

cADPR, AMP, ADPR, and NAD in a single chromatographic step. Fractions were collected and counted for radioactivity.

35. NADase activity was measured by separation of released [carbonyl-¹⁴C]nicotinamide from NAD with Bio-Rad AG X4 anion exchange resin. The assay mixture (50 μ l) contained 10 μ l of protein fraction and 200 μ M [carbonyl-¹⁴C]NAD (50,000 cpm) in buffer A (16) and was incubated at 37°C for 5 min. The reaction was terminated by addition of 1 ml of 0.1% SDS. The sample was applied to a 0.4-ml column of AG X4 resin, which was subsequently washed with 4 ml of deionized water. The flow-through and wash fractions were combined, and [¹⁴C]nicotinamide was determined by liquid scintillation counting.

36. ADPR cyclase activity was determined by addition of 10 μ l of enzyme fraction to 40 μ l of 250 μ M [³²P]NAD (0.5 \times 10⁶ cpm) in buffer A (16). After incubation at 37°C for 10 min, the reaction mixture was treated with phosphodiesterase (0.1 U), diluted, and applied to a 0.4-ml column of DHB Bio-Rex 70 equilibrated with buffer B (16). After washing of the column with 30 ml of buffer B, [³²P]cADPR was eluted with 5 ml of deionized water and quantitated by liquid scintillation counting.
37. Supported in part by NIH grant CA43894 and NSF grant DMB 891724. We thank D. M. Payne for helpful discussions.

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TECHNICAL COMMENTS

Determining Whitefly Species

The statement in the report by T. M. Perring *et al.* (1) that the “superbug” is not a strain of the sweetpotato whitefly, *Bemisia tabaci* (Gennadius), but a new species, seems premature. When more than 25 pairs of males and females of both strains were placed together, interstrain mating resulted in the production of viable, hybrid females (2). Field collections made in the Imperial Valley of California in 1992 revealed that feral populations of the two strains had interbred. Hybrid whiteflies that had fixed (not induced) esterase loci from both “A” and “B” strain parents were clearly identified (2–4).

Perring *et al.* used single primer polymerase chain reaction amplification (RAPD-PCR) and found that genetic differences between the strains were at a “species” level, but RAPD-PCR fragments have revealed only arbitrary differences between the DNAs. “Genetic distances” of a size similar to those between *B. tabaci* strains are likely to be observed if either strain is compared to RAPD-PCR fragments generated from any number of randomly selected taxa (for example, another whitefly strain or species, dogs, or nematodes). The RAPD-PCR results in the report by Perring *et al.* are of potential diagnostic value, but of little phylogenetic utility.

When one of us (B.C.C.) compared more than 2000 nucleotides of genes in the ribosomal RNA (rRNA) transcript from *B. tabaci*, which included three variable expansion regions, the rDNA in those strains was identical (5, 6). Sequences of 28S rDNA D2 expansion regions (550 nucleotides) have been found to be identical in the *B. tabaci* strains, whereas 40 nucleotide substitutions have been found in ash and greenhouse whiteflies (5). The D2 expansion region has been used to deduce phylogenies of subgenera and sibling species of *Drosophila* (7). Whiteflies also have uncom-

monly elongated (\approx 2450 nucleotides) 18S rDNAs (6). This extra length stems from two internal, variable expansion regions (8). The 18S rDNA of the two *B. tabaci* strains has been found to be identical, whereas 60 to more than 100 nucleotide substitutions have been found in ash, iris, and greenhouse whiteflies (5, 6). Sternorhynchans (for example, aphids and whiteflies) have maternally heritable, procaryotic endosymbionts (9). An earlier study of endosymbiont 16S rDNA found that aphid endosymbiosis resulted from a singular infection of a primordial ancestor during the Triassic. Since that time, aphids and their endosymbionts have cospeciated, resulting in congruent phylogenies (10). Whitefly endosymbiosis follows a similar congruency, wherein endosymbiont 16S rDNA distinguishes whitefly species (6, 11). Both strains of *B. tabaci* have two endosymbionts. The nucleotide sequences of 16S rDNAs (\approx 1600 nucleotides each) of the respective endosymbionts have been found to be identical in the *B. tabaci* strains, whereas 70 nucleotide substitutions have been found in greenhouse and ash whiteflies (11). In summary, our mating and phylogenetic studies do not support the conclusion of Perring *et al.* that the “superbug” is a new species of whitefly.

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Perring *et al.* (1) interpret the results of experiments on two forms of *B. tabaci* to mean that the two forms are distinct species. This conclusion seems premature because the results are not only preliminary, but unconvincing. Their estimation of differences between populations, based on the genetic distance measurements, is not appropriate for taxonomic purposes. The standard error of the Nei statistic depends on both the numbers of loci sampled (eight polymorphic loci were reported) and the sample size (which was not reported). It is not possible to judge the significance of the reported value without some estimate of the standard error of the calculated genetic distance. Perring *et al.* give a range of genetic distances between whitefly species, of 0.24 to 0.83. Which value really separates whitefly species? Perring *et al.* give no indication of whether the populations were in Hardy-Weinberg equilibrium (an assumption for the calculation of the Nei statistic), and the genetic differences they report could be a result of laboratory selection or founder effects.

Perring *et al.* find no evidence of mating between the A and B biotypes (1). Other researchers (2) have observed A \times B hybrids when mating was attempted under conditions different from those used by Perring *et al.*

Products of RAPD-PCR are useful as genetic markers and for the construction of molecular genetic maps (3). However, we are not aware of any agreement in the literature about the interpretation of RAPD data for insect taxonomy. Our own unpublished data indicate that whiteflies (morphologically typical of *B. tabaci*) collected from several areas of the world show RAPD-PCR differences as great as those found between the A and B forms [as defined

by esterase patterns (4)]. We do not know if these differences define taxonomic status, but the data show that complex genetic differentiation exists in whitefly populations. An examination of the 18S rDNA nucleotide sequence indicates no specific difference between the A and B forms (5).

