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Genetic diversity of the Helsinki area rabbits before and after the 2016 rabbit haemorrhagic disease epidemic

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Tiivistelmä – Referat – Abstract

The European rabbit (*Oryctolagus cuniculus*) is a small mammal native to the Iberian Peninsula, but introduced by humans to all continents except Antarctica. The rabbit has been a remarkably successful invasive species due to its generalist nature and fast reproduction. Its spreading has mostly been destructive to the local nature, and humans have used fatal rabbit diseases such as rabbit haemorrhagic disease (RHD) to control harmful populations.

The rabbit population in Helsinki is one of the most northern annually surviving rabbit populations in the world. It is believed to have originated from escaped pet rabbits in the late 1980s, and in the early 2000s, the rabbits spread rapidly around the Helsinki area. RHD spread unintentionally to Finland in 2016, and the disease caused a significant reduction in the Helsinki rabbit population.

Rabbit population genetics has previously been studied in several countries, but never before in Finland. The aim of the thesis was to examine the genetic diversity and population structure of the Helsinki rabbit population before and after the RHD epidemic, and to compare the results to similar preceding rabbit population genetic studies.

Rabbit populations have previously been found to recover from major population crashes without a notable loss in genetic diversity using DNA microsatellite markers. The recent RHD epidemic in Helsinki provided an opportunity to study, whether a rabbit population can recover from a population crash even in a harsher environment without losing genetic diversity. To conduct genetic analysis, fourteen DNA microsatellite loci were genotyped from individuals caught during two distinct time periods, in 2008-2009 (n=130) and in 2019-2020 (n=59).

Population structure was observed in both temporal rabbit populations with small but significant F_{ST} values. The 2019-2020 population was more diverse than the 2008-2009 population in terms of allele numbers and expected heterozygosity. This result was unexpected considering the recent RHD-epidemic but could be explained by gene flow from new escaped rabbits. Compared to other wild rabbit populations around the world, the Helsinki area rabbits exhibit significantly lower genetic diversity.

Bottleneck tests showed a significant signal separately in both temporal populations, but the RHD bottleneck cannot be distinguished based on the tests. The results could be biased by new gene flow, or the initial bottleneck caused by the founder effect of only a few pet rabbits.

The rabbits have demonstrated their adaptation and survival skills in the cold climate of Helsinki. The population has significantly lower genetic diversity compared to other wild populations, yet recovered from a major RHD epidemic without reduction in genetic diversity under these more extreme environmental conditions. It has been proven again; the rabbit is a thriving invasive species.

Avainsanat – Nyckelord – Keywords

The European rabbit, *Oryctolagus cuniculus*, population genetics, population structure, genetic diversity, genetic bottleneck, Rabbit Haemorrhagic Disease, RHD, invasive species

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Vuoden 2016 kanin verenvuototautiepidemian vaikutus Helsingin kanipopulaation geneettiseen monimuotoisuuteen

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Tiivistelmä – Referat – Abstract

Kaniini (*Oryctolagus cuniculus*) on Iberian niemimaalta kotoisin oleva nisäkäs, joka on levinnyt ihmisen toiminnan ansiosta ympäri maailmaa. Kani on erittäin menestynyt vieraslaji nopean lisääntymiskykynsä ja generalistisen taipumuksensa ansiosta. Kanit ovat aiheuttaneet suuria muutoksia paikalliseen luontoon useissa maissa, ja siksi haitallisia populaatioita on yritetty kontrolloida mm. levittämällä kanienverenvuototautia (rabbit haemorrhagic disease, RHD).

Suomessa kaneja tavataan ainoastaan pääkaupunkiseudulla ja muutamissa muissa taajamissa. Helsingin kanipopulaation uskotaan syntyneen karanneista lemmikkikaneista 1980-luvun lopulla. Kanien määrä kasvoi Helsingissä merkittävästi 2000-luvulla, ja ne ovat levinneet myös Espooseen ja Vantaalle. Kanien verenvuototauti levisi tahattomasti suomeen keväällä 2016, mistä seurasi populaation koon merkittävä romahdus kesän aikana.

Kanien populaatiogenetiikkaa on tutkittu useissa eri maassa, mutta ei koskaan Suomessa. Pro graduni tavoitteena oli tutkia Helsingin kanien geneettistä monimuotoisuutta ja populaation rakennetta ja verrata tuloksia aiemmin julkaistuihin tutkimuksiin. Kanipopulaatioiden on todettu selviävän suuristakin populaatiokoon romahduksista eli pullonkauloista menettämättä merkittävästi geneettistä monimuotoisuutta. Suomen kylmä ilmasto itsessäänkin jo koettelee kanien selviytymisen rajoja, ja siksi oli kiinnostavaa selvittää, miten pohjoisen ilmaston ja RHD-epidemian aiheuttaman populaatiokoon romahduksen yhteisvaikutus ilmeni Helsingin kanikannassa.

Tutkimuksen aineistona oli kanikudosnäytteitä ennen ja jälkeen RHD-epidemiaa, vuosilta 2008-2009 (n=130) sekä 2019-2020 (n=59). Populaatiogeneettisiä analyyseja varten genotyypattiin neljätoista kaneilla varioivaa DNA-mikrosatelliittilokusta.

Populaatio rakennetta oli havaittavissa sekä vanhassa että uudessa populaatiossa pienillä, mutta merkitsevillä F_{ST} arvoilla. Uusissa näytteissä oli enemmän alleeleja ja suuremmat heterotsygotia-arvot vanhoihin näytteisiin verrattuna, mikä oli yllättävä tulos RHD-epidemian jälkeen. Yksi mahdollinen selitys on, että populaatioon on tullut geenivirtaa uusista karanneista kaneista. Muiden maiden kanipopulaatioihin verrattuna Helsingin kanit ovat monimuotoisuudeltaan selkeästi köyhempiä.

Pullonkaulatestit antoivat merkitsevän signaalin molemmille populaatiolle, joten testeillä ei pystytty erikseen vahvistamaan juuri RHD-epidemian aiheuttamaa geneettistä pullonkaulaa. Testien tulokseen on voinut vaikuttaa migraatio sekä populaation syntymähetkellä perustajanvaikutuksesta seurannut pullonkaula.

Kanit ovat osoittaneet poikkeuksellisen adaptaatiokykynsä Helsingin kylmässä ilmastossa, ja populaatio selvisi äärioloista huolimatta myös RHD-epidemian aiheuttamasta populaation romahduksesta menettämättä merkittävästi geneettistä monimuotoisuutta.

Avainsanat – Nyckelord – Keywords

Kaniini, Oryctolagus cuniculus, populatiogenetiikka, populaatio rakenne, geneettinen monimuotoisuus, geneettinen pullonkaula, Kanin verenvuototauti, RHD, vieraslaji

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Abbreviations

AMOVA = Analysis of Molecular Variance

DAPC = Discriminant Analysis of Principal Components

DF= discriminant function

I.A.M. = infinite alleles model

PC = principal component

RHD = rabbit haemorrhagic disease

S.M.M. =stepwise mutation model

T.P.M. = two-phase model

Table of contents

1. Introduction

1.1. The European rabbit

The European rabbit, *Oryctolagus cuniculus* (Linnaeus, 1758) (Figure 1) belongs to the order Lagomorpha with two extant families, the Ochotonidae (pikas) and the Leporidae, which includes 63 species of hares and rabbits (Andrew *et al.* 2018). The European rabbit is the only species of its genus, with two subspecies *O. c. cuniculus* and *O. c. algirus*.

Figure 1. A wild rabbit with typical greyish-brown fur
(Picture from https://en.wikipedia.org/wiki/European_rabbit)

Rabbits are burrowing animals that form small social and territorial breeding groups, typically including one to three adult males and one to six adult females (Webb *et al.* 1995). Habitat and population density affect the group size, which can be up to 20 individuals, but rabbits live also solitarily (Lees & Bell 2008). Rabbits generally tend to stay close to the entrances of the group warren, but their home range size can vary greatly among different habitats. Researchers have reported home ranges of 0.01-0.4 ha in the Netherlands (Dekker *et al.* 2006), 0.5-2 ha in Spain (Lombardi *et al.* 2007), 0.7-2 ha in France (Devillard *et al.* 2008), and 2.1-4.2 ha in Australia (Moseby *et al.* 2005).

Within a group, there is a linear dominance hierarchy among both male and female individuals, which results in the dominant individuals having generally higher reproductive success (Webb *et al.* 1995). Females compete for the best nesting sites inside the warren, and males compete for access to females (Surridge *et al.* 1999a). Both sexes defend the warren, but females are usually more aggressive (Lockley 1961; Southern 1948).

Young male rabbits tend to leave the breeding group before the first breeding season, and this male-biased natal dispersal creates gene flow between groups (Webb *et al.* 1995). Female rabbits typically stay in the native group and the female philopatry leads to a high relatedness among female group members, and increases co-operation. However, dominant females may banish young females to control the number of kittens in the warren since too many can attract more predators (Surridge *et al.* 1999a). The social structuring and low level of gene flow between groups results in the breeding groups becoming genetically distinct units. In addition, the effect of genetic drift is stronger in a small group, and thus genetic structure is often observed in rabbit populations (Surridge *et al.* 1999a).

1.2. The European rabbit as an invasive species

The native range of the European rabbit is the Iberian Peninsula and southern France (Figure 2), but they have been introduced to all continents except Antarctica, and to over 800 islands worldwide (Flux & Fullagar 1992; Lees & Bell 2008). The Phoenician traders and Romans are thought to be the first who transferred rabbits from the Iberian Peninsula to many parts of the Mediterranean including North Africa starting over 3000 years ago (Flux & Fullagar 1992). Rabbits were bred in enclosures for meat and fur, but dug their way out and spread to the wild in many places. In the Middle Ages rabbits were introduced to northern Europe, including the British Isles in the 11th century (Surridge *et al.* 1999b).

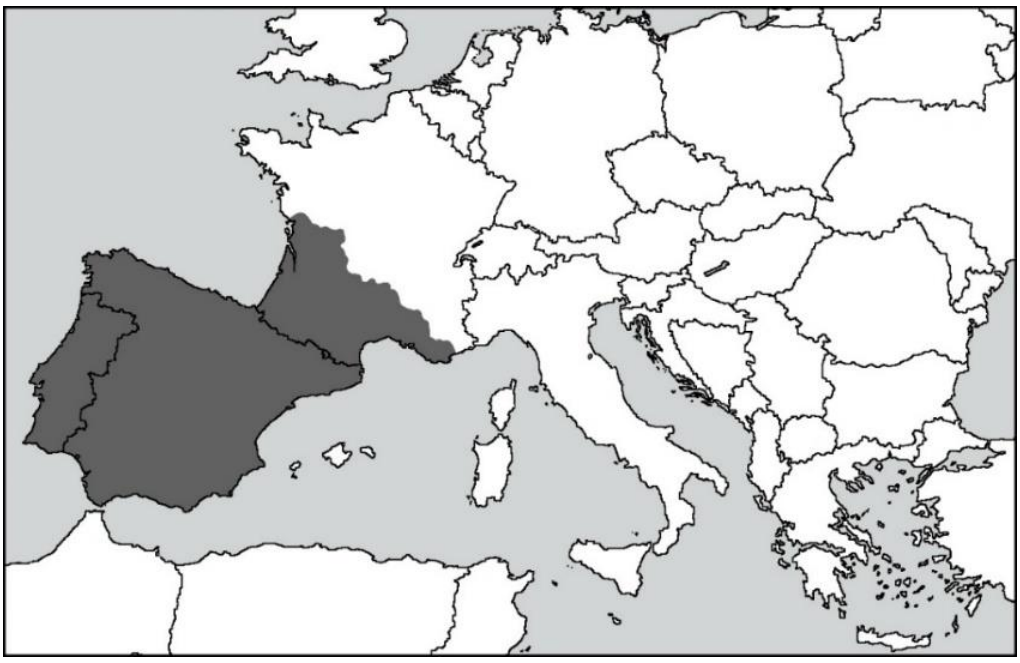

Figure 2. Native range of the European rabbit, adopted from Irving-Pease *et al.* 2018

The worldwide intentional introductions of the rabbit began on a large scale after the 18th century (Carneiro *et al.* 2011) when Europeans continued to explore and colonise new areas. Rabbits were introduced to New Zealand in the 1850s (King 2017) and South America (Chile) in 1936 (Lees & Bell 2008). Thirteen rabbits were transported from England and released in Victoria, Australia, in 1859, where the species spread unexpectedly fast and occupied most of the continent by 1910 (Zenger *et al.* 2003). Rabbit meat remained a prominent reason behind the introductions, but rabbits were also released in the wild for leisure hunting, to attract tourists, and to control vegetation in some places (Flux & Fullagar 1992). A distribution map (Figure 3) from the GBIF database (Global Biodiversity Information Facility, https://gbif.org/, accessed 15.3.2021) shows an indicative present-day distribution of rabbits around the world based on human observations.

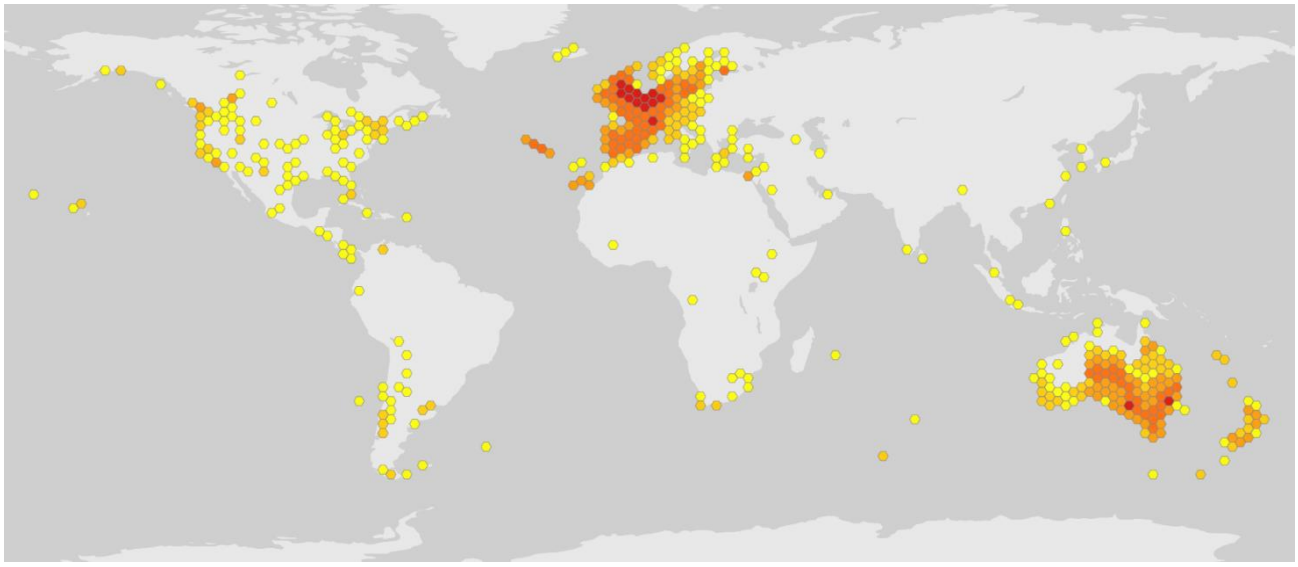

Figure 3. Rabbit observations in GBIF database from 1700 to 2021. Darker colour indicates more observations.

