Bulletin of Entomological Research (2000) **90**, 391–396

Genetic relationships among biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae) based on AFLP analysis

M.T. Cervera[1, 2], J.A. Cabezas[1, 2], B. Simón[3], J.M. Martínez-Zapater[1,2], F. Beitia[2] and J.L. Cenis[3*]

[1]CNB-CSIC, Campus de Cantoblanco, 28049 Madrid, Spain: [2]SGIT-INIA, Ctra. de La Coruña, Km 7.5, 28040 Madrid, Spain: [3]CIDA, c/Mayor s/n, 30150 La Alberca (Murcia), Spain

Abstract

Genetic similarities between 13 samples belonging to nine reference biotypes and two field populations of *Bemisia tabaci* (Gennadius), one field population of *B. medinae* Gómez-Menor and another of *B. afer* Priesner & Hosny, were evaluated using amplified fragment length polymorphism (AFLP) markers. The results indicate that *B. tabaci* biotypes can be grouped together with a minimum similarity coefficient of 0.32 and separated from the two other species with a similarity coefficient of 0.07. *Bemisia tabaci* biotypes were grouped in four clusters which comprised: (i) Near East and Indian subcontinent biotypes; (ii) B and Q biotypes plus a Nigerian population from cowpea; (iii) New World A biotype; and (iv) S biotype and a Nigerian population from cassava. These results were consistent with a previous grouping of biotypes based on RAPD-PCR analysis. The AFLP assay allowed the scoring of a total of 354 polymorphic bands in two reaction events with the use of two primer combinations.

Introduction

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) exhibits a large amount of biological and genetic variation among natural populations (Costa & Brown, 1991; Brown *et al*., 1995b). The presence of biotypes or host races of this whitefly was first recognized in the 1950s, when it was discovered that morphologically indistinguishable populations of *B. tabaci* showed different biological traits with respect to host range, host-plant adaptability and plant virus-transmission capabilities (Bird, 1957). Soon it was discovered that polymorphisms at the esterase locus were frequent and that these polymorphisms could be used as biotype markers (Costa & Brown, 1991; Bedford *et al*., 1992; Wool *et al*., 1993; Brown *et al*., 1995a). Up to the present time, approximately 19 distinct esterase phenotypes have been characterized in populations of *B. tabaci* worldwide and named with a letter code from A to S. These letters have subsequently been used also to name the respective biotypes (Bedford *et al*., 1992; Brown *et al*., 1995a; Banks *et al*., 1999). These biotypes can differ in multiple traits, such as host-plant adaptation, induction of phytotoxic reactions and insecticide resistance (Bethke *at al*., 1991; Costa & Brown, 1991; Burban *et al*., 1992; Bedford *et al*., 1993, 1994; Costa *et al*., 1993; Byrne *et al*., 1995). Most biotypes have a limited host and geographic range, transmitting indigenous geminiviruses of local distribution and are of low agricultural importance. By contrast, the B biotype is highly polyphagous and has spread globally with the trade in ornamentals. This spread has made the movement of plant viruses into new areas easier with serious economic consequences (Bedford *et al*., 1994; Brown *et al*., 1996). These facts highlight the importance of assessing the biotype status of *B. tabaci* populations.

The development of molecular markers based on nucleic acids technology has provided new insights in the study of *B. tabaci* variation. The random amplified polymorphic DNA technique (RAPD–PCR, Williams *et al*., 1990) is useful in differentiating biotypes (Gawel & Bartlett, 1993; Perring *et al*., 1993; Guirao *et al*., 1997). Molecular phylogenetic studies

* Author for correspondence
Fax: +34 968 366 792
E-mail: JoseL.Cenis@carm.es

based on comparative sequence analysis of a region of the 16S ribosomal subunit and mitochondrial cytochrome oxidase I (COI) genes have also been carried out to determine the genetic relationships among a number of *B. tabaci* populations (Brown *et al.*, 1995b; Frolich *et al.*, 1999). Another approach with great potential in the study of genetic variation is amplified fragment length polymorphism (AFLP), which is based on the selective polymerase chain reaction (PCR) amplification of a fraction of the fragments obtained after DNA restriction allowing a higher resolution of genetic differences (Vos *et al.*, 1995). The selection is achieved by the design of primers, which anneal at the ends of the restriction fragments by means of adaptors previously ligated to the fragments. Typically, more than one hundred amplified bands can be visualized with each AFLP assay. This technique has been widely used to assess genetic similarities on many different species. However its use on insects is so far restricted to the study of genetic diversity within and among gypsy moth *Lymantria dispar* (Linnaeus) (Lepidoptera: Lymantriidae) populations (Reineke *et al.*, 1999).

