

Review. Rapid evolution of the population of begomoviruses associated with the tomato yellow leaf curl disease after invasion of a new ecological niche

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Abstract

Epidemics of tomato yellow leaf curl disease (TYLCD) caused by monopartite begomoviruses (family *Geminiviridae*) result in devastating damage to tomato (*Solanum lycopersicum* L.) crops in Spain since early 1990's. Initially, the ES strain of the species *Tomato yellow leaf curl Sardinia virus* (TYLCSV) was reported as the causal agent. The population of this virus exhibited a high genetic stability. However, introduction of new virus types also associated with TYLCD occurred which rapidly altered the virus population structure and diversity. Thus, isolates of the Mld and the IL strains of *Tomato yellow leaf curl virus* (TYLCV) were introduced, which resulted in a progressive displacement of TYLCSV. Single, undifferentiated subpopulations were observed for each virus type, compatible with founder effects. Mixed infections were detected in single plants, rationalizing the occurrence of recombinants. In fact, natural recombinants between TYLCSV and TYLCV with selective advantage over the parental genotypes rapidly emerged and spread in the virus population. These data evidenced the great dynamism of the begomovirus population associated with the TYLCD after the invasion of a non-native area and the contribution of genetic migration and recombination to the genetic diversification.

Additional key words: geminivirus, genetic diversity, genetic migration, recombination.

Resumen

Revisión. Rápida evolución de la población de begomovirus asociados con la enfermedad del rizado amarillo del tomate tras la invasión de un nuevo nicho ecológico

Las epidemias de la enfermedad del rizado amarillo del tomate (tomato yellow leaf curl disease, TYLCD) causadas por begomovirus (familia *Geminiviridae*) monopartitos ocasionan importantes pérdidas económicas en los cultivos de tomate (*Solanum lycopersicum* L.) españoles desde inicios de los años 1990s. En un principio estas epidemias se encontraron asociadas con la cepa ES de la especie *Tomato yellow leaf curl Sardinia virus* (TYLCSV). La población de este virus presentaba una alta estabilidad genética en ausencia de influencias externas. Sin embargo, la migración de nuevos tipos genéticos de begomovirus asociados con TYLCD resultó en una rápida alteración de la diversidad y estructura de la población viral existente. Así, la introducción de las cepas Mld e IL de la especie *Tomato yellow leaf curl virus* (TYLCV) resultó en un desplazamiento progresivo de TYLCSV en las epidemias. La población quedó estructurada en subpoblaciones únicas e indiferenciadas para cada tipo viral, compatible con efectos fundadores. Además, se detectaba la presencia de infecciones mixtas de virus en plantas individuales, lo que permitía la generación de tipos recombinantes. De hecho, en poco tiempo se observó la emergencia en la población de recombinantes naturales entre TYLCSV y TYLCV, con ventajas selectivas frente a los virus parentales. Por tanto, nuestros datos sugieren un gran dinamismo de la población de begomovirus asociados con TYLCD después de la invasión de un área no nativa, así como la contribución de la migración genética y la recombinación en la diversificación genética de dicha población.

Palabras clave adicionales: diversidad genética, geminivirus, migración genética, recombinación.

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Introduction¹

This work is in memory of José María Malpica Romero. His enthusiasm with genetic population studies was contagious and encouraged us to progress in our work. When we talked with him shortly after the first outbreak of tomato yellow leaf curl disease (TYLCD) epidemics in Spain, he predicted the rapid evolution of the virus population. After the introduction into a new ecological niche, viruses need to evolve rapidly to adapt and to compete successfully, he said, and he was right. In fact this is the conclusion of our studies a decade after our talk.

The ability of viruses to colonize new ecological niches is well documented and has been frequently associated with the development of important epidemic outbreaks. During the last decades virologists have done considerable effort to understand the evolutionary changes accompanying the expansion of virus populations. As a result, an increased knowledge of evolutionary mechanisms used by viruses exists, which may facilitate epidemiological predictions for the control of viral diseases (Domingo and Holland, 1997; Roossinck, 1997; Gibbs *et al.*, 1999). The recent introduction of viruses associated with TYLCD in Spain provided an interesting study case to analyze aspects of genetic adaptation and evolution of an invading plant virus. Here, we review the data on the evolution of TYLCD-associated virus populations in Spain and the western Mediterranean basin and discuss about forces that are driving this process.

The tomato yellow leaf curl disease and associated viruses

The tomato yellow leaf curl disease is one of the most devastating viral diseases affecting tomato (*Solanum lycopersicum* L.) crops in tropical and subtropical regions of the world, and recently in more temperate regions (Moriones and Navas-Castillo, 2000). Epidemics of TYLCD cause severe damage to field and greenhouse tomato crops in affected areas. The disease was first

described in Israel in 1931 (Cohen and Antignus, 1994) and since then, TYLCD has been reported causing devastating damage to tomato crops in many regions of Africa, the Middle East, south-east Asia, Europe (Czosnek and Laterrot, 1997; Moriones and Navas-Castillo, 2000), and more recently America (Brown and Idris, 2006; Zambrano *et al.*, 2007). TYLCD infections in tomato result into severe stunting of plants with virtually no yield if early infection occurs. Symptoms of TYLCD in tomato consist in a more or less prominent upward curling of leaflet margins and reduced size of young leaflets that can exhibit a yellow chlorosis (Fig. 1A and 1B). Also, flower abortion is common in infected plants. Although no symptoms are observed in fruits, infected plants are less vigorous and produce fruits with reduced market value. In addition to tomato, some TYLCD-associated viruses also have been involved in infections of common bean (*Phaseolus vulgaris* L.) (Navas-Castillo *et al.*, 1999) and pepper (*Capsicum annuum* L. and *C. chilensis* Jacq.) (Morilla *et al.*, 2005; Polston *et al.*, 2006) crops. Host range of TYLCD-associated viruses is short and data available including natural and experimental hosts are compiled in other works (Mansour and Al-Musa, 1992; Cohen and Antignus, 1994; Moriones and Navas-Castillo, 2000; Jordá *et al.*, 2001).

