

Molecular Characterisation of Begomoviruses Associated With Chilli Leaf Curl Disease

Dinesh Tiwari¹ ●, Ioanna Theodorou² ●, Nikolaos Papadakis³ ●

¹ Assistant Professor, Department of Plant Pathology, Kaveri Delta Centre for Animal Sciences, Thanjavur, India. Email: d.tiwari@kdcas.ac.in | ORCID: 0000-0001-0411-4599

² Assistant Professor, Department of Seed Science and Technology, Thessaly Plain Institute of Agriculture, Larissa, Greece. Email: i.theodorou@tpia.gr | ORCID: 0000-0001-0044-7767

³ Senior Scientist, Department of Genetics and Plant Breeding, Thessaly Plain Institute of Agriculture, Larissa, Greece. Email: n.papadakis@tpia.gr | ORCID: 0000-0001-3427-4518

ABSTRACT

Chilli leaf curl disease is one of the most damaging constraints on chilli and pepper production across South Asia, and the begomoviruses behind it are usually described as if a single named virus were responsible. This narrative review asks how firmly that description is supported. It draws on published work on begomovirus genome organisation, betasatellites and alphasatellites, whitefly transmission, recombination, sequence-based classification and diagnostic methods, and it reports no new sequences or data. The literature shows a disease that is better described as a complex than as a single agent: several begomovirus species, often together with a betasatellite, have been recovered from affected chilli, and recombination blurs the boundaries between them. Which virus appears to be causal therefore depends on where and when plants were sampled, which primers and amplification method were used, and which classification threshold was applied. Reports that rest on a short fragment, or on a single plant per site, can name a virus confidently without being able to exclude its neighbours. The review concludes that the causal agent of chilli leaf curl is best treated as site-dependent, sets out a minimum description that would make molecular reports comparable, and identifies where better sampling and full-genome work would help most.

Keywords: Chilli leaf curl disease; Begomovirus; Betasatellite; Whitefly; Recombination; Virus classification; Narrative review

Citation: Tiwari, Theodorou and Papadakis [2019]. Molecular Characterisation of Begomoviruses Associated With Chilli Leaf Curl Disease. DOI: 10.62649/v7.i03.2019.pp47-56

Copyright: © 2019 by the authors. Open access under CC BY 4.0 license.

Article Information: Received: December 2, 2018 Accepted: April 10, 2019 Published: August 24, 2019

Article Class: Review Article

1. Introduction

Few diseases make a chilli grower's heart sink as quickly as leaf curl. Leaves roll and pucker, the plant stops growing properly, flowers drop, and what fruit does set is small and sparse. In much of South Asia the disease has become one of the main reasons a chilli crop fails, and reports of it have accumulated for decades alongside a wider rise in whitefly-transmitted viruses [1, 2]. The agents behind it belong to the genus *Begomovirus*, the largest genus in the family *Geminiviridae* [3].

Papers on the subject often speak of chilli leaf curl virus as if the matter were settled: one disease, one virus. The molecular literature tells a less tidy story. Begomoviruses evolve fast, exchange sequence readily, and frequently travel with small satellite molecules that change how disease looks and behaves [4, 5]. A survey that recovers one virus from one plant at one site has found something real, but it has not necessarily found the cause of leaf curl in the next field, let alone the next state. The aim is a fair account of what has been shown and what has merely been repeated.

This review therefore asks a narrow question and tries to keep it narrow. How well does the published molecular evidence support the idea that chilli leaf curl disease has a single, identifiable causal virus, and what do the complications of species boundaries, recombination and method mean for how reports should be read? It covers genome organisation, satellites, whitefly transmission, recombination, classification and diagnosis, and it reports no new sequences. Where the chilli-specific literature is thin we say so, instead of filling the gap with figures we cannot check.

2. Literature Review

2.1 Begomovirus Genomes and Their Satellites

Begomoviruses have small circular single-stranded DNA genomes packaged in paired, or geminate, particles, which gives the family its name [3, 6]. Some species, mostly in the Americas, carry two genome components called DNA-A and DNA-B. Many Old World species are monopartite, and the DNA-A component alone carries the genes for the coat protein, for replication and for the proteins that deal with the host's defences [7, 8]. The layout is sketched in Figure 1. The genes sit on both strands, either side of a common region that contains the origin of replication and the promoters.

Replication runs through rolling-circle and recombination-dependent routes that rely heavily on host machinery, and the viral proteins reprogramme the plant cell to make it permissive [7]. Several of the proteins also interfere with RNA silencing, the plant's main antiviral defence [9]. That overlap matters for what follows: the same small genome handles replication, movement, transmission and counter-defence, so changes anywhere in it can have consequences for symptoms and host range. This is one reason the same disease can seem to have a different cause in each region.

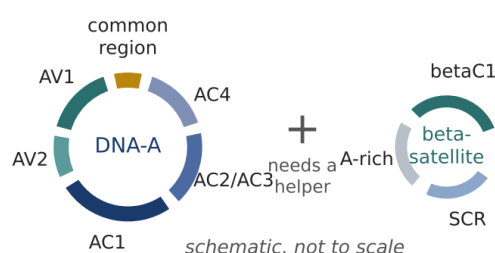


Figure 1. Layout of a begomovirus genome and its satellites (schematic, not to scale, not data).