This study aimed to assess the performance of AFLPs in the genetic study of a collection of *B. tabaci* populations and biotypes from different locations in four continents.

Materials and methods

Insect populations

A total of 11 samples of *B. tabaci* were included in the study (table 1). Five of them (Biotypes A, H, K, M and S) came from established laboratory collections (John Innes Centre, Norwich, UK and University of Arizona, Tucson) and were fully characterized with respect to esterase phenotype (Brown *et al.*, 1995a; Banks *et al.*, 1999) and other biological features (Costa & Brown, 1991; Bedford *et al.*, 1994). Samples of B and Q biotypes came from the collection of F. Beitia (Instituto Nacional de Investigaciones Agrarias, Madrid, Spain), originally isolated on tomato crops from southern Spain. They were fully characterized in the course of previous studies (Guirao *et al.*, 1997). Two populations from Pakistan came from a survey of cotton fields made in 1997. Each of these Pakistan populations was representative of the two main genetic groups found within 25 populations from the survey, analysed by RAPD–PCR (Cenis, unpublished). The two populations analysed from Nigeria were selected from the collection of I. Abdullahi (Plant Quarantine Service, Ibadan, Nigeria). One was collected from cowpea and the other from cassava. Finally, two samples corresponding to two different species of *Bemisia* were used as outgroups, namely *B. medinae* Gómez-Menor, and *B. afer* Priesner & Hosny (Hemiptera: Aleyrodidae). Both species were collected in the Canary Islands by A. Carnero (Centro de Investigación y Tecnología Agraria, Tenerife, Spain). These two populations were identified by the morphology of the pupal case in the course of a thorough survey of the whitefly fauna from the Canary Islands. All the samples, composed only of adults, were fixed and stored in 70% ethanol. Prior to the AFLP assay, at least 15 individuals of each sample were analysed by RAPD–PCR in order to verify the homogeneity of samples and to eliminate the possibility of contamination with other biotypes.

AFLP assay

A preliminary experiment was undertaken in order to assess the reproducibility of the amplification and the minimal amount of DNA needed for the reactions. For this experiment, we extracted DNA from single whiteflies (approximately 25 ng) by scaling down the protocol described by Cenis *et al.* (1993) and assayed samples diluted 3, 4 and 6-fold or pooled in different ways. Pools were made either by crushing together 20 individual adults or by mixing four aliquots of DNA solution extracted from three individuals of biotype Q and one from biotype B. In a second experiment for assessing genetic relationships among biotypes and species, 20 individuals from each population were pooled together and DNA was extracted exactly as described in the protocol previously mentioned.

AFLP analysis was performed according to Vos *et al.* (1995) with the modifications described by Cervera *et al.* (1996). The DNA was digested using *Mse*I (New England Biolabs) and *Eco*RI (Pharmacia) restriction enzymes. Digestion was carried out in a final volume of 35 µl in 10 mM Tris-H acetate, 10 mM Mg acetate, 50 mM DTT, pH 7.5, 10 U *Eco*RI, 8 U *Mse*I with DNA ranging from 1/6 of total DNA from a single insect up to 150 ng of total DNA when using

Table 1. Origin of the populations of *Bemisia* used in the study.

