Chapter 8
Archaeology of Human Pathogens: Palaeopathological Appraisal of Palaeoepidemiology

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Abstract The recent introduction of the new field of research of palaeomicrobiology has established new links between microbiological and archaeological sciences by using molecular techniques on archaeological material. However, although the material under study appears to be shared by both these fields, some of the methods, concepts, expectations and paradigms are not. The goal of this chapter is to present, from the bioanthropological and palaeopathological point of view, what ancient bones can tell us concerning the reconstruction of past infectious diseases from a palaeoepidemiological perspective.

8.1 Introduction: the Evolutionary Paradigm

The general framework of the history of human pathogens is inscribed into the evolutionary paradigm – scientifically introduced in its modern form by Charles Darwin in 1859 (Darwin 1859)[1]. This paradigm is necessary and sufficient to explain, in the field of human infection, phenomena such as the extinction of human diseases ('suette', *lues maligna praecox*, Spanish flu), the appearance of new ones (AIDS, Legionnaire's and Mad Cow diseases), and the re-emergence of others [tuberculosis (TB)]. Based on this strong paradigm, it has been possible to build models of co-evolution, clearly illustrated by the Reed Queen Theory (Van Valen 1973), that have been invaluable to the understanding of host–pathogen interactions (Combes 2001).

From this perspective, the possibility of accessing primary data (i.e. human remains) allows us to examine the history of human infections more directly, in order to better understand their present-day evolution over a longer time scale, and to reexamine the phenomenon of re-emergence in terms of its real evolutionary significance.

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[1]Surprisingly, despite its fundamental importance for all living species, this paradigm is still disputed (directly or insidiously) at the beginning of this new millennium.

D. Raoult and M. Drancourt (eds.), *Paleomicrobiology: Past Human Infections*.

Palaeopathology, palaeoradiology and palaeomicrobiology (Drancourt and Raoult 2005), including palaeoparasitology, thus play a key role in the retrospective diagnosis of infectious diseases in ancient human remains.[2]

8.2 From Epidemiology to Palaeoepidemiology

The tentative leap from retrospective diagnosis (by analogy in palaeopathology and palaeoradiology, or demonstratively in palaeomicrobiology) to global evaluation of pathological conditions in past populations is attempted by the field of palaeoepidemiology (for a general introduction, see Cohen and Crane-Kramer 2003). Palaeoepidemiology can be defined as the '*Use of epidemiologic methods to infer how certain diseases might have been distributed in ancient times; how, why and where they originated and, armed with this information, to predict possible futures of communicable and other diseases, possible trends in the emergence of new diseases, and reemergence of old ones. Evidence comes from contemporary accounts and from archaeological studies (evidence derived from bones, teeth, stomach contents)*' (MediLexicon 2007).

As this definition refers to methods of investigation in epidemiology, it is of interest to summarise these. After collecting data on a population (more often on a statistically representative sample of the population under study), modern descriptive epidemiology can calculate incidence and prevalence rates (Gerstman 2003). Prevalence measures the total number of cases of a disease in a given population; incidence corresponds to the rate of occurrence of new cases in this population. It should be noted that *Incidence* (i.e. number of new cases of a disease during a given time interval) is often used to mean *Incidence rate* (incidence divided by the number of people at risk, often expressed as the incidence per 1,000). Incidence can be called the 'absolute risk' (AR), and incidence rate the 'relative risk' (RR). Thus, incidence rate provides information about the risk of contracting the disease, whereas prevalence is a measure of how common the disease is (typically expressed as a percentage). It is also of interest to distinguish two types of prevalence: *point* and *period* prevalence. Point prevalence measures the proportion of people in a population who have a disease at a particular time; it represents a 'snapshot' of the disease in time. Period prevalence evaluates the proportion of people in a population who have the disease over a specific period of time (e.g. a season or a year). Period prevalence is distinct from incidence, because it concerns *all* affected individuals, (regardless of the date of contraction); whereas incidence concerns only those individuals who have *newly* contracted the disease during the same specified time interval.

Lifetime prevalence is the number of individuals (expressed as a percentage) in a statistical population that, compared to the total number of individuals, have

[2] Although generally not considered as palaeopathology or palaeomicrobiology research (probably because of its applied consequences) the 'resurrection of the Spanish flu virus' clearly falls into this category.

experienced the disease at some point in their life (up to the time of assessment). Lifetime morbidity risk is the theoretical prevalence of a disease at any point in life for anyone, regardless of time of assessment.

Duration of a disease influences both prevalence and incidence: a disease with a long duration may have a high prevalence but a low incidence rate; a disease with a short duration (but easily transmitted) may have a low prevalence but a high incidence rate. In other words, prevalence is a useful criterion for evaluation of long-lasting diseases, but incidence is a more relevant parameter when discussing diseases of short duration.

These parameters are well defined and are commonly used in clinical epidemiology; the diachronic approach opens fundamental new perspectives on our knowledge of the evolution of disease. Thus epidemiology can inform palaeoepidemiology. However, some clouds obscure the blue serenity of this sky. Measuring disease frequency in the past is far from the relatively straightforward procedure it is in present populations (Waldron 1994; Dutour et al. 1998, 2003). The main reason is that past populations are represented mainly by skeletal populations, and these do not properly represent past populations as they existed when alive. In fact, skeletal series are the worst type of sample for an epidemiologist. Paradoxically, it could be stated that palaeoepidemiology has very little to do with the epidemiology of past populations as it concerns the epidemiology of skeletal samples only.

It is obvious that when describing disease frequency in palaeopathology, some of the measures, such as incidence rate, commonly used by modern epidemiologists are impossible to attempt. Appropriate rates that can be used in palaeoepidemiology include prevalence, period prevalence, proportional morbidity rates, and age-specific prevalence rates (Waldron 1994). More recently, Boldsen (2001) defined 'point prevalence at death', and suggested that this rate could be obtained from a formula using sensitivity and specificity rates.

