Supplementary Material

Clinal distribution of human genomic diversity across The Netherlands despite archaeological evidence for genetic discontinuities in Dutch population history

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Supplementary Note 1.

To be able to interpret and understand the genomic data and analyses presented in this paper, we regard it crucial to understand the Dutch population history in somewhat more detail than typical for a genomics paper. Therefore, we decided to present this information to the reader via the supplementary material, and only provide a brief summary in the main text. What follows here is a brief overview of events that we consider to have been most influential on the current Dutch genetic landscape. This overview is by no means exhaustive nor complete, but illustrates and describes the complexity of the Dutch population history to an extent that we consider appropriate for the purpose of understanding the genomic data presented in this paper. As usually applied: dates presented as BP (before present) indicate non-calibrated ^{14}C years (which are not similar to calendar years), unless specifically mentioned. Dates presented as BC/AD (before Christ/after Christ) indicate calibrated (cal) ^{14}C years, which are similar to calendar years. In the accompanying Table S1, crucial terms, dates and population size estimates are summarised and figure S1 depicts population size estimates in a chart.

The oldest physical evidence of the presence of anatomically modern humans (AMH) in north-western Europe dates back to at least 43-42 thousand years (kyr) cal BP [1], a period generally known as the Palaeolithic. This is well before the last glacial maximum (LGM; roughly 26- 15 kyr BP). There is no physical evidence, however, of AMH in The Netherlands before the LGM and even an inferred presence at that time based on artefacts is uncertain. As in the rest of northwest Europe, there is no proof of human presence during the LGM, by the end of the geological period, the Pleistocene [2].

By the end of the LGM, northwest Europe was re-colonized by hunter-gatherers, presumably from a number of refugee areas in southern Europe. The earliest evidence of returning hunter-gatherers in The Netherlands dates around 12 kyr to 11 kyr BP, during the Late Palaeolithic era (12.8 kyr -9.7 kyr BP) [2]. By the end of the Palaeolithic era, the geological period the Holocene (11,700 yr BP) starts, during which the Pleistocene landscape is covered by new layers of sediment under the influence of rising sea and river levels. Note that at this point in time England is still connected to the European mainland via landmass [3]. Based on artefact studies it is assumed that during this period people were very mobile.

During the Mesolithic period (~9000-5300 BC) people keep residing on hunting-gathering but there are indications of reduced mobility [2]. Around 7000 BC The Netherlands and England became separated by the North Sea. By 6500 BC this sea reached approximately the current Dutch coastline and vast areas of peat land were formed in the west and north of The Netherlands [3]. Reliable estimates for population sizes during the Late Palaeolithic and Mesolithic are difficult to obtain, but are expected to be around 2,000 individuals [2].

Around 5300 BC Limburg (the southeast of The Netherlands) was the first Dutch region to be colonized by Neolithic farmers. The rest of the country resided on hunting-gathering until 4200 BC, and it lasted until 3400 BC before the Neolithic transition was complete in the whole region of The Netherlands [2]. Debates are still on-going as to what extent the Neolithic transition in Europe was based on migration of people or cultural diffusion and, related to that, to what extent this resulted in the replacement of local hunter-gatherers. Ancient human DNA (aDNA) studies show different results for different regions of Europe [4-8] demonstrating that at least in some European regions the Neolithic transition did involve people. These aDNA studies have not yet been conducted on ancient Dutch human samples, but Neolithic skeletons from Germany show a strong genetic relation with modern Near East populations, the designated origin of the Neolithic lifestyle [4]. At this time, the formation of peat lands in the west continues and the coastline of the central-west moved several kilometres of land inwards [3]. The estimated population size for the Neolithic period is around 10,000 individuals, who inhabited both the dry southeast and the wetter areas in the rest of the country [2]. There is a substantial variety in material culture during the middle Neolithic period (4200-2900 BC) with clear geographic boundaries. In the Late Neolithic period (2900-2000 BC) this variety disappears and makes way for a more uniform material culture, although the south seems to be more culturally related to the Belgian, French and English regions and the north more to Scandinavia.

