Roscoff (France), 26-30 septembre 2009

Maladies infectieuses émergentes : focus sur les nouvelles approches expérimentales et théoriques de l'évolution virale

Understanding Emergence of Infectious Diseases: Focus on New Experimental and Theoretical Approaches to Virus Evolution

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Rapport sur la Conférence

Conference Report

RESUME DU RAPPORT

Conférence Jacques Monod intitulée : Maladies infectieuses émergentes : focus sur les nouvelles approches expérimentales et théoriques de l'évolution virale

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L'objectif principal de cette conférence était de présenter les développements et résultats expérimentaux et théoriques les plus récents dans le champ de l'émergence et de l'évolution virale. Les travaux présentés et discutés par les intervenants invités portaient sur les processus écologiques et évolutifs ainsi que les bases génétiques sous-jacents à l'émergence ainsi que la description de nouvelles stratégies possibles permettant de diminuer l'impact des émergences virales et leur expansion. Cette conférence a mis en lumière de nouveaux concepts théoriques qui ont émergés durant les dernières années et a permis d'identifier des domaines dans lesquels la recherche reste nécessaire.

Les sujets et intervenants pressentis ont très clairement attiré l'intérêt de la communauté scientifique mondiale s'intéressant au thème de la conférence, comme l'indique le grand nombre de participants, au total de 112 venant de 11 pays différents (Irlande, Angleterre, Italie, France, Allemagne, Espagne, Suisse, Pays-Bas, Afrique du Sud, Brésil et Etats-Unis d'Amérique). Cette conférence a permis de réunir de nombreux spécialistes les plus reconnus dans leurs domaines. Par ailleurs, le relatif petit nombre de participants (une centaine de personnes) et l'atmosphère intime et paisible ainsi que les excellentes prestations offertes par la station de recherche CNRS de Roscoff a permis de stimuler de nombreuses discussions en profondeur et très certainement d'initier de futures collaborations. Ces atouts sont à mettre en premier lieu au crédit de Mme D. Lidoreau mais aussi des personnels de Roscoff qui ont pris part à l'organisation pratique (administration, réception, logement, salle des posters, installation informatique). Nous souhaitons aussi faire part de notre gratitude quant à la qualité des repas et des services. L'événement social organisé un des après-midi sur l'île de Batz a été particulièrement apprécié.

Un aspect particulièrement intéressant de la conférence a été de réunir des scientifiques de cultures et formations différentes (écologie, physique statistique, biologie évolutive, biologie théorique, ingénierie chimique, épidémiologie et virologie) travaillant sur des systèmes biologiques différents (animaux, bactériens, végétaux). La communication entre les participants en a été d'autant plus vive. En planifiant cette conférence, nous avions cherché à favoriser la diversité des approches. Nous sommes convaincus qu'une telle couverture multidisciplinaire a été l'une des « plus-value » de cette conférence.

En suivant cette directive que nous nous étions donnée, nous avons organisé les contributions autour de neuf sessions :

1. Création de la variabilité génétique : données empiriques
2. Création de la variabilité génétique : les approches de mutagenèse létale
3. Contournement des mécanismes de résistance de l'hôte
4. Bases génétiques de l'adaptation
5. Infections multiples
6. Impact de la transmission sur l'évolution virale
7. Co-évolution virus-hôte
8. Evolution des génomes viraux et de l'organisation génomique

9. Evolution moléculaire et phylogénomiques

Au total, 26 intervenants ont été invités à donner une présentation de trente minutes. Parmi eux, neuf provenaient de laboratoires français, onze européens et six américains. En plus, 14 participants ont été sélectionnés pour donner une présentation orale de quinze minutes, sélection basée sur l'originalité et le caractère novateur du résumé soumis. Deux sessions posters (deux heures chacune, pour un total de 66 affiches) ont complété ce programme scientifique. Nous avons pris soin de permettre autant de question et de discussion que possible, tant dans les sessions posters que durant les présentations orales, les pauses et durant les repas. Enfin, nous avions prévu une plage horaire pour une discussion générale durant laquelle les sujets ayant retenus l'attention de l'ensemble des participants ont été mis en exergue et discutés abondamment. Les présentations orales et affichées étaient toutes d'une très bonne qualité et, bien que le programme était relativement serré, le retour direct des participants concernant la conférence a été enthousiaste et nous paraît donc avoir été un succès.