The European rabbit has been a remarkably successful invasive species, and the result of their introductions have mostly been destructive to local nature, especially in Australia and New Zealand. Rabbits are generalists and can adapt to broadly varying environments from temperatures as high as 50°C to cold snowy winters (Lees & Bell 2008). Only a few rabbits are needed to establish a new population, and they are efficient reproducers; female rabbits can have up to seven litters per year and the mean litter size is 4-6 kittens (Tablado & Palomares 2009). Factors that limit population size include predation pressure, pathogens, lack of suitable nesting sites, and the quality and quantity of green vegetation (Lees & Bell 2008).

Escaped or released domesticated rabbits can also survive in the wild, which further enables the emergence of new feral annually surviving populations. Domesticated rabbit breeds are phenotypically highly variable, but rabbits will revert to the wild-type phenotype appearance in only a few generations in the wild (Thulin *et al.* 2017). Domestication of the rabbit started relatively recently, c. 1400 years ago in France according to historical records and many genetic studies (Alves *et al.* 2015; Carneiro *et al.* 2014). A major genetic bottleneck occurred during domestication, but only a small number of single-nucleotide polymorphisms (SNPs) have been fixed in the genomic regions affected by selection during domestication, which explains the rabbit's fast ability to de-domesticate (Carneiro *et al.* 2014).

1.3. Myxomatosis and rabbit haemorrhagic disease

Many attempts have been made to control harmful rabbit populations with varying success. Fatal viral rabbit diseases such as myxomatosis and rabbit haemorrhagic disease (RHD) have been introduced as biocontrol agents to eradicate harmful populations for instance in Australia, New Zealand, and France (Kerr 2012). The *Myxoma* virus originates from the Americas and causes mild symptoms in the local cotton-tail rabbits (genus *Sylvilagus*), but induces a fatal disease in the European rabbit (Kerr 2012). The virus is mainly transmitted by blood-sucking or biting insects but also by direct contact. The first wave of myxomatosis had a mortality rate of over 90% (Queney *et al.* 2000).

The rabbit haemorrhagic disease (RHD) is a highly contagious and fatal rabbit hepatitis that causes 70-90% mortality in adult rabbits, although the disease is nearly asymptomatic in young kittens up to two months old (Abrantes *et al.* 2012; Isomursu *et al.* 2018). A newer strain of the virus (RHDV2) was identified in France in 2010 (Le Gall-Recule *et al.* 2013), which also affected young rabbits older than 15 days and those vaccinated against RHD, but had an overall mortality rate of 5-80% (Isomursu *et al.* 2018). The RHD viruses can be transmitted in multiple ways, for instance, through direct contact, by insects, or from the soil (Abrantes *et al.* 2012).

Despite the high mortality of both diseases, they have not had a permanent effect on introduced populations, and wild rabbits remain a problem in many countries, especially in Australia. However, the viruses together with overhunting and habitat loss are causing a serious decline in the native rabbit populations in the Iberian Peninsula, where the European rabbit is an important keystone species (Lees & Bell 2008).

1.4. The European rabbit in Finland

The Finnish fauna includes three species of lagomorphs: the Mountain hare (*Lepus timidus),* the European hare (*Lepus europaeus),* and the European rabbit (*Oryctolagus cuniculus)*. The European rabbit was uncommon in Finland before the 20th century, but large-scale rabbit husbandry began in 1919-1920 to produce meat and fur (Pihlström 2016). Rabbits were an important food source, especially during the Second World War, since rabbits do not require a lot of space and grow relatively fast. Later rabbits became popular pets, and nowadays their importance as a meat source is minor. Rabbits are notorious for digging their way out of enclosures, and thus it is likely that they escaped into the wild occasionally ever since they were brought to Finland. The history of the European rabbit in Finnish nature is poorly recorded and sustainable populations have been documented only since the late 20thcentury. Rabbits have never been released into the wild with a permission from the game authorities, as in many other countries, and therefore all Finnish wild rabbits originate from escaped domesticated rabbits. Wild rabbit populations are highly localized, and they are only found in urban and suburban areas. For this reason, Finnish wild rabbits are often referred to as city-rabbits. Rabbits are not adapted to tolerate cold winters, and this restrains their spreading to rural areas. Rural areas also have more predators for rabbits. The most notable wild rabbit population is found in the Helsinki area, but wild rabbits are also found in a few other larger cities, for example in Turku, Vaasa (https://vieraslajit.fi), Porvoo (Leikas & Rautiainen 2010) and Hyvinkää (https://hyvinkaa.fi/).

1.4.1. The Helsinki rabbit population

The rabbit population in the Helsinki area is the biggest and best known in Finland. Sporadic rabbit sightings have been reported at least since the 1970s, but it was not until the mid-1980s when the first permanent population was observed in the Kyläsaari neighborhood (Leikas & Rautiainen 2010). This small number of rabbits lived in a wasteland area where they did not attract much attention. The area was used to store compost and twig piles which provided a good food source and shelter, and enabled the rabbits´ survival even during cold winters. The rabbits stayed in the Kyläsaari area and the nearby Arabianranta neighborhood for the next ten years (Leikas & Rautiainen 2010).

In the early 2000s, rabbits started to spread rapidly to new areas, and they were observed as far as 10 km north of the city center. Helsinki is a green city with many gardens and public parks, which offer well-suited habitats for rabbits and made their spreading easy. In 2007 the rabbits had taken

over almost all areas south of the Kehä I ring road, and from 2008 onwards rabbit sightings were also reported in the neighboring cities of Espoo and Vantaa (Figure 4), where they have been permanent residents ever since (Leikas & Rautiainen 2010).

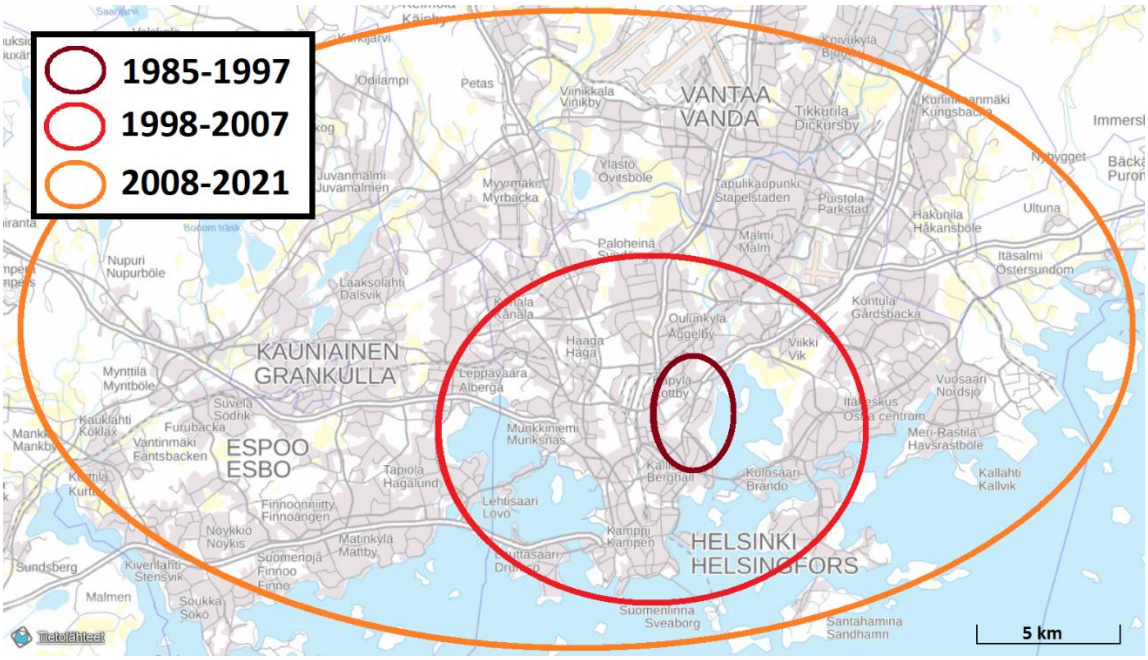

Figure 4. Spreading history of the European rabbit in the Helsinki area. The 1985-1997 and the 1998-2007 ranges are adopted from Leikas & Rautiainen 2010. The 2008-2021 range is adapted from rabbit observations in https://laji.fi/observation/map, accessed 14.4.2021.

The number of rabbits varied greatly among areas based on the availability of proper shelter and food sources. Rabbits were most frequently seen in allotment gardens and in the Helsinki city center around Töölönlahti bay. It was estimated that there could be up to 600 individuals/km^2 in the most densely populated areas during the peaks of rabbit occurrence (Leikas & Rautiainen 2010). However, estimates of the number of rabbits and their distribution are strongly tied to human proximity, since no regular or long-term monitoring schemes have been organized.

Horticultural damage caused by rabbits is much higher than that of hares, and their effect on the city environment became apparent after the number of rabbits grew strongly. Rabbits can feed on almost all plants, even toxic ones, like yew (Leikas & Rautiainen 2010). In the summer, rabbits feed mostly on hay and grass, but they can also consume summer flowers and perennial plants, causing economic and aesthetic damage to both private and city gardens. Rabbits can also be a personal nuisance, especially in the allotment gardens where citizens grow their own vegetables. The worst damage is caused during winter when there is limited plant material available. Rabbits can gnaw the bark of tree trunks and shrubs, eventually killing the plants. In addition to the damage for

horticulture, rabbits can break building foundations and structures in parks by digging their burrows. During the peak years, the annual economic loss caused by the European rabbit in Helsinki was estimated to be several hundred thousand euros (Leikas & Rautiainen 2010). From 2002 onwards the population was controlled by hunting to limit this economic cost. In the 2004-2005 season less than 50 rabbits were hunted, but the number increased to over 700 in 2008 and almost 4000 in 2009 (Leikas & Rautiainen 2010).

1.4.2. The RHD epidemic in 2016

In April 2016, many dead rabbits were suddenly observed in Helsinki. The Finnish Food Safety Authority Evira (from 2018 known as Ruokavirasto, Finnish Food Authority) determined the cause of death as RHD. This was the first time RHD was detected in Finland, and the RHD virus was characterized as the newer strain RHDV2 (Isomursu *et al.* 2018). During April and May 2016, the virus spread fast and killed a massive number of rabbits. For instance, dozens of dead rabbits were removed by biology students from the Kumpula Botanic Garden during one weekend (Figure 5).

Figure 5. Biology students removing dead rabbits from the Kumpula Botanic Garden at the turn of April and May 2016

At the end of the summer, neither dead nor alive rabbits were no longer observed, which suggests a significant reduction in the population size. However, it is difficult to estimate the mortality rate due to the lack of monitoring of the population. The Natural Resources Institute Finland (LUKE) has

reported the number of hunted rabbits in Finland from 2005 to 2019 (Figure 6). This statistic provides an estimate of the population size variation based on hunting pressure in different years, since most of the rabbits in Finland live in the Helsinki area. The RHD epidemic stands out in the statistics since there was less need to hunt rabbits during the epidemic year 2016 and the following year 2017.

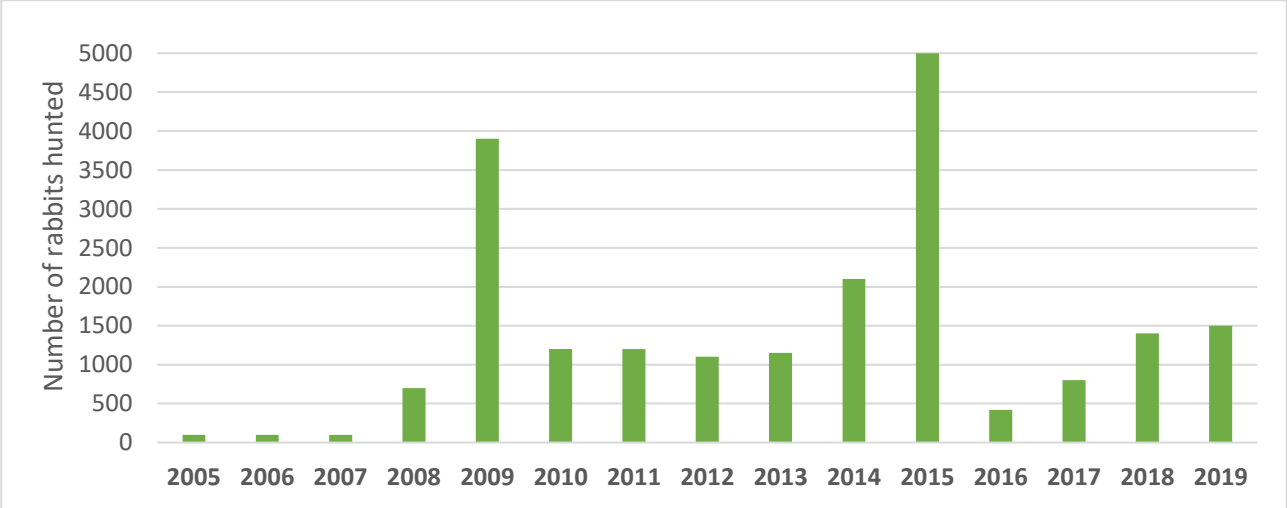

Figure 6. The number of rabbits hunted per year in Finland 2005-2019. Numbers are taken from LUKEs hunting statistics (https://luke.fi/en/ accessed 10.4.2021)

The virus epidemic affected the size and distribution of the rabbit population remarkably in the following years. The city center, where the rabbits were most often observed, has remained quiet to this day. For instance, rabbits used to be a major problem in the Kaisaniemi Botanic Garden, but there were no sightings in the winter 2019. Instead, the population is growing rapidly in northern and eastern Helsinki, and especially in the city of Espoo in the West. The LUKE rabbit hunting data (Figure 6) indicates that the population has recovered fast, since already in 2018 up to 1400 rabbits were hunted, and the hunting continues to this date.

In the summer of 2020, myxomatosis was diagnosed in wild rabbits in Espoo for the first time in Finland (Finnish Food Authority announcement 2020). Rabbit deaths were reported constantly in the local news during summer and autumn in the Helsinki area, and the *Myxoma* virus was also detected in pet rabbits. It remains to be seen how these two viruses will affect the Helsinki area rabbits in the future.