Considering prevalence, we can examine how the numerator and denominator differ in palaeoepidemiology from the modern situation, in order to establish, if possible, more relevant comparisons of past and present infectious conditions. What we must appreciate is what the ratio n/N (in which 'n' is the number of palaeopathological cases of a given disease observed in ancient human remains, and 'N' the number of individuals constituting the archeological population sample under study) really represents.

The 'n' question refers to palaeopathological diagnosis, and the 'N' question to the nature of the sample (represented by a collection of human remains).

8.3 Palaeopathological Diagnosis: the '*n*' Question

According to Brothwell (1961), in palaeopathology '…diagnosis is by far the greatest problem'. This is due to the specificities of the subject, including (1) the retrospective diagnosis, (2) the use of modern diagnosis criteria, (3) the scarcity of pathognomonic lesions, and (4) the incomplete nature of ancient material. In a

concept introduced by some physicians such as William Osler, in modern medical practice the patient is a collective of signs and symptoms to be characterised and analysed algorithmically in order to reach a diagnosis. This process of identifying a pathological condition is based on a set of diagnostic criteria, including a spectrum of various types of information and observations as well as the results of different investigations. This includes anamnesis, complete examination and complementary analyses (medical imaging, lab tests, etc.). In modern medicine, diagnosis may be achieved using analogical (e.g. association of symptoms such as sub-acute or chronic asthaenia, vesper fever, weight loss, and radiological thoracic opacity could indicate several diseases, among them tuberculosis) and/or demonstrative (e.g. PCR analyses demonstrating the presence of *Mycobacterium tuberculosis*) procedures. In palaeopathology, retrospective diagnosis is silent (no anamnesis, no medical history), static (no evolution of signs and symptoms) and limited (mainly to skeletal expression). Indeed, mummified tissues are exceptional – the majority of ancient human remains are represented only by bones and teeth. This means that many diseases are under-represented or completely lacking because they leave no, or only minimal, imprints on bone, and many diseases that do affect bones may be confused with each other as they do so in a similar manner. In addition, many diseases can cause death before enough time has elapsed for bone to be affected (Ubelaker 1998).

Natural processes (physical, chemical, and biological) – so-called taphonomic processes (Mays 1992) – acting upon ancient skeletal remains will interfere with the '*n*' question of diagnosis in two ways: firstly the preservation state of the skeletal material, which can be fragmentary, incomplete or intermingled, will influence the quality of observations; secondly, taphonomic alterations can mimic disease conditions and induce interpretation errors (so-called pseudopathology), sometimes even for experienced palaeopathologists.

Two other comments should be added to complete the picture of the '*n*' question for palaeopathological diagnosis. The first is the 'attraction force' of the typical form of a disease; the second is 'forgotten diagnoses'. Palaeopathology has, as did medicine in early modern times, focussed its interest on 'typical' cases. Much as biological anthropology did in the first half of the twentieth century, by studying individual 'type' rather than population variability, palaeopathology at that time was interested mainly in 'casuistic reasoning' rather than in the actual 'health status' of past populations. From a certain point of view, to base a diagnosis on only the typical expression of a disease is reasonable. On other hand, as we know from clinical experience, diseases are rarely, if ever, represented by a single typical symptom, but rather by a set of major and minor signs; thus the scoring of only 'pathognomonic changes' will underevaluate the past prevalence of a given disease. If we consider the theoretical '*n*' as the sum of pathognomonic changes (n_0) and other minor symptoms representing various clinical expression of the same disease (n_1+n_2+n_3….), assessing only the n_0/N rate will clearly minimise the real presence of the disease in the past population under study. The n_0/N rate should be interpreted as the 'minimal' prevalence of the disease. The practical example of tuberculosis clearly illustrates this point. From the palaeopathological point of view,

only the typical skeletal changes of Pott's disease are reliable for retrospective TB diagnosis, with precise diagnostic criteria: involvement of one to four vertebrae in the same area, destructive lesions, vertebral collapse producing angular kyphosis, posterior involvement uncommon, and anterior concavity of several adjacent vertebrae corresponding to the presence of a cold abscess (Aufderheide and Rodriguez-Martin 1998; Ortner 2003). However, other extraspinal skeletal involvements due to TB are also frequent (osteoarthritis of joints, especially hip and knee; osteomyelitis of long and short bones, especially femora, tibia, and foot bones) and may represent the only skeletal lesions attributable to a tuberculous infection in a palaeopathological specimen. Moreover, some minor palaeopathological changes, such as rib internal lesions (Santos and Roberts 2006) or endocranial serpiginous lesions (Hershkovitz et al. 2002; Schultz 1999) have recently been correlated with TB infection, as confirmed by using palaeomicrobiological techniques (Maczel et al. 2005). Taking into account all of the less typical changes associated with skeletal TB (extra-spinal and minor changes) will strongly modify estimates of TB prevalence. For example, tuberculosis changes were scored on 1,294 Hungarian skeletons from the medieval–modern period: the prevalence of tuberculosis estimated using only typical changes is about 0.2%; this rises to 3.8% (about 20 times more) if all skeletal expressions of TB are considered (Maczel, 2003).

The second comment concerns 'forgotten diagnoses'. In 1888, the French physician Victor Ménard published a book in which he summarised the courses given by Professor Lannelongue at the Faculty of Medicine in Paris (Ménard 1888). In his book, he pointed out that the term 'vertebral tuberculosis' refers not only to the 'classic' form known as Pott's disease[3], where the typical vertebral collapse can be seen, but should also include other manifestations: superficial 'carious' lesions or 'superficial vertebral tuberculous osteoperiostitis' (Fig. 8.1). He distinguished the two anatomical forms of vertebral tuberculosis (classical Pott's and superficial vertebral lesions) by the fact that they might appear separately. He pointed out that superficial vertebral 'caries' are frequently associated with visceral lesions; vertebral lesions are characterised by the lack of reparation, and the affected individuals frequently die of TB. The extension of these superficial lesions, appearing as small excavations on the anterior surface and lateral sides of vertebrae, is often considerable (they generally affect 5–6 to 12 vertebrae). The denuded surface shows variable aspects: sometimes it is smooth and plain, but generally it is rough, irregular, mined by small sinuous excavations, covered at the sides by newly formed bone layers, and infiltrated by 'fungosity'.

