*Bemisia*: Bionomics and Management of a Global Pest
Bemisia: Bionomics and Management of a Global Pest
This book is dedicated to Dr. Jacquelyn (Jackie) L. Blackmer (1954–2008), a co-author of Chapter 5 and a renowned entomologist in the field of insect behavior, insect-plant interactions, and insect dispersal and migration. During postdoctoral work at The University of Arizona and her most recent tenure with the United States Department of Agriculture, Agricultural Research Service in Arizona she made numerous advances in understanding the behavior, physiology and ecology of *Bemisia tabaci*. Dr. Blackmer conducted some of the first and still the most comprehensive studies on the migratory and short-range flight behavior of *Bemisia tabaci* including the role of environment, physiology, morphology, life history and host quality. She advanced our understanding of host quality factors in whitefly life history, developed an artificial diet system, and examined the flight behavior of whitefly parasitoids. She was a tireless, innovative and inspirational researcher as well as being a generous collaborator, colleague and friend to all with whom she worked. Her significant contributions to entomology will be long remembered and will continue to inspire advances in whitefly biology, ecology and management.
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Introduction

Philip A. Stansly and Steven E. Naranjo

*Bemisia tabaci* (Gennadius) has distinguished itself from the more than 1,000 whitefly species in the world by its adaptability, persistence and potential to damage crops of all types. Its only rival for this distinction is perhaps the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood, which fills the niche of polyphagous whitefly pest in temperate regions, while *B. tabaci* reigns supreme in the tropics and subtropics. Nevertheless, proliferation of protected agriculture and mass movement of plants and produce have extended the range of *B. tabaci* into most of the temperate regions of the world as well. *B. tabaci* stands out against any whitefly rival in a number of other aspects: host range, virus vectoring ability, damage potential that includes physiological disorders and honeydew associated quality issues, an ability to adapt morphologically to its host, and the existence of multiple sibling species, just to name a few. These and many other characteristics of *B. tabaci* are examined here in depth.

First named *Aleyrodes tabaci* and described from tobacco in Greece (Gennadius 1889), the insect now known as *Bemisia tabaci* was soon afterward reported from Florida as *A. inconspicua* (Quaintance 1900). The “tobacco whitefly” later solidified its pest status in that crop as the vector of tobacco leaf curl disease in East Africa (Storey 1931) and Indonesia (Thung 1932). At the same time, the self same “cotton whitefly” was recognized in West Africa and Sudan as the vector of cotton leaf curl disease (Golding 1930; Kirkpatrick 1931), which had been reported from Nigeria two decades earlier (Farquharson 1912). The most effective control for both these epidemics was found to be timely removal of the principal inoculum source, the previous year’s crop residue (Hopkins 1932; Tarr 1951).

The cotton/tobacco whitefly soon began to be reported as a pest in its own right, first in India (1929), and subsequently in the Sudan (1950s), El Salvador (1961), Mexico (1962), Brazil (1968), Turkey (1974), Israel (1976), Thailand (1978) and Arizona and California (1981), mostly on cotton (Horowitz 1986). In many cases, whitefly infestations reached outbreak proportions, a situation attributed to various causes including improper use of insecticides, climate, and increased agricultural intensification.

The number of plant species known to host *B. tabaci* also proliferated, especially after Takahashi (1936) and Russell (1958) synonymized it with some 18 other described species. The large number of synonyms was in part due to host induced
morphological variation (Mound 1963; Neal and Bentz 1999). Mound and Halsey (1978) listed 302 species in 74 families to which Greathead (1986) added 204 more to make the widely quoted total of 506. The list continues to expand as the insect invades new areas and more records are collected in regions where it has established. The largest numbers in Greathead’s list are in Leguminosae (56) and Compositae (33), but these are also the two largest plant families. The greatest percentage of species within plant families known to serve as hosts for B. tabaci is attributed to the Malvaceae (3.5%) followed by the Curcubitaceae (2.7%), Euphorbiaceae (1.7%), and Convolvulaceae (1.7%). With the addition of the Cruciferae, this list includes most crops on which B. tabaci is a serious pest.

It was not until 1986 that Florida experienced outbreaks of what is now known as Bemisia tabaci biotype “B”, first in greenhouse poinsettia, then in a wide diversity of vegetable, ornamental and agronomic crops throughout the state. The number of reported outbreaks soon accelerated around the globe. The old biotype “A” of more limited host range, totally disappeared in the USA and damage attributable to the new biotype was estimated at 500 million $US in 1993. The subsequent worldwide outbreak of biotype B stimulated greater attention to whiteflies in general, and B. tabaci in particular, from the agricultural, research and extension communities.

