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DNA fingerprinting of cereal aphids using $(GATA)_4$

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Abstract. The role of DNA fingerprinting as a tool for studying cereal aphid populations along with the principals behind fingerprinting and the process of obtaining fingerprints using the multilocus probe, ^{32}P labelled $(GATA)_4$ are discussed. The successful application of this technique in obtaining fingerprints capable of distinguishing between different clones of *Sitobion avenae* (F.) is presented.

The population genetics of clonal species of migratory and introduced pests is a poorly understood area of population ecology. Aphids are perhaps the best known example of a clonal species of insect and several, including *Sitobion avenae* (F.) are important pests of cereal crops in a number of countries. Conventional studies can detect the arrival or introduction of these species, but the genetic constitution of the immigrants and the fitness of the different clones with respect to their ability to colonise and exploit a new resource is unknown and may vary considerably. Therefore, depending on the clonal make-up of immigrants, the likelihood of an outbreak or establishment may vary between sites and years. Knowledge of such variation may have significant implications for forecasting outbreaks of pests, determining the likely impact of newly introduced species, and interpreting the development of insecticide-resistant strains of pests.

Conventional ecological methods, used in the past to provide some insight into these processes, are limited in their ability to resolve these processes as they are unable to distinguish between morphologically identical clones. Consequently, they deal with a population as a group of insects belonging to the same species and not as a collection of clones which may be quite variable in their ability to perform one or more of the above processes. In some circumstances this level of resolution may be unnecessary, but in most research relating to aphid population ecology, forecasting and control whether through chemical, biological or integrated pest management, a knowledge of clonal diversity may be invaluable. In short, an ability to separate populations into a series of clones may allow us to answer some ecological questions better and to answer questions which in the past we have not been able to ask.

Listed below are a series of simple ecological questions relating to the survival of aphids in refuge areas and their subsequent emigration and exploitation of cereal crops. These are by no means exhaustive, but if answered will provide an as yet unattainable level of resolution for the study of cereal aphid ecology.

Survival in refuge areas (oversummering and overwintering habitats) raises several questions regarding the genetic diversity of the species in these habitats. They include, what are the refuge areas for a given species and where are they located? These are two

basic questions which have not been answered definitively in terms of the connection between aphids in known refuge areas and their arrival later in crops. Furthermore, how many clones are present in these areas and do all persist equally well? Are all clones equally suited to all species within their host range? Are all clones equally good emigrants or are some better and if so, are some better colonists? Once aphids colonise a crop, how many clones are involved and is the number dependent upon the clonal diversity in nearby refuge areas? What are the spatial and temporal dynamics of clones within a crop and are clones present in crops in one region more similar to each other than to those found in more distant regions? Answers to these would not only provide invaluable information on which to construct and modify pest control and forecasting systems, but also essential data on aphid biology which may provide a useful insight into such problems as the development and spread of pesticide resistance in aphid populations.

Various conventional methods such as tagging and trapping aphids have been used, without success to provide definitive answers to these questions. Answers to questions relating to aphid migration and clonal diversity have also been sought using allozyme electrophoresis. Such studies have, however, only been possible in species exhibiting sufficient levels of enzyme polymorphism so providing the allelic variants for genotypic discrimination. In aphids, low enzyme polymorphism and the resultant low heterozygosity has led to insufficient differences within and between populations to allow a detailed picture of the temporal and spatial clonal diversity to emerge (Loxdale et al., 1985). Similarly, the use of mitochondrial DNA as a means of distinguishing between populations has also foundered (Martinez et al., 1992).

The recent demonstration that tandemly repeated sequences of DNA analogous to those described by Jeffreys et al. (1985) are both present and polymorphic in aphids, coupled with the ability to characterise clones from individuals (Carvalho et al., 1990), indicate that DNA fingerprinting should be especially useful for monitoring the diversity and dynamics of aphids in the field. These tandemly repeated sequences of DNA occur in blocks containing variable numbers of the tandem repeats (VNTR). The process by which the repeated sequences are obtained and visualised is presented in Fig. 1. The probe used for this study was the 16-mer oligonucleotide, $(GATA)_4$ discovered by Epplen (1992). This probe is homologous with sequences located at more than one locus within the aphid genome and so is termed a multilocus probe and the technique, multilocus DNA fingerprinting. We have adapted Epplen's methodology and are now able to clearly visualise as bands, VNTR's within the aphid genome. Oligonucleotides were chosen instead of Jeffreys probes as used by Carvalho et al. (1990) because they are manufactured and so of guaranteed purity. The process is also faster, the lengthy processes of Southern blotting and blocking is avoided thereby saving 1 to 1.5 days, and considerably cheaper. Several species including *S. avenae*, *Metopolophium dirhodum* (Walker) and *Rhopalosiphum padi* (L.) have now been fingerprinted.