This cultural uniformity lasts until the Middle Bronze Age (1800 BC), when new cultural boundaries appeared between the northeast (related to north Germany and Denmark), the central and south (related to northern France and south Britain) and a small regional group in eastern west Frisia. This division between north and south lasts until the beginning of the Roman period (12 BC) [2]. This boundary between north and south does not follow the central Dutch river streams. The rivers function as easy migration and trade routes rather than as natural barriers.

For the Iron Age (800-12 BC) there is less evidence for long distance trade compared to previous periods [2, 9]. During this period, peat formation reached a maximum. More than half of the country is covered with peat, mostly in the west but also the north and south. Already during the Iron Age, people started to reclaim land [3]. Halfway through the Bronze Age the central-west coastline expanded westwards again and reached its current position. There are not many reliable archaeological indications to estimate the population size during the Bronze and Iron Ages, but the maximum population size for the Late Bronze Age (1100-800 BC) and Early Iron Age (800-500 AD) is estimated at 15,000 to 30,000 individuals [2]. During the Bronze and Iron Ages habitation occurred mainly outside the peat lands, with a preference for the central river area and the northern tidal lands and to a lesser extent the eastern and southern Pleistocene sandy soils [2-3].

In 56 BC The Netherlands were invaded by the Romans for the first time, although at that time they had no authority yet in the area. Around the same period another group of people, the Batavians, migrated into the central Dutch river area from the region of Hessen, Germany [9]. Around 12 BC the Romans conquered the Dutch area for a second time, which finally resulted in a fortified boundary along the river Rhine, as part of the Limes of the Roman Empire. From this time onwards, the Dutch area below the Rhine was officially part of the Roman province Germania Inferior, although there is also clear evidence of substantial Roman cultural influence north of the Rhine [10-11]. However, soldiers were often recruited locally; about one third to one half was of ‘German’ origin. Other legions that are known to have resided in the Dutch area originated from Gallia and the Danube area [10-11]. It is therefore not likely that significant groups of Mediterranean people migrated to the Dutch area. The Dutch population expanded fast during the Roman period, especially in the river area and in the southeast, but also outside the Roman territory. It reached a peak during the Middle Roman period (47-260 AD) with approximately 150,000 individuals [2, 12].

By the end of the third century the Romans started losing power, which coincides with a temporal decrease of the populations in the west and the river area of The Netherlands

at both sides of the Limes [11]. Towards the end of the Roman period the population further decreased and occupational gaps were observed in many archaeological sites all over the country [3, 9, 11-14]. Estimates of the population size are not available, however. During the Roman period, reclamation of land had strongly increased which, in combination with natural factors, resulted in flooding and the disappearance of vast tracts of land in the southwest, including the whole of Zeeland [3].

Relatively little information is available for the Early Medieval (450-950 AD) era, but it was a dynamic period in terms of new power establishments after the Romans left and there are many indications of significant and successive migrations during the fifth and sixth centuries [9, 11-14]. Population estimates for the Early Medieval period suggest approximately 40,000 individuals, when the population had already started to expand again [14]. During the Carolingian period (750-950 AD) the river area and the north coast (Frisia) were relatively densely populated. The eastern Pleistocene sand areas, the Limburg Meuse area and the coastal dunes in the west, including Zeeland again, were more scarcely populated, and the peat lands were supposedly scarcely populated or not inhabited at all [3]. Along the rivers in the central area several trade ports with international networks, such as Dorestad, Tiel, Utrecht and Deventer, flourished throughout the Early Medieval age, but the same area also suffered from repeated attacks by Vikings who are reported to specifically abduct local women [10].

During the twelfth century, agricultural yields increased significance, land was being reclaimed on a large scale and trade increased. Agricultural surplus allowed for a faster population growth. This enabled a rapid rise of cities, perhaps much more so than in other parts of Europe. The newly reclaimed lands, trade and urbanisation also increased the mobility of the population. Due to continuous land reclamations from the 13th century until the second half of the 20th century, new land was occupied by new settlers on a continuous basis [3, 15].

During the 14th century the population decreased, due to both famine and epidemics of the bubonic plague. Economic and demographic growth recovered, however, and continued until the 18th century [15]. Around the beginning of the 16th century the Dutch population is estimated to amount to approximately 2 million individuals [15]. The majority of them lived in the northwest, with 50% living in cities. In the south, about 30% lived in cities, but in the other areas urbanisation was less than 20% [10].

