

Article

The Origins of the Royal Spanish Surname Castilla: Genetics and Genealogy

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Abstract: In most Western European societies, surnames pass from generation to generation and in cases where surnames are shared by fathers to children, the Y chromosome passes down from fathers to male offspring in the same way as surnames do. The aim of this study was to ascertain the patrilineal relationship between individuals with the surname “Castilla” and their respective Y-chromosome haplotypes. The toponymic surname “Castilla” is part of the Spanish royal family. Genealogical studies of this surname have allowed the formulation of different hypotheses about its origin, most of which were centered in Burgos. To shed some light on the origin of the surname Castilla and to investigate the possible co-ancestry behind the living carriers of this surname, markers located in the Y chromosome-specific region were analyzed in a sample of 102 men whose paternal surname was Castilla. The study aimed to establish the minimum number of founders and the expansion time of the lineages from our sample. Two major haplogroups were identified: R1b and E1b1b-M81. The high frequency of the E1b1b-M81 haplogroup in comparison to that of the general Spanish population, its low haplotype diversity, and its young TMRCA (323+ / – 255 years CE) are compatible with the historical timing of the obligation to use surnames. However, the coincidence of the most common haplogroup in the Castilla sample and the most frequent haplogroup in the Spanish general population, R1b, makes it difficult to identify founder haplotypes/haplogroups in the history of the Castilla surname.



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1. Introduction

The origin of the “Castilla” surname has been a subject of debate among genealogists for many years. Males bearing the “Castilla” surname (referred to as Castilla men in this article) have been part of the Spanish royal family. This was the case for Pedro I de Castilla, el Cruel, and Isabel de Castilla (Isabel La Católica). Currently, there are different theories regarding the origin of this surname, which are not exclusive.

Documentary sources indicate that the origin of this surname was King Pedro I de Castilla, el Cruel, born in Burgos (north of Spain) in 1334 and died in Montiel, Valladolid (central Spain) in 1369. The son of Alfonso XI and María de Portugal, Pedro I was king in 1350. He had nine children, two of whom were males with offspring: Juan de Castilla y de Castro (1355–1405) and Diego de Castilla y de Sandoval (?–1458). Other documents indicate that the first male to bear this surname was Tello, the first count of Vizcaya. Tello was an illegitimate son of the king Alfonso XI and Leonor de Guzmán. Thus, the two men had a common ancestor, Alfonso XI.

Another possibility is that the surname refers to a specific place. The County of Castilla was one of the medieval counties of the Kingdom of León (850–1035), and the Kingdom of Castilla was one of the medieval kingdoms of the Iberian Peninsula (1035–1230). With

the promulgation of the Spanish Constitution of 1978, two autonomous communities were recognized: Castilla-La Mancha and Castilla y León. According to some specialists, 77% of Spanish surnames are toponyms (Álvarez 1968).

The third possibility is that some Castilla men bear the surname because of modifications to the other surnames. For instance, there are documents about a family that changed the surname Ruiz Castilla to Castilla (Lucas 2008).

The study of different documents (civil records, parish records, etc.) has allowed the compilation of hundreds of genealogies. Generally, these genealogies indicate that there are, at least, three lineages (Lucas 2008; López-Parra et al. 2005).

- (1) Lineage of descendants of Pedro I el Cruel.
- (2) Lineage of Vadocondes (southern Burgos). These Castilla men have a common ancestor, Juan de Castilla de Vadocondes, born about 1550.
- (3) Lineage of Rubena (northern Burgos). Castilla men have two possible ancestors: Pedro de Castilla de Rubena (1510) and Blas de Castilla de Rubena (1580).

Moreover, this surname is present in South America: in “Catálogo de Pasajeros a Indias”, there is a reference to Don Pedro de Castilla in 1560.

At present, this surname is widely represented in Spain (15,255 people with the first surname Castilla reported by the Spanish National Statistical Institute 1 January 2021), although it is not among the 100 most frequent surnames in Spain; there are 26,223 surnames with more than 100 bearers, men and women living in Spain, and the Castilla surname is in position 336. Nowadays, in the Iberian Peninsula, the largest number of people whose first surname is Castilla is located in Huelva (2.51%).

