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The identity of the African pine woolly aphid: a multidisciplinary approach[1]

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A pine woolly aphid of the genus *Pineus* was inadvertently introduced to Zimbabwe in 1962, and now damages plantations of exotic pines in eight countries of eastern and southern Africa. Identification of the species and its area of origin were needed to facilitate collection of potential control agents. Samples of *Pineus* were collected from Africa, Europe, USA, Australia and New Zealand. These were subjected to cytological, multivariate morphometric and DNA (RFLP) analyses. The African *Pineus* showed close correspondence with some of the Australian samples, confirming suspicions that Australia was the source of the introduction. However, there are no native pines in Australia. On morphological and cytological grounds, the African pine woolly aphid also shows affinity with samples from California and Hawaii, and seems likely to be conspecific with *P. boerneri*, originally described from *Pinus radiata* in California. Pine woolly aphid populations in Australia and New Zealand were found to include both *P. boerneri* and a species of European origin, *P. pini*.

Introduction

Conifer woolly aphids (Homoptera, Adelgidae) are related to true aphids (Aphididae) but retain certain primitive features, such as parthenogenetic females that lay eggs rather than produce live young. The genus *Pineus* comprises species that typically host-alternate in the northern hemisphere between *Picea* spp. (primary hosts) and *Pinus* spp. (secondary hosts). As is frequent in aphids, the sexual phase of the life cycle on the secondary host may be lost, and many species are only known from their parthenogenetic generations on the secondary host. Adelgids have a complex polymorphism, and the apterous females on pines not only differ markedly from the *Picea*-feeding morphs, but they also have relatively few stable, species-specific morphological characters of their own. Taxonomic characterisation and identification of *Pineus* populations on pines is therefore very difficult.

Since about 1968, an introduced species of *Pineus* has become a serious pest of exotic pine plantations in eastern and southern Africa (Mills, 1990). Barnes *et al.* (1976) considered that it may have been introduced first into Zimbabwe, in 1962, on *Pinus taeda* scions from Australia, although there is no firm evidence of this. Very similar insects have infested conifers in Australia and New Zealand for many years, but in those countries also the pine woolly aphids are not indigenous — there being no native Australasian pines — and the number of species introduced and their origins are still in doubt (Eastop, 1966; Tanton & Alder, 1977).

Before attempting biological control of the African pine woolly aphid, it is advisable to know its correct identity and area of origin, so that the most suitable potential biocontrol agents can be located. With this objective in view, we used a multidisciplinary approach combining chromosome studies (performed by the first author), multivariate morphometrics (by the

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second author) and RFLP analysis (by the third author). Samples of eggs and adult insects for this work were collected from various *Pinus* species in Africa (Malawi, Kenya), Europe (UK), USA (California, Hawaii), Australia and New Zealand. What follows is a summary of results that will be published in greater detail elsewhere. Watson (1995) has published a preliminary report.

Chromosomal analysis

Somatic metaphase spreads were prepared from embryonic cells obtained from eggs fixed in 3:1 methanol/acetic acid shortly (1–2 days) after oviposition. Chromosomes were measured using a Kontron Videoplan image analyser. A total of 40 samples provided chromosome preparations that could be karyotyped.

The eight African samples were collected from various *Pinus* spp. (*kesiya, maximinoi, patula, radiata*; five samples from Kenya and three from Malawi). They all had an apparently identical, structurally heterozygous (aneuploid) karyotype, comprising 17 chromosomes, the most

