The Black Legend on the Spanish presence in the Low Countries: verifying shared beliefs on genetic ancestry

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Abstract

Objectives
War atrocities committed by the Spanish army in the Low Countries during the 16th century are so ingrained in the collective memory of Belgian and Dutch societies that they generally assume a signature of this history to be present in their genetic ancestry. Historians claim this assumption is a consequence of the so-called ‘Black Legend’ and negative propaganda portraying and remembering Spanish soldiers as extreme sexual aggressors. The impact of the presence of Spaniards during the Dutch Revolt on the genetic variation in the Low Countries has been verified in this study.

Materials & Methods
A recent population genetic analysis of Iberian-associated Y-chromosomal variation among Europe is enlarged with representative samples of Dutch (N = 250) and Flemish (N = 1087) males. Frequencies of these variants are also compared between donors whose oldest
reported paternal ancestors lived in – nowadays Flemish – cities affected by so-called Spanish Furies (N = 116) versus other patrilineages in current Flemish territory (N = 971).

Results

The frequencies of Y-chromosomal markers Z195 and SRY2627 decline steeply going north from Spain and the data for the Flemish and Dutch populations fits within this pattern. No trend of higher frequencies of these variants has been found within the well-ascertained samples associated with Spanish Fury cities.

Discussion

Although sexual aggression did occur in the 16th century, these activities did not leave a traceable “Spanish” genetic signature in the autochthonous genome of the Low Countries. Our results support the view that the ‘Black Legend’ and historical propaganda on sexual aggression have nurtured today’s incorrect assumptions regarding genetic ancestry.

Keywords: Ancestry testing, Collective history, Spanish army, Y chromosome, genetic genealogy, 16th century

Introduction

A genetic revolution is coursing through the field of human history, which was up until the last decade mainly trodden by historians and archaeologists (Krause and Paabo 2016). Large population genome studies and innovative ancient genome methods are providing new insights into human migration routes and population admixtures, and pinpoint when individuals received certain heritable physical characteristics in the past (Llamas et al. 2017). Today, population genetic research is especially focused on long-standing hypotheses about prehistorical migrations across the continents during e.g. the Neolithic and the Bronze Age (Lazaridis et al. 2014; Pagani et al. 2016). However, the genome is a palimpsest that also sheds light on the impact of historical and more recent demographic events on human populations (Jobling 2012). Therefore, the value of human history research increases considerably when a
reciprocal collaboration exists between geneticists, historians and archaeologists (Jobling 2012; Nature 2016; Vander Linden In press). This does not only mean that geneticists need to interrogate historians to elucidate observed population genetic patterns but also that historians have to address geneticists to validate particular hypotheses formulated within their discipline.

One heated controversy among historians is to what extent the propaganda against the presence of Spanish rulers and soldiers in the at that time united Low Countries has influenced the current collective memory of the nowadays divided Belgium and the Netherlands. In the 16th century, a rebellion started in the Low Countries, partly ignited by the political and religious hegemony of their overlord, Philip II, also King of Spain, and partly inspired by the new conflict between Catholics and Protestants (Parker and González de León 1999). Tensions increased further when the King sent an army of 10,000 soldiers under the command of the Duke of Alba from Spain to his most northern provinces in 1566 (Parker 1990a). Particularly in the period 1572-1578, several cities in the Low Countries fell victim to the so-called Spanish Furies, with mutinous and underpaid troops plundering, raping and murdering on a large scale (Parker 1973; Soen 2008). Philip II regained control over most of the rebelling regions in the south, which left most of present-day Belgium under the reign of Spain until the Peace of Utrecht in 1713 (Parker 1990b). The northern provinces continued their resistance that eventually led to the Dutch independence, recognized by the Spanish King in 1648.