The satellites deserve a separate word. Betasatellites are circular molecules that depend

on a helper begomovirus for replication, movement and packaging, and they carry a single gene, betaC1, which is a pathogenicity factor and a suppressor of silencing [10, 11]. Their discovery changed how several diseases were understood. In ageratum yellow vein disease, a virus alone could cause only mild symptoms, and the full disease required the satellite [12]. Experiments with other begomovirus-betasatellite pairs showed that the satellite could be required for typical symptoms and that betaC1 alone could reproduce much of the effect [13, 14, 15]. Alphasatellites, a third class, replicate on their own but still rely on a helper for movement and spread [4].

Table 1 lists the components that may be present in a single infected plant. The practical consequence is that a plant can carry a begomovirus, a betasatellite and an alphasatellite together, and that any diagnosis aimed only at the first will describe a third of the picture. A betasatellite recovered from okra in India, and its association with yellow vein mosaic disease, was an early example from the same region and a warning of what to expect in other crops [16].

Table 1. Genome components that can be present in a chilli leaf curl infection.

Component	Main role	Status in chilli reports
DNA-A	Replication, coat protein, vector transmission, silencing suppression [3, 7]	Present in every case
DNA-B	Movement in bipartite viruses [8]	Only with some species
Betasatellite	Symptom determinant; depends on helper virus [13, 14]	Frequently recovered [20]
Alphasatellite	Self-replicating; role unclear [4]	Reported less often
Recombinant segments	Mosaic of parental sequence [30, 31]	Common in surveys

Note: Qualitative summary of the cited literature. Not every component occurs in every infected plant, and the set recovered depends on the method used.

2.2 Disease Complexes and the Idea of a Cause

The term disease complex describes a disease in which the symptoms arise from several interacting agents instead of one. The cotton leaf curl disease of Pakistan and north-western India was an influential case, in which a changing set of begomoviruses and a betasatellite shared the credit and the blame [17, 18]. Reviews of begomovirus ecology make the same point in general terms: where several viruses, a satellite and a common vector meet in a crop, the disease is a property of the assemblage [5, 19].

In chilli the position is similar. A widely cited survey of chilli leaf curl disease in India recovered a mixed population in which more than one begomovirus species and several betasatellites occurred, with evidence of recombination among the viral sequences [20]. A species named from the foothills of northern India was reported together with its own betasatellite [21]. Taken as isolated reports these look like competing answers to the question of what causes the disease; read together, they look like samples from one shifting population. The differences look small on a map, yet they decide which resistance source a breeder should trust.

2.3 Whiteflies as Vectors

Begomoviruses are transmitted by the whitefly *Bemisia tabaci* in a persistent, circulative manner: the virus is taken up with phloem sap, crosses the gut wall, moves through the insect and is released with saliva, without multiplying in the usual sense [22, 23]. The passage involves specific interactions between insect proteins and the virus particle, and it takes hours, not minutes, which is why quick, casual feeding is less efficient than prolonged access to an infected plant.

Bemisia tabaci is not one entity. Early work distinguished biotypes with different host ranges and different transmission abilities [24, 25], and later work argued that the group is a complex of cryptic species that look alike but are genetically distinct [26, 27]. Genetic groups differ in how widely they colonise crops, how readily they displace one another, and how well they transmit particular viruses. The whitefly has been called a supervector for just this reason: it is the common thread in the emergence of many unrelated plant diseases [28]. For chilli, it means that a change in the local whitefly population can change which virus is moved, even when the viruses themselves have not changed. A plant sampled early in infection can give a different picture from one sampled late.

2.4 Recombination, Mutation and Emergence

Begomoviruses change by two main routes. Point mutation is frequent, and the estimated rates for a well-studied species are comparable with those of RNA viruses, which is surprising for a DNA virus [29]. Recombination is the other route, and it appears to be common in the family; early work argued that new viruses could emerge by frequent recombination [30], and later analyses showed that recombination patterns preserve interacting regions of the genome [31]. Recombination has been linked to host switches and to the global

spread of important species [32, 33].

For classification this is a serious complication. A recombinant has two or more ancestors, so a single phylogenetic tree built from one region can place it with either parent depending on which region was used. A sequence that appears to sit between two species in a distance table may be a recombinant, a divergent member of one species, or a member of a new species, and the three possibilities call for different conclusions.

2.5 Taxonomy and Species Boundaries

The criteria for naming begomovirus species have changed over time. A revision proposed in 2008 used pairwise comparison of complete DNA-A sequences with a lower demarcation value [34], and a later revision raised the species threshold to 91 per cent identity and set a separate, higher value for strains [8]. The change was not a discovery that species had moved; it was a decision about where to draw a line across a continuum, made to improve consistency. Software now exists to compute and display such comparisons in a uniform way [35], so that two groups working on the same sequences can reach the same answer.

Before full genomes were easy to obtain, a short region of the coat protein gene was used for provisional identification [36]. That approach is quick but it carries all the risks of reading one gene: it can group recombinants wrongly, and it cannot see a satellite at all. The taxonomy literature itself is clear that a short fragment can only support a provisional name.

2.6 How Begomoviruses Are Detected and Sequenced

Detection began with degenerate primers designed to amplify a fragment of the coat protein region from a wide range of whitefly-transmitted geminiviruses [37, 38]. These are fast and sensitive, and they became the standard first screen. Their weakness is the same as their strength: they are built on conserved sequence, so a lineage that departs from the expected pattern may be missed or only partly amplified.