Species	Biotype[a]	Origin	Collection	Host
B. tabaci	H	India	JIC[b]	Watermelon
B. tabaci	K	Pakistan	JIC[b]	Cotton
B. tabaci	unknown (I)	Pakistan	Field[c]	Cotton
B. tabaci	unknown (II)	Pakistan	Field[c]	Cotton
B. tabaci	M	Turkey	JIC[b]	Cotton
B. tabaci	A	Arizona	Univ. of Arizona[d]	Pumpkin
B. tabaci	unknown	Nigeria	Field[e]	Cassava
B. tabaci	unknown	Nigeria	Field[e]	Cowpea
B. tabaci	S	Spain (Malaga)	JIC[b]	*Ipomoea indica*
B. tabaci	Q	Spain (Murcia)	INIA	Tomato
B. tabaci	B	Spain (Malaga)	INIA	Tomato
B. medinae		Spain (Tenerife, Canary I.)	Field[f]	Tomato
B. afer		Spain (La Palma, Canary I.)	Field[f]	Tomato

[a]Biotype as defined by esterase banding patterns (Bedford *et al.*, 1992); [b]supplied by I.D. Bedford; [c]collected by F. García-Arenal; [d]supplied by J.K. Brown; [e]supplied by S. Winter; [f]supplied by A. Carnero.

pooled sets of individuals, for 3 h at 37°C. Both *Eco*RI and *Mse*I adaptors, were ligated to the DNA fragments by adding to the digestion 5 µl of a mix containing 5 pmol *Eco*RI adaptor, 50 pmol *Mse*I adaptor, 8 mM ATP, 10 mM Tris-H acetate, 10 mM Mg acetate, 50 mM DTT, pH 7.5 and 1.4 U T4 DNA ligase (Boehringer). The ligation was incubated for 3 h at 37°C and overnight at 4°C. The *Eco*RI adaptor consisted of the combination of primers 5′-CTCGTAGACTGCGTACC and CTGACGCATGGTTAA-5′. The *Mse*I adaptor consisted of the combination of primers 5′-GACGATGAGTCCTGAG and TACTCAGGACTCAT-5′.

Digested-ligated DNA fragments were diluted 7-fold to be used as a template for the first amplification reaction, the pre-amplification step. This pre-amplification consisted of a PCR reaction using primers which are complementary to the adaptors *Eco*RI and *Mse*I with an additional selective 3′ nucleotide in order to reduce the complexity of the amplified DNA fragment collection (only 1/16 fragments are amplified). PCR reactions were performed in a 20 µl volume of 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.2 mM of each dNTP, 30 ng of each primer (GENSET) *Eco*RI + A and *Mse*I + C, 0.4 U *Taq* DNA polymerase (Boehringer) and 3 µl of diluted DNA fragments. PCR amplifications were carried out in a Perkin Elmer 9600 using 28 cycles, each cycle consisting of 30 sec at 94°C, 1 min at 60°C, and 1 min at 72°C.

Ten microlitres of the pre-amplification products were diluted by adding 100 µl of water. This was used as the starting material for the selective radioactive amplification. For this reaction, only *Eco*RI primers were labelled. Both *Eco*RI primer and *Mse*I primer used in each analysis contained the same sequences as those used in the pre-amplification but with three additional selective nucleotides at the 3′ end. The PCR reaction was performed in a 20 µl volume of 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.08 mM of each dNTP, 4 ng [^{33}P]-*Eco*RI primer, 24 ng *Mse*I primer, 0.4 U *Taq* DNA polymerase (Boehringer), and 5 µl of diluted preamplified DNA. The selective amplification was carried out using the following cycling parameters: one cycle of 30 sec at 94°C, 30 sec at 65°C, 1 min at 72°C then 12 cycles in which the annealing temperature decreases 0.7°C per cycle, followed by 23 cycles of 1 min at 94°C, 30 sec at 56°C, and 1 min at 72°C.

Two primer combinations were used in this analysis: *Eco*RI + AAG/*Mse*I + CTG and *Eco*RI + ACT/*Mse*I + CTG. At the end of the selective radioactive PCR, the samples were denatured by adding an equal volume of formamide-buffer (98% formamide, 10 mM EDTA, pH 8.0, 0.05% bromophenol blue and 0.05% xylene cyanol), heated for 3 min at 94°C, then 3 µl of each sample were loaded on 4.5% acrylamide/bisacrylamide 19:1, 7.5 M urea and 1 × TBE gels.