À la suite de cette conférence, nous pouvons conclure que le champ de l'évolution virale est en pleine expansion et très actif. Face à la menace permanente imposée par les maladies émergentes d'origine virale et face à aux échecs de contrôles des maladies virales émergentes voire notre incapacité de prédire quand et où de nouveaux virus vont émerger, il convient de désigner la nécessité d'engager plus de recherches prennant en compte tant les composantes écologiques qu'évolutives des processus d'émergence. Cette conférence a permis de démontrer que l'étude de l'émergence des maladies virales ne peut pas se limiter à la description des épidémies mais au contraire, la compréhension des causes du saut d'espèces hôtes ou du changement de la dynamiques des populations virales intra-hôtes comme inter-hôtes. Cette connaissance ne pourra venir que dans la collaboration entre les écologistes, évolutionnistes, épidémiologistes, chimistes, physiciens, mathématiciens et virologistes. Développer de nouvelles thérapies antivirales sans prendre en considération l'impressionnante dynamique adaptative des virus conduira irrémédiablement à l'échec. Pareillement, mettre en place des stratégies de contrôles des populations hôtes sans prendre en compte les propriétés écologiques à petites échelles spatiales et des hôtes alternatifs réservoirs ne permettra pas d'enrayer l'émergence de nouveaux virus dévastateurs. Toute tentative future de développer des stratégies de contrôle ou de prévention nécessite une approche multidisciplinaire. La conférence Jacques-Monod fût certainement l'un des lieu pour la construction de telles interactions.

Pour finir, et afin de préparer au mieux l'organisation d'une prochaine conférence Jacques-Monod, l'ensemble des personnes présentes ont désigné avec une très chaleureuse et enthousiaste participation, Rémy Froissart et Edwards C. Holmes comme potentiels Président et vice-président (respectivement).

CONFERENCE REPORT

Final report from the Jacques-Monod Conference entitled: Understanding Emergence of Infectious Diseases: Focus on New Experimental and Theoretical Approaches to Virus Evolution

Roscoff, September 26-30, 2009

Summary

The main objective of this meeting was aimed at covering the most recent experimental and theoretical developments and exciting results in the field of virus emergence and evolution. The works presented and discussed by the selected speakers addressed both ecological and genetic mechanisms behind emergence as well as depicted possible new strategies to minimize the impact of viral emergence and spread. This conference highlighted new theoretical concepts that have emerged over the last few years and also identified areas where new research is desperately needed.

The selected topics and speakers were of high interest to the international scientific community, as indicated by turnout of applicants, a total of 112 from 11 different countries (Brazil, Ireland, Italy, France, Germany, Spain, Switzerland, The Netherlands, South Africa, UK, USA). This conference featured many of the most highly recognized specialists in the field. The relatively small scale of the meeting, the intimate and peaceful atmosphere, and the excellent facilities provided by the CNRS at Roscoff, stimulated many in-depth discussions and most likely initiated future collaborations. Much of this must be credited to Ms. Dominique Lidoreau and the personnel of the Roscoff center who took care of all the practical organization (administration, lodging, poster room, computer facilities). We also want to emphasize the very good quality of housing and the excellent food. On one of the afternoons, an excursion was organized to Batz Island. This was a highly appreciated social event.