Rolling circle amplification with a phage polymerase made it possible to amplify whole circular genomes from small amounts of tissue without prior knowledge of the sequence [39, 40]. Small-RNA deep sequencing offered another route, assembling viral genomes from the fragments that the plant itself produces in response to infection [41]. Both have been used to discover new viruses and to reveal infections that targeted primers missed. They bring their own problems, discussed

below, with mixed templates and assembly. Read this way, failure to find a satellite is not evidence that none was present.

3. Materials and Methods

3.1 Scope and Question

The review asks one question in two parts: does the published evidence support a single causal begomovirus for chilli leaf curl disease, and if not, how should molecular reports on the disease be read and written? The question was broken into the steps a molecular report must take: which plants were sampled and how many, how nucleic acid was amplified, whether the whole genome and any satellites were recovered, how recombination was handled, and how names were assigned. None of these steps is difficult, but each one is easy to leave out.

3.2 Literature Assembly

We drew on peer-reviewed work in four areas: begomovirus biology, genome organisation and satellites; whitefly biology and virus transmission; recombination, evolution and taxonomy; and the methods of detection, sequencing and sequence comparison. Sources were chosen for relevance, standing in the field and clarity of method. Work on viruses of other crops was used for what it shows about mechanisms and methods, not as evidence about chilli.

We did not run a formal systematic search and do not claim one. This is a narrative synthesis, and the choice of sources reflects the authors' judgement. It is therefore a better guide to how the question should be asked than to the precise proportions in which each virus occurs in any region, and it avoids quoting prevalence figures from individual surveys that could not be checked against the primary papers for this article.

3.3 Appraisal Framework

To judge how far a molecular report can be trusted to name a cause, we asked five questions. How many plants and sites were sampled [20]? Was the whole DNA-A sequenced, or only a fragment [36, 37]? Were satellites looked for [4]? Was recombination tested before the sequences were compared [42]? And were the species names assigned by a stated method and cut-off [8, 35]? Table 2 collects the main reasons surveys can disagree, and Table 3 lists the methods themselves.

Table 2. Why different surveys can name different viruses in chilli leaf curl.

Source of difference	Effect on the answer	Remedy
Species complex	Several species coexist and co-infect [19, 20]	Sample many plants per site
Recombination	Single gene trees mislead [33, 42]	Test for breakpoints first
Short fragment	Cannot separate close relatives [36]	Sequence the whole DNA-A
Primer choice	Some lineages are missed [37]	Use circular amplification [39, 40]
Classification threshold	A cut-off, not a natural boundary [8, 34]	State the method and cut-off
Satellite not tested	Symptom cause misassigned [4]	Test for satellites too

Note: Sources of disagreement are drawn from the methodological and taxonomic literature cited; they are not attributed to any single survey.

4. Results

4.1 What the Literature Establishes About the Disease Agent

Three points are secure. First, chilli leaf curl disease is associated with begomoviruses; the molecular evidence for this is consistent across reports [20, 21]. Second, whitefly transmission is the route of spread in the field, and the biology of that route is well described [22, 23]. Third, betasatellites contribute to symptoms in several begomovirus-host systems and have been found in chilli [13, 20]. Each of these is a statement about a class of agent. None of them names a single virus.

The step from class to name is where the literature divides. Reports from different regions and years name different species as the main agent, and the review found no basis in the published sources for treating any one name as universally correct. A short-fragment survey of one district might return one species at every site while a full-genome survey of a neighbouring state returns several. Both can be right about their own samples. This is one reason the same disease can seem to have a different cause in each region.

4.2 The Species Complex

The evidence for a species complex rests on the surveys already described [20, 21] and on the general behaviour of begomoviruses in crops where several are present [18, 19]. Mixed infection is not unusual in this family, and it creates the conditions for recombination, because exchange needs two genomes in one cell [30, 31]. Mixed infection also favours pseudo-recombination in the bipartite viruses, in which components are

swapped between related species, though this is of limited relevance for the monopartite species that make up most Old World chilli reports [8].

What follows for the grower is awkward. If a field holds a mixture, then a diagnostic result that names one virus may describe the most abundant, the easiest to amplify, or the one the primers were designed for. The question of which virus is the cause is, in a mixture, a question about which of several contributes how much, and an answer needs experiments with infectious clones, not just sequence recovery [12, 13]. The point is not that a survey is poor, only that its conclusion travels less far than its title suggests.

Sampling design makes this worse. A survey that takes one symptomatic plant from each field will, by construction, report one virus per field, and the apparent uniformity of a region may simply reflect how few plants were looked at. Conversely, a survey that processes many plants at each site will find mixtures that the smaller survey could not see. The two do not contradict each other, but a reader comparing them without noticing the difference in sampling could be misled into thinking that the virus population itself had changed [19, 20].

4.3 Betasatellites and What They Change

Betasatellites complicate the picture in a particular way. Betasatellites of different origin may associate with different helper viruses, and the association between a betasatellite and its cognate helper reflects a degree of co-evolution [43]. In experimental systems the satellite can be necessary for severe symptoms [13, 14]. A survey that sequences only the helper virus will therefore find the same virus in plants with quite different symptoms and may reach the wrong conclusion about what drives severity.

The reverse can also occur. A satellite may be recovered from plants that show mild symptoms, and in the absence of controlled tests its contribution cannot be known [4, 11]. The literature on chilli does not yet allow the contribution of each betasatellite species to be separated from that of its helper. Table 1 is useful here as a checklist of what should be looked for, and a report that tests only for DNA-A should say so.