In the 16th century, Protestantism emerged in the otherwise Catholic Netherlands, resulting in a major religious clash in 1566. This initiated the 80-year war with Spain (1568-

1648), during which several cities were (repeatedly) under siege and occupied by Spanish armies. In 1648 the 80-year war ended and The Netherlands officially became Protestant, attracting large groups of religious refugees from elsewhere such as the south. In practice, however, the southeast remains predominantly Catholic and also in the northwest and central-east, Catholicism remained popular. This marked religious division is still present today [10].

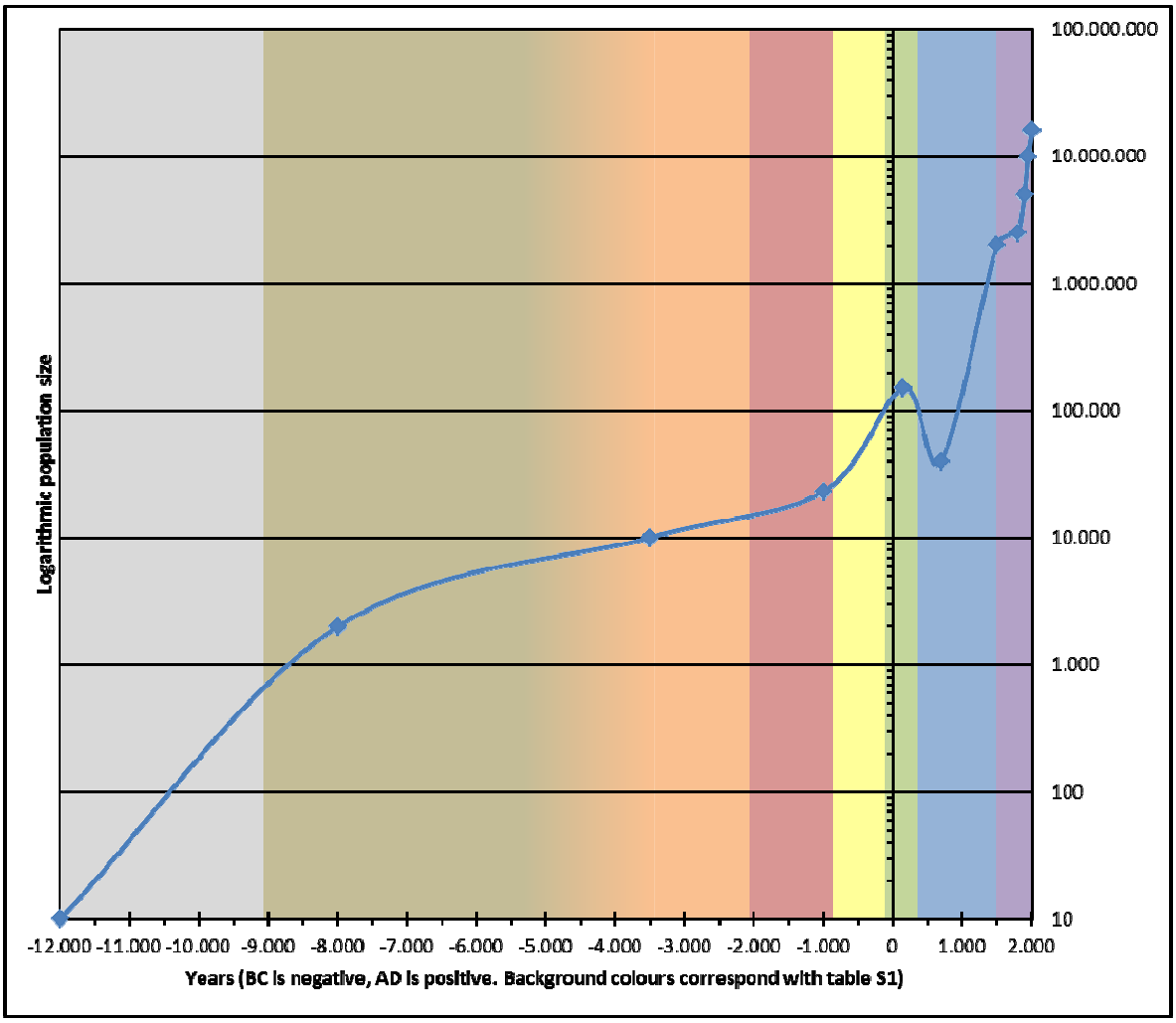
By the end of the 18th century the Dutch economy stagnated as a result of which population growth stagnated too; from 2 million in the 16th century to only 2.5 million in 1800. Cities showed shrinking population sizes, but population concentration was still highest in the northwest [10, 15]. However, only a century later, in 1900, according to the first official population census by the government [16], the Dutch population had doubled to just over 5 million. Population growth was strongest in the northwest, followed by present day Dutch provinces of Utrecht, Zeeland, Groningen and Limburg. Drenthe, as in most previous periods, was the least densely populated region [10]. Before 1900 general mobility was low; around 1850 about 70% of the population lived in the municipality where they were born. These percentages were highest in the south, central east and in Friesland. Industrialisation in the south and central east in the second half of the 19th century also attracted many migrants from abroad, as did the cities and international harbours in the west. At the same time, land reclamation in Drenthe attracted new settlers, not only from other Dutch regions, but also from Germany [17].