At least nine different virus species more or less related phylogenetically, and strains of them, have been associated with TYLCD (Fig. 2), among others, *Tomato yellow leaf curl virus*, TYLCV (Moriones and Navas-Castillo, 2000; Fauquet and Stanley, 2005; Stanley *et al.*, 2005). These viruses belong to the genus *Begomovirus* which includes geminiviruses (family *Geminiviridae*) that are transmitted in a circulative persistent manner by the whitefly *Bemisia tabaci* Gennadius (*Hemiptera: Aleyrodidae*). Their genome consists of monopartite or bipartite circular single-stranded DNAs. Monopartite TYLCD-associated viruses have a genome of about 2.8 kb that contains six partially overlapping open reading frames (ORFs), bi-directionally organized in two transcriptional units that are separated by an intergenic region (IR) of about 300 nt (Stanley *et al.*, 2005) (Fig. 1C). On the virus sense strand there are

¹ Abbreviations used: C (complementary sense strand), IR (intergenic region), ORF (open reading frame), REN (replication enhancer protein), Rep (replication associated protein), SSCP (single-strand conformation polymorphism), ToLCV (*Tomato leaf curl virus*), TrAP (transcriptional activator protein), TYLCAXV (*Tomato yellow leaf curl Axarquia virus*), TYLCCNV (*Tomato yellow leaf curl China virus*), TYLCD (tomato yellow leaf curl disease), TYLCGuV (*Tomato yellow leaf curl Guangdong virus*), TYLCKaV (*Tomato yellow leaf curl Kanchanaburi virus*), TYLCMaV (*Tomato yellow leaf curl Málaga virus*), TYLCMLV (*Tomato yellow leaf curl Mali virus*), TYLCSV (*Tomato yellow leaf curl Sardinia virus*), TYLCthV (*Tomato yellow leaf curl Thailand virus*), TYLCV (*Tomato yellow leaf curl virus*), V (viral sense strand).

two overlapping ORFs, V1 and V2, and on the complementary sense strand, ORFs C1 to C3 partially overlap and ORF C4 is contained in the ORF C1. Encoded proteins are the CP (V1 ORF), the capsid protein; the replication-associated protein (Rep, C1 ORF), a protein essential for replication; the transcriptional activator protein (TrAP, C2 ORF), involved in the activation of the transcription of virus sense genes; the replication enhancer protein (REn, C3 ORF), which interacts with C1 protein enhancing viral DNA accumulation; and protein products encoded by the V2 «precoat» and C4 ORFs, that have been involved in symptom expression and virus movement. The non-coding IR region contains key elements for the replication and transcription of the viral genome including the origin of replication, within a stem-loop structure conserved in the geminiviruses (Jupin *et al.*, 1994; Wartig *et al.*, 1997; Noris *et al.*, 1998).

Emergence of plant viral diseases causes considerable economical and social problems (Rybicki and Pietersen, 1999). However, despite their importance, the attributes responsible for the establishment and spread of invaders are often difficult to pinpoint or are unknown. Ecological studies have provided a body of information concerning factors that are important for invasive parasites (Schrage and Wiener, 1995). Also, genetic approaches provide essential information to understand virus success because genetic variation can be determinant for invaders. For successful emergence, introduced parasites need to evolve rapidly to circumvent loss of genetic variation normally associated with founder effects. Here are summarized results about TYLCD emergence in Spain and the western Mediterranean basin and forces that seem to play a major role in their evolution and adaptation, influencing their success after the invasion of a non-native area.

Genetic stability in the population of a TYLCD-associated viral species in the absence of external alteration

The spread of TYLCD in Spain is well documented (Sánchez-Campos *et al.*, 1999, 2002). The first reports of infections were of the early 1990's. Since then, epidemics of TYLCD have caused devastating damage to field and greenhouse tomato crops. The spread of TYLCD paralleled the outbreaks of the natural insect vector *B. tabaci*. Emergence of TYLCD epidemics at the major growing areas was associated with the presence of the ES strain of the species *Tomato yellow*

leaf curl Sardinia virus (TYLCSV-ES) as the causal agent (Noris *et al.*, 1994; Reina *et al.*, 1994). It was an opportunity the possibility to monitor TYLCD epidemics in Spain since the first outbreak in 1992 and to perform systematic samplings in affected areas of three major tomato producing areas (Almería, Málaga, Murcia) during several years. Thus, samples collected during an eight-year period were analyzed for TYLCSV presence and a population genetic study was conducted to assess the evolution of this virus (Sánchez-Campos *et al.*, 2002). Variation within two coding (ORFs V2 and C2) and the non-coding IR regions of TYLCSV isolates collected was assessed by single-strand conformation polymorphism (SSCP) analysis. Interestingly, it was found that a low genetic diversity existed within the population with no evident geographical or temporal structures. The SSCP analysis showed that the population was composed of few common genetic types plus several infrequent ones suggesting a gamma distribution of the frequency of genetic types (Gibbs *et al.*, 1999). Therefore, the TYLCSV population showed to be genetically uniform. Moreover, the analysis of sequences from the IR region, which exhibited the highest variability, confirmed the limited genetic diversity revealed by the SSCP analysis. In fact, the average nucleotide diversity (Nei, 1987) in the IR for the entire population was 0.0087, a value ten times smaller than that obtained for a plant pathogenic non-coding RNA (the satellite RNA of *Cucumber mosaic virus*) or four times smaller than the value obtained for the plant RNA virus *Tobacco mild green mosaic virus*, which is considered to be relatively genetically stable (reviewed by García-Arenal *et al.*, 2001). Therefore, it was concluded that the TYLCSV population studied was a unique, undifferentiated population with a low genetic diversity, compatible with a founder effect associated with a population bottleneck during the transmission to a new area. Although genetic stability was evident, a tendency to a lineal increase in diversity over time was observed in some TYLCSV subpopulations (Table 1), but no accumulation of mutations in single isolates occurred. A similar situation was also observed after the introduction of a TYLCD-associated virus in other geographical areas (Delatte *et al.*, 2007). Thus, results are consistent with data available for plant viruses which indicate that genetic stability seems to be the rule in their populations (Gibbs *et al.*, 1999), probably associated with selection against less fit variants arising by mutation. Therefore, stability in TYLCSV populations in the absence of external influences is