Although the Romans brought surnames to the Iberian Peninsula, they were not used until the end of the 9th century. Patronymics were the most used surnames, and the nobles were the only ones to use them. In documents from the end of the 12th century CE, surnames related to place of origin can be found (Salazar and Acha 1991). In the 15th century, hereditary surnames were relatively consolidated, mainly due to the initiative of Cardinal Cisneros, who made it mandatory to register in the parishes the marriages, births, and deaths of all the inhabitants, with information of their parents.

In most European and Hispano-American societies, surnames pass from generation to generation, from fathers to children, as the Y chromosome does. Males who share the same surname can be expected to belong to the same Y-chromosome haplogroup (Jobling and Tyler-Smith 2003). Therefore, the comparison of DNA results and data in archives can, in theory, be useful for solving problems in the reconstruction of genealogies. However, this correlation only holds true if specific assumptions are made, namely: the surname has a unique origin, there were no illegitimacies (or adoptions), there were no deliberate surname changes responsible for the introduction of new Y-chromosome types, belonging to another surname group, and finally, Y chromosomes associated with different surnames must have been unrelated at the time of surname establishment (Jobling 2001; King and Jobling 2009). Mutations and genetic drift are also factors that need to be considered (King and Jobling 2009). Mutations change the alleles of a haplotype of the Y chromosome in rapidly mutating markers (Gusmão et al. 2005). Changes in the frequencies of the haplotypes by chance (genetic drift), together with the type of surname, for example, from a region or a profession, could favor the phenomena of associations of different haplotypes/haplogroups with the same surname.

Few molecular studies have been published on the analysis of a specific surname and its relationship with Y-chromosome haplotypes. The study of the surname Jefferson in a case of paternity of Thomas Jefferson (King et al. 2007), the research regarding four microsatellites of Y chromosomes in 48 men bearing the surname Sykes (Sykes and Irven 2000), surname Ye in China (Zeng et al. 2019), and the case of the surname Colom in Spain are some examples (Martínez-González et al. 2012).

The main goal of this study was to shed some light on the origin of the surname Castilla and to investigate the possible co-ancestry behind the living carriers of this surname. Genetic markers located in the Y chromosome-specific region were used to characterize

the male lineages present in a sample of individuals whose paternal surname was Castilla. Thus, we pretended to establish the minimum number of founders and the expansion time of the lineages in our sample.

2. Results

Haplotype, haplogroup, and geographic origin for each individual can be found in Table S1 and Table 1. By analyzing Y-STRs, 55 different haplotypes were identified in the overall sample of 102 individuals. Of these, 71% were unique. This value is much lower than that of a general series of Spaniards in which 125 haplotypes were found in 148 individuals (91.2%).

Table 1. Haplogroup frequencies in Castilla samples and the Spanish reference population. N: sample size, Freq: frequency of haplogroup, K: number of haplogroups. HD: haplotype diversity.

HG	Castillas				Spain			
	N	Freq	K	HD	N	Freq	K	HD
E1b1b > V13	0	0.000			2	0.014	2	1.000
E1b1b V257 > PF2431	1	0.010			0	0.000		
E1b1b V257 > M81	14	0.137	3	0.473	6	0.041	4	0.800
G1a CTS11562	1	0.010			0	0.000		
G2a2a PF3147 >> L91	0	0.000			1	0.007		
H1a1a M82	1	0.010			1	0.007		
I1	2	0.020	1	0.000	0	0.000		
I2a1a M26	1	0.010			4	0.027	4	1.000
I2a2a M223	0	0.000			3	0.020	2	0.668
J1a2a1a2 P58	0	0.000			2	0.014	2	1.000
J2a1 M319	0	0.000			1	0.007		
J2b2a M241	0	0.000			1	0.007		
R1a M198	1	0.010			1	0.007		
R1b M269	47	0.461	30	0.967	89	0.601	81	0.998
T-L131 > CTS933	3	0.029	2	0.667	0	0.000		
T >> CTS11451 > PF7455	0	0.000			2	0.014	2	1.000
Others	31	0.304			35	0.236		
TOTAL	102		60	0.978	148		135	0.999

The Castilla groups, according to their origin, presented HD values similar to those observed in Spanish populations (Martin et al. 2004) (Table S1). The lowest values were observed for Burgos, Avila, Salamanca, and Granada. However, HMP in Castilla as a group was more than double that in the Spanish population (Table S1).