Data analysis

AFLP bands showing only high or medium relative intensity were visually scored as present (1) or absent (0). Genetic similarity (GS) between pairs was estimated according to Dice coefficient (Sneath & Sokal, 1973) [$GS(ij) = 2a/(2a + b + c)$], where GS(ij) is the genetic similarity between individuals i and j, a is the number of polymorphic bands that are shared by i and j, b is the number of bands present in i and absent in j, and c is the number of bands present in j and absent in i. Genetic relationships among genotypes were represented in a dendrogram based on the unweighted pair-group method of arithmetic averages (UPGMA). Goodness of fit of cluster analysis (between the similarity matrix and the dendrogram obtained) was measured by calculating the co-phenetic correlation between the similarity matrix and the co-phenetic matrix.

In order to compare the genetic similarities obtained from AFLPs with those obtained from RAPDs, we analysed a common set of six biotypes (A, B, H, K, M and Q) that were previously studied by Guirao *et al*. (1997) and also used in the present work. Matrix comparisons to determine the agreement between AFLP and RAPD based similarity matrices were made using the Mantel test (Mantel, 1967). Calculations were carried out using the NTSYS-PC software package, version 1.8 (Rohlf, 1993).

Results

The quality of the amplification made with DNA from a single individual insect was the same as that with DNA obtained from 20 individuals (fig. 1A). When comparing three different dilutions of the same individual, the amplification obtained with the 1/6 dilution gave the same

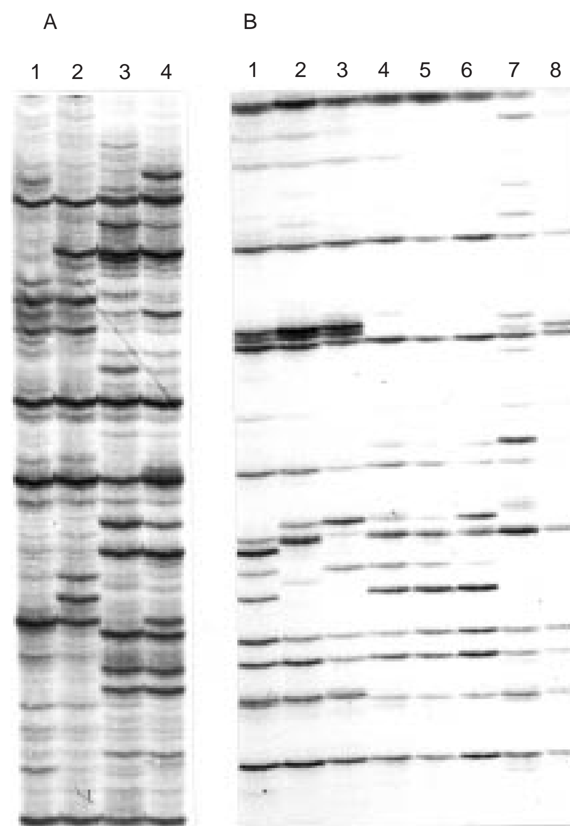

Fig. 1. AFLP patterns obtained with single and pooled adults of *Bemisia tabaci*. Panel A: lanes 1 and 3, reaction with DNA of a single individual of B and Q biotypes respectively; lanes 2 and 4, reaction with DNA extracted from a pool of 20 insects crushed together of B and Q biotypes, respectively. Panel B: lanes 1–3, reactions with DNA of the same Q biotype individual diluted 1/3, 1/4 and 1/6 respectively; lanes 4–6, reactions with three different adults of Q biotype; lane 7, reaction with a single adult of B biotype; lane 8, reaction with DNA of a pool formed by 1/4 aliquots of the individuals of lanes 4–7.

quality as that when using a complete individual, although some differences were observed in the intensity of the bands at different dilutions (fig. 1B). In the pool made by mixing the DNA solutions of three individuals of biotype Q and one of biotype B, most of the bands of the two biotypes were present. However, a small proportion of bands were too weak to be compared in the amplification of this pool. By contrast, the amplification of the pools made by crushing together 20 individuals was of a better quality. As a consequence, we decided to use this type of pool in the comparison among biotypes.

