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Is the European spatial distribution of the HIV-1-resistant CCR5-Delta32 allele formed by a breakdown of the pathocenosis due to the historical Roman expansion?

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Abstract

We studied the possible effects of the expansion of ancient Mediterranean civilisations during the five centuries before and after Christ on the European distribution of the mutant allele for the chemokine receptor gene *CCR5* which has a 32-bp deletion (*CCR5-Δ32*). There is a strong evidence for the unitary origin of the *CCR5-Δ32* mutation, this it is found principally in Europe and Western Asia, with generally a north-south downhill cline frequency. Homozygous carriers of this mutation show a resistance to HIV-1 infection and a slower progression towards AIDS. However, HIV has clearly emerged too recently to have been the selective force on *CCR5*. Our analyses showed strong negative correlations in Europe between the allele frequency and two historical parameters, i.e. the first colonization dates by the great ancient Mediterranean civilisations, and the distances from the Northern frontiers of the Roman Empire in its greatest expansion. Moreover, other studies have shown that the deletion frequencies in both German Bronze Age and Swedish Neolithic populations were similar to those found in the corresponding modern populations, and this deletion has been found in ancient DNA of around 7,000 years ago, suggesting that in the past, the deletion frequency could have been relatively high in European populations. In addition, in West Nile virus pathogenesis, *CCR5* plays an antimicrobial role showing that host genetic factors are highly pathogen-specific. Our results added to all these previous data suggest that the actual European allele frequency distribution might not be due to genes spreading, but to a negative selection resulting in the spread of pathogens principally during Roman expansion. Indeed, as gene flows from colonizers to European native populations were extremely low, the mutational changes might be associated with vulnerability to imported infections. To date, the nature of the parasites remains unknown; however, zoonoses could be incriminated.

Keywords: Chemokine receptor *CCR5*; $\Delta 32$ allele; geographic distribution; ancient Mediterranean civilisations; Roman Empire

1. Introduction

C-C chemokine receptor 5 (CCR5) is the principal entry co-receptor for macrophage-tropic Human Immunodeficiency Virus type 1 (HIV-1) strains, which predominate at the early stages of the HIV-infection (Lusso, 2006). A mutation of the *CCR5* gene, termed $\Delta 32$, consists of a 32 bp deletion that occurs at a site of a repeated motif in this gene and results in a frameshift in the coding sequence that produces a non-functional protein, and as a result it is not expressed in the cell membrane (McNicholl et al., 1997). Individuals homozygous for the mutation are resistant to infection, even after repeated high-risk exposure (Liu et al., 1996; Paxton et al., 1996), but this resistance does not appear absolute since isolated cases of HIV-positive deletion homozygotes are emerging (Biti et al., 1996). These infections could have occurred with T-tropic strains that use the CXCR4 molecule as coreceptors to enter the target cells. Heterozygotic carriers have reduced susceptibility to infection and reveal a slowed progression towards AIDS, which is delayed for an additional 2 or 3 years (Samson et al., 1996; Eugen-Olsen et al., 1997; Huang et al., 1996; Michael et al., 1997; Zimmerman et al., 1997). However, the studies on these aspects of disease progression are contradictory (Dean et al., 1996; Hoffman et al., 1996; Huang et al., 1996; Samson et al., 1996; reviewed in Arenzana-Seisdedos and Parmentier, 2006), for example, Samson et al. (1996) have reported a 35% decrease in heterozygotes among HIV-1 positive individuals relative to seronegative controls, whereas Dean et al. (1996) did not report significant difference.

The *CCR5*- $\Delta 32$ allele is mainly present in Europeans (10% on average), and the allele frequency is highest (>15%) in the areas surrounding the Baltic and White Seas, and in Central Russia near Novosibirsk (Balanovsky et al., 2005). According to these last authors, from these maximum, the frequency gradually decreases in all directions across Europe; however, there are some additional peaks of frequency in samples from the Northern Coasts of France and some parts of Russia (Dean et al., 1996; Liu et al., 1996; Samson et al., 1996; Lucotte, 1997, 2001a, 2002; Martinson et al., 1997, 2000; Zimmerman et al., 1997; Libert et al., 1998; Lucotte and Mercier,

1998a, b; Stephens et al., 1998; Lucotte and Dieterlen 2003a; Balanovsky et al., 2005). In Europe, the lowest values have been found in the Mediterranean area and more especially in islands (Corsica, Crete, Cyprus, Sardinia, Sicily, but not in Balearics). Outside Europe, the mutation is found at low frequencies in neighbouring regions (North Africa, Middle East, Central Asia); it is absent in Sub-Saharan Africa, East and South-East Asia, and in indigenous populations of the Americas and Oceania (Fig. 1).

Such a pattern is unusual for genes in human populations; and it became really striking when data on mutation age appeared. The age of the *CCR5-Δ32* allele has been estimated to be between 700 and 3,500 years BP based on linkage disequilibrium data (Stephens et al., 1998; Slatkin, 2001). Based on the diversity of microsatellites linked with the *CCR5* gene, Libert et al. (1998) showed that the *CCR5-Δ32* mutation arose about 2,000 years BP, but in a recent study using 32 small nuclear polymorphisms (SNPs) markers, the estimated deletion age increased to 5,075 years (Sabeti et al., 2005). Moreover, this last study is consistent with an ancient DNA evidence which suggests that the allele is at least 2,900 years old in Germany (Hummel et al., 2005) and with a recent study of Scandinavian Mesolithic DNA which has pushed the date of the first occurrence back to around 5000 BC (Lidén et al., 2006). However, the extent of heterozygosity, differentiation across populations, and linkage disequilibrium in the *CCR5* region, which is not dissimilar to other human genomic regions, added to the supposed age of the origin of the mutation (around 5,000 years), would challenge claims of recent positive selection and could be consistent with neutral evolution (Sabeti et al., 2005; Hedrick and Verelli, 2006). Nevertheless, such results cannot rule out the possibility that some selection may have occurred at *CCR5*, the absence of evidence being not evidence of absence. Positive selection may also cause a rapid rise of an allele frequency, creating a disparity in the age of an allele estimated from its high frequency in the population (characteristic of an old allele) and its long-range linkage disequilibrium (characteristic of a young allele); according to several authors (reviewed in de Silva and Stumpf, 2004; Stumpf and Wilkinson-Herbots, 2004; Galvani and Novembre, 2005; Cohn and Weaver, 2006), the pattern of genetic variation occurring

at *CCR5-Δ32* might be the consequence of adaptive changes to older pathogens or ecological variations.