Between the First and Second World War, the Dutch society became strongly polarised; Catholics, Protestants, socialists and liberals had their own networks influencing many aspects of their life. The geographic spread of Catholics and Protestants remained as before, but socialists and liberals were mainly found in the north. This system more or less continued until shortly after the Second World War (1940 - 1945), and started to fade around the 1960's [11,16]. In 1950 the Dutch population size was just over 10 million; nowadays it is heading towards 17 million [16]. During the second half of the 20th century relatively large groups of people migrated within the country in a complex pattern of different directions [17].

Supplementary Table 1. Schematic overview of geological and cultural periods with corresponding dates and population size estimates for the Dutch territory ([1]: [3], [2]: [2, 14], [3]: [2], [4]: [14], [5]: [11], [6]: [17]).

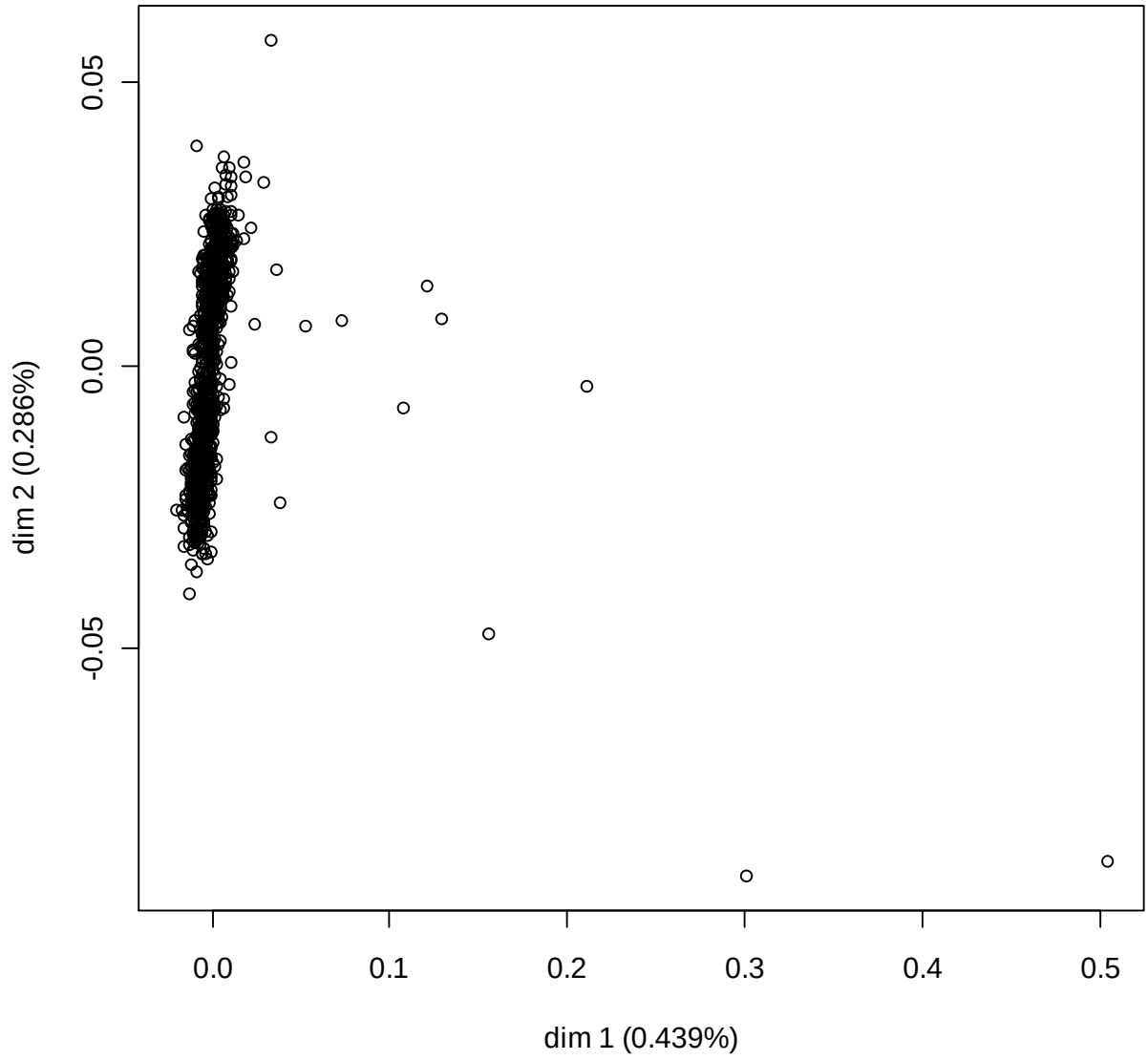
Geological period[1]	Cultural period		Date[2]	Population size x 1,000
Pleistocene	Paleolithic			~0[3]
Holocene	Late		12800 BP - 9700 BP	~2[3]
	Mesolithic		9000 - 5300/3400 BC	
	Neolithic	Early	5300 - 4200 BC	~10[3]
		Middle	4200 - 2900 BC	
		Late	2900 - 2000 BC	
	Bronze Age	Early	2000 - 1800 BC	15-30[3]
		Middle	1800 - 1100 BC	
		Late	1100 - 800 BC	
	Iron Age	Early	800 - 500 BC	
		Middle	500 - 250 BC	
		Late	250 - 12 BC	
	Roman Period	Early	12 BC - 47 AD	~150[3]
		Middle	47 - 260 AD	
		Late	260 - 450 AD	
	Medievals	Early	Merovingian 450 - 750 AD	~40[4]
			Carolingian 750 - 950 AD	
		Late	950 -1500 AD	
	Modern period		1500 AD	~2,000[5]
			1800 AD	~2,500[5]
			1900 AD	~5,000[6]
			1950 AD	~10,000[6]
			2011 AD	~16,000[6]

Supplementary Figure 1. Estimated population size on Dutch territory over times, based on Supplementary Table 1.