Two different primer combinations were used for the analysis of 11 populations of *B. tabaci* along with representative populations of *B. medinae* and *B. afer*. The use of the primer combinations *Eco*RI + AAG/*Mse*I + CTG and *Eco*RI + ACT/*Mse*I + CTG allowed the identification of 354 scorable polymorphic bands (99% of the total bands). Faint amplified bands were not considered in this study. AFLP patterns shown by each pooled population are illustrated in fig. 2.

The dendrogram based on genetic similarity data (fig. 3) grouped all the populations of *B. tabaci* in a separate cluster from the other two species, *B. medinae* and *B. afer*. Co-phenetic correlation between the similarity matrix and the co-phenetic matrix was very high (0.95, $P = 0.001$) indicating a very good fit of the cluster analysis. Four groups could be distinguished in the cluster of *B. tabaci*. One formed by populations from the Near East and Indian subcontinent populations, another group formed by biotypes B, Q and the Nigerian cowpea population, a third group formed by the S biotype from Spain and the Nigerian cassava population and a fourth group composed solely of the A biotype from Arizona.

The correlation (using the Mantel test) between the genetic similarity matrices obtained in the present AFLP study and in a previous study made with RAPD–PCR for the biotypes A, B, H, K, M and Q (Guirao *et al.*, 1997) was 0.70 ($P < 0.05$).

Discussion

The results obtained from this study demonstrate the usefulness of AFLPs for the differentiation of *B. tabaci* biotypes. Usually, good quality DNA is required for an AFLP assay (Reineke *et al.*, 1998). Our results show that, although some reproducibility is lost, a six times dilution of the DNA extracted from a single insect with a simple minipreparation is enough to provide good amplification. This simplifies the application of AFLP to the genetic analysis of populations of this insect. The comparison of AFLP and RAPD–PCR indicates that the two techniques provide a similar level of information on genetic relationships. However, AFLP offers better yield and reproducibility.

The results of this work corroborate previous information on the genetic relationship among *B. tabaci* biotypes. The clear separation between *B. medinae* and *B. afer* in relation to *B. tabaci* is quite consistent with the morphological differences shown by these species. *Bemisia medinae* is a species described by Gómez-Menor (1954) and at present it has only been found on Tenerife Island (Canary Islands). On the other hand, *B. afer* is a species with two synonymies (*B. hancocki* Corbett and *B. citricola* Gómez-Menor). It has a worldwide distribution and it is considered to be a complex formed by several populations with different morphologies