When the distribution of haplogroups in the Castilla sample was considered, it was similar to that of the Spanish population (Flores et al. 2004) (Table 1). The R1b haplogroup was present in 49% of men with the surname Castilla; however, the frequency of the E1b1-M81 haplogroup (13.9%) was much higher than that found in the Iberian Peninsula population (4.03%) (López-Parra 2008). Haplogroups O1b1 and Q-M346 were more frequent in the Asian and American populations. These haplogroups were present among the samples collected in this study, although at a low frequency, similar to the general Spanish population (Flores et al. 2004).

The surname Castilla can be considered a frequent surname, with a number of bearers of 15,255 individuals in the Spanish population (INE 2021). Haplotype diversity was compared with the frequency of the surname. When considering other Spanish surnames for which information is available for the same markers (Martínez-Cadenas et al. 2016), the pattern resembles that of frequent surnames ($R^2:0.402$) (Figure 1a). In addition, when representing the distribution of the samples collected according to their province of origin, a strong correlation was observed ($R^2:0.683$) (Figure 1b). This shows that areas where Castilla is less frequent have higher HMP values.

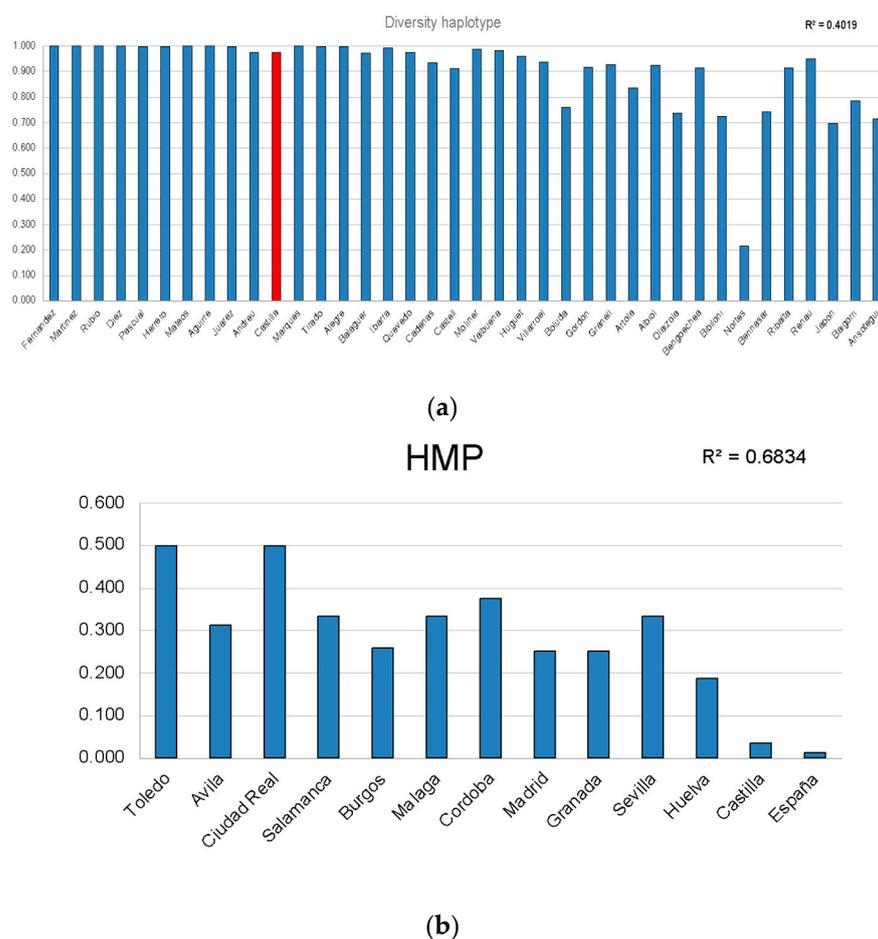


Figure 1. (a) Distribution of the HD (haplotype diversity) in Spanish and Castilla men. Surnames ordered by reverse frequency rank. Red HD of the Castilla sample. The HD of the Spanish population was calculated for the same markers analyzed in Castilla. (b) Distribution of haplotype match probability (HMP) in Castilla men according to their geographical origin. Surnames ordered by reverse frequency rank.