A particularly valuable aspect of the meeting was that it brought together scientists from widely different cultures and scientific backgrounds (ecology, statistical physics, evolutionary biology, theoretical biology, chemical engineering, epidemiology, and virology) that were communicating vividly. In planning this conference, we favored diversity and interdisciplinary approaches, and such a broad coverage was certainly appreciated and considered to be an "added value" to this conference. Along these lines, we took care to balance contributions and dispatched them into 9 sessions:

1. Generation of genetic variability: basic mechanisms.
2. Generation of genetic variability: lethal mutagenesis.
3. Escaping from host resistance mechanisms.
4. The genetic basis of adaptation.
5. Multiple infections.
6. Impact of transmission on viral evolution
7. Virus-host coevolution.
8. Evolution of viral genomes and genome organization.
9. Molecular evolution and phylogenomics.

A total of 26 speakers were invited to give 30' full talks. Of these, 9 come from French laboratories, 11 from other European laboratories and 6 from the USA. In addition, 14

applicants were selected to give 15' oral presentations, based on the originality and novelty of their abstracts. Two poster sessions (2 hours each, adding up to 66 posters) completed the scientific program. There was time and opportunity for questions and discussions in both poster sessions and general presentations as well as during the breaks and over lunch and dinner. Furthermore, we reserved an afternoon for a general discussion into which the more interesting topics were highlighted and deeper discussed. The oral and poster presentations were of outstanding quality and, although the schedule was tight, the feedback from participants indicated their enthusiasm for the meeting.

Scientific report

Session I. Generation of genetic variability: Basic mechanisms

Simon WAIN-HOBSON (Pasteur Institute, France) presented data on the recently described mechanism of epigenetic variability on HIV-1. The action of cytidien deaminases such as the mammalian APOBEC3 and ADAR1 leads to inflation of the overall mutation rate of retroviruses. These enzymes edit viral RNA increasing its mutation rate. Ironically, many of these genes are up regulated by interferons, presumably to help control viral spread, which they certainly do. Yet by inducing a mutator phenotype, they may help the virus adapt to the highly hostile inflammatory environment, particularly in a chronic infection. Once again, inflammation can be seen as a double-edged sword. Unlike the ADAR loci, the number of APOBEC genes varies greatly among species.

José M. CUEVAS (Universitat de València, Spain) showed that the amount of genetic variability and the rate of molecular evolution at three different regions of Hepatitis C virus depended on the degree of secondary structure present at these regions. Paired regions tolerated less genetic variability and evolved more slowly, a correlation that also held within regions.

Siobain DUFFY (Rutgers University, USA) presented results on the rates of evolution and presence of positive selection and/or genetic drift on the evolution of plant ssDNA viruses. She showed evidence of sustained selection in the long-term evolution. She presented evidence that ssDNA viral genomes show signatures of DNA damage that commonly occurs when nucleic acids spend time in a single-stranded state. That is, that cytosine to thymine transitions are overrepresented relative to the reverse substitution, and that is frequently caused by spontaneous deamination of cytosine to uracil, which calls for an adenine during complementary strand synthesis.

Marco VIGNUZZI (Pasteur Institute, France) reviewed his recent studies using fidelity variants of poliovirus that have shown that moderate increases in fidelity resulted in observable changes in population diversity and compromised the virus' ability to adapt to changing environments.

Session II. Generation of genetic variability: lethal mutagenesis

One of the most intriguing recent empirical observations is that replication of picornaviruses in the presence of the mutagenic nucleoside analogue ribavirin may result in either virus extinction or selection of viral mutants with decreased sensitivity to ribavirin. Biochemical and structural studies suggest that multiple sites in the viral RNA-dependent RNA polymerases, located close or away from the catalytic domain, can affect ribavirin resistance through different mechanisms. Esteban DOMINGO (CSIC, Spain), a pioneer on these studies

gave an overview of the empirical evidences supporting the notion of lethal mutagenesis, highlighting the potential therapeutic implications of his research.

