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Towards a quantitative perception of human-microbial co-evolution

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1. ABSTRACT

The influence of changes in population structures of modern humans and human pathogens is likely reciprocal. In my opinion, a quantitative approach to study this co-influence in a historical perspective requires, in particular, adequate estimators of genetic distances that are well developed for human but not yet for microbial populations. Here, I propose a simple measure of genetic distance between geographic populations within a microbial species based on the observed difference in the frequencies of its genotypes. Further, I apply the proposed method to principal components analysis of *Mycobacterium tuberculosis*, and interpret the geographic distribution of its VNTR haplotypes in the light of human historical and recent migrations. The proposed approach may be helpful for a quantitative understanding of human-microbial interactions that constitute an integral part of the global history.

2. INTRODUCTION

The evolutionary histories of the human and microbial species are, at least partly, co-mirrored and co-shaped. Thirty years ago, W. H. McNeill demonstrated how human history was influenced by various local and global epidemics, the Black Death being the most notorious example (1). More recently, comparative studies attempted (i) timing of specific events in the genome evolution of *M. tuberculosis* (2) and (ii) tracing hidden patterns of human migrations in the geographic distribution of the *Helicobacter pylori* (3, 4) and polyomavirus JC (5-7) genotypes.

A quantitative approach to understand a human-microbial spatiotemporal cross-talk may be based on (i) the direct comparison of geographic populations within a microbial versus human species followed by (ii) a subsequent comparison with available data on human

history, demography and linguistics. In my opinion, this kind of analysis requires, in particular, an adequate estimation of genetic distance between populations. To date, various statistics have been developed to calculate genetic distances between human populations (8-11). These distances are consequently used for a graphical visualization of the interpopulation relationships, e.g., using principal components analysis. However, these measures/statistics are not applicable to the haploid microbial pathogens and especially those with mainly or exclusively clonal population structure without lateral gene transfer.

Whereas the *M. tuberculosis* complex includes mycobacteria widely differing in terms of their host specificities, *M. tuberculosis sensu stricto* (*M. tuberculosis*) is exclusively a human pathogen. The tubercle bacillus has the remarkable ability to persist in the human host in the form of a long-term asymptomatic infection referred to as latent tuberculosis (TB). One third of the world population is estimated to have a latent TB infection. The latent or dormant TB was perhaps the predominant mode of *M. tuberculosis* co-existence with its human host in a pre-industrialized time when transmission of the pathogen was historically vertical, i.e., mainly family/household-linked. Among several genetic families identified within *M. tuberculosis* (12, 13), the Beijing (Bj) genotype is marked by genetic homogeneity and geographical omnipresence (14, 15). Taken together, these data likely reflect its rapid global spread during the last century, if not the last few decades. For the first time, it was identified in the *M. tuberculosis* strains isolated in the Beijing area in China hence the name (16). Subsequent studies have shown that these strains are endemically prevalent in East Asia, South Africa, and Northern Eurasia (14, 15). The Bj strains demonstrate some important pathogenic features, rapidly being disseminated world-wide, and, in a great part, are responsible for a current global TB epidemic.

In the present study, I proposed a simple and straightforward measure of genetic distance between microbial geographic populations and applied it to the phylogeographic analysis of the *M. tuberculosis* Beijing family.

3. MATERIALS AND METHODS

3.1. Calculation of genetic distance

Firstly, a few definitions need to be introduced. Type or genotype within a species comprises strains that have identical structure of one or a combination of genomic regions (molecular markers). Area is a region, city, or country sub-region, considered as a separate geographic unit. Area co-shared types include strains from at least two areas whereas area-specific types are found only in one area.

Secondly, I suggest that Observed Distance (OD) between microbial populations A and B can be calculated using the following formula:

$$OD(A,B) = \sum_{i=1}^{N} |A_i - B_i|$$

where, N is the total number of different types identified in the combined sample of populations A and B, and A_i and B_i are frequencies of the ith type in these populations. The proposed formula takes into account not only area-specific types but also difference in the frequencies of the area co-shared types. In one extremity, consider two populations with no shared types, then, this OD will be 2. Contrarily, consider two populations with identical types' frequencies, then, this OD, also logically, will be zero. Finally, in the exact intermediate case (50% overlapping), population A and population B specific types would contribute, respectively, |0.5-0| and |0-0.5| absolute difference values, thus giving OD=1. In fact, this OD measure can be looked at as a snapshot distance between populations without disclosing how and what forces contributed to generate it. However, exactly for this reason it seems most suitable to compare complex populations by using principal components (PC) analysis as PCs are statistically independent from one another and can isolate independent expansions (9).