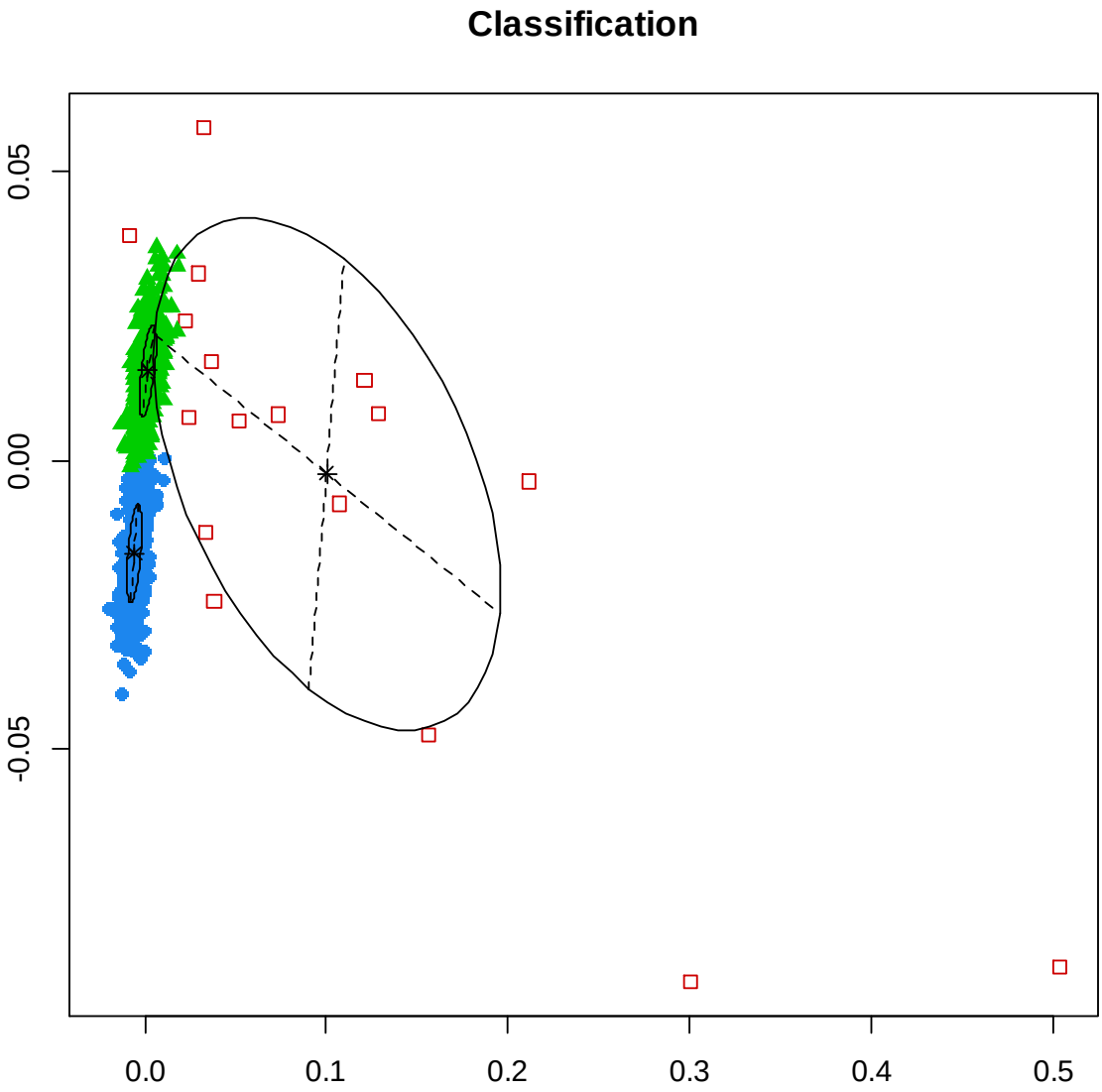


Supplementary Figure 2. A) Classical MDS on genome-wide autosomal data performed with 969 individuals of 54 Dutch subpopulations after data cleaning. B) Mclust analysis performed in the first two dimensions. Mclust detects three main clusters.