It was only possible to create networks for the haplotypes of the R1b and E-M81 haplogroups, as the other haplogroups did not meet the criteria for performing the network since there were not at least three different haplotypes. Regarding the E-M81 haplogroup, it was observed that 50% of the haplotypes corresponded to individuals from Burgos, and that all but one belonged to the same cluster. The two haplotypes that presented a lower frequency were only one step away, in one case in *DYS389I* and in the other *DYS389II* (Figure 2).

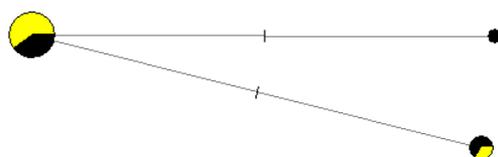


Figure 2. Reduced network for the E-M81 haplogroup. Color legend: yellow: haplotypes of Castilla from the south of Burgos, and black: haplotypes of Castilla from other origins.

Regarding the network of the R1b haplogroup, one possible ancestral descent cluster was observed, which comprised 58% of the total Castilla R1b population (Figure 3). This descent cluster was compatible with the founder effect. It is also characterized by individuals whose origin is in the north of the Iberian Peninsula (Figure 4), specifically in Ávila,

Salamanca, and Burgos. Since two smaller descent clusters were observed, grouping five and four haplotypes, more than 77% of the haplotypes R1b obtained were within these three main clusters or in descent clusters.

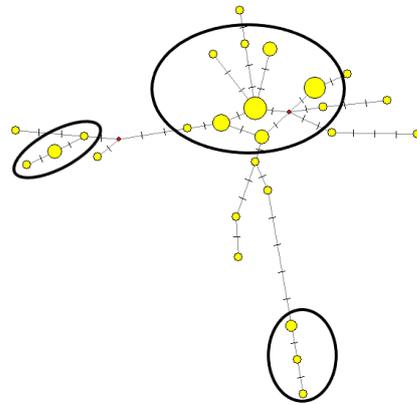


Figure 3. Reduced network for the R1b haplogroup.

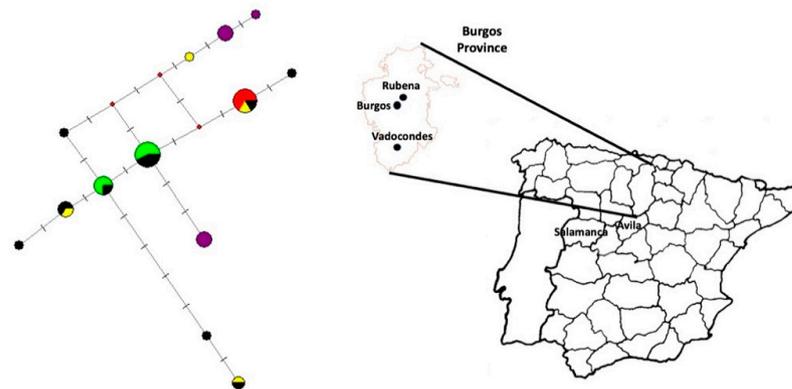


Figure 4. Reduced network for the R1b haplogroup with samples from Burgos and haplotypes from other origins with the same haplotypes and one or two steps. Color legend: yellow: haplotypes of Castilla from south of Burgos, orange: haplotypes of Castilla from north of Burgos, green: haplotypes of Castilla from Salamanca, purple: haplotypes of Castilla from Avila, and black: haplotypes of Castilla from other origins.

To test the hypothesis of an alleged origin of the Castilla surname in Burgos (see introduction), a network was performed with haplotypes whose origin was in Burgos and haplotypes of people who were not from Burgos but coincided with the previous ones or were at 1–2 mutation steps (Figure 4). Haplotypes that were within one or two mutational steps were also included.

Among the samples whose origin was Burgos, three haplogroups were identified: R1b, E1b1b-M81, and G. Regarding haplogroup G, there was only one individual with that haplogroup among all the samples from Castilla. In the case of the E1b1b-M81 haplogroup, the obtained haplogroup coincides with the one that corresponds to all Castilla, since 7 out of the 14 samples come from South Burgos.

Regarding the R1b-M269 haplogroup, 34 individuals were included. Practically, all the haplotypes whose origin is Salamanca and Avila are integrated into the network, seven out of eight in both cases. It is observed that the samples that correspond to individuals from Burgos are grouped into four clusters located in remote positions in the network (Figure 4). The clusters that relate to two of the four clusters fundamentally correspond to individuals from Salamanca. The third cluster constituted a descent cluster of individuals from Avila.