The AIDS pandemic is too recent to change allele frequencies. Factors of historical selection should therefore be pointed out; they could be epidemic infections (Cohn and Weaver, 2006 and references therein) or even autoimmune diseases (Ajuebor et al., 2006; Otaegui et al., 2007) and age-related diseases (Balistreri et al., 2007). Based on the fact that, besides HIV-1, chemokine receptors interact with other pathogens, it was supposed that the *CCR5-Δ32* mutation might induce resistance to a set of infectious diseases. Bubonic plague was initially proposed as the selective agent (Stephens et al., 1998); however, such a hypothesis is not supported by the historical evidence, the Black Death did not strike Europe alone but spread from the East to the West, devastating regions in other continents as much or even more than Europe (Cohn and Weaver, 2006). Further, the gradient of Black Death mortality sloped in the opposite direction from that of present-day genotypes: the heaviest casualties were in the Mediterranean, the very regions whose descendents account for the lowest incidences of the HIV-1 resistant allele. In addition, forensic examination of German victims of the 14th century bubonic plague pandemic indicates that *CCR5-Δ32* carriers did not have an altered mortality risk (Hummel et al., 2005; Kremeyer et al., 2005). Similarly, analyses of DNA collected at archaeological sites in Poland dated back to 11–14th centuries suggested that historic pandemics had little effect on its present-day frequency (Witas and Zawicki, 2006; Zawicki and Witas, 2008); consistent with this fact, *CCR5*-deficient mice were not protected against infection with *Yersinia pestis* (Meccas et al., 2004). Moreover, although it has generally been accepted that the great epidemic of the 14th century, often referred to as the Black Death, was the bubonic plague, this view has been challenged by scholars from various disciplines. One of the principal criticisms was that the plague did not by itself cause the high mortality observed during the Black Death but it is nevertheless admitted that plague has well played a major role in the large number of deaths (for discussion see, Noymer, 2007; Theilmann and Cate, 2007). In addition, the genetic mutation should not be attributed to a single disease strike, but due to

recurrence diseases over several centuries (Scott and Duncan 2001, 2004; Galvani and Slatkin, 2003; Duncan and Scott 2005; Duncan et al., 2005), and other diseases like smallpox (Klitz et al., 2001; Lalani et al., 1999; Galvani and Slatkin, 2003; Novembre et al., 2005), or even haemorrhagic diseases due to an Ebola-like virus (Scott and Duncan 2004), could be more plausible candidates. These claims continue to be made (Duncan and Scott 2005; Duncan et al., 2005) even after new scientific evidence of the allele's more ancient origins and possible earlier rate of increase (Schliekelman et al., 2001; Novembre et al., 2005; Sabeti et al., 2005) as well as conflicting historical facts. Moreover, the CCR5 null mutation could also play a role in absence of infections; for example, a protect role has been suggested against autoimmune diseases (Burns et al., 2005; Ajuebor et al., 2006; Prahalad, 2006) or asthma (Walker et al., 2006). However, some of the correlations described were not confirmed in subsequent studies, and many of these data remain therefore controversial.

Previous discussion on the geographic distribution of *CCR5-Δ32* has also focused on the north–south cline in frequency. Lucotte (2001a) and Lucotte and Dieterlen (2003a) suggested that the cline and other features of the geographic distribution imply a Viking origin. In particular, they proposed that the allele was present in Scandinavia before the Viking expansion in the 800s and then was carried by Vikings northward to Iceland, eastward to Russia, and southward to Central and Southern Europe. An alternative hypothesis has been proposed by Balanovsky et al. (2005) which have suggested the following scenario: the mutation would have occurred in an Uralic population, and then spread among other Uralic-speaking populations; the raising of *CCR5-Δ32* in North-Eastern Europe would be a result of selection and/or genetic drift, and finally the migrations of Northern Europeans across the globe allowed the spread of mutation (with selection continued due to the gene flow). These two hypotheses of a northern origin, together with typical levels of dispersal in Europe, may explain partly of the geographic distribution of *CCR5-Δ32* but not the overall spatial distribution. Even if generally the factors which caused the spread of the *CCR5-Δ32* mutation are not fully understood so far (Galvani and Novembre, 2005), Lucotte's hypothesis, since

its seminal paper in 2001a, and that of Balanovsky et al. (2005) show evidences that analyses of European migrations could help to understand the actual distribution of this genetic mutation. Besides disease-related hypotheses, some other explanations have been put forward to explain the *CCR5-Δ32* spatial pattern. Since some factors, such as climate, provide selective pressure that differs across the globe, the possible impact of climate has been studied and a negative correlation between *CCR5-Δ32* mutation and temperature factors has been revealed (Balanovsky et al., 2001; Limborska et al., 2002). Another alternative is that the allele may have arisen in Central Europe and increased to a higher frequency in the North because of a geographical gradient in selection intensity (Limborska et al., 2002). However, further analyses have indicated that the influence of climate on *CCR5-Δ32* distribution was, if anything, indirect (Balanovsky et al., 2005).

Maps displaying geographic patterns of an allele distribution are useful to understand specific facts to that allele, including its evolutionary history and the effects of evolutionary factors like mutation and natural selection (Cavalli-Sforza et al., 1994). The geographic distribution of a particular allele may give information on the origin area of the genetic change that generated it. Correlations of the distributions of gene frequencies with environmental and/or historical parameters at the geographic level have been instrumental in the discovery of specific genetic adaptations. The sickle-cell anaemia gene was the first example, because its geographic distribution showed a correlation with that of malaria (Haldane, 1949). The hypothesis that this gene may confer resistance to malaria was later confirmed by more direct tests (Cavalli-Sforza et al., 1994). In our study, an alternative hypothesis concerning the north to south downhill gradient of *CCR5-Δ32* frequencies has been explored using several set of geographic and historical maps: if we suppose that the global frequency was very high in the past in all the European populations, could some historical events suggest an underselection of this mutation?

2. Materials and methods

We have compiled a global database on *CCR5-Δ32* frequency distribution using data from 28 published sources and ALFRED database, with a total of 18,563 samples from 69 European populations. We focused our analysis on the region extending from approximately 34°N to 72°N and 25°W to 45°E. Expansion of ancient Mediterranean peoples came from numerous historic atlas (principally: Talbert, 1988, 2000; Cornell and Matthews, 1991; Scarre, 1995; Morkot, 1999).

Computations were performed with the R freeware that provides an environment for statistical analysis (R Development Core Team, 2006; <http://www.r-project.org/foundation/>). As the *CCR5-Δ32* allele frequencies have been estimated from samples of different sizes, they do not have the same precision, a larger sample size yields better frequency estimation. The classical least squares regression assumes that every observation should be treated equally. But this procedure that treats all of the data equally would give less precisely measured points more influence than they should have and would give highly precise points too little influence. This is the reason why weighted least squares regression has been chosen, where weights are the sample sizes.

In this paper, we aim to explain allele frequency by several factors. In order to know which factors have more influence, AICc-based model selection (Burnham and Anderson, 1998) and BIC methods (Shumway and Stoffer, 1999) have been used. We next compute a multiple linear regression to develop a complete model.

