FROM THE ACADEMY: COLLOQUIUM INTRODUCTION

In the light of evolution IX: Clonal reproduction: Alternatives to sex

Michel Tibayrenc[a,1], John C. Avise[b,1], and Francisco J. Ayala[b,1]

[a]*Infectious Diseases and Vectors: Ecology, Genetics, Evolution, and Control, Institute of Research and Development 224, CNRS 5290, Universities of Montpellier 1 and 2, 34394 Montpellier, France; and* [b]*Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697-2525*

Evolutionary studies of clonal organisms have advanced considerably in recent years, but are still fledgling. Although recent textbooks on evolution or genetics might give the impression that nonsexual reproduction is an anomaly in the living world, clonality is the rule rather than the exception in many viruses, bacteria, and parasites that undergo preponderant asexual evolution in nature. Asexual reproduction is also common in insects, pathogenic helminthes, crustacea, and plants, and is found even in vertebrates. Clonality is thus of crucial importance in basic biology as well as in studies dealing with transmissible diseases.

The primary focus of the Colloquium is on the balance between sexuality and clonality, because many so-called clonal organisms benefit from both evolutionary modes. Apart from its classic advantage of generating new genetic variants, sexual reproduction (genetic exchange between two different cells) plays a major role in DNA repair, which could represent its ancestral function. Clonal evolution, by contrast, permits the proliferation of high-fitness, multilocus associations and the avoidance of recombinational load (generation of low-fitness recombinants). Moreover, clonal evolution plays a crucial role in cancer biology, where the propagation of cancer cells displays striking analogies with that of microparasites.

The study of clonal reproduction raises many theoretical, experimental, and technological challenges that may yield considerable pay-offs in microbiology and parasitology (e.g., in human medicine, veterinary medicine, and agronomy), artificial cloning, and the study of cancer. This "In the Light of Evolution" Colloquium made it possible to bring together specialists in various disciplines, including genetics, evolution, statistics, bioinformatics, and medicine. A balance is sought between the various disciplines, including clonal animals and plants, animal and human cloning, pathogens, and cancer studies. Active cross-fertilization is expected among scientists who have previously worked mostly separately. The Colloquium publication should be of considerable interest to the scientific community, including graduate students and postdoctorates.

General Considerations

Dave Speijer, Julius Lukeš, and Marek Eliáš (9) point out that clonal reproduction is often perceived as exceptional because the focus is on metazoans. When unicellular organisms are considered, the picture is different. The authors argue that the debate on the relative significance of sex and clonality in eukaryotes requires distinguishing between multicellular and unicellular organisms. The authors propose the somewhat provocative view that eukaryotes in general can be seen as clonally propagating cell lines with episodic bouts of sex, triggered by external or internal clues. Speijer et al. argue that eukaryotic sex may have developed as a cellular survival strategy, possibly linked to internal reactive oxygen species and stresses generated by a proto mitochondrion. In the framework of the symbiogenic model of eukaryotic origin, sex might have directly resulted from the same evolutionary process by which eukaryotic cells arose.

Zhao-Rong Lun et al. (10) advance the very original and provocative view that infections by the parasitic protozoans *Trypanosoma brucei* (the agent of human sleeping sickness and several cattle diseases) and by *Toxoplasma gondii* (responsible for toxoplasmosis) may be equivalent to cancer processes from an evolutionary point of view. These protozoans have complex life cycles, some components of which (such as derivatives of *T. brucei* no longer transmitted by vectors) may be seen as cancerous forms of the original parasite.

According to Ignacio Rodriguez-Brenes and Dominik Wodarz (11), clonal processes may be driving pathogenesis in human diseases, as is the case with cancer, a clear example of clonal evolution. Their goal is to finely analyze, by means of mathematical models, the dynamics of cancer cells during their proliferation as well as under treatment. The authors discuss the relationships between cancer processes, cell evolution, and the induction of replicative senescence through telomere shortening. Then, Rodriguez-Brenes and Wodarz consider cancer clonal evolution under therapy, focusing on the treatment of chronic lymphocytic leukemia with tyrosine kinase inhibitors. Evolutionary mathematical models have the potential to make patient-specific predictions about treatment efficiency. Evolutionary models could thus become important clinical tools in the growing field of personalized medicine.

The last article of the first section of the Colloquium, by Claus-Peter Stelzer (12), seeks to settle a simple question: why aren't we all clonal, given the evident costs of sex? The author argues that the classic costs of sex are highly variable, may have been overemphasized by theoretical approaches, and might be tightly linked to specific advantages conferred to given lineages. The so-called sex paradox (that sex is widespread despite its high evolutionary cost) may be only apparent.