During the revolt, the royal army consisting of mainly Spanish soldiers received the reputation of being a band of murderers, robbers and rapists. Historians have long pointed out that opponents to the royal regime in the Low Countries adopted the earlier existing ‘Black Legend’ to describe the Spanish as extremely barbarous and cruel (Airey 2012; Swart 1975). As early as the end of the 15th century in a time of strong rivalry between the main European political powers, this intensive propaganda against Spain started to spread after their acts towards the native inhabitants of the Americas, the Jews and Moors in Spain and towards Protestants elsewhere on the European continent (Hillgarth 2000; Pérez 2009; Schneider 2008). The negative propaganda was successfully adopted by the revolting provinces in the form of numerous pamphlets, literature and paintings (Eekhout 2014; Thomas 1990). Especially depictions of sexual violence were accentuated in this long-term campaign to vilify the Spanish
(Pipkin 2009; Swart 1975), which might have added to current collective memory in Belgium and the Netherlands arguing that occasional dark hair and skin colour or pronounced eyebrows stem from the 16th-century rapes. Although the phenotypic variation in these populations fit well within the North-South cline in Europe (Frost 2006), it is not exceptional that many Belgian and Dutch individuals take part in genetic ancestry testing specifically to verify the contribution of Spanish DNA to their genome (Larmuseau 2017).

Whether there is a Spanish genetic signature in the Low Countries associated with the events in the 16th century or not is an interesting research question due to its academic controversy and the strong collective ideas about genetic ancestry. As of yet, European-wide population genetic studies do not indicate that the autochthonous Belgian and Dutch people are more linked to the Spaniards in comparison with neighbouring populations. The individuals in the Low Countries can be placed exactly between the genomes from autochthonous individuals of France, Germany, Denmark and the UK when looking at two-dimensional summaries of genetic variation in Europeans (Lao et al. 2008; Novembre et al. 2008). Also more in-depth genomic analyses show a clear North-South cline within the Netherlands which is part of the much broader cline over the European continent in accordance to their geographical position (Abdellaoui et al. 2013; Francioli et al. 2014; Lao et al. 2013). Moreover, no admixture history between both populations is yet inferred by genomic data and appropriate statistical methods to date (Hellenthal et al. 2014). In contrast to these results, a recent study interpreted the occurrence of a specific founder mutation associated with a heart disease in several families from the South of the Netherlands directly with the Spanish interventions during the 16th century (Claes et al. 2016). Next to the presence in several Dutch families, this mutation was also observed in one Spanish family besides a German family. Since the migration time nor direction of this specific mutation was determined, it is hardly clear whether there is a Spanish footprint in the genetic history of the Low Countries.

Recent studies described deep phylogenetic Y-chromosomal lineages which are strongly associated with the Iberian Peninsula (Solé-Morata et al. 2017; Villaescusa et al. 2017). Samples from the Low Countries were not included in these European-wide studies despite them being highly informative in testing any Spanish signature present in the Dutch and Flemish populations due to war crimes in the 16th century. The Y chromosome has a high
informative value when looking at male-biased historical migration events (Jobling and Tyler-Smith 2017), especially since the Y-chromosomal variation might be linked to historical populations by using in-depth patrilineages (Larmuseau et al. 2013a). Therefore, our research hypothesis is that, if there is indeed a Spanish signature present in the autochthonous genetic variation of the Low Countries, the frequencies of the Y-chromosomal lineages which are strongly associated with the Iberian Peninsula, namely R1b-Z195 and R1b-SRY2627, should show an aberrant pattern among the autochthonous Belgian and Dutch families according to the distribution clines across Europe described by Solé-Morata et al. (2017). Moreover, by linking in-depth genealogical data with the Y-chromosomes, population samples of males who are patrilineally associated to particular communities in current Flanders historically affected by the Spanish Furies (Parker 1973), namely Mechelen (1572), Aalst (1576), Antwerp (1576) and Zichem (1578), would expose more Iberian-specific Y-chromosomal variants in comparison with samples from other regions of current Flanders, since the Spanish heritage is expected to be higher in those Spanish Furies communities as a result of ‘rape’ and ‘bastard children’ (Parker 1990a; 1990b). As such it will be tested whether the Spanish army did indeed leave a demographic impact on the population of such extent as they are credited for in the collective memory.