[3] It should be noted that Sir Percival Pott described, in 1779, 'morbific alterations' of vertebrae of unclear origin, and that, in 1816, Jacques-Mathieu Delpech proposed that these lesions be called 'tuberculous infection of vertebrae', pointing out the fact that this was the first time that this disease had been assigned a characteristic name: *L'état de la science sur ce point est tel qu'il convient aujourd'hui d'appeler cette maladie infection tuberculeuse des vertèbres, et ce sera la première fois qu'elle aura reçu une dénomination caractéristique* (J.M. Delpech 1816)

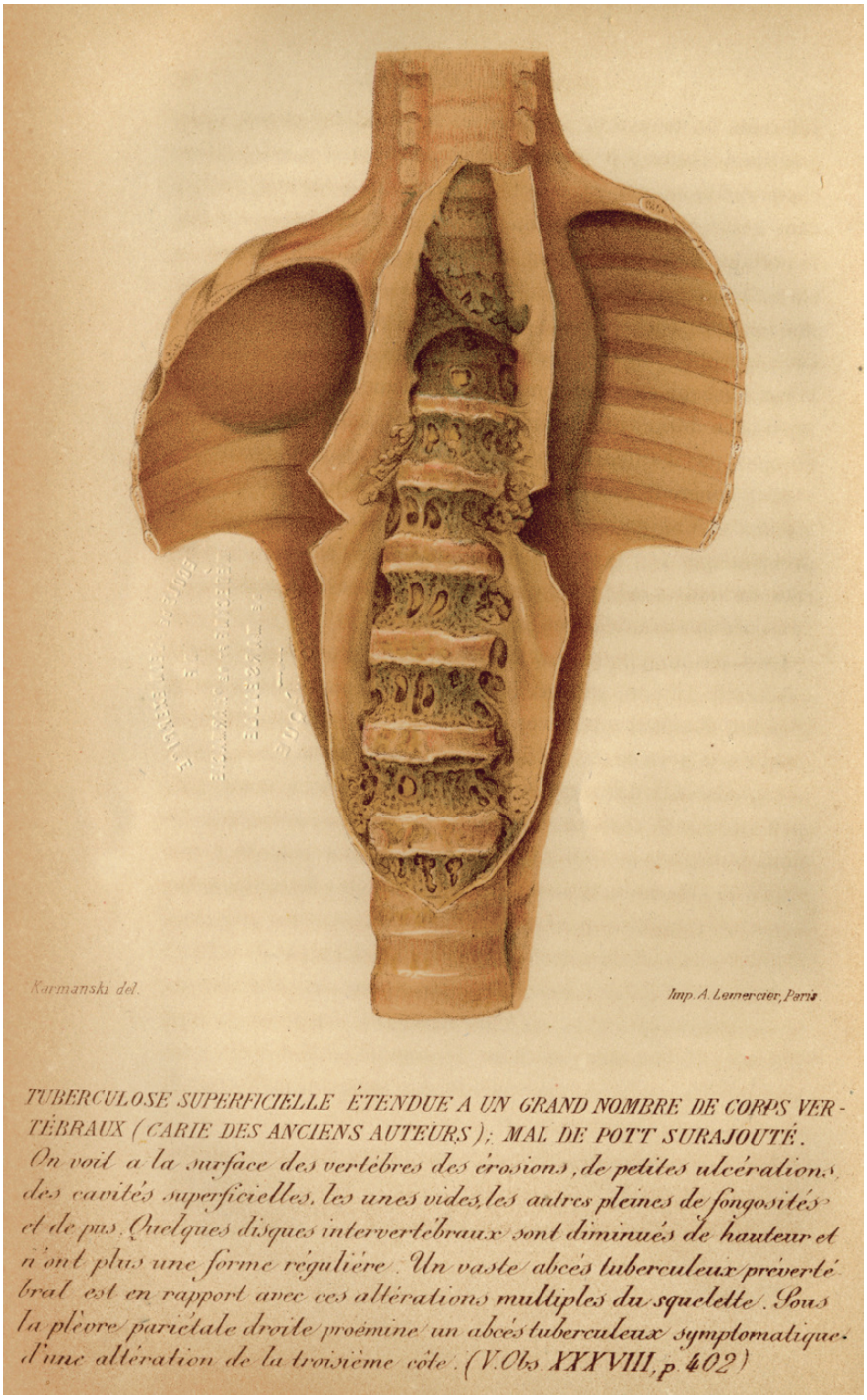

Fig. 8.1 Anatomical lesions described by Lannelongue as "superficial vertebral tuberculous osteoperiotitis" (Ménard 1888). Reproduced courtesy of the Library of the University of la Mediterranée, Collection of Ancient Medical Books, Faculty of Medicine of Marseille

The infection is suggested to progress along the blood vessels entering the vertebra, represented by the enlargement of vascular channels (see details in Maczel 2003). This description has totally disappeared from modern literature on skeletal tuberculosis – it was last mentioned by Sorrel and Sorrel-Dejerine (1932). This could mean either that this clinical expression of TB no longer exists in modern populations or that, because of its scarcity or difficulties in observing such features by medical imaging, it is ignored by modern clinicians. The re-discovery of these vertebral lesions by palaeopathologists is quite recent. Baker (1999) suggested that the 'smooth walled resorptive lesions/severe circumferential pitting', observed in some vertebral columns of four osteoarcheological series, might be of tubercular origin. Her hypothesis, ignoring Ménard's description, was based on the co-occurrence of these vertebral changes with other pathological conditions indicating TB. Among osteological collections with known cause of death, a frequent association has been found between these vertebral lesions and tuberculosis, especially in younger age groups (Pàlfi et al. 2000[4]; Ortner 2003). Haas et al. (2000) were the first to use molecular techniques to establish the relationship between these superficial vertebral alterations and tuberculosis.

