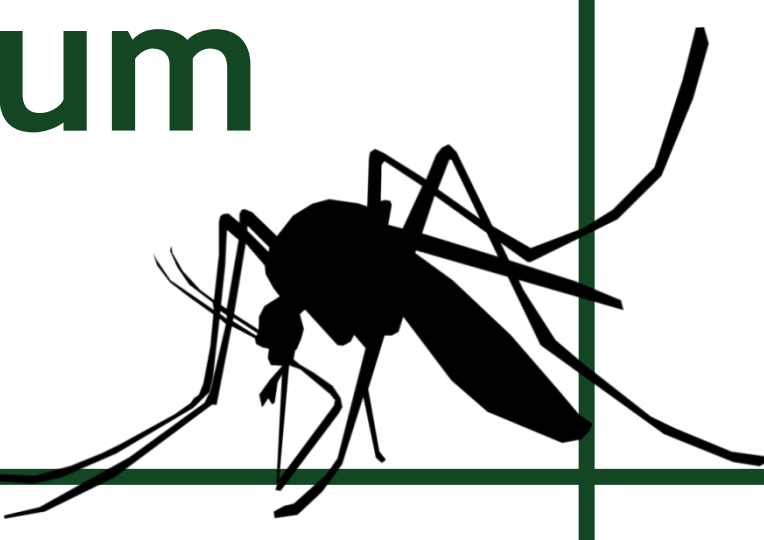


Population genetic structure of *Aedes japonicus* in Belgium suggests multiple introductions



N. Smitz¹, K. De Wolf², I. Deblauwe², H. Kampen³, F. Schaffner⁴, J. De Witte², A. Schneider², I. Verlé², A. Vanslembrouck^{2,5}, W. Dekoninck⁵, K. Meganck¹, S. Gombeer⁵, A. Vanderheyden⁵, M. De Meyer¹, T. Backeljau^{5,6}, D. Werner⁷, R. Müller², W. Van Bortel^{2,8}

¹ Royal Museum for Central Africa, BopCo, Leuvensteenweg 17, 3080 Tervuren, Belgium, ² Institute of Tropical Medicine, The Unit of Entomology, Nationalestraat 155, 2000 Antwerpen, Belgium, ³ Friedrich Loeffler Institut, Südufer 10, 17493 Greifswald, Germany, ⁴ Francis Schaffner Consultancy, Lörracherstrasse 50, 4125 Riehen, Switzerland, ⁵ Royal Belgian Institute of Natural Sciences, BopCo & Scientific Heritage Service, Vautierstraat 29, 1000 Brussel, Belgium, ⁶ University of Antwerp, Evolutionary Ecology Group, Universiteitsplein 1, 2610 Antwerpen, Belgium, ⁷ Leibniz Centre for Agricultural Landscape Research, Eberswalder Straße 84, 15374 Müncheberg, Germany, ⁸ Institute of Tropical Medicine, Outbreak Research team, Nationalestraat 155, 2000 Antwerpen, Belgium

Corresponding author e-mail: nathalie.smitz@africamuseum.be

Aedes japonicus japonicus has expanded beyond its native range and has established in multiple European countries, including Belgium. Since 2002, an established population is known to exist at Natoye, Belgium. Between 2012 and 2015, an intensive elimination campaign was undertaken at Natoye, after which the species was declared to be eradicated. Yet in 2017 the species was re-detected at the Natoye location. A first objective therefore was to sample for specimens at Natoye and along the Belgian border with Germany to investigate the origin of captured *Aedes japonicus* specimens, assuming them to be related to the established population in western Germany. An additional objective was to investigate if the population at Natoye resulted from new introductions and/or from a few undetected specimens that had escaped the elimination campaign.