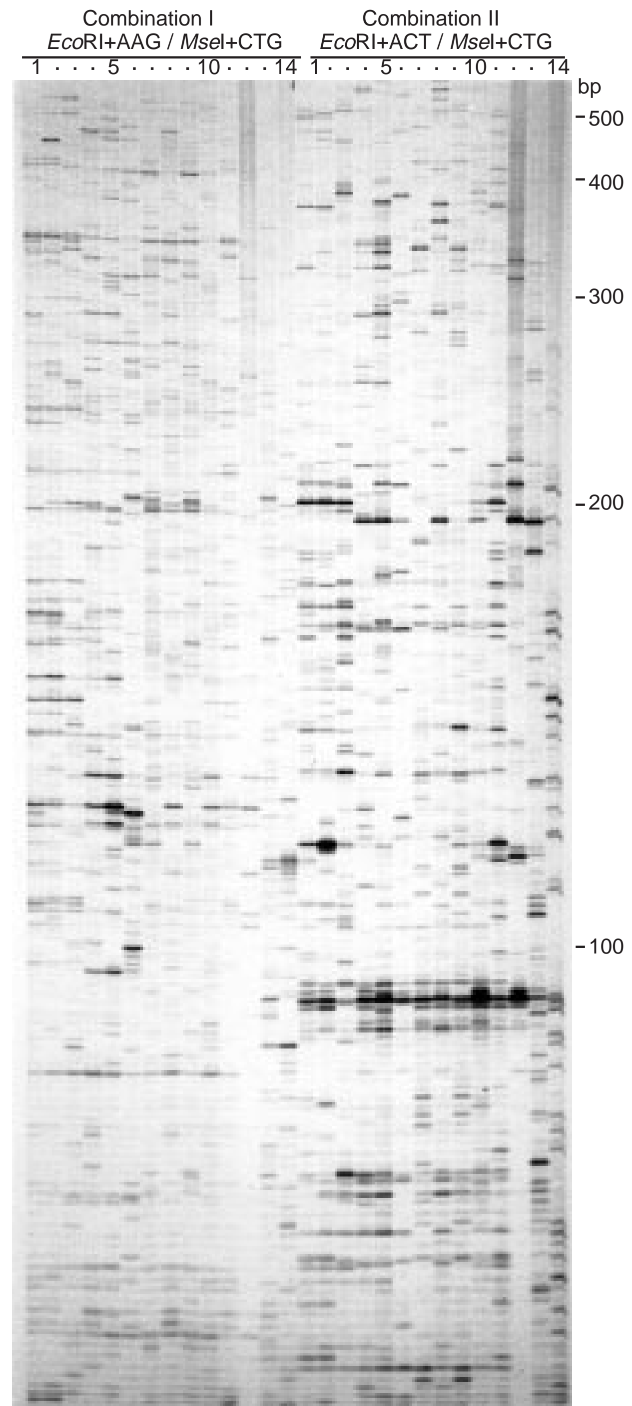

Fig. 2. AFLP patterns obtained with two primer combinations for 11 biotypes and populations of *Bemisia tabaci*, one sample of *B. medinae* and one sample of *B. afer*. Lanes 1–11, reactions with *B. tabaci* populations (in the same order that in table 1); lane 13, *B. medinae*; lane 14, *B. afer*. Lane 12 failed and was excluded from the analysis.

(J. Martin, The Natural History Museum, London, personal communication).

Most of the *B. tabaci* biotypes studied here seem to group

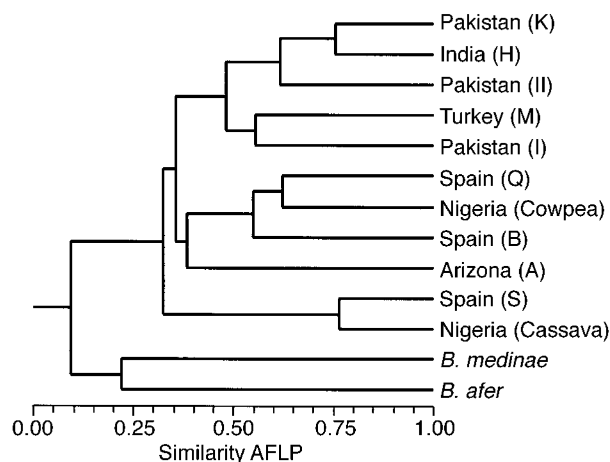

Fig. 3. UPGMA dendrogram based on Dice similarity among 11 biotypes and populations of *Bemisia tabaci*, one of *B. medinae* and one of *B. afer*.

into one of four different geographical areas: the Near East and Indian subcontinent (H, K and M), the Mediterranean Basin (B and Q), equatorial Africa (Nigeria-cassava) and the New World (A). This grouping is consistent with the results obtained after analysis of mitochondrial 16S and cytochrome oxidase I (COI) sequences from *B. tabaci* populations (Frohlich *et al.*, 1999).

The information obtained in this study allows the placement of the two populations collected in Nigeria in relation to previously characterized biotypes. Burban *et al.* (1992) reported the presence in West Africa of one biotype which is almost monophagous, feeding only on cassava and wild eggplant and another that has a broad range of hosts including sweet potato, okra and tomato. These two biotypes could correspond to our cassava and cowpea populations, respectively. A previous study had already shown that the cowpea population, together with other populations collected from sweet potato and tomato, gave a different isoenzyme pattern from those isolated from cassava (Abdulahi *et al.*, 1998). According to the AFLP data, the cowpea population is included in a group with the B biotype and the Q biotype, which is predominant along the south coast of the Iberian Peninsula (Guirao *et al.*, 1997). The cassava population constitutes a different group with the genetically related S biotype, which was collected originally by I.D. Bedford from a wild plant of *Ipomoea indica* (Convolvulaceae) in Nerja (Malaga, Spain) in 1995 and kept as a laboratory colony at the John Innes Centre, Norwich, UK (Banks *et al.*, 1999). However, the distribution in Spain of the S biotype appears to be very limited, since it has not been found elsewhere. The S biotype has also been shown to be genetically similar by mitochondrial DNA studies to other populations collected on cassava in Kenya and Uganda (G. Banks, personal communication).