Our goal with this volume is to follow up on two previous books edited by Dan Gerling: Whiteflies: their Bionomics, Pest Status and Management (1990, ISBN 0-946707-16-2) and Bemisia: 1995 – Taxonomy, Biology, Damage, Control and Management (1996, ISBN 1 898298 33.5). The latter, co-edited by Richard Mayer, consisted of proceedings from the first International Bemisia Workshop that took place in Israel in 1994. The present volume is a somewhat belated follow-up of the 4th International Bemisia Workshop that took place in Florida, December 2006.

It was perhaps fitting that we met in Florida, 20 years after the onset of the latest and perhaps most dramatic chapter in the history of encounters between whiteflies and humans. Much has been learned about Bemisia in the interim, but as is usually the case, we only find that there is much more yet to discover. The arrival to the USA, Australia and China of biotype “Q”, originally from in the Middle East and southern Europe and thought to be especially resistant to insecticides, sets the stage for another epic struggle among biotypes, the outcome of which may again be mitigated by effective management. Only a deepening knowledge of genetics, biology, interactions with hosts, natural enemies, vectored viruses, and responses to chemicals and environmental conditions (bionomics) will provide the tools needed for satisfactory management. Thus, the broad focus of this book, from basic to applied.

Our specific objective is to provide a review of Bemisia taxonomy, genetics, biology, ecology and management, focusing mostly on progress during the last 10–15 years, and directed at workers in the field as well as the informed professional who may not necessarily specialize in whitefly research. We have divided this review into 5 sections: (1) Taxonomy and Genetics, (2) Biology and Ecology, (3) Epidemiology of Whitefly Transmitted Viruses, (4) Management, and (5) Genomics, in a total of 18 chapters. This structure is in keeping with our goal of providing a broad but thorough review of the subject matter. Each section has its own section editor(s), all leaders in their fields. Section editors were responsible for writing an introduction
to each section, and working with the chapter authors on content and style. It is our
pleasure to help bring this work to fruition and also provide this short introduction
to what has been truly a group effort.

Following an introduction by Brown, Section One opens with an overview by
Gill and Brown of whitefly systematics at the generic level, including an evolu-
tionary history and description of the \textit{B. tabaci}, and \textit{B. afer} complexes as well as \textit{Lipaleyrodes} and other \textit{Bemisia}-like species, posing the question: Can molec-
ular techniques solve the \textit{Bemisia tabaci} complex conundrum? Chapter 2 by
Brown examines the phylogenetic biology of the \textit{B. tabaci} sibling species group
that has so occupied evolutionary biologists as well as management practition-
ers since the first biotype B outbreaks were recorded in the 1980s. Chapter 3 by
Hadjistylli et al. describes the toolbox of molecular methods that are being used
to unravel the genetic complexities and relationships within the \textit{B. tabaci} species
group.

Section Two provides an overview of the biology and ecology of this fascinating
and complex insect. After an introduction by editors Naranjo and Legg, we find a
thorough discussion of life history, functional anatomy, feeding and mating behavior
by Walker et al (Chapter 4) which includes many outstanding illustrations, but still
comes to the unsettling conclusion (as in many of the chapters) that although much
is known, much remains to be discovered. Rosell et al. (Chapter 5) take us into the
bewildering world of whitefly symbionts and their myriad effects on whitefly life
history, behavior and virus transmission. They also review our current understand-
ing of the interaction of \textit{Bemisia} with other herbivores, mediated by the host plants
they share. Chapter 6 by Naranjo et al. places our whitefly into its environment,
describing demographics as revealed by laboratory and field-based life tables, spa-
tial and temporal dynamics, sampling methods, population models and invasion and
outbreak mechanisms.

The worst damage from \textit{B. tabaci} infestation is usually as a consequence of
its role as virus vector. Editors Lapidot and Polston open Section Three with an
introduction to set the stage for a discussion of these relationships with empha-
sis on biology and epidemiology of different whitefly transmitted viruses. The
\textit{Geminiviridae} are covered by Legg (Chapter 7) with a review of the cassava
mosaic viruses, Moriones and Navas-Castillo (Chapter 8) who discuss \textit{Tomato yel-
low leaf curl virus} (TYLCV), and Morales (Chapter 9) who provides a general
review of begomoviruses in Latin America. Wintermantel (Chapter 10) reviews
the \textit{Criniviridae}, and Adkins and colleagues (Chapter 11) briefly discuss the
\textit{Ipomoviridae}. The section concludes with a review of evidence for transovarial
transmission of TYLCV by Accotto and Sardo (Chapter 12), a truly daunting
prospect.

Following an introduction to Section Four by editors Gerling and Horowitz, the
authors examine progress made during recent years in perfecting the components of
\textit{Bemisia} IPM: optical manipulation (Anignus, Chapter 13), host plant resistance
(Nombela and Muñiz, Chapter 14), biological control (Arnó et al., Chapter 15) and
insecticides examined through the lens of resistance in ecological terms (Castle
et al., Chapter 16). Stansly and Natwick (Chapter 17) wrap up the section by
reviewing the integration of these components into biologically based management systems for protected and open field crops.