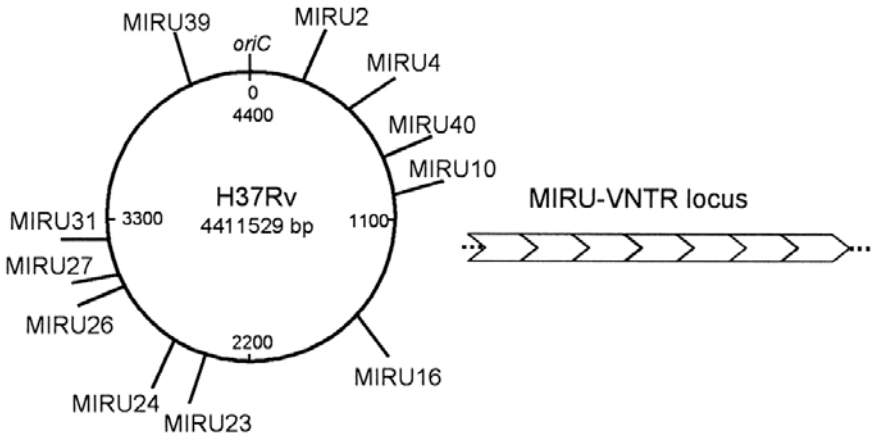
3.2. VNTR database of the *M. tuberculosis* Beijing family

A model of the *M. tuberculosis* Bj genotype family was used to test the above proposed method. The characteristic structure of one genome region (DR [Direct Repeat] locus) defines the Bj genotype and distinguishes it from other families within *M. tuberculosis* (14-16). DR locus consists of minisatellite alternating exact direct repeats and variable spacers. In the Bj genotype, most of the DR units were deleted during evolution, perhaps by a single event mediated by IS*6110* recombination (17). The Bj strains may be further discriminated by minisatellite VNTR (variable number of tandem repeats) loci (2, 18, and references therein). The VNTR loci are scattered throughout the bacterial chromosome; the number of repeat copies per locus may vary among strains, and the use of several such loci allows interstrain differentiation (Figure 1). The VNTR profiles are presented as multi-digit numerical codes, each digit representing the copy number in a locus.

Search of the VNTR profiles of the Bj strains from published studies was done by using the Entrez-Pubmed and Google engines, followed by inspection of the retrieved articles for the presence of information on Bj isolates. These data were compared with earlier versions of the Bj VNTR database (2, 19). Finally, the 11 VNTR loci (18) database has been created on 993 *M. tuberculosis* Bj strains whereas all Bj strains were subdivided into types with unique combinations of the 11 VNTR loci (Figure 1). Here, the analysis was performed on the 857 Bj strains from nine geographic areas (2, 18-27) where these strains constitute a noticeable proportion of the local *M. tuberculosis* populations (Figure 2).

3.3. Principal components analysis

The observed distances between nine geographic *M. tuberculosis* Bj populations were calculated using the above formula and based on the VNTR types' distribution. The resulting OD matrix was used for principal



Strain	VNTR profile	Origin	VNTR type	Ref
6-hk-7	2 2 3 3 5 1 7 3 4 3 3	China, Hong Kong	M1	1
8-hk-6	2 2 3 3 5 1 7 3 5 3 4	China, Hong Kong	M107	1
22-hk-2	2 2 3 3 5 1 7 3 5 4 2	China, Hong Kong	M108	1
10-hk-5	2 2 3 3 5 1 7 3 5 5 3	China, Hong Kong	M109	1
1-hk-77	2 2 3 3 5 1 7 3 5 3 3	China, Hong Kong	M11	1
12-hk-5	2 2 3 4 5 1 7 3 5 3 2	China, Hong Kong	M114	1
23-hk-2	2 2 3 3 5 1 8 3 5 5 3	China, Hong Kong	M126	1