3. Results

As suggested by various authors, the gradient of *CCR5-Δ32* frequency in Europe could be due to the spread of a particular pathogen through European population (Cohn and Weaver, 2006 and references therein). Moreover, it is well known that ancient civilisations (such as for example, the Greek and Roman Empires) came into military and commercial contacts, ca. 3000-2000 years ago,

swapping their dominant infections (McMichael, 2004). Several studies have evidenced that colonization has generated a breakdown in the European pathocenosis. The concept of pathocenosis was introduced by Mirko D. Grmek (1969); the idea is that the frequency and the overall distribution of each disease, above and beyond various endogenous and ecological factors, depend on the frequency and distribution of all other diseases in the same population (Gremk, 1991; Gourevitch, 2005). For example, a study has identified differences in the demographic and paleopathological characteristics of the ancient Adriatic populations before and after the Roman conquest (Capasso et al., 2003). Similarly, the plague bacterium, *Yersinia pestis*, accompanied Roman legions returning from the Middle East, and the Justinian plague of 542 AD devastated Constantinople and the Roman Empire (McNeill, 1976). As the Roman Empire has spread far and wide across Europe and has established a durable colonization, it had probably the most adverse health effects in this continent (the term "Roman Empire" is used to denote the entire period in ancient times in which there was a major power with its capital at Rome, whether republic or empire). However, Rome's rivals in the second part of the 1st millennium BC have also probably had an impact on the spread of diseases in Europe, which could concern mainly Greeks, Etrurians and Phoenicians (including Carthaginians) and their trading-colonies. Interestingly, in our analyses of an extensive set of historical maps, only the spread of the great ancient Mediterranean civilisations of approximately the five centuries BC and AD could partially overlap maps of the allele frequency distribution (data not shown). Moreover, two arguments are in favour of this hypothesis: one of them is spatial; in 2001a, Lucotte had already shown that a clear north-south decreasing cline was evident for *CCR5-Δ32* frequencies; the other is temporal, i.e. the possibly ancient origin for this allele (>5000 years) (Sabati et al., 2005; Hedrick and Verelli, 2006; Lidén et al., 2006).

Figure 2 shows that there is some correspondence between the *CCR5-Δ32* frequency in Europe and the Roman colonization spread; more precisely, it suggests a negative correlation. Moreover, Etrurian, Greek, Phoenician and Carthaginian territories or colonies, which have been

occupied before the Roman expansion, exhibit generally the lower *CCR5-Δ32* frequency (for example, Greece and most of the Mediterranean islands). However, the two types of data on the map do not correspond perfectly, and we can only conclude that these patterns are not inconsistent with the hypothesis that allele frequency and the spread of ancient Mediterranean civilisations in Europe during the 1st millennium BC and the beginning of the 1st millennium AC are causally related.

To test this hypothesis, we looked for a correlation between *CCR5-Δ32* frequency and the expansion of the ancient Mediterranean civilisations. Firstly, we performed a formal correlation analysis between the *CCR5-Δ32* frequencies and the first colonization dates of European countries by Mediterranean civilisations (Fig. 3A). The correlation with the dates when European people were invaded is in congruence with our hypothesis. Moreover, a remarkable correlation can be pointed out, with a highly significant ($p = 2.7 \times 10^{-16}$) correlation coefficient ($r = 0.88$); moreover, $s_e = 9.0 \times 10^{-6}$ and equation of regression is $y = 1.1 \times 10^{-4} x + 0.0971$. Figure 3B shows a strong correlation between the *CCR5-Δ32* allele frequencies and the distances from the Northern frontiers of the Roman Empire in its greatest expansion, which corroborates our hypothesis. If we denote by z the distance versus the frontiers (with negative values inside and positive values outside), we get the following significant ($p = 3.7 \times 10^{-15}$) equation $y = 3.2 \times 10^{-5} z + 0.0971$, with correlation coefficient $r = 0.78$ and $s_e = 3.1 \times 10^{-6}$. However, analyses of the distances inside the frontiers are more supported than those outside the borders. In the first case, the correlation coefficient $r = 0.65$ is highly significant ($p = 4.6 \times 10^{-7}$), $s_e = 6.7 \times 10^{-6}$ and the equation of regression is $y = 0.104 + 3.9 \times 10^{-5} z$, whereas in the second case $r = 0.38$ with a p value of 0.091, $s_e = 7.3 \times 10^{-6}$ and the equation of regression is $y = 0.111 + 1.3 \times 10^{-5} z$. Note that in the first case, an inverse correlation is observed between *CCR5-Δ32* allele frequency and (absolute) distances from frontiers. Taken together, these analyses show evidence that both distances and dates may explain allele frequencies. In order to know which of these factors had the greatest influence on allele frequency distribution, AICc and BIC criteria have been used. These analyses show that allele frequency variability is

better explained by dates (AICc = - 236.8 and BIC = - 231.7) than by distances (AICc = - 193.0 and BIC = - 187.9). In addition, a multiple linear regression analysis has been performed to get a complete model. The corresponding equation is $y = 0.0987 + 1.1 \times 10^{-4} x + 2.1 \times 10^{-6} z$; moreover, the correlation coefficient adjusted R-squared = 0.76 is highly significant $p = 4.0 \times 10^{-15}$; this shows evidence that at least one of the factors may explain allele frequency variability. However, in this equation, the coefficients differ significantly for dates versus distances respectively, $s_e = 1.3 \times 10^{-5}$ and 6.2×10^{-6} , and $p = 1.7 \times 10^{-10}$ and 0.73, respectively. Lastly, correlation between the colonization dates and the distances from the frontiers of the Roman Empire in its greatest expansion has been found with a significant correlation coefficient $r = 0.40$ ($p = 0.0051$; $s_e = 0.364$), and the equation of regression is $z = - 625.9 + 1.069 x$ (figure not shown). Whereas in the univariate analyses these two variables had influences; in multiple regression analyses, the influence of colonization dates is significantly larger than that of distances.

For the calculation of the equations of the regression and the R^2 values, all the European populations have been included. However, "outliers" populations, even if the regressions are practically unaffected by the inclusion of them, exhibit some original characteristics which warrant further discussion. The Romanian sample size is very low ($n = 11$) (Martinson et al., 2000); moreover, although Dacia, a Roman province which included most of the modern Romanian regions, has been conquered by Romans in 106 AD, a great part of the littoral of the nowadays Romania belonged to the Achaemenian Empire at the end of the Vth century BC since the invasion of Scythian territories by King Darius the Great of Persia in 512 BC; so this last date has been used in our analyses. Concerning Basques and Lapps, Cavalli-Sforza (2002) has underlined that, in Europe, these populations have unique genetic makeup and languages and are separated themselves from other Europeans. The *CCR5-Δ32* allele frequency of the Lapps is around 0.08 in spite of their northern location (Libert et al., 1998). Surprisingly, whereas for the Spanish Basques, the *CCR5-Δ32* allele frequency is around 0.062 (Libert et al., 1998) to 0.086 (Martinson et al., 1997), it is 0.018 for French Basques (Lucotte and Mercier, 1998b). However, this result coincides with those

of previous studies in which heterogeneity was observed among certain Basque districts or provinces (Manzano et al., 2002). On the other hand, the values of the allele frequency of the two Croatian island populations analyzed exhibit great discrepancies in spite that they are not geographically distant (≈ 220 km) (Smoljanović et al., 2006). The values of the allele frequency are respectively 0.065 for the northern population (Island of Rab, Lopar) and 0.015 for the Southern one (Island of Vis, Komiža). This last island has the smallest area of our dataset (≈ 90 km²), and the low allele frequency value observed for its population could be due to the founder effect and subsequent genetic drift which would act together to strongly separate their frequencies, this could explain the quasi-extinction of the allele in an isolated island community (Smoljanović et al., 2006). Moreover, island Croatian populations represent one of the best-characterized isolate resources in Europe; indeed, unusual autochthonous diseases and specific medical conditions on these islands result from the reproductive isolation and specific genetic structure of their populations, characterized by high degree of genetic isolation, consanguinity, and inbreeding (Saftić et al., 2006; Vitart et al., 2006). In addition, it is evidence that some islands had under Greek influence before the fourth century; Issa on the Island of Vis, which could be one of the two oldest Greek colonies on the Eastern Adriatic, was probably founded during the sixth or fifth centuries BC; however, as it is well known that the tyrant of Syracuse, Dionysius the Elder established a colony on Vis in 397 years BC (Beaumont, 1936); so this date has been used in our analyses.