The final Section Five consists of a single chapter by Czosnek and Brown, which in some sense projects the previous 17 chapters into the future by describing the potential of genomic methods to further illuminate many of these same areas of research. Overall, we hope our efforts may serve to advance basic and applied research on Bemisia for some time to come, leading ultimately to solutions for one of the most significant agricultural pests, worldwide.

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Introduction

The *Bemisia tabaci* (Gennadius) complex is one of the most agriculturally important insect pests worldwide, predominately as a vector of the plant virus genus *Begomovirus* (Family, Geminiviridae). The increased importance of begomoviruses as emerging viral pathogens is directly related to the adaptability of certain *B. tabaci* haplotypes – and biotypes – to agricultural cropping systems, which are increasingly expanding into whitefly habitats.

This whitefly complex has attracted attention because of its unusually plastic or variable phenotypic traits, including host range, environmental adaptation, fecundity, and variable dispersal behaviors. Also, the *B. tabaci* complex has long confounded systematists owing to lack of morphological characters that can be linked to diverse phenotypes. Members of the *B. tabaci* complex further vary with respect to composition of secondary endosymbionts that are thought to contribute to certain aspects of fitness. The suite of phenotypic characters of certain biological types closely aligns with recognized invasive behaviors. Particularly invasive *B. tabaci* often exhibit resistance to certain insecticides used in agricultural production systems, which similarly, seems related to the inherent plasticity of the species.

Although distinct biotypes and haplotypes may be equally plastic and adaptive in different environments, some have a greater capacity to become invasive; others are moderately problematic, or even benign. Likewise, certain biotypes/haplotypes have an extensive or only a moderate range of host species, while others are host-specific, or nearly so. Monophagous and polyphagous biological variants are potentially equally damaging as virus vectors, depending on vector phenotypes and crop (or non-crop) hosts involved. The *B. tabaci* complex is poorly studied in genetic and genomics terms; whether behavioral traits are solely expressed in a gene for gene manner, and/or if the different phenotypes are the result of differential gene expression and probably epigenetic regulation is not known, mainly because tools are lacking with which to conduct functional genomics analyses.
A comparison of the sequences for the mitochondria cytochrome oxidase I gene (mtCO1; 780 bases) and a nuclear intron from the whitefly nuclear voltage-gated sodium channel gene (850 bases) for the Sida (moderately fecund, polyphagous) and Jatropha (monophagous, low fecundity) biotypes sympatric in the Caribbean region, diverge by less than 1% (mtCO1), and are otherwise indistinguishable (unpublished data). Although not entirely conclusive, these haplotypes (biotypes) appear to be very closely related but are known to be differentially host-adapted, although these differences are not correlated with notable genetic variation.

Unexpectedly, phylogenetic variation (mtCO1) for worldwide B. tabaci was found to range between 0 and 26%, making the gene somewhat useful for haplotype taxonomic classification despite its saturation, which precludes assignment of a basal clade for the B. tabaci complex. Phylogenetic inferences based on the mtCO1 sequence delineate ~6 major clades (depending on nodes used for demarcation) of B. tabaci that group phylogeographically, each with sister clades.

Although much emphasis has been leveraged on the invasiveness of the B. tabaci complex, only a few haplotypes have been truly invasive following their transport to exotic locales by people. As expected, they are not phylogenetically aligned with the haplotypes endemic to the region. These invasive haplotypes are the B (introduced worldwide, 1980s) and Q (introduced, 2005-onward) biotypes, and the (holotype) B. tabaci from Greece (first recorded outbreak, 1889), the latter, responsible for the first documented outbreak in a cultivated crop – e.g. tobacco – and the impetus for naming the species. Unexpectedly, the Greek mtCO1 haplotype of adults recovered from leaves archived in the British Museum may have originated in Asia, perhaps arriving as a stow-away aboard a cargo ship bringing goods from Asia to Europe via the Mediterranean Sea (Gill and Brown Chapter 1; Brown Chapter 2).

From the turn of the century to the 1960s, outbreaks of B. tabaci became increasingly more frequent in vegetable and fiber crops in the tropics and subtropics. Virus-like diseases became problematic in cotton in Sudan, in vegetable crops in India, and in cassava throughout Sub-saharan Africa. In Brazil, whitefly-transmitted viruses caused damage to soybean and tomato crops, and throughout Latin America and the Caribbean Basin bean crop yields were reduced by Bean golden yellow mosaic virus infection. In the Southwestern USA, Cotton leaf crumple virus negatively affected lint quality and reduced cotton yields. The literature reveals that much attention was centered on pesticide solutions to reduce whitefly and virus-induced crop damage, and reports of the ineffectiveness of certain pesticides emerged as a common theme. Since then, the economic importance of B. tabaci as a pest and vector has continued to increase, and research efforts began to provide new knowledge about this phloem-feeding insect.