Figure 1. A: symptoms of leaf deformation and upward curling of leaflet margins in leaves of tomato yellow leaf curl disease (TYLCD)-affected tomato plants. B: severe stunting and yellowing characteristic of TYLCD infection during early growth stages of tomato plants. C: schematic representation of the genome organization of monopartite TYLCD-associated viruses; arrows show the open reading frames and the black box in the circle shows the intergenic region containing the stem-loop structure common in all members of the family *Geminiviridae*.

suggested from these results. However, natural virus populations are not closed systems, and can be subjected to external forces of variation that can derive in unexpected genetic diversification as shown below.

Migration as source of genetic diversity in TYLCD-associated virus populations

An interesting question arises from invasion biology: how do introduced populations, whose genetic variation

probably has been reduced by population bottlenecks, persist and adapt to new conditions? In fact, conservation genetics show that reduced genetic variation due to genetic drift and founder effects can limit the ability of a population to adapt (Sakai *et al.*, 2001). However, many introduced species can persist and expand their ranges, evolving rapidly and become invasive. Moreover, genetic analyses have shown that in some occasions in contrast to expected, substantial genetic variation was observed in introduced populations. One key for invasion success is the occurrence of multiple introductions that can transform among-population variation in native ranges

Table 1. Nucleotide diversity within *Tomato yellow leaf curl Sardinia virus* populations inferred from IR sequences for each location and their R^2 values of lineal regression over time^a

Province	1992	1993	1994	1995	1996	1997	1998	R^2
Almería	0.0000 (4)	0.0028 (9)	0.0052 (7)	—	0.0080 (4)	0.0098 (7)	—	0.9804 ^b
Málaga	—	—	—	0.0018 (7)	0.0058 (4)	0.0106 (5)	—	0.9973
Murcia	0.0066 (8)	0.0043 (3)	0.0085 (3)	—	0.0101 (4)	0.0085 (3)	0.0096 (4)	0.4789

^a Nucleotide diversities were estimated as proposed by Nei (1987). In parenthesis is indicated in each case the number of isolates randomly selected from the whole population, for which the sequence of the IR was obtained. ^b In each location R^2 resulted from lineal regression analyses (SPSS, 1999) of nucleotide diversity values over time. Source: Sánchez-Campos *et al.* (2002).