2. Aim of the thesis

Genetic structure and diversity of wild rabbit populations have been studied in many countries, but not before in Finland. Rabbits are famous for their extreme adaptation ability, but they do not favor wet and cold ecosystems, and snow depth is one of the limiting factors of their survival (Lees & Bell 2008). The Helsinki rabbit population is one of the most northern annually surviving rabbit populations in the world and the climate of Helsinki approaches the survival limits of the rabbits. These factors make the Helsinki population a unique study subject.

Previously, rabbit populations have been found to recover from major population crashes without a notable genetic bottleneck using DNA microsatellite markers (Queney *et al.* 2000; Zenger & Vachot-Griffin 2003). The recent RHD epidemic in Helsinki provides an opportunity to study, whether a rabbit population can recover from a population crash even in a harsher environment without losing genetic diversity.

The aims of the thesis are:

1) To provide general population genetic knowledge of the Helsinki area rabbits and compare the results to previous similar studies.

2) To compare the genetic diversity and the population structure of the Helsinki population at two temporal points: before and after the RHD epidemic.

3) To test whether the RHD epidemic caused a genetic bottleneck.

3. Materials and methods

3.1. Ethics statement

The European rabbit has been classified as a game animal in Finland since 1993 (Leikas & Rautiainen 2010) and the same hunting legislation applies to it as to the European and Mountain hare. According to the law, a hunting license and landowner's permission are required to hunt rabbits and extra caution is needed when hunting in a city. All the rabbits in this study were hunted by legitimate hunters and were hunted according to the regulations. None of the rabbits were purposely killed for this study.

3.2. Sampling strategy

This master´s thesis includes rabbits sampled from the Helsinki area from two time periods, 2008-2009 and 2019-2020, which will be hereafter called the old population and the new population, respectively. Mammal specialist Heidi Kinnunen (Finnish Museum of Natural History LUOMUS) organized the collection of the first set of samples in 2008-2009. Muscle tissue samples from 149 rabbits were preserved in ethanol, labelled with locality and other information of the individual (capture location, hunting method, fur color, weight, and sex). From these samples, 130 were chosen for this thesis (See Appendix Table 1), and were added to the LUOMUS Genomic resources collection (searchable at https://laji.fi/theme/luomusgrc/search/list). Most of the individuals were caught in the city center area around Töölönlahti bay or in the Kaisaniemi Botanic Garden (Figure 7 & Table 2). None of the old samples were from Espoo or near Vantaa, even though rabbits had already spread to those areas.

The new rabbits were hunted between September 2019 and April 2020 using ferrets. The aim was to sample roughly 100 individuals from the same areas as the old samples. However, no rabbits were caught in the city center area where the rabbits used to be abundant, and a total of 59 individuals were sampled (See Appendix Table 2). Most of the individuals were caught around the Herttoniemi neighborhood in eastern Helsinki and around the Kannelmäki neighborhood in northern Helsinki (Figure 7 & Table 2).

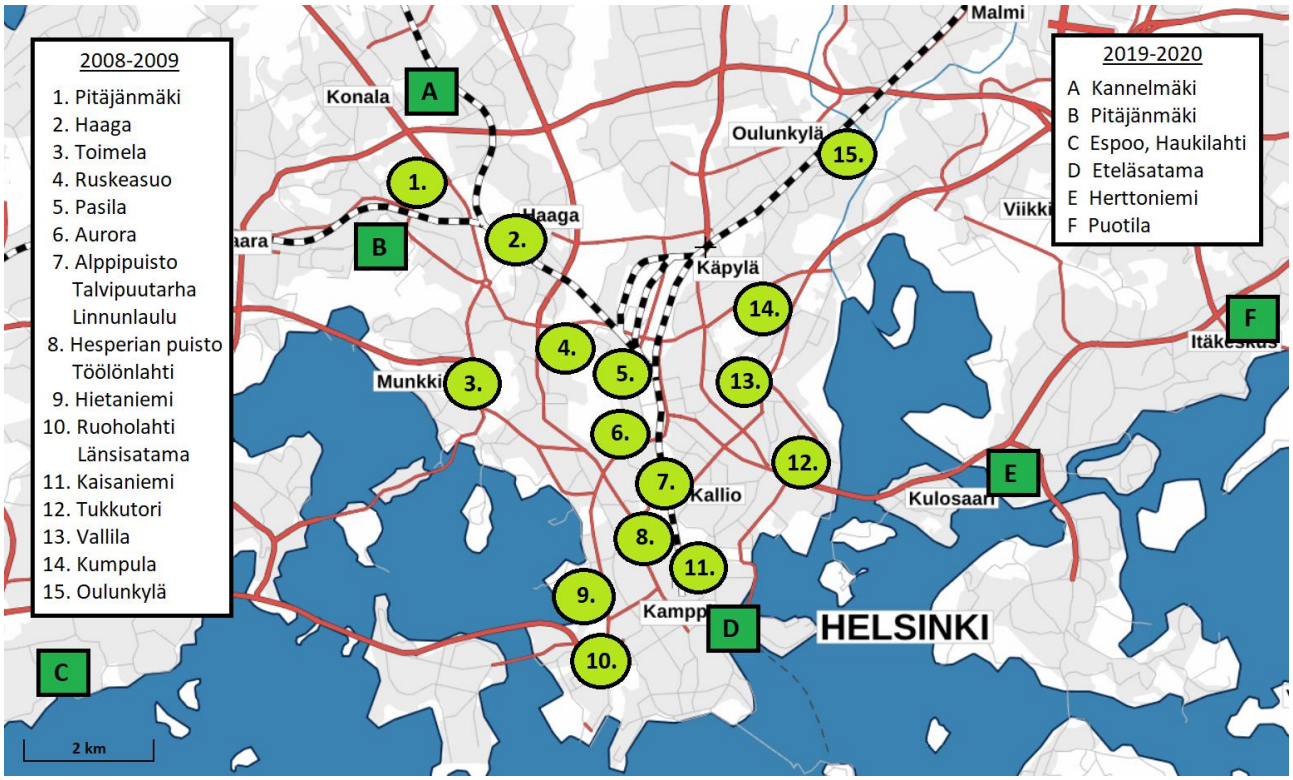

Figure 7. Rabbit capture locations. 2008-2009 locations 1.-14. in light green circles, 2019-2020 locations A-F in dark green squares.

Tissue sampling was done on frozen individuals, and therefore two small pieces of ear were cut off from each rabbit. The first piece was stored in 96 % ethanol deposited in Luomus' Genomic resources collection, and the second was placed in an empty Eppendorf tube for DNA extraction for this study. All tissue samples were stored in a freezer after sampling. Fur color and markings were documented during sampling, and an approximate hunting location and date were obtained from the hunter.

3.3. DNA extraction

DNA extractions were performed in the DNA-lab of LUOMUS using the NucleoSpin Blood & Tissue DNA extraction kit (Macherey-Nagel). The old tissue samples were preserved in ethanol, and a small piece (~3 mm^3) was cut for DNA extraction. Ethanol was allowed to evaporate from the tissue before adding the first lysis buffer. The new tissues were preserved without ethanol and an approximately 4x4 mm piece of ear tissue was cut for DNA extraction. The standard extraction protocol provided by the manufacturer was followed using an overnight pre-lysis, and DNA was eventually eluted using 100 µl of ultrapure water. DNA concentration and A260/280 ratio were checked with a NanoDrop spectrophotometer (Thermo Scientific), and 10% of the extractions were also checked with a Qubit

fluorometer (Invitrogen) to validate concentration. All DNA extractions had adequate quality for the GRC collection, and they can be browsed at https://laji.fi/.

3.4. DNA microsatellite genotyping

Thirty-nine potential candidate DNA microsatellite markers developed for the European rabbit were evaluated based on the number of alleles and size variation detected in multiple rabbit population genetic studies (Abdel-Kafy *et al.* 2018, Alda & Doadrio 2014, Alves *et al.* 2015, Chantry-Darmon *et al.* 2005, Jochova *et al.* 2017, Mougel *et al.* 1997, Queney *et al.* 2000, Queney *et al.* 2001, Rico *et al.* 1994, Surridge *et al.* 1999b and Surridge *et al.* 1999c). Fourteen markers (Table 1) were chosen for this thesis based on the observed number of alleles, use in the above-mentioned studies, and PCR success rate if reported. Three multiplex PCRs (Table 1) were designed based on the size variation in the previous studies and the PCR annealing temperatures. Forward primers were labelled with a fluorescent label (Table 1). The primers (Appendix Table 3) were ordered from Metabion international AG (https://metabion.com/).

Table 1. The fourteen DNA microsatellite markers used in the thesis, and the corresponding multiplex, fluorescent label, and PCR annealing temperature for each marker.

Thesis code	Original code	Reference	Multiplex	Label	PCR annealing °C
INRA192	INRACCDDV0192	Chantry-Darmon *et al.* 2005	1	FAM	57
INRA228	INRACCDDV0228	Chantry-Darmon *et al.* 2005	1	FAM	57
INRA169	INRACCDDV0169	Chantry-Darmon *et al.* 2005	1	TAMRA	57
INRA087	INRACCDDV0087	Chantry-Darmon *et al.* 2005	1	HEX	57
INRA104	INRACCDDV0104	Chantry-Darmon *et al.* 2005	1	HEX	57
INRA201	INRACCDDV0201	Chantry-Darmon *et al.* 2005	2	FAM	57
INRA119	INRACCDDV0119	Chantry-Darmon *et al.* 2005	2	FAM	57
INRA140	INRACCDDV0140	Chantry-Darmon *et al.* 2005	2	TAMRA	57
INRA102	INRACCDDV0102	Chantry-Darmon *et al.* 2005	2	HEX	57
SAT13	SAT13	Mougel *et al.* 1997	2	HEX	57
SOL8	SOL8	Rico *et al.* 1994	3	FAM	60
SAT7	SAT7	Mougel *et al.* 1997	3	FAM	60
SAT8	SAT8	Mougel *et al.* 1997	3	TAMRA	60
SAT3	SAT3	Mougel *et al.* 1997	3	HEX	60

DNA microsatellite genotyping was done in the Molecular Ecology and Systematics Laboratory (MES-lab) in Biocenter 3 at the Viikki campus. The PCR amplifications were done with the multiplex PCR kit (Qiagen) using a standard protocol that was scaled down to 10 µl reaction volume and Q-solution was left out (See Appendix Table 4). 1 µl of undiluted DNA was used in all reactions. PCR reactions were run in following conditions: initial denaturation at 95 °C for 15min, 30-32 cycles of

94 °C for 30 s (denaturation), 57 or 60 °C for 90 s (annealing) and 72 °C for 60 s (extension), and final extension at 60 °C for 30 min. Annealing temperatures were chosen for each multiplex reaction according to table 1. DNA microsatellite genotyping was performed with ABI 3730 DNA Analyzer (Applied Biosystems) using GeneScan™ 500 ROX™ size standard. 1 µl of diluted (1:50-1:200) PCR product was used in the genotyping. All unclear or unusual results were repeated to get a reliable result for all markers in all samples. All results were manually checked with GeneMapper 5 software (Applied Biosystems).

3.5. Data analysis

DNA microsatellite data was confirmed to not contain null alleles, large allele drop-out, or mis-scoring due to "stutter-bands", using MICRO-CHECKER 2.2.3 software (Van Oosterhout *et al.* 2004). Deviation from expected Hardy-Weinberg equilibrium was tested with a probability test, and linkage disequilibrium between all loci pairs was tested with log likelihood ratio statistics in the online version of GENEPOP 4.7. (Raymond & Rousset 1995, https://genepop.curtin.edu.au/). All tests were carried out separately for the old and new populations without subpopulation division.

3.5.1 Genetic diversity and population structure

Genetic diversity was evaluated as allele frequencies, number of alleles per locus, number of private and effective alleles, and observed (H_O) and expected heterozygosity (H_E), which were calculated using the excel add-on package GenAlEx 6.5. (Peakall & Smouse 2006, 2012).

Population structure was analysed using a Discriminant Analysis of Principal Components (DAPC). DAPC defines the relationships of pre-defined groups so that it maximizes between-group variance and minimizes within-group variance. DAPC also gives membership probabilities which indicates how likely the individuals are to belong to the pre-defined group and if the groups are distinct or mixed. DAPC was run in R-studio 3.6.3 (R Core team 2020) using the *adegenet* 2.1.3 package (Jombart 2008). The optimal number of PCs to retain for each DAPC run was estimated with *xvalDapc()* and *optim.a.score()* functions.

Population structure was examined at two different levels: a) temporal difference between the old and the new populations and b) a subpopulation structure within the two time periods. The subpopulation divisions for DAPC were determined based on the hunting location of the rabbits (Figure 7) and the sample size in each location (Table 2). In the old population, subpopulations were

further divided to three groups: South (S), West (W), and North & East (N&E) (Table 2). The two Pasila samples were kept as an own subpopulation in the first subpopulation division, but excluded as outliers from the second, since they differed markedly from all other samples in a preliminary DAPC run. The subpopulation divisions (Table 2) were used as the pre-defined groups in DAPC runs.

Table 2. The number of captured rabbits (n) from the 2008-2009 and the 2019-2020 locations, with subpopulation divisions. Code=site number/letter in Figure 7.

2008-2009					2019-2020			
Code	**n**	**Location**	**1. subpop. division**	**2. subpop. division**	**Code**	**n**	**Location**	**1. subpop. division**
1.	2	Pitäjänmäki	Ruskeasuo	West	A	29	Kannelmäki	Kannelmäki
2.	1	Haaga			B	6	Pitäjänmäki	Pitäjänmäki
3.	5	Toimela			C	8	Espoo Haukilahti	Espoo
4.	12	Ruskeasuo			D	2	Eteläsatama	Eteläsatama
5.	2	Pasila	Pasila	-	E	13	Herttoniemi	Herttoniemi
6.	2	Aurora	Töölönlahti	South	F	1	Puotila	Puotila
7.	18	Talvipuutarha						
	10	Alppipuisto						
	2	Linnunlaulu						
8.	2	Töölönlahti						
	4	Hesperian puisto						
9.	3	Hietaniemi	Hietaniemi					
10.	1	Ruoholahti						
	2	Länsisatama						
11.	30	Kaisaniemi	Kaisaniemi					
12.	3	Tukkutori	Vallila	North & East				
13.	8	Vallila						
14.	2	Kumpula						
15.	21	Oulunkylä	Oulunkylä					

Population structure was also estimated using a hierarchical Analysis of Molecular Variance (AMOVA), which was performed with GenAlEx. AMOVA calculates the distribution of the genetic variation in the samples and then estimates how much different hierarchical levels explain that variation. Moreover, AMOVA provides estimates for associated F statistic values for each hierarchical level, and estimates their departure from zero by bootstrapping. The hierarchies are among region, among subpopulation, among individuals, and within individuals. The two time periods were assigned as regions, the second subpopulation division was chosen for the old samples, and the first subpopulation division for the new samples (Table 2). The Eteläsatama and

Puotila populations were excluded from all hierarchies since they had only two and one samples, respectively.