Alphasatellites add a further layer. They replicate by themselves but depend on a helper for movement and for transmission by the vector, and in many systems their effect on symptoms is slight or unclear [4, 11]. They are rarely tested for in chilli surveys, so their absence from a report says

little about their absence from the field. A thorough survey would treat the three kinds of molecule as parts of one infection and say which of them were looked for.

4.4 Recombination and the Meaning of a Name

Recombination operates on the same sequences that classification uses. The recombination-detection programs now available allow breakpoints and likely parents to be inferred from alignments [42], and the sequences are usually aligned and compared with standard tools [44, 45]. A virus named from a complete DNA-A that was never tested for recombination may carry a segment from another species, and the identity figure that gave it a name may be an average over regions with different histories.

This has a direct consequence for resistance work. A breeder who selects chilli lines against a named virus is selecting against the sequences of one isolate, and if the virus population is a shifting mosaic the resistance may be tested against a target that is not representative of the next season's field. That does not make resistance screening futile, but it does mean the isolates used should be fully described and, ideally, drawn from several sites [5, 19]. Reviews of this kind are more useful when they say where the evidence stops than when they smooth it over.

4.5 Reading and Writing a Molecular Report

Methods shape answers, and Table 3 sets out what each class can establish. Figure 2 summarises the same idea as a matrix. Degenerate primers detect and give a provisional name, but cannot see what lies outside the amplified fragment [37, 38]. Rolling circle amplification yields whole genomes but struggles when several similar genomes are present in the same sample [39, 40]. Cloning and Sanger sequencing give clean genomes but usually of whichever form is most abundant. Deep sequencing sees mixtures but is hardest to interpret when recombinants must be assembled [41].

A fair way to name a virus from a full sequence is sketched in Figure 3. Recombination is checked before identity is compared, the comparison uses the stated cut-off, and a sequence that falls near the cut-off is reported as a candidate and not forced into a species [8, 35]. This is conservative, but it matches how the classification itself is defined.

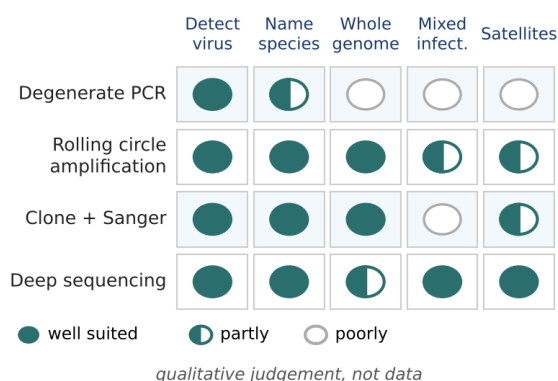


Figure 2. Which questions each class of method can answer (qualitative judgement, not data).

A reader can turn these points into a short list of questions. Was the plant material dated and located, so that the sampling can be repeated? Did the authors sequence beyond the region amplified by the primers? Was a satellite sought, and by which method? Were the sequences tested for recombination, and what was done when breakpoints were found? And did the authors say which cut-off they used for naming species, and whether the sequences fell close to it [8, 42]? A report that answers all five lets its conclusion be weighed; one that answers none should be read as an observation, not as a finding about the cause of the disease.

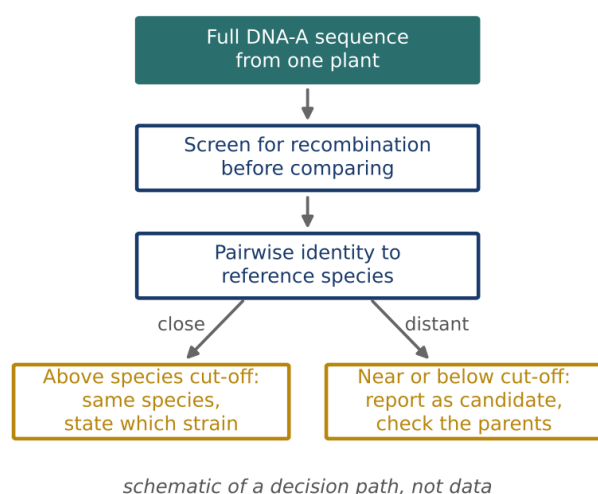


Figure 3. Decision path for naming a virus from a full sequence (schematic, not data).

Put plainly, the literature supports a cautious statement. Chilli leaf curl disease is caused by begomoviruses, often with a betasatellite; the species recovered vary with place, year and method; and recombination means that species boundaries in this group are themselves a matter of convention. Which virus counts as the cause is, for now, a property of the site and of the survey, and the best summary is a distribution of agents, not one name.

It is worth saying what this does not mean. It does not mean that nothing is known, or that molecular surveys have been wasted. The surveys have established that begomoviruses and satellites are involved, they have supplied the sequences on which later comparisons depend, and they have shown how much diversity there is to explain [20, 21]. The argument is only that the step from sequence to cause needs more than most reports have so far supplied, and that readers should adjust how much weight they give a single name accordingly.

5. Discussion

5.1 Interpretation and Practical Reading

The main finding is a conditional one. Chilli leaf curl is best understood as a disease associated with a shifting set of begomoviruses and satellites, and the identity of the virus found at a site is sensitive to sampling and method. This fits what has been learned about begomovirus complexes in other crops [17, 19], and it implies that the useful question for a grower or breeder is not which virus causes leaf curl but which viruses and satellites are present locally and how that set is changing. Nothing in this reading argues against naming viruses; it argues for saying how the name was reached.

