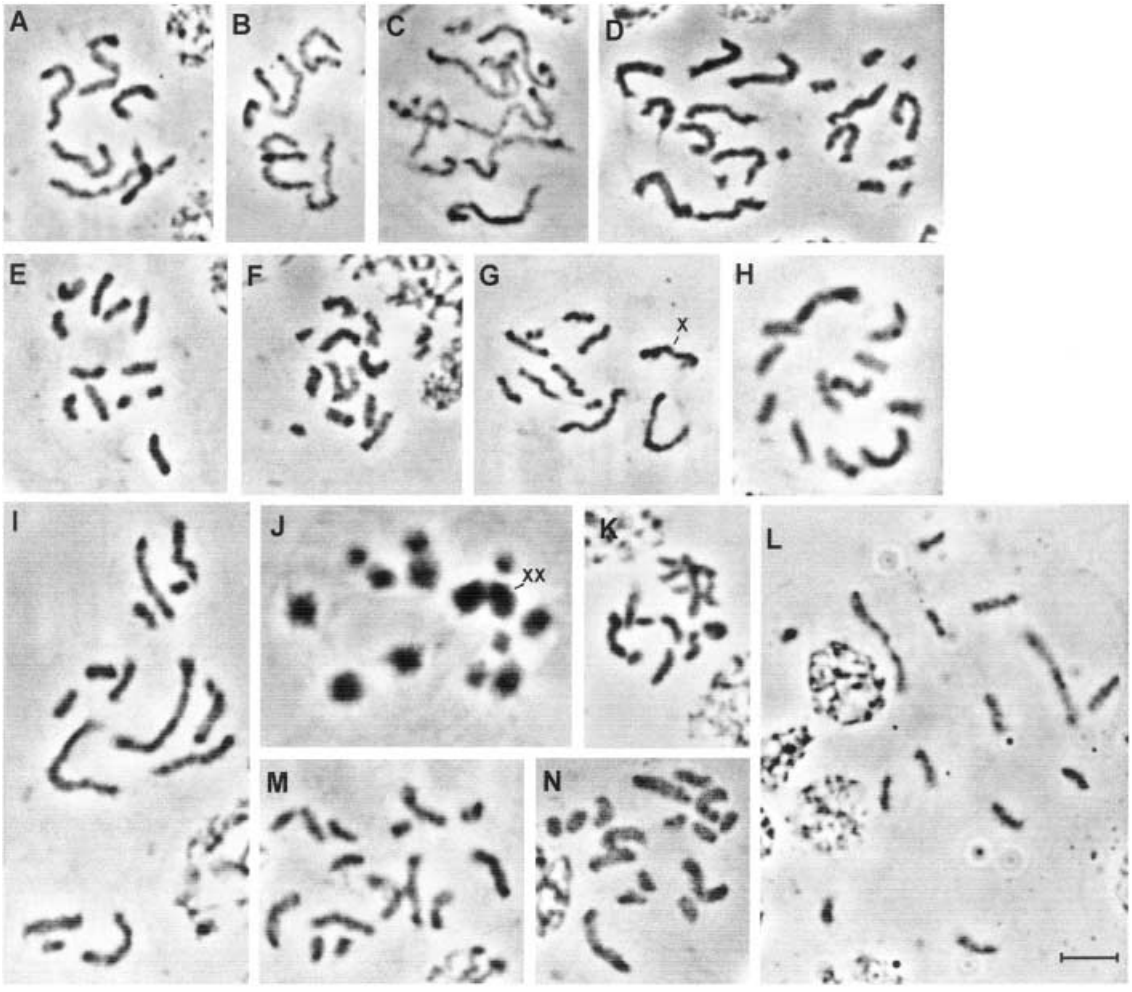

Fig. 1. Prometaphase chromosome spreads of somatic mitotic cells from 12 species of *Neuquenaphis*. **(A)** *N.* (*N.*) *palliceps*, 2n = 6; **(B)** *N.* (*N.*) *valdiviana*, 2n = 6; **(C)** *N.* (*Spicaphis*) *chilensis*, 2n = 10; **(D)** *N.* (*S.*) *chilensis*, tetraploid cell, 4n = 20; **(E)** *N.* (*N.*) *edwardsi*, 2n = 12; **(F)** *N.* (*N.*) *schlingeri*, 2n = 12; **(G)** *N.* (*S.*) *essigi*, 2n = 11 (male); **(H)** *N.* (*N.*) "sp. A", 2n = 12; **(I)** *N.* (*N.*) *similis*, 2n = 14; **(J)** *N.* (*N.*) *similis*, 2n = 14 (oocyte, with X chromosomes associated); **(K)** *N.* (*N.*) *staryi*, 2n = 14; **(L)** *N.* (*N.*) *bulbicauda*, 2n = 14; **(M)** *N.* (*N.*) *sensoriata*, 2n = 16; **(N)** *N.* (*N.*) "sp B", 2n = 16. Bar in (L) represents 5 μm (all photographs).

ences (Fig. 2B). The X chromosomes could not be identified in *bulbicauda* or sp. B.

