Acquisition, circulation and transmission of begomoviruses by their whitefly vectors

Henryk Czosnek
The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture
Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot 76100
Israel

Abstract

Some geminiviruses are transmitted by the whitefly Bemisia tabaci in a circulative manner. These geminiviruses are assigned to the genus Begomovirus within the family Geminiviridae. The route followed by begomoviruses in their insect vector and the velocity of translocation of the various viruses seem to be intrinsic to the whitefly, not to the virus. Subsequent to ingestion during feeding on an infected plant, viruses are first associated with the stylet food canal and then to the proximal part of the descending...
midgut. Transmittable viruses cross the gut barrier into haemolymph from where they reach the accessory salivary glands. A GroEL homologue produced by the whitefly endosymbiotic bacteria facilitates this journey. The virus is then egested with the saliva into the phloem of a host plant. The capsid seems to be the only viral determinant involved in particle translocation. Unidentified receptors interacting with the viral particles permit their passage across membranes of the insect digestive system and salivary glands. Begomoviruses remain associated with their vector for various periods of time, sometimes during their entire adult life. The long-term presence of some begomoviruses has deleterious effects on the longevity and the fertility of the insect host. Some begomoviruses have been shown to be transovarially transmitted to adult progeny. Monopartite as well as bipartite begomoviruses can also be transmitted during mating. A functional genomic project of the whitefly Bemisia tabaci initiated about three years ago may provide answers to questions related to cellular determinants involved in virus translocation in its vector, effect of virus on the insect host and others related to whitefly development, resistance to insecticide, and host plant preference.

Introduction

The whitefly Bemisia tabaci is a major pest to major agricultural plants in tropical and subtropical regions worldwide. B. tabaci consists of a complex of biotypes [1] which can be sorted with polymorphic DNA markers [2]. The different biotypes differ in plant-host range, fecundity and ability to transmit viruses. For example, the B biotype colonizes over 500 plant species and a single female may lay approximately 400 eggs during her lifetime. In contrast, the A type biotype colonizes about 200 species and a female may lay about 100 eggs only. Unfertilized eggs give raise to haploid males; fertilized eggs develop into diploid females (arrhenotoky). Mated females can regulate the sex of their progeny by selectively fertilizing some of their eggs. B. tabaci develops into a flying adult from an egg, through four instars [3]. B. tabaci instars are able to ingest and transmit begomoviruses [4,5], however flying adults are those who spread the disease in the field. The ratio of male to female naturally changes throughout the course of the year, in fields and in insectaries [6].

Whiteflies, especially those of the B type, vector a large number of viruses infecting many important agricultural plants, ornamentals and weeds [7]. Geminiviruses constitute the most important class of pathogens transmitted by this insect. They are small plant viruses characterized by a 22 x 38 nm geminate particle consisting of two joined incomplete icosahedra [8,9,10]. Geminiviruses transmitted by the whitefly B. tabaci are assigned to the genus Begomovirus within the family Geminiviridae [11]. Begomoviruses infect many important agricultural plants worldwide including bean, cassava, cotton,
Interactions of geminiviruses with the whitefly vector

melon, pepper, potato, squash, tobacco, tomato and watermelon. They are considered as one of the major emerging threats to agricultural crops [12]. Most begomoviruses possess a bipartite genome consisting of two ~ 2,600 nucleotide covalently closed circular single-stranded DNA molecules, named DNA-A and DNA-B, each encapsidated in a geminate particle. DNA-A encodes one major open reading frame (ORF) on the virion genome strand (AV1) and three on the complementary strand (AC1 to AC3). AV1 encodes the coat protein (CP); AC1, the replication associated protein (Rep); AC2, a transcriptional activator protein (TrAP); and AC3, replication enhancer protein (REn). DNA-B encodes two ORFs: BV1, on the virion strand and BC1, on the complementary strand. BV1 encodes a nuclear shuttle protein (NSP) and BC1 a cell-to-cell movement protein (MP). Several begomoviruses belonging to the tomato leaf curl complex have a monopartite genome organization similar to that of the DNA-A of bipartite viruses. The genome of monopartite viruses encodes six genes, two on the virion genome strand (V1 and V2) and four on the complementary genome strand (C1 to C4). V1 encodes the CP and V2 may control symptoms and movement. C1 encodes the Rep protein, C2 the TrAP, C3 the REn and C4 may affect host-range, symptom severity and movement [13,14].