Figure 1. Position of the 11 MIRU-VNTR loci (18) on the *M. tuberculosis* strain H37Rv chromosome, a structure of the VNTR locus, and first rows of the VNTR database of the *M. tuberculosis* Beijing genotype, as an example. The 11-digit code in the 'VNTR profile' column shows a number of repeats per locus in 11 loci: ## 2, 4, 10, 16, 23, 24, 26, 27, 31, 39, 40, respectively.

components analysis. In brief, principal components analysis does not reconstruct a history as a phylogenetic tree does, and in fact it does not give a history at all, but it represents a whole set of data in a very simple graphical way, and reveals latent patterns if they exist in the mass of apparent nonsense that the original data seem at the beginning (9). To use a concise description, it simplifies the data matrix reducing the number of dimensions with which one can represent the data, i.e. distances between the objects (populations) with a minimum loss of information. It may be said that the total variation is decomposed into its "components"; the word "principal" refers to the fact that there would be many more components, but we have been chosen only the most important ones, i.e. those that explain the largest fraction of the variation (9).

Here, the PC analysis has been carried out by means of its variety, an approach called multidimensional scaling (MDS). The MDS is a statistical algorithm that allows to investigate how the objects (i.e., populations) relate to each other and to uncover hidden structure that might be residing in a complex data set. Finally, it shows these relationships in the form of a map based on the object-to-object proximity information. The Permap software was used for this purpose as described in detail in its manual (28). The data were entered in the form of lower-triangular dissimilarity matrix (available on request). This software allows to represent relations between objects (populations) as a "solution" in up to four dimensions. In the present analysis, the numeric values were generated for three dimensions giving coordinates of the studied populations. Further, these coordinates were plotted to the three two-dimensional graphs (Figure 3). A percent ratio of each of the three PCs was calculated based on their maximal and minimal values, the PC1, logically, accounting for the largest diversity (compare three graphs in Figure 3).

4. RESULTS AND DISCUSSION

The proposed OD measure of genetic distance was tested with *M. tuberculosis*. This strictly human pathogen is devoid of horizontal gene transfer and consequently it has a clonal population structure. Several genetic families have been identified within this species, e.g., Haarlem, Beijing, EAI, CAS, Manu, and LAM (12, 13, 29). Most likely, these families could have initially been endemic within specific geographical areas. Some of

