Proposal to sequence the genome of the grape Phylloxera (*Daktulosphaira vitifoliae* Fitch)



Three entomologists (JE Planchon, J Lichtenstein, CV Riley) visiting a phylloxera infested vineyard in the South of France - frontpage of "Le Journal Illustré, 28th September 1878".

Phylloxera Genomics Initiative§

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Abstract

Grape phylloxera (*Daktulosphaira vitifoliae* Fitch) is a historical pest of grapevine with worldwide economic and ecological importance. Phylloxera is native to the eastern United States of America where its natural hosts are American *Vitis* species. These natural hosts show varying levels of resistance to the insect and a few accessions of *Vitis* spp. have been used to produce rootstocks for use in commercial viticulture worldwide. The use of such rootstocks is, to-date, the only means by which economically viable grape production can be reliably maintained from *Vitis vinifera* in a phylloxera-infested vineyard. If the recent release of the grape genome has accelerated rootstock breeding programmes, the long-term stability of the host-plant resistance is conditioned to the non emergence of virulent phylloxera strains. However, there has been recent evidence in Australia of the emergence of two apparently resistant phylloxera clones, which suggests that previously host-resistant plants can now be susceptible to phylloxera.

In contrast with the accurate and detailed historic studies on the biology of this insect, genetic information is still quite limited on this potentially dangerous species for a major crop. We propose the sequencing of the phylloxera (*Daktulosphaira vitifoliae* Fitch) genome through an integrated approach. Phylloxera, because of its basal phylogenetic relationship to aphids, provides an interesting model for comparative genomics between aphids (in the broad sense). The phylloxera sequence will also fill the gap between aphids and related taxa such as bugs, cicadas and leafhoppers, psyllids and whiteflies.

Knowledge of the genome of the phylloxera will considerably improve our understanding of many of the specific biological features of this invasive pest. This include identifying genes for complex traits including those with relevance to the genetic basis of host-plant interaction, to leaf gall and root formation, to nutrition on grape, and to the developmental causes of extreme phenotypic plasticity. Finally, knowledge of the phylloxera genome is also relevant to human and economic well being by participating to reduce environmental cost and risk in viticulture.

(A) Importance/Benefits of the grape Phylloxera genome

An invasive historical pest. Phylloxera is an historic pest species with worldwide economic and ecological importance. The insect is native to North America and indigenous on *Vitis* species. It was accidentally introduced into Europe around 1850 where in the absence of American *Vitis* species it became a devastating pest of the highly susceptible European grapevine species *V. vinifera*. Therefore, phylloxera caused the collapse of the European viticulture industry. Its subsequent spread to vineyards around the globe proceeded rapidly and it is now found in almost all viticulture regions of the world (North America, Europe, Australia, South Africa, South America, Asia and the Middle East, etc). Some American *Vitis* species show resistance mechanisms to root-feeding by this insect, and these have been used as rootstocks at the end of the 19th century for continued commercial production of *V. vinifera*. Today, phylloxera is still a major threat for viticulture imposing the grafting of *V. vinifera* and quarantine regulations in areas (e.g. Australia, Chile, Armenia and China) where varieties are predominantly grown own-rooted.

The key 'basal' phylogenetic position of grape phylloxera. *D. vitifoliae* (grape phylloxera) belongs to the Phylloxeroidea (Figure 1), a group that includes the pine and spruce 'aphids' (Adelgidae) and the phylloxerans (Phylloxeridae). The Phylloxeroidea is a small monophyletic superfamily of the Hemiptera closely related to the Aphidoidea (the true aphids), its nearest sister taxon. The Aphidoidea and the Phylloxeroidea probably diverged in the Jurassic or earlier from some aphidiform ancestor whose origin can be traced back up to about 250 my ago (Heie, 1987). While not an aphid *sensu stricto*, phylloxera, because of its basal phylogenetic relationship to aphids and because it shares a subset of the biological adaptations associated with aphids, provides an interesting model for comparative genomics.