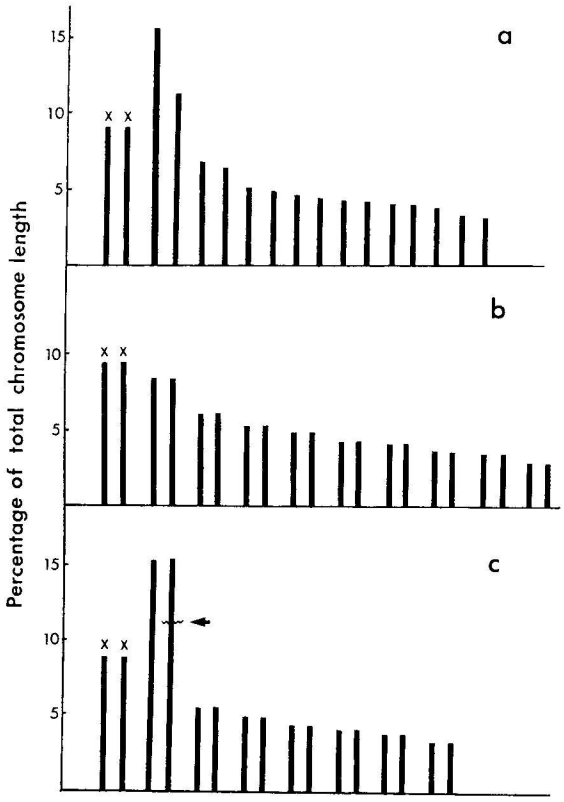

Fig. 1. Relative lengths of chromosomes in (a) 17-chromosome *Pineus* from Africa; (b) 2n = 20 from Europe; and (c) 2n = 16 from Hawaii. The arrow in (c) indicates the hypothetical breakpoint to give rise to the 17-chromosome karyotype.
Longueur relative des chromosomes chez les *Pineus* à: a) chromosomes d'Afrique; b) 20 chromosomes d'Europe; c) 16 chromosomes de Hawaii. La flèche dans c) indique le point supposé de rupture qui a conduit au karyotype de 17 chromosomes.

obvious characteristic being a long, unpaired chromosome of about 15.5% of the total length of the chromosome complement (Fig. 1a). This chromosome was longer than the X chromosomes, which each comprised about 9% of total chromosome complement length in metaphase cells and were recognisable by their differential condensation and association with nucleoli in prophase cells.

All three samples from the UK were from *Pinus sylvestris*, and had a karyotype of 2n = 20 (Fig. 1b) with apparent structural homozygosity (i.e. an apparently normal diploid set of 10 pairs of homologous chromosomes). The X chromosomes were the longest pair and each comprised about 9% of total complement length, as in the 17-chromosome karyotype from Africa.

Of the eight Australian samples karyotyped, six had a 17-chromosome karyotype indistinguishable from that of the African populations; five of these were from *P. radiata* and one from *P. pinea*. One of the four New Zealand samples (from *P. pinea*) also had a 17-chromosome karyotype like that of the African *Pineus*. The other samples from Australia and New Zealand, all from *P. radiata*, had nuclei with more chromosomes, but the preparations were too poor for detailed study. They all resembled the UK 2n = 20 form in having the X chromosomes as the longest elements in the chromosome complement; but the two Australian samples appeared to have a 21-chromosome complement, whereas 19 chromosomes could be counted in nuclei of the other three samples from New Zealand.

The single sample from Hawaii (from *Pinus pinaster*) had 2n = 16, an apparently diploid set of chromosomes, with one autosome pair longer than the X chromosomes, each member of this pair comprising about 15.5% of total chromosome length (Fig. 1c). Analysis of relative length measurements indicates that the 17-chromosome form could have been derived from this karyotype by a single dissociation of one member of the longest autosome pair. Such dissociations seem to play a large part in karyotype evolution in aphids, and are especially common in permanently parthenogenetic populations (Blackman, 1980).

The Californian material included one sample with 2n = 16 as in Hawaii, and two samples with the 17-chromosome karyotype. These were all collected on *P. radiata*, which is native to California. All the other Californian samples, including four from *P. radiata*, had a 2n = 20 karyotype resembling that of the UK populations.

To summarize the results of chromosome studies, the African *Pineus* has a characteristic, aneuploid 17-chromosome karyotype which also occurs in North America, Australia and New Zealand, and could easily have been derived from a 2n = 16 form currently present in California and Hawaii. *Pineus* with a distinctly different karyotype, 2n = 20, occur in Europe (UK) and North America (California), and aneuploid karyotypes possibly derived from 2n = 20 occur in Australia and New Zealand. There is no clear association between the karyotype and the pine species colonised.

Multivariate morphometrics

Sixteen morphological characters were measured on samples of 7–10 specimens from each collection locality, using a Videoplan image analyser. These samples were used as the groups in a canonical variates analysis (CVA), a method that has proved very useful in distinguishing closely related aphid species (Blackman, 1992). The first canonical variate (CV1) clearly separated the samples into two groups which corresponded with the main groupings established by chromosome studies; the samples with 16 or 17 chromosomes all had much lower scores on CV1 than those with 19 or more chromosomes (Watson, 1995).

There is thus good evidence, using two distinct methods, that at least two species are present in the material studied, although only one of these has been found in Africa. In the Californian material there is also possibly a third species; six of the Californian samples with 2n = 20 were well separated from the rest by their low scores on the second canonical variate.