As a complement to Prof. Domingo's experimental results, Claus O. WILKE (University of Texas, USA) and Susanna C. MANRUBIA (CSIC-INTA, Spain) presented mathematical theories for lethal mutagenesis. After discussing some of the general misunderstandings about lethal mutagenesis (e.g., equating it to error catastrophe) Dr. Wilke presented results on how it works on structured spaces representing e.g. hosts containing multiple organs and tissues to which the mutagen has different penetrability. Dr. Manrubia introduced the new concept of lethal defection by which the replication of wildtype viruses is jeopardized in presence of defector genomes produced by error prone replication.

Guillaume MARTIN (CNRS, France) presented new mathematical models for the evolution of viral populations in constant environments that represent a single fitness peak. These developments built up on the classic quantitative genetics theory for morphological and fitness traits and allow for continuous changes in traits. Building up on this approach, Sylvain GANDON (CNRS, France) explored the epidemiological and within-host dynamics of viruses whose mutation rate is exogenously augmented. These new developments bring the lethal mutagenesis into an epidemiological and demographic framework.

Session III. Escaping from host resistance mechanisms

Evolving viral population that may harbor escape mutants at noticeable frequency constantly jeopardizes the durability of new antiviral therapies. Santiago F. ELENA (CSIC, Spain) explored the durability of resistance conferred by transgenically expressing artificial microRNAs (amiR) designed to target key components of the viral genome. Viral populations evolving in the susceptible host generate escape mutants that are maintained at the mutation-selection-drift balance. These mutants break the resistance imposed by the amiR after short periods of time. Combinations of several amiRs may improve durability. Indeed, the results presented suggest that single nucleotide substitutions that affect the binding affinity of the amiR and the target sequence may favor viral escape and the fixation of subsequent mutations further reducing binding.

Following on the topic of durability, Benoît MOURY (INRA, France) is exploring whether polygenic resistance may be a better approach than resistances based on single genes. Monogenic resistances to plant viruses were defeated at high frequency when introgressed in a susceptible genetic background whereas they were not when combined to partial resistance quantitative trait loci. The suppression of emergence of virulent mutants due to the genetic background resulted both from a differential selection effect and the necessity of the virus to generate multiple mutations. The virus adaptation to the polygenic resistance was possible by a step-by-step selection with a primary selection for virulence towards the major gene, followed by selection for adaptation to the genetic background.

Carlos BRIONES (CSIC-INTA, Spain) touched on another controversial and appealing concept recently introduced in the virological literature: the memory of viral quasispecies. Experiments designed with specific mutants of FMDV in cell culture showed that one of the relevant biological consequences of the quasispecies nature of RNA viruses is the possible presence of memory genomes as minority components of their mutant spectra. Minority memory genomes descend from those variants that were dominant at an earlier phase of quasispecies evolution, and arise when viral populations are subjected to discontinuous selective pressures. Dr. Briones presented data compatible with the existence *in vivo* of memory genomes in HIV-1 populations sampled from treated patients.

To close this session, Susan CARPENTER (Iowa State University, USA) presented results examining the existence of trade-offs between immune evasion and replication fitness during persistent lentivirus infections.

Session IV. Genetic basis of adaptation

Sebastian BONHOEFFER (ETH, Switzerland) discussed recent work on a quantitative analysis of a very large data set consisting of 60,000 HIV sequences with *in vitro* fitness estimates in absence of drugs or presence of 15 individual retroviral inhibitors in order to estimate the fitness effects of individual mutations and the role of epistatic interactions between mutations. A new statistical approach was developed to evaluate the impact of epistasis in fitness. The ruggedness of fitness landscapes depends on the amount, sign and intensity of epistasis.

Disentangling the contribution of mechanistic predisposition to recombination and of selection acting on recombinants is complicated by the difficulty of obtaining information on recombinant variants generated before selection acts. Matteo NEGRONI (CNRS, France) studied recombination in the HIV-1 envelope gene between primary isolates of subtypes that frequently recombine in the pandemic using a three-step approach: (*i*) determine the distribution of breakpoints found in recombinants generated in cell culture before any selection is applied, (*ii*) subsequently characterized the functionality of the individual recombinants in cell culture infection tests and (*iii*) corrected the pattern of recombinants on the basis of the probability of generating functional clones depending on the location of the breakpoints. Using this approach, he showed that the combined effects of mechanistic processes and purifying selection acting against dysfunctional recombinants explains the distribution of breakpoints found in nature.