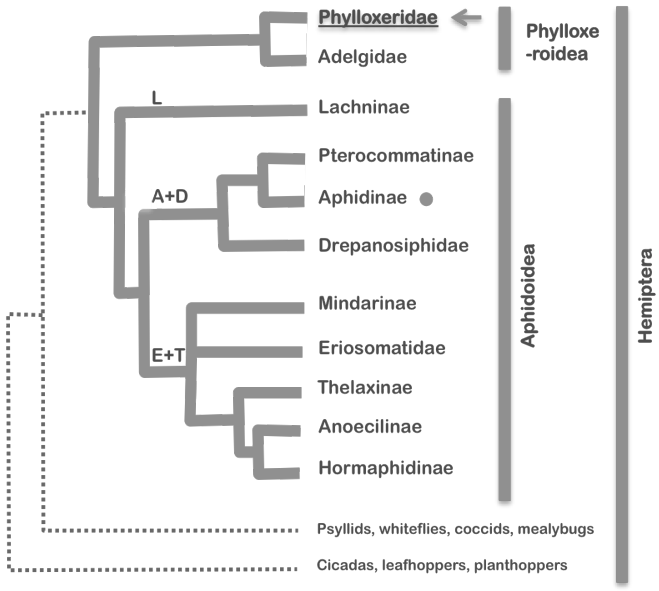


Figure 1. Simplified phylogenetic relationships among aphid subfamilies and tribes as proposed by Ortiz-Rivas and Martínez-Torres (2010). The filled circle indicates the Aphidinae subfamilly (80% of the aphid species) that includes the pea aphid *Acyrthosiphon pisum* that has been recently sequenced (IAGC, 2010).

One genome of aphid (the pea aphid; see IAGC 2010) is already available and several efforts of the International Aphid Genomics Consortium (IAGC) concentrate on other aphid genomes of the same family as they represent major pests of annual crops. However, no genome project on Phylloxeroidea has been proposed yet; the present project will therefore provide essential comparisons between aphid (in the broad sense) genomes and an extraordinary resource for understanding major feature of aphid evolution, such as the rapid radiation after host shifting from gymnosperms to angiosperms. Eventually, the phylloxera sequence will also fill the gap between aphids and related taxa such as bugs, cicadas and leafhoppers, psyllids and whiteflies allowing

to study genome evolution at the evolutionary scale of the Hemiptera order (see Figure 1).

An 'aphid' that lacks endosymbionts. The food source and nutritional physiology of *D. vitifoliae* clearly differ from that of the Aphididae which ingest phloem sap, have primary endosymbionts and require essential amino acids. *Daktulosphaira vitifoliae* instead uses its relatively strong stylets to penetrate directly into the parenchymatic zone and imbibe the cellular content. Thus, phylloxera take up a protein-rich diet and do not appear to need bacterial endosymbionts for synthesis of essential amino acids or proteins.

Therefore, contrary to true aphids (Aphidoidea) that exhibit a mutualistic association with intracellular bacteria (*Buchnera* sp.), their primary symbionts, *D. vitifoliae* appears to lack *Buchnera*. However, an association has been postulated between grape phylloxera and bacteria *Pantoea agglomerans* (Vorwerk et al. 2007). Although this association may have been established over a long evolutionary period, the role of *P. agglomerans* in grape phylloxera still remains to be defined. Potentially it may impact on host-plant interactions between phylloxera genetic strains and *Vitis* species. The availability of the phylloxera genome sequence will allow elucidating the evolution of its association with *Pantoea*, and by using the comparative approach will certainly bring new insights into the co-evolution of the association of Aphidoidea with their symbionts. As part of a eukaryotic genome project, it often occurs that microorganism genomes can be also totally or partially sequenced since their DNA are co-extracted. Sequencing the Phylloxera genome might thus lead to the description of associated microorganisms as a by-product.

An 'aphid' with a specialised digestive system. In comparison with aphid feeding from phloem sap, the food source of grape phylloxera is high in protein and low in sugar and water. Therefore, there might be some important evolutionary adaptations such has the ability to digest proteins for providing the energy requirements during the adult life stage. Grape phylloxera does appear to have a complete digestive system (Kingston et al. 2007). It appears to lack the capacity for anal waste excretion due to a lack of anal dorsal muscles making honeydew excretion improbable, despite having an anal opening this appears to be non-functional. The alternative mechanism of waste excretion although as yet unclear, may include periodic elimination of waste into the gall via salivary glands and/or during oviposition. The insects high reproductive capacity means that energy requirements for egg production are high and storage of food may also assist in the extend survival of later instars of phylloxera, which can survive for up to nine days in the absence of diet.