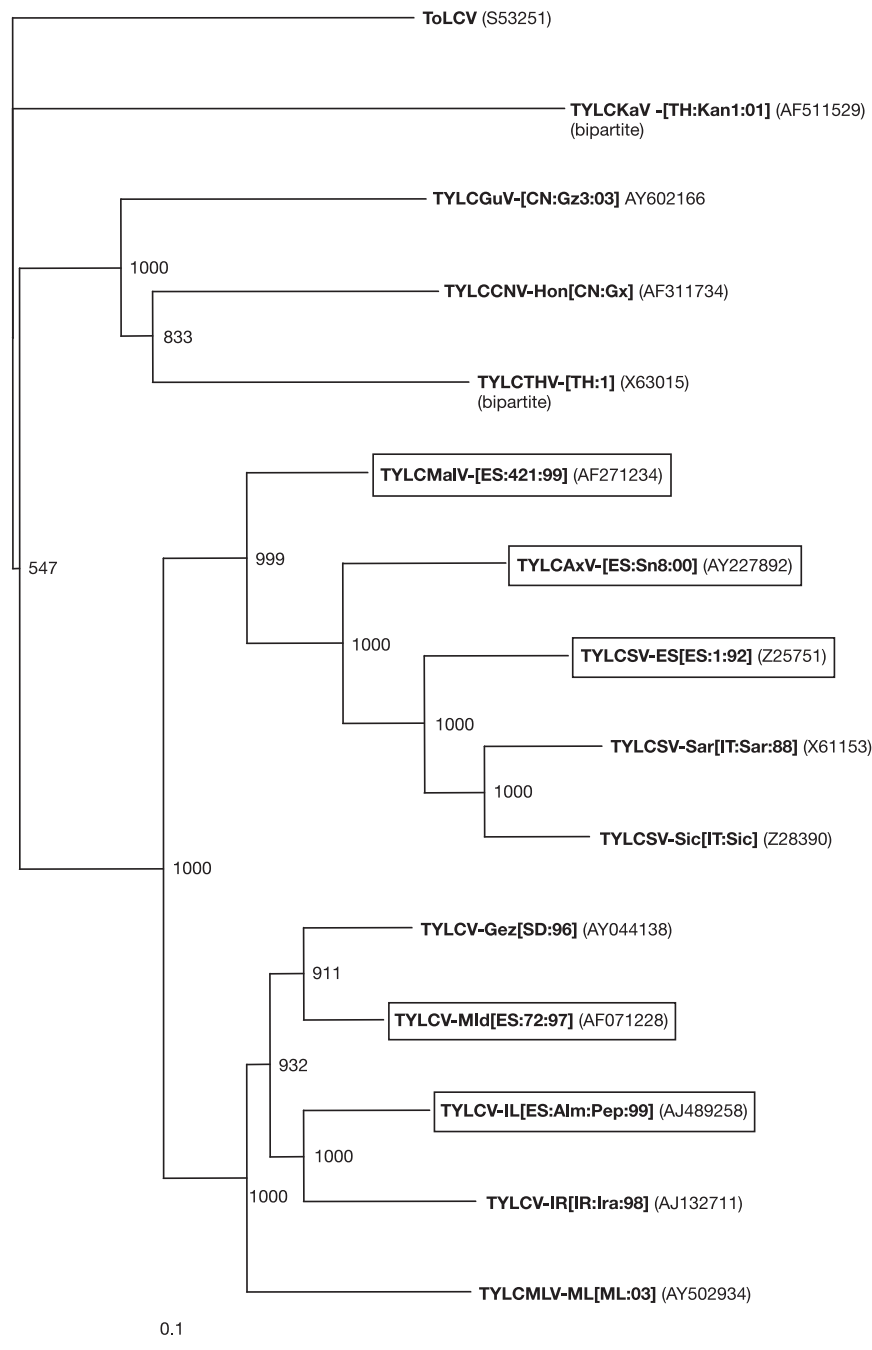


Figure 2. Phylogenetic relationships between viruses associated with the tomato yellow leaf curl disease (TYLCD) based on the complete genome (or DNA A) sequence. Relationships were inferred by neighbor-joining analysis. Support for nodes in a bootstrap analysis with 1000 replicates is shown for figures over 800. Vertical distances are arbitrary, and horizontal distances reflect number of nucleotide differences between branch nodes; scale bar indicates the horizontal distance equivalent to 0.1 replacements per position. Sequences of representative isolates of each TYLCD-associated species are included: *Tomato yellow leaf curl Axarquía virus* (TYLCAxV), *Tomato yellow leaf curl China virus* (TYLCCNV), *Tomato yellow leaf curl Guangdong virus* (TYLCGuV), *Tomato yellow leaf curl Kanchanaburi virus* (TYLCKaV), *Tomato yellow leaf curl Málaga virus* (TYLCMaIV), *Tomato yellow leaf curl Mali virus* (TYLCMLV), *Tomato yellow leaf curl Sardinia virus* (TYLCSV), *Tomato yellow leaf curl Thailand virus* (TYLCTHV), *Tomato yellow leaf curl virus* (TYLCV). The sequence of an isolate of *Tomato leaf curl virus* (ToLCV) was used as outgroup. Bipartite viruses are indicated, and the GenBank accession number of the complete genome (or DNA A) sequence used for comparison is indicated between brackets. Genotypes reported in Spain are boxed (adapted from Moriones and García-Andrés, 2007).

to within-population variation in introduced areas, as demonstrated in other systems (e. g. Kolbe *et al.*, 2004).

The colonization of TYLCD-associated viruses in the western Mediterranean basin is an example of success in which an increase of the genetic diversity resulted from successive genetic migrations. Thus, for example in Spain as shown above, after the initial introduction of TYLCSV, a relatively stable period was observed in the population, with a reduced genetic diversity typical of a population bottleneck upon its introduction. Reduction in genetic diversity could have been detrimental for the success of this TYLCD-associated virus population. However, subsequent introductions of TYLCV strains provided opportunities to increase genetic diversity. Ecological circumstances dramatically changed in southern Spain for TYLCD epidemics after the introduction of the Mld strain of TYLCV (TYLCV-Mld) at about 1996 (Navas-Castillo *et al.*, 1999). In addition to an increase in the genetic diversity of the population, the spread of TYLCV resulted in the progressive displacement of TYLCSV in the epidemics (Table 2) (Sánchez-Campos *et al.*, 1999). Displacement of TYLCSV by TYLCV was associated with a better ecological fitness of the latter virus. Ecological factors were analyzed, suggesting that the highest rate of transmission of TYLCV by *B. tabaci* in addition to its host range including common bean, a bridge crop between tomato crops, might have been involved in such a displacement (Sánchez-Campos *et al.*, 1999). This could have direct consequences for the evolution of the TYLCSV population, because the decrease in the population size might result in accumulation of mutations according to Muller's ratchet mechanism (Chao, 1990) as observed in other cases (Fraile *et al.*, 1997). Interestingly, an additional introduction of a new TYLCV variant was reported some time later

in southern Spain. In this occasion, the strain IL of TYLCV (TYLCV-IL) was detected (Morilla *et al.*, 2003). This strain was found to be well adapted to the ecological circumstances present in the invaded area showing that soon after its introduction it was the predominant virus type in TYLCD epidemics. Moreover, evolution of TYLCV-IL frequencies in several geographical region sampled suggested that a progressive colonization of the different regions occurred, first Almería, then Murcia, and finally Málaga (García-Andrés *et al.*, 2007a). It should be noted that striking molecular differences existed between TYLCV-Mld and TYLCV-IL genomes in the region covering the 5'-part of the IR and the 5' two thirds of the Rep ORF (Fig. 3A), resulted from a genetic exchange through recombination with a begomovirus associated with the tomato leaf curl disease (Fig. 3B) (Navas-Castillo *et al.*, 2000). This genetic difference might result into biological differences that can determine disparities in their epidemiological success. Thus, for example, differences exist between these two viral types in the ability to infect genotypes of plants selected for resistance to TYLCD (Fig. 3C). Therefore, data suggested a great dynamism for the population of viruses associated with TYLCD, related to the successive introductions of different genetic types. Similar conclusions also were obtained in a parallel study conducted in this same geographical area (Font *et al.*, 2007).

After more than ten years of continuous TYLCD epidemics in the western Mediterranean basin, a complex virus population was observed. Thus, as shown in Figure 4, based on samplings performed in that area (García-Andrés *et al.*, 2007a), phylogenetic analyses revealed presence of isolates that grouped with four distinct TYLCD-associated begomovirus species, and strains of the same virus species: the Sar, Sic and ES

Table 2. Results of a systematic survey conducted during 1996 to 1998 in field-grown commercial tomato crops of southern Spain and analysis of the samples for TYLCSV and/or TYLCV infection

Year	No. of fields sampled	No. of samples collected	Incidence of TYLCD (%) ^b	Incidence (%) of samples positive for: ^a		
				TYLCSV	TYLCV	TYLCSV + TYLCV
1996	20	1,000	68.6	39.8	7.1	21.9
1997	12	605	81.3	17.0	38.2	26.1
1998	11	554	96.7	1.8	72.7	22.2

^a Samples were tested by tissue blot hybridization on positively charged nylon membranes using digoxigenin-labeled DNA probes specific to TYLCSV or TYLCV. Percentages were calculated over the total number of collected samples. ^b Incidence of TYLCD was calculated over the number of samples collected based on the total number of samples positive for TYLCSV, TYLCV, and for TYLCSV + TYLCV.