The information obtained on the populations from Pakistan, together with additional studies made on individual insects of a larger set of populations using RAPD–PCR (Cenis, unpublished), suggests the existence of two additional genetic types to the previously described K biotype (Bedford *et al.*, 1992) in that country. However, these results are still preliminary since the analysed samples included adults collected directly in the field which may have comprised a mixture of biotypes or species.

In conclusion, the results presented here show the usefulness of the AFLP assay in the study of genetic variation of whiteflies, and contribute support to the general picture which is developing on the genetic relationships within the *B. tabaci* species complex.

Acknowledgements

We thank I.D. Bedford (John Innes Centre, Norwich, UK), J.K. Brown, (University of Arizona, Tucson, USA), A. Carnero (Centro de Investigación y Tecnología Agraria, Tenerife, Spain), F. García-Arenal (Universidad Politécnica, Madrid, Spain) and S. Winter (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany), for supplying whitefly populations and helpful comments. This work was funded by the project AGF98–0439-C05–01, from Comisión Interministerial de Ciencia y Tecnología. M.T. Cervera was funded by a postdoctoral fellowship from Comunidad Autónoma de Madrid and B. Simón by a predoctoral fellowship from Fundación SENECA (Murcia, Spain). Support for research at Centro Nacional de Biotecnología was provided through a specific agreement between CSIC and INIA.

References

Abdullahi, I., Atiri, G., Winter, S. & Thottappilly, G. (1998) Differentiation of whitefly (*Bemisia tabaci*) biotypes from different regions in Africa and their relation to the occurrence of cassava mosaic geminiviruses (in German). *Mitteilungen an die Biologische Bundesanstalt Braunschweig* **357**, 284.

Banks, G.K., Bedford, I.D., Beitia, F.J., Rodríguez-Cerezo, E. & Markham, P.G. (1999) A novel geminivirus of *Ipomoea indica* (Convolvulaceae) from Southern Spain. *Plant Disease* **83**, 486.

Bedford, I.D., Briddon, R.W., Markham, P.G., Brown, J.K. & Rosell, R.C. (1992) *Bemisia tabaci:* biotype characterization and the threat of this whitefly species to agriculture. *Proceedings 1992 British Crop Protection Conference – Pests and Diseases* **3**, 1235–1240.

Bedford, I.D., Briddon, R.W., Markham, P.G., Brown, J.K. & Rosell, R.C. (1993) A new species of *Bemisia* or biotype of *Bemisia tabaci* (Genn.) as a future pest of European agriculture. *Plant Health and the European Single Market, BCPC Monograph* **54**, 381–386.

Bedford, I.D., Briddon, R.W., Brown, J.K., Rosell, R.C. & Markham, P.G. (1994) Geminivirus transmission and biological characterisation of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. *Annals of Applied Biology* **125**, 311–325.

Bethke, J.A., Paine, T.D. & Nuessly, G.S. (1991) Comparative biology, morphometrics, and development of two populations of *Bemisia tabaci* (Homoptera: Aleyrodidae) on cotton and poinsettia. *Annals of the Entomological Society of America* **84**, 407–411.

Bird, J. (1957) A whitefly-transmitted mosaic of *Jatropha gossypifolia*. *Agricultural Experimental Station University of Puerto Rico* **22**, 1–35.

Brown, J.K., Coats, S.A., Bedford, I.D., Markham, P.G., Bird, J. & Frohlich, D.R. (1995a) Characterization and distribution of esterase electromorphs in the whitefly, *Bemisia tabaci*

(Genn.) (Homoptera: Aleyrodidae). *Biochemical Genetics* **33**, 205–214.

Brown, J.K., Frohlich, D.R. & Rossell, R.C. (1995b) The sweetpotato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex? *Annual Review of Entomology* **40**, 511–534.