A gall-forming species. *Daktulosphaira vitifoliae* induces formation of leaf and root galls on *Vitis* species. Phylloxera is thus a valuable model for studying the biology of obligate gall-forming species in Hemipterans. Galling ability on leaves and roots has been documented to be highly variable between *Vitis* species. Moreover, leaf and root galling can be influenced by both environmental conditions and the kind of phylloxera strains. For instance, native strains of grape phylloxera that form leaf galls on *V. girdiana* in southern California and on *V. arizonica* in Arizona have not been found on roots (Granett et al. 2001). Some Australian strains of phylloxera in Victoria are able to induce leaf galling and are genetically distinct from neighbouring phylloxera strains that lack this capacity and can only induce root galls. In addition other Australian strains have the capacity to induce both root and leaf galls depending on the host genetic background. The induction of leaf galling under Australian conditions also appears to be influenced by environmental conditions. The comparison of the pea aphid (and other

non-gall forming aphid species) and phylloxera genomes will thus provide new insights into this unexplored aspect of aphid biology.

The interaction between Phylloxera and *Vitis*. A major focus concerns the interaction of phylloxera with its host plant. The use of resistant rootstocks has largely prevented phylloxera-related damage but this genetic stability implies that resistance for rootstocks will not be overcome by the evolution of aggressive phylloxera strains. A major issue for viticulture is thus to gain a better understanding of the genetic determinants involved in the compatible interaction of phylloxera with grapevine. To date, consistent dominant biotypes (defined by host-performance parameters) have been described to occur in Australia and California, but could not be confirmed in Europe. Of major importance in the interaction with *Vitis* species is the function of the salivary glands and gut: it is indeed hypothesized that salivary factors are essential for gall induction and maintenance, and possibly also for host adaptation and for host plant resistance. Secretory and excretory functions are likely to be very specialized in this taxon, and therefore the secretome and metabolome subject to specific adaptive processes could be compared between phylloxera and other aphid species. The secretome of the pea aphid genome is under investigation already.

It is worth noting that the recent release of the grapevine genome (*Vitis vinifera* var. Pinot noir, Jaillon et al. 2009) is providing a unique opportunity to study the molecular dialogs between phylloxera and grapevine. Grape and phylloxera genomes availability will allow functional genomics approaches on both the plant and the insect, a strategy that has shown its value and efficacy for the study of complex interaction in other host-parasite interactions.

Life cycle and polyphenism in Phylloxeridae. Phylloxeridae share with aphids complex life cycles that are characterized by the alternation between different morphs (sexual/asexual, winged/apterous) generated by clonal reproduction, the so-called polyphenism. Asexual individuals forming galls on leaves are called gallicoles (leaf-feeding wingless), and on roots, radicicoles (root-feeding, wingless). The eggs hatch into instars that are mobile and can move between roots and leaves to establish new feeding sites. After the first instar gallicoles and radicicoles tend to feed in one place. Sexuparae (winged females) are generated on roots and emerge from the ground prior to adult eclosion. After eclosion these winged forms disperse and then lay male and female eggs asexually. The newly hatched sexuales (wingless) moult four times before they mate, and each female lays a single overwintering egg. The fundatrix for the succeeding year's gallicoles hatches from the overwintering egg. Overwintering of radicicoles also occurs as first instar hibernants (Figure 2).

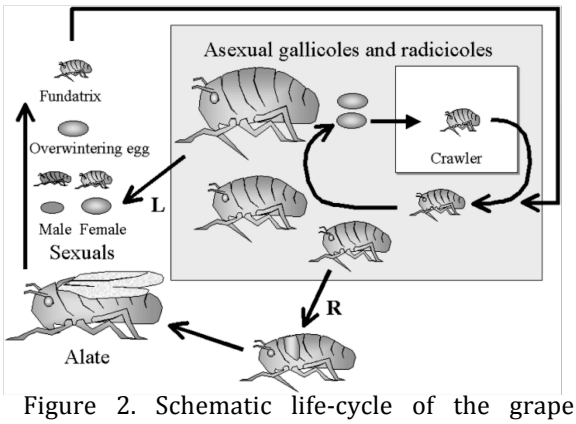


Figure 2. Schematic life-cycle of the grape phylloxera (from Granett et al. 2001).