The second conditional concerns the vector. Because the vector is itself a complex of genetic groups [26, 27], changes in the local whitefly population can alter which virus predominates without any change in the viruses. A report of a new dominant species may therefore reflect an insect change as much as a viral one, and neither explanation should be assumed before the vector has been identified.

A third consideration is how names are used downstream. Quarantine decisions, seed health testing and resistance breeding all draw on virus names, and each assumes the name stands for something stable. Where the name is a convention applied to a shifting population, those uses need the underlying sequence behind them, so that a decision taken on one name can be checked when the taxonomy moves again [3, 8].

A fourth point concerns evolution. A group of viruses that mutates quickly [29] and recombines often [33] will not stay still while surveys are being published. Names assigned a few seasons ago may describe lineages that have since been replaced or reassorted, and a review that appears to settle the question will date quickly. This is not a reason to avoid naming viruses but to date the sampling and state the genome region used, so that future

comparisons are possible. These points apply with equal force to any virus complex, not only to the one affecting chilli.

Host range adds one more moving part. Begomoviruses move between crops and wild plants, and the vector feeds on both, so a field of chilli is rarely isolated from other sources of virus [2, 5]. Tomato, papaya, weeds and neighbouring vegetable crops can all carry begomoviruses and satellites, and exchange between them is part of how new combinations arise [1, 33]. A survey confined to chilli can therefore see only one slice of a larger network, and a virus named in chilli may be one that arrived from elsewhere.

Three findings would change this reading. A large, structured survey with complete genomes and satellite testing, showing the same single virus across contrasting regions and years, would support a single causal agent. Infectious clone experiments showing that one virus alone reproduces full disease in chilli, without a satellite, would simplify the picture. And evidence that sequence-based species boundaries track biological differences in host range or transmission would justify giving species names more weight [8, 12].

5.2 Limitations

This is a narrative review and has the limitations of that form. The selection of sources reflects judgement, no formal search or scoring was carried out, and no numerical results from individual chilli surveys are quoted, because they could not be checked against the primary reports for this article. A reader who needs the frequency of a given species in a given region should consult the original surveys, applying the appraisal questions above.

The review also leans on work from other crops, such as cotton, okra and tomato, to explain mechanisms [16, 17, 32]. That is reasonable for biology that is shared across the genus, but not for claims about which species predominate in chilli, and the conclusions should be read in that light. A name that cannot be checked against a sequence is a label, not a finding.

5.3 Implications for Reporting and Survey Design

A survey able to answer the question would sample several plants per site and several sites per region, record symptoms and date, and amplify by a method that does not depend on a presumed sequence [39, 40]. It would sequence whole genomes, look for satellites by their own methods [10], test for recombination before comparing

identities [42], and state the demarcation criteria used [8]. It would also identify the whitefly genetic group present [27].

Reports could be made much more comparable by a short minimum description: genome region and length obtained, number of plants and sites, amplification method, satellite status, recombination test and result, and classification method with cut-off. None of this requires new technology. It requires only that the methods paragraph answer the questions a reader will ask, and that databases hold complete sequences with enough metadata to be reused.

5.4 Directions for Future Work

Three needs stand out. First, structured, multi-site surveys with whole-genome and satellite recovery, so that the distribution of agents can be described rather than guessed. Second, infectious-clone experiments in chilli that separate the contribution of each helper and satellite, building on methods already used in other hosts [12, 13]. Third, work linking vector genetic groups to the viruses they carry in chilli fields, so that changes in virus populations can be read against changes in the insect [26, 28].

For breeders, the practical need is isolates that represent the variation in the field, fully sequenced and described, so that resistance can be judged against a realistic target. For extension workers, the need is a plain message that leaf curl may have more than one cause at once, and that vector management is likely to matter whatever the virus is called [1, 2].

5.5 Practical Reading for Growers and Advisers

For a grower the sensible response does not depend on the name of the virus. Whitefly management, early removal of badly affected plants, clean nursery stock and attention to weeds and neighbouring crops all act on the shared route of spread, and they are justified whichever species is in the field [2, 28]. Insecticide use needs care, because the vector is a complex whose genetic groups can differ in how they respond, and it should be guided by local advice rather than by assumptions drawn from other regions [24, 26].

For an adviser, the useful stance is neither to dismiss molecular reports nor to treat them as final. A diagnosis based on a short fragment is a good indication that a begomovirus is present and a weak basis for saying what else is present [36, 37]. When a laboratory result is needed to support a decision, such as the choice of resistant material, it is worth asking for whole-genome confirmation

and for a statement about satellites, since both change how the result should be read [11, 39]. Such a request is reasonable, and it will become easier to meet as sequencing becomes more routine.

6. Conclusion

Chilli leaf curl disease is a begomovirus disease, but the published molecular evidence does not support naming a single causal virus. Species, satellites and recombinants occur together, the whitefly vector is itself a complex, and classification rests on a threshold that is a convention instead of a natural boundary. The answer to which virus causes the disease depends on where and when plants were sampled and on how they were analysed.

For the practitioner the message is practical rather than discouraging. Treat the cause as local, test for satellites as well as viruses, describe isolates fully and date the sampling. For the researcher, the opportunity is a set of structured, whole-genome surveys and clone experiments that turn a collection of local names into a picture of the population.