Such 'forgotten diagnoses' should remind us that (1) old clinical descriptions are interesting, (2) the natural expression of infectious diseases is strongly influenced by our modern preventive and curative arsenal, and (3) modern clinical diagnostic criteria are, consequently, not the most appropriate way to establish diagnoses of infectious diseases in old bones.

In order that, as put by Waldron (1994), the attempt to establish retrospective diagnosis in palaeopathology will not become as difficult as 'trying to navigate through a minefield with the aid of the sun and a Mickey Mouse watch', we would do well to bear these points in mind.

8.4 The Nature of the Sample: the '*N*' Question:

A skeletal 'population' is in fact a sub-sample of several other samples. Of course, the sampling is not randomised. The main extrinsic factors contributing to the constitution of osteoarchaeological series are (1) burial assemblage (influenced by cultural practices), (2) duration (time of constitution of the sample, sometimes extending over several centuries), (3) taphonomic processes (chemical or biological), and (4) condition of the archaeological excavation.

[4] Palaeopathology of tuberculosis. Contribution to the knowledge of the evolution of the disease. Oral presentation by Pálfi Gy, Dutour O, Ortner DJ given in Budapest at the 1st European Region Conference of the International Union Against Tuberculosis and Lung Disease, 12–15 April 2000.

8.4.1 Burial Assemblage

Burial assemblage, which can influence the sample structure (Sellier 1996) and consequently the reconstruction of prevalence of diseases, depends on cultural practice. For example, some ancient civilisations buried their children separately (Watts 1989; Blaizot et al. 2003). If the disease under palaeoepidemiological study presents age-specific prevalence rates (which is, for example, the case for tuberculosis), it will be of interest to determine if the youngest individuals are missing or under-represented in the skeletal series because of specific burial practices.

In other cases, burial practices concern gender selection (e.g. monastic cemeteries), where the skeletal material obviously displays a very specific age and sex distribution (for instance mainly old men), inducing an over- or under-estimation of age-/sex-specific prevalence rates of some diseases (Waldron 1985). Thus, the burial assemblage must be precisely known in order to define the skeletal sample; the ideal sample is a non-selected population.

8.4.2 Time Effect

As Waldron suggested, period prevalence seems to be the most adapted rate in palaeoepidemiology – the period frequently being a very long one (Waldron 1994).

Large skeletal samples frequently come from excavations of a hypogea, necropolis, or cemetery that was in use over several centuries. In such cases, the skeletal population is the sum of the dead portions of the successive living populations. Although it is usually difficult to date the burials archaeologically, precisely separating the chronological sub-samples is easier (Boldsen 2001), and the 'population' is defined more by the burial place than by its chronological range. It is unlikely that a population would have remained static in structure and origin over a period of several centuries (except in the uncommon case of a genetically isolated community with a stable economic status); however, this bias of heterogeneity remains outwith our control (Wood et al. 1992).

The reconstructed prevalence of diseases, which can be single or recurrent events, chronic or acute, will generally be minimised, tending to a mean prevalence for the total period, especially for acute or sporadic phenomena (Dutour et al. 2003). Even if the prevalence cannot really be predicted, it must be taken into consideration when studying the epidemiology of skeletal series. The shorter the period involved in the constitution of the skeletal sample, the better the sample for palaeoepidemiology.

8.4.3 Taphonomy

The effect of taphonomy on palaeoepidemiology is twofold. On a general level, a poor state of preservation of a skeletal sample will reduce its interest for

palaeoepidemiology. Preservation indices (Dutour 1989; Bello 2000) can quantify the overall preservation state of a skeletal series and provide information on the intensity of taphonomic processes. Clearly, the number of individuals alone is insufficient to prescribe the material available for palaeoepidemiological studies, and each skeletal population presents its own general preservation profile. On a more detailed level, differences in preservation can occur within the same skeletal population, depending on gender or age – female and juvenile skeletons seem to be frailer and tend to be destroyed more often than the male and adult skeletons (Masset 1973; Dutour 1989; Bello et al. 2006).

The palaeodemographic structure of the osteoarchaeological series needs to be known in palaeoepidemiology, especially when studying the prevalence of diseases having a gender- or age-specific prevalence rate, as is the case for some infectious diseases.

Preservation also depends on anatomical localisation; some parts of the skeleton (hand and foot, ribs, spine) are more delicate and, consequently, more often missing than other parts (Fig. 8.2). This differential preservation must be compared with the skeletal distribution of the diseases studied, taking into account the preferential localisation of a given infectious disease. For example, as the osseous involvement of TB frequently concerns the spine and extremities, it is of interest to know something about the preservation of these skeletal elements in the series. For leprosy, information about the preservation state of the facial skeleton (especially the nasal aperture and palate areas) and hand and foot bones is necessary to evaluate the material on which the calculation of prevalence was made. For treponematosis, although preferentially localised to a more robust part of the skeleton, the evaluation of prevalence must take into account the preservation state of tibiae and skulls.