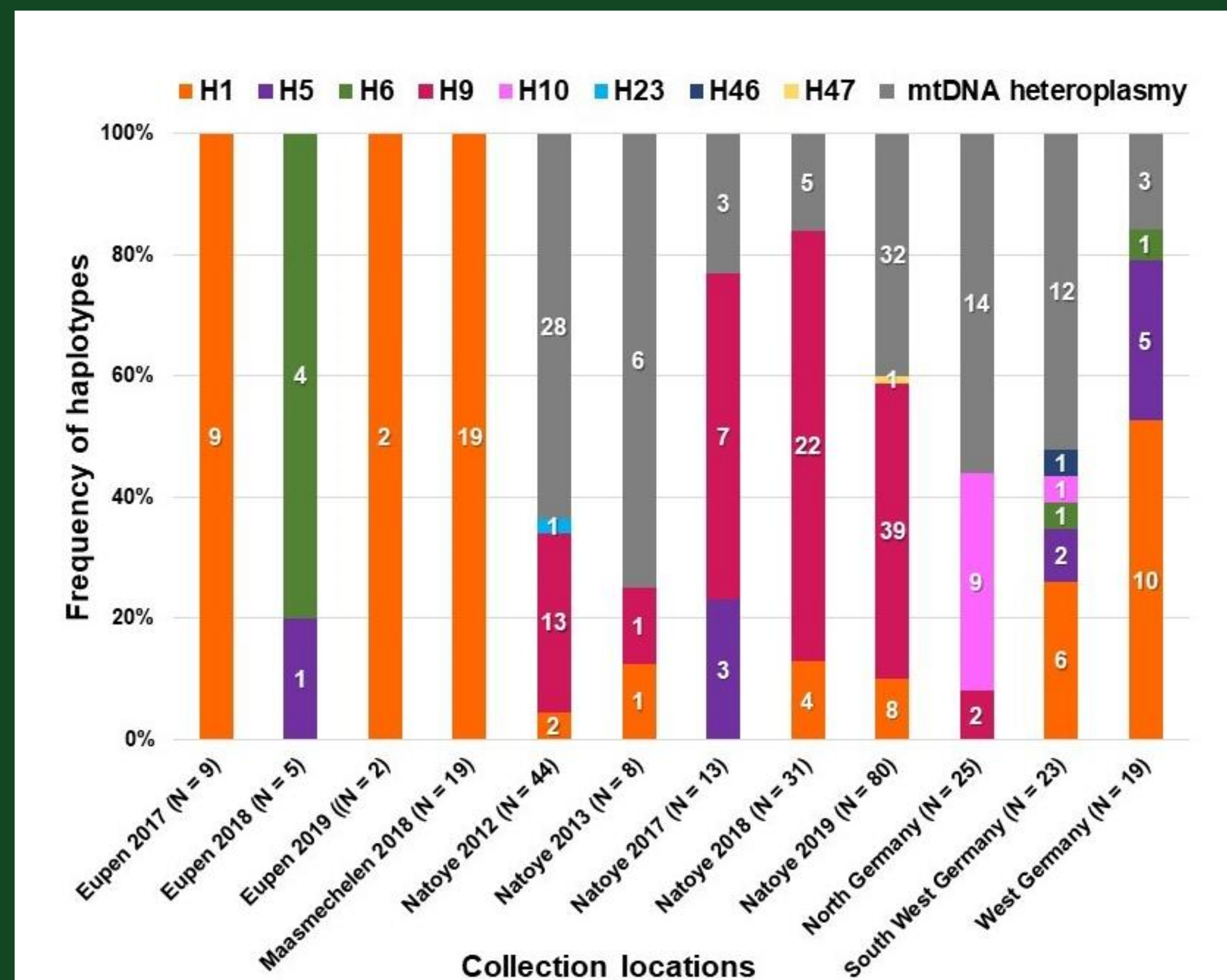
Eradication campaign

Continuous / repeated introductions

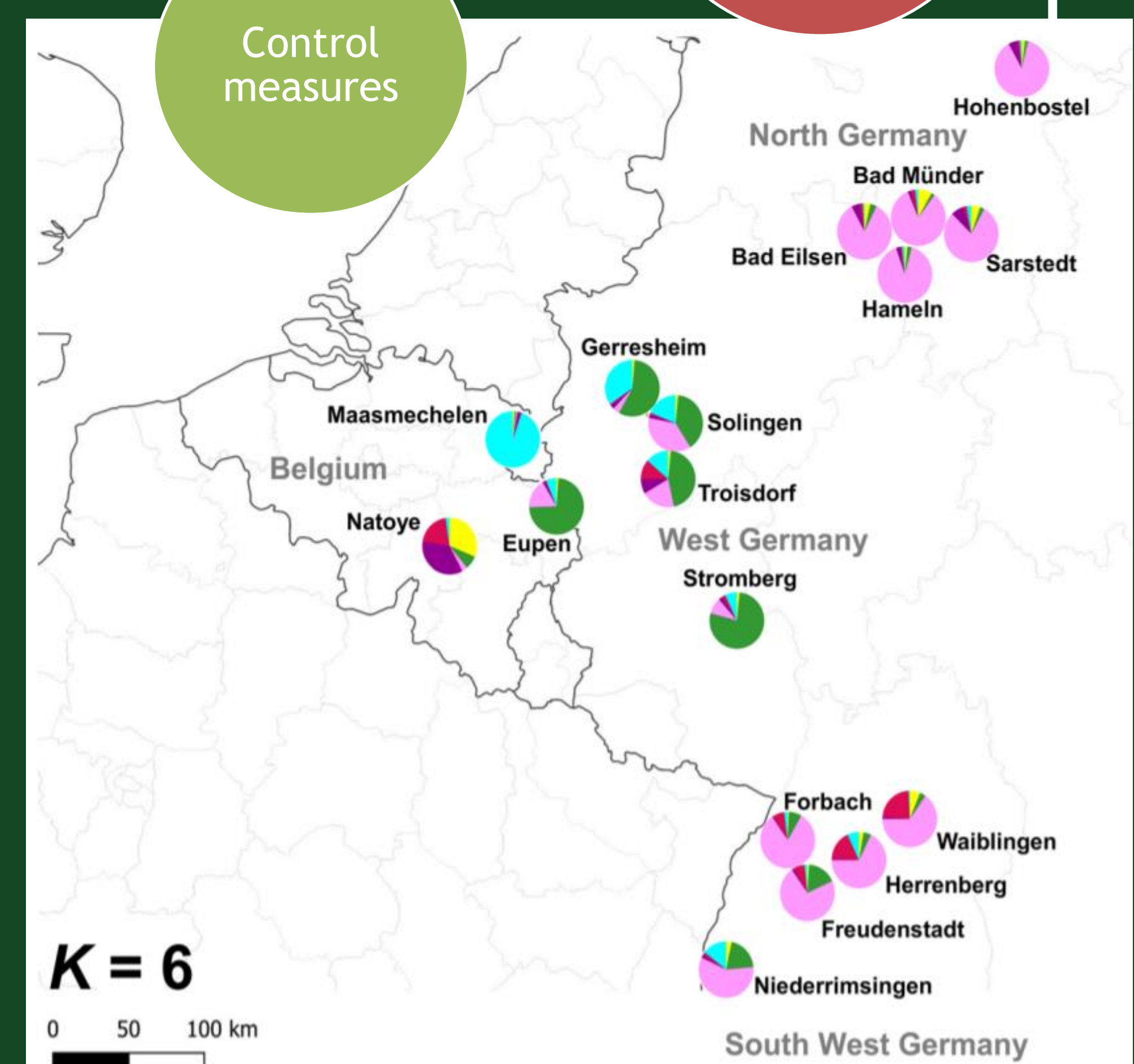


Control measures

To investigate the introduction source(s), population genetic variations at seven microsatellite loci and the mitochondrial NADH dehydrogenase subunit 4 (*nad4*) locus were surveyed. At Natoye, specimens were collected before and after the elimination campaign, to investigate temporal changes in the genetic composition and diversity and German samples were included as reference material.



Frequencies of the *nad4* locus haplotypes over the different sampling locations. Numbers in each barplot graph indicate the number of samples.