Taken together, Figures 2 and 3 could suggest that, in the past, all the European populations had a relatively high *CCR5-Δ32* allele frequency; that would not be unusual either, many other polymorphisms found in similar frequencies (7%-9%) in Europeans do not occur in the other populations (Gross, 2005). However, the possible decrease of the ancestral *CCR5-Δ32* allele frequency was probably not due directly to the military or colonization spreads; indeed, gene flows from colonizers to European native populations were extremely low (Cavalli-Sforza et al., 1994). However, a recent meta-analysis suggested that the actual patterns of 35delG connexin 26 prevalences could be the result of Ancient Greek colonizations of the "Magna Grecia" in historical

times (Lucotte, 2007), showing that gene flux cannot be ruled out. Moreover, Figure 3B suggests that the factor responsible of the decrease could have diffused beyond the borders of the Roman Empire. The diffusion of a factor excludes the role of climatical changes, the most probable hypothesis being that the decrease of the allele frequency could be due to the spread of human parasites or zoonoses which could affect human populations.

4. Discussion

4.1. Role of the Roman spread in the European CCR5-Δ32 allele frequency

Previous studies have hypothesized that the geographic distribution of the null allele implies Viking (Lucotte, 2001a; Lucotte and Dieterlen, 2003a) or Uralic origins (Balanovsky et al., 2005). The role of these peoples in the spread of the allele frequency is plausible (see below, paragraph 4.2); however, due to the relatively low number of individuals, the gene flow has been probably relatively weak; moreover, this cannot explain the whole European allele frequency distribution. We propose an alternative hypothesis which is rather complementary than opposite to these studies: a strong level of the null allele frequency in all the Paleo-Caucasian and Paleo-Uralic peoples follow up by a progressively decrease of the frequency southwards. Because of the strong evidence for the unitary origin of the *CCR5-Δ32* mutation (Libert et al., 1998; Klitz et al., 2001), the null allele could have been already present in the ancestors of the European populations (in spite of their present language differences) at a relatively high frequency, probably > 10% as suggested by analysis of ancient DNA from Bronze age (Hummel et al., 2005) and Neolithic (Lidén et al., 2006). Moreover, this last study pushed the observed age of the allele back to at least 7,000 years ago which is congruent with the estimation of Sabeti et al. (2005). In addition, in population genetics, it is generally assumed that the most frequent genetic marker is the oldest (Watterson and Guess,

1977), and quantitative studies have concluded that heterozygous carriers of *CCR5-Δ32* in the past had a fitness advantage of at least 5% and probably as high as 35% (Stephens et al., 1998; Slatkin, 2001).

According to Balanovsky et al. (2005), the influence of climate on *CCR5-Δ32* distribution was, if anything at all, indirect; thus *CCR5-Δ32* conferred an advantage against some non ecological but recurrent and strong selective factors, possibly epidemic. Based on several recent publications, important aspects of the epidemic implications like bubonic plague or smallpox seem to be unravelling (Elvin et al., 2004; de Silva and Stumpf, 2004; Stumpf and Wilkinson-Herbots, 2004; Galvani and Novembre, 2005). In the context of pathogenic threats, it is interesting to mention another example where human null alleles are selected in certain geographic areas: a null allele at the Duffy blood group locus was shown to be beneficial in some Africans, probably because it confers resistance to malaria (Hamblin et al., 1998). The comparison with the distribution of the *caspase 12* null allele is also instructive. It has been driven by positive selection, probably because it confers protection from severe sepsis; nevertheless, it has been lost in non-Africans and is nearly lost in Africans (Wang et al., 2006).

Our results suggest, in Europe, the *CCR5-Δ32* allele frequency is negatively correlated with the expansion of Mediterranean civilizations and principally Roman spread. Moreover, multiple regression analysis show evidences that the influence of colonization dates is significantly larger than the distances versus the frontiers. This suggests that the influence of distances may be due to some extent to its covariation with colonization date and might be less influential than previously thought. This bias is due to the fact that the Roman expansion has not been uniform across space or across time. In some areas, the distances versus the frontiers have not changed significantly for centuries, whereas for example after the conquest of Gaul by Julius Caesar, which has lasted eight years, the Roman Empire border has been pushed forward to a distance of more than 1000 km (Julius Caesar: Gallic Wars).

Figure 3B could suggest a diffusion of a factor which could be human or animal parasites which affected human populations; indeed, as the gene flows from colonizers to European native populations were extremely low within the conquered territories, their genetic impact outside the borders were negligible. In Europe, the Romans were the cause of some permanent changes in the distribution of birds and beasts (Jennison, 1937); several animals have been voluntarily introduced throughout Europe, such as cat and donkey (Clutton-Brock, 1981, 1987) and others involuntarily, such as malaria vector mosquito species (Gremk, 1989). Our hypothesis is that a human disease or a zoonosis, for which the *CCR5-Δ32* mutation can be favourable, could have played a role in the decrease of the mutation frequency or absence of maintenance of the null allele if it would have appeared. Interestingly, recent studies suggest that the lack of CCR5 is dispensable for infection control in humans. Indeed, it has been reported that *CCR5-Δ32* homozygosis was strongly associated with symptomatic West Nile virus (WNV) infection (Glass et al., 2006), consistent with a previous finding that CCR5 was a crucial antiviral and survival factor in WNV infection in mice (Glass et al., 2005).

4.2. Focus on the null allele frequency in the Uralic-speaking and Island peoples

The highest frequencies are observed in two Finno-Ugric populations (Estonians and Finns), and in Northern Russians who are supposed to have a strong Finno-Ugric background (Bunak, 1964). Balanovsky et al. (2005) have proposed that the mutation have occurred in a Uralic population and has spread in neighbouring areas due to migrations and to gene flow. This is also probably the case for Hungary; this country was occupied in the 9th century AD by 100.000-500.000 Magyar invaders, principally herders who spoke a Uralic language. They formed a fraction of 10-50% of the total population after the invasion; subsequent gene flow from neighbours is likely to have decreased this initial proportion. The current estimate of admixture is 13% (Guglielmino et al.,