8.4.4 Crude and Corrected Prevalences

Few methods that take taphonomy into account in calculating past prevalences have been developed. In most studies, estimations correspond to the ratio of the number of cases / total number of skeletons – termed "crude prevalence" by Waldron (1994). This measure of prevalence does not really consider the preservation state of the osseous remains. As mentioned above, each skeletal collection has its own preservation profile, and this crude method thus weakens the validity of comparative work. If we consider a theoretical skeletal collection of 200 individuals to evaluate the prevalence of tuberculosis, the identification of three cases of Pott's disease in this sample gives a prevalence rate of 1.5%. Taking into consideration the fact that 50 spines are almost totally missing, and that 50 others are too incomplete or fragmentary to yield valuable observations, the prevalence corresponds in fact to three observations on 100 spines, i.e. 3%. We recommend a correction of the prevalence using the formula: corrected prevalence, $C_rP = n/N–a$ (where a is the number of bones affected by disease that are *not* observable). We call this method correction by representation. Reducing the denominator increases the prevalence rate. Waldron suggests that prevalence in the missing parts can be assumed to be proportional to that

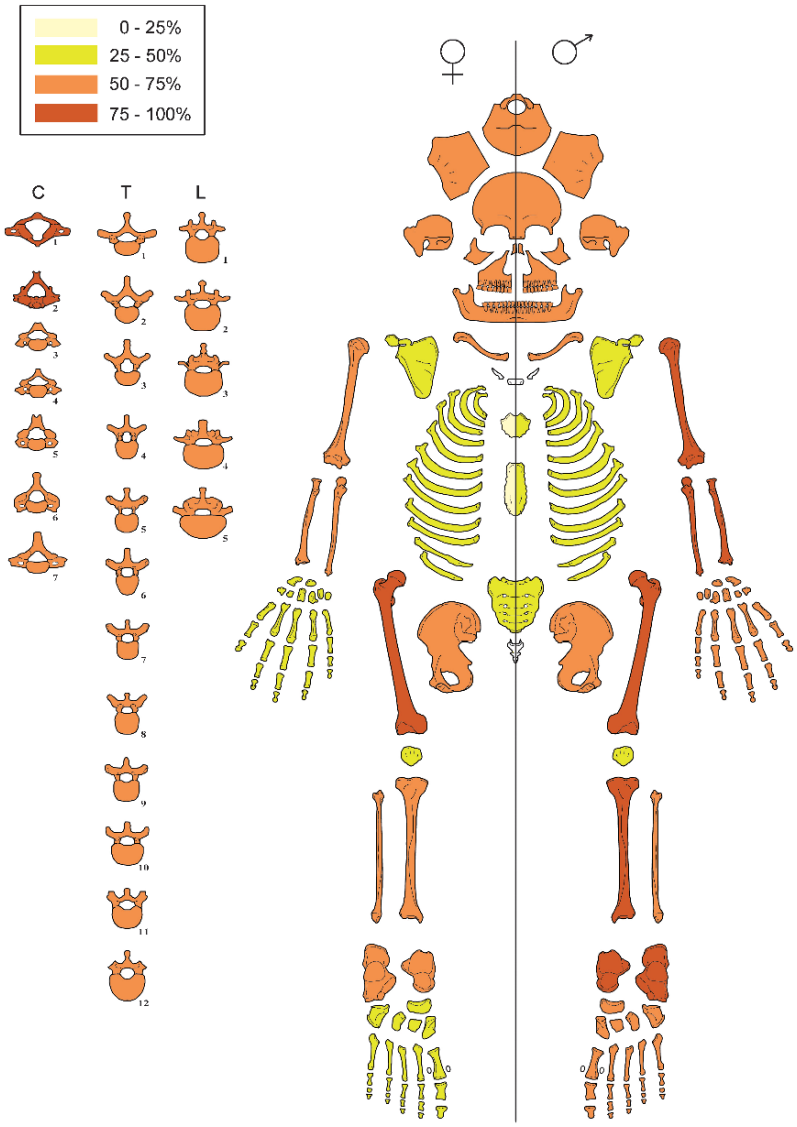

Fig. 8.2 Variation of skeletal preservation in function of the localisation and gender in a given skeletal collection (Bello 2000; Bello et al. 2006)

in the preserved parts, which validates C_rP for the totality of the sample. He also proposes considering the crude prevalence n/N (including *a*) as a minimal rate (*none* of the missing spines was affected) and a maximal prevalence rate of n+a/N (*all* of the missing spines were affected), the true rate lying somewhere in between. We propose another possibility to re-evaluate the crude ratio, which is to counterbalance the crude prevalence by a factor, *F*. The idea is to take into account the number of observable skeletal elements (vertebrae in this case), which are essential for the