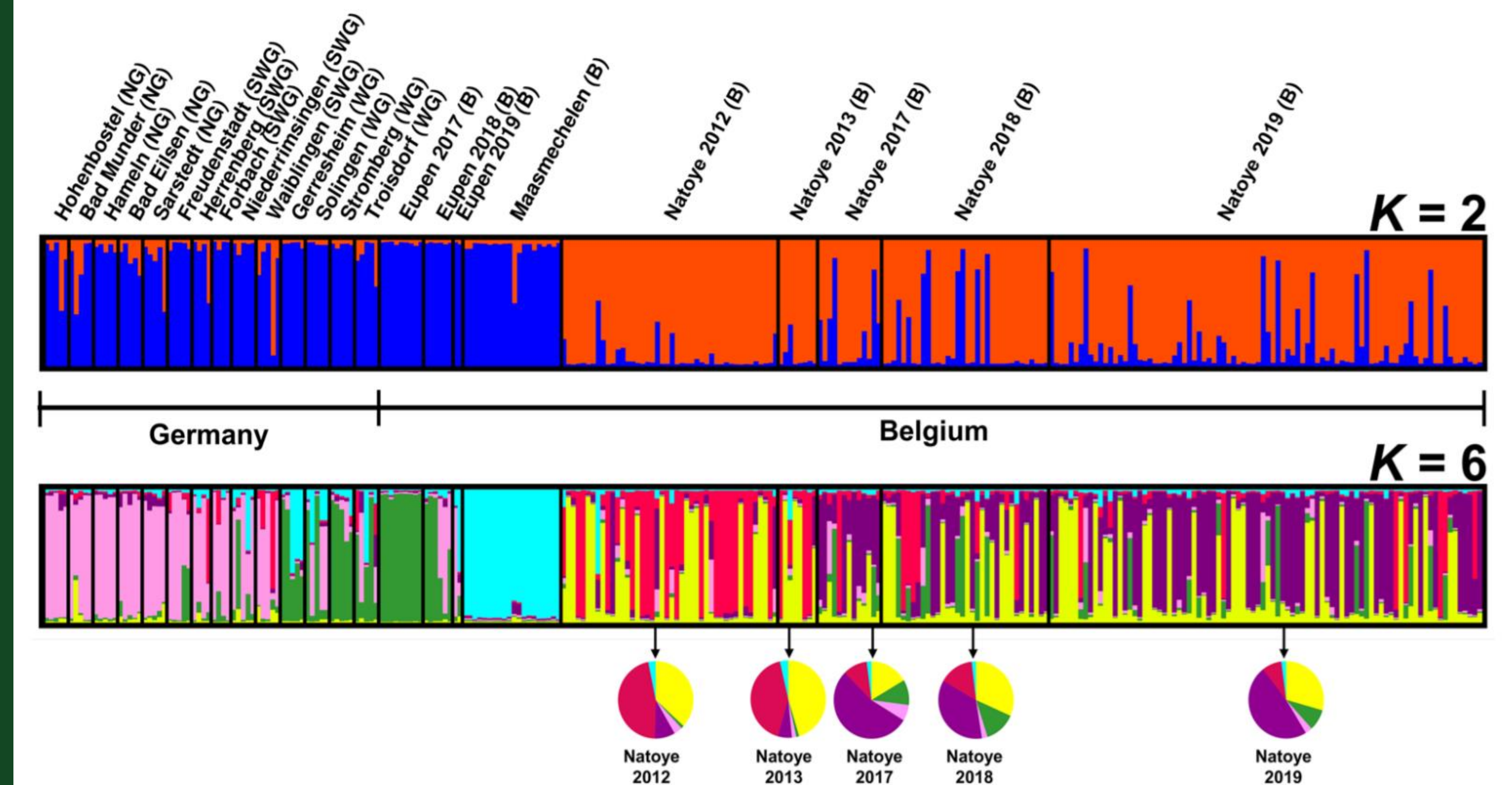


Map of Belgium and Germany population displaying the Bayesian cluster analysis results for $K = 6$ per sampling locality, based on seven microsatellite loci. Each pie chart represents one sampling location. Colors of the pie chart represent the mean assignment probabilities for all individuals collected at that location to each clusters.

The genetic study indicates that the Natoye population is significantly differentiated from all other populations included, both for *nad4* (high prevalence of haplotype H9) and the microsatellite data. The population also showed a clear difference between its genotypic microsatellite make-up in 2012-2013 and 2017-2019, i.e., before and after the eradication in 2014-2015. These results would indicate that there may be a new introduction from an external source after the eradication process. The population present in 2012-2013 is, however, believed to have survived the eradication since admixture between the two genotype clusters based on microsatellite data was identified. This suggests the elimination campaign undertaken over years at Natoye was not completely successful, which underlines the complexity of controlling invasive species.

The results suggest a relation between the collected individuals at Eupen and Maasmechelen with and from different populations in West-Germany, the invasion front of *Ae. japonicus* to the west.

Clusters for both $K = 2$ and $K = 6$, inferred with Structure® v2.3.4 software. The cluster membership of each individual is shown by the color composition of the vertical lines, with the length of each color being proportional to the estimated membership coefficient.



Considering the international movement of goods and people, and the colonising behavior of *Ae. japonicus* in Germany, it is to be expected that further introductions will occur in Belgium. To further investigate the population genetic relationships and changes in the allelic frequencies over time in the frame of surveillance programmes, thorough sampling of all *Ae. japonicus* populations, including representatives of its native and invasive ranges, additionally to the use of genome wide genetic data, would be required.

INTRODUCTION

METHODS AND RESULTS

CONCLUSION