In subsequent years, both endemic and introduced B. tabaci have continued to increase in importance in agricultural systems as their magnitude and prevalence in irrigated crop production systems have become commonplace, particularly in tropical and subtropical regions and in temperate greenhouse production areas. Spill-over from the field into ornamental crops resulted in the infestation of additional plant species and their addition to the host list for B. tabaci, presently topped off at over 500 species. In addition, at least two haplotypes have reached invasive status,
after having been transported to exotic locales on ornamental plants. The worldwide introduction and rapid establishment of the pesticide resistant biotype B beginning in ∼1980 (B. Kumashiro and R. Gill personal communication) further underscored the potential significance of *B. tabaci*. Most recently, whitefly-transmitted viruses have been introduced into non-native locations where they have established, and in most instances, have been found to cause greater damage than their endemic counterparts.

Consequently, *B. tabaci* and the viral pathogens it transmits are no longer restricted to native habitats or contained by natural geographic boundaries. Increased monoculture cropping, reduced genetic variation in cultivated species, and widespread use of insecticides in agriculture, together with international transport of infested plants are substantial contributing factors to the global significance of both indigenous and exotic *B. tabaci* and the plant viruses they transmit.

The three chapters in Section I address the current state of knowledge about the systematic of *B. tabaci* with biological-genetic, and classical and molecular taxonomy perspectives (Gill and Brown Chapter 1). Also addressed is the status of population genetics research, which is essential for revealing important insights in population structure, clarifying evolutionary patterns, and providing new insights into the extent of gene flow within the complex (Brown Chapter 2; Hadjistylli et al. Chapter 3). In all arenas, greater research and understanding is needed.

The invasiveness of several main exotic and/or indigenous biotypes of *B. tabaci* – e.g. A and B, and most recently Q biotype – has contributed widely to raised awareness of the economic importance of this whitefly and viruses it transmits to food, fiber, and ornamental crops. The unique biology of the *B. tabaci* complex, and a quest to elucidate the basis for conserved virus-vector interactions have become of increasing interest to the scientific community. Whether *B. tabaci* will continue to be thought of as a “complex of genetic and phenotypic variants”, or as a number of separate species, is still not clear. Even so, comparative biological investigations, together with molecular and population biology approaches, are now crucial to addressing a number of central questions. Recent studies have engaged multiple disciplines with diverse perspectives, each contributing new and exciting knowledge. The authors hope that these writings will inspire continued transdisciplinary collaborations and creative dialog.

The last Section (V; Chapter 18) in this book is particularly relevant to Section I in that it underscores the crucial need for a *The Whitefly Genome Project* and the associated functional genomics tools to facilitate answers to many unresolved questions. It is anticipated that the collective knowledge and dedication expressed in this book will garner enthusiasm, provide clearer rationale, and forge in-roads to acquire the support needed to develop a genomics toolbox, now essential for devising new technologies and advancing old paradigms. That the functional genomics (and other “omics”) studies are hindered because the *B. tabaci* genome sequence has not been determined seems unfathomable. At a time of world food shortage and with the impending effects of global change on food production, it seems unlikely that a more relevant biological and genetic indicator could be identified. We envision an undertaking analogous to “The *Drosophila species*” sequencing project, with *B. tabaci*
serving as the first “agricultural model study system”, a tractable and exciting undertaking given the abundant phenotypic and genetic attributes already known and the threat of prevalent pest outbreaks. Collectively, we look forward to solution-driven research directed by such a full blown genome project for the *B. tabaci* complex, and to highlighting those significant inroads in future communications.
Chapter 1
Systematics of Bemisia and Bemisia Relatives: Can Molecular Techniques Solve the Bemisia tabaci Complex Conundrum – A Taxonomist’s Viewpoint

Raymond J. Gill and Judith K. Brown

Introduction

The whitefly, Bemisia tabaci (Gennadius), has a long history as a serious pest of agriculture worldwide. One of the most serious attacks began in 1985–1986 in Florida where very heavy populations infested ornamental and crop plants. By 1990, it had reached the fertile desert croplands of southern Arizona and California, where clouds of whiteflies could be seen flying over the desert valleys. Crops were destroyed before they could produce fruit, and the resultant infestation totally changed the cropping procedures and methods that had been used in these desert valleys for years. However, sweetpotato whitefly had previously caused economic losses to many crops in these valleys in the years 1980–1985, largely due to increased population levels and the transmission of closteroviruses to the plants. But in 1990, the situation changed. Much heavier populations of the whitefly developed, and although closteroviruses disappeared from the scene, conditions in the valleys became extremely serious with losses to growers of many millions of dollars, in addition to loss of jobs and livelihoods for farm workers and others associated with agriculture in these areas. For further information on these events see Gill (1992).