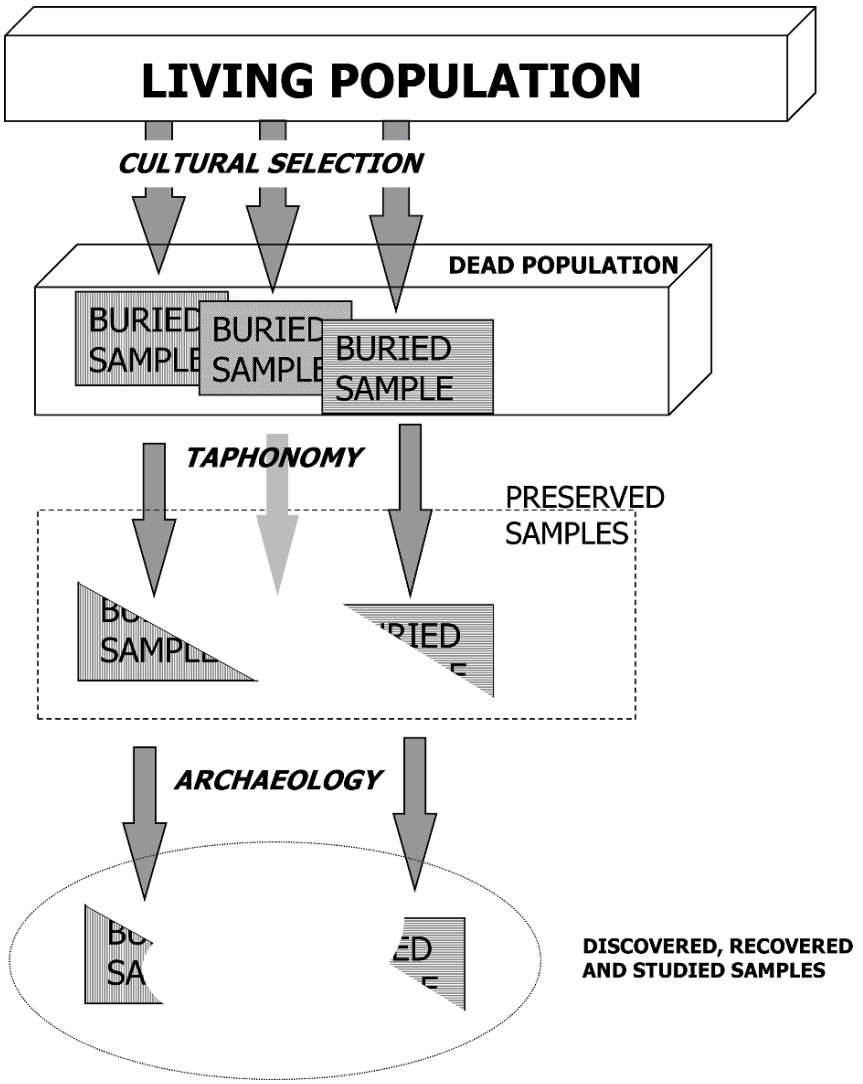

Fig. 8.3 Scheme of the three steps (cultural, taphonomic, archaeological) going from an ancient living population to its remains

diagnosis of the disease – TB in our example (Dutour et al. 2003). The ratio of the theoretical effective number / observable effective number represents the factor *F*. To take a real example, on the Hungarian collection from Balcsamas dating from the seventeenth century, Maczel (2003) found evidence of 15 cases of vertebral TB, giving a crude prevalence rate of 14%. However, among the 2,675 possible vertebrae (theoretical number), only 2,408 were represented. Thus, our counterbalance factor *F* is equal to 1.11. The counterbalanced prevalence is thus 15.5%.

The purpose of the above is to provide a method to calculate prevalence adapted to each sample that will be valid for comparative studies. However, according to

Bello et al. (2006) reliable results can be obtained if one compares prevalence in series showing a similar preservation pattern.

8.4.5 Archaeology and Related Studies

The constitution of a skeletal series depends mainly on archaeology (Fig. 8.3). Frequently, only part of a cemetery has been discovered, and/or excavations may have been carried out on no more than a segment of the unearthed part of the cemetery. The recovery may then concern only part of the excavated area, or only parts of the skeletons (e.g. skull and long bones, which were, until recently, considered the most informative elements for anthropologists). Anthropological study following excavation may be limited (e.g. to sex and age distribution only), and the storage of these skeletal series can make them difficult to study in their totality by palaeoepidemiologists. Hence we see the implications of other parameters of sample selection. The ideal case would be the excavation of a site in its totality, without any selection in the recovery of the osteological sample, with appropriate storage, contributing to open skeletal libraries.

We can thus appreciate the challenge more clearly: palaeoepidemiology mainly concerns the study of diseases in different skeletal samples, the latter having been to a greater or lesser degree selected from past populations by different factors in quite variable and unknown proportions.

Another important point concerning the sample structure, called intrinsic factor by Waldron (1994), in the characterisation of the nature of the sample should be highlighted.

8.5 Intrinsic Factor: Structure of the Sample

Theoretically, human skeletal remains are representative of a variable part of a dead population, which itself derives from the living population. This constitutes a major distinction from epidemiology, as palaeoepidemiologists study diseases in a community of the dead. According to Waldron (1994) ‘it is surprising how often this fact is overlooked’.

The dead population differs in sex and age distribution from the living population. In less developed parts of the world – which we presume more closely resemble the past than more developed societies – the demographic structure of the mortality curve is the reverse of that for the living population. Over 40% of the living population is 15 years old or younger. The mortality curve, on the other hand, shows high mortality of the 0- to 5-year-old cohort, relative stability between the ages of 5 and 35, a progressive increase to age 55, and a dramatic increase after age 55, i.e. a typical U-shaped profile (Fig. 8.4).

Ideally, the entire dead part of a given population would be preserved in a single cemetery, enabling us to reconstruct the structure of the living population from the age distribution of the skeletal population. However, the relationships between the

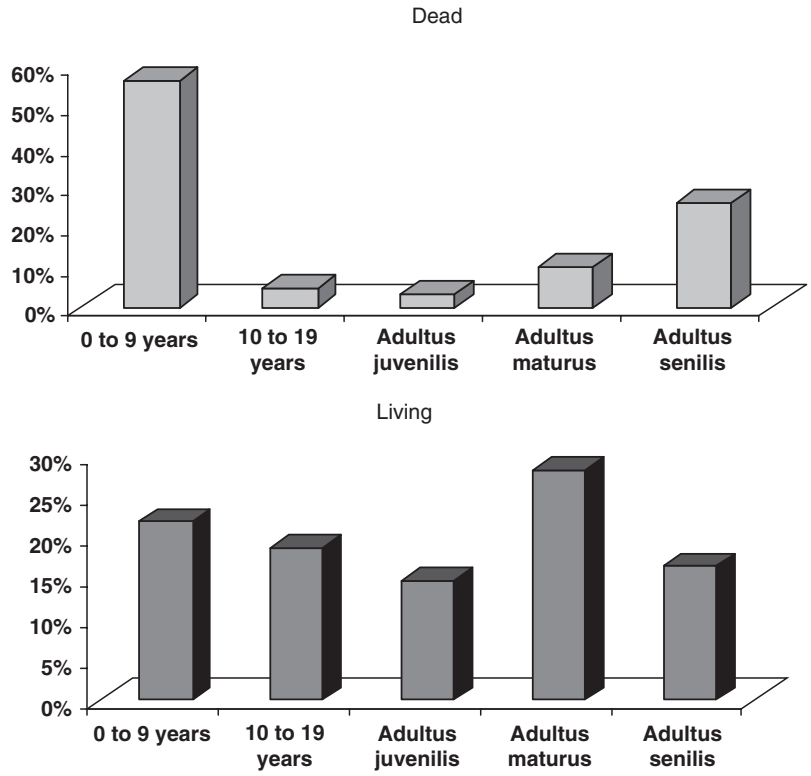

Fig. 8.4 Distribution of age categories for mortality and living profiles (pooled data from several historical pre-Jennerian populations and from the present populations of several undeveloped countries)

two curves depend on other parameters as well. An improved economy, for example, will modify the demographic pattern, making it older and reducing the mortality of its youngest members. Although the application of the demographic pattern of an undeveloped society to past populations is probably adequate in many cases, we must nonetheless consider other patterns, especially those in developing countries.