In the pea aphid genome, a very large number of genes have been predicted (more than 34,000), a similar situation recently observed in *Daphnia duplex*, another arthropod which display several polyphenisms. It is intriguing to underline the observation that in both species, the large number of predicted genes is correlated with several gene duplications. Thus, it is tempting to hypothesize the role of gene

duplication in the extension of polyphenism. Similar to Aphididae, Phylloxeridae life-cycles present a large number of discrete phenotypic forms: parthenogenetic females that feed on root or leaf, asexual winged forms, sexuales (reviewed by Forneck & Huber 2009). They also include some specific characteristics such as oviparity of asexual females, the lost of rostrum in winged asexuals and apterous sexuales. Finally, it has been documented that polyphenism is under the control of environmental conditions and, in some cases, by the host-plant itself. Furthermore, phylloxera reproduces only by oviparity (sexual or asexual) whereas Aphidinae (including the pea aphid) alternates between oviparity (sexual reproduction) a viviparity (asexual reproduction). Therefore, the phylloxera genome will allow us to confirm if duplications of development-related genes could have facilitated the evolution of polyphenism in Aphidomorpha.

Role of phylloxera in the ecological network associated to grapevine. The parasite community associated with grapevine leaves and roots covers multiple interactions with fungi, bacteria and virus (figure 3) but information on the molecular interaction is still sparse. Uncovering the phylloxera genome will provide the basis for the study of gene-gene interactions of parasite relations.