To attempt to answer the questions posed it was necessary to determine whether the probe selected could distinguish between different clones. This is somewhat arbitrary as the ability to separate clones depends not only on the 2 individuals compared being different, but also on the ability of the probe restriction enzyme combination used (see Fig. 1), to detect these differences. Therefore, the term clone should be used bearing this in mind. To gauge the level of variability amongst clones of *S. avenae* and so determine the usefulness of the $(GATA)_4$ probe, 16 individuals, 6 from to locations in Southampton (lanes 1 &

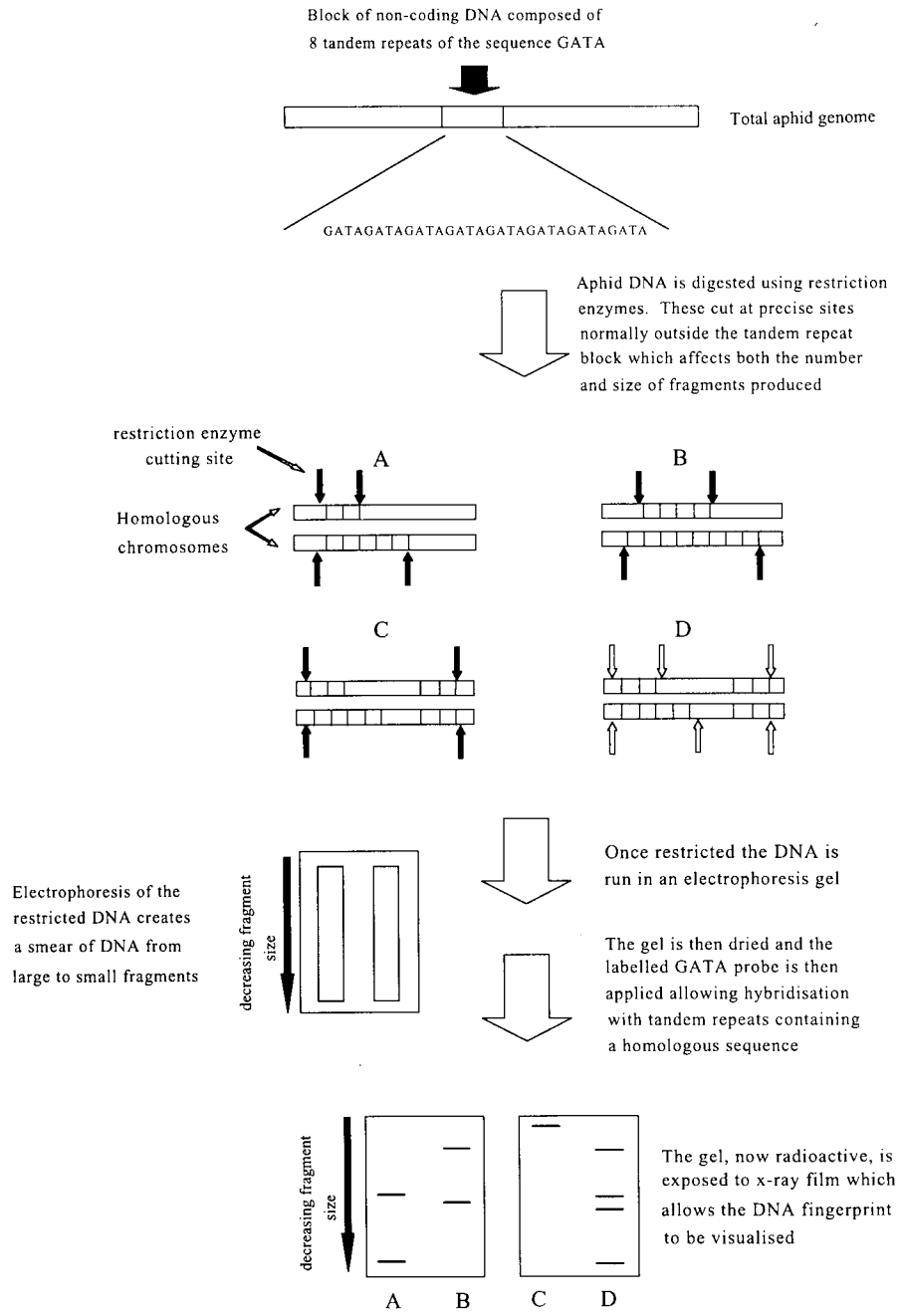

Fig 1. The basic steps involved in obtaining a DNA fingerprint.