History of the Problem

The different responses of these two separate population explosions in the southwestern desert suggested that possibly two species were involved under the name B. tabaci. By 1991 at least, molecular research and differing biologies had begun to suggest that there were in fact major differences between these two populations that eventually became known as biotype A for those that had occurred in the southwestern desert areas of the United States prior to 1990, and the B biotype for populations

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that occurred in Florida starting in 1985–1986, and found on poinsettia plants in Arizona in 1986–87, and after 1990 in the desert valleys. This eventually led to the species description of biotype B as the silverleaf whitefly, *Bemisia argentifolii*, by Bellows et al. (1994). At the same time, several laboratories were already demonstrating the existence of many different populations around the world, referred to as biotypes in addition to the A and B populations. Therefore the naming of this new species has been soundly refuted, with eventually the species being suggested by Brown et al. (1995) as a member of a sibling species complex, and by DeBarro et al. (2005) as a race of *tabaci* and thus a synonym of it. Part of the reasoning behind the synonymy is that there are basically no morphological characteristics of *tabaci* whitefly populations that can distinguish them as distinct species. Part of the basis for the original description of *B. argentifolii* was a slight difference in the occurrence of a single minute seta on the cephalothoracic submargin of the pupal usually being present in A populations and usually not in B populations. This seta is also usually lacking in most other biotypes of *tabaci*, as will be explained later.

**The Problem**

Similar issues with setal placement and population separation exist within another species, *Bemisia afer* Priesner and Hosny, as well as many other details regarding the species and generic placement of other whiteflies. Since morphology is considered the foremost basis for species separation by all systematists, one would assume that the case for synonymy within the *B. tabaci* group of populations should be an easy decision. However, this is not the case, as several important taxonomic problems exist because, as a result of morphological studies of *Bemisia* species and others, it appears that morphological characteristics of many whitefly species are very poorly understood (Martin 2003).

Much has been discussed over the years concerning plasticity in the morphology of whitefly pupae. Several authors have mentioned this observation, including Husain and Trehan (1933) and Deshpande (1933). Russell (1948) showed morphological variation within species of the genus *Trialeurodes* based on the host plant, and Mound (1963) conducted rearing experiments with *B. tabaci* showing that pupal morphology varied with the character of the leaf surface. David (1987) summarized these findings and added other examples, particularly in species occurring in India, and Sundararaj and David (1992) discussed host-correlated variation in *Dialeurodes kirkaldyi* (Kotinsky). In the same light, Mohanty and Basu (1986) suggested that pupal morphological variations could be affected by temperature and humidity as well. There is some evidence, based on *B. afer*-like forms from the Macaronesian Islands, that host plants may affect species morphology in ways other than through leaf hair topology.

With the advent of molecular based investigations into whitefly species relationships and phylogeny, it has come time to analyze some of the problems with the current genus/species relationships within the Aleyrodidae. Due in part to this
pupal plasticity, it appears that there is a lot of confusion, not only regarding species limits, but generic levels of classification as well. Mound (1984) suggested that several larger generic groups represent arbitrary units consisting of similar appearing species rather than evolutionarily related forms, and further adds that many species are ill defined and probably represent host-induced forms or localized strains. While this problem occurs throughout the family, much work is being done currently on the genus *Bemisia* and its apparent relatives and on other species and genera that have at least a close similarity in pupal case morphology.

In research stemming from the introduction of the B biotype of *B. tabaci* into the United States and the subsequent astronomical losses in agriculture in that country, it was shown by Neil and Bentz (1999) that morphological changes in the pupal development of *B. tabaci* and *Trialeurodes vaporariorum* are influenced by the environment surrounding the hatching first instar nymphs. If the surrounding leaf surface of the host is smooth and clean, *tabaci* nymphs show little or no development of long dorsal setae. However, if the leaf surface is hairy, if there are a lot of dirt particles, or there are other instars or pupae and the as yet unsettled nymph bumps into these objects, long dorsal setae of varying numbers probably will be produced. For *T. vaporariorum*, long wax spine-like extrusions are produced by papilla-like dorsal pores if the host has many leaf hairs, but the extrusions and papillae do not necessarily form if the host leaf is smooth. The resulting differences in morphological appearance of *B. tabaci* are considered to be the cause of the large amount of synonymy of *tabaci* over the years. Russell (1957) published a list of these synonyms, and this list was followed by a list in Mound and Halsey (1978) in their catalog work.

With the introduction of the B biotype (Costa and Brown 1990, 1991; unpublished data) into the United States, and its appearance in economically damaging populations in Florida in 1986, taxonomists were aware that this whitefly was acting differently from populations of *B. tabaci* that had occurred in that state prior to the arrival of B, but that there appeared to be no discernable morphological differences. It was not until later that work by Brown et al. (1992, 1995a, b, 2000), Coats et al. (1994), Costa and Brown (1990, 1991), Costa et al. (1993), Perring et al. (1992, 1993), and others, showed molecular differences (Gawel and Bartlett 1993), with the designation then of the A and B biotypes for those populations in the USA (Brown et al. 1995a, b; based on Costa and Brown 1991, Costa et al. 1993). Later, numerous other biotypes were added to the list, with the Q biotype also causing considerable economic problems to agriculture (Dennhey et al. 2006).