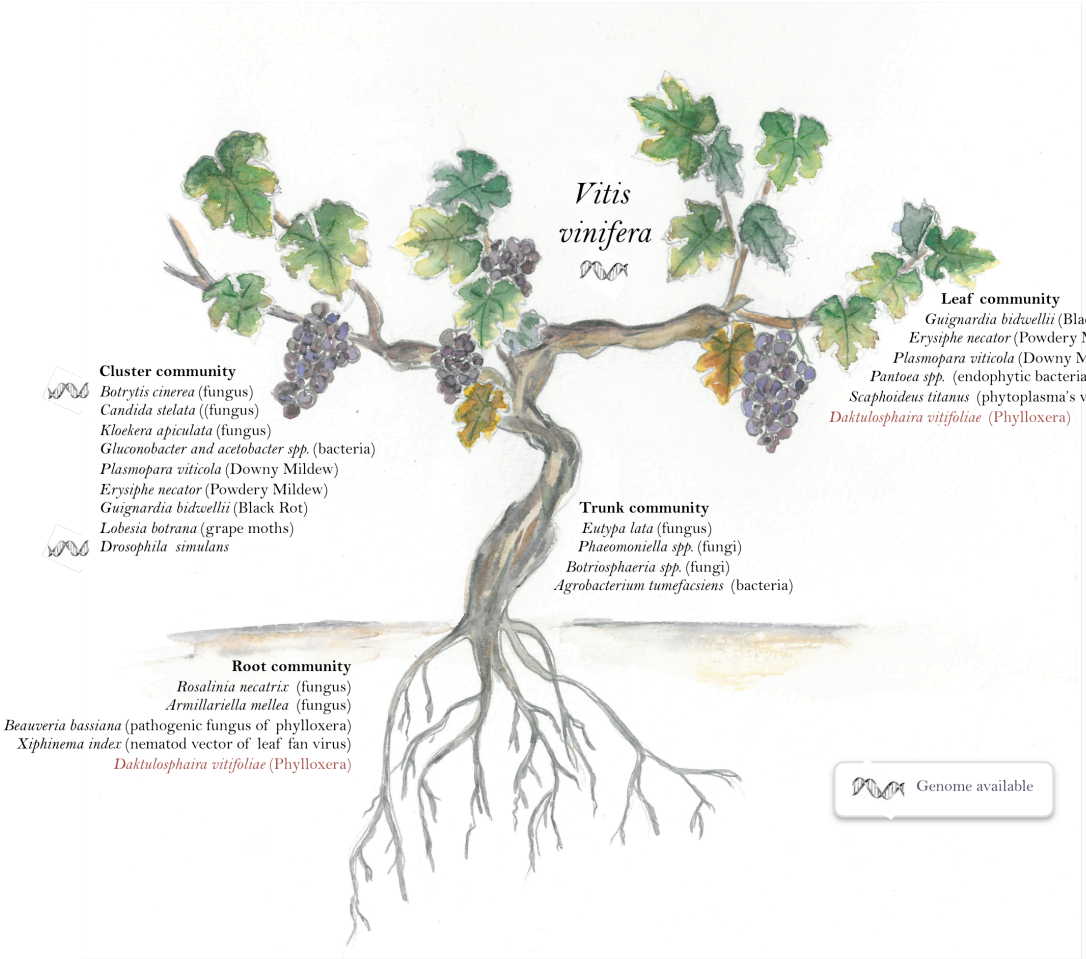


Figure 3: Ecological network associated to *Daktulosphaira vitifoliae.*

Relevance to human and economic well-being. A full genome sequence of phylloxera is required to address a broad range of human health-related problems. Indeed, phylloxera biology is relevant to human health and economic well-being:

- As a quarantine pest phylloxera causes millions of dollars in expenses to detect and control this pest.
- Pesticide treatments to control phylloxera greatly interacts with ecology and may affect fruit quality; This is especially the case for treatments of soil-borne phylloxera which may modify rhizosphere ecology.
- Breeding for grape rootstock resistant to phylloxera is a long-term and costly effort. The identification of the genetic determinants involved in the compatible interaction with grape is essential for rootstock breeding purposes in order to ensure the long-term stability of the host-plant resistance.
- Phylloxera modifies the plant by inducing a set a genes that results in the production of starch granules into nodosities (whereas all other part of the root do not). The Phylloxera/Root Interaction can be used as a model to understand gene function and ultimately transform rootstocks.
- Understanding the functioning of phylloxera's metabolism that does not rely on primary endosymbionts (*Buchnera*) may provide critical information on effective or maybe more primitive metabolic mechanisms in aphids.

(B) Sequencing and research strategies

In order to fully tap the information from this project and amass essential information on coding gene sequences and gene expression, we propose to accompany the genome sequence project with a set of RNA-Seq runs as well as the development of functional analyses tools such as RNAi. We propose to integrate the development of these genomic resources in AphidBase, a centralized bioinformatic resources website already available for aphids.

1. Organisation and project management. The phylloxera initiative (see annex 1) will be part of the International Aphid Genome Consortium (IAGC) that recently stated in its new White Paper (http://www.aphidbase.com/aphidbase/news/aphid_white_paper_ii) the importance of developing several aphid genome projects including phylloxera. By integrating the IAGC, our phylloxera community follows the ethical rules of this consortium by releasing in the public databases the different sequences produced by this genome project. The Phylloxera Genomics Initiative will also take advantage of the different experts of aphid biology within the IAGC members.

A first meeting about phylloxera genomics was held by Astrid Forneck in September 2010 in Vienna where it has been decided to enter the IAGC, write this White Paper and organize our strategy for this genome project, such as sharing RNA-Seq conditions to be tested. Furthermore the necessity of updated and continuing coordinated research reflects the interests of the community which has recently established a working group (P10) within the ISHS (International Society of Horticultural Sciences). Conference and visio calls can be easily organized and an annual meeting is envisaged. This meeting could be associated with the annual IAGC meeting (next one in June 2011, Kansas City). A mailing list of the phylloxera community has been established, but our community gains also at using the aphid genomics mailing list from IAGC (more than 200 people).

2. Preliminary tasks and biological material.

Genome sizing. Estimation of the phylloxera genome size by flow cytometry was circa 400 Mb, as described below.

In June 2010, phylloxera leaf galls were collected from Petit-Arvine (*Vitis vinifera*) grafted on Fercal rootstock at INRA Château Couhins (Pessac-Léognan, Bordeaux AOC, France). A total of twenty infected leaves with more than ten galls each were used to infect a Harmony cultivar (rootstock). The phylloxera population was maintained in an insect-proof cage in greenhouse conditions and after four months the population was held at 4°C. In January 2011, 90 root-feeding larvae were collected for the flow cytometry analysis. The genome size estimation was performed on nine different samples of insects from this established population. For each sample, cell suspensions were prepared from pooled whole bodies from 10 larvae. Insect samples were finely chopped with a razor blade in 600 mL of cold Galbraith buffer supplemented as usual with 1% (w/v) polyvinylpyrrolidone 10.000 and 40 mg/mL RNase A (Roche). The samples were analyzed on a FACScalibur cytometer. All samples were compared against one *Medicago truncatula* cv A17 (2C = 1,125 Mbp) control sample of known genome size. For each phylloxera sample, at least 3000 nuclei were analyzed.