7. Acknowledgements

The authors thank colleagues who commented on earlier drafts of this review.

8. Funding

This work received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

9. Conflict of Interest

The authors declare no conflict of interest.

10. Author Contributions

D.T. conceived the review and wrote the sections on begomovirus biology and disease. I.T. wrote the sections on vectors and methods. N.P. wrote the sections on recombination, classification and reporting. All authors revised the manuscript and approved the final version.

11. Ethics Statement

This is a review of published literature. No plant or animal experiments were performed and no ethical approval was required.

12. Data Availability

No new data were generated. All sources are cited in the reference list.

13. References

- [1] Varma, A., & Malathi, V. G. (2003). Emerging geminivirus problems: A serious threat to crop production. *Annals of Applied Biology*, 142(2), 145–164. DOI: [10.1111/j.1744-7348.2003.tb00240.x](https://doi.org/10.1111/j.1744-7348.2003.tb00240.x)
- [2] Navas-Castillo, J., Fiallo-Olivé, E., & Sánchez-Campos, S. (2011). Emerging virus diseases transmitted by whiteflies. *Annual Review of Phytopathology*, 49, 219–248. DOI: [10.1146/annurev-phyto-072910-095235](https://doi.org/10.1146/annurev-phyto-072910-095235)
- [3] Zerbini, F. M., Briddon, R. W., Idris, A., Martin, D. P., Moriones, E., Navas-Castillo, J., Rivera-Bustamante, R., Roumagnac, P., & Varsani, A. (2017). ICTV virus taxonomy profile: Geminiviridae. *Journal of General Virology*, 98(2), 131–133. DOI: [10.1099/jgv.0.000738](https://doi.org/10.1099/jgv.0.000738)
- [4] Briddon, R. W., & Stanley, J. (2006). Subviral agents associated with plant single-stranded DNA viruses. *Virology*, 344(1), 198–210. DOI: [10.1016/j.virol.2005.09.042](https://doi.org/10.1016/j.virol.2005.09.042)
- [5] Rojas, M. R., Hagen, C., Lucas, W. J., & Gilbertson, R. L. (2005). Exploiting chinks in the plant's armor: Evolution and emergence of geminiviruses. *Annual Review of Phytopathology*, 43, 361–394. DOI: [10.1146/annurev-phyto.43.040204.135939](https://doi.org/10.1146/annurev-phyto.43.040204.135939)
- [6] Jeske, H. (2009). Geminiviruses. *Current Topics in Microbiology and Immunology*, 331, 185–226. DOI: [10.1007/978-3-540-70972-5_11](https://doi.org/10.1007/978-3-540-70972-5_11)
- [7] Hanley-Bowdoin, L., Bejarano, E. R., Robertson, D., & Mansoor, S. (2013). Geminiviruses: Masters at redirecting and reprogramming plant processes. *Nature Reviews Microbiology*, 11(11), 777–788. DOI: [10.1038/nrmicro3117](https://doi.org/10.1038/nrmicro3117)
- [8] Brown, J. K., Zerbini, F. M., Navas-Castillo, J., Moriones, E., Ramos-Sobrinho, R., Silva, J. C. F., ... Varsani, A. (2015). Revision of *Begomovirus* taxonomy based on pairwise sequence comparisons. *Archives of Virology*, 160(6), 1593–1619. DOI: [10.1007/s00705-015-2398-y](https://doi.org/10.1007/s00705-015-2398-y)
- [9] Vanitharani, R., Chellappan, P., & Fauquet, C. M. (2005). Geminiviruses and RNA silencing. *Trends in Plant Science*, 10(3), 144–151. DOI: [10.1016/j.tplants.2005.01.005](https://doi.org/10.1016/j.tplants.2005.01.005)
- [10] Briddon, R. W., Bull, S. E., Amin, I., Idris, A. M., Mansoor, S., Bedford, I. D., ... Markham, P. G. (2003). Diversity of DNA beta, a satellite molecule associated with some monopartite begomoviruses. *Virology*, 312(1), 106–121. DOI: [10.1016/S0042-6822\(03\)00200-9](https://doi.org/10.1016/S0042-6822(03)00200-9)
- [11] Zhou, X. (2013). Advances in understanding begomovirus satellites. *Annual Review of Phytopathology*, 51, 357–381. DOI: [10.1146/annurev-phyto-082712-102234](https://doi.org/10.1146/annurev-phyto-082712-102234)
- [12] Saunders, K., Bedford, I. D., Briddon, R. W., Markham, P. G., Wong, S. M., & Stanley, J. (2000). A unique virus complex causes *Ageratum* yellow vein disease. *Proceedings of the National Academy of Sciences*, 97(12), 6890–6895. DOI: [10.1073/pnas.97.12.6890](https://doi.org/10.1073/pnas.97.12.6890)