Clonality in Multicellular Organisms

Plants display a great diversity of mechanisms for addressing sexual and asexual reproduction, as pointed out by Spencer Barrett (13). Barrett explores unisexual reproduction in plants and the concepts of genets (parental genotypes) and ramets (vegetative modules

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[1]To whom correspondence may be addressed. Email: Michel.Tibayrenc@ird.fr, javise@uci.edu, or fjayala@uci.edu.

Box 1. In the Light of Evolution

In 1973, Theodosius Dobzhansky penned a short commentary titled "Nothing in biology makes sense except in the light of evolution" (24). Most scientists agree that evolution provides the unifying framework for interpreting biological phenomena that otherwise can often seem unrelated and perhaps unintelligible. Given the central position of evolutionary thought in biology, it is sadly ironic that evolutionary perspectives outside the sciences have often been neglected, misunderstood, or purposefully misrepresented. Biodiversity—the genetic variety of life—is an exuberant product of the evolutionary past, a vast human-supportive resource (aesthetic, intellectual, and material) of the present, and a rich legacy to cherish and preserve for the future. Two challenges, as well as opportunities, for 21st century science are to gain deeper insights into the evolutionary processes that foster biotic diversity and to translate that understanding into workable solutions for the regional and global crises that biodiversity currently faces. A grasp of evolutionary principles and processes is important in other societal arenas as well, such as education, medicine, sociology, and other applied fields, including agriculture, pharmacology, and biotechnology. The ramifications of evolutionary thought extend into learned realms traditionally reserved for philosophy and religion. The central goal of the "In the Light of Evolution" series is to promote the evolutionary sciences through state-of-the-art colloquia and their published proceedings. Each installment will explore evolutionary perspectives on a particular biological topic that is scientifically intriguing but also has special relevance to contemporary societal issues or challenges. Individually and collectively, the "In the Light of Evolution" series aims to interpret phenomena in various areas of biology through the lens of evolution, address some of the most intellectually engaging as well as pragmatically important societal issues of our times, and foster a greater appreciation of evolutionary biology as a consolidating foundation for the life sciences.

The organizers and founding editors of this effort (J.C.A. and F.J.A.) are the academic grandson and son, respectively, of Theodosius Dobzhansky, to whose fond memory this "In the Light of Evolution" series is dedicated. May Dobzhansky's words and insights continue to inspire rational scientific inquiry into nature's marvelous operations.

produced by genets). As is the case for other organisms, the availability of highly discriminant genetic markers will help to understand better the functional interactions between sexual and clonal reproduction in phanerogams (plants that produce seeds). These interactions may happen to be antagonistic and thus lower the fitness of clonal plants.

John Avise (14) reviews the different forms of whole-animal vertebrate clonality, both in nature and in the laboratory. These forms include human cloning via nuclear transfer, as well as parthenogenesis, gynogenesis, kleptogenesis, hybridogenesis, polyembryony, selfing, and inbreeding. Understanding clonal reproduction in vertebrates makes it possible to better assess the ecological and evolutionary significance of sexuality. Avise analyses the impressive development of human experimental clonality, and then proceeds to a nuanced analysis of natural populations of clonal fishes, amphibians, reptiles, and mammals.

Carol Keefer (15) explores the growing field of artificial cloning in domestic animals. Embryo splitting can produce a few genetically identical animals, whereas nuclear transfer of donor nuclei into recipient oocytes could theoretically generate large numbers of cloned offspring. However, this theoretical potentiality is severely limited by technical difficulties. Importantly, somatic cell nuclear transfer research has advanced knowledge about epigenetic regulation during embryonic development and about how to reprogram cells (for example, to induce pluripotent stem cells). Artificial cloning of animals should be viewed as a valuable research tool.

Francisco Ayala (16) points out that cultural evolution now predominates over biological evolution in humans, although natural selection still persists. More than 2,000 human diseases have a genetic cause. Genetic therapy and healthcare may increase the incidence of hereditary diseases, although the rate of increase is very low over the generations. Germ-line genetic therapy could prevent this increase; however, it is presently not technically feasible. Some authors have proposed the cloning of eminent persons as a way to improve the genetic endowment of humankind. Ayala points out that it is possible to clone genomes but it is not possible to clone human individuals, for the obvious reason that a person's character is importantly determined by the individual's lifetime experiences, not only by the individual's genotype. However, therapeutic cloning could considerably augment the efficiency of organ transplantation and tissue healing in the future.