A

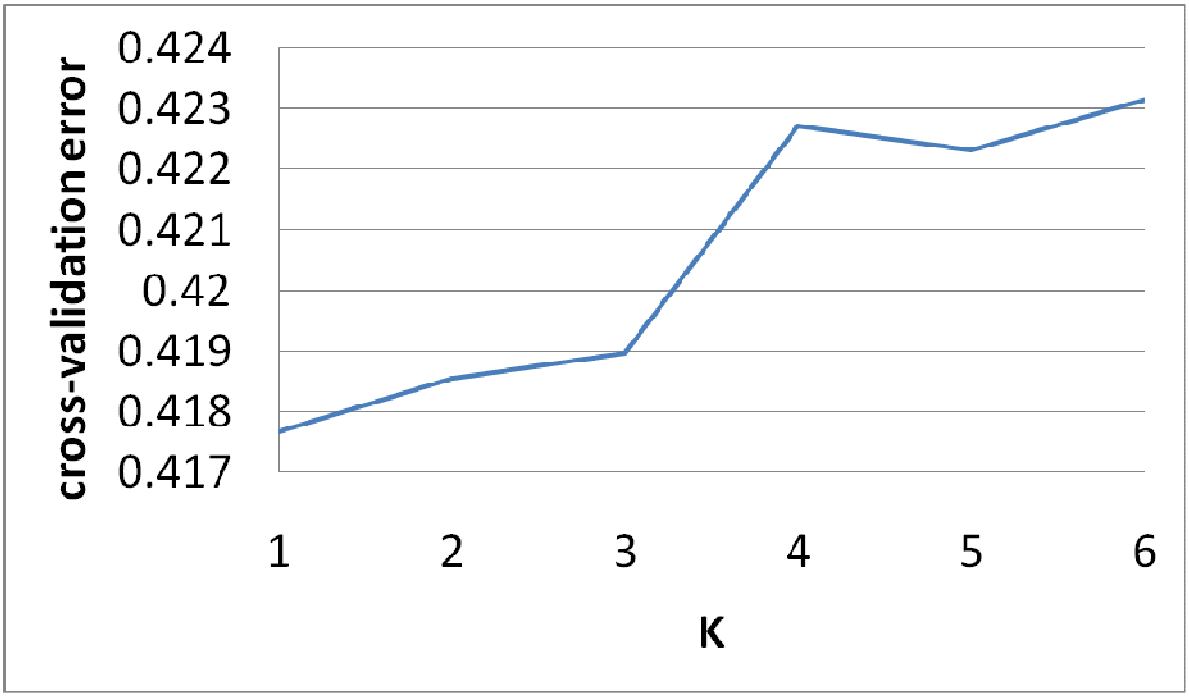


B

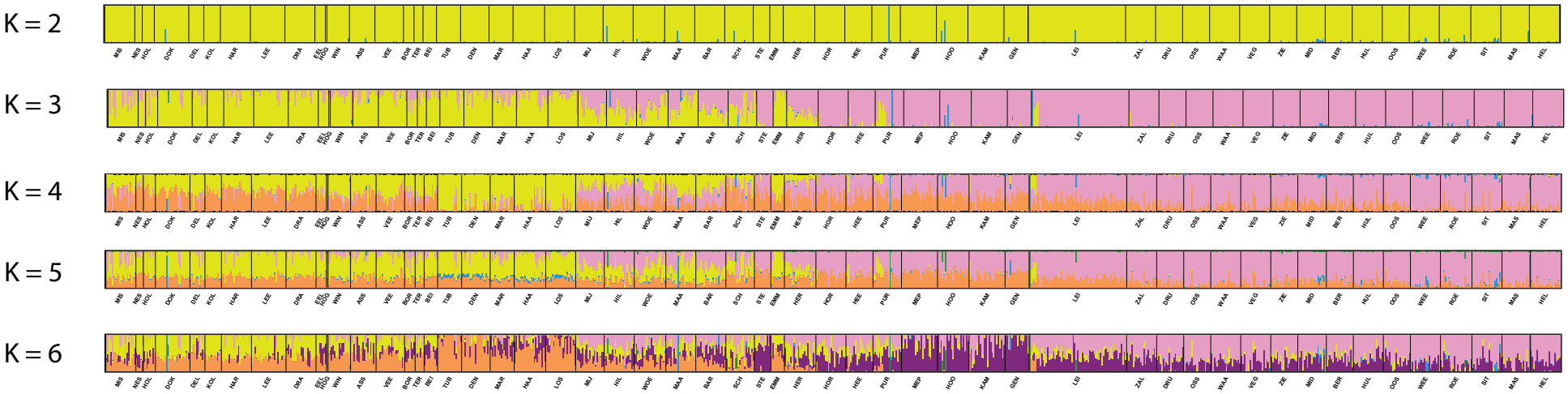


Supplementary Figure 3. A) Cross-validation plot obtained from Admixture (K=1 to K=6) using 969 individuals and the LD pruned set of autosomal markers. B) Admixture clustering from K=2 to K=6 based on 10 replicates each one.