At a comparative level, networks corresponding to the R1b and E-M81 haplogroups with haplotypes from Spain were built (Figure 5). In network R1b, a majority cluster was

identified. The descent cluster was integrated into 54% of the R1b haplotypes. Similarly, the network obtained in the E-M81 haplogroup presented a figure identical to that of Castilla, although with a lower number of haplotypes. In the series from Spain, six E-M81 haplotypes were identified, and in the case of Castilla, 14 E-M81.

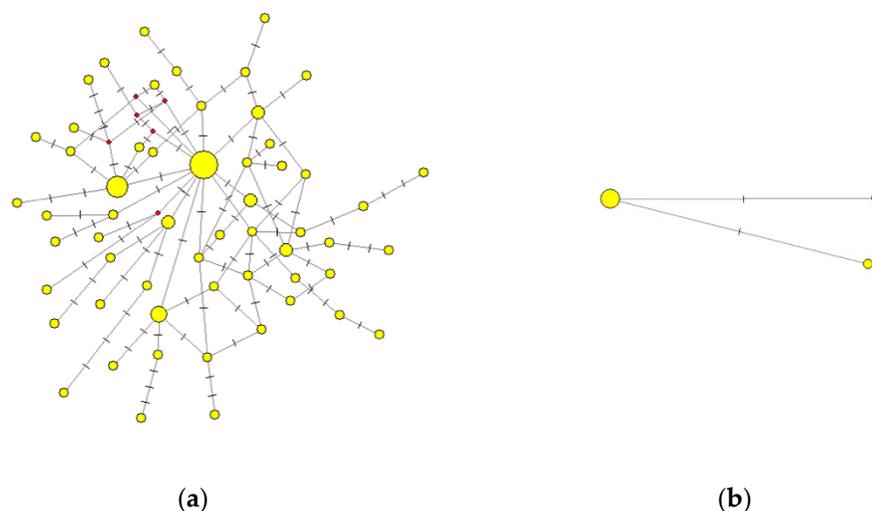


Figure 5. (a) Network of R1b haplogroup of Spanish reference population. (b) Network of E-M81 haplogroup of Spanish reference population.

The ages of the clusters corresponding to haplogroups E1b1b-M81 and R1b-M269 were calculated. A cluster with a greater number of individuals was considered an ancestral cluster (Table 2).

Table 2. TMRCA in major lineages found in Castilla Sample. R-M269* corresponds to network of Figure 4.

HG	Frequency Core	Age in Years	sd	Geographical Origin Core	Frequency Derivate Cluster	Geographical Origin Derivate Cluster	Frequency Total
E-M81	10	322.857143	255.240983	Burgos	3	Burgos (1), Colombia (1), Jaen (1)	13
R-M269	7	1506.6667	462.2688	Argentina (2), Salamanca (4), Spanish (1)	20	Burgos (6), Salamanca (4), Avila (4), Huelva (2), Spanish (1), Galicia (1), Ciordoba (1), Granada (1)	27
R-M269*	7	1412.5	532.687108	Argentina (2), Salamanca (4), Spanish (1)	22	Burgos (7), Salamanca (3), Avila (3), Spanish (2), Huelva (2), Coedoba (1), Argentina (1), Granada (1), La Rioja (1)	29

Regarding the E1b1b-M81 haplogroup, where all haplotypes have been included, a relatively young date has been obtained, 323+/- 255 years CE. In the case of the R1b-M269 haplogroup, when considering the descent cluster that includes a greater number of individuals, an older age was obtained, 1507+/- 462 years CE. In the network of Figure 4, corresponding to the individuals from Burgos, and the haplotypes of other origins coinciding with them, or of one or two mutation steps, one large descent cluster was obtained that grouped most of the individuals (31 individuals) and a much smaller one (5 individuals). Regarding the largest descent cluster, the age obtained was slightly younger than that of general R1b, 1411+/- 533 years CE. The minor descending cluster is much younger, 452+/- 320 years CE. If we consider the standard deviation, all descended clusters score older than the established hereditary surnames in Spain.