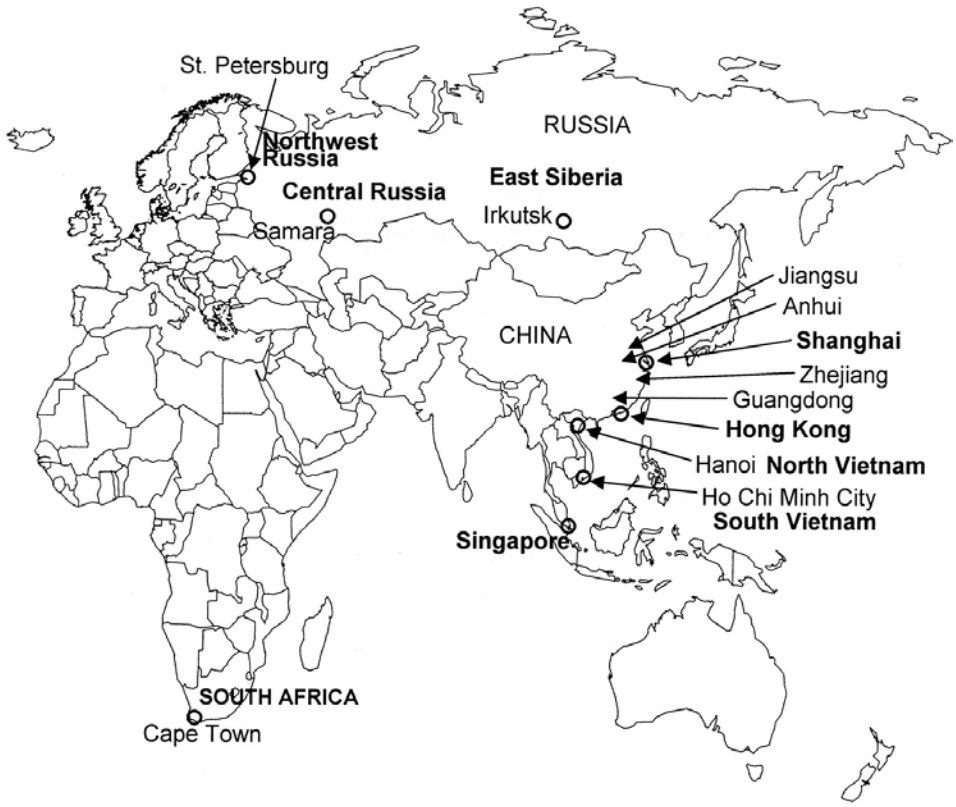


Figure 2. Areas of origin of the studied *M. tuberculosis* strains and other geographic names mentioned in the text. Names in bold refer to those in Figure 3. In this study, the VNTR data on *M. tuberculosis* strains from nine areas have been included: Shanghai (China [20]), Hong Kong (China [21]), Singapore (22), North Vietnam (Hanoi [2]), South Vietnam (Ho Chi Minh city [2]), Northwest Russia (mainly St. Petersburg and surrounding provinces [19, 23]), Central Russia (Samara [24]), East Siberia (mainly Irkutsk [25, 26]), South Africa (Cape Town [18, 27]).

these remain circumscribed to the particular regions, whereas others have become omnipresent. The Beijing family, probably the most characterized *M. tuberculosis* lineage, is an example of the latter group. These strains are endemically prevalent in South-East Asia, the area of origin and primary dispersal, as well as in Northern Eurasia and South Africa, areas of their secondary dispersal (2, 14, 16, 30). These strains have been found in the countries as distant as Argentina (31), Malawi (32), and Australia (33) and new unexpected routes of their apparently recent transmission are being uncovered (34). The Bj strains have accounted for several severe outbreaks of the multidrug-resistant tuberculosis (35, 36); the notorious New York City strain W also belongs to this genotype (37). These strains attract great attention worldwide because some, albeit not all, studies demonstrated their high transmissibility (38, 39), association with multiple-drug resistance (40, 41), increased risk of febrile response in patients (42), increased virulence in the BCG vaccinated mice (43), the ability to more rapidly multiply in human macrophages (44), and a presumably easier adaptation to changing environments due to mutator alleles of the *mutT* genes (45). Currently, the Bj strains are being disseminated from the two principal foci, East Asia and countries of the former Soviet Union; for these latter the spread of the multi- and extensively drug-resistant strains appears to have the most worrying impact on the global situation of TB.

4.1. Genetic geography of the *M. tuberculosis* Beijing family and human migrations

The performed analysis of the genetic geographic diversity of the *M. tuberculosis* Bj family has re-confirmed a general rule of isolation by distance (Figures 2 and 3). In the first PC set (Figure 3a), both Vietnam *M. tuberculosis* Bj populations are closely located. The same is also true for Russian Bj populations although with the smallest among-population variation. Nevertheless, a clear geographic gradient is noticed for all three Russian Bj populations (Figure 3a). In my opinion, these observations support a general robustness of the analysis. In its turn, this indirectly supports a reliability of the less expected affinities, likely representing hidden patterns of human migrations reflected in the patterns of *M. tuberculosis* geographic diversity. For example, an affinity between Northwest Russia and East Siberia, especially evident in PC2/PC3 coordinates is intriguing.

Genetic drift occurs rapidly in small populations, particularly in those that are also isolated; consequently, these groups quickly accumulate distinctive type frequencies. *M. tuberculosis* Bj strains were likely brought