In this review the characteristics of acquisition, transmission and retention of begomoviruses by the whitefly vector *B. tabaci* B type will be discussed, concentrating on a selected number of monopartite and bipartite geminiviruses.

**Ingestion and inoculation of begomoviruses**

**Acquisition and transmission**

Most begomoviruses are restricted to the phloem of infected plants. Hence, to acquire a begomovirus from an infected plant or to transmit a begomovirus to a host plant, the stylets of *B. tabaci* need to find their way between the epidermal and parenchymal cells before penetrating the vascular tissues and reaching the phloem they feed on [15,16]. The parameters of acquisition and transmission of a begomovirus were first defined for the monopartite begomovirus *Tomato yellow leaf curl virus* (TYLCV) and were based on biological tests [4,17]. Single insects are able to acquire TYLCV and transmit it to tomato plants. The reported minimum acquisition access period (AAP) and inoculation access period (IAP) of Middle eastern TYLCV isolates varied from 15 to 60 min and from 15 to 30 min, respectively [17-19]. Similar values were reported for other monopartite geminiviruses infecting tomato such as *Tomato yellow leaf curl Sardinia virus* (TYLCSV) from Italy [20]. Efficient AAP and IAP of bipartite begomoviruses infecting tomato such as *Tomato yellow leaf curl Bangalore virus* (ToLCBV) from India are not drastically different [21].

The development of molecular tools has allowed the refinement of these studies. The genome of TYLCV was readily detected by Southern blot
hybridization in DNA extracted from a single viruliferous whitefly [22]. The frequency of detection increased as the length of the AAP increased, from 10-20% after 30 min to 100% of the insects tested after 8 h. The hybridization signals indicated that insects that had access to the same tissues for the same period of time could acquire variable amounts of viral DNA [22]. A similar study conducted previously with the bipartite Squash leaf curl virus (SLCV) showed a similar albeit slower increase in the frequency of virus detection with time [23].

PCR allowed detecting amounts of TYLCV DNA in a single insect below the threshold of infectivity [24]. Using print-capture PCR, we have detected TYLCV DNA in 20% of the individuals tested as early as 5 min after access to the infected plant [25]. Monitoring the electronic waveforms produced during insect feeding allows dissection the virus transmission process [26]. Analysis of electrical penetration graphs during transmission of TYLCV by B. tabaci indicated that, following a short probing period, the minimum phloem contact period for successful inoculation of TYLCV was 1.8 min [27]. Anatomical differences between virus source plant and target host plants (e.g. accessibility of the phloem in the leaf) may be reflected in differences in acquisition and transmission parameters associated with various begomoviruses.

**Latent period**

Once ingested, begomoviruses are not immediately available for infection. They need to translocate from the digestive tract to the salivary glands from which they are excreted with the saliva during feeding. The time it takes for a begomovirus to complete this path and to infect susceptible plants is called the latent period. The latent period may not reflect the speed of virus translocation but rather the time it takes for an insect to accumulate enough virions to be able to transmit the disease to plants. For some begomoviruses this threshold may be reached much earlier than for others. For example SLCV has been detected by PCR in the saliva 8 h after the beginning of the AAP [28] while the minimal latent period was approximately 19 h [29]. In contrast TYLCV has been detected in the salivary glands of B. tabaci 7 h after the beginning of the AAP, only 1h before the insects were able to infect tomato plants [30]. The estimated latent period for a given virus may vary due to the experimental conditions or to changes in virus and/or vector with time. For example the latent period of TYLCV from Israel was reported to be 21 h in the early 1960s [4] while it was found to be 8 h thirty five years later [30].

**Transmission efficiency of begomoviruses: The effect of gender and age**

It has been reported that a single insect was able to infect a tomato plant with TYLCV following a 24 h AAP; efficiency of transmission reached 100%
when 5 to 15 insects were used [4,18,19]. A similar number of insects were necessary to achieve 100% transmission of the bipartite SLCV [29]. However in most cases the age and/or the gender of the insects used is ignored. It has been previously reported that female whiteflies transmit the monopartite TYLCV [4] and the bipartite ToLCBV [21] with higher efficiency than males.