After a major meeting of scientists in Dallas, Texas in 1990 to discuss control methods for this new problem with *tabaci*, it was determined that a study should be made into the morphology of *tabaci* and its apparent relatives and simultaneously look at molecular aspects of *tabaci* and other closely related species. This study would hopefully shed some light on the possible origins of this particular biotype, and could determine if in fact more than one species was present as a complex. Partial results in this study of the molecular nature of *tabaci* and other whiteflies were published by Campbell et al. (1994, 1995), and will be discussed later. The
morphological studies were also aimed at finding significant and reliable characters for separating genera and species, and which characters might be a product of convergence and not related to phylogenetic sequences, and thus not particularly useful in determining whitefly hierarchy and generic placements.

Specimens of various species of *Bemisia* have been assembled and illustrated. Collections of specimens with *tabaci* characteristics have been studied from all over the globe, and many have been illustrated, although most of these illustrations have yet to be published, as the study is still ongoing. Illustrations from published descriptions of most known species of whiteflies around the world were studied to determine whether other similar appearing genera might contain species that are actually closely related to *Bemisia* or might even belong in the genus. Most of these illustrations will soon be published in a refereed systematics journal.

Representative species of whiteflies that were considered to belong to the genus *Bemisia*, as well as species similar in appearance to *Bemisia*, were studied morphologically. This included the careful mapping of dorsal setae and pore groups as well as other morphological structures that occur on pupal stages. These structures were then illustrated for later comparison purposes. Specimens were gathered from various collections, and included syntype specimens of *B. tabaci* and paratypes of some other species. Recent collections of specimens for molecular testing were also checked for the proper identification and illustrated if necessary. Adults were also studied when available.

**Problems in Whitefly Systematics**

Early work by Sampson (1943, 1947) and Sampson and Drews (1956) included the first family-wide keys to the genera of world whiteflies. Basically, these keys break apart some major groupings of genera based on the important characters found in the vasiform orifice or associated with it in the pupal stage. Important considerations include whether or not the operculum nearly or completely covers the orifice, whether the posterior end of the orifice is closed or open, and whether or not the lingula extends posteriorly beyond the operculum and the end of the orifice (Fig. 1.1). Also important is whether or not there is a dorsal furrow extending from the rear of the orifice to the posterior body margin. Length of the orifice in relation to the distance to the posterior margin might also have importance, as could the relative lengths of the lingulae. In the genus *Bemisia*, the operculum does not fill the orifice completely, the orifice is open posteriorly and the lingula is long enough to extend beyond the operculum and sometimes beyond the posterior end of the orifice. Some other species and genera outside of *Bemisia* also have these characteristics to a degree, so separation of these can rely on other characters, or they can be open to question as being part of the *Bemisia* generic group or not. Many of these species have been studied morphologically and hopefully most will be studied molecularly in the future.
It has become even more apparent that the systematics of whiteflies is in a bit of disarray. Whitefly taxonomists are aware of problems in the placement of species in a number of the world genera (Mound 1984) and this study and others are beginning to emphasize this very strongly. A prime example of this is the species *Pealius azaleae* (Baker and Moles). It has a vasiform orifice that is open posteriorly, with the lingula having two apical setae and a spatulate shape. These characters are in complete opposition to the type species of the genus, *Pealius maskelli* (Bemis), which has the posterior margin of the orifice closed like the lip of a bowl, with the lingula blunt and covered with microsetae instead of having one set of long setae (Fig. 1.1). The overall vasiform orifice characteristics of *azaleae* would then suggest that it might possibly be a *Bemisia*, but definitely it belongs in some other genus than *Pealius*. Hopefully more molecular work will help solve this placement dilemma.

While samples of *maskelli* have not been available for molecular testing, note the position in Fig. 1.2 of another North American species, *Pealius kelloggi* (Bemis), morphologically similar and thus probably congeneric with *P. maskelli*. The results of cytochrome oxidase 1 analysis shown in the tree (Fig. 1.3) indicated that
Fig. 1.2 Phylogenetical tree based on 18S ribosomal RNA gene (rDNA). Data simplified from Campbell et al. (1994) and showing possible relationships of *Bemisia tabaci* to some other common species of whiteflies.

*P. azaleae* is basal to other *Bemisia* species tested at that time. However, see also Fig. 1.4 where in another analysis, it is basal to both the *B. tabaci* and *B. afer-hancockii* complexes.