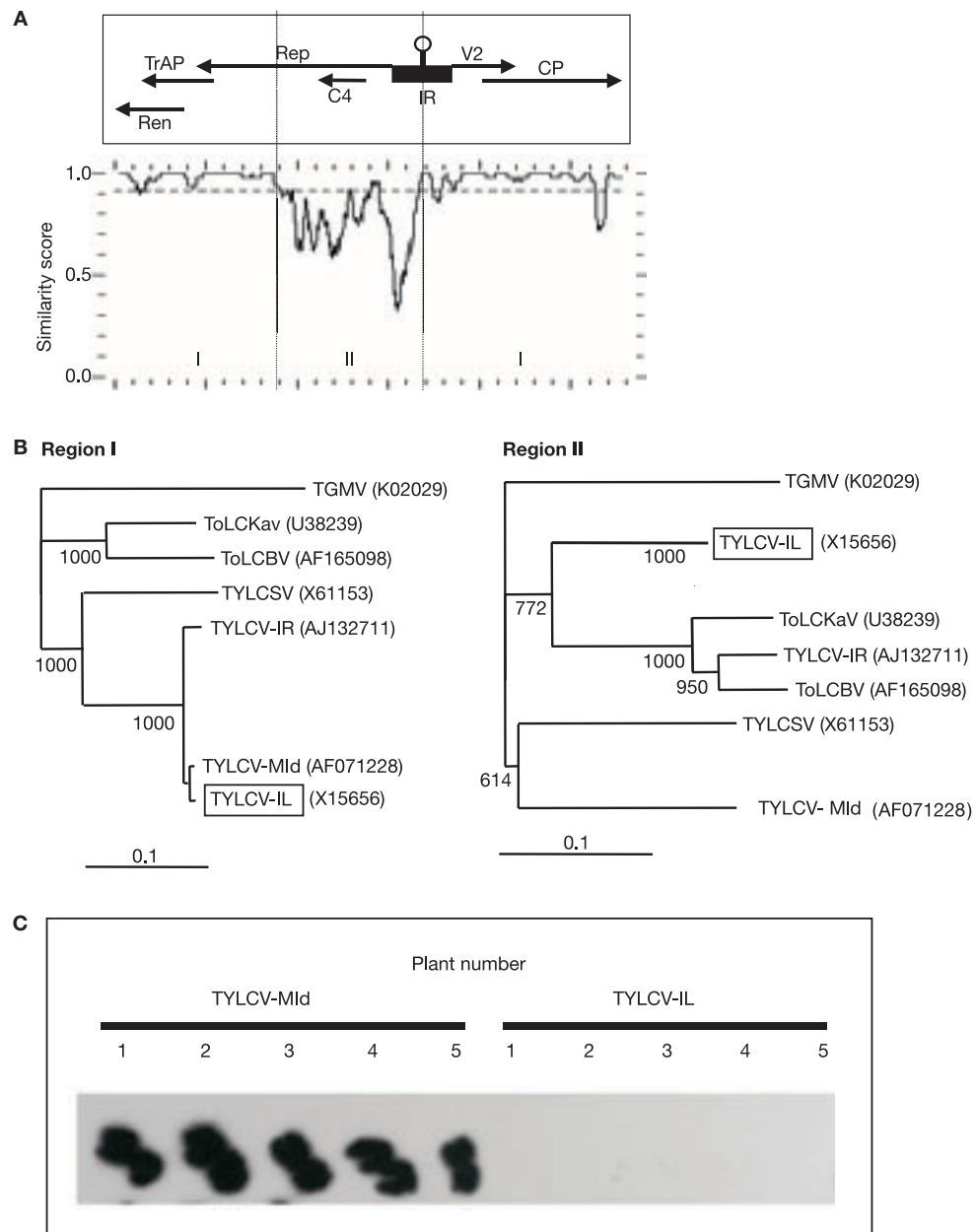
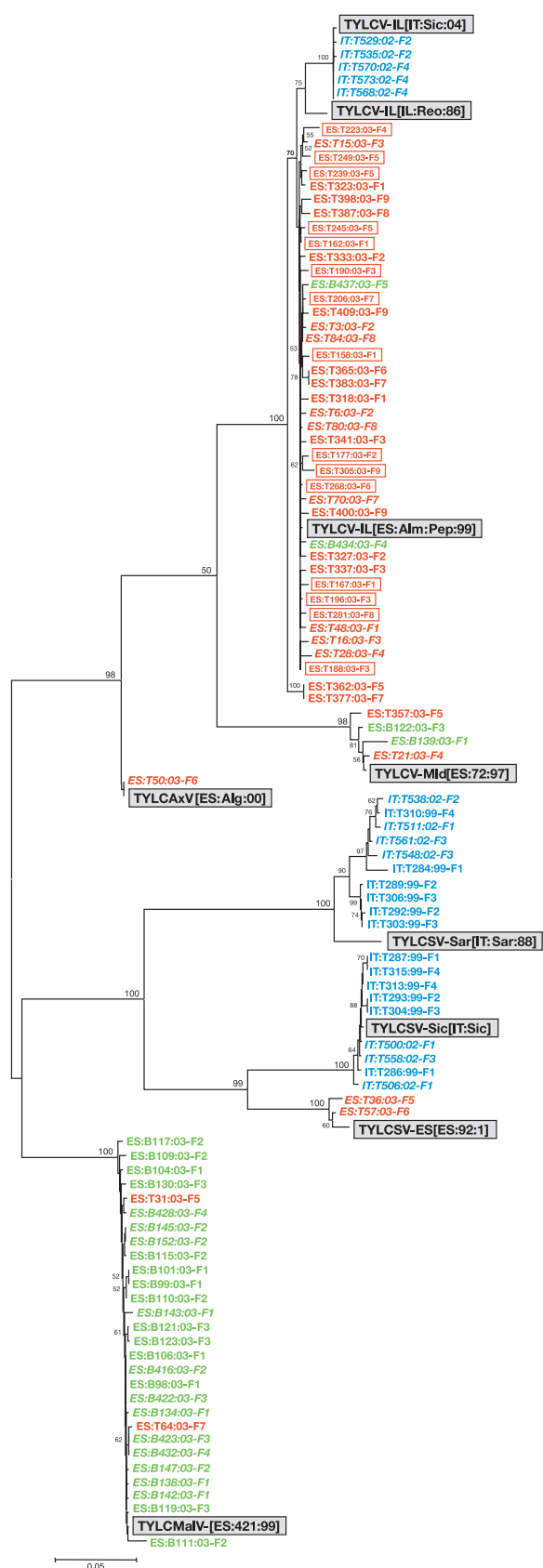