We have studied the effect of the gender and of the age of synchronized populations of adult *B. tabaci* on the efficiency of transmission of TYLCV acquired during a 48 h AAP [31]. Nearly all the 1 to 2 week-old adult females were able to infect tomato plants during a 48 h IAP. In comparison, only about 20% of the males of the same age were able to infect plants. Infection capacity decreased with age. While 60% of the 3 week-old females infected plants, the males were totally unable to infect tomato plants. Only 20% of the 6 week-old females were able to infect tomato plants. Aging insects acquire fewer virus than younger individuals: 17 day-old adult females ingested less than half the virus ingested by 10 day-old insects and 24 day-old adults ingested only about 10% [32]. It has to be noted that female and male *B. tabaci* transmitted SLCV with the same efficiency [23]. The reason for these differences is unclear.

The path of geminiviruses in the whitefly host
Organs and cells involved in circulative transmission of begomoviruses

Once ingested, begomoviruses follow a path that has been described in some detail. The extensive anatomical analysis of the begomovirus non-vector whitefly *Trialeurodes vaporariorum* performed in the 1930s still serves as a reference for analyzing the internal anatomy of whitefly species [33]. The description of *B. tabaci* mouthparts [34], anterior alimentary canal [35], digestive tract, filter chamber and salivary glands [36-38] has helped define the pathway of begomoviruses in their insect vector.

*B. tabaci* feeds on phloem sap of infected plants by inserting its stylets into plant tissue and locating the vascular tissue. The stylet bundle is composed of three stylets: the maxillary stylet, which contains the food canal (through which phloem is acquired) and the lateral salivary canal (through which saliva is injected into the plant), and two mandibulary stylets [34]. The stylet food canal empties into the precibarium and cibarium. The cibarium joins the esophagus, which runs along the dorsal side of the thorax before entering the filter chamber [38]. The internal esophagus expands within the filter chamber where it is united with the continuous lumen, extends into the connecting chamber, caecae, and descending and ascending midguts. The descending midgut exits the connecting chamber and maintains a large diameter where it meets the ascending midgut. The descending midgut is composed of thick epithelial cells surrounding a large lumen, with large nuclei and microvilli
extending into the lumen. The ascending midgut narrows until it enters the filter chamber where it meets both the continuous lumen and the internal ileum. It is formed by very thick epithelial cells with an extensive brush border of microvilli surrounding a rather small lumen. The hindgut terminates with the rectal sac [38]. In females with developing eggs, the ovaries fill the abdomen, crushing the hindgut against the dorsal surface of the insect and sometimes pushing the midgut into the thorax. The viral particles penetrate the gut membranes into the haemolymph, crossing the epithelial cells of the whitefly digestive tract which bridge between the gut lumen and the haemolymph, or hemocoel, a primitive blood system that circulates around the body cavity between the various insect organs. From there, begomoviral particles reach the salivary glands. A pair of primary salivary glands is located in the prothorax. The paired accessory glands are much smaller and slightly anterior to the primary glands. The primary salivary glands are made of at least 13 nearly symmetrical large cells surrounding a central lumen lined with microvilli, which empties into a duct at the base of the gland. This duct joins the accessory salivary gland duct and the medial duct. Each accessory gland is composed of four large cells similar to one another, encircling a central lumen lined with extensive microvilli [38]. The primary and accessory gland ducts on either side fuse to form the lateral salivary ducts. The two lateral ducts fuse to form a single, dual-channeled, medial salivary duct [36]. The salivary canal is contained almost entirely within one stylet, while the food canal is centrally located and is formed by the apposition of the food grooves in both stylets. The food and salivary canals end at the stylet tip [34]. Viral particles reach the salivary glands and finally enter the salivary duct from where they are egested with the saliva. Translocation of begomoviruses from the digestive tract to the haemolymph and from the haemolymph to the salivary gland is thought to be mediated by still un-identified receptors.

**Visualization of begomoviruses in B. tabaci**

Two bipartite begomoviruses (*Tomato mottle virus*, ToMoV, and *Cabbage leaf curl virus*, CaLCV) have been immunolocalized in the *B. tabaci* filter chamber and in the anterior part of the midgut, with ToMoV detected in the salivary glands [39]. We have immunolocalized TYLCV to the stylets [40]. Label was associated mainly with the stylet food canal and the signal was conspicuous all along the lumen. Similarly, we have immunolocalized the virus to the proximal part of the descending midgut. Label was associated with food in the lumen, with the microvilli and with electron-dense materials in the gut wall epithelial cells. In another study, we have immunolocalized TYLCV to the filter chamber and the distal part of the descending midgut [7]. Using *in situ* hybridization we have detected TYLCV in the nucleus of 3 of 14 of the cells of the *B. tabaci* primary salivary glands [7].
Velocity of TYLCV translocation in *B. tabaci*