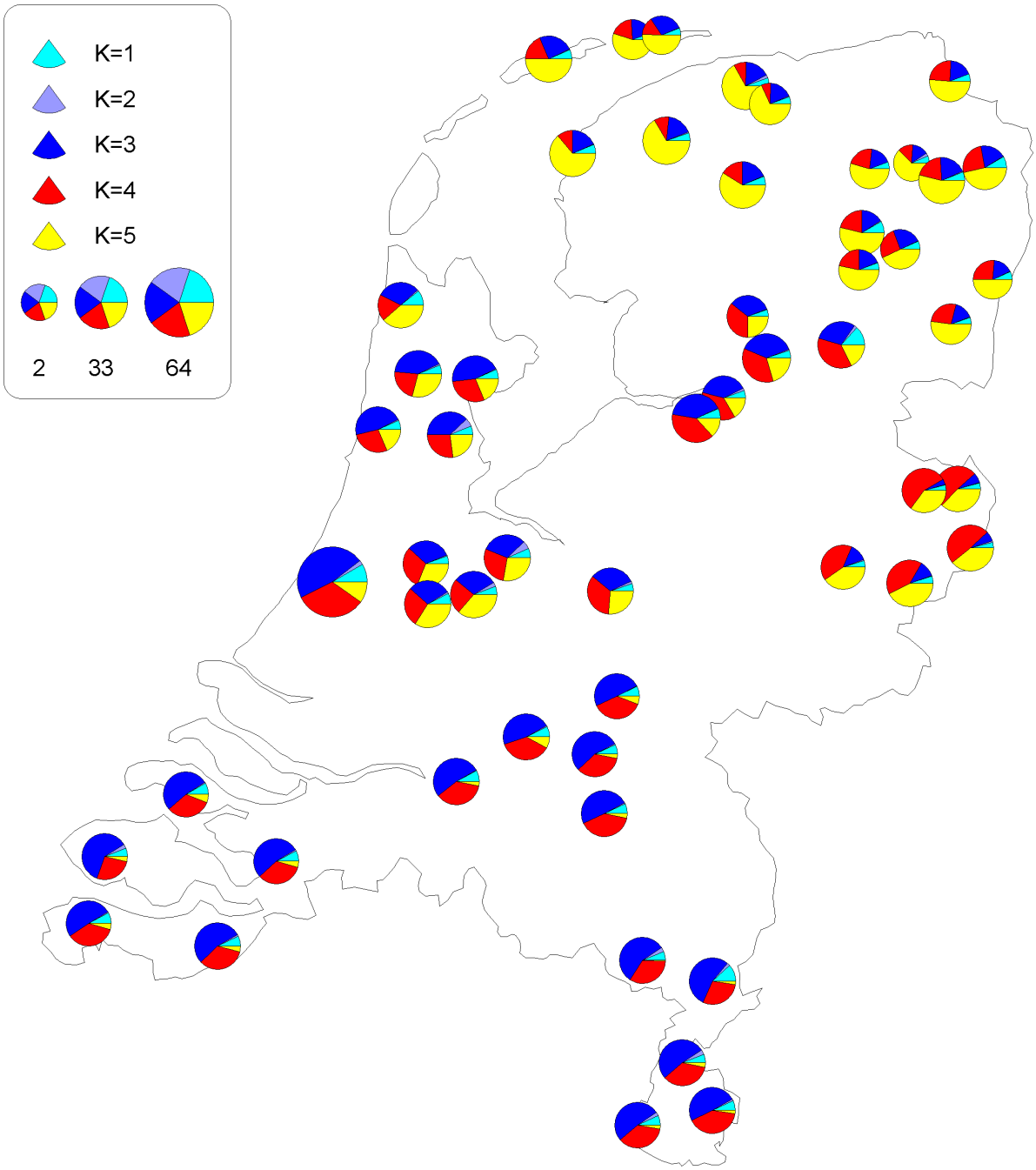
A



B


K = 2
K = 3
K = 4
K = 5
K = 6
MIB NES HOL DOK DEL KOL HAR LEE DRA EEL ROD WIN ASS VEE BOR TER BEI TUB DEN MAR HAA LOS MJ HIL WOE MAA BAR SCH STE EMM HER HOR HEE PUR MEP HOO KAM GEN LEI ZAL DRU OSS WAA VEG ZE MID BER HUL OOS WEE ROE SIT MAS HEL

Supplementary Figure 4. Pie chart map of the genome-wide ancestry assignment in the 54 Dutch subpopulations estimated by FRAPPE using K = 5 assumed parental populations. For subpopulations see Table 1 and Figure 1.



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