The ideal situation in palaeoepidemiology is encountered when the dead population has the same structure as the living one, for example when a non-selected part of the population (or the population in its entirety) suddenly disappears. This is what we called the 'Pompeii model' (Dutour et al. 1998, 2003). Working on other types of material corresponding to massive death occurring over a short time period, with no biological or cultural selection, constituted by skeletal series coming from plague epidemics, we assumed (Dutour et al. 1994) and subsequently demonstrated (Dutour et al. 1998, 2003) that such samples correspond to the criteria of the 'Pompeii model' and are very well suited to palaeoepidemiological analyses. Indeed, any peculiarities exhibited can minimise or even cancel out some of the common extrinsic or intrinsic biases observed in skeletal collections. Thus, such

series can provide a more accurate picture of the palaeoepidemiological situation of some diseases than can be observed in more common types of material. The palaeoepidemiology of tuberculosis can be considered as an example.

8.6 Palaeoepidemiology of Tuberculosis

Tuberculosis is a good example of a re-emerging disease. Its prevalence is once more on the rise, and recent statistics place its mortality rate higher than that of AIDS. TB might become a major problem, especially if we take into account antibiotic-resistant germs, which are on the increase, and its future may very well be similar to its past. What we know of its past is limited mainly to mortality records of the nineteenth and early twentieth centuries. The classical data are that TB infection increased in the nineteenth century, its spread facilitated by urbanisation and overcrowding. In late nineteenth century France, the mortality rate from phthisis was between 3.08 and 3.69 per 1,000 (Bello et al. 1999); during the same period in Germany, mortality from TB was 2.6 per 1,000 (Alfer 1892, quoted in Ortner 2003). Our knowledge of the situation prior to this period is very poor, being limited to some rare historical records of mortality, such as the London Bills of Mortality beginning in the seventeenth century, which indicated that death by "consumption" (pulmonary tuberculosis or primary lung infection) accounted for 20% of all deaths during non-plague years (Clarkson 1975). The accuracy of diagnosis in the seventeenth century, however, was poor. A more reliable gauge is skeletal populations.

Since there is considerable uncertainty concerning the assignation of TB as the causative agent of the macro-morphological bone changes on which detection of TB infection has been mainly based in osteoarchaeological material (Waldron 1999), attention has focussed on the molecular level in search of a more reliable diagnosis and, consequently, more reliable disease frequencies in past populations. As a consequence, molecular biological techniques developed during the last decade have greatly broadened the diagnostic horizon in palaeopathology, not only by confirming the macroscopical diagnosis as a result of providing direct, demonstrative proof of tuberculous infection, but also by helping to identify new criteria for differential diagnosis.

Morphological techniques often do not allow the recognition of TB lesions, and the more specific identification of the disease agents is even more difficult, since human- and bovine-hosted TB, the two main human-affecting members of the *Mycobacterium tuberculosis* complex (MTC), produce anatomically similar bone changes (Ortner 1999). However, despite the fact that members of the MTC share many common characteristics, they, as well as other *Mycobacteria*, can be differentiated on the biomolecular level. The biomolecular analysis of archaeological human remains for TB has proved to be efficient. Such studies have been conducted in mummies (Salo et al. 1994; Nerlich et al. 1997; Crubézy et al. 1998; Pap et al. 1999; Zink et al. 2001), bone remains (Spigelman and Lemma 1993; Baron et al. 1996; Taylor et al. 1996, 1999; Fearman et al. 1999; Dutour et al. 1999; Haas et al. 2000) and even in calcified tissues (Donoghue et al. 1998; Pálfi et al. 1999), proving that fragments of ancient mycobacterial DNA can survive for

long periods, probably due to their tough cell wall, and can provide direct evidence of TB infection. Such studies furnished evidence, from distinct genetic loci, for the presence of DNA fragments from Mycobacteria (65 kDa antigen gene) and more specifically from organisms belonging to the MTC (IS6110, rpoB) (Taylor et al. 1999; Haas et al. 2000; Mays et al. 2001). With the help of such biomolecular analyses, more reliable diagnosis of both typical and atypical morphological alterations can be developed, thus determining new diagnostic criteria involving more minor changes such as vertebral hypervascularisation (Ménard 1888; Baker 1999), rib periostitis (Kelley and Micozzi 1984; Roberts et al. 1994), and endocranial changes (Schultz 1999; Hershkovitz et al. 2002). An important source of tuberculous alterations can be found in anatomical collections where the cause of death is recorded. The search for new diagnostic criteria was extended to the United States [the Hamann-Todd (Kelley and Micozzi 1984) and Terry Collections (Roberts et al. 1994)] as well as to Portugal, where *Mycobacterium tuberculosis* infection in the Coimbra Identified Skeletal Collection was confirmed by the use of biomarkers (Santos and Roberts 2001).

However, mycobacterial DNA can be detected even in bones without morphological changes (Fearman et al. 1999; Zink et al. 2001). This point leads to the question of infection versus exposure to infection, which is especially relevant in molecular palaeoepidemiology. The question of the meaning of negative or positive molecular results is still broadly open from a palaeoepidemiological point of view: a negative result can signify either lack of infection or a molecular taphonomical problem; a positive result, with the exception of contamination, is not necessarily related to disease, as it can also provide testimony of exposure to the infection.