1990). Moreover, a part of the present Hungary is the most southern region of the occidental Europe which has never been colonized by Romans. The best example of rapid spread of this null allele is suggested by Ashkenazi Jews which have high frequencies of *CCR5-Δ32* (Kantor and Gershoni, 1999; Lucotte et al., 1999; Maayan et al., 2000; Klitz et al., 2001; Lucotte and Smets, 2003); as numerous evidences show that the mutation has not been present before the Jewish Diaspora, the *CCR5-Δ32* mutation must have moved into the Ashkenazi by admixture with their Northern European neighbours, suggesting a strong advantage of this allele; especially these studies have shown that the gene pool of Jewish populations has remained relatively isolated from neighbouring non-Jewish communities after the Diaspora (Klitz et al., 2001). In addition, in Ashkenazi, the null allele frequency could be accentuated compared to the non-Jews neighbouring populations; this could be the result of genetic drift, a serie of dispersals to and within Europe, endogamy, and/or rapid population growths. Balanovsky et al. (2005) have also shown that the *CCR5-Δ32* pattern is interrupted by local maxima > 12% (in Tatars and in Shorians). Even if these two Turkic speaking people have a complicated population history, their allelic frequencies suggest gene flow due to Finno-Ugric background since the allele frequency in Turkic peoples is around 5-6% or less (Limborska et al., 2002). The examples of Ashkenazi and, to a lesser degree, of Tatars and Shorians, evidenced that high gene flow occurred rapidly, probably in a few centuries or less, throughout the two last millennia, and suggest that the loss of the null allele could be so rapid. The future challenge will be why these disparate allele frequency patterns were not transformed? Another interesting feature is the allele frequency in Finns (0.16) which have a proportion of 90% European genes and 10% Uralic (Guglielmino et al., 1990). We could consider that the *CCR5-Δ32* mutation occurred in some Uralic populations and then spread at relatively high frequencies in quite a large area of Uralic and related populations; however, the hypothesis of the presence of the mutation in the ancestors of all the European and North-East Asian peoples is, according to us, most parsimonious. Moreover, the genetic distance could be incongruent with language affinities (Cavalli-Sforza et al., 1994); it must be stressed that linguistic heritage, while it may tend to

correspond with cultural continuity, does not imply genetic or biological descent. The transmission of language by conquest, assimilation, migration, or any other ethnic movement is a complex and enigmatic process; for example, in the case of Indo-European, no genetic conclusions can or should be drawn. According to Comrie (2002), when Indo-Europeans arrived in Europe, this continent was already peopled by farmers, but as Indo-Europeans had technological or social organization advantages, their language had such a prestige that it replaced the autochthon languages without replacement of population. A similar scenario for Uralic-speaking peoples is plausible. If this hypothesis is true, this is in favour of a pre-existing presence of the null allele in the European populations.

Moreover, in numerous studies, discrepancies in the expected north-south cline frequency has been found in populations from islands and mountains, areas which combine ecological and cultural special features; for example, for Basques (French: 0.018 and Spanish Pyrenees: 0.068) (Limborska et al., 2002), Croatian islands populations (0.065 and 0.015) (Smoljanović et al., 2006), and Balearians (0.089 versus from 0.027 to 0.040 for other larger Mediterranean island populations) (Piras et al., 2005). Similarly, the population of the Azores Islands has one of the highest *CCR5-Δ32* allele frequencies (0.165), in spite of its southern latitude (39.5° N) (Freitas et al., 2006). In these isolated populations, the founder effect together with subsequent genetic drift could have played together a role in the frequency level. Moreover, these communities with small populations will not sustain endemic transmission and, due to their relative isolation, may not regularly be exposed to epidemics, and their introduction often resulted in intense, rapidly spreading outbreaks involving all age groups born since the previous outbreak (Youngkin et al., 1949; Christensen et al., 1951; Black, 1966; Willis and Warburton, 1974), and rapid selection of favourable alleles. Further analyses of isolated island communities might eventually provide more substantial support for the epidemic role in the allele selection or perhaps contrarily rule it out.

Numerous other apparently surprising *CCR5-Δ32* allele frequencies could be explained at least partially by some historical traits as, for example, difference between Norwegian and Swedish

frequencies that could be due to the slave gene flow during the Viking period. Slaves came from neighbouring tribes, Germanic, British and other Northern European tribes; however, Norwegians got their European slaves from more southern countries (which have a lower level of the null allele frequency). Moreover, whereas Y-chromosome and microsatellite analyses suggested that 20%–25% of Icelandic founding males had Gaelic ancestry, with the remainder having Norse ancestry (Helgason et al., 2000a), mtDNA variation studies suggested that the majority of Icelandic founding females may have originated from the British Isles (Helgason et al. 2000b). The analyses of the actual allele frequencies of Gaelic, Irish (0.08), Iclander (0.015), Dane (0.012), Norwegian (0.012) and non-Saami Swedish populations (0.014) (Martinson et al., 1997; Libert et al., 1998; Stephens et al., 1998; Magierowska et al., 2000, ALFRED database), strongly suggest that the Vikings have been instrumental in disseminating the *CCR5-Δ32* allele in Iceland, as previously suggested by Lucotte (2001a). In addition, analysis of another human mutation can reinforce this hypothesis; indeed, the mutation responsible for most cases of genetic haemochromatosis in Europe (*HFE C282Y*) appears to have been a unique event often described as a "Celtic mutation" - originating in a Celtic population in Central Europe and spreading west and north by population movement (Lucotte, 2001b; Lucotte and Dieterlen, 2003b). It has been calculated that this mutation occurred in mainland Europe before 4,000 BC (Distante et al., 2004) and it has also been suggested that Viking migrations were largely responsible for the distribution of this mutation (Milman and Pedersen, 2003).

Conclusion

Following previous studies suggesting a role of Vikings or Uralic peoples in the geographical distribution of the *CCR5-Δ32* allele frequency, we propose an alternative scenario explaining the modern *CCR5-Δ32* spatial pattern. According to us, the frequency of the null allele was relatively

high in the ancient European populations, and has changed rapidly both in time (during the centuries around the Christian Era) and in space (across the South of Eurasia). Map of the spread of Roman legions partially overlaps those of the null allele distribution. In addition to this qualitative observation, quantitative results on correlation between *CCR5-Δ32* frequency and a set of parameters (colonization dates or distances from the borders) are in favour of a role of Romans in the decrease of this allele frequency in spite of the historical migrations and ethnic mixtures in Europe since the two last millennia. As the Roman Empire spread from Italy across Europe, so did these adverse health effects. The role of Romans and of some other colonizers would have been indirect and possibly due to a human disease or (cryptic) zoonosis. Indeed, the CCR5 receptor can function for or against the host, depending on the pathogen, whereas in HIV pathogenesis, CCR5 is promicrobial; in West Nile Virus pathogenesis, CCR5 plays an antimicrobial role, showing possible deleterious effects of the null allele mutation. However, the exact scenario implies probably a more complicated pattern of mutation spread. For example, in our hypothesis, a mechanism has maintained the low null allele frequency in the South during about the last 1.5 millennium, suggesting that the parasitic agent(s) spread by ancient colonizers which caused the negative selection continued throughout the centuries to stabilize the *CCR5-Δ32* allele frequency among the Southern European populations. In addition, the exact pathogens that were responsible for the spread and/or the decrease of the *CCR5-Δ32* allele are still unknown, probably several agents are implicated. However, recent studies have led the demonstration of CCR5 involvement in a growing number of specific pathophysiological situations.