- [13] Cui, X., Tao, X., Xie, Y., Fauquet, C. M., & Zhou, X. (2004). A DNAbeta associated with Tomato yellow leaf curl China virus is required for symptom induction. *Journal of Virology*, 78(24), 13966-13974. DOI: [10.1128/JVI.78.24.13966-13974.2004](https://doi.org/10.1128/JVI.78.24.13966-13974.2004)
- [14] Saeed, M., Behjatnia, S. A. A., Mansoor, S., Zafar, Y., Hasnain, S., & Rezaian, M. A. (2005). A single complementary-sense transcript of a geminiviral DNA beta satellite is determinant of pathogenicity. *Molecular Plant-Microbe Interactions*, 18(1), 7-14. DOI: [10.1094/MPMI-18-0007](https://doi.org/10.1094/MPMI-18-0007)
- [15] Yang, J.-Y., Iwasaki, M., Machida, C., Machida, Y., Zhou, X., & Chua, N.-H. (2008). betaC1, the pathogenicity factor of TYLCCNV, interacts with AS1 to alter leaf development and suppress selective jasmonic acid responses. *Genes & Development*, 22(18), 2564-2577. DOI: [10.1101/gad.1682208](https://doi.org/10.1101/gad.1682208)
- [16] Jose, J., & Usha, R. (2003). Bhendi yellow vein mosaic disease in India is caused by association of a DNA beta satellite with a begomovirus. *Virology*, 305(2), 310-317. DOI: [10.1006/viro.2002.1768](https://doi.org/10.1006/viro.2002.1768)
- [17] Mansoor, S., Briddon, R. W., Zafar, Y., & Stanley, J. (2003). Geminivirus disease complexes: An emerging threat. *Trends in Plant Science*, 8(3), 128-134. DOI: [10.1016/S1360-1385\(03\)00007-4](https://doi.org/10.1016/S1360-1385(03)00007-4)
- [18] Mansoor, S., Zafar, Y., & Briddon, R. W. (2006). Geminivirus disease complexes: The threat is spreading. *Trends in Plant Science*, 11(5), 209-212. DOI: [10.1016/j.tplants.2006.03.003](https://doi.org/10.1016/j.tplants.2006.03.003)
- [19] Seal, S. E., vandenBosch, F., & Jeger, M. J. (2006). Factors influencing begomovirus evolution and their increasing global significance: Implications for sustainable control. *Critical Reviews in Plant Sciences*, 25(1), 23-46. DOI: [10.1080/07352680500365257](https://doi.org/10.1080/07352680500365257)
- [20] Vinoth Kumar, R., Singh, A. K., Singh, A. K., Yadav, T., Basu, S., Kushwaha, N., Chattopadhyay, B., & Chakraborty, S. (2015). Complexity of begomovirus and betasatellite populations associated with chilli leaf curl disease in India. *Journal of General Virology*, 96(10), 3143-3158. DOI: [10.1099/jgv.0.000254](https://doi.org/10.1099/jgv.0.000254)
- [21] Kumar, Y., Hallan, V., & Zaidi, A. A. (2011). Chilli leaf curl Palampur virus is a distinct begomovirus species associated with a betasatellite. *Plant Pathology*, 60(6), 1040-1047. DOI: [10.1111/j.1365-3059.2011.02466.x](https://doi.org/10.1111/j.1365-3059.2011.02466.x)
- [22] Rosen, R., Kanakala, S., Kliot, A., Pakkianathan, B. C., Farich, B. A., Santana-Magal, N., ... Ghanim, M. (2015). Persistent, circulative transmission of begomoviruses by whitefly vectors. *Current Opinion in Virology*, 15, 1-8. DOI: [10.1016/j.coviro.2015.06.008](https://doi.org/10.1016/j.coviro.2015.06.008)
- [23] Czosnek, H., Hariton-Shalev, A., Sobol, I., Gorovits, R., & Ghanim, M. (2017). The incredible journey of begomoviruses in their whitefly vector. *Viruses*, 9(10), 273. DOI: [10.3390/v9100273](https://doi.org/10.3390/v9100273)
- [24] Brown, J. K., Frohlich, D. R., & Rosell, R. C. (1995). The sweetpotato or silverleaf whiteflies: Biotypes of *Bemisia tabaci* or a species complex? *Annual Review of Entomology*, 40, 511-534. DOI: [10.1146/annurev.en.40.010195.002455](https://doi.org/10.1146/annurev.en.40.010195.002455)
- [25] Bedford, I. D., Briddon, R. W., Brown, J. K., Rosell, R. C., & Markham, P. G. (1994). Geminivirus transmission and biological characterisation of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. *Annals of Applied Biology*, 125(2), 311-325. DOI: [10.1111/j.1744-7348.1994.tb04972.x](https://doi.org/10.1111/j.1744-7348.1994.tb04972.x)
- [26] De Barro, P. J., Liu, S.-S., Boykin, L. M., & Dinsdale, A. B. (2011). *Bemisia tabaci*: A statement of species status. *Annual Review of Entomology*, 56, 1-19. DOI: [10.1146/annurev-ento-112408-085504](https://doi.org/10.1146/annurev-ento-112408-085504)
- [27] Boykin, L. M., & De Barro, P. J. (2014). A practical guide to identifying members of the *Bemisia tabaci* species complex: And other morphologically identical species. *Frontiers in Ecology and Evolution*, 2, 45. DOI: [10.3389/fevo.2014.00045](https://doi.org/10.3389/fevo.2014.00045)
- [28] Gilbertson, R. L., Batuman, O., Webster, C. G., & Adkins, S. (2015). Role of the insect supervectors *Bemisia tabaci* and *Frankliniella occidentalis* in the emergence and global spread of plant viruses. *Annual Review of Virology*, 2, 67-93. DOI: [10.1146/annurev-virology-031413-085410](https://doi.org/10.1146/annurev-virology-031413-085410)
- [29] Duffy, S., & Holmes, E. C. (2008). Phylogenetic evidence for rapid rates of molecular evolution in the single-stranded DNA begomovirus Tomato yellow leaf curl virus. *Journal of Virology*, 82(2), 957-965. DOI: [10.1128/JVI.01929-07](https://doi.org/10.1128/JVI.01929-07)
- [30] Padidam, M., Sawyer, S., & Fauquet, C. M. (1999). Possible emergence of new geminiviruses by frequent recombination. *Virology*, 265(2), 218-225. DOI: [10.1006/viro.1999.0056](https://doi.org/10.1006/viro.1999.0056)
- [31] Martin, D. P., Lefeuvre, P., Varsani, A., Hoareau, M., Semegni, J.-Y., Dijoux, B., ... Shepherd, D. N. (2011). Complex recombination patterns arising during geminivirus coinfections preserve and demarcate biologically important intra-genome interaction networks. *PLoS Pathogens*, 7(9), e1002203. DOI: [10.1371/journal.ppat.1002203](https://doi.org/10.1371/journal.ppat.1002203)
- [32] Lefeuvre, P., Martin, D. P., Harkins, G., Lemey, P., Gray, A. J. A., Meredith, S., ... Heydarnejad, J. (2010). The spread of tomato yellow leaf curl virus from the Middle East to the world. *PLoS Pathogens*, 6(10), e1001164. DOI: [10.1371/journal.ppat.1001164](https://doi.org/10.1371/journal.ppat.1001164)
- [33] Lefeuvre, P., & Moriones, E. (2015). Recombination as a motor of host switches and virus emergence: Geminiviruses as case studies. *Current Opinion in Virology*, 10, 14-19. DOI: [10.1016/j.coviro.2014.12.005](https://doi.org/10.1016/j.coviro.2014.12.005)
- [34] Fauquet, C. M., Briddon, R. W., Brown, J. K., Moriones, E., Stanley, J., Zerbini, M., & Zhou, X. (2008). Geminivirus strain demarcation and nomenclature. *Archives of Virology*, 153(4), 783-821. DOI: [10.1007/s00705-008-0037-6](https://doi.org/10.1007/s00705-008-0037-6)