Table 1. C-values estimates for the nine samples of grapevine phylloxera.
Each sample consisted of 10 radicicoles larvae.

Sample	Sample intensity (2C)	Control intensity (2C)	Ratio (sample/control)	C Value (Mb)
1	593	843	0.703	395.7
2	628	874	0.719	404.2
3	565	793	0.712	400.8
4	577	815	0.708	398.2
5	621	854	0.727	409.0
6	665	918	0.724	407.5
7	610	849	0.718	404.2
8	562	817	0.688	386.9
9	609	860	0.708	398.3
Mean	603	847	0.712	**400.5**
SD	33.1	36.8	0.01	**6.76**

Haploid genome sizes estimates for the nine samples analysed are given in Table 1. Mean C-value for D. is 400.5 Mb with a standard deviation (SD) that appeared to be relatively low (<2%) indicating that the phylloxera population analysed revealed low intraspecific variability for genome size.

Acknowledgments**:** D. Papura (INRA Bordeaux) established the Phylloxera population which was held in greenhouse. She also prepared the insects for the flow cytometry analysis with the technical assistance of L. Delbac (INRA Bordeaux). Flow cytometry analysis was performed by O. Catrice (INRA Toulouse) with the technical assistance of S. Richart-Cervera (INRA Bordeaux).

3. Decision of the Phylloxera lineages to be sequenced. This proposal aimed at developing genomic resources for two different lineages of Phylloxera. Several clonal lineages have been described by biotype from root- and leaf-feeding habitats. These have been all sampled in economically important wine regions apart from the native habitat range covering France, Hungary, Germany, Australia and the United States (California). Most strains are genotyped by a set of six SSR markers, available for the scientific community and serve as identification. However few of this clonal lineages are long-term maintained under controlled conditions; among them lineages in Australia and Austria. The Austrian lineages are field-sampled from leaf galls of abandoned rootstocks and selected for fitness on rootstock hosts (roots). Two lineages (sampled in 2006) are currently sustained under controlled greenhouse conditions on Teleki 5C. The genetic background of the Australian and Austrian (as most European) lineages vary in terms of the genetic diversity which is significantly higher in Europe due to migration (of infected material), multiple infection, rare sexual events, than in Australia. In contrast to Europe, some Australian Biotypes remain stable in vineyards and can be

traced spatially and temporally for ongoing screenings and trials. In Australia six (of an identified 83) clonal lineages have been maintained for years and have been used to study host-plant interactions under laboratory and glasshouse conditions and compare these with field observations, where edaphic and climatic conditions appear to influence host-plant susceptibility and relative virulence of genetic strains. The Australian lineages were field sampled from root galls of *V. vinifera* (on which they are maintained) and have been used for comparative host-plant fitness studies on >10 commercially available rootstock studies and >20 novel rootstock hybrids. One of two predominant clonal lineages has also been used in electrophysiological feeding interaction studies and histological studies to characterise the insects gut morphology.

To maximize the genetic traits (& resources) of a clonal lineage one would suggest the crossing of two lineages (economic biotypes & sampled from native habitat) breeding a F1 and a S1 respectively. However, this strategy will be complicated to implement because: i) of the difficulties and yet not solved problems of rearing sexual offspring, ii) such crosses often in F1 progenies that have important fitness decrease. This is supported by genetic studies that have shown excess of heterozygosity in all populations tested so far in both native and economic populations (Corrie et al 2002, Lin et al. 2006, Vorwerk & Forneck 2006). We therefore suggest employing an existing clonal lineage from an economic habitat rather than using a new cross and selfed lineage.

Several phylloxera field-sampled strains from Australia and Europe have been phenotyped before and show differences in host performance and fitness when bio-assayed. Therefore, in the first step, we suggest sequencing of both an Australian lineages (e.g. G1) and an European lineage to allow coverage of all prospective genes. Using the 10 SSRs already available (Corrie et al. 2002, Lin et al. 2006, Vorwerk & Forneck 2006) and the new SSRs that are developed at INRA Bordeaux, the heterozygosity level of the lineages to be sequenced will be assessed. The selected phylloxera lineages will be provided to all groups for biological and genomic testings.