3. Discussion

3.1. Castilla Surname, Other Spanish Surnames, and Spanish Population

Haplotypes and haplogroups of 102 Castilla Y chromosomes were identified. Due to the low mutation rate of SNPs, it is very improbable that individuals with the same surname

and paternal lineage present mutations in the SNPs and yield different haplogroups during the short period after the introduction of surnames. However, it is possible to find different haplotypes in the same haplogroup with low haplotype diversity because the mutation rates of the STRs were comparatively higher. In the present study, when compared with other surnames with frequencies like the Castilla surname, such as the Spanish surnames Juarez or Marques (Figure 1a), the Castilla surname presents a similar pattern (Spearman's $\rho = 0.010$, $P = 0.879$); therefore, when the number of surname bearers increases, HD increases (Figure 1a, Table 1). When compared with Castilla from different geographical origins, when the number of bearers of the surname increases, the HMP decreases (Figure 1b). That is, the frequency of bearers of the surname is decisive in establishing the degree of co-ancestry, as has already been observed in other studies (King and Jobling 2009; Solé-Morata et al. 2015; Martínez-Cadenas et al. 2016). Surnames with many bearers present many different haplotypes and haplogroups that behave like samples collected from a general population of a specific geographic origin. Although the opposite effect has been observed in the Irish population (McEvoy and Bradley 2006), this relationship has not been observed in Andalusian surnames (Calderón et al. 2015).

When comparing the Castilla series with the general series for Spain, it was observed that the HD is similar. However, the Castilla samples had a higher percentage of shared haplotypes. This is consistent with the bottleneck effect when the Castilla surname appears. In addition, the presence of haplotypes belonging to distant haplogroups within the Castilla sample seems to suggest a polyphyletic origin of the surname Castilla. These two scenarios do not exclude the existence of a principal founder haplogroup of the surname, to which other haplogroups are later incorporated as Castilla.

3.2. Founding Haplotypes

The descent clusters identified in the networks were analyzed to identify the founding haplotypes/haplogroups (Figures 2–4). A conventional approach is to consider the most frequent haplogroup in the surname to be the founder lineage. In this case, haplogroups R1b and E1b1b-M81 would be the founding haplogroups. However, other haplogroups, now at low frequencies, cannot be discarded as founders, given that very dramatic and unknown demographic events could have happened in the past.

When the frequency of the R1b haplogroup was compared between Castilla men (0.461) and the Spanish general population (0.601), the obtained values were almost equal ($p = 0.085$). The TMRCA calculated for the cluster descent of the R1b haplogroup (1507+/- 462 years CE) seemed to indicate that the use of surnames began long before their mandatory use. Thus, there are sources that report its use in the 9th or 10th century, but at the same time, consider that it was something exceptional. These results raise the possibility that it is more common than previously thought. Nevertheless, it cannot be forgotten that the defined cluster descent depends on the criteria defined by the researcher, in this case, up to two steps. In addition, the mutation rate used (one mutation every 1130 years) favored a greater age of the cluster. A more frequent mutation rate or a more restricted criteria, only haplotypes at 1 step, would give younger clusters. Therefore, these results are compatible with clusters established after obligatory use of the surnames. In the studies of Catalan and Colom surnames, faster mutation rates were used: one mutation every 777 years and every 600 years, respectively. In both cases, TMRCA were closer to the mandatory period of the surnames. However, when considering the number of haplotypes as a criterion to consider the founder cluster, it can only be assessed as the minimum number of founders. In the simulations carried out by King and Jobling (2009), they found that the chance of survival of a lineage from one founder after 20 generations was 9.6%. Because of drift, many founder haplotypes have been lost and do not reach the present. Finally, the possibility of overestimating age should be indicated, as haplotypes of different subhaplogroups may be considered.

The fact that one of the most frequent haplogroups in Castilla is also the most frequent in the Spanish population makes it difficult to establish this haplogroup as the founder of

the surname. The networks obtained for the R1b haplogroup in the Castilla and Spaniards groups indicate that with the present results, it is not possible to determine whether this haplogroup is the founding haplotype/haplogroup of the surname. Other studies of the Spanish general population (Martínez-Cadenas et al. 2016) and Catalan surnames (Solé-Morata et al. 2015) seem to be able to define a minimum number of founders in infrequent surnames, although not in Andalusian surnames, where there is a low correlation between surnames and Y chromosome markers (Calderón et al. 2015) or Italian surnames (Boattini et al. 2021).