Using DNA from extracts of *B. tabaci* raised in Arizona as substrates for PCR, the bipartite SLCV DNA was detected in insect extracts after a 30 min AAP on infected pumpkin, and was found in the haemolymph after 2 h and in the saliva and honeydew after 8 h [28]. We have measured the velocity of translocation of TYLCV DNA and coat protein (CP) in whiteflies from Israel. Stylets, head, midgut, haemolymph and salivary glands dissected from a single insect were used as substrate for PCR and immunocapture-PCR [30]. TYLCV was detected in the head 10 min after the beginning of the AAP and in the midgut after 40 min. The virus reached the haemolymph 90 min after the beginning of the AAP and was detected in the salivary glands approximately 5.5 h thereafter, approximately 1 h before the insects were able to infect tomato plants. TYLCV translocation timing obtained by PCR and by immunocapture-PCR overlapped, suggesting that the viral DNA is within virions. Hence begomoviruses transit in the body of *B. tabaci* according to an invariable sequential path: head-midgut-haemolymph-salivary glands [30]. Moreover, it is likely that the path and the velocity of begomovirus translocation are independent of the identity of the begomovirus (as long as it is transmissible) and of the geographical origin of the *B. tabaci* vector.

Fate of non-transmittable begomoviruses

During the transit of begomoviruses in their whitefly vector, the capsid is the structure that is exposed to the whitefly tissues and interacts with insect receptors and chaperones [41]. Vector specificity of geminiviruses is determined by the CP and there is no evidence for the involvement of other virus-encoded proteins in transmission.

Loss of begomovirus transmission by *B. tabaci* can be caused by a small number of amino acid replacements in the CP. Natural TYLCSV mutants have been isolated which are ingested but not transmitted by *B. tabaci*. Loss of TYLCSV transmission was due to the replacement of two amino acids at positions 129 and 134 in the CP [42]. This region of the CP is also implicated in transmission of the bipartite *Watermelon chlorotic stunt virus* [43]. *Abutilon mosaic virus* (AbMV) is another bipartite begomovirus that has lost the ability to be transmitted [44], probably because it has been maintained and propagated by cuttings. Mutagenesis of AbMV CP showed that exchange of three amino acids at positions 124, 149 and 174 restored transmissibility by whiteflies [45]. Replacing the CP of AbMV with that of the closely-related transmissible *Sida golden mosaic virus* (SiGMV) produced a whitefly-transmissible chimeric AbMV [46].

Although not transmittable, the pattern of association of AbMV with *B. tabaci* was similar to that of TYLCV. Following a 4-day AAP on infected abutilon plants, AbMV DNA remained associated with *B. tabaci* during the 15
day experiment, while the CP was detectable only for up to 7 days [47]. AbMV was detected in the vector digestive system, but not in the haemolymph, indicating that this virus was unable to cross the gut/haemolymph barrier [40]. We speculate that following acquisition, AbMV binds to the putative \textit{B. tabaci} receptors present in part of the digestive tract. However, because of a change in the conformation of the capsid due to mutations in the CP, AbMV cannot be internalized in the epithelial cells by the microvilli system and delivered to the haemolymph.

**Begomoviruses may affect the fitness of their whitefly host**

**Long-time association of begomovirus with the whitefly vector**

Following a 1-2 day AAP, begomoviruses may be retained in their whitefly vector for several weeks and sometimes for the entire life of the insect. SLCV and TYLCV remain associated with \textit{B. tabaci} during the entire life of the vector [32,48] while TYLCSV is undetectable after approximately 20 days [27].

In most instances the viral DNA remained associated with the insects much longer than infectivity indicated. For example while TYLSCV DNA was detectable up to 20 days after the end of the 48 h AAP, infectivity was retained for up to 8 days only [5]. Viral DNA and CP are not retained in \textit{B. tabaci} for the same time periods. Following the end of the 48 h AAP, TYLCV DNA remained conspicuous during the 5 week life span of the insect while the amount of TYLCV CP steadily decreased until it was undetectable at day 12 [32]. The disappearance of the virus CP was associated with a fast decrease in whitefly ability to infect host plants, as shown for TYLCV [32] and SLCV [48]. It is possible that most of the viral DNA dissociated from the capsid, left the circulative pathway and invaded insect tissues.