The fixation indices F_{IS}, $F_{ST,}$ and F_{IT} were also calculated for the old and the new subpopulations with frequency-based method in GenAlEx, since it uses a different approach compared to AMOVA. The first subpopulation divisions were chosen for both temporal populations, but subpopulations with 1-2 samples were left out (Pasila, Puotila, and Eteläsatama).

3.5.2 Genetic bottleneck

The third aim of the thesis was to test whether the RHD epidemic caused a genetic bottleneck in the Helsinki rabbit population. A genetic bottleneck means a severe reduction of effective population size, which causes loss of genetic diversity, because the remaining individuals carry only a proportion of the former variation. A bottleneck especially causes the loss of low frequency alleles. Factors that affect the magnitude of the bottleneck are, for example, the percentage of lost individuals, how many generations the bottleneck lasts, and how quickly the population size recovers afterwards (Cornuet & Luikart 1996).

During a bottleneck, the number of alleles is reduced relatively faster than heterozygosity decreases (Piry *et al.* 1999). Therefore, heterozygosity becomes greater than expected according to the mutation-drift equilibrium. For this reason, a recent bottleneck can be detected if there is an excess of heterozygosity in the majority of studied polymorphic loci. This was tested with the three statistical tests available in the BOTTLENECK 1.2.02 (Piry *et al.* 1999) software: a sign test, a standardized differences test, and a one-tailed Wilcoxon sign rank test as well as a mode-shift test of allele frequencies. BOTTLENECK tests three different mutation models: an infinite alleles model (I.A.M.) (Kimura & Crow 1964), a stepwise mutation model (S.M.M.) (Kimura & Ohta 1978), and a two-phase model (T.P.M.) (Dirienzo *et al.* 1994). All tests were run with default settings. The infinite alleles model is not recommended for DNA microsatellite data (Putman & Carbone 2014) but since the evolutionary timescale in the thesis is relatively short and the allele sizes were often widely separated (Figure 8), the I.A.M. model was also included.

4. Results

4.1. Evaluation of DNA microsatellite markers

DNA microsatellite genotyping was successful for all fourteen amplified loci in all 189 samples. Every sample had a unique multilocus genotype. Two of the loci were monomorphic (INRA228 and SAT13), and these were therefore omitted from further analyses. The locus INRA102 was monomorphic in the old samples, but was polymorphic in the new samples. This locus was thus not excluded, and a total of twelve loci were used in the analyses. Among polymorphic loci, there were 2-7 alleles per locus within all samples (Figure 8), with an average of 3.75. Allele frequencies by subpopulations are shown in Appendix Table 5.

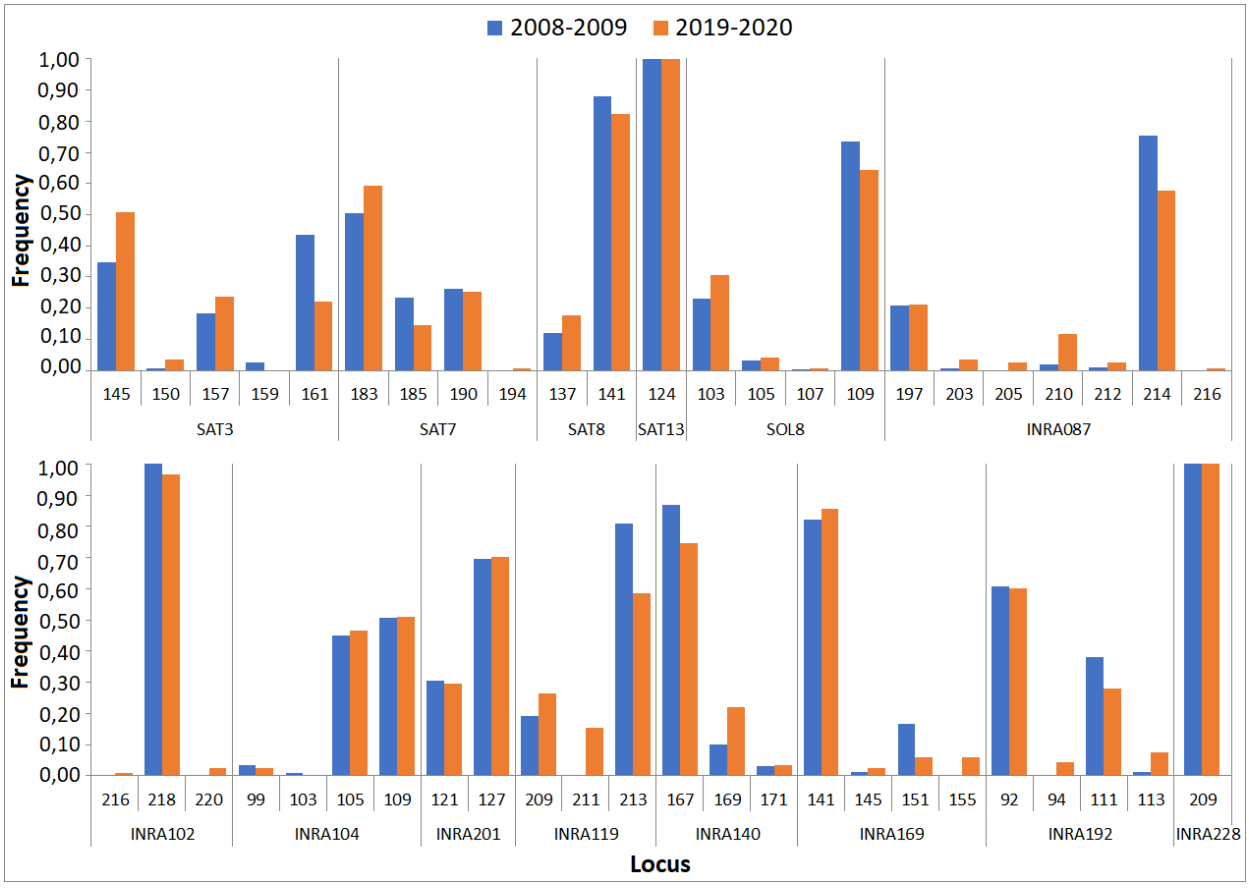

Figure 8. Allele frequencies of all 14 loci in the 2008-2009 and 2019-2020 samples

There was no evidence for null alleles, large allele dropout, or scoring error due to stuttering in any of the loci. No deviation from the Hardy-Weinberg equilibrium was detected in the old or new populations, except the locus INRA119, which differed from the expected with a P-value of 0.003 in the new population. This is most likely due to a single allele (211), which had a high frequency (0.54) in the Herttoniemi group but low frequencies (0-0.07) in all other groups (Appendix Table 5). No significant linkage disequilibrium was detected between any locus pair.

4.2. Temporal diversity and structure of the Helsinki population

All measurements of genetic diversity (Table 3) show that the new population is more diverse than the old population. The new population has higher observed and expected heterozygosity and more alleles in all categories. Fixation index F has a value close to zero in both populations indicating that the difference between observed and expected heterozygosity does not differ significantly in either population. Locus specific values for both populations are presented in Appendix Table 6.

Table 3. Mean values over all 12 loci for the old and the new populations: SE=standard error, n=sample size, Total=total number of alleles, Per locus=average number of alleles in loci, Private= number of private alleles, Effective=average effective number of alleles, H_O=observed heterozygosity, H_E=expected heterozygosity, uH_E=unbiased expected heterozygosity, and F=Fixation Index.

			Number of alleles							
Population		**n**	**Total**	**Per locus**	**Private**	**Effective**	**H_O**	**H_E**	**uH_E**	**F**
2008-2009	**Mean**	130	37	3,083	2	1,764	0,379	0,381	0,382	0,021
	SE	0		0,358		0,165	0,059	0,054	0,054	0,026
2019-2020	**Mean**	59	43	3,583	8	1,947	0,456	0,447	0,451	0,010
	SE	0		0,379		0,148	0,054	0,049	0,049	0,056

In the DAPC analysis on the combined data, twenty principal components (PC) were retained, and they cumulatively explained 97.2% of the total variance. A scatterplot of the first discriminant function (Figure 9) shows an overlap between temporal populations, and most of the samples are located in the shared area, which implies close similarity between the groups. The new population is more diverse compared to the old one, indicated by the samples being in a wider range in the x-axis. Assignment probabilities for the pre-defined groups were 95.4% (the old) and 61.0% (the new).

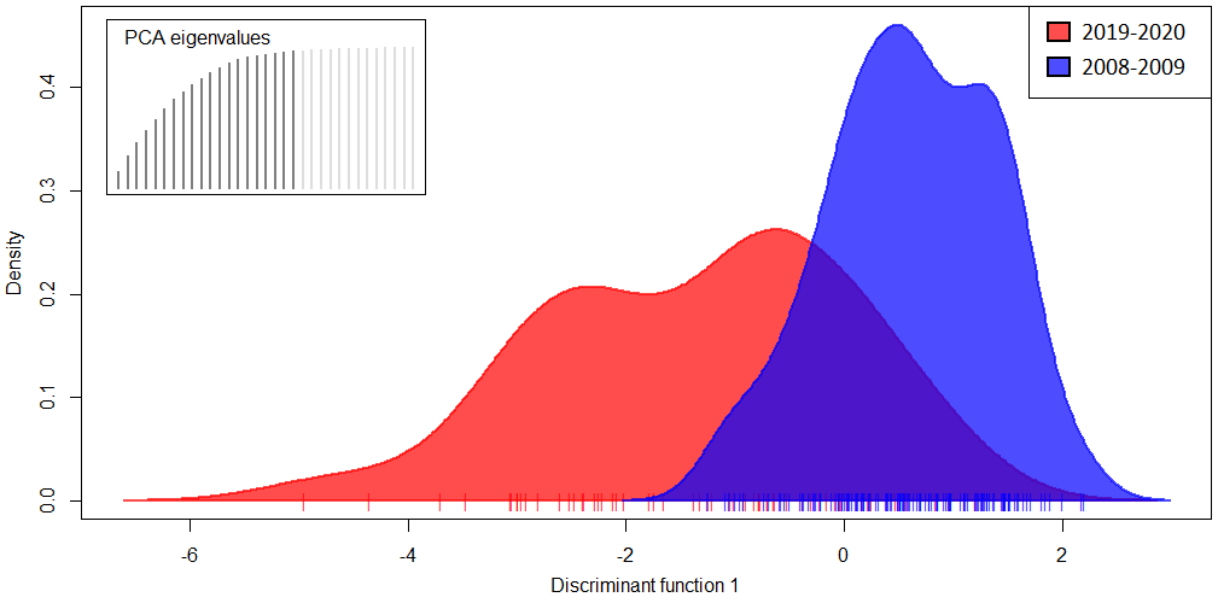

Figure 9. Scatterplot of the DAPC performed on the combined data. Values of the first discriminant function (DF) are on the X axis and the density of samples for the DF values on the Y axis.

4.3. Spatial population structure of the Helsinki population

4.3.1. The 2008-2009 population

In the DAPC analysis on the old data, fifteen PCs were retained, and they cumulatively explained 96.9% of the total variance. The scatterplot of the two first discriminant functions (Figure 10) shows that the subpopulations are not separated clearly and are mostly overlapping. An average assignment probability for the subpopulations was 54.6%, which also indicates that the groups are not clear-cut. The lowest assignment probabilities were in the Kaisaniemi group (33.3%) and the Hietaniemi group (50.0%). Pasila had the highest probability (100%) and Vallila the second highest (69.2%). The two samples from Pasila are significantly different from all other samples and were therefore excluded from the second DAPC as outliers.

The DAPC test does not separate subpopulations clearly, but the F_{ST} value 0.071 among subpopulations (Table 4) indicates that there is a significant population structure in the old samples. A negative F_{IS} value (−0.050) indicates that on average there is excess heterozygosity within subpopulations and no inbreeding.

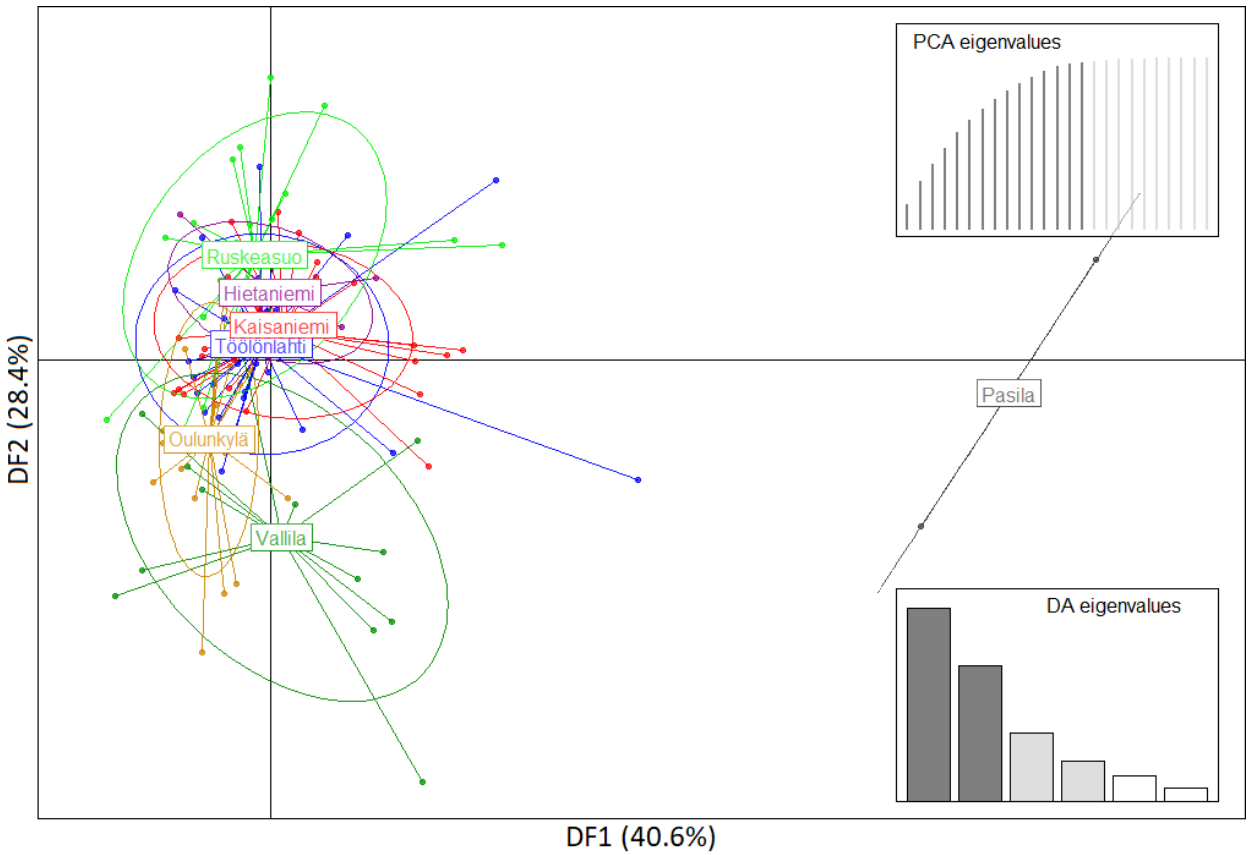

Figure 10. Scatterplot of the DAPC performed on the 2008-2009 subpopulations (subpop. division 1 in Table 2). The X- and Y-axes are the first two discriminant functions (DF1 & DF2). Percentages represent the proportion of total genetic variation calculated from eigenvalues.