4. Genome sequencing of *D. vitifoliae*

Sequencing. We propose that the *D. vitifoliae* genome will be produced on new generation sequencing platforms such as Solexa/Illumina rather than 454. As stated by Hugh Robertson (University of Illinois): "different groups have experienced various levels of difficulties with 454 homopolymer length errors, so a movement is underway to migrate entirely to ILLUMINA sequencing to avoid these difficulties. This movement has been made possible by two key developments. First, the current ILLUMINA Genome Analyzer II machine with modifications is capable of generating high quality reads up to 125 bases in length, and each lane of the seven available in a flowcell can generate ~35 million paired end reads, which can be various distances apart. Second, assembly of these large numbers of relatively short reads has been made possible by the development of several assembly packages, most notably SOAPdenovo by the BGI (Beijing Genome Institute) in China (Li et al. 2010)." Therefore, it is usually recommended to have an 80x coverage of the genome to allow a correct assembly.

Assembly of NGS reads is not yet completely resolved and this depends on different parameters. Quality of the assembly depends on the degree of repeats and transposable elements: the pea aphid genome contains approx. 30% of repeats and transposable elements. Composition of the phylloxera genome is not known but we might expect the same range of complexity. *De novo* assembly of the phylloxera genome could be performed with the guidance of Hugh Robertson's group; there are different options

such as those based on SOAPdenovo assembly or using other algorithms like ABYSS. DNA libraries will be prepared from whole body adult females: we propose several DNA libraries differing by insert size: 500 bp, 3 kb, 8 kb and 12 kb.

Genome annotation can be divided into two sequential steps: *in silico* predictions and manual annotation. The quality of *in silico* annotation depends mainly on expression evidence for genes encoding proteins or non-coding RNAs. This genome project includes therefore several RNA-Seq runs in order to produce as much as possible of partial transcript sequences from different tissue sources and different conditions (morphs for instance). Different bioinformatic tools may be combined in order to optimize the description of gene models from EST sequences data.

Database. After sequencing, assembly and automatic annotation, a database allowing to store, display, browse, and seek features within the genome is necessary. AphidBase has been developed by INRA Rennes (France) in the frame of IAGC (http://www.aphidbase.com). It contains the annotated pea aphid genome but is dedicated to house other aphid genomes. AphidBase will thus be the database housing the phylloxera genome project. This will allow comparative genomics between different aphid species. Moreover, AphidBase contains the Apollo tool that is used for manual annotation. Manual annotation of the pea aphid genome has been performed by IAGC, using Apollo, after a workshop where users learnt how to use this annotation tool. The IAGC will organise a similar manual annotation for phylloxera.

AphidBase is also connected to other dedicated databases such as Cycads (for metabolic networks) and PhylomeDB (for phylogenomics). Groups handling those two databases are willing to load future phylloxera genome data on their database to allow i) an analysis specific to phylloxera and ii) an inter-species comparison.

Finally, the use of genomic resources to understand many of the specific biological traits of phylloxera (see below) requires the development of functional analysis tools. Stable transgenic phylloxera is probably too far to be reality: for the moment, transgenics are available for very few insect species. However, transient RNAi after injection of interfering RNAs has been developed for several insect species including aphids (Jaubert-Possamai et al. 2007) and other Hemiptera.

5. Exploitation of the genome sequence

Transcriptomic and functional approaches. To study some specific biological traits or particular aspects in the biology of the phylloxera (changes of morphs or alimentary modifications that occur at some key points of the biological cycle), transcriptomic and functional approaches will be developed. For instance, high-throughput RNAseq will allow analysing and comparing the differential expression of the transcripts between root-feeding (underground) and galls-feeding (aerial) morphs, between male and female forms, winged versus apterous forms, or sexual versus asexual morphs. This transcriptomic approach will help in identifying candidate genes responsible for the phenotypes under study. Besides, functional approaches - such as RT-PCR, RNAi, etc - will be used to study specific functions of phylloxera.