**Effect of begomoviruses on longevity and fertility of \textit{B. tabaci}**

The long-term association of TYLCV with female \textit{B. tabaci} raised on TYLCV non-host eggplants was correlated with a decrease in longevity [32]. Following a 48 h AAP on TYLCV-infected tomato plants, the difference at the 50\% mortality point between viruliferous and non-viruliferous insects was between 5 and 7 days, depending on the time of the year the experiment was conducted. These results showed that the life expectancy of viruliferous insect populations was significantly lower (approximately 20\%) than that of the non-viruliferous controls. In another study, it was shown that the life span of female whiteflies fed for 24 h on SLCV-infected plants was on average 25\% shorter than that of whiteflies fed on the same virus source for 4 h only [48].
The long-term association of TYLCV with female *B. tabaci* was also correlated with a decrease in fertility [32]. Following a 48 h AAP on TYLCV-infected tomato plants, the mean number of eggs laid either on tomato or on eggplant during a 7 or 20 day period was significantly lower than that laid by non-viruliferous insects of the same age. The decrease in fertility was not observed during the first 24 h following AAP, indicating that the target was maturing eggs. The percentage of eggs that developed into instars was similar, whether they were laid by infected or non-infected insects. Therefore TYLCV influenced the number of eggs laid but not the emergence of the instars. In a similar experiment the effect of a TYLCV isolate from China (TYLCCNV) on two *B. tabaci* biotypes (invasive B and local ZHJ1) was appraised [49]. Following a 48 h AAP on TYLCCNV-infected tobacco plants the mean longevity and fertility of viruliferous B and ZHJ1 insect biotypes on cotton were significantly lower than that of non-viruliferous insects. In the same study, the effect of another monopartite geminivirus, the Tobacco curly shoot virus (TobCSV) on the two biotypes was appraised following a 48 h AAP on TobCSV-infected tobacco and transfer to cotton plants. The results were just the opposite of those obtained with TYLCCNV. Viruliferous B biotype whiteflies exhibited higher longevity and fertility than non-viruliferous whiteflies, while the effect of TobCSV on ZHJ1 insects was minor.

In contrast to TYLCV the bipartite begomovirus ToMoV does not affect fertility [50]. Whiteflies infected with ToMoV deposited significantly more eggs on healthy tomato leaves than non-viruliferous whiteflies. There was no significant difference between viruliferous and non-viruliferous whiteflies for the number of adults emerged or the proportion of those adults surviving from the egg stage. There was no significant correlation between the number of eggs deposited per viruliferous and non-viruliferous females and progeny survival rates on healthy tomato. These observations indicate that some begomoviruses have deleterious effects on their insect host while others do not.

**Fate of begomoviruses in their whitefly host**

**Association of viral particles with insect chaperones**

As discussed above, begomoviral particles need to cross the gut wall into the haemolymph on their way to the salivary gland. The haemolymph consists of plasma in which haematocytes digest foreign proteins, microorganisms and tissue debris [51]. Hence transiting virions face a particularly hostile environment. A GroEL homologue produced by the primary endosymbionts of aphids has been shown to play a crucial role in the transmission of luteoviruses [52]. Similarly, endosymbiotic bacteria housed in the whitefly mycetocytes have a cardinal role in protecting begomoviruses in the haemolymph [53]. As demonstrated for TYLCV, the GroEL homologue seems to bind to and protect
begomoviruses from degradation in the haemolymph. Disrupting the GroEL-TYLCV association leads to the degradation of the virus and to a marked decrease in transmission efficiency [41,47]. We have shown that in the yeast two-hybrid system, B. tabaci GroEL interacted with the CP of TYLCV as well as with the CP of the non-transmissible AbMV [41], indicating that the amino acid residues at position 124, 149 and 174, which prevented AbMV from crossing into the insect haemolymph [45] did not prevent binding to GroEL. It has been suggested that viruses belonging to unrelated taxonomic groups have taken advantage of endosymbiotic bacterial proteins produced by their insect vector to avoid degradation in the haemolymph [53].

Replication?