Materials and Methods

Sampling selection

For Belgium, we focused on the population in contemporary Flanders, the northern half of Belgium, since most of the Spanish Furies happened in this region and the general willingness to participate in genetic research is highly limited in Wallonia, the southern part of the country (Larmuseau et al. 2015). To avoid any confusion with anachronisms, we define ‘Flanders’ in this study by the political region which is so-called nowadays. Therefore, the term does not refer to the historical ‘County of Flanders’ which corresponds mainly to the present provinces West Flanders and East Flanders. Samples for the Flemish population were selected from the genetic genealogical databank at the KU Leuven (University of Leuven, Belgium) with currently 1609 samples in total (July 2017 - without the sample collection of the project ‘De Gen-iale
Stamboom’ which was collected in a way that was not appropriate for this study. The analysis was approved by the ‘Medical Ethical Committee’, the official institutional review board (IRB) of the KU Leuven – University of Leuven (procedure number S55864). All the samples were collected with written consent from the donors who gave their permission for Y-chromosomal analysis, storage of the samples, genealogical research, and scientific publication of their anonymized DNA results. The legal in-depth patrilineages of the male donors were collected within a citizen science project (Soen and Huyse 2016) and afterwards checked individually with the copies of the parish records and the civil registry of the State Archives in Belgium (www.arch.be). We selected those DNA donors with available patrilineal genealogical data up until an oldest reported paternal ancestor (ORPA) born within the present borders of Flanders before the year 1800. When samples of other related persons in direct paternal line or persons with the same surname (or spelling variants) were present in the dataset, only one individual for each patrilineage and surname was selected at random to avoid any familial bias in the analysis. Possible over- or underrepresentation of a certain region within current Flanders was accounted for as well, to provide a representative genetic genealogical sample for the total population.

In this genetic genealogical sample of Flanders, we further selected those individuals which had their genealogical roots in one of the four cities where a well-known Spanish Fury occurred within the period 1576-1578, namely in Antwerp, Mechelen, Aalst or Zichem. A DNA donor was associated with one of the four cities according to the criteria for communal samples given in Larmuseau et al. (2015), namely (a) their surname was present among the inhabitants of that city in circa 1600 based on archival documents (e.g. parish records, burgher lists, penningkohieren, gichten, etc) and (b) their ORPA was born before 1700, in a place less than 5 km away from the centre of that city.

Finally, for the Dutch sample, we collected the publicly available Y-SNP data of the fathers of 250 trios from the Genome of the Netherlands Project (Francioli et al. 2014). The places of residence of those unrelated trios are evenly distributed among the Netherlands, constituting a representative sample of the entire autochthonous population (Boomsma et al. 2014).
Y-chromosomal analysis

The selected Flemish population sample is a combination of samples which were already in-depth SNP-typed for the Y chromosome in previous studies (Larmuseau et al., 2012a, 2014b) and newly collected samples from the ongoing genetic genealogical project of the KU Leuven (Supplementary Table S1). After DNA extraction of the buccal swabs with SwabSolution (Promega, Madison, WI, USA), the additional samples were genotyped by using multiplex SNAPSHOT mini-sequencing assays (Thermo Fisher Scientific, Waltham, MA, USA) to assign the subhaplogroup at the highest resolution level of the latest Y-chromosomal tree reported by Karafet et al. (2008), including extra Y-SNPs within haplogroup R-M269 as given in Supplementary Figure S1.

Population differentiation

Reported frequencies of Y-SNP markers Z195 and SRY2627 in European populations were collected from Solé-Morata et al. (2017) and joined with the ones in Flanders (including the frequencies for the Spanish Fury cities versus the rest of current Flanders) and the Netherlands. Haplogroup frequency maps were drawn with SURFER v. 12 (Golden Software, Golden CO, USA). Differences in the frequencies of Z195 and SRY2627 between the samples were assessed by Fisher exact tests implemented in R (The R Foundation for Statistical Computing 2011) with Bonferroni corrections for pairwise calculations (Rice 1989).