Table 4. F statistics over the old and the new subpopulations, respectively. Values are means across all loci with standard deviations (SE).

Population		F_{IS}	F_{IT}	F_{ST}
2008-2009 subpopulations	Mean	−0.050	0.024	0.071
	SE	0.030	0.031	0.014
2019-2020 subpopulations	Mean	−0.092	−0.004	0.087
	SE	0.063	0.068	0.020

To better distinguish the relationships among areas, another DAPC was run with three groups based on geography (Figure 11): South (S), West (W), and North & East (N&E). Fifteen PCs were saved, and they cumulatively explained 97.3% of the total variance. Average assignment probability increased to 78.1%, and per group probabilities were 91.9% (S), 64.7% (W), and 50.0% (N&E). There was no clear population structure between neighbourhoods based on DAPC, but isolation by distance produced regional differences in the West to East axis.

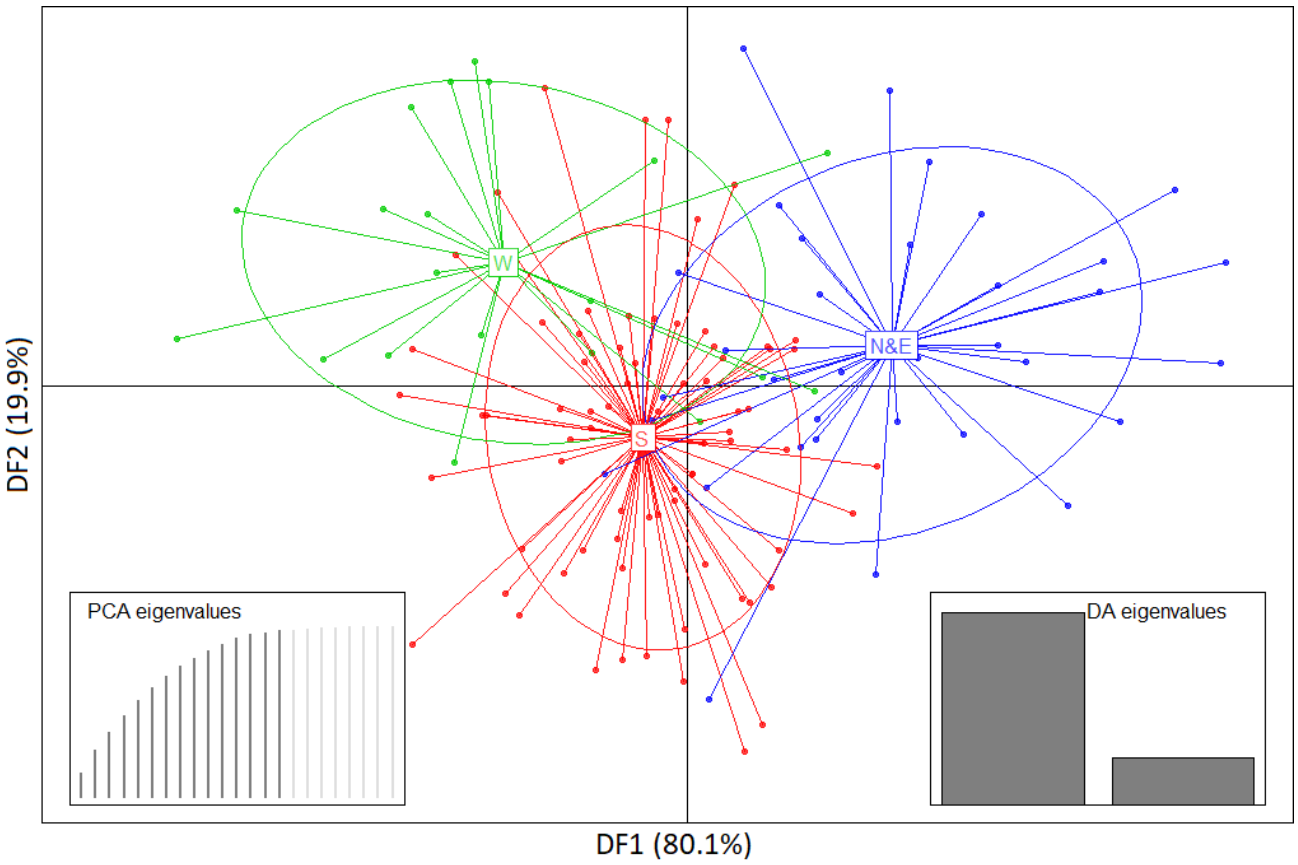

Figure 11. Scatterplot of the DAPC performed on the three groups based on geography (subpop. division 2 for the old population in Table 2). The X- and Y-axes are the first two discriminant functions (DF1 & DF2). Percentages represent the proportion of total genetic variation calculated from eigenvalues.

4.3.2. The 2019-2020 population

In the DAPC for the new subpopulations, twenty PCs were saved, and they cumulatively explained 97% of the total variance. Three out of five discriminant functions were saved and the first two were plotted (Figure 12). An average assignment probability for the groups was 89.8% which indicates that the pre-defined groups are well separated, and that it is likely that individuals belong in the group they were assigned to. Eteläsatama had the lowest assignment probability of 50.0%, and in Herttoniemi, Kannelmäki, and Puotila assignment probability ranged from 92.3-100% per group. The DAPC result suggests that subpopulations are genetically differentiated and the Herttoniemi subpopulation is most separated from the other groups. The F_{ST} value of 0.087 (Table 4.) indicates also that there are significant genetic differences between subpopulations. A negative F_{IS} value (−0.092) indicates that on average there is excess heterozygosity within subpopulations and no inbreeding.

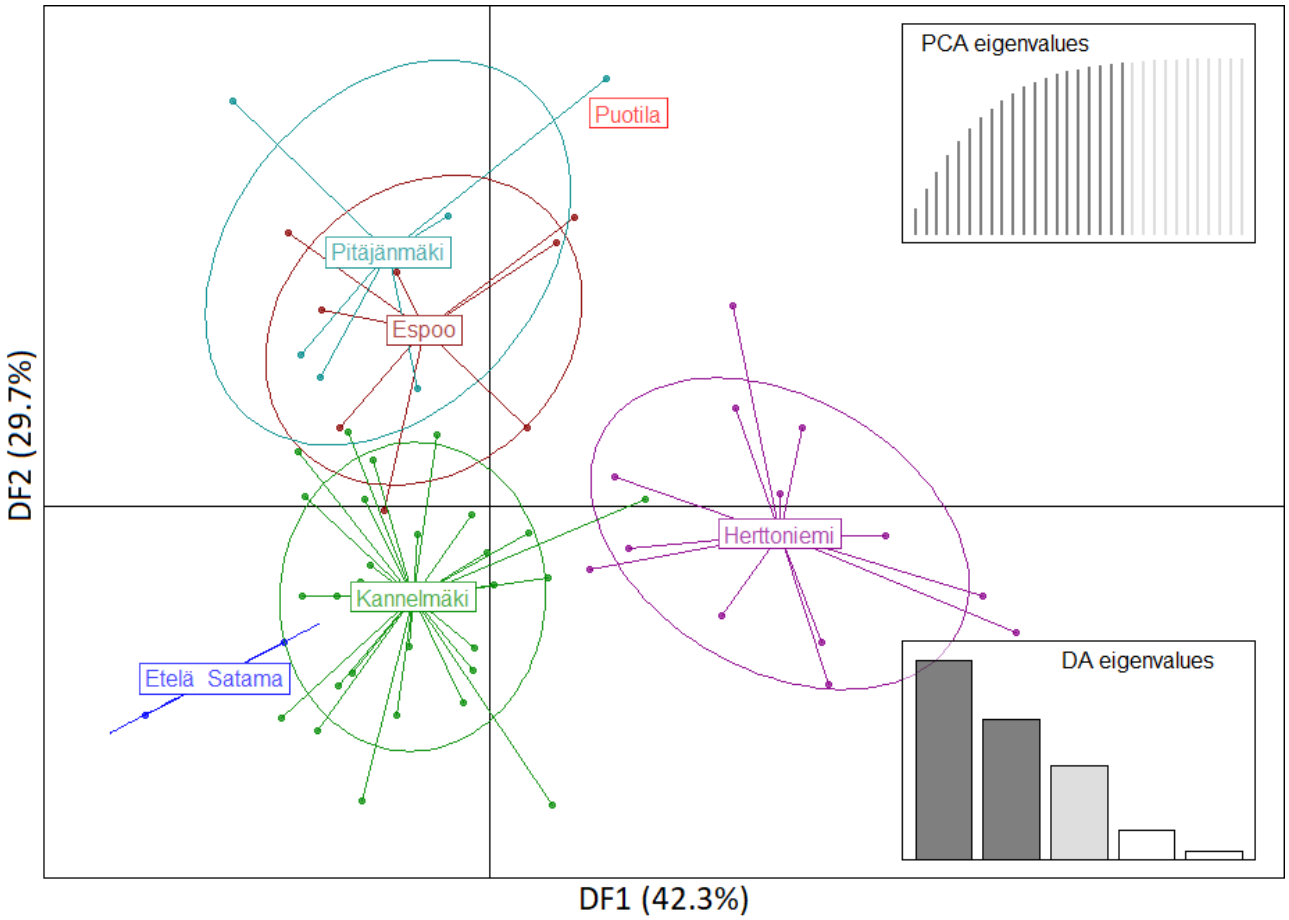

Figure 12. Scatterplot of the DAPC performed on 2019-2020 subpopulations. The x- and y-axes are the first two discriminant functions (DF1 & DF2). Percentages represent the proportion of total genetic variation calculated from eigenvalues.

4.3.3. AMOVA analysis

In the hierarchical AMOVA, 94.8% of the total variation is explained by the variation found within the individuals and none (0.0%) is explained by the differences among individuals (Table 5a). Only 0.7% of the total variation is explained by the difference between the two time periods. This is in line with other results, suggesting that the differentiation between the old and the new samples is small but significant. However, the F_{RT} value 0.007 is very close to zero.

A larger proportion of the total variation (4.5%) is explained by the differences between subpopulations within time periods (Figure 13). The F_{ST} value of 0.053 and the F_{SR} value of 0.046 are still quite small, but the corresponding P-values are significant (Table 5b). These results suggest that there is some population structure among the subpopulations, but the differences are minor.

Table 5. AMOVA results a) Source of variation (df=degrees of freedom, Est.Var.=Estimation of Variation) b) F-statistics

a) Source of variation	df	Est. Var.	%
Among Regions	1	0.018	0.70 %
Among Pops	5	0.114	4.50 %
Among Indiv	177	0	0.00 %
Within Indiv	184	2.399	94.80 %
Total	367	2.531	100 %

b) F-Statistics	Value	P (rand ≥ data)
F_{RT}	0.007	0.023*
F_{SR}	0.046	0.001*
F_{ST}	0.053	0.001*
F_{IS}	-0.026	0.874
F_{IT}	0.029	0.107

* Significant P-values <0.05

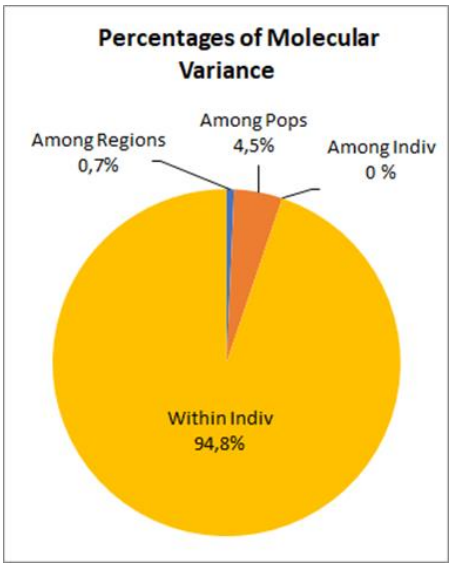

Figure 13. AMOVA percentages of molecular variance

4.4. Genetic bottleneck

All the tests show evidence of bottleneck in both populations, but the signal differs among mutation models and statistical tests (Table 6). The sign test returned significant P values for both populations with I.A.M., but non-significant results with T.P.M. and S.M.M. The number of loci with heterozygosity excess ranged from six to nine out of eleven in the old population, and five to ten out of twelve in the new population.

The standard differences test gave a significant P value in the old population only with I.A.M., but in the new population with I.A.M. and S.M.M. The Wilcoxon's one-tailed test for heterozygosity excess produced a significant P-value with I.A.M. and T.P.M for the old population. The new population had significant P value with I.A.M.

A mode-shift in the distribution of allele frequencies is expected after a bottleneck, but the mode-shift test gave normal L-shaped distribution of allele frequencies for both populations. Locus specific heterozygosity excess or deficiency under the three mutation models for both populations are presented in Appendix Table 7.

Table 6. P values for the bottleneck tests in the old and new populations

	2008-2009			2019-2020		
Test	**I.A.M.**	**T.P.M.**	**S.M.M.**	**I.A.M.**	**T.P.M.**	**S.M.M.**
Sign test	0.020*	0.317	0.607	0.023*	0.150	0.217
Number of loci with heterozygosity excess	9/11	7/11	6/11	10/12	9/12	5/12
Standard differences test	0.011*	0.134	0.188	0.035*	0.380	0.028*
Wilcoxon one-tailed test	0.006*	0.012*	0.711	0.039*	0.259	0.715

* Significant P-values <0.05

5. Discussion and conclusions

5.1. Helsinki area rabbits exhibit low genetic diversity

My studies showed that the Helsinki area rabbits have significantly lower expected heterozygosity (0.38 & 0.45; Table 3) compared to other wild rabbit populations, even lower than in domesticated rabbit breeds. Previous population genetic studies using DNA microsatellite markers have shown that the native rabbit populations in the Iberian Peninsula are the most diverse and have the highest expected heterozygosity (H_E) ranging 0.71-0.86 (Alda & Doadrio 2014; Alves *et al.* 2015; Queney *et al.* 2001; Zenger *et al.* 2003). Wild rabbit populations have been studied in many other countries as well, and expected heterozygosities have been recorded, for instance, in France (0.50-0.72), Egypt (0.69-0.72), Australia (0.65-0.72), and Germany (0.5-0.6) (Abdel-Kafy *et al.* 2018; Alves *et al.* 2015; Queney *et al.* 2000; Queney *et al.* 2001; Zenger *et al.* 2003; Ziege *et al.* 2020). Domestic rabbit breeds have lower expected heterozygosity (breed average 0.46) than wild populations because of the domestication bottleneck (Alves *et al.* 2015). The studies mentioned above had also higher allele numbers for all fourteen DNA microsatellite loci, and the monomorphic loci SAT13 and INRA228 were not monomorphic in any of the reference studies used, which also reflects the lower genetic diversity in the Helsinki area rabbits.