Begomovirus replication in its vector remains a controversial issue. It has been postulated that geminiviruses do not replicate in their insect vectors [54]. However studies to determine virus titer over time in whiteflies have shown that TYLCV DNA persists in the insects longer than infectivity would suggest [20,32,55]. Hence, the persistence of begomoviruses in B. tabaci as infective entities for longer than the latent period, sometimes for the entire life of the insect, raises the question of replication of the virus in the insect.

Accumulation of viral DNA in B. tabaci reared on a TYLCV non-host plant, after first feeding on plants infected with a TYLCV isolate from Egypt, has been interpreted as multiplication of TYLCV in its vector [19]. We have found that after a short AAP the amount of TYLCV DNA associated with whiteflies detectable by Southern blot hybridization steadily increased after a lag period of 8 h, reaching maximum levels after approximately 16 h and decreasing thereafter [31]. These results could be explained by the ingestion of viral replicative complexes which complete their replication cycle in the insect. It has to be noted that following acquisition of the closely related TYLCSV, accumulation of viral DNA was not observed [20].

Transcription?

Transcriptional activity of two begomoviruses in the B. tabaci vector, the monopartite TYLCV and the bipartite ToMoV, has been evaluated [55]. After feeding on virus-infected tomato plants and after subsequent transfer to cotton, a ToMoV and TYLCV non-host plant, real-time RT-PCR was performed using specific primers for three ToMoV genes (AV1, BC1 and BV1) and three TYLCV genes (V1, V2 and C3). The ToMoV gene transcripts rapidly became undetectable in whiteflies following transfer from tomato to cotton, probably because degradation was not accompanied by new synthesis. On the other hand, TYLCV transcripts increased after transfer of whiteflies to cotton, and were readily detected after 7 days indicating active TYLCV transcription. Interestingly, the difference observed in ToMoV and TYLCV transcripts in the
vector parallel observations on the different biological effects of these viruses on whiteflies, i.e. TYLCV, but not ToMoV, reduced whitefly fitness [32,50].

**Acquisition of begomovirus by whiteflies independently of the infected plant virus source: Transovarial inheritance and transmission during mating**

**Transovarial transmission**

Transovarial transmission of plant viruses by their insect vector is a rare event and has been associated with replication and with deleterious effects on the insect host [56,57]. Usually, the virus was transmitted to some, but not to all progeny. Geminiviruses have not been considered to be transmitted transovarially to progeny [54]. Using PCR, Southern blot hybridization and transmission tests, we have found that TYLCV was transmitted to the progeny of viruliferous insects with various efficiency. Moreover the progeny of viruliferous insects was able to infect tomato test plants. Dissection and analysis of the reproductive system of viruliferous whiteflies showed that both the ovaries and the maturing eggs contained TYLCV DNA [58]. The closely related TYLCSV was also found to be transmitted transovarially to the first generation progeny. Similarly to TYLCV [58], TYLCSV was detected in eggs and nymphs as well as in adults [59]. However, in contrast to TYLCV, the adult progeny of viruliferous insects were unable to infect tomato plants [59]. It is interesting to note that in the later experiments, while TYLCSV DNA was associated with eggs, instars and adults of the first generation progeny, TYLCV was detected neither in instars nor in adult progeny of viruliferous females. These divergent results may be due to intrinsic differences in the highly inbred insect colonies raised in the laboratory and used in these experiments.

The way in which TYLCV [58] and TYLCSV [59] enter the whitefly reproductive system is unknown. It is possible that during the maturation of eggs in the ovaries, geminiviral particles penetrate the egg together with the endosymbionts, via an aperture in the membrane [60]. Invading TYLCV may affect the development of some of the eggs, causing a decrease in fertility [32].

**Transmission during mating**

Another route of acquisition of begomoviruses. We have shown that TYLCV can be transmitted between whiteflies in a sex-dependant manner, in the absence of any other source of virus [61]. TYLCV was transmitted from viruliferous males to females and from viruliferous females to males, but not between insects of the same sex. Transmission took place when insects were caged in a feeding chamber or on TYLCV non-host cotton plants. TYLCV was
detected in the haemolymph of the recipient insects about 1.5 h after caging, but was detected neither in the midgut nor in the head. Hence the virus bypassed the pathway followed after feeding on infected plants and probably infected the recipient insect by means of haemolymph exchange. From there TYLCV translocated in the salivary glands, but never crossed the gut membranes back into the digestive system [61].