Ben BERKHOUT (University of Amsterdam, The Netherlands) reviewed experimental work from his group illustrating how HIV-1 escapes from all sources of antiviral inhibitors. His results suggest that the most promising avenue of antiviral therapy would necessarily be a combination of different drugs.

Understanding the ecological and evolutionary processes promoting host shifts by viruses would aid current disease management strategies, and would help future efforts to predict emergence events. Prior ecological history shapes the genetics underlying adaptive traits in viruses, and should determine the likelihood that viruses can fortuitously exploit novel hosts. Rémy FROISSART (CNRS, France) presented the results of an evolution experiment in which *Cauliflower mosaic virus* was evolved in either of two single hosts (*Arabidopsis thaliana* and *Nicotiana bigelovii*) or in a correlated fluctuation of both hosts. The evolutionary process was described both in terms of phenotypic and genotypic changes. Genomic data revealed that parallel mutations appeared differentially depending on environmental contexts: (*i*) their distribution among genes was different among environments, (*ii*) a majority of the mutations were accumulating in constant environments; (*iii*) no identical mutations were detected in different environments, and (*iv*) populations evolving in variable environments contained more polymorphic loci than those evolving in constant environments. Compared to the ancestor, virus accumulation and virulence of populations evolved in constant environments increased in the host species where evolution took place and correlatively decreased in the alternative host. These results suggest the existence of a cost associated with host switching.

Following up on the previous talk, Paul E. TURNER (Yale University, USA) presented data testing the hypothesis that viruses historically evolved on multiple hosts are better capable of emerging on un-encountered hosts. Using an elegant *in vitro* evolution experiment, he

showed that multi-host evolution fostered RNA virus emergence under environmental change, indicating an informative risk factor for predicting future pathogen emergence.

Christina BURCH (University of North Carolina, USA) described a series of experiments designed to characterize the effects of and the interactions between spontaneous mutations in RNA bacteriophage ϕ6. The presented data challenged the emerging paradigm that very few mutations have large effects on fitness, and confirm that the mutation effect distribution changes shape when fitness is reduced by the fixation of deleterious mutations.

Session V. Multiple infections

Recombination, complementation and competition are examples of intracellular interactions among viral genomes. Such interactions often generate new variants responsible for emerging viral diseases. The key parameter determining whether recombination and/or complementation may be important is the multiplicity of infection (MOI). Despite its relevance, estimates of MOI during natural infections are scarce. Serafín GUTIÉRREZ (INRA, France) presented new data on evaluating how MOI changed along the curse of infection of a plant virus. He showed that, despite spatial and temporal differences, MOI is always greater than 1, thus making recombination and complementation feasible phenomena.

Minus VAN BAALEN (CNRS, France) argued about what should be considered as the unit of selection in viral populations. The definition should take into consideration that within- and between-host processes do not occur at the same time scales and that mutation and recombination affect the definition.

Finally, Thomas W. BERNGRUBER (CNRS, France) discussed on another important question that, in general, has is not being correctly considered in the study of virus emergence and evolution, namely the phenomenon of superinfection inhibition. In the case of vertically transmitted viruses, superinfection inhibition is fundamental to prevent cheater high virulence genotypes to spread into the population.