Clonality in the Microbial World

Transmissible diseases caused by pathogenic microorganisms are the main cause of disease and mortality in humankind, if one considers the whole world. The medical relevance of research on the evolution of clonal parasites is, therefore, considerable. Celia Perales, Elena Moreno and Esteban Domingo (17) consider clonality in virus evolution. Viruses possess efficient recombination machinery that allows them to generate new variants. In many instances, recombination is not indispensable for replication cycles, which leads to the propagation of viral lineages. Recombination produces new viral pathogens, which may provide a fitness advantage to certain viruses. The authors propose a model of continuous mutation and recombination. In this model, clonality is the standard mode of viral evolution, whereas recombination is considered nonessential.

Bacterial evolution has been a focus of research since the early 1980s. It is about bacteria that the "clone concept" was first elaborated at the dawn of molecular population genetics during the era of isoenzymes. Louis-Marie Bobay, Charles Traverse, and Howard Ochman (18) emphasize that limited recombination events in bacteria are very difficult to detect, even with large samples and advanced techniques, such as whole-genome sequencing. Their paper focuses on *Escherichia coli*, a model for bacterial population genetics for more than 40 y. Within this span of time, the clonality status of *E. coli* has evolved as different and more discriminant sequencing methods have been used.

John Taylor et al. (19) review current knowledge on recombination and clonality in fungi and yeasts. These organisms include several important pathogens, such as *Candida albicans*, *Aspergillus fumigatus*, and *Cryptococcus neoformans*. The authors criticize the traditional view that many fungi are exclusively clonal and that some of them have been so for hundreds of millions of years. Taylor et al. examine the relative impacts of clonality and recombination on several major species. According to the authors, there is need for a measure of the "clonality/sexuality" ratio, as well as for a more homogeneous terminology. The lack of a consistent language confuses population genetic interpretations.

The article by Michel Tibayrenc and Francisco Ayala (20) proposes a synthetic

view of pathogen population genetics, including viruses, bacteria, microparasites, and fungi. Clonality is defined as strongly restrained recombination on an evolutionary scale, with only occasional bouts of genetic recombination/hybridization. The main consequences of clonality on pathogen population structure are linkage disequilibrium (nonrandom association of genotypes at different loci) and stable genetic clustering ("near-clades"). These features are observed in many important species, including *E. coli*, *Trypanosoma cruzi* (the parasite responsible for Chagas disease), and *T. gondii*.

Clonality, Cancer, and Evolution

Andrii Rozhok and James DeGregori (21) seek to design a general evolutionary model of cancer. The paradigmatic multistage model of carcinogenesis by Nordling, Armitage, and Doll was a major progress, but many observations still do not fit it. As an example, the so-called "Peto's paradox" (larger mammals do not have more cancers than smaller ones, although they have much larger underlying stem cell pools), remains unexplained. Because fitness is highly environment-dependent, the impact of oncogenic mutations on somatic cells should vary with age and tissue microenvironment. Cancer age-dependence could be a result of systemic processes regulated above the cell level, altered by aging.

Irving Weissman (22) addresses stem cells as units of selection, in particular in the framework of cancer development. An example is taken from the model *Botryllus schlosseri* (a colonial tunicate). In this organism, germ-line stem cell predation is limited by a single locus, a highly polymorphic histocompatibility gene that has hundreds of alleles. In mice and humans, stem cells that generate other cells compete for niches in bone marrow. The process that leads from these stem cells to acute leukemia through multiple genetic and epigenetic events involves selection and competition among clones, which Weissman proposes as a general theme for cancer research.

Wendy Van Duren, Mark van Kleunen, and Marcel Dorken (23) consider the problem of interference between sexual fitness and clonality in sessile organisms. The movement of gametes within a given clone should reduce sexual fitness through mate limitation of male reproductive success and through self-fertilization, leading to high rates of inbreeding in the offspring. With the help of spatially explicit models, the authors show that clonal propagation increases sexual fitness when the genet expands outward. The main conclusion is that clonality often increases sexual fitness instead of interfering with it, whenever clonal offspring extend over a larger area than nonclonal phenotypes.

The papers that follow offer a fine sample of state-of-the-art research on clonal evolution. They should continue to give this subject the high importance it deserves in the framework of evolutionary studies, considering in particular the considerable relevance of clonality to research in cancer, transmissible diseases, and artificial cloning. This field of research has been until now highly compartmentalized. As a typical example, cancer specialists are often ignorant of research dealing with clonality in infectious agents, and cancer research is ignored by those investigating infectious diseases. Collaborative programs could contribute to develop fruitful multidisciplinary approaches with productive cross-fertilization.

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