The prevalence of skeletal lesions can thus help evaluate the frequency of the disease versus exposure to the disease; the latter is thought to be very high, even generalised, if TB infection was present in a given ancient population.

We attempted to estimate the minimal prevalence of TB on plague skeletal material and other material studied. As mentioned above, we encountered several methodological problems:

1. Reconstruction of global TB prevalence from skeletal lesions depends on the frequency of TB skeletal involvement over the total number of TB infections. According to the literature (Nathanson and Cohen 1941; Lafond 1958; Kelley and Micozzi 1984; Davies et al. 1984; Aufderheide and Rodriguez-Martin 1998; Ortner 2003), this varies from 3% to 9%. Hence, to avoid overestimation, we can assume a minimal prevalence from the minimal frequency of skeletal involvement.
2. TB skeletal infection mainly affects the youngest part of the population (Sorrel and Sorrel-Dejerine 1932), with more than 60% of all victims being under the age of 20. Poor preservation or absence of the youngest individuals in an osteoarchaeological series will underestimate the reconstructed frequency of skeletal TB.
3. Bone repartition of TB involvement mainly affects the spine (25–50% of skeletal TB cases; Steinbock 1976) and the extremities. These parts of the skeleton are, unfortunately, often poorly preserved.
4. A palaeopathological diagnosis is established on morphological (osteological and radiological) criteria, defined by comparison of clinical, radiological, and pathological records (Sorrel and Sorrel-Dejerine 1932). The significant TB

prevalence in the past, as evidenced by documentary sources (Cronje 1984) contrasts with the paucity of palaeopathological evidence (Stirland and Waldron 1990). It has been suggested that the usual palaeopathological diagnostic criteria for skeletal tuberculosis are inadequate (Roberts et al. 1994). Biomolecular analysis of *Mycobacterium tuberculosis* DNA in presumed palaeopathological cases may confirm the diagnosis (Spigelman and Lemma 1993; Baron et al. 1996; Taylor et al. 1996, Dutour et al. 1999, Pàlfi et al. 1999, Salo et al. 1994, Taylor et al. 1996, Nerlich et al. 1997, Crubézy et al. 1998, Haas et al. 2000, Zink et al. 2001, Mays et al. 2001).

If we take a rough look at some large osteoarchaeological collections (numbering a total of 5,848 skeletons) from Hungary, dating from the seventh to the seventeenth centuries, a reconstruction from skeletal lesions of the minimal prevalence of TB infection in the population showed variations depending on chronology (Pàlfi and Marcsik 1999): between the seventh and eighth centuries (the Avar era), it represents 23% (crude prevalence rate: 0.7); during the tenth century Hungarian conquest, 0% (but some cases of leprosy have been described; Pàlfi 1991); between the eleventh and thirteenth centuries, 8.6% (crude prevalence rate: 0.26); and for the period from the fourteenth to the seventeenth centuries, 31% (crude prevalence rate: 0.95). This osteoarchaeological material does not provide us with the desired criteria for palaeoepidemiology, i.e. short periods of time, absence of selection, Pompeii-like palaeodemographic structure.

On our plague material, for one of our series (L'Observance), diagnosis was established both morphologically and molecularly on 3 individuals out of the 179 that can be observed (crude prevalence rate: 1.67%). These three samples also gave positive

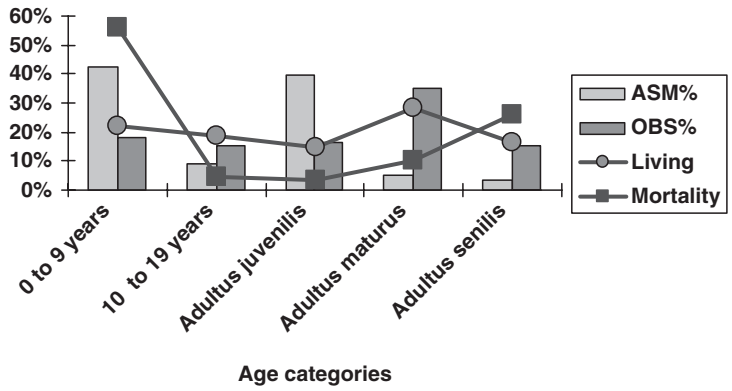

Fig. 8.5 Differences in sample structure. A sample from a plague mass grave (OBS) dating from the eighteenth century is much more similar to the age category distribution of a contemporaneous living population (historical demographic data). Sample ASM (slave cemetery, from the same period) exhibits clear differences in its structure, even in comparison with a mortality profile (same data): over-representation of young adults is obvious. This difference in structure is sufficient to explain the results obtained by reconstructing minimal prevalences for tuberculosis in these two samples, as well as the poorly realistic result for ASM (55% and over 100%, respectively)

molecular results (Zink et al. 2001); other 'control' samples with no lesions remained negative. In the same manner, if we base our estimation on 3% of skeletal involvement, the minimal prevalence of TB infection in the population in 1722 was about 55%. This prevalence seems to be very high for eighteenth century material; however, we should bear in mind the frequency of tubercular infection observed in undeveloped countries 40 years ago, e.g. 37% in Phnom-Penh in 1966 (Nguyen 1988).

Moreover, a study of a contemporaneous eighteenth–nineteenth century slave cemetery in the French West Indies (Courtaud et al. 2005) revealed six cases of vertebral tuberculosis on 148 preserved spines (crude prevalence: 4%). The minimal prevalence of TB is over 100%, suggesting a generalised TB infection in this population of slaves or, more likely, an effect of sample structure in the cemetery population, due to the addition of successive dead parts during a period with high TB prevalence (over about one century) in the living population of slaves (Fig. 8.5).

Such results highlight the need for reliable palaeopathological material for palaeoepidemiology, especially in the reconstruction of past infectious diseases.

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