Furthermore, it is known that introgression estimates in other surnames of haplotypes whose coincidence is due to state and not ancestry are between 1–3.4% (King and Jobling 2009; Solé-Morata et al. 2015). These rates have been calculated in surnames with low frequencies or those located in a very defined local area. Although this is not the case for the Castilla surname, the results obtained in the R1b haplogroup for the frequent Castilla surname indicate a larger introgression rate.

A special case is the Castilla E1b1b-M81. This haplogroup had higher frequencies in Castilla (13.9%) than that in the Spanish population (4.1%), with a statistically significant difference ($p = 0.005$). In addition, this haplogroup presented higher frequencies among the populations of North Africa. This most recent TMRCA (323+/- 255 years CE) could be compatible with the obligation to use surnames, but it would also be compatible with the expulsion of both Jewish and Islamic populations from Spain at the end of the 15th century. Data indicate that those people who wanted to avoid expulsion changed their surnames to more Christian surnames (Obradó 2006). The very compact descent cluster, with very low haplotype diversity, together with the high frequency of this haplogroup in Castilla, seems to suggest the acquisition of the surname of males whose origin was not Iberian.

3.3. Geographical Hypothesis

When analyzing the samples from Burgos, because the information provided by the analysis of the genealogical trees suggests Burgos as the possible origin, four different haplotypes were obtained with respect to the R1b haplogroup. Two of these four haplotypes differed in two ways. In the network shown in Figure 2, other haplotypes identical to these four and also those 1–2 steps away were all incorporated, despite having a different origin outside Burgos. Thus, it is observed that a connection is established with three out of the four haplotypes, from those Castilla, whose origin is in Salamanca and Avila, regions that are between 240–265 km away. The incorporation of these haplotypes allows the haplotypes of northern Burgos (Rubena) to move to those from the south (Vadocondes). Again, the TMRCA of the majority-descent cluster presents a value that precedes the introduction of surnames.

In relation to the E1b haplogroup, it was observed that all Castillas with this Burgos haplogroup were from the south.

In general, it can be indicated how a greater diversity of haplogroups and/or haplotypes is observed in the Castillas of southern Burgos than in the Castillas of northern Burgos, which all carry the same haplotype and haplogroup.

3.4. Surname Castilla and Castilla Kingdom

An important difficulty in the study of this surname is its idiosyncrasy. Castilla refers to a large region of Spain, to a historical kingdom, and is therefore an obvious toponymic surname for the time being. On the other hand, its relationship with royalty allowed genealogists to assume that its use was uncommon.

Different genealogical studies suggest that during the Middle Ages, family names were used more than surnames. The family name refers to a family, whereas the surname serves to differentiate individuals according to them belonging to a family (de Salazar y Acha 1991). For example, the family of the Count of Trastámara was known as the Trastámara, although none of them had the last name Trastámara. During the Reconquest of Spain, many Castilla-Leonese men participated in the repopulation of the rest of the

peninsula. [Calderón et al. \(2015\)](#) found that the majority of their samples from Andalucía had the Castilian-Leonese surname. Therefore, it would not be strange that people from the kingdom of Castilla acquire the surname “de Castilla” to indicate their origin.

The studies carried out in the documentary sources suggest that the family name has been widely used since the Middle Ages, and it is still used in towns in Spain. These studies suggest that until the Council of Trento (1545–1563), there were many changes in surnames for different reasons ([Salinero and Testón 2010](#)). Sometimes, it was because the last name only passed to the eldest son, in other cases, as a way of indicating the geographical origin (from the region of Castilla), for economic interests or social importance, etc. [Ryskamp \(2012\)](#) considers a “circular pedigree model”, where the individual could receive his/her surname from any of his/her relatives, even from those who were several generations away, for example, a grandmother or a grandfather.

According to these studies, it would be very difficult to find some type of relationship between a surname and Y-chromosome haplotype/haplogroup. The analysis of SNPs that allow the subtyping of haplogroups may be of interest in those surnames that have a lower frequency and a lower diversity of haplogroups. RM markers can lead, however, to an individualization of each sample, thus blurring any genetic relation among haplotypes. On the one hand, this would explain the great diversity of haplogroups found among Castilla. In addition, the effect of genetic drift and/or introgression, either due to coincidence by state, non-paternity, and other reasons, must be considered.