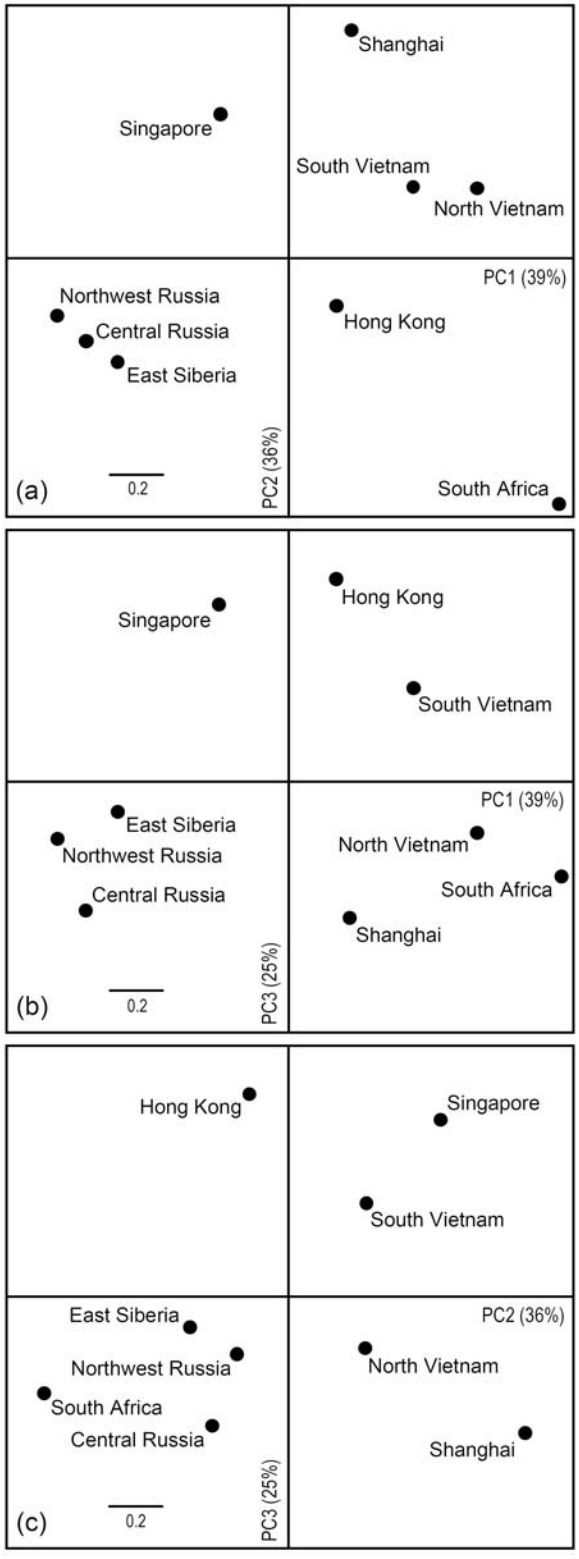


Figure 3. Relationships of the nine geographic populations of the *M. tuberculosis* Beijing family strains estimated as MDS graph based on the VNTR-OD matrix.

to South Africa following Indian Ocean slave-trade route since the 17th century (2, 46, 47) hence a distal position of South Africa (Figure 3a) reasonably reflecting a founder effect of the initially small *M. tuberculosis* Bj population brought here from Indonesia. The VNTR data on the Indonesian *M. tuberculosis* strains are not published and a relative affinity of South Africa to North Vietnam and Shanghai *M. tuberculosis* Bj populations in PC1/PC3 coordinates (Figure 3b) should be interpreted with a caution. Intriguingly, in PC2/PC3 coordinates (Figure 3c), South Africa and all three Russian Bj populations are located in the same lower-left part of the graph; perhaps, this illustrates their counter-position of the younger and secondary Bj populations versus ancient, primary and more divergent East Asian Bj populations (2).

Let us look closer at three Chinese populations, also keeping in mind the suggested Chinese origin of the *M. tuberculosis* Beijing family (2, 16). Singapore, a sparsely populated island, was ceded to the British East India Company in 1819; now it is a relatively modern amalgam of an indigenous Malay population with a third generation Chinese majority (77%) (48). Analysis of *M. tuberculosis* Bj strains reveals a weak proximity of Singapore to Shanghai in the first PC set, and to Hong Kong in the second PC set whereas both Bj populations from mainland China, Hong Kong and Shanghai, are well separated from each other in all three PC sets (Figure 3). In my opinion, this may reflect the more diversified origins of the Singaporean Chinese population, partly from southern China (hence affinity to Hong Kong), and eastern China (hence affinity to Shanghai), both primarily reachable from Singapore by a sea route.