Phylloxera population genomics and resequencing phylloxera strains to identify genes responsible for adaptations (host races, obligate parthenogenesis, insecticide resistance). The availability of two different phylloxera strains will provide many polymorphic markers (SNP) and will open the door to population genomics approaches on phylloxera. With the re-sequencing of phylloxera lineages, the genetic basis of

adaptation will be unravel directly at the genome level, without any prerequisites about the selectively advantageous genes or traits. Re-sequencing strains having distinct phenotypes will also allow distinguishing between the neutral genetic background and outlier loci (e.g. showing a higher than expected genetic differentiation between populations). This approach will be used to address host specialisation of phylloxera on different *Vitis* spp., transitions to obligate parthenogenesis, or insecticide resistance.

Phylloxera/*Vitis* interaction. Understanding the mechanisms of gall induction to roots and leaves of *Vitis* are important to learn about the changes occurring in the plant physiology when infested by phylloxera. Using metabolomic approaches the analysis of the insects' saliva, function of the salivary glands and gut are essential as are analysis of the gall tissues to learn about the potential impacts to host-performance during the gall induction and gall maturation phase. In complement to RNA-Seq approach we plan projects involving metabolomic profiling of the phylloxera salivary glands including the analysis of the potential effects of "associated microorganisms (e.g. *P. agglomerans*)" abundant in the salivary glands and the effects of phylloxera inducing root galls in rootstocks.

References

Corrie AM, Crozier RH, van Heeswijck R, Hoffmann AA (2002) Clonal reproduction and population genetic structure of grape phylloxera, *Daktulosphaira vitifoliae*, in Australia. Heredity, 88:203–211.

Downie DA (2002) Locating the sources of an invasive pest, grape phylloxera, using a mitochondrial DNA gene genealogy. Molecular Ecology, 11(10): 2013-2026.

Forneck A, Huber L (2009) (A)sexual reproduction - a review of life cycles of grape phylloxera, *Daktulosphaira vitifoliae*. Entomologia Experimentalis et Applicata, 131(1):1-10.

Granett J, Walker MA, Kocsis L, Omer AD (2001) Biology and Management of Grape Phylloxera. Annual Review of Entomology, 46:387-412.

Heie OE (1987) Palaeontology and phylogeny. Aphids: Their biology, natural enemies, and control, vol 2A. Eds. A. K. Minks and P. Harrewijn. Elsevier, Amsterdam.

IAGC consortium (2010) Genome sequence of the pea aphid *Acyrthosiphon pisum*. PLoS Biology, 23:8(2): e1000313.

Jaillon O, Aury J-M, Noel B, Policriti A, Clepet C, Casagrande A, Choisne N, Aubourg S, Vitulo N, Jubin C, Vezzi A, Legeai F, Hugueney P, Dasilva C, et al. (2007) The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature, 449:463-467.

Jaubert-Possamai S, Le Trionnaire G, Bonhomme J, Christophides JK, Rispe C, Tagu D (2007) Gene knockdown by RNAi in the pea aphid *Acyrthosiphon pisum*. BMC Biotechnol. 7:63.

Kingston KB, Powell KS and Cooper PD (2009) Grape phylloxera: new investigations into the biology of an old grapevine pest. Acta Horticulturae, 816:63-70.

Li R, et al. (2010) The sequence and de novo assembly of the giant panda genome. Nature, 463:311-317.

Lin H, Walker MA, Hu R, Granett J (2006) New simple sequence repeat loci for the study of grape phylloxera (*Daktulosphaira vitifoliae*) genetics and host adaptation. American Journal of Enology and Viticulture, 57(1):33-40.

Ortiz-Rivas B, Martínez-Torres D (2010) Combination of molecular data support the existence of three main lineages in the phylogeny of aphids (Hemiptera: Aphididae) and the basal position of the subfamily Lachninae. Molecular Phylogenetics and Evolution, 55(1):305-17.

Vorwerk S, Martinez-Torres D, Blaich R, Forneck A (2007) *Pantoea agglomerans* associated bacteria in grape phylloxera (*Daktulosphaira vitifoliae* Fitch). Agriculture and Forest Entomology, 9:57-64.

Vorwerk S, Forneck A (2006) Reproductive mode of grape phylloxera (*Daktulosphaira vitifoliae*, Homoptera: Phylloxeridae) in Europe: molecular evidence for predominantly asexual populations and a lack of gene flow between them. Genome, 49(6):678-687.