- [35] Muhire, B. M., Varsani, A., & Martin, D. P. (2014). SDT: A virus classification tool based on pairwise sequence alignment and identity calculation. *PLoS ONE*, 9(9), e108277. DOI: [10.1371/journal.pone.0108277](https://doi.org/10.1371/journal.pone.0108277)
- [36] Brown, J. K., Idris, A. M., Torres-Jerez, I., Banks, G. K., & Wyatt, S. D. (2001). The core region of the coat protein gene is highly useful for establishing the provisional identification and classification of begomoviruses. *Archives of Virology*, 146(8), 1581-1598. DOI: [10.1007/s007050170081](https://doi.org/10.1007/s007050170081)
- [37] Rojas, M. R., Gilbertson, R. L., Russell, D. R., & Maxwell, D. P. (1993). Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. *Plant Disease*, 77(4), 340-347. DOI: [10.1094/PD-77-0340](https://doi.org/10.1094/PD-77-0340)
- [38] Briddon, R. W., & Markham, P. G. (1994). Universal primers for the PCR amplification of dicot-infecting geminiviruses. *Molecular Biotechnology*, 1(2), 202-205. DOI: [10.1007/BF02921559](https://doi.org/10.1007/BF02921559)
- [39] Haible, D., Kober, S., & Jeske, H. (2006). Rolling circle amplification revolutionizes diagnosis and genomics of geminiviruses. *Journal of Virological Methods*, 135(1), 9-16. DOI: [10.1016/j.jviromet.2006.01.017](https://doi.org/10.1016/j.jviromet.2006.01.017)
- [40] Inoue-Nagata, A. K., Albuquerque, L. C., Rocha, W. B., & Nagata, T. (2004). A simple method for cloning the complete begomovirus genome using the bacteriophage phi29 DNA polymerase. *Journal of Virological Methods*, 116(2), 209-211. DOI: [10.1016/j.jviromet.2003.11.015](https://doi.org/10.1016/j.jviromet.2003.11.015)
- [41] Kreuze, J. F., Perez, A., Untiveros, M., Quispe, D., Fuentes, S., Barker, I., & Simon, R. (2009). Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs. *Virology*, 388(1), 1-7. DOI: [10.1016/j.virol.2009.03.024](https://doi.org/10.1016/j.virol.2009.03.024)
- [42] Martin, D. P., Murrell, B., Golden, M., Khoosal, A., & Muhire, B. (2015). RDP4: Detection and analysis of recombination patterns in virus genomes. *Virus Evolution*, 1(1), vev003. DOI: [10.1093/ve/vev003](https://doi.org/10.1093/ve/vev003)
- [43] Zhou, X., Xie, Y., Tao, X., Zhang, Z., Li, Z., & Fauquet, C. M. (2003). Characterization of DNAbeta associated with begomoviruses in China and evidence for co-evolution with their cognate viral DNA-A. *Journal of General Virology*, 84(1), 237-247. DOI: [10.1099/vir.0.18608-0](https://doi.org/10.1099/vir.0.18608-0)
- [44] Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32(5), 1792-1797. DOI: [10.1093/nar/gkh340](https://doi.org/10.1093/nar/gkh340)
- [45] Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33(7), 1870-1874. DOI: [10.1093/molbev/msw054](https://doi.org/10.1093/molbev/msw054)