In Shanghai, the vernacular dialect is Shanghainese that is an inseparable part of the Shanghainese cultural identity (49). A specific feature of the Shanghai human population is that a majority of its residents are descendants of immigrants from the adjacent eastern China provinces of Jiangsu, Zhejiang and Anhui (Figure 2) who moved to Shanghai in the late 19th up until mid-20th century (50). These are regions that generally speak the same family of dialects as Shanghainese dialect - Wu Chinese. The present-day continuation of this historic, demographic and linguistic situation (51) explains a remarkable divergence between *M. tuberculosis* Bj populations from the two Chinese regions, Shanghai and Hong Kong, in all three PC sets (Figure 3).

Finally, Singapore and Hong Kong *M. tuberculosis* Bj populations exhibit a weak affinity in the second and third PCs that is reasonably explained by their co-shared Chinese descent. Still, they sufficiently differ in the first PCs (Figure 3a) as indeed they had essentially different sources of origin of their respective Chinese human populations. In Hong Kong, most of 96% Chinese population are Cantonese dialect speakers (52), i.e. linguistically originating from the Guangdong province in southern China, whereas the origins of Chinese population were much more diversified for Singapore with only 6% accounting for Cantonese Chinese speakers (53).

A history of the Beijing genotype in Vietnam appears more complex. In this country, the present day Chinese minority (Hoa) speak predominantly Cantonese Chinese dialect and are mainly urban dwellers, mainly in the Ho Chi Minh City, South Vietnam (54). Until the 1979 census, the Hoa were the largest minority of Vietnam. Thus, the Hoa's contribution to the weak affinity of South Vietnam and Hong Kong (Figure 3b) cannot be completely ruled out. However, since 1975, many Hoa communities have left Vietnam after South Vietnam was defeated by the North and after a border war with China in 1979, so that according to the 1999 census the Hoa were only the fifth-largest minority. Therefore, the effect of the present Hoa (Chinese Vietnamese) human population was likely negligible to contribute to the divergence between *M. tuberculosis* Bj populations from South and North Vietnam observed in the second and third PC sets (Figure 3ab). Contrarily, looking deeper back into the ancient history, Vietnam had been ruled intermittently by Chinese dynasties for about 1000 years, and achieved independence in the 10th century (55). Consequently, the *M. tuberculosis* Bj strains might have been brought to Vietnam from China during that long period and this distant co-ancestry might account for some very general and weak proximity of the both Vietnam populations to the Chinese populations in the *M. tuberculosis* Bj PCA graphs. On the other hand, it may be possible that a weak affinity of the South (but not North) Vietnam to Hong Kong (Figure 3a), and, in a lesser extent, to Singapore (Figure 3c) may reflect a real demographic and migratory history in South East Asia in the relatively less distant past. Finally, one should also remember that in 1954, following its separation into North and South Vietnam, 900,000 people migrated south (54, 56). Thus the difference between the two *M. tuberculosis* Bj populations from this country may also partly lie in the historically recent events in 1954-1975, involving mass migrations within Vietnam itself without external human (and *M. tuberculosis*) flow.

4.2. Concluding remarks

Summing up, the above comparative analysis revealed strong geographic specificities of the local clonal variants of *M. tuberculosis*, in particular, underlining an insignificant human exchange between southern and eastern China (Hong Kong versus Shanghai) and primarily sea-routes in the Chinese settlement of Singapore in the 19th-20th centuries. Contrarily, a significant proximity of the three geographically distant Russian *M. tuberculosis* populations may reflect a recent dissemination of the Bj strains fuelled by mass human migration in the twentieth-century Russia. Finally, some weak and less expected affinities of the distant *M. tuberculosis* Bj populations (North Vietnam and South Africa; Northwest Russia and East Siberia) are intriguing and undoubtedly require an additional detailed investigation.

In my opinion, the implications from the use of the proposed method to calculate distances between microbial populations are various. It may become a tool to help in a comparative history of humans and human pathogens, as well as human natural commensals. A relatively novel concept of "Big History" places human history within an overview of the entire known history, from the beginning of the Universe up until life on Earth today (57, 58). Consequently, the proposed method may be useful for a quantitative understanding of human-microbial interactions and co-evolution in a broad historical perspective as a part of global natural, including human, history.

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