Brown, J.K., Bird, J., Frohlich, D.R., Rosell, R.C., Bedford, I.D. & Markham, P.D. (1996) The relevance of variability within the *Bemisia tabaci* species complex to epidemics caused by subgroup III geminiviruses. pp. 77–89 *in* Gerling, D. & Mayer, R.T. *(Eds)* Bemisia: *1995. Taxonomy, biology, damage, control and management*. Andover, UK, Intercept.

Burban, C., Fishpool, L.D.C., Fauquet, C., Fargette, D. & Thouvenel, J.C. (1992) Host associated biotypes within West African populations of the whitefly *Bemisia tabaci* (Genn.), (Hom., Aleyrodidae). *Journal of Applied Entomology* **113**, 416–423.

Byrne, F.J., Bedford, I.D., Devonshire, A.L. & Markham, P.G. (1995) Esterase variation and squash induction in B-type *Bemisia tabaci* (Homoptera: Aleyrodidae). *Bulletin of Entomological Research* **85**, 175–179.

Cenis, J.L., Perez, P. & Fereres, A. (1993) Identification of aphid (Homoptera:Aphididae) species and clones by random amplified polymorphic DNA. *Annals of the Entomological Society of America* **86**, 545–550.

Cervera, M.T., Gusmao, J., Steenackers, M., Peleman, J., Storme, V., van den Broeck, A., van Montagu, M. & Boerjan, W. (1996) Identification of AFLP molecular markers for resistance against *Melampsora larici-populina* in *Populus*. *Theoretical and Applied Genetics* **93**, 733–737.

Costa, H.S. & Brown, J.K. (1991) Variation in biological characteristics and esterase patterns among populations of *Bemisia tabaci* Genn. and the association of one population with silverleaf symptom induction. *Entomologia Experimentalis et Applicata* **61**, 211–219.

Costa, H.S., Brown, J.K., Sivasupramaniam, S. & Bird, J. (1993) Regional distribution, insecticide resistance and reciprocal crosses between the A and B biotypes of *Bemisia tabaci*. *Insecticide Science and its Application* **14**, 255–266.

Frohlich, D.R., Torres-Jerez, I., Bedford, I.D., Markham, P.G. & Brown, J.K. (1999) A phylogeographical analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. *Molecular Ecology* **8**, 1683–1691.

Gawel, N.J. & Bartlett, A.C. (1993) Characterization of differences between whiteflies using RAPD-PCR. *Insect Molecular Biology* **2**, 33–38.

Gómez-Menor, J. (1954) Aleuródidos de España, Islas Canarias y Africa Occidental. 3ª nota. *Eos* **30**, 363–377.

Guirao, P., Beitia, F. & Cenis, J.L. (1997) Biotype determination in Spanish populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Bulletin of Entomological Research* **87**, 587–593.

Mantel, N. (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research* **27**, 209–220.

Perring, T.M., Cooper, A.D., Rodríguez, R.J., Farrar, C.A. & Bellows, T.S.J. (1993) Identification of a whitefly species by genomic and behavioral studies. *Science* **259**, 74–77.

Reineke, A., Karlovsky, P. & Zebitz, C.P.W. (1998) Preparation and purification of DNA from insects for AFLP analysis. *Insect Molecular Biology* **7**, 95–99.

Reineke, A., Karlovsky, P. & Zebitz, C.P.W. (1999) Amplified fragment length polymorphism analysis of different geographic populations of the gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantridae). *Bulletin of Entomological Research* **89**, 79–88.

Rohlf, F.J. (1993) *NTSYS-PC numerical taxonomy and multivariate analysis system*. Version 1.8, Setauket, Nueva York, Exeter.

Sneath, P.H.A. & Sokal, R.R. (1973) *Numerical taxonomy*. San Francisco, California, Freeman and Company.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. & Zabeau, M. (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* **23**, 4407–4414.

Williams, J.G.K., Kubelik, A.R., Livack, K.J., Rafalsky, J.A. & Tingey, S.V. (1990) DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18**, 6531–6535.

Wool, D., Gerling, D., Belloti, A.C. & Morales, F.J. (1993) Esterase electrophoretic variation in *Bemisia tabaci* (Genn.) (Hom., Aleyrodidae) among host plants and localities in Israel. *Journal of Applied Entomology* **115**, 185–196.

(Accepted 19 May 2000)
© CAB *International*, 2000