Figure 3. A: PLOTSIMILARITY diagram (scanning window = 50) comparing the nucleotide sequences of *Tomato yellow leaf curl* (TYLCV) isolates of the Mld (isolate [ES:72:97], GenBank accession number AF071228) and IL (isolate [IL:Reo:86], GenBank X15656) strains. Separation between regions I and II for which differential distribution of nucleotide identity is observed are indicated by vertical dotted lines. Positions of the open reading frames and of the intergenic region (IR) are indicated at the top of the figure. Horizontal broken line shows the mean similarity between the sequences compared. B: neighbor joining phylogenetic trees depicting topology and distance relationships of sequences in regions I and II defined in panel (A). Trees were inferred using programs in the CLUSTAL W software package. The significance of the nodes in a bootstrap analysis with 1,000 replicates is shown. Vertical lengths are arbitrary while horizontal lengths reflect genetic distance between branch nodes. Scale bars indicate the horizontal distance equivalent to 0.1 replacements per position. DNA-A sequences of isolates of TYLCV (TYLCV-IL is boxed) and related begomoviruses [*Tomato golden mosaic virus*, TGMV; *Tomato leaf curl Bangalore virus*, ToLCBV; *Tomato leaf curl Karnataka virus*, ToLCKaV; *Tomato yellow leaf curl Sardinia virus*, TYLCSV] are included in the comparison. The sequence of the DNA-A of an isolate of TGMV was used as an outgroup. GenBank accession numbers of sequences used for comparison are indicated between brackets. C: squash blot hybridization of petiole cross sections of young newly emerged leaves of plants of a *Solanum habrochaites* resistant accession inoculated with TYLCV-IL or TYLCV-Mld, at 30 days post inoculation; a probe that equally recognizes both virus types was used for hybridization.



strains of TYLCSV, the IL and Mld strains of TYLCV, *Tomato yellow leaf curl Axarquía virus* (TYLCAxV), and *Tomato yellow leaf curl Málaga virus* (TYLCMaV). Interestingly, spatial differentiation was observed in the population. For example, differences were evident in the population structures when comparing tomato subpopulations from Italy or Spain (isolates in blue and red, respectively, in Fig. 4). In fact, Italian and Spanish subpopulations only shared isolates of the IL strain of TYLCV, and even those grouped separately (Fig. 4). Different histories of virus spread might have determined the differences observed. Thus, the population structure found is compatible with several introductions and spread of different virus types in the region compatible with founder effects (García-Andrés *et al.*, 2007b). Therefore, migration seemed to have contributed notably to the genetic diversification of the TYLCD-associated virus populations present in the western Mediterranean basin, and the resulting genetically variable population probably have gained a greater potential for local adaptation (Lively and Dybdahl, 2000).

Figure 4. Phylogenetic relationships for a sequence of about 780 nucleotides encompassing the non-coding intergenic region and 5'-proximal parts of Rep and V2 open reading frames derived for tomato yellow leaf curl disease (TYLCD)-associated begomovirus-like molecules cloned from tomato samples collected in Sicily (Italy) during 1999 and 2002 (normal and italics blue text, respectively) or from tomato and common bean (red and green text, respectively) samples collected in Spain during 2003 in Almería, Málaga, or Murcia provinces (normal, italics, and boxed text, respectively). Relationships were inferred by neighbor-joining analysis. Support for nodes in a bootstrap analysis with 1000 replications is shown for values over 50%. Horizontal branch lengths are drawn to scale with the bar indicating 0.05 nucleotide replacements per site. Vertical distances are arbitrary. GenBank accession numbers for sequences shown are DQ317696 to DQ317784, and DQ317797 to DQ317806. Isolates are identified using a code that refers to country (ES, Spain; IT, Italy), host species origin (T, tomato; B, common bean), sample number:year, and field (F1, field 1). Equivalent regions of representative isolates are included (boxed and shadowed text) of begomoviruses associated with TYLCD in the Mediterranean area: the Sar, Sic and ES strains of *Tomato yellow leaf curl Sardinia virus* (TYLCSV), IL (isolates from Israel —IL—, Spain, and Italy) and Mld strains of *Tomato yellow leaf curl virus* (TYLCV), *Tomato yellow leaf curl Málaga virus* (TYLCMaV), and *Tomato yellow leaf curl Axarquía virus* (TYLCAxV) (GenBank accession numbers X61153, Z28390, Z25751, X15656, AJ489258, DQ144621, AF071228, AF271234, and AY227892, respectively) (adapted from García-Andrés *et al.*, 2007a).

Recombination as source of genetic diversity in TYLCD-associated virus populations

Gene flow provided by sex and/or recombination is exploited by parasites to increase their evolutionary potential and to enhance local adaptation (Bürger, 1999), and viruses are not an exception (Roossinck, 1997; García-Arenal *et al.*, 2001). During mixed infections, viruses can exchange genetic material through recombination or reassortment of segments (when the parental genomes are multipartite) if present in the same cell context of the host plant. Hybrid progeny viruses might then arise, some of them well adapted in the population that can cause new emerging diseases. Recombination appears to be common among members of the family *Geminiviridae* (Padidam *et al.*, 1999). In this group of viruses, more notably among members of the genus *Begomovirus*, recombination seems to have contributed greatly to the genetic diversification of viral populations (Sanz *et al.*, 2000; Berrie *et al.*, 2001; Pita *et al.*, 2001). Therefore, the potential of begomoviruses to generate genetic diversity through recombination can be relevant for their ecological fitness, and recombination should be taken into account among forces driving evolution in this group of viruses.