DISCUSSION

Aphid chromosomes are holocentric, like those of all other Hemiptera (WHITE 1974). At cell divisions, the spindle fibres attach along the whole length of the chromosome, rather than at a single site (the centromere), with the consequence that if any chromosomes should become fused or dissociated they will still divide and segregate normally into daughter cells. This is in contrast to the situation in organisms with localised centromeres, in which fusions and dissociations will result respectively in dicentric and acentric fragments. These will segregate abnormally into daughter cells, resulting in cell death. The greater ability of fusions and dissociations of holocentric chromosomes to survive cell division may be the reason why this type of chromosomal change seems to play a prominent part in aphid karyotype evolution, leading to changes in chromosome number (BLACKMAN 1980).

Another feature of aphid biology that favours karyotypic evolution is cyclical parthenogenesis. New chromosomal rearrangements are initially heterozygous and, in order to evolve into evolutionarily stable new karyotypes, they have to become "fixed" in homozygous condition, usually as a result of mating between two heterozygotes. This is a major barrier to survival of any new chromosomal rearrangement in sexually-reproducing organisms, as any new rearrangement will initially be rare, so there is little chance of mating between two individuals

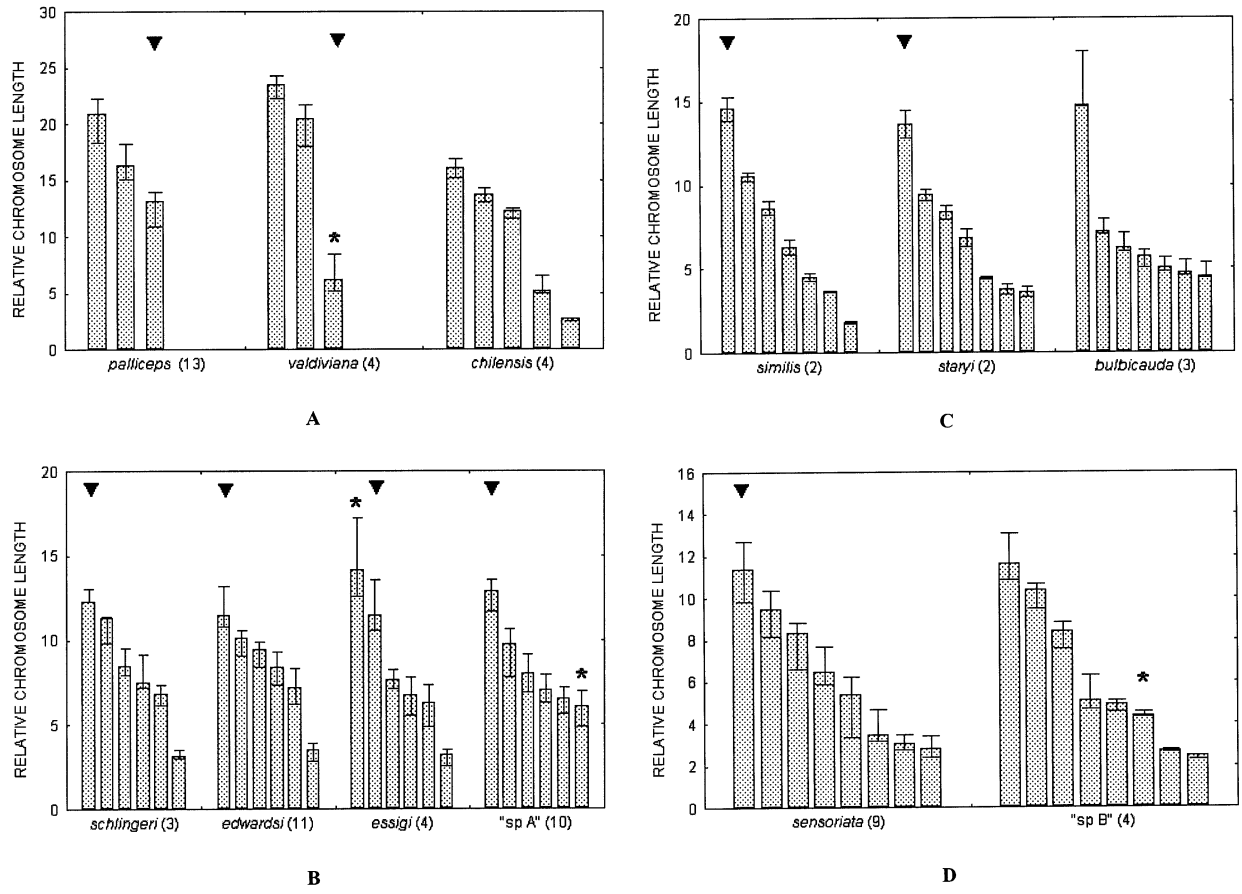

Fig. 2. Idiograms of haploid chromosome sets of *Neuquenaphis* spp. X chromosomes (where identified) are indicated by an arrowhead, and chromosomes differing significantly in length at the 1 % significance level ($P < 0.01$), from all others of the same rank in species with the same chromosome number, are indicated by an asterisk. Bars shows range of variation (i.e. maximum and minimum values). Numbers in brackets after species names are numbers of cells measured. **A,** species with $2n = 6$ and $2n = 10$ (statistical comparison was between the $2n = 6$ species only); **B,** species with $2n = 12$; **C,** species with $2n = 14$; **D,** species with $2n = 16$.

carrying the same rearrangement, except in small, inbreeding populations. Even then, individuals that are homozygous for a new fusion or dissociation will often be inviable. However, between each annual bisexual generation, aphids have a sequence of parthenogenetic generations. Work on other aphid species has shown that both fusions and dissociations can occur and be inherited during parthenogenetic reproduction (HALES et al. 2000; SPENCE and BLACKMAN 2000). In the course of the parthenogenetic phase, a female that is heterozygous for a new fusion or dissociation can produce many progeny, so that in the subsequent sexual phase there is a much better chance of sib-mating between males and mating females that both carry the new rearrangement. Thus, cyclical parthenogenesis must increase the chance that a lineage homozygous for a viable new chromosomal rearrangement will arise.