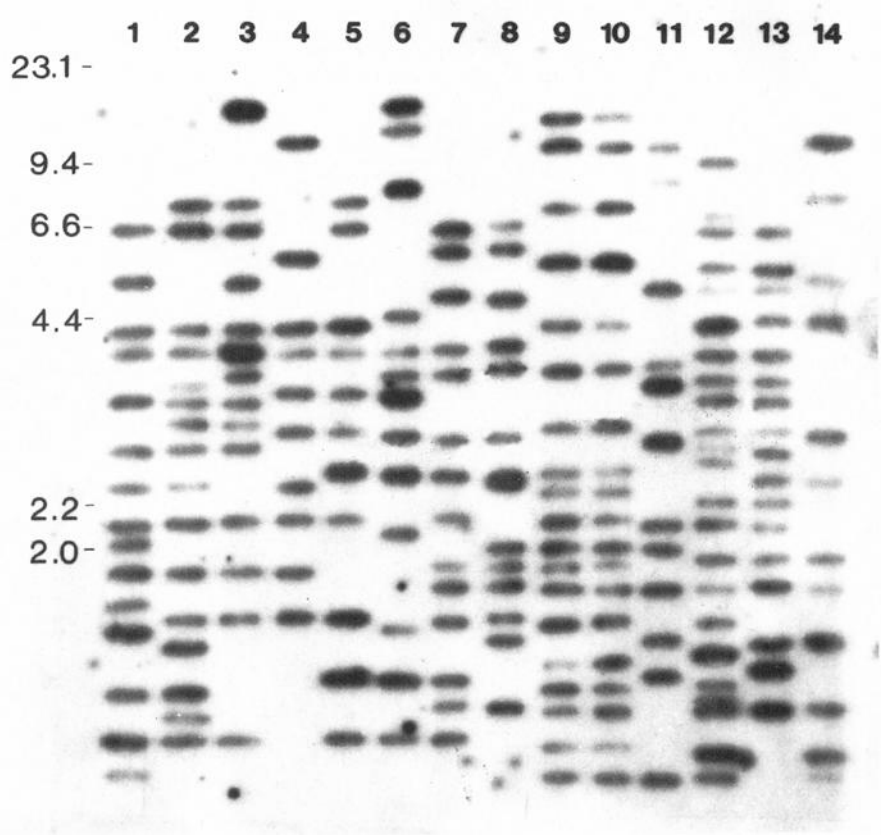

Fig 2. The $(GATA)_4$ probe has been used to obtain unambiguous fingerprints from 14 putative clones of *S. avenae* collected from several locations across England. Lanes 1 & 4; 2 & 3; 5 & 6; 7 & 8; 9, 10 & 11; 12, 13 & 14 are individual aphids from the same population.

4, 2 & 3, 5 & 6), 4 from fields at Oxfordshire (7 & 8, 9, 10 & 11) and 3 from fields near Rothamsted were fingerprinted (Fig. 2). Each aphid yielded 4 to 5 µg of DNA which was extracted using phenol/chloroform. DNA from single aphids was then restricted using either EcoRI, HaeIII, HindIII, HinfI, MboI, MspI or RsaI. The quality and quantity of the DNA was measured using minigels. Aphid DNA along with the molecular weight marker lambda HindIII was then run in a 0.7% agarose gel for 21 h. This allows DNA fragments >1.8kb (kilobases) to be examined. After electrophoresis, gels were photographed under UV light and the position of the markers noted. Gels were then dried and hybridized overnight with ^{32}P labelled $(GATA)_4$. After washing, gels were exposed to x-ray film for 4 to 10 days.