Analysis of restriction enzyme fragment length polymorphisms (RFLPs) in nuclear ribosomal RNA genes (rDNA)

Thirty-two cryopreserved samples were used for RFLP analysis. The probes were agarose gel-purified fragments of Dipteran rDNA clones from a sandfly (*Phlebotomus papatasi*) and a complete unit (pDm 238) of *Drosophila melanogaster*, labelled by random priming or nick translation (for methods see Sambrook *et al.*, 1989, and Ready *et al.*, 1991). The restriction enzymes used all had sites in those coding regions of rDNA that are usually well conserved among closely related populations of insects. The differences found among the *Pineus* samples were due to length and restriction site variations in the intergenic spacer. There was a clear difference between the 17-chromosome samples from Africa and Australia, with units of 17.0–18.0 kb, and those with 19 or more chromosomes (from California, England, Australia and New Zealand) which mostly yielded much smaller units. In particular the intergenic spacer in the 19- to 21-chromosome samples had a *Pst I* site (or sites) that was absent from the 17-chromosome African and Australia samples. When two 17-chromosome samples from California were analysed by this method, however, their rDNA RFLP patterns differed from both those described above, and the 16-chromosome sample from Hawaii yielded a fourth distinct pattern.

The results of RFLP analysis indicate that, of the samples examined, those with 17 chromosomes from Australia were most similar to the African *Pineus*. Indeed, two of the Australian samples were indistinguishable from the three samples from Kenya. All the differences found were of a form that might be expected within a closely related group of species or conspecific populations.

Conclusions

These three very different approaches to a single problem all gave results in close agreement with the proposition that the pine woolly aphid was introduced to Africa from Australia, as has been suggested previously (Barnes *et al.*, 1976). The results of RFLP analysis leave little room for doubt that this is the case. The karyotype and morphology of the African *Pineus* indicate further that it has a close taxonomic relationship with 16- and 17-chromosome populations in California and Hawaii, and is distinct from the European species *P. pini*. We consider that on present evidence it is likely to be conspecific with *P. boerneri*, described from *Pinus radiata* in California (Annand, 1928). Annand's description agrees fully with the 17-chromosome form. Differences in RFLP patterns between the US 16- and 17-chromosome samples and the African/Australian 17-chromosome samples are not incompatible with a conspecific status.

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Approche multidisciplinaire à l'étude de l'identité d'un chermes du pin

Un chermes du pin du genre *Pineus* a été introduit par inadvertance au Zimbabwe en 1962 et provoque aujourd'hui des dégâts dans les plantations de pins exotiques dans huit pays de l'est et du sud de l'Afrique. L'identification de l'espèce et de sa région d'origine étaient nécessaires pour faciliter la recherche d'agents potentiels de lutte. Des échantillons de *Pineus* ont été collectés en Afrique, en Europe, aux Etats-Unis, en Australie et en Nouvelle-Zélande, et ont été soumis à des analyses cytologiques et morphométriques à critères multiples, ainsi qu'à l'étude de leur

ADN par RFLP. Les *Pineus* africains présentent de grandes similitudes avec certains échantillons australiens, confirmant les soupçons quant à une introduction à partir de l'Australie. Cependant, il n'y a pas de pins natifs d'Australie. D'après les critères morphologiques et cytologiques, le chermes du pin présente également des affinités avec les échantillons de Californie et d'Hawaii, et semble conspécifique de *P. boerneri*, décrit à l'origine sur *Pinus radiata* en Californie. Les populations de chermes du pin d'Australie et de Nouvelle-Zélande incluent à la fois *P. boerneri* et une espèce d'origine européenne, *P. pini*.

Подлинность африканской волосатой сосновой тли: разносторонний подход

Волосатая сосновая тля рода *Pineus* была случайно занесена в Зимбабве в 1962 году и в настоящее время повреждает плантации экзотических сосен в восьми странах восточной и южной Африки. Идентификация вида вредителя и области его происхождения требовали упрощения подбора потенциальных агентов для борьбы с ним. Образцы *Pineus* были получены из Африки, Европы, США, Австралии и Новой Зеландии и были подвержены цитологическим и морфометрическим многомерным анализам, а также исследованию их ДНК методом определения полиморфизма рестрикционных фрагментов (RFLP). Африканский вид *Pineus* проявляет значительное сходство с некоторыми австралийскими образцами, а это подтверждает подозрение, что Австралия является источником ввоза вредителя. Однако местных видов сосны в Австралии не существует. По морфологическим и цитологическим параметрам африканская волосатая сосновая тля также имеет сходство с организмами из калифорнийских и гавайских проб и, вероятно, принадлежит к тому же виду , что и *P. boerneri*, первоначально описанной на *Pinus radiata* в Калифорнии. Популяции волосатой сосновой тли в Австралии и Новой Зеландии объединяют одновременно два вида, а именно *P. boerneri* и вид европейского происхождения *P. pini*.

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