Our analyses also suggest that only a holistic approach which combines various fields of investigation (molecular biology, parasitology including virology, evolutionary and population genetics, paleoepidemiology including pathocenosis aspects) has enabled us to understand the interrelations between parasites and the *CCR5-Δ32* resistance allele. Moreover, the correlations discovered by geneticists and epidemiologists between present-day genotypes in human populations, and varying levels of resistance to diseases, now demand a new cooperation between

scientists and historians (Cohn and Weaver, 2006). Together, they can explore the connections between events, environment, biological change, and possible selective pressures that have occurred in the historical and pre-historical past and none sources cannot be neglected by either. Previous scientific evidence for the ancient spread of resistance alleles or pathogenic agents could become available through research on ancient DNA (Sonoda et al., 2000; Sallares and Gomzi, 2001; Hummel et al., 2005; Lidén et al., 2006; Zawicki and Witas, 2008) and this research field could be determinant in the comprehension of the interrelations with human genome and pathogenic agents in the last millennia.

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Table

Frequency	Date of colonisation	Distance/ frontier (km)	Sample size	Population	References
0.039	-600	-1290	576	Greece	Magierowska et al., 1998; Stephens et al., 1998; Martinson et al., 2000; Papa et al., 2000
0.032	-600	-1690	200	Greece, Crete	Apostolakis et al., 2005
0.027	-600	-1570	1368	Cyprus (Greeks)	Christodoulou et al., 1997; Martinson et al., 2000; Salem et al., 2007
0.096	-19	-1530	250	Spain, Oviedo	Alvarez et al., 1998
0.095	-218	-1880	100	Spain, Murcia	Libert et al., 1998
0.074	-214	-1190	145	Spain, Catalonia	Martinson et al., 1997; Lucotte and Mercier, 1998b
0.050	-44	-1220	100	Spain, San Sebastian	Magierowska et al., 1998
0.068	-44	-1310	118	Spain (Basques)	Martinson et al., 1997; Libert et al., 1998
0.038	-600	-1910	208	Spain, Sevilla	Ruiz et al., 2001
0.089	-237	-1380	45	Spain, Balears	Piras et al., 2005
0.073	-154	-1520	82	Spain (Spaniards)	Stephens et al., 1998; Su et al., 2000
0.064	-138	-1970	101	Portugal, Lisbon	Libert et al., 1998
0.052	-60	-1850	124	Portugal, Porto	Lucotte and Mercier, 1998b
0.047	-370	-920	484	Italy (Italians)	Stephens et al., 1998; Romano-Spica et al., 2000; Su et al., 2000
0.056	-218	-490	62	Italy, North	Lucotte and Mercier, 1998b
0.057	-400	-920	639	Italy, Rome	Martinson et al., 1997; Battiloro et al., 2000
0.047	-226	-400	371	Italy, Padua	Zamarchi et al., 1999
0.087	-222	-490	98	Italy, Milan	Libert et al., 1998
0.027	-600	-1200	700	Italy, Sardinia	Libert et al., 1998; Magierowska et al., 1998; Battiloro et al., 2000; Piras et al., 2005
0.040	-598	-1680	901	Italy, Sicily	Sidoti et al., 2005
0.108	-54	-580	101	France, Lille	Lucotte and Mercier, 1998b
0.087	-57	-510	276	France, Reims	Lucotte and Mercier, 1998b
0.111	-57	-350	291	France, Nancy and Strasbourg	Lucotte and Mercier, 1998b
0.122	-57	-630	344	France, Paris	Lucotte and Mercier, 1998b; Magierowska et al., 1998
0.089	-56	-680	230	France (Frenchs)	Stephens et al., 1998
0.123	-56	-1120	207	France, Brest	Libert et al., 1998; Lucotte and Mercier, 1998b
0.099	-121	-850	425	France, Montpellier	Libert et al., 1998; Lucotte and Mercier, 1998b
0.018	-56	-1200	111	France, Biarritz (Basques)	Lucotte and Mercier, 1998b
0.068	-121	-1010	102	France, Perpignan (Catalans)	Lucotte and Mercier, 1998b
0.052	-300	-700	124	France, Nice	Lucotte and Mercier, 1998b
0.032	-535	-920	229	France, Corsica	Lucotte and Mercier, 1998b; Piras et al., 2005
0.119	-57	-430	310	Belgium, Leuven (Flanders)	Struyf et al., 2000
0.092	-55	-490	704	Belgium, Brussels	Libert et al., 1998
0.085	-58	-420	64	Switzerland, Bern	Lucotte and Mercier, 1998b
0.045	106	-180	11	Romania	Martinson et al., 2000
0.100	6	-370	364	Netherlands	Dean et al., 2002
0.106	-12	-160	99	Germany, Mulheim	Lucotte and Mercier, 1998b
0.108	-12	0	208	Germany (Germans)	Stephens et al., 1998
0.115	125	-470	705	Great Britain (Britains)	Martinson et al., 1997; Stephens et al., 1998
0.104	-9	0	456	Hungary (Hungarians)	Libert et al., 1998; Magierowska et al., 1998; Szalai et al., 1998
0.083	-49	-310	495	Slovenia	Poljak et al., 1998; Stephens et al., 1998
0.082	-165	-540	73	Albanian	Stephens et al., 1998
0.051	-342	-610	104	Bulgaria	Magierowska et al., 1998; Stephens et al., 1998
0.089	14	0	36	Austrian	Stephens et al., 1998
0.071	14	-270	303	Croatia	Ristić et al., 2005
0.065	-59	-370	98	Croatia, Island of Rab, Lopar	Smoljanović et al., 2006
0.015	-228	-590	100	Croatia, Island of Vis, Komiža	Smoljanović et al., 2006
0.05	-438	-180	90	Ukraine, Crimea (Tatars)	Limborska et al., 2002
0.096		210	88	Ukraine, Lvov (Ukrainians)	Limborska et al., 2002
0.110		180	83	Ukraine, Kiev (Ukrainians)	Limborska et al., 2002
0.090		50	86	Ukraine, Lugansk (Ukrainians)	Limborska et al., 2002
0.14		120	335	Slovakia	Dean et al., 2002
0.107		160	547	Czech	Stephens et al., 1998; Petrek et al., 2000; Dean et al., 2002
0.142		1930	204	Sweden, Umea (Swedes)	Libert et al., 1998
0.140		1340	225	Sweden, Stockholm	Magierowska et al., 1998; Stephens et al., 1998
0.080		2250	120	Sweden, Lapland (Saamis)	Libert et al., 1998
0.120		700	183	Norway, Oslo	Libert et al., 1998; Magierowska et al., 1998
0.116		1310	329	Finland (Finns)	Libert et al., 1998; Stephens et al., 1998; ALFRED database
0.107		730	283	Lithuania, Vilnius (Lithuanians)	Libert et al., 1998
0.083		170	191	Ireland	Martinson et al., 1997; Stephens et al., 1998; ALFRED database
0.118		280	408	Denmark (Danes)	Libert et al., 1998; Lucotte and Mercier, 1998b; Stephens et al., 1998; ALFRED database
0.144		1220	662	Estonia	Stephens et al., 1998; Kalev et al., 2000
0.147		1410	102	Iceland	Martinson et al., 1997
0.138		870	133	Russia, Moscow (Russians)	Libert et al., 1998; Stephens et al., 1998
0.166		1480	33	Russian, St. Petersburg	Magierowska et al., 1998
0.120		660	78	Russia, Ryazan (Russians)	Slominsky et al., 1997
0.118		740	1005	Poland	Magierowska et al., 1998; Stephens et al., 1998; Jagodzinski et al., 2000; Su et al., 2000
0.16		760	80	Belarus (Belarusians)	Slominsky et al., 1997
0.080		50	56	Moldavia, Kishinev (Moldavians)	Limborska et al., 2002
North African and Near Eastern populations					
0.015			167	Morocco (Moroccans)	Elharti et al., 2000
0.010			145	Tunisia (Tunisians)	Barbouche et al., 2001
0.005			259	Leban (Lebaneses)	Stephens et al., 1998; Karam et al., 2004
0.012			175	Syria	Voevodin et al., 1999; Salem et al., 2007
0.054			194	Turkey	Libert et al., 1998; Stephens et al., 1998; Piras et al., 2005