4. Materials and Methods

4.1. Samples and Methods

A list of men bearing the Castilla surname was obtained from public telephone directories. A postal request for a cheek-cell sample was sent to 400 people. Other samples, especially Argentine Castillas, were collected, as well, through an announcement on the website: www.apellidocastilla.es (<http://www.apellidocastilla.com/> (accessed 6 February 2003)). All participants signed an informed consent form prior to sample donation. Thus, a total of 102 samples were obtained. The first classification was based on the place of birth of volunteers. Among the samples collected in Spain, four samples from individuals born in Argentina were pooled with the rest of the Argentinean samples. In all cases, the specific origins of parents and grandparents were solicited. There was no close kinship among the samples (first and second degree, e.g., brothers, father-son, cousins, or uncle-nephew). The overall sample taken from Castilla was classified according to the place of origin of their relatives by the oldest paternal lineage. Sometimes, donors had genealogical data corresponding to four or five generations. In other cases, the samples were classified according to the place of birth of the volunteers.

Buccal swabs and blood samples were collected in 903 Protein Saver Cards (Whatman®). DNA was extracted using a standard phenol/chloroform method ([Sambrook et al. 1989](#)). Eleven Y-specific microsatellites (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, and DYS385a/b) were analyzed according to [López-Parra et al. \(2005\)](#) and were confirmed by [Palha et al. \(2007, 2012\)](#). Genotyping was performed using ABI 3730 and named according to ([Gusmão et al. 2006](#)).

4.2. Statistical Analysis

Summary statistics were calculated using Arlequin ver. 3.5.2 (<http://cmpg.unibe.ch/software/arlequin3>). Haplotype diversity (HD) was calculated using HapYDive ([Alves et al. 2006](#)). The haplotype match probability (HMP) was calculated. The calculation of significance when comparing frequencies, such as Spearman’s correlation, was performed using SPSS version 27.

Each Castilla sample and samples from reference populations were assigned to haplogroups using the Bayesian approach implemented in Y-DNA Haplogroup Predictor-NEVGEN (<https://www.nevgen.org/>) (accessed 8 January 2022). A general sample from

Spain (Martin et al. 2004) was used for Y-STRs haplotypes, using the same 11 STRs for which Castilla data were available. Haplotypes with a probability <90% were left unclassified.

Network analysis (Bandelt et al. 1999) of haplogroup-associated haplotypes was performed using NETWORK 10.2.0.0 software (<http://www.fluxus-engineering.com/sharenet.htm>). The reduced median (RM) algorithm was applied to the files of median joining or MJ-algorithm obtained.

Y-STRs were weighted in inverse proportion to their variance (Qamar et al. 2002).

Clusters within surname networks were identified by considering the ad hoc rules proposed by King and Jobling (2009). Thus, within each haplogroup, haplotypes that differed by one or two steps were grouped and considered descendants of a common founder. In addition, DYS385a/b was amplified simultaneously and was excluded from the network. The number of repeats in DYS389I was subtracted from that in DYS389II in all analyses.

Time to the most recent common ancestor (TMRCA) was estimated in the network from the rho statistic, using a 25-year generation time. The overall genetic mutation rates for the ten Y-STRs were 2.21×10^{-2} , obtain from YHRD (Y-chromosome haplotype reference database, release 68). A male generation time of 25 years was used, and we obtained one mutation per 1130 years, according to Martínez-González et al. (2012). Considering this value, we only estimated TMRCA among clusters separated in two steps.

5. Conclusions

The idiosyncrasy of the Castilla surname points to a complex history where non-patrilineal transmissions (illegitimacies and other forms of transmission out of the genealogical standards) and the effect of genetic drift could have played a remarkable role. Although the results for other surnames can differ, the coincidence of the most common haplogroup in the Castilla sample and the most frequent haplogroup in the Spanish general population makes it difficult to identify founder haplotypes/haplogroups in the history of the surname Castilla.

It is very possible to find an association between haplogroup/haplotype and surnames when the surname is not common and when a surname carries an uncommon haplogroup. However, the criteria used to define founders based on the frequency of haplotypes/haplogroups in the present study may be imprecise, and other indicators are required to establish the founder lineages of a surname.

Supplementary Materials: The following Supporting Information can be downloaded at: <https://www.mdpi.com/article/10.3390/genealogy7030052/s1>, Table S1: Haplogroup frequencies and HD in Castillas samples and Spain reference population. N: sample size, K: number of different haplogroups. HD: haplotype diversity. Freq Castilla: Frequency of the surname Castilla. SD: standard deviation.

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