The key role of the haemolymph was demonstrating by caging non-viruliferous B. tabaci males with females fed on AbMV-infected abutilon plants. AbMV DNA was never detected in the males. Identical results were obtained in the reciprocal mating scheme (unpublished). Since AbMV remains in the digestive tract and is unable to cross the gut barrier into the haemolymph, these results confirmed that virus cannot be acquired from the feeding solution and that mating is the obligate route for sexual transmission of TYLCV, which probably occurs by exchange of haemolymph during intercourse. Transmission of SLCV and WmCSV during mating was also observed by detecting viral DNA-A and DNA-B in the recipient insects (unpublished).

Viral and cellular determinants involved in begomovirus circulative transmission
Whitefly-begomovirus co-adaptation

Whiteflies and begomoviruses have a long history of co-habitation which has left various traces. On the one hand fossils anatomically similar to modern whiteflies have been found in ~ 120 million year (MY) old amber from Lebanon [62]. On the other hand, multiple repeats of geminiviral DNA sequences highly homologous to regions of the modern bipartite Tomato golden mosaic virus seem to have integrated into the genome of some tobacco ancestors during Nicotiana speciation, about 25 MY ago [63]. In this context it is interesting to note that the endosymbiotic bacteria that produce the GroEL homologue on which depends the survival of begomoviruses in their insect vector [47], have been associated with whiteflies for the last 200 MY [66].

During this long-lasting virus-vector relationship begomoviruses might have optimized the conformation of their capsid to fit the receptors that mediate their circulation in the insect host and to interact with the chaperonins produced by the whitefly endosymbiotic bacteria. It is interesting to note that the adaptation of the local vector to the local begomovirus is reflected in the parameters of acquisition and transmission. Transmission of a begomovirus by an insect from the same geographical region is more efficient than in the case where virus and insect originated from two different regions [65]. Whiteflies may have also adapted to begomoviruses. Circulation of the virus may be one mechanism developed to avoid invasion of insect tissues by harmful viruses. In
In the latter case, it is clear that these efforts have been only partially successful since many begomoviruses remain associated with the insect vector for many days following a short AAP [4,5,23,32], and some begomoviruses are able to invade the reproductive system [58,59] and affect vital parameters [32].

Insect determinants of begomovirus transmission

While begomoviruses are able to infect many plant species they are vectored by a single insect species. The begomoviral capsid and insect receptors in the gut and salivary glands seem to govern the specificity and efficacy of efficient begomovirus circulative transmission. Sequence comparisons of begomovirus CPs has allowed these viruses to be grouped according to their geographical origin: 1) Americas, 2) Western Mediterranean basin, 3) Middle East, 4) Indian subcontinent, 5) East and Southeast Asia and Australia Similarly, the whitefly B. tabaci complex could be resolved into five major groups based on polymorphic mitochondrial DNA markers, essentially overlapping the geographical distribution of begomoviruses [2]. Analysis of CP mutants has allowed the amino acids necessary for efficient circulative transmission to be determined [42-46]. All the mutants describe so far confine the virus in the whitefly digestive system. The insect receptors which are thought to mediate translocation of begomoviruses from the gut to the haemolymph and from the haemolymph to the salivary glands are unknown. Also unknown are the genes that are affected by the virus, whether during circulative transmission or during long-term storage in the insect tissues.

The whitefly genome project

We have proposed a genomic approach to better understand the genetic make-up of B. tabaci. The B. tabaci genome has been hardly explored. It has been reported that nuclei of haploid males contain 10 chromosomes [65]. Using flow cytometry we have estimated the DNA content of nuclei from haploid B. tabaci males as 1,020 million base-pairs [67], which is approximately five times that of the fruitfly Drosophila melanogaster.

A functional genomics approach has been taken to understand the patterns of gene expression during whitefly development and during association of whiteflies with begomoviruses. We have constructed three cDNA libraries for non-viruliferous whiteflies (eggs, immature instars, and adults) and two from adult insects that fed on tomato plants infected by two geminiviruses: the monopartite TYLCV and the bipartite ToMoV. The sequences of approximately 20,000 clones have been determined [68]. Comparisons with public databases indicated that the libraries contained genes involved in cellular and developmental processes. Some sequences were specific to developmental stages while others were specific to viruliferous insects. The functional analysis of these genes is in progress.
References

23. Polston, J E, Al-Musa, A, Perring, T M, and Dodds, J A. 1990, Phytopathology, 80, 850.
Interactions of geminiviruses with the whitefly vector