Table 1

CCR5-Δ32 allelic frequencies in different locations in Europe, Middle East, and North Africa. Approximate colonization dates by Achaemenids, Greeks, Phoenicians-Carthaginians, Etrurians and Romans, and distances from the frontiers of the Roman Empire at its maximal extent are shown. As the colonization dates are not sure before the middle of the first millennium BC, the oldest dates retained are 600 BC. The distances are calculated from the towns when known; otherwise, they are calculated from the geographical centre of each country. Moreover, land distances have been prioritized versus marine.

Figures

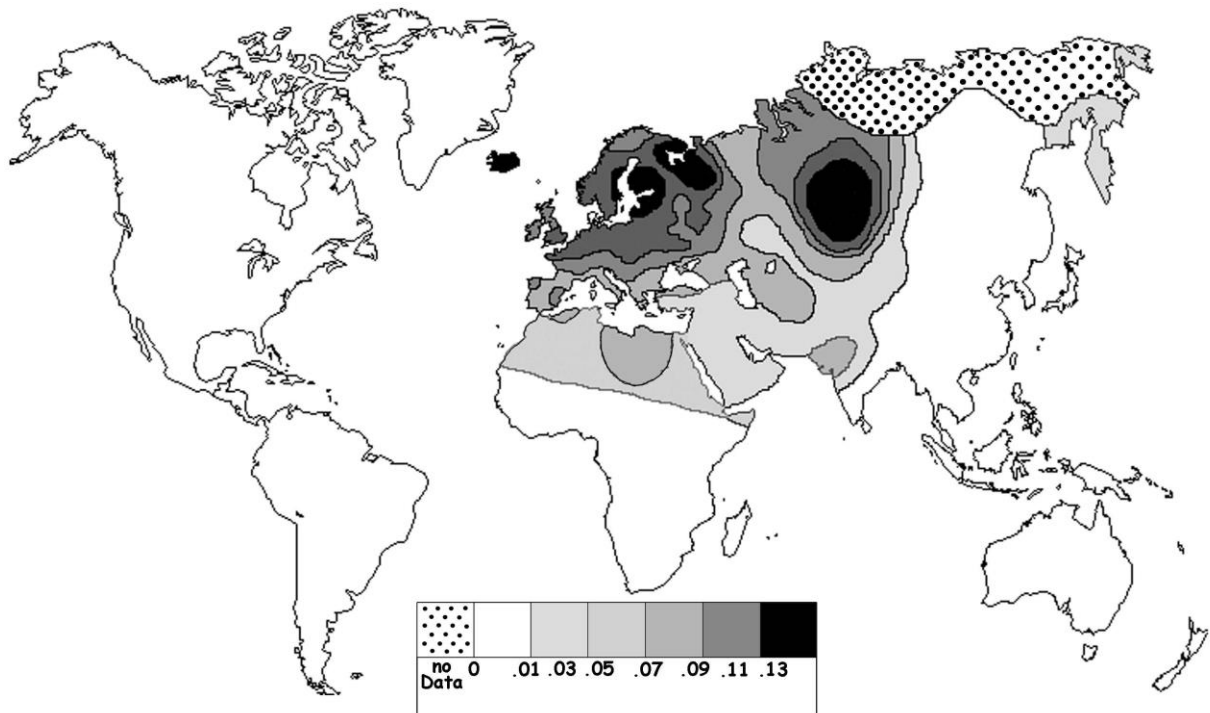


Fig. 1. Current world-wide frequency distribution of *CCR5-Δ32* allele frequencies. Only the frequencies of Native populations have been evidenced in Americas, Asia, Africa and Oceania. Map redrawn and modified principally from Balanovsky et al. (2005).