Nevertheless, the chromosome numbers of species within genera usually vary little, suggesting that there is normally strong stabilising natural selection. The extent of intrageneric variation in *Neuquenaphis* is unusual, and may reflect the fact that the genus is probably a relatively ancient one. However, instances are known of extensive karyotype variation within an evolutionarily recent aphid group, the prime example being the *Rubus*-feeding species of the genus *Amphorophora*, in which diploid chromosome numbers range from 4 to 72 (BLACKMAN 1980).

Differences in chromosome number between species probably represent only a small proportion of the karyotypic variation in *Neuquenaphis*. Without any reference points along the length of a chromosome, such as centromeres or DNA markers, it is difficult to quantify this variation at all accurately. However, comparisons of relative lengths make it clear that species with the same chromosome number do not necessarily have the same karyotype. Thus, chromosome number data on its own does not provide any reliable information about the pathways by which the

various karyotypes have evolved, or about phylogenetic relationships.

Four of the 12 species have $2n = 12$, including species in both subgenera, and two species which do not seem to be very closely related on morphological grounds (*edwardsi* and *schlingeri*) have $2n = 12$ karyotypes that are indistinguishable. This suggests that $2n = 12$ could be the primitive number for the genus, with smaller and larger numbers arising as a result of both fusion and dissociation events.

In *Neuquenaphis*, as in other aphid groups, most of the karyotype variation seems to involve the autosomes. Species with autosomes differing in number and relative length mostly have similar X chromosomes. Where these could be identified, they contributed 11–15 % of total chromosome length (Fig. 2). The exception is *N. valdiviana*, in which the X chromosomes are much smaller, indicating that that some of the original X chromatin has either been lost or translocated onto an autosome. The X chromosomes, and any changes to them, must be under different selection pressures from the autosomes, because of their role in sex determination. The genes that they carry have to be viable as single copies in the XO males, and must therefore be subject to dosage compensation.

Although karyotype variation in *Neuquenaphis* may yield little information of phylogenetic significance, it will be a useful taxonomic tool for species recognition. One problem with this group is that the apterous and alate parthenogenetic females differ greatly in morphology, and may even be described as different species (QUEDNAU and REMAUDIÈRE 1994). Karyotypic data can help to confirm whether two such morphs are conspecific.

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