Session VI. Impact of transmission on viral evolution

Tony L. GOLDBERG (University of Wisconsin, USA) presented a large-scale field study seeking to determine the fine-scale ecological processes driving the epidemiological dynamics of *West Nile virus* (WNV) in a transmission hotspot in Chicago's urban area. Spatial autocorrelation analyses of viral sequences recovered from mosquito pools demonstrated a pattern of distance-limited transmission, with negative autocorrelation at distances greater than 4 km. Viral genetic diversity in mosquitoes varied by land cover type (higher in residential than in natural sites) and by vector type (higher in non-Culex than in Culex mosquitoes). Bayesian analysis of evolutionary rates demonstrated an approximately 10-fold higher rate of envelope gene evolution during the summer months than between years. Bayesian phylodynamic analyses of sequences collected between 2005 and 2007 yielded a complex pattern of increasing and then decreasing effective numbers of infections that is apparently "decoupled" from the seasonal dynamics of local amplification

Cécile DESBIEZ (INRA, France) provided an example of the impact of transmission on a plant virus system, *Watermelon mosaic virus* (WMV). WMV has been described in France for more than 30 years causing mild symptoms on zucchini squash. However, since 1999, severe strains have been responsible for important damages in zucchini crops. Molecular studies revealed that only one molecular group of strains was present before 1999, whereas a second group, closely related to isolates from Eastern Asia, was also observed afterwards. To understand why the virulent strain is displacing the mild one, experimental and modeling

approaches have been undertaken to estimate the relative fitness of classic and virulent strains.

In the case of HIV-1, the duration of infection and the rate of transmission are both dependent on the set-point viral load. George SHIRREFF (Imperial College London, UK) showed that a correlation exists in set-point between transmission pairs, suggesting that it is a heritable trait that may be evolving under natural selection.

John YIN (University of Wisconsin, USA) described new technical approaches to study *in vitro* the dynamics of virus infection. Using cell reactors and microfluidic methods, he estimated *Vesicular stomatitis virus* growth parameters for single cells. Interestingly, he showed that viral yield per cell spanned a broad range from 8000 to less than 10 infectious particles. He also showed how these techniques were applied to study the arising of defective interfering particles (DIPs).

Flaviviruses are predominantly maintained in a trasmission cycle between mosquitoes and birds and have caused major epidemic encephalitis in the USA. The need for arboviruses to replicate in disparate hosts is thought to result in constrains on both evolution and host-specific adaptation, suggesting that this class of viruses are generalist paying a cost of suboptimal adaptation on each alternative host. Alexander T. CIOTA (New York State Department of Health, USA) described an experiment testing this hypothesis for WNV and *St. Louis encephalitis virus* (SELV). Passage of WNV in mosquitoes resulted in adaptation to mosquitoes in terms of both infectivity and replication kinetics, yet did not result in any apparent fitness cost in chickens. A lack of both genetic and phenotypic change was measured in WNV following 20 passages in chickens, suggesting further host specialization was not attainable. Similarly, no additional specialization to mosquitoes was attainable for SLEV following 20 passages in mosquitoes and, although a strain more infectious to chicks did emerge following chick passage of SLEV, no cost was measured for this strain in terms of mosquito infectivity.

Session VII. Virus-host coevolution

Rapid bacteria-phage coevolution and its consequences have been extensively demonstrated in the laboratory. However, its significance in natural population is unclear. Angus BUCKLING (Oxford University, UK) reported extensive phage local adaptation in two natural bacteria-phage systems over a range of spatial scales. These data demonstrate rapid evolution of phage to their bacteria host, and suggest coevolution may play an important role over “ecological” time scales in natural populations.

Parasite infection has a negative impact on host fitness, which has been defined as virulence. Virulence evolution may drive parasite evolution and host-parasite coevolution, and consequently, it is at the root of fundamental phenomena such as pathogen emergence. Despite a wealth of literature on theoretical aspects of virulence evolution, experimental analyses are scarce, particularly for plant parasites. Fernando GARCÍA-ARENAL (Universidad Politécnica de Madrid, Spain) used *Cucumber mosaic virus* (CMV) and *Arabidopsis thaliana* to study which plant traits are involved in minimizing CMV virulence. The analysis of several traits in relation to CMV infection of 21 *Arabidopsis* genotypes has shown that all traits, including virulence, depended on the host-virus genotype x genotype interaction. Host genotypes varied in their susceptibility to CMV, measured as virus multiplication, and the virulence of CMV genotypes depended on *Arabidopsis* genotype. Virus multiplication and virulence were not correlated, at odds with assumptions of most models of virulence evolution. Finally, Prof. García-Arenal showed that the combined effects of plant density and virus infection might result either in a reduction or an increase of the competitive ability of the host.