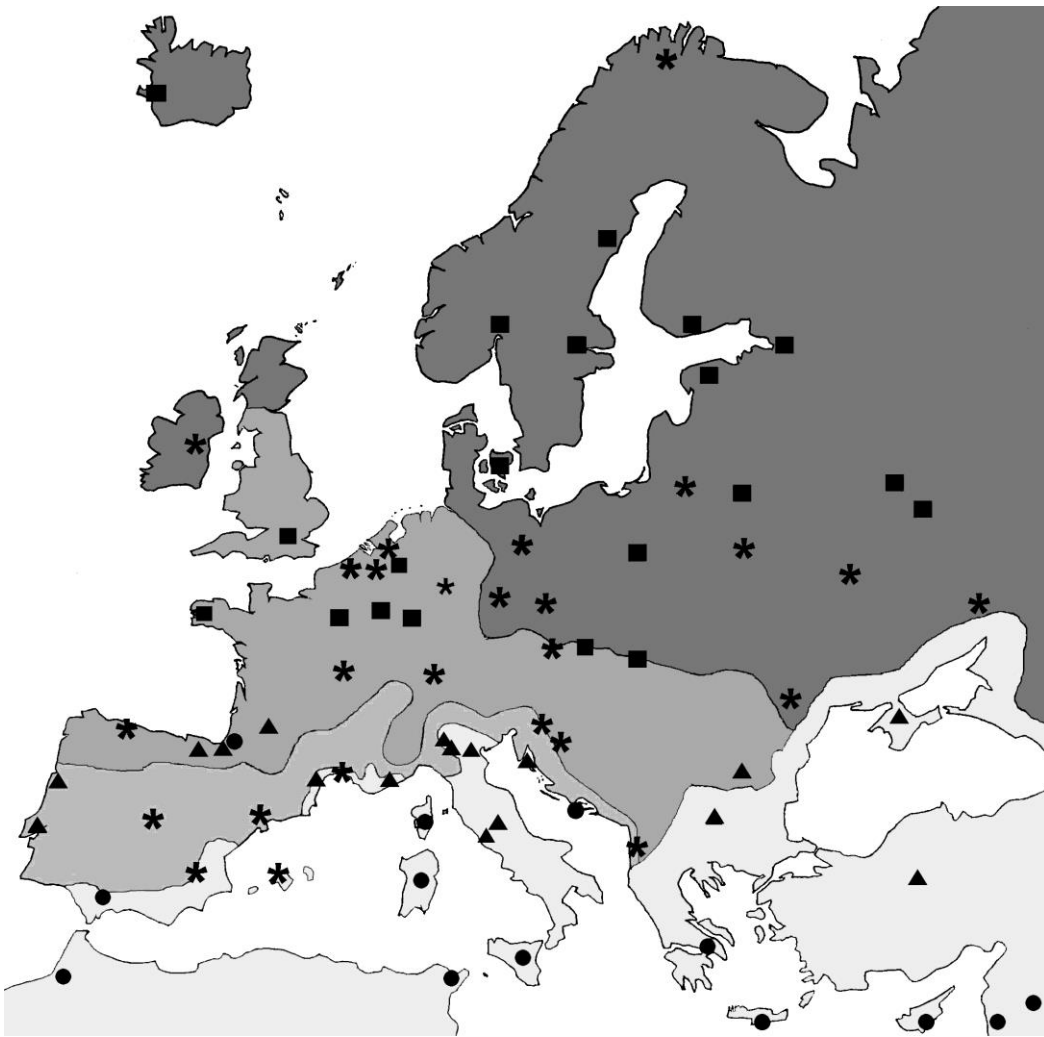
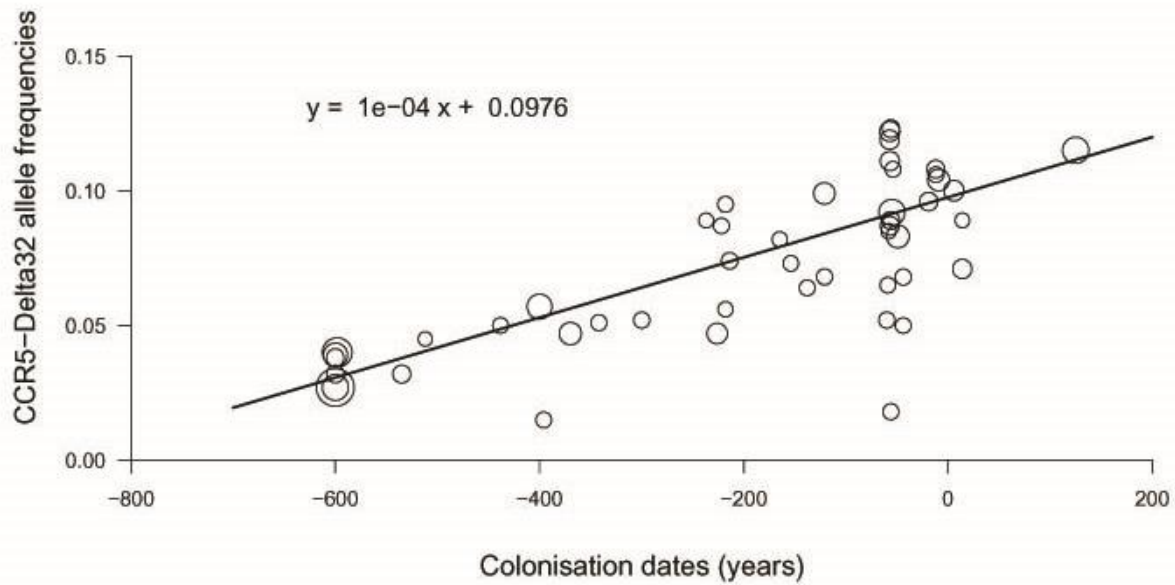


Fig. 2. Geographical maps showing the principal stages of the conquests of the ancient Mediterranean and Near Eastern Empires and the *CCR5-Δ32* allele frequency distribution. All the Roman, Etrurian, Greek, Phoenician, Carthaginian and Achaemenian territories and colonies conquered, even during a short period, such as Nova Britannia (nowadays South of Scotland), Magna Germania (approximately the former West Germany) and Iazygia (East part of Hungary) are shown. Moreover, at each stage, the territories conquered in the past and even those lost are represented. In very pale grey, pale grey and dark grey maximal extension in, respectively, 264 AD, 60 BC and around 163 BC; areas which never colonized by the Empires cited above are in very dark grey. The sampling locations are marked by black points; their form depends on the *CCR5-Δ32* frequency, i.e. closed circles (>0.01 to 0.04), triangles (>0.04 to 0.07), stars (>0.07 to 0.11) and squares (>0.11).

A



B

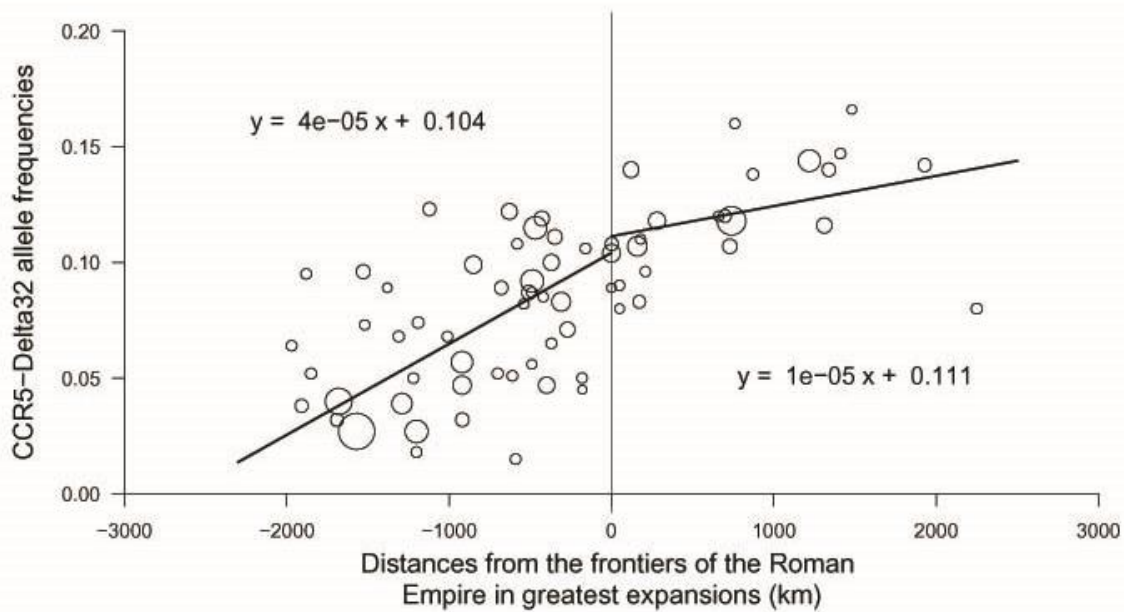


Fig. 3. Correlation plot between frequency of the *CCR5-Δ32* allele and European colonization events. Values are in table 1. (A), Correlation between the allele frequency and the first colonization date by Achaemenids, Greeks, Phoenicians-Carthaginians, Etrurians or Romans; (B), Correlation between the allele frequency and approximate distance from the Northern frontiers of the Roman Empire at its maximal extent (see Fig. 1). Inside (negative values) and outside (positive values) distances are considered separately. Dot size is proportional to the population size and represents the weight of each data point into the fitting criterion.

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