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Multi-author Reviews

Ancient DNA

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Ancient DNA: an introduction

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The explosion of molecular technologies over the past two decades has fostered a revolution, both in the public and scientific perception, as to how genetics will impact on knowledge of ourselves and our environment. One of the more curious, but far from least interesting, avenues made accessible by this new technology is the molecular analysis of DNA from preserved organisms of the recent or distant past: ancient DNA studies. With its modest beginnings in the pre-PCR era of the early and middle 1980s, until the First International Congress on Ancient DNA, in Nottingham, England, in 1991, this emergent field had little visibility and proportionally little impact on important genetic questions. After 1991, however, with the realization that problems of sample authenticity could be resolved by careful quality control, the field has gained both in numbers of competent practitioners and in the variety of biological questions being approached. It seems timely, therefore, at this early point in the history of ancient DNA studies, to survey both the organismal range of preserved DNA currently being analyzed, as well as the breadth of biological questions being asked. This, therefore, is the primary focus of this assemblage of reports. As such, it is not intended to be an exhaustive survey of all work in the field, but rather should give the reader an appreciation for the variety of disciplines that are valid subjects for genetic analysis of ancient DNA.

Based on reports from the Second International Conference on Ancient DNA, held in Washington, D.C., in 1993, it appears that given suitable preservation conditions virtually any life-form containing DNA, even simple unicellular organisms and possibly viruses, are, at least in theory, targets for such studies. Given such a vast array of potential genetic analyses, it is not surprising that early efforts as presented here impact on virtually all subdisciplines of modern biology, including evolution, conservation biology, animal and plant biology, anthropology, and even human health, particularly as related to infectious diseases.

The first two reports describe key technical issues of ancient DNA analysis. Handt, Höss, Krings and Pääbo focus on problems common to all ancient DNA analyses which employ PCR technology. Issues of authenticity and artifacts impact so fundamentally because analysis relies almost totally on the PCR amplification of very small amounts of heavily damaged DNA. In fact, the avoidance of PCR artifacts and verification of sample authenticity are now an obligatory portion of any report involving ancient DNA. Interestingly, some PCR artifacts that clearly have the potential to confound analysis of modern DNA samples, for example jumping PCR, were initially recognized through analysis of ancient DNA. The methodology described here provides a solid foundation for both new and experienced workers, and will help them to avoid the many pitfalls frequently encountered. The contribution by Tuross, focusing on the biochemistry of DNA in ancient bone, is pivotal to all but a few ancient human studies and many animal studies, because preserved bone is by far the most predominant repository for ancient DNA. In addition, effective procedures for extracting DNA without substantial damage to bone surface features or overall morphology are described in detail and are essential for many anthropological studies where bone morphology cannot be sacrificed. Equally importantly, DNA polymerase inhibitors which co-isolate from virtually all ancient bone DNA are analyzed and techniques for its elimination or minimization described. It is hoped that these first two contributions will provide any newcomer to the field with a starting point, outlining the technologies and precautions and thus optimizing the chances of success with the particular ancient sample of interest.

The next group of four contributions cover a vast range of life-forms and is intended to give the reader a sense of the current and potential impact of ancient and preserved DNA on the fields of evolutionary and conservation biology. The initial contribution in this group by Poinar is the only one in this series that does not specifically analyze ancient DNA. Nevertheless, it may be the most significant, because it brings into perspective the extraordinary diversity of life-forms available in amber deposits. Initial successes at genetic analysis of insects in amber have fostered the very real possibility that quite ancient DNA from organisms as diverse as fungi and vertebrates – some preserved for over one hundred million years – will be possible. Also included is a discussion of many practical considerations when attempting to collect amber specimens. The chapter by DeSalle approaches the broad question of how genetic analysis of ancient samples will supplement current phylogenetic information. Clearly, if extremely old, potentially unique fossil species contain DNA, they could be extremely useful in resolving ambiguities in evolutionary relationships based currently on analysis of extant samples. Using both theoretical and experimental examples, DeSalle highlights the way in which fossil genetics will impact on such questions and, equally importantly, where they may not add significantly to our understanding. This is an important point when the evolutionary biologist considers destructive analysis of a rare or unique sample. Roy, Girman and Wayne discuss applications of much more recent sources of preserved DNA, those from museum collections. Their primary focus is on questions of conservation biology and how preserved DNA analysis can impact on the study of current groups of endangered species. Using four examples from their own recent work, estimates of genetic variability in declining populations and reconstruction of the hybrid origins of extant species are described. Both are critical to enlightened management of endangered groups of animals. As with the diversity of genetic samples potentially preserved in amber, the museum, as a repository for preserved DNA, is emphasized. Although museum collections of mammalian species are extensive, they are exceeded by the variety and numbers of avian specimens archived worldwide. Cooper provides one example from this resource by describing genetic analysis of a Pacific Island avian species containing both extinct and extant members in order to reconstruct this portion of an ecosystem at a time before significant impact by humans. The result suggests new relationships among New Zealand wrens and is a nice example of how ancient DNA studies can benefit from phylogenetic understanding as discussed earlier by DeSalle. Clearly the vast number and diversity of museum specimens from which DNA may be rescued, as implied by these four contributions, portends the significant influence ancient DNA analysis may have on evolutionary and conservation biology.