As far as we can determine, there have been no scientific studies showing that *B. tabaci* exhibits haplo-diploid reproduction. Perring *et al.* cite Byrne and Bellows (6) and Mound (7) in this respect. Byrne and Bellows cite Mound. Mound does not give data supporting this point and, in fact, states that the mechanism controlling sex ratio is unclear (7, p. 305).

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Response: We welcome the opportunity to reply to the comments of our colleagues. We presented (1) data from four independent experiments (two were designed to investigate putative reproductive isolation between the whiteflies and two were designed to elucidate genetic differences), all of which corroborated our conclusion of two distinct species.

Campbell *et al.* and Bartlett and Gawel say that interstrain mating of whiteflies results in viable, hybrid females. To prove this, males of one known type would have to be paired with virgin females of the other type (much like the work in our study), and the viability of "hybrid" F1 females would need to be evaluated by individually mating virgins to males from both whitefly types to determine whether females are produced in the subsequent F2 generation. A "batch" crossing study of the type mentioned by Cam-

pbell *et al.* does not answer this question.

Campbell *et al.* state that field collections revealed that the two strains of whiteflies interbred, as indicated by esterase patterns. Two of the three references cited for this work (their references 2 and 3), are in nonrefereed proceedings from conferences, and we do not know their content. Their reference 4 states that both species were found in the field in the fall of 1990, but only the silverleaf whitefly the following spring (2). These results are consistent with our allozymic analyses of whiteflies collected throughout the southern United States, Mexico, Egypt, and Spain, in which we have found only two whitefly types.

Campbell *et al.* used only esterase banding patterns to identify "hybrids." With electrophoresis most enzymes exhibit a simple co-dominant Mendelian pattern of inheritance, which makes the patterns ideal for use as genetic markers. A few enzymes, such as the esterases and general proteins, lack this genetic reliability, as noted by Richardson *et al.* (3); others have found five variants of banding patterns for sweetpotato whitefly and one similar pattern for silverleaf whitefly (4, p. 215).

Campbell *et al.* argue against the use of PCR data and discuss their results with rDNA analyses. Their presentation (5) of homologous 16S and 18S regions of the endosymbiotic bacterial and whitefly genomes, respectively, in the two whiteflies are not necessarily inconsistent with our findings. These genes are highly conserved and may not distinguish closely related whiteflies that may have speciated recently. We know of no data on the separation of closely related whitefly species obtained with rDNA analyses.

RAPD-PCR has been used to delineate taxa of bacteria (6), fungi (7), nematodes (8), and other life (9). In the organisms analyzed so far, intraspecific similarity ranges from 80 to 100%, while interspecific similarities range from 0 to 30%. Analysis by RAPD-PCR (1) of different populations of the two whiteflies showed intraspecific similarities greater than 90%; interspecific similarity was less than 10%.

The initial point raised by Bartlett and Gawel concerns the estimation of population differences based on genetic distance measurements. We used (1, 10) 14 enzyme stains to examine 18 loci; polymorphisms were present for eight of the enzymes. For each enzyme, 10 individual whiteflies from each of 17 populations were analyzed. We found an interspecific genetic distance value of 0.24, which was calculated by pooling allele frequency values of populations within each species (11 populations of silverleaf whitefly and 6 of sweetpotato whitefly) and comparing these pooled values. Because populations were pooled, we did not calcu-

late a standard error. Intraspecific values were 0.040 between populations of the sweetpotato whitefly and 0.014 between those of the silverleaf whitefly. Again, because we took only the most disparate populations (providing the most extreme measure of intraspecific variation), we did not estimate variance around the value for genetic distance.

To address the concerns of Bartlett and Gawel, we have performed an analysis without pooling the data (11). None of these figures were markedly different from the values published in our report; they fell well within the ranges established by Nei (12) for species and populations, respectively. More important is the fixed allelic differences that were present at three separate loci (EST IV, PGI, and PGM). Fixed differences appear when two species do not share any alleles at the locus and indicate that gene transfer is not occurring (3).

Bartlett and Gawel raise the issue of whether the populations used in our studies were in Hardy-Weinberg equilibrium, suggesting that genetic differences could have resulted from laboratory selection or founder effects. The best evidence against this is the similarity that we found in allelic frequency across the potentially diverse silverleaf whitefly populations; this diversity was described in table 2 of our report (1, p. 75). We expected genetic differences between the JD colony and our other five colonies as a result of selection pressures of the different culture conditions and possible founder effects. Yet, although there was a slight difference in allelic frequencies of the polymorphic loci, the JD population had the same fixed alleles as the others. Individuals from these spatially separated cultures copulated, producing female offspring. Individuals from these populations could not successfully cross with the silverleaf whiteflies.

Bartlett and Gawel point out a citation error in our report with respect to haplo-diploid reproduction. The incorrect citation (contained in note 16 of our report) of a study by Mound (13) was a result of our using information in a paper by Lopez-Avila (14, p. 11), who stated that

Virgin females of *B. tabaci* lay eggs which give rise only to males (Azab, Megahed & El-Mirsawi 1972; Husain & Trehan 1933; Mound 1983; Sharaf & Batta 1985).

Mound's 1983 paper should not have been part of this list; the other citations are properly placed.

Bartlett and Gawel question whether *B. tabaci* exhibits haplo-diploid reproduction. We obtained only male offspring from unfertilized females and only female offspring from fertilized females. Our data corroborate the results of many other studies (15).

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