Results

The genetic genealogical sample for Flanders contained 1087 Y-chromosomal profiles in total after rigorous selection. This group includes 709 previously genotyped samples (Larmuseau et al., 2012a, 2014b) and 378 newly collected samples (see Supplementary Table S1, including the list of subhaplogroups with their nomenclature according to van Oven et al. (2014)). From these profiles and according to the stringent criteria, 116 samples were genealogically associated to one of the four cities in current Flanders where a Spanish Fury occurred. The frequencies of R1b-Z195 and R1b-SRY2627 in these samples and in the Dutch sample are given
in Table 1 and illustrated by the contour maps in Figure 1, together with the frequencies of
the other European populations which were already analysed in Solé-Morata et al. (2017). For
Z195, the frequencies in Flanders and the Netherlands were 4.1% and 4.4% respectively (non-
significant difference, p>0.05), and were significantly (p<0.01) lower than in Nord-Pas-de-
Calais (12.5%) and Île-de-France (10.3%), and non-significantly lower than in Great-Britain
(8.7%; p>0.05). For SRY2627, a lower frequency was found in the Netherlands (0.4%) versus
Flanders (1.0%), both lower than in Northern France (1.2-3.2%) and Great Britain (4.4%). All
these pair-wise differences for SRY2627 were non-significant (p>0.05). Furthermore, there
were no significant differences observed in the frequencies of Z195 and SRY2627 between the
samples associated with the Spanish Fury cities versus the rest of Flanders (p>0.05). More so,
even no trend of a higher occurrence of these subhaplogroups was found between both well-
ascertained samples (Table 1).

Discussion

In this study, the occurrence of two Y-chromosomal subhaplogroups which are associated with
the Iberian Peninsula, namely R1b-Z195 and R1b-SRY2627, has been investigated within the
Flemish and Dutch populations. Both lineages are present in the autochthonous populations,
however, the substantially lower frequencies in comparison with the ones in neighbouring
Northern French populations are consistent with the North-South cline of both lineages in
Europe as earlier reported by Solé-Morata et al. (2017). Even a significantly lower frequency
of Z195 was found in Flanders and the Netherlands (both ca 4%) versus neighbouring regions
in Northern France (10-12%), indicating that there is no higher occurrence of Iberian-specific
variants associated with a historical gene flow event in the Low Countries. Moreover, no
difference or even trend of higher Iberian Y-chromosomes was found between well-
ascertained patrilineages of cities where Spanish Furies occurred in the 16th century versus
the rest of current Flanders. Since the genetic genealogical approach associates Y-
chromosomes to specific locations based on archival records and in-depth patrilineages
(Larmuseau et al. 2013a), it is now possible to analyse the Y-chromosomal variation within
those specific communities close to the events in the 16th century, thereby avoiding
disturbances in any population genetic pattern due to more recent migration events like the
Industrial Revolution in the 19th century (Larmuseau et al. 2012a). Therefore, a significant difference or even trend for a higher frequency of these Iberian haplogroups was expected if the Spanish army had indeed left a genetic signature in the population. Although the Royal army was heterogeneous, with soldiers hailing from different regions and countries, about three quarters of the soldiers sent to the Low Countries originated from Spain and especially the Basque Country figured prominently, with the frequencies of both markers being among the highest in Europe (Fig. 1) (Parker 1990a; 1990b; Solé-Morata et al. 2017). The earlier observed trend of higher frequency for marker Z195 in Nord-Pas-de-Calais, that belonged in part of the Habsburg Netherlands, than in middle French regions was not indicative for the rest of the Low Countries, as expected based on earlier Y-chromosomal analysis (Larmuseau et al. 2014; 2012b).