In the case of TYLCD epidemics in Spain, the presence of different virus types was an excellent substrate for recombination to occur. In fact, the existence of mixed infections in nature was demonstrated (e.g. Sánchez-Campos *et al.*, 1999). Moreover, it was demonstrated that TYLCD-associated viruses can co-localize in the same cell and share nuclei of infected cells (Morilla *et al.*, 2004) where replication occurs, which is a prerequisite for recombination to take place. Furthermore, experimental evidence was provided for the occurrence of multiple recombinants after experimental co-inoculation of tomato plants (García-Andrés *et al.*, 2007b). Therefore, emergence of recombinant genotypes in nature was highly predictable. Specific aspects about occurrence of recombination among viruses of the TYLCD complex have been reviewed recently by Moriones *et al.* (2007). It was found that recombinant genotypes quickly emerged and spread in the population after the novel introduction of TYLCV virus strains into Spanish epidemics. During field surveys two types of recombinants were found, TYLCMaV and TYLCAxV (Monci *et al.*, 2002; García-Andrés *et al.*, 2006). The former resulted from a genetic exchange between

TYLCSV-ES and TYLCV-Mld, and the latter also involved TYLCSV-ES, but the genetic exchange occurred with TYLCV-IL (Fig. 5). The survival and establishment of these recombinants in nature will probably depend on them having selective advantages, as demonstrated for another plant virus (Bonnet *et al.*, 2005). Interestingly, for these two recombinants, novel pathogenic properties were demonstrated that suggested enhanced ecological adaptation to the invaded area. Thus, in addition to be readily transmissible by *B. tabaci*, they exhibited a host range wider than either parental virus (Table 3), which is consistent with selection for a better natural fit. Notably, as shown in Figure 4, it was found for TYLCMaV that soon after its first detection in the epidemics, it displaced almost completely TYLCV in common bean, suggesting its better adaptation to this host (García-Andrés *et al.*, 2007a). TYLCAxV was found at high frequencies in the population of the wild host plant *Solanum nigrum* L. (García-Andrés *et al.*, 2006) and its spread into cultivated host plants was confirmed (García-Andrés *et al.*, 2007a). The novel biological properties suggested that it is a step forward in the ecological adaptation to the invaded area (García-Andrés *et al.*, 2006). Based on the singular genetic and biological properties of these two recombinant viruses, and following species demarcation criteria proposed for begomoviruses (Fauquet *et al.*, 2003), the *Geminiviridae* Study Group of the International Committee on Taxonomy of Viruses considered them as two new virus species in the *Begomovirus* genus (Fauquet and Stanley, 2005).

Collectively, the above data provide evidence of the key role of recombination in the evolution of TYLCD-associated virus populations. The outbreak of recombinant begomoviruses with enhanced pathogenicity within the TYLCD complex was demonstrated. Since the appearance of virus variants with better fit can have dramatic consequences in begomovirus epidemics (Zhou *et al.*, 1997; Pita *et al.*, 2001), studies are under way to elucidate the possible effect of the outbreak of these viruses on the population of TYLCD-associated viruses.

Concluding remarks

The colonization of TYLCD-associated begomoviruses in Spain is an example of invasion success following multiple introductions, similar to those reported for animals or plants (Novak and Mack, 2001; Kolbe *et al.*, 2004). As shown in some of such invasions of new areas, after the initial introduction of TYLCSV