5.2. Evidence of new gene flow to the Helsinki area population

The rabbits in the Helsinki area are presumably descend from domesticated rabbits, and thus it was expected that the population would not be particularly diverse. However, it was surprising that after the RHD bottleneck, the new population had higher expected heterozygosity and more private and total amount of alleles than the old population. This could be explained by sampling error, since the old and new rabbits were not from the same areas, but may be also caused by new gene flow.

All rabbits in the Helsinki area supposedly originate from the original population in Kyläsaari, but it is possible that there have been other rabbit introductions. This is even likely since 10-20 recently escaped pet rabbits are captured in the Helsinki area every year (Anu Rosti, personal information 2007 as in Leikas & Rautiainen 2010). Moreover, all escapees are probably not captured and might survive and join the resident population. In addition, the rabbits transform to a wild-type appearance in few generations, but the Helsinki rabbits exhibit often unusual colour variation and

domestication marks in fur. In fact, ~15% of the new sampled rabbits had domestication marks in their fur, and this might be a sign of gene flow from newly escaped domesticated rabbits.

5.3. Isolation by distance creates geographical differences in Helsinki area rabbits

Population structure is often observed in rabbit populations due to the dispersal behaviour and tendency to live in groups. Population structure was observed in Helsinki in both temporal rabbit populations with small but significant F_{ST} values (Table 4 & 5). However, other population genetic studies on rabbits have reported higher F_{ST} values, for instance, 0.150 in East Anglia, England (Surridge *et al.* 1999b) and 0.100 in Frankfurt, Germany (Ziege *et al.* 2020). The sampling in this thesis was not ideal for a fine-scale population structure study for either time period, as there was no possibility to influence the catching locations and number of rabbits from each location. This could potentially be the reason why the observed F_{ST} values were lower compared to other studies.

The data was better suited for a general view, and the DAPC analyses denote that isolation by distance creates geographical differences in Helsinki. The old samples were very similar to each other and the pre-defined groups were overlapping in the scatterplot (Figure 10). However, the relationship between the groups matches with the geography of Helsinki. Kaisaniemi and Töölönlahti groups are overlapping in the scatterplot, as these areas are located near to each other in Helsinki. The Oulunkylä and Vallila groups are on opposite sides to the Ruskeasuo group in the scatterplot, correspondingly, the neighbourhoods in these groups are located on opposite sides of Töölönlahti bay also geographically. The scatterplot of the three geographical groups (Figure 11) visualises this result and shows that isolation by distance produces regional differences in the West to East axis. The geography of Helsinki is not as evidently visible in the scatterplot of the new population (Figure 12), but the pre-defined groups were better separated and showed more difference between neighbourhoods, compared to the old population.

The data may have included related individuals in both populations, and this can amplify the positive signal in the DAPC. The analysis uses pre-defined groups and maximizes between-group variance, and thus relative individuals in a group will amplify the differences between groups. This needs to be considered especially in the new population, since the samples were hunted with ferrets. In ferret hunting, the ferret is released inside the rabbit warren, and the aim is to eliminate all the rabbits in the warren. For this reason, it is likely that at least some samples in the new dataset are from the same warrens, and thus are probably related. The old samples were hunted with multiple

different methods, and the rabbits from the same location were caught at different times, which decreases the risk of relatives in the old population.

Because of the non-ideal sampling in the new population, it would be interesting to repeat the population structure tests with more systematic sampling strategy. Ideally, there would be more locations, and at least 15 samples from each location, but not from the same warren. It would be better to use more markers to increase the analytical power. In addition, it would be interesting to add samples from other cities and compare them to the Helsinki area rabbits. There is anecdotal information, that rabbits have sought shelter and warmth in freight trains during winter, and thus been transferred to other cities in Finland. It would be intriguing if the additional studies would provide support for this.

5.4. Fast population growth prevents the loss of genetic diversity after a bottleneck

The bottleneck tests showed weak but significant signals in both temporal populations. The signal in the old population could be a remnant of the initial bottleneck caused by the founder effect of a few pet rabbits. Rabbits have been observed to recover even from severe bottlenecks without a significant reduction in genetic diversity due to their fast reproduction. For example, no evidence of a genetic bottleneck was observed in Australia, although the initial foundation was only thirteen rabbits (Zenger *et al.* 2003). The rabbits spread around 200 km in the first ten years in Australia, but for the first ten years in Helsinki, the population remained in low numbers in an area smaller than 10 km^2. The absence of a bottleneck was also observed in France after a RHD-epidemic, and this was also explained by fast population growth (Queney *et al.* 2000). The lack of fast population growth in the beginning in Helsinki might explain the bottleneck signal in the old population, although the rabbits did spread fast eventually.

The 2016 RHD epidemic in Helsinki caused a significant reduction in the rabbit population size. The bottleneck tests also gave significant P values for the new population, but the RHD bottleneck cannot be distinguished based on the tests, since the signal was similar in the old population. The initial bottleneck or other events that resulted in a bottleneck signal in the old population could also be the cause of the signal in the new population. In addition, after a bottleneck, low frequency alleles are expected to disappear, but the new population was more diverse than the old population, based on both allele numbers and expected heterozygosity. The population recovered fast after the RHD epidemic based on both the LUKE hunting statistic (Table 6) and personal observations, and

this could explain the absence of a distinct genetic bottleneck in the new population. These results are in line with the fact that after a bottleneck the population growth rate has a major impact on the amount of lost genetic diversity.

On the other hand, the bottleneck tests do not take migration into account and this might bias the results. There is evidence of new gene flow in the new population, and thus it is possible that a genetic bottleneck did occur, but the new gene flow biases the bottleneck test results. The rabbits in the old population had domestication marks in their fur, and therefore new gene flow could be present also in the old samples, and thus bias the bottleneck results also for the old population.

5.5 Origin of the Helsinki area rabbits

Alves *et al.* (2015) studied genetic relationships between wild rabbits from Spain and France, and domesticated rabbit breeds. Their data is open access, and another potential aim for the thesis was to combine a dataset from the shared DNA microsatellite markers (n=13), and test whether the Helsinki area rabbits would group with the domesticated rabbits. This would have provided evidence that the Helsinki area rabbits are indeed descended from domesticated rabbits, and not from wild individuals. Unfortunately, the alleles were not comparable to each other, and this test could not be executed in this thesis. However, it would be interesting to conduct this in the future if tissue samples or DNA extractions from wild and domestic individuals could be obtained. Alves *et al.* (2015) reported also that rabbit breeds can be distinguished from each other using DNA microsatellite markers. Such test might also show if the Helsinki area rabbits are descended from particular rabbit breeds.

5.6. Conclusions

The European rabbit has spread worldwide through human action, and the introduction of the species has caused a dramatic impact on local nature in many countries. In Finland, the consequences have thus far been restricted to economic losses, since the rabbits are found only in urban and suburban areas. However, if the rabbits were to spread to rural areas in the future, they may cause unexpected effects to Finnish ecosystems as well. For example, the viral rabbit diseases could possibly spread to native hare populations, since the rabbit viruses have also been found to infect hares (Barlow et al. 2014; Velarde et al, 2017). The depth of snow is an important factor that

limits the spreading of rabbits to Finnish rural areas, but global warming may affect the amount of snow in the future, especially in southern Finland.

Rabbit population genetics has not been studied in Finland before, and this thesis provided both general knowledge of the Helsinki rabbit population, and an unexpected result regarding possible gene flow from newly escaping domesticated rabbits. In addition, the unintentional spread of RHD in Finland during 2016 provided a great opportunity to study whether a rabbit population can recover from a population crash even in a harsher environment. The rabbit viruses have been used to control harmful populations in many countries, but it has been often observed that even a few surviving rabbits can allow a population to revive quickly. Despite the northern climate in Finland, the Helsinki population recovered from a major reduction in population size. If the aim would be to eliminate harmful rabbit populations in the future, it should be considered that the viruses alone will not probably cause permanent effect.

The rabbits have demonstrated their adaptation and survival skills in the cold climate of Helsinki for over 30 years. The population was found to have significantly lower genetic diversity compared to wild populations studied in other countries, yet recovered from a major RHD epidemic without a notable genetic bottleneck even under the more extreme environmental conditions. It has been proven again; the rabbit is a thriving invasive species.

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8. Appendix

Table 1. Part of the original information of the 2008-2009 tissue samples from Heidi Kinnunen. A lab code was added (HK + original rabbit number) and colour remarks were translated from Finnish to English. All rabbits were typical greyish-brown colour unless stated otherwise. More information of the samples can be found: http://id.luomus.fi/GJAA.1086 and "show extra info"

Labcode	Laji.fi code	Hunting location	Hunting date	Hunter	Colour remarks
HK2	GJAA.1086	Kaisaniemi	6.1.2008	Pesu, Marko	
HK3	GJAA.1087	Kaisaniemi	6.1.2008	Pesu, Marko	
HK4	GJAA.1088	Kaisaniemi	6.1.2008	Pesu, Marko	
HK5	GJAA.1089	Kaisaniemi	6.1.2008	Pesu, Marko	
HK6	GJAA.1090	Kaisaniemi	6.1.2008	Pesu, Marko	white front paw, star on forehead and white chest
HK7	GJAA.1091	Kaisaniemi	6.1.2008	Pesu, Marko	
HK8	GJAA.1092	Kaisaniemi	6.1.2008	Pesu, Marko	
HK9	GJAA.1093	Kaisaniemi	6.1.2008	Pesu, Marko	
HK10	GJAA.1094	Kaisaniemi	6.1.2008	Pesu, Marko	
HK11	GJAA.1095	Kaisaniemi	6.1.2008	Pesu, Marko	
HK12	GJAA.1096	Kaisaniemi	6.1.2008	Pesu, Marko	
HK13	GJAA.1097	Kaisaniemi	6.1.2008	Pesu, Marko	
HK14	GJAA.1098	Kaisaniemi	6.1.2008	Pesu, Marko	white spot on forehead
HK15	GJAA.1099	Kaisaniemi	6.1.2008	Pesu, Marko	
HK16	GJAA.1100	Kaisaniemi	6.1.2008	Pesu, Marko	
HK17	GJAA.1101	Kaisaniemi	6.1.2008	Pesu, Marko	
HK18	GJAA.1102	Kaisaniemi	6.1.2008	Pesu, Marko	
HK20	GJAA.1103	Pasila	9.11.2008	Maavuori, Jaakko-Ilkka	
HK21	GJAA.1104	Pasila	9.11.2008	Maavuori, Jaakko-Ilkka	
HK22	GJAA.1105	Kumpula	19.11.2008		white star on forehead
HK23	GJAA.1106	Talvipuutarha (Kaupunginpuutarha)	18.11.2008	Nuutinen, Kari	
HK24	GJAA.1107	Kaisaniemi	27.10.2008	Pesu, Marko	
HK25	GJAA.1108	Kaisaniemi	27.10.2008	Pesu, Marko	
HK26	GJAA.1109	Ruskeasuo	18.11.2008	Seuna, Veikko	white star on forehead
HK28	GJAA.1110	Ruskeasuo	18.11.2008	Seuna, Veikko	white neck
HK29	GJAA.1111	Linnunlaulu	17.11.2008	Tammi, Jyrki	
HK30	GJAA.1112	Vallila	19.11.2008	Seuna, Veikko	
HK31	GJAA.1113	Linnunlaulu	17.11.2008	Tammi, Jyrki	
HK32	GJAA.1114	Vallila	19.11.2008	Seuna, Veikko	
HK33	GJAA.1115	Oulunkylä	21.11.2008	Luoto, Hannu	
HK34	GJAA.1116	Oulunkylä	21.11.2008	Luoto, Hannu	
HK36	GJAA.1117	Kaisaniemi	4.9.2008	Pesu, Marko	
HK37	GJAA.1118	Kaisaniemi	4.9.2008	Pesu, Marko	
HK38	GJAA.1119	Kaisaniemi	4.9.2008	Pesu, Marko	
HK39	GJAA.1120	Länsisatama	21.11.2008	Tiainen, O.	