Lena BAYER-WILFERT (Cambridge University, UK) studied the vertical transmission and long-term coevolution between the sigma virus and its host *Drosophila melanogaster*. Resembling a gene-for-gene pattern of interactions, major-effect resistance polymorphisms in host populations is matched by polymorphisms in the viral population that overcome resistance.

Leonor PALMEIRA (Université Lyon I, France) presented a bioinformatic analysis of virus-host coevolution aimed to determine whether viruses evolve as a pool of interchangeable genetic modules that allow them for rapid evolution. She presented a database of families of homologous proteins between fully sequenced viruses, human and insect vectors.

Session VIII. Evolution of viral genomes and genome organization

Edward C. HOLMES (Pennsylvania State University, USA) discussed on the most recent comparative genomics data of *Influenza A virus* (IAV). Recent developments in comparative genomics, particularly the Influenza Genome sequencing Project begun in 2005 have provided a vital new perspective on the epidemiology and evolution of human IAV. He showed how large-scale genome sequence data is altering our view of IAV evolution. He focused on the following areas: (*i*) the nature of viral evolution within epidemic seasons, (*ii*) the evolutionary genomics of IAV, particularly its population dynamics and the evolutionary role played by reassortment, and (*iii*) the frequency and importance of mixed infection in influenza. Together, these analyses reveal that the genome-wide evolution of IAV is characterized by a complex pattern of frequent reassortment interspersed by dramatic reductions in genetic diversity most likely reflecting selective sweeps, some of which affect multiple segments.

Metagenomic studies have analyzed viruses from several marine environments as well as some mammalian gut samples. However, all of these studies have examined only bacterial viruses, and they have not been able to link viruses with their hosts. To cover this gap, Marilyn ROOSSINCK (Samuel R. Noble Foundation, USA) undertook a different type of study in which individual plants were sampled and the presence of RNA viruses assessed by high throughput multiplex sequencing. She has been working on two different ecosystems widely differing in plant diversity (Costa Rica rain forest and Oklahoma tall grass prairie). She found a high incidence of virus-infected plants, most of which are asymptomatic and may act as reservoirs for new emerging viruses.

Marianne DEPAEPE (INSERM, France) highlighted the existence of correlations between virus decay rates and structural characteristics of virions. She showed results on burst size for 8 phages infecting *Escherichia coli*. Genome size appears as the major constraint for the evolution of lysis time and burst size.

The fitness effects of random mutations are central to evolution, yet there are few experimental determinations of these effects. In the case of viruses, site-directed mutagenesis provides a powerful tool for accurately estimating the effects of single mutations. Rafael SANJUÁN (Universitat de València, Spain) reviewed existing evidences gathered from animal, plant and bacterial viruses indicating that RNA viruses show very little tolerance to mutations. Ongoing experiments in his laboratory are extending this result to ssDNA viruses. He highlighted the relationship between robustness and evolvability that RNA and ssDNA viruses might not fulfill the conditions for robustness to promote evolution.

Session IX. Molecular evolution and phylogenomics

Christine CHEVILLON (IRD, France) presented an analysis of the consecutive epidemics and intra-epidemic periods of *Dengue virus* type 2 (DENV-2) in the French Guiana. Despite

evidence for an ongoing DENV-2 circulation among Greater Antilles, Lesser Antilles and Amazonian Basin, the micro-evolutionary patterns observed in Amazonian Basin contrasted from previous observations performed in Puerto Rico (Greater Antilles) regarding the occurrence of recombination, the nature of selection and the relative timing of immigration and epidemics. The resemblance of such contrast to that observed within the Old World leads to consider that the host geography overcome the impact of transmission rate in determining viral micro-evolutionary patterns.