That the molecular versus morphological approach to systematics of whiteflies has been partially successful at least, is illustrated by the fact that species in the genus *Aleyrodes* have very similar pupal morphology to the *B. afer* complex of species (discussed below), but as seen in the Campbell tree (Fig. 1.2) is distinct from *Bemisia* and not particularly related to it based on 18S ribosomal DNA (rDNA) sequences. Species in the genus *Aleyrodes*, for example, meet the requirements of the genus *Bemisia* and are very similar in appearance to the *B. afer* group of species in respect to the shape and structure of the vasiform orifice and lingula, setal placements, variable development of long dorsal setae, overall body shape and adult structures. *Aleyrodes* species differ in pupal morphology from the *afer* complex only in having relatively shorter lingulae in the vasiform orifice, and the 7th and 8th abdominal segments subequal in width on the midline ahead of the orifice. In *afer* species, the lingulae are long and narrow, and the width of the 7th abdominal segment on the midline is much shorter than that of the 8th. The adults of most *Aleyrodes* species are very similar to some *afer* complex adults in having completely divided upper and lower compound eyes, but differ in being more sclerotized, having some diffuse dark markings along the wing veins, and most importantly, having the lengths of antennal segments 4 and 5 subequal. In the known adults of *Bemisia* species, and for most other Aleyrodine species, antennal segment 4 is always much shorter than segment 5. Apparently then, the only reliable morphological characters that tend to separate the genus *Aleyrodes* from *Bemisia* are the widths of abdominal segments 7 and 8 in the pupae, and the lengths of the 4th and 5th antennal segments in the adults. Therefore, these particular characters represent valid generic level separations between the two genera. Without molecular data, this genus level concept could not be proven either way.
A condensed and simplified tree based on the mitochondria cytochrome oxidase I sequence (780 bases) molecular data showing inferred (ML, PAUP) phylogenetic relationships of *Bemisia tabaci* populations with other related species [selected acronyms referred to are: *Bemisia centroamericana* from Belize (sample courtesy J. Martin, UK); for *B. tabaci* ABA from Benin on *Asystasia* (Bedford et al. 1994) and Sweet potato Uganda (UgSwPot) (courtesy J. Legg), GRE-1889 pupae from the paratype collection at the British Museum or USDA-collection, Beltsville, MD]; the other *B. tabaci* haplotypes/biotypes are well-known reference sequences, available in the Genbank database; *Bemisia* spp undescribed from New Zealand (NZEAL); *Lipaleyrodes emiliae* Taiwan (LEP); *Pealius azaleae* (AZALCA) from Azalea, USA (orig Asia); Outgroups are: *Trialeurodes vaporariorum* (TVAP) and *T. variabilis* (TVAR)
Separation of *Bemisia* species from other genera based on morphology alone is difficult. Adults, when known, are less useful even than pupae in most cases, nor are they much use in separating species within *Bemisia* itself (although see comments on the *afer* complex of species discussed below). Similar issues occur with many other species of whiteflies, not only in *Bemisia*- and *Pealius*-like species, but many other generic groups as well. In addition, many original descriptions are woefully inadequate by modern standards, adults are known for only a very small percentage of described species, and in many cases type material is not available or adequate for further study.
Morphological Studies: A Versus B Biotype

The first phase of *Bemisia* morphological studies involved the search for differences between the A and B biotypes. Careful study of the pupal cases of both biotypes revealed no major differences, although it was noted that a submarginal seta (ASMS4) occurred on almost all specimens collected from the New World prior to 1980 (Fig. 1.5). However, this seta is very difficult to find on specimens with long setal development and margins that are indented from proximity to leaf hairs [see comments on the significance of this setal pair in Roselle et al. (1997)]. This

![Fig. 1.5 Outline of left side of a *Bemisia tabaci* complex specimen showing positions of normal anterior and posterior submarginal setae (ASMS, PSMS) as well as the supernumerary setae.](image-url)
ASMS4 setal arrangement included specimens from the United States and Central and South America, including types of the possible synonym B. poinsettiae Hemple from Brazil and the Jatropha biotype of B. tabaci from Puerto Rico (Bird 1957, Bird and Maramorosch 1978). This setal position is absent in the B biotype except that a setal base would occasionally be present on some specimens although a seta would almost never appear to be present. The ASMS4 setal position is essentially absent from specimens of all biotypes known from Europe and Africa except for its presence in the type specimens of tabaci from Greece. Among other things, this could suggest that the original type population of tabaci may have been introduced into Greece from the New World. However, work by Brown et al. (2004) using Bayesian analysis and maximum likelihood analyses of mtCO1 sequences (see the position of the haplotype “GRE” in Fig. 1.3) suggests that the original type specimens of tabaci have affinities with populations from China, India, and Nepal and therefore may have originated in Asia, or that this locale represents a cross-roads (hybrid zone?) for the Asian and Mediterranean haplotypes.