The next three contributions, although dealing with preserved animal and plant DNA, all have as one common focus their ability to reveal unique aspects of the behavior of ancient human populations. The first, by Hardy, Casane, Vigne, Callou, Dennebouy, Mounolou and Monnerot, focuses on the origin of modern European rabbits by analyzing DNA from Bronze Age bone. This work marks one of the first efforts to utilize ancient DNA for tracing the co-migration of prehistoric human populations and their associated agricultural stocks. Practical considerations are also discussed with regard to collection and extraction of DNA from animal bones at archeological sites. As such, the report should provide an initial guide for further analysis of co-migrations between man and animals, as well as providing an avenue for understanding the origin of domesticated animals. The next two studies focus on analysis of plant genetic material in association with archeological sites. Brown, Allaby, Brown, O'Donoghue and Sallares, describe analysis of wheat DNA from a first-millennium-B.C. Iron Age archeological site. Also presented is a new approach for establishing the ploidy of ancient wheat species. Ploidy determination is likely to be a key element in reconstructing the complex pattern of selective breeding which has resulted in the development of modern hexaploid wheats. It thus has an impact on questions relating directly to plant domestication as well as to man's effect on plant diversity. In contrast to such broad questions, Rollo, Asci, Antonini, Marota and Ubaldi, provide an example of the caution that must be exercised when examining archeological material for ancient DNA. Namely, the researcher must be aware of the possibility that ancient and modern microorganisms may be present on an otherwise uncontaminated relic, in this case grass clothing from the Tyrolean Ice Man. Eventual identification of the plant species associated with preserved humans should provide important information regarding habits and origins from samples of even just one individual.

The final two contributions to this review focus on analysis of human remains and highlight the type of genetic information that can be derived from both within-population as well as continental-wide analyses. Hauswirth, Dickel, Rowold and Hauswirth, describe genetic profiling of DNA from preserved brain tissue in a North American peat bog. Such analysis on multiple individuals from a single burial site, spanning more than fifty human generations, has allowed implications regarding the genetic continuity of ancient societies as well as the first estimates of rates of DNA evolution based on radiocarbon years. Additionally, the ability to estimate genetic diversity at medically important loci, such as HLA, suggests eventual relevance to understanding human disease origin and evolution. In contrast, Merriwether, Rothhammer and Ferell focus on questions of large-scale human migration into the New World by comparative analysis of modern and ancient

Native American Indians. The single geographic point of entry across the Bering land bridge makes the pattern of human colonization of the Western Hemisphere a potentially resolvable issue, and ancient DNA has already begun to play a substantial role. For example, the initial proposal that four mitochondrial haplotypes migrated into the New World from Asia is substantiated here by genetic analysis from widely separated North and South American archeological sites. Importantly, Merriwether et al. establish region-specific patterns of these four markers which should be critical in establishing more detailed migration patterns and their timing. Clearly, any theory of ancient human behavior based on genetic analysis of modern humans must survive a test of comparable genetic analysis on ancient populations. Thus, molecular archeology should have a fundamental impact on our understanding of our ancestral origins.

Although broad in the diversity of subjects covered, this review of the areas currently impacted by ancient DNA analysis should provide the reader with a concise overview of where opportunities lie to apply analysis of preserved DNA to current biological problems. It also provides a technical introduction to effective procedures for isolating DNA from a variety of materials of human and non-human origin and instruction on how to verify the authenticity of an ancient sample and minimize artifactual results. The genetic study of preserved biological tissue is clearly just beginning. However, fundamental techniques and criteria for scientific validity have already been largely established and many areas of biology in which such analysis will be useful have been identified. Nevertheless, the ultimate utility of ancient DNA studies remains to be fully established; its potential is fascinating and encompasses nearly the full range of modern biological disciplines.