The so-called 'measurably-evolving' pathogens such as HIV-1 and *Hepatitis C virus* (HCV) can evolve on the timescale of a single infection, with new variants transmitted to susceptible individuals. Simon D. W. FROST (Cambridge University, UK) presented two examples of how within-host viral evolution can affect patterns of genetic variation at the level of the population of infected hosts. First, he considered how fluctuating selection pressure exerted by, for example, CTL responses can result in transient polymorphisms of wildtype and escape mutants within the host, exhibited by the abundance of ambiguous nucleotide calls in sequence data. When there is 'toggling' between wildtype and escape mutants over time, short inter-transmission intervals can lead to dramatically elevated frequencies of escape mutants at the population level. Secondly, Dr. Frost considered how ongoing viral evolution within individuals affects inference of the population dynamics of infected hosts over evolutionary time. For simple epidemiological models, the shape of the phylogeny is determined not only by the number of infected individuals (i.e. the prevalence), but also by the incidence, and due to the different timescales for population size and genetic variation to equilibrate, it may be extremely difficult to capture declines in prevalence using phylogenetic analysis of sequence data.

Following up from the previous presentation, Samuel ALIZON (ETH, Switzerland) presented the problem caused by the fact that HIV-1 life-history traits of an infection can be poorly related from one infected patient to the next. He used the RNA sequence data obtained from different untreated patients to estimate the transmission chain. Each branch of the tree corresponds to a patient whose infection life-history traits are known. By analyzing this tree, they showed that traits that are known to be linked to virulence can have a strong viral component and that the probabilities of resistance to drugs exhibit a higher signal than more complex traits such as viral load.

Continuing on transmission trees, Gaël THEBAUD (INRA, France) suggested that this epidemiological tool makes only sense when genetic information is incorporated into the picture. To illustrate his point, he used *Foot-and-mouth disease virus* (FMDV) data from the 2001 UK outbreak and showed that predictions of the spread of the virus become much more precise.

Mario A. FARES (Trinity College Dublin, Ireland) has been exploring the fixation of various types of mutations in RNA viruses with different epidemiological behaviors. He presented data from two studies, FMDV and HIV-1. To understand how mutations fixed in RNA viruses influence one another, he studied their distribution at the molecular level and used various theoretical methods to explore their dependency. An important conclusion from the analyses performed is that drift and selection balance through the accumulation of slightly deleterious and advantageous mutations that lead the epidemiology of these viruses.

Conclusion and recommendations

This conference emphasized that the field of virus evolution is an expanding and very active one. Given the continuous threat imposed by new emerging diseases of viral origin and our failure to control them, even less, to predict when and where a new virus will emerge, more research is needed that takes into consideration both the ecological and evolutionary

components of the process. This conference has served to demonstrate that the study of emerging viral diseases cannot be only limited to the description of the epidemics but, instead, we need to really understand what are the causes of a virus to jump the species barrier or to change its population dynamics. This understanding will only came from a tight collaboration between ecologists, evolutionary biologists, epidemiologist, chemicists, physicists, mathematicians, and virologists will join expertise and efforts. Developing new antiviral therapies without taking into consideration the highly dynamic nature of viruses, or devising population control strategies that do not take into consideration the fine-scale ecological properties of the virus and their reservoir hosts will be irremediably condemned to failure. Any future attempt to develop new preventive and control strategies needs to incorporate a multidisciplinary approach. And the place to build such interactions is a conference such as the Jacques Monod.

In conclusion the feedback from participants indicated their enthusiasm for the meeting. They were overall happy with the content and the stimulating atmosphere of the conference. We have already heard several comments from participants indicating strong support for the perspective of a future Jacques Monod Conference on virus evolution. Finally, we obtained a strong and enthusiastic approval of the participants for the designated next president, Dr. Rémy Froissart, and the newly elected vice-president, Prof. Edward C. Holmes, and feel confident that they will be able to put together a very strong, exciting and cutting-edge program.