Johnson and Bowden (1973) and Mound (1983) suggested that both B. tabaci and B. hancocki Corbett may have been transported across the Atlantic by man during the seventeenth or eighteenth centuries, so there is no reason why New World populations couldn’t move the other direction, considering the amount of traffic between Europe and Central and South America during those periods as well as from the Orient. Multiple different scenarios can obviously be suggested here. Similarly, the South American species B. tuberculata may be populations of hancocki (Mound 1983), a possible synonym of B. afer. In contrast, Anderson et al. (2001) suggest that afer has only recently been identified from Peru; perhaps the recently discovered specimens resulted from a recent introduction from Europe or Africa.

The significance of placement of this tiny ASMS4 seta in some tabaci populations that are otherwise indistinguishable from other populations around the World is open to question. Because the setal positions in tabaci sensu strictu are variable anyway, the presence of the ASMS4 seta may not have any significance at all, and further study of Bemisia morphology has shown some interesting and often confusing morphological plasticity. What this all means is that pupal morphological plasticity is making it very difficult to determine just how many species of whiteflies there are in the genus Bemisia itself, in addition to whether there may or may not be more than one species within the tabaci assemblage of populations.

Aside from the extra ASMS4 submarginal setae found in New World populations and the tabaci syntypes, European and African populations of tabaci always have three pairs of submarginal setae on the cephalothorax (ASMS 1 to 3) and five pairs on the abdomen, for a total of eight pairs. Several species of Bemisia have been described from Africa including afer (Preisner and Hosny), combreticula Bink-Moenen, hirta Bink-Moenen, and guieriae Bink-Moenen that have 14 pairs of submarginal setae, as does Bemisia berbericola (Cockerell), known from the western United States. This number of submarginal setae is expressed in a number of other genera as well. In afer at least, these setae may be elongate or short, and are in addition to long or short dorsal setae. The 14 submarginal setae in these particular species suggest a primitive condition, and this is shown to be correct in part in
molecular-based trees developed by Campbell et al. (1995) (see Fig. 1.2) and from data adapted from Brown (Figs. 1.3 and 1.4). This then suggests that the loss of some of the marginal setae in \textit{tabaci} could be a more recent event in evolutionary time. However, it appears that the genes necessary to control the missing setae are still present, but may be lying dormant. Such is the case with the ASMS4 setae of the A biotype, which apparently has the gene for setal expression at that location turned on. It is interesting at this point that populations of \textit{tabaci} have been collected in Hong Kong and in Malaysia that have at least 11 pairs of submarginal setae instead of eight, three more than any other Euro-African population studied so far (Fig. 1.5). Unfortunately, these populations have not been molecularly characterized, but it may be part of the more primitive, less derived Asian forms that will be discussed here later. It is therefore apparent that, while the plasticity of the morphology of \textit{tabaci} pupal cases is affected by host plant substrates and other environmental factors, there are also several subtle differences in morphology between populations of \textit{tabaci} that are likely gene-linked rather than environmentally driven.

Another confusing characteristic found among \textit{tabaci} relatives is the species \textit{Bemisia formosana} Takahashi. The species \textit{Bemisia graminus} David and Winstone was synonymized under \textit{tabaci} by David and David (2001), but has been further synonymized under \textit{formosana} by Martin and Mound (2007). In specimens identified as either \textit{formosana} or \textit{graminus} there appear to be no differences in chaetotaxy among pupae from other Euro-African \textit{tabaci} biotypes including the B biotype. In addition, an intact, un-emerged adult specimen inside one of the \textit{formosana} pupae studied here has the upper and lower compound eye connected by one ommatidium, a character that is rarely found in other whitefly adults that is frequently used to separate adult \textit{tabaci} individuals from adults of other commonly encountered economic species. Possibly these are the reasons for the synonymy under \textit{tabaci} by David and David (2001). However, specimens of both \textit{formosana} and \textit{graminus} are so far found only on grasses, and in all cases the shape of the pupal cases differ from those of \textit{tabaci} only in that they are elongated, rather than having the typical oval shape of \textit{tabaci} pupae. This pupal shape suggests a long and monophagous association with grasses, and suggests further that either of these entities, synonyms or not, are closely related to \textit{tabaci} but are not the same species. It is common among species within the Sternorrhyncha including aphids, scale insects, and other whitefly species that are associated primarily with grasses, to have an elongated shape. Some whitefly examples of the grass-feeding habit are members of the genera \textit{Aleurocybotus} and \textit{Vasdavidius}, the species \textit{Aleurolobus barodensis} (Maskell) and the species \textit{Aleurocyperus humus} Ko and Dubey, feeding on grass-like Cyperaceae. Occasional access to grasses does not illicit the elongated shape, which is borne out by specimens of the B biotype with pupae of the typical oval shape taken from sorghum leaves in the Imperial Valley of California during a very heavy valley-wide population explosion. It should be noted, however, that other species of whiteflies may have elongated puparia that are not associated with grasses, such as \textit{Bemisia giffardi}, known from \textit{Citrus} and many other plants, as well as \textit{Peraccius durantae} Lima and Racca-Filho on Verbenaceae and \textit{Dialeurolonga hoyti} Mound on \textit{Coffea},