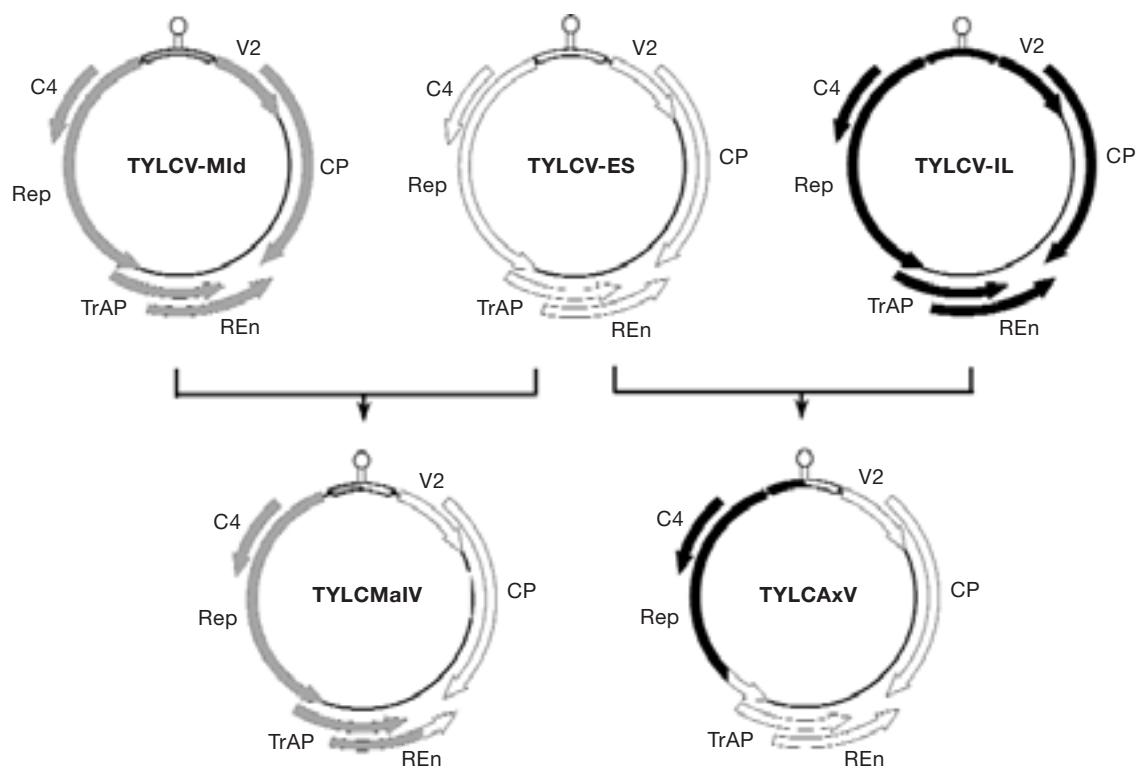


Figure 5. Schematic representation of genetic exchanges occurred between isolates of the ES strain of *Tomato yellow leaf curl Sardinia virus* (TYLCSV-ES) and of the strains Mld (TYLCV-Mld) or IL (TYLCV-IL) of *Tomato yellow leaf curl virus* that resulted in the recombinant viruses *Tomato yellow leaf curl Málaga virus* (TYLCMaIV) and *Tomato yellow leaf curl Axarquía virus* (TYLCAxV). Arrows indicate the putative proteins coded by these viruses. The hairpin conserved in the intergenic region of all geminiviruses is represented at the top of each circular molecule.

in the early 1990's, a relatively stable period was observed, with reduced genetic diversity typical of a population bottleneck upon its introduction. Reduction in genetic diversity could have been detrimental for the success of the virus population, however, the sub-

sequent migrations of TYLCV strains resulted in increased diversity and provided conditions for recombination to occur, giving rise to new genetic diversity. It is well known that recombination helps evolution to create high fitness genotypes rapidly (Bürger, 1999).

Table 3. Ability to infect several plant host species by *Tomato yellow leaf curl Sardinia virus* (TYLCSV), *Tomato yellow leaf curl virus* (TYLCV), and the recombinant viruses derived from them, *Tomato yellow leaf curl Málaga virus* (TYLCMaIV) and *Tomato yellow leaf curl Axarquía virus* (TYLCAxV)^a

Plant species	Parental viruses		Recombinant viruses ^b	
	TYLCSV	TYLCV	TYLCMaIV	TYLCAxV
Tomato	+	+	+	+
Common bean	—	+	+	+
<i>Solanum nigrum</i>	+	— ^c	+	+

^a «+» indicate detection, and «—» indicate not detection in plants 30 days after inoculation analyzed by hybridization of tissue blots from petiole cross-sections of young non-inoculated leaves. Plants were inoculated via *Agrobacterium tumefaciens* using infectious clones. ^b TYLCMaIV resulted from a genetic exchange between isolates of TYLCSV-ES and of TYLCV-Mld and TYLCAxV resulted from a genetic exchange between isolates of TYLCSV-ES and of TYLCV-IL. ^c Presence of TYLCV virus traces in young non-inoculated leaves of *S. nigrum* plants could be detected by polymerase chain reaction amplification but not by hybridization.

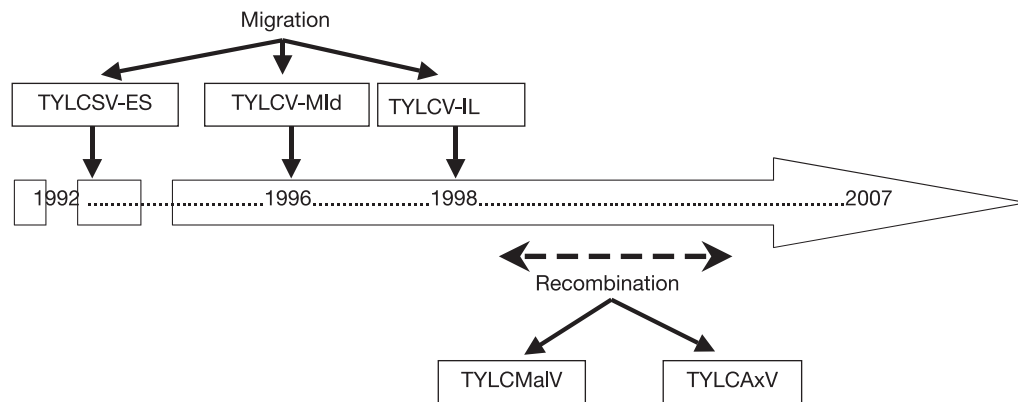


Figure 6. Schematic representation of the temporal evolution of the population of viruses associated with tomato yellow leaf curl disease present in Spain, showing approximate dates of introduction of the ES strain of *Tomato yellow leaf curl Sardinia virus* (TYLCSV-ES) and of strains Mld (TYLCV-Mld) and IL (TYLCV-IL) of *Tomato yellow leaf curl virus*, and of emergence of the recombinant viruses derived from genetic exchanges between them, *Tomato yellow leaf curl Málaga virus* (TYLCMaV) and *Tomato yellow leaf curl Axarquía virus* (TYLCAxV).

Data provided evidence for emergence of recombinant virus types and for the key role of recombination as a force driving the evolution of TYLCD-associated virus population in the invaded non-native area. Thus, in a relatively short time period lapse, the combination of genetic migration and recombination notably increased genetic diversity in the virus population associated with TYLCD in Spain (Fig. 6). The resulting genetically variable population probably has gained a greater potential of adaptation. It is also interesting to note the possible role of native weeds as reservoirs of the genetic diversity for this begomovirus population (García-Andrés *et al.*, 2006), which should be examined in more depth. The existence of multiple infections in long-lasting wild hosts might ensure that novel begomovirus genotypes emerge after several rounds of recombination, in a symbiogenesis-like evolution process (Roossinck, 2005). These new genotypes can help the success of the invader population. Therefore, it would be of great interest to examine the adaptive significance of the new begomovirus genotypes that could emerge after recombination. Also, it remains the question for future research of how the intensive human influence on this agro-system, such as host changes (new tomato varieties), can modulate the evolution of this dynamic TYLCD-associated virus population selecting better adapted virus genotypes.

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