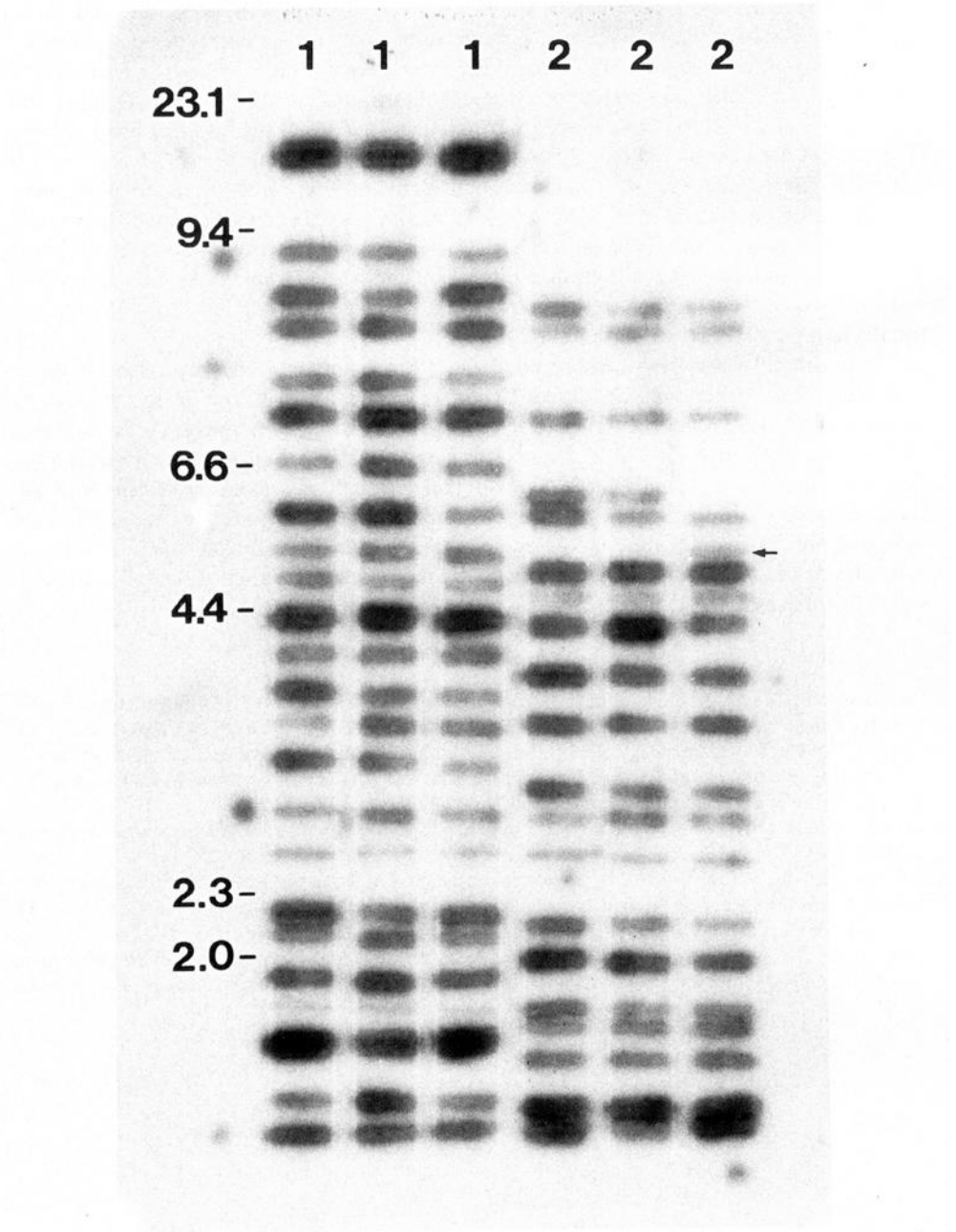

Fig 3. Clonal stability of 2 clones of *S. avenae* from generations 1, 6 and 12. Clone 1 shows continuous clonal stability, but clone 2 shows a single band shift in the twelfth generation.

RsaI, MspI, MboI all gave bands, but in each case signals were weak and indistinct. EcoRI and HaeIII gave good bands, but variability between putative clones was poor. HaeIII also suffered from high background interference which tended to obscure the fainter bands. HindIII gave both good clear signals with little background interference and all, but one of the 16 putative clones were distinguishable on the basis of band sharing (Fig. 2). Between 10 and 25 bands ranging from 1.8 up to 23.1kb (mean = 15.8 ± 1.1, n = 16) are clearly visible (Fig. 2). Bands are considered different if they are >0.5mm apart. The similarity coefficient ($2N_{AB}/(N_A + N_B)$, where N_{AB} is the number of bands between 2 individuals while N_A and N_B is the number of bands exhibited by each individual, was then calculated. Similarity ranges from 0.11 up to 1.00 and while sample sizes are too small to test for variability, it is encouraging to note that the highest coefficients of similarity are found among individuals from the same source.

Clonal stability was also demonstrated for 2 Southampton clones by taking a single individual from generations 1, 6 and 12 (Fig. 3). For Clone 1, continuous stability is shown, but Clone 2 shows stability over the first and sixth generations only as there is a single band difference (5.0kb) in the twelfth generation. This suggests either contamination or a mutation at a single locus. Contamination is unlikely given the rearing arrangements in which clones are kept separated in Blackman boxes. This leaves mutation as the only real possibility and while not being detrimental to the overall results is interesting as it illustrates the development of new clones and highlights the ability of the probe to readily distinguish between different clones.

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