The lack of a Spanish DNA signature based on the global Y-chromosomal variation in the populations is consistent with full genome analyses and advanced admixture methods (Abdellaoui et al. 2013; Francioli et al. 2014; Hellenthal et al. 2014; Lao et al. 2008; Novembre et al. 2008). The only published designation of a genetic link between Spain and the Netherlands for the 16th century is a recently described mutation associated with a heart disease that is found in several families from the southern part of the Netherlands as well as in one German and one Spanish family (Claes et al. 2016). Nevertheless, only the time frame when the mutation arose has been calculated so far to establish their claim, namely between 1060 and 1580 with a generation time of 25 years, and between 670 and 1405 with a generation time of 35 years. This result indicates a founder mutation linking the families together on a historical time scale; however, it gives no indication when and in which direction the mutation migrated across Europe. Associating the mutation distribution directly to the presence of the Spanish troops in Southern Netherlands and not suggesting any other viable hypothesis, is exemplary for the historical cherry-picking that leads to the arbitrariness in deciding what kind of past events could have given rise to modern genetic diversity (Jobling 2012).

Until additional data are available for this specific mutation, one of the most promising possibilities to verify any genetic impact of the Spanish army on the native population is by studying numerous ancient genomes from just before and after the Dutch revolt. The large-
scale excavation of the 13th-17th century churchyard in the Dutch city Eindhoven and the associated ancient DNA analysis is one of the research projects that will provide further insights on this topic, as Eindhoven is the region where the particular mutation has been associated with the presence of Spanish troops (Claes et al. 2016). Nevertheless, preliminary results of Y-chromosomal variation before and after the 16th century do not show any specific discontinuity associated to a specific admixture event (Altena et al. 2013), suggesting again that there was indeed no Spanish impact on the population-wide genetic variation in the Low Countries.

Based on the historical records it is clear that the Spanish army effectively committed war atrocities including rape in the Low Countries during the 16th century and especially during the Spanish Furies (Pipkin 2013; Soen 2008). Moreover, it could be expected that some native women would have preferred the company of particular Spanish soldiers (Swart 1975), and that in the wake of the Spanish rule over the Southern Netherlands until 1713, mixed marriages occurred between Spaniards and the natives, to which a limited number of families and surnames bare witness until today, e.g. Desmadryl and Serrano (Soen et al. 2017). Nevertheless, the impact of these circumstances on the genetic variation was not so substantial as to have a recognisable signature in the current autochthonous population, not even within a genetic genealogical sample of those communities that became victims of a Spanish Fury in the second half of the 16th century. Therefore, it is important to understand why this collectively expected signature is not confirmed in the genetic ancestry.

A collectively expected signature due to historical propaganda

Historiography has always mainly focused on politics, battles and the higher socio-economic classes in society (Vandenbroeke 1986). However, only demographic events have had an impact on population genetic patterns (Jobling 2012). Due to this imbalance, the impression exists that dominant events in national and local history also left a footprint in the global society and therefore resulted in a population-wide DNA signature. Published genetic signatures of historical migration events, however, showed that this is almost always the result of an extensive settlement of the migrant group containing both males and females, which definitely had a demographic impact, e.g. Phoenician merchant colonies among the
Mediterranean Sea (Zalloua et al. 2008), Viking settlements in Northwest England (Bowden et al. 2008), and Greek colonists in Sicily (Tofanelli et al. 2016). According to simulations, there should have been at least some thousands of breeding men and hundreds of breeding women settled to observe the current genetic signature of the Greek colonists in Sicily (Tofanelli et al. 2016). A similar demographic impact of the Spanish army on the Low Countries populations cannot be assumed by rape only during the Spanish Fury, even on a huge scale. This is especially the case because rape does not always lead to fertilisation and since women were often murdered afterwards during the Spanish Furies (Airey 2012). Moreover, if ‘Spanish’ children were born after all, it is also very likely that they might have been stigmatised and less well cared for. Therefore, they would not have the same chances of survival as other children, which might be analogous to the lower fitness and higher mortality observed for bastard children born without a legal father in the past, as demonstrated in several historical populations (Brandstrom 1996; Gardarsdóttir 2000; Kok et al. 1997). The fact that a hostile army generally does not have a strong genetic impact through means of sexual aggression alone is also indicated by the close link between Y-chromosomes, surnames and patrilineages in many European populations (King and Jobling 2009; Larmuseau et al. 2013b; Solé-Morata et al. 2015). Nevertheless, future research on historical extra-pair paternity (EPP) with a specific focus on regions which were often occupied in the past, will further provide insights on the impact of sexual aggression on population-wide genetic variation (Larmuseau et al. 2016).