HK40	GJAA.1121	Ruskeasuo	27.9.2008		
HK41	GJAA.1122	Länsisatama	20.11.2008	Tiainen, O.	
HK42	GJAA.1123	Ruskeasuo	27.9.2008		Black
HK43	GJAA.1124	Ruskeasuo	27.9.2008		
HK44	GJAA.1125	Ruskeasuo	27.9.2008		
HK45	GJAA.1126	Ruskeasuo	27.9.2008		white star on forehead
HK46	GJAA.1127	Ruskeasuo	27.9.2008		
HK47	GJAA.1128	Ruskeasuo	28.9.2008		
HK48	GJAA.1129	Ruskeasuo	27.9.2008		lighter in colour than usual
HK49	GJAA.1130	Ruskeasuo	27.9.2008		
HK50	GJAA.1131	Ruskeasuo	27.9.2008		
HK51	GJAA.1132	Oulunkylä	7.12.2008	Luoto, Hannu	
HK52	GJAA.1133	Oulunkylä	7.12.2008	Luoto, Hannu	
HK53	GJAA.1134	Oulunkylä	7.12.2008	Luoto, Hannu	white star on forehead
HK54	GJAA.1135	Oulunkylä	7.12.2008	Luoto, Hannu	
HK55	GJAA.1136	Oulunkylä	7.12.2008	Luoto, Hannu	
HK56	GJAA.1137	Vallilla	3.12.2008	Seuna, Veikko	
HK57	GJAA.1138	Vallilla	3.12.2008	Seuna, Veikko	
HK58	GJAA.1139	Vallilla	3.12.2008	Seuna, Veikko	white star on forehead
HK59	GJAA.1140	Talvipuutarha	1.12.2008	Seuna, Veikko & Nuutinen	
HK60	GJAA.1141	Talvipuutarha	1.12.2008	Seuna, Veikko & Nuutinen	
HK61	GJAA.1142	Talvipuutarha	1.12.2008	Seuna, Veikko & Nuutinen	
HK62	GJAA.1143	Talvipuutarha	1.12.2008	Seuna, Veikko & Nuutinen	
HK63	GJAA.1144	Talvipuutarha	1.12.2008	Seuna, Veikko & Nuutinen	
HK64	GJAA.1145	Talvipuutarha	1.12.2008	Seuna, Veikko	very small white spot on forehead
HK65	GJAA.1146	Talvipuutarha	1.12.2008	Seuna, Veikko & Nuutinen	
HK66	GJAA.1147	Talvipuutarha	1.12.2008	Seuna, Veikko & Nuutinen	
HK67	GJAA.1148	Talvipuutarha	1.12.2008	Seuna, Veikko & Nuutinen	
HK68	GJAA.1149	Talvipuutarha	1.12.2008	Seuna, Veikko & Nuutinen	
HK69	GJAA.1150	Kaisaniemi	12.7.2008	Pesu, Marko	black, white stripe on forehead, left front paw had white spot
HK70	GJAA.1151	Kaisaniemi	12.7.2008	Pesu, Marko	very small white spot on forehead
HK71	GJAA.1152	Kaisaniemi	12.7.2008	Pesu, Marko	
HK72	GJAA.1153	Kaisaniemi	18.8.2008	Pesu, Marko	white socks on front paws, small white spot on chest
HK73	GJAA.1154	Kaisaniemi	18.8.2008	Pesu, Marko	
HK74	GJAA.1155	Kaisaniemi	18.8.2008	Pesu, Marko	white star on forehead
HK75	GJAA.1156	Kaisaniemi	18.8.2008	Pesu, Marko	
HK76	GJAA.1157	Kaisaniemi	18.8.2008	Pesu, Marko	very small white spot on forehead
HK77	GJAA.1158	Töölönlahti	1.12.2008	Seuna, Veikko	white star on forehead, white spot on nose and chest, white socks in front paws
HK78	GJAA.1159	Töölönlahti	1.12.2008	Seuna, Veikko	
HK79	GJAA.1160	Vallila	18.11.2008	Seuna, Veikko	very small white spot on forehead
HK80	GJAA.1161	Vallila	18.11.2008	Seuna, Veikko	
HK81	GJAA.1162	Vallila	18.11.2008	Seuna, Veikko	
HK82	GJAA.1163	Oulunkylä	3.2.2009	Luoto, Hannu	white stripe on forehead
HK83	GJAA.1164	Oulunkylä	3.2.2009	Luoto, Hannu	

HK84	GJAA.1165	Oulunkylä	3.2.2009	Luoto, Hannu	
HK85	GJAA.1166	Oulunkylä	3.2.2009	Luoto, Hannu	white stripe on forehead
HK86	GJAA.1167	Oulunkylä	3.2.2009	Luoto, Hannu	
HK87	GJAA.1168	Oulunkylä	22.11.2008	Luoto, Hannu	
HK88	GJAA.1169	Oulunkylä	22.11.2008	Luoto, Hannu	white stripe on forehead
HK89	GJAA.1170	Oulunkylä	22.11.2008	Luoto, Hannu	
HK90	GJAA.1171	Oulunkylä	13.11.2008	Luoto, Hannu	
HK91	GJAA.1172	Oulunkylä	2.2.2009	Luoto, Hannu	
HK92	GJAA.1173	Oulunkylä	2.2.2009	Luoto, Hannu	
HK93	GJAA.1174	Oulunkylä	2.2.2009	Luoto, Hannu	
HK94	GJAA.1175	Oulunkylä	2.2.2009	Luoto, Hannu	
HK95	GJAA.1176	Oulunkylä	2.2.2009	Luoto, Hannu	
HK96	GJAA.1177	Kumpula	2.12.2008		
HK97	GJAA.1178	Talvipuutarha (Kaupunginpuutarha)	28.11.2008	Ahlbeck & Mäkeläinen	white star on forehead
HK98	GJAA.1179	Talvipuutarha (Kaupunginpuutarha)	28.11.2008	Ahlbeck & Mäkeläinen	
HK99	GJAA.1180	Talvipuutarha (Kaupunginpuutarha)	28.11.2008	Ahlbeck & Mäkeläinen	
HK100	GJAA.1181	Talvipuutarha (Kaupunginpuutarha)	28.11.2008	Ahlbeck & Mäkeläinen	
HK101	GJAA.1182	Talvipuutarha (Kaupunginpuutarha)	14.11.2008	Seuna, Veikko	white star on forehead
HK102	GJAA.1183	Talvipuutarha (Kaupunginpuutarha)	14.11.2008	Seuna, Veikko	
HK103	GJAA.1184	Talvipuutarha (Kaupunginpuutarha)	14.11.2008	Seuna, Veikko	white star on forehead
HK104	GJAA.1185	Toimela	6.2.2009	Koskinen, Jari	white star on forehead
HK105	GJAA.1186	Toimela	6.2.2009	Koskinen, Jari	
HK106	GJAA.1187	Toimela	6.2.2009	Koskinen, Jari	
HK107	GJAA.1188	Toimela	6.2.2009	Koskinen, Jari	
HK108	GJAA.1189	Toimela	6.2.2009	Koskinen, Jari	
HK193	GJAA.1190	Hesperianpuisto	24-25.2.2009	Luoto, Hannu	white stripe on forehead
HK194	GJAA.1191	Hesperianpuisto	24-25.2.2009	Luoto, Hannu	
HK195	GJAA.1192	Hesperianpuisto	24-25.2.2009	Luoto, Hannu	
HK196	GJAA.1193	Hesperianpuisto	24-25.2.2009	Luoto, Hannu	
HK522	GJAA.1194	Tukkutori	28.3.2009	Luoto, Hannu	
HK607	GJAA.1195	Tukkutori	25.3.2009	Luoto, Hannu	black
HK608	GJAA.1196	Tukkutori	25.3.2009	Luoto, Hannu	
HK619	GJAA.1197	Ruoholahti	15.4.2009	Paananen, Rentokil	
HK620	GJAA.1198	Alppipuisto (Linnanmäki)	4.3.2009	Silvennoinen	white stripe on forehead
HK621	GJAA.1199	Alppipuisto (Linnanmäki)	4.3.2009	Silvennoinen	white small stripe on forehead
HK622	GJAA.1200	Alppipuisto (Linnanmäki)	4.3.2009	Silvennoinen	
HK623	GJAA.1201	Alppipuisto (Linnanmäki)	4.3.2009	Silvennoinen	white stripe on forehead
HK625	GJAA.1202	Alppipuisto (Linnanmäki)	4.3.2009	Silvennoinen	
HK755	GJAA.1203	Haaga	6.5.2009	Koskinen, Jari	white nose
HK761	GJAA.1204	Pitäjänmäki	8.9.2009	Mäkinen, Jussi	gold aquti, white stripe on forehead and spot on chest
HK762	GJAA.1205	Pitäjänmäki	4.7.2009	Mäkinen, Jussi	white long stripe on forehead and spot on chest
HK769	GJAA.1206	Hietaniemi	15.8.2009	Koskinen, Jari	white spot on chest and small star on forehead
HK770	GJAA.1207	Hietaniemi	15.8.2009	Koskinen, Jari	white stripe on forehead
HK771	GJAA.1208	Hietaniemi	15.8.2009	Koskinen, Jari	

HK772	GJAA.1209	Alppipuisto	28.7.2009	Rautiainen, Antti	
HK773	GJAA.1210	Alppipuisto	28.7.2009	Rautiainen, Antti	white star on forehead
HK774	GJAA.1211	Alppipuisto	28.7.2009	Rautiainen, Antti	
HK775	GJAA.1212	Alppipuisto	28.7.2009	Rautiainen, Antti	
HK776	GJAA.1213	Alppipuisto	28.7.2009	Rautiainen, Antti	
HK777	GJAA.1214	Aurora	10.7.2009	Koskinen, Jari	
HK778	GJAA.1215	Aurora	10.7.2009	Koskinen, Jari	

Table 2. Additional information of the 2019-2020 tissue samples. All rabbits were typical greyish-brown colour unless stated otherwise. More information of the samples can be found: http://id.luomus.fi/GJAA.1027 and "show extra info"

Labcode	Laji.fi code	Hunting location	Hunting date	Hunter	Color remarks	Tissue sampling date
EL1	GJAA.1027	Espoo	8.8.2019	Koskinen, Jari		30.8.2019
EL2	GJAA.1028	Espoo	8.8.2019	Koskinen, Jari		30.8.2019
EL3	GJAA.1029	Espoo	8.8.2019	Koskinen, Jari		30.8.2019
EL4	GJAA.1030	Espoo	8.8.2019	Koskinen, Jari		30.8.2019
EL5	GJAA.1031	Espoo	8.8.2019	Koskinen, Jari		30.8.2019
EL6	GJAA.1032	Espoo	8.8.2019	Koskinen, Jari		30.8.2019
EL7	GJAA.1033	Espoo	8.8.2019	Koskinen, Jari		30.8.2019
EL8	GJAA.1034	Espoo	8.8.2019	Koskinen, Jari		30.8.2019
EL9	GJAA.1035	Herttoniemi	26.9.-8.10.2019	Koskinen, Jari		10.10.2019
EL10	GJAA.1036	Herttoniemi	26.9.-8.10.2019	Koskinen, Jari	black otter	10.10.2019
EL11	GJAA.1037	Herttoniemi	26.9.-8.10.2019	Koskinen, Jari		10.10.2019
EL12	GJAA.1038	Herttoniemi	26.9.-8.10.2019	Koskinen, Jari		10.10.2019
EL13	GJAA.1039	Herttoniemi	26.9.-8.10.2019	Koskinen, Jari		10.10.2019
EL14	GJAA.1040	Herttoniemi	26.9.-8.10.2019	Koskinen, Jari		10.10.2019
EL15	GJAA.1041	Herttoniemi	26.9.-8.10.2019	Koskinen, Jari		10.10.2019
EL16	GJAA.1042	Herttoniemi	26.9.-8.10.2019	Koskinen, Jari		10.10.2019
EL17	GJAA.1043	Herttoniemi	26.9.-8.10.2019	Koskinen, Jari		10.10.2019
EL18	GJAA.1044	Herttoniemi	26.9.-8.10.2019	Koskinen, Jari		10.10.2019
EL19	GJAA.1045	Herttoniemi	26.9.-8.10.2019	Koskinen, Jari		10.10.2019
EL20	GJAA.1046	Herttoniemi	26.9.-8.10.2019	Koskinen, Jari		10.10.2019
EL21	GJAA.1047	Herttoniemi	26.9.-8.10.2019	Koskinen, Jari		10.10.2019
EL22	GJAA.1048	Kannelmäki	31.10-6.11.2019	Koskinen, Jari		7.11.2019
EL23	GJAA.1049	Kannelmäki	31.10-6.11.2019	Koskinen, Jari		7.11.2019
EL24	GJAA.1050	Kannelmäki	31.10-6.11.2019	Koskinen, Jari		7.11.2019
EL25	GJAA.1051	Kannelmäki	31.10-6.11.2019	Koskinen, Jari		7.11.2019
EL26	GJAA.1052	Kannelmäki	31.10-6.11.2019	Koskinen, Jari		7.11.2019
EL27	GJAA.1053	Kannelmäki	31.10-6.11.2019	Koskinen, Jari		7.11.2019
EL28	GJAA.1054	Kannelmäki	31.10-6.11.2019	Koskinen, Jari		7.11.2019
EL29	GJAA.1055	Kannelmäki	31.10-6.11.2019	Koskinen, Jari		7.11.2019

EL30	GJAA.1056	Kannelmäki	31.10-6.11.2019	Koskinen, Jari		7.11.2019
EL31	GJAA.1057	Kannelmäki	31.10-6.11.2019	Koskinen, Jari		7.11.2019
EL32	GJAA.1058	Kannelmäki	31.10-6.11.2019	Koskinen, Jari	white spot on forehead	7.11.2019
EL33	GJAA.1059	Kannelmäki	31.10-6.11.2019	Koskinen, Jari	white stripe on forehead, white sock on left front paw and white chest	7.11.2019
EL34	GJAA.1060	Kannelmäki	31.10-6.11.2019	Koskinen, Jari		7.11.2019
EL38	GJAA.1061	Kannelmäki	31.10-6.11.2019	Koskinen, Jari		7.11.2019
EL39	GJAA.1062	Kannelmäki	31.10-6.11.2019	Koskinen, Jari		7.11.2019
EL40	GJAA.1063	Kannelmäki	31.10-6.11.2019	Koskinen, Jari	black otter, white stripe on forehead	7.11.2019
EL41	GJAA.1064	Kannelmäki	31.10-6.11.2019	Koskinen, Jari	black otter, white sock in right front paw	7.11.2019
EL42	GJAA.1065	Kannelmäki	31.10-6.11.2019	Koskinen, Jari	black otter	7.11.2019
EL43	GJAA.1066	Puotila	30.1.2020	Koskinen, Jari		30.1.2020
EL44	GJAA.1067	Eteläsatama	1.1.-25.1.2020	Koskinen, Jari	white spot on forehead	30.1.2020
EL45	GJAA.1068	Eteläsatama	1.1.-25.1.2020	Koskinen, Jari		30.1.2020
EL46	GJAA.1069	Kannelmäki	15.11.-20.12.2019	Koskinen, Jari	white spot on forehead	30.1.2020
EL47	GJAA.1070	Kannelmäki	15.11.-20.12.2019	Koskinen, Jari	black otter	30.1.2020
EL48	GJAA.1071	Kannelmäki	15.11.-20.12.2019	Koskinen, Jari	white small spot on forehead	30.1.2020
EL49	GJAA.1072	Kannelmäki	15.11.-20.12.2019	Koskinen, Jari		30.1.2020
EL50	GJAA.1073	Kannelmäki	15.11.-20.12.2019	Koskinen, Jari		30.1.2020
EL51	GJAA.1074	Kannelmäki	15.11.-20.12.2019	Koskinen, Jari		30.1.2020
EL52	GJAA.1075	Kannelmäki	15.11.-20.12.2019	Koskinen, Jari		30.1.2020
EL53	GJAA.1076	Kannelmäki	15.11.-20.12.2019	Koskinen, Jari		30.1.2020
EL54	GJAA.1077	Kannelmäki	15.11.-20.12.2019	Koskinen, Jari		30.1.2020
EL55	GJAA.1078	Kannelmäki	15.11.-20.12.2019	Koskinen, Jari	white stripe on forehead	30.1.2020
EL56	GJAA.1079	Kannelmäki	15.11.-20.12.2019	Koskinen, Jari		30.1.2020
EL57	GJAA.1080	Pitäjänmäki	4.4.2020	Koskinen, Jari		3.4.2020
EL58	GJAA.1081	Pitäjänmäki	4.4.2020	Koskinen, Jari		3.4.2020
EL59	GJAA.1082	Pitäjänmäki	27.3.2020	Koskinen, Jari	black otter	3.4.2020
EL60	GJAA.1083	Pitäjänmäki	27.3.2020	Koskinen, Jari		3.4.2020
EL61	GJAA.1084	Pitäjänmäki	27.3.2020	Koskinen, Jari		3.4.2020
EL62	GJAA.1085	Pitäjänmäki	27.3.2020	Koskinen, Jari		3.4.2020

Table 3. Primer sequences and repeat patterns of all fourteen DNA microsatellite markers.