Consequently, it is remarkable that a fixation on a possible Spanish signature in Flemish and Dutch DNA persists in the collective memory, as compared to a much lower interest in traces of other historical foreign opponents. This fixation has mainly its origin in schoolbooks and popular literature such as comics which propagate the stereotype of the extremely cruel Spanish soldier in the 16th century (Eekhout 2014). Because of this, the historical propaganda campaign still has an impact on the collective memory in both Dutch and Belgian populations until this day. This success is the result of adopting the already popular ‘Black Legend’ to create a national identity in newly created countries. It is well known that in the new Dutch republic of the late 16th and early 17th century, opponents tried to refrain from the Spaniards by picturing them especially as ‘raping tyrants’ as a means of fostering identity in the new republic (Pipkin 2009; 2013; Swart 1975). Also in Belgium a similar propaganda appeared much
later in time, namely in the 19th century after the birth of the country, when liberal forces
wanted to create a Belgian identity based on historical grounds by imposing a negative image
on former oppressors (Tollebeek et al. 1998). Due to the success of these political strategies,
historians in Belgium and the Netherlands are still fighting against the many prejudices and
wrong assumptions about what happened exactly in the Low Countries in the 16th century
(Swart 1975; Thomas 1992). In the last decades, historians have started numerous initiatives
to provide much more data about this period, and the genetic results will further foster these
efforts in an attempt to change collective perceptions about the demographic impact of these

Conclusion

In the current postgenomic and ancient genome era, human population geneticists are mainly
focused on (pre)historical migrations and admixture events to explain observed genetic
patterns. With this study on the collectively expected Spanish impact on Low Countries’ DNA,
we have shown that the lack of a genetic signature in a population might still be interesting
from a biological as well as historical viewpoint. Firstly, it provides information on whether a
historical event had a demographic impact on the population under study. This demographic
impact is an important issue on which genetics can shed light in addition to the historical
record (Jobling 2012; Nature 2016). Next to verifying the credibility of historical stories,
popular sagas and folk tales (Gibbons 2017), the lack of an expected genetic footprint might
be informative when it illustrates how particular political propaganda became part of the
collective memory (Barash 2016). The current genetic falsification of a collectively expected
Spanish signature present in the Dutch and Flemish populations is an important new element
in the heated controversy among historians about the ongoing influence of the ‘Black Legend’
related to the Spanish war crimes in the 16th century. Nonetheless, changing a collective idea
about the historical past will be highly challenging when only communicating raw genetic
results to the broad public. This is especially the case in the current era of highly popular direct
to consumer (DTC) genetic companies who widely advertise the idea that DNA easily informs
the client of an unexpected ancestry (Moray et al. 2017; Phillips 2016; Su 2013). Therefore,
such research requires close interdisciplinary collaborations between geneticists, historians
and experts in social communication. Only the successful union of the fields of science and humanities will provide further insights in our species’ history, which then might be recorded in our collective memory.

**Data archiving**

The Y-SNP data of all Flemish samples are available on the YHRD database (www.yhrd.org) with accession numbers YA003651, YA003652, YA003653, YA003738, YA003739, YA003740, YA003741, YA003742, YA003739, YA003740 and YA003742.

**Acknowledgments**

The authors would like to thank Prof. Dries Raeymaekers, Dr. Gert Gielis, Dr. Werner Thomas and Drs. Sophie Verreyken for inspiring discussions, Kelly Nivelle for reading a previous version of the manuscript, and Familieke Vlaamers vzw for assisting in the sampling and data collection. MHDL is a postdoctoral fellow of the Fund for Scientific Research – Flanders (FWO-Vlaanderen). Funding was provided by KU Leuven (BOF-C1 grant C12/15/013) and the Fund for Scientific Research – Flanders (Research grant number 1503216N), and by Agència de Gestió d’Ajuts Universitaris i de la Recerca (Generalitat de Catalunya) grant 2014 SGR 866.

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