Marker name	Forward primer	Reverse primer	Repeat pattern	Reference
INRACCDDV0087	5' GATCTGGGACTCCAGAGTGTG 3'	5' GAACACCGGTCTGGATGG 3'	$(TG)_{14}$	Chantry-Darmon *et al.* 2005
INRACCDDV0102	5' GCCAAACTTCCTTCAGCCTAT 3'	5' ACAGCTGTTCGTGCTTTCAGT 3'	$(AC)_{18}$	Chantry-Darmon *et al.* 2005
INRACCDDV0104	5' AGATTTGGCACCCTTGTTCTT 3'	5' TATTCCCCTGGCAATGAAACT 3'	$(GT)_{15}$	Chantry-Darmon *et al.* 2005
INRACCDDV0119	5' CGGAGAAGAGGTTACCACGA 3'	5' ATGACCCTGCTTGTCCTCTG 3'	$(GT)_{16}$	Chantry-Darmon *et al.* 2005
INRACCDDV0140	5' TCTCTGTTGGCCATCTCCTAA 3'	5' TCTACTACCCAGCCCCATACC 3'	$(TG)_{14}$	Chantry-Darmon *et al.* 2005
INRACCDDV0169	5' AGCACCCACATGATGAAAGTC 3'	5' GAGCGACAAATCCAGCTCAT 3'	$(CA)_{17}$	Chantry-Darmon *et al.* 2005
INRACCDDV0192	5' TGCAATAGGTGGAGGCTTAGA 3'	5' TCCACAGAGGAGATATAGTGGTCTT 3'	$(TG)_{11}$	Chantry-Darmon *et al.* 2005
INRACCDDV0201	5' AGGCAGGTAAGGGGGAAAG 3'	5' GCATTTGGGGAAGTAACCAGT 3'	$(TG)_{14}(AG)_{10}$	Chantry-Darmon *et al.* 2005
INRACCDDV0228	5' ACTCCCAGCCTCAGCTGTT 3'	5' ATGCTGCTGTGGGACAGACT 3'	$(TG)_{12}$	Chantry-Darmon *et al.* 2005
SAT3	5' GGAGAGTGAATCAGTGGGTG 3'	5' GAGGGAAAGAGAGAGACAGG 3'	$(TC)_{22}$	Mougel *et al.* 1997
SAT7	5' GTAACCACCCATGCACACTC 3'	5' GCACAATACCTGGGATGTAG 3'	$(TG)_{14}$	Mougel *et al.* 1997
SAT8	5' CAGACCCGGCAGTTGCAGAG 3'	5' GGGAGAGAGGGATGGAGGTATG 3'	$(CT)_{14}(GT)_{8}TT(GT)_{5}$	Mougel *et al.* 1997
SAT13	5' CAGTTTTGAAGGACACCTGC 3'	5' GCCTCTACCTTTGTGGGG 3'	$(GT)_{13}$	Mougel *et al.* 1997
SOL8	5' GGATTGGGCCCTTTGCTCACACTTG 3'	5' ATCGCAGCCATATCTGAGAGAACTC 3'	$(TG)_{19}(N)_{15}(TG)_{5}$	Rico *et al.* 1994

Table 4. QIAGEN®Multiplex PCR reaction mix applied from standard protocol.

multipex PCR reaction mix (1x)	µl
2x QIAGEN MultiplexPCR Master Mix	5
Primer mix (2 µM each primer*)	1
RNase-free water	3
Template DNA	1
Total volume	10

* 2,1-2,5 µM INRA087, INRA140, INRA169 and SAT8

Table 5. Allele frequencies of the 12 polymorphic loci by subpopulations (the first divisions in Table 2). n=sample size

		2008-2009							2019-2020					
	n	38	30	21	20	13	6	2	29	13	8	6	2	1
Locus	**Allele**	**Töölönlahti**	**Kaisaniemi**	**Oulunkylä**	**Ruskeasuo**	**Vallila**	**Hietaniemi**	**Pasila**	**Kannelmäki**	**Herttoniemi**	**Espoo**	**Pitäjänmäki**	**Eteläsatama**	**Puotila**
SAT3	**145**	0,421	0,400	0,238	0,275	0,385	0,167	0,250	0,466	0,692	0,563	0,167	0,500	1,000
	150	0,000	0,033	0,000	0,000	0,000	0,000	0,000	0,034	0,038	0,063	0,000	0,000	0,000
	157	0,197	0,150	0,310	0,150	0,077	0,167	0,250	0,259	0,154	0,250	0,333	0,250	0,000
	159	0,013	0,000	0,000	0,125	0,000	0,083	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	161	0,368	0,417	0,452	0,450	0,538	0,583	0,500	0,241	0,115	0,125	0,500	0,250	0,000
SAT7	**183**	0,526	0,433	0,524	0,600	0,500	0,500	0,000	0,586	0,692	0,438	0,667	0,500	0,500
	185	0,237	0,233	0,310	0,050	0,385	0,250	0,250	0,138	0,231	0,000	0,167	0,250	0,000
	190	0,237	0,333	0,167	0,350	0,115	0,250	0,750	0,259	0,077	0,563	0,167	0,250	0,500
	194	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,017	0,000	0,000	0,000	0,000	0,000
SAT8	**137**	0,118	0,133	0,071	0,150	0,038	0,250	0,250	0,172	0,308	0,125	0,083	0,000	0,000
	141	0,882	0,867	0,929	0,850	0,962	0,750	0,750	0,828	0,692	0,875	0,917	1,000	1,000
SOL8	**103**	0,224	0,133	0,286	0,200	0,308	0,500	0,250	0,397	0,269	0,063	0,250	0,500	0,000
	105	0,000	0,000	0,095	0,000	0,154	0,000	0,000	0,069	0,038	0,000	0,000	0,000	0,000
	107	0,013	0,000	0,000	0,000	0,000	0,000	0,000	0,017	0,000	0,000	0,000	0,000	0,000
	109	0,763	0,867	0,619	0,800	0,538	0,500	0,750	0,517	0,692	0,938	0,750	0,500	1,000
INRA087	**197**	0,224	0,217	0,024	0,450	0,077	0,250	0,000	0,276	0,231	0,063	0,083	0,250	0,000
	203	0,000	0,000	0,000	0,000	0,000	0,000	0,500	0,052	0,000	0,000	0,083	0,000	0,000
	205	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,052	0,000	0,000	0,000	0,000	0,000
	210	0,000	0,017	0,000	0,000	0,077	0,167	0,000	0,086	0,077	0,188	0,167	0,500	0,000
	212	0,026	0,017	0,000	0,000	0,000	0,000	0,000	0,034	0,000	0,000	0,083	0,000	0,000
	214	0,750	0,750	0,976	0,550	0,846	0,583	0,500	0,500	0,692	0,750	0,500	0,250	1,000
	216	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,083	0,000	0,000
INRA102	**216**	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,017	0,000	0,000	0,000	0,000	0,000
	218	1,000	1,000	1,000	1,000	1,000	1,000	1,000	0,983	1,000	1,000	0,750	1,000	1,000
	220	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,250	0,000	0,000
INRA104	**99**	0,026	0,050	0,000	0,025	0,077	0,000	0,250	0,052	0,000	0,000	0,000	0,000	0,000
	103	0,013	0,000	0,000	0,000	0,000	0,000	0,250	0,000	0,000	0,000	0,000	0,000	0,000
	105	0,592	0,450	0,262	0,525	0,462	0,000	0,250	0,397	0,538	0,500	0,500	0,500	1,000
	109	0,368	0,500	0,738	0,450	0,462	1,000	0,250	0,552	0,462	0,500	0,500	0,500	0,000
INRA119	**209**	0,158	0,233	0,071	0,350	0,038	0,417	0,250	0,207	0,154	0,313	0,750	0,000	0,500
	211	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,069	0,538	0,000	0,000	0,000	0,000
	213	0,842	0,767	0,929	0,650	0,962	0,583	0,750	0,724	0,308	0,688	0,250	1,000	0,500

INRA140	**167**	0,882	0,867	0,976	0,775	0,808	1,000	0,500	0,707	0,731	0,938	0,667	0,750	1,000
	169	0,118	0,083	0,024	0,200	0,115	0,000	0,000	0,259	0,269	0,063	0,167	0,250	0,000
	171	0,000	0,050	0,000	0,025	0,077	0,000	0,500	0,034	0,000	0,000	0,167	0,000	0,000
INRA169	**141**	0,737	0,767	0,881	0,925	1,000	0,917	0,250	0,793	0,962	0,938	0,833	0,750	1,000
	145	0,013	0,000	0,000	0,000	0,000	0,000	0,500	0,034	0,038	0,000	0,000	0,000	0,000
	151	0,250	0,233	0,119	0,075	0,000	0,083	0,250	0,121	0,000	0,000	0,000	0,000	0,000
	155	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,052	0,000	0,063	0,167	0,250	0,000
INRA192	**92**	0,566	0,667	0,619	0,500	0,692	0,750	0,500	0,569	0,692	0,625	0,583	0,250	1,000
	94	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,115	0,063	0,083	0,000	0,000
	111	0,434	0,333	0,381	0,500	0,269	0,083	0,500	0,362	0,192	0,125	0,250	0,500	0,000
	113	0,000	0,000	0,000	0,000	0,038	0,167	0,000	0,069	0,000	0,188	0,083	0,250	0,000
INRA201	**121**	0,171	0,333	0,381	0,350	0,538	0,083	0,250	0,397	0,231	0,063	0,333	0,250	0,000
	127	0,829	0,667	0,619	0,650	0,462	0,917	0,750	0,603	0,769	0,938	0,667	0,750	1,000

Table 6. Locus specific values of genetic diversity for the old and the new populations: n=Sample Size, Na=number of alleles, Npa= number of private alleles, Ne= number of effective alleles, Ho=observed heterozygosity, He=expected heterozygosity, uHe=unbiased expected heterozygosity, and F=Fixation Index.

	2008-2009								2019-2020							
Locus	**n**	**Na**	**Npa**	**Ne**	**H_O**	**H_E**	**uH_E**	**F**	**n**	**Na**	**Npa**	**Ne**	**H_O**	**H_E**	**uH_E**	**F**
SAT3	130	5	1	2,911	0,715	0,656	0,659	-0,09	59	4		2,743	0,627	0,635	0,641	0,013
SAT7	130	3		2,65	0,662	0,623	0,625	-0,062	59	4	1	2,286	0,61	0,563	0,567	-0,085
SAT8	130	2		1,266	0,177	0,21	0,211	0,158	59	2		1,414	0,288	0,293	0,295	0,015
SOL8	130	4		1,684	0,423	0,406	0,408	-0,042	59	4		1,962	0,458	0,49	0,494	0,067
INRA087	130	5		1,634	0,346	0,388	0,39	0,108	59	7	2	2,541	0,525	0,606	0,612	0,134
INRA102	130	1		1	0	0	0		59	3	2	1,071	0,034	0,066	0,066	0,486
INRA104	130	4	1	2,167	0,515	0,538	0,541	0,043	59	3		2,099	0,593	0,524	0,528	-0,133
INRA119	130	2		1,451	0,338	0,311	0,312	-0,09	59	3	1	2,303	0,475	0,566	0,571	0,161
INRA140	130	3		1,305	0,215	0,233	0,234	0,078	59	3		1,651	0,441	0,394	0,398	-0,118
INRA169	130	3		1,419	0,277	0,295	0,296	0,061	59	4	1	1,351	0,288	0,26	0,262	-0,11
INRA192	130	3		1,944	0,508	0,486	0,487	-0,046	59	4	1	2,233	0,712	0,552	0,557	-0,289
INRA201	130	2		1,733	0,377	0,423	0,425	0,109	59	2		1,716	0,424	0,417	0,421	-0,015

Table 7. Locus specific heterozygosity excess or deficiency under the mutation models. n= 2x number of samples, Na=Number of alleles, Heq= expected heterozygosity under the mutation model at equilibrium, S.D.= standard deviation, DH/sd=standardized difference, Prob=probability.

		observed			under the I.A.M.				under the T.P.M.				under the S.M.M.			
locus	**population**	**n**	**Na**	**He**	**Heq**	**S.D.**	**DH/sd**	**Prob**	**Heq**	**S.D.**	**DH/sd**	**Prob**	**Heq**	**S.D.**	**DH/sd**	**Prob**
INRA192	2008-2009	260	3	0.487	0.278	0.188	1.116	0.200	0.344	0.181	0.791	0.281	0.427	0.143	0.425	0.437
INRA104	2008-2009	260	4	0.541	0.370	0.190	0.901	0.212	0.462	0.160	0.490	0.369	0.567	0.112	-0.235	0.327
INRA169	2008-2009	260	3	0.296	0.284	0.192	0.065	0.484	0.340	0.182	-0.237	0.422	0.433	0.141	-0.976	0.180
INRA087	2008-2009	260	5	0.390	0.459	0.177	-0.391	0.335	0.551	0.135	-1.197	0.138	0.649	0.094	-2.745	0.021
INRA201	2008-2009	260	2	0.425	0.158	0.165	1.613	0.131	0.178	0.166	1.489	0.133	0.195	0.169	1.357	0.159
INRA140	2008-2009	260	3	0.234	0.280	0.188	-0.243	0.471	0.343	0.179	-0.606	0.306	0.443	0.136	-1.537	0.098
INRA119	2008-2009	260	2	0.312	0.152	0.159	1.000	0.210	0.179	0.168	0.791	0.258	0.205	0.171	0.625	0.315
SOL8	2008-2009	260	4	0.408	0.364	0.189	0.232	0.463	0.463	0.156	-0.358	0.321	0.567	0.111	-1.429	0.104
SAT3	2008-2009	260	5	0.659	0.452	0.182	1.137	0.130	0.553	0.139	0.762	0.243	0.653	0.087	0.071	0.449
SAT8	2008-2009	260	2	0.211	0.165	0.170	0.272	0.331	0.177	0.169	0.201	0.357	0.188	0.169	0.136	0.387
SAT7	2008-2009	260	3	0.625	0.276	0.184	1.891	0.016	0.345	0.181	1.552	0.035	0.431	0.142	1.372	0.054
INRA192	2019-2020	118	4	0.557	0.417	0.180	0.778	0.256	0.486	0.156	0.452	0.397	0.582	0.111	-0.225	0.343
INRA104	2019-2020	118	3	0.528	0.318	0.182	1.150	0.145	0.382	0.171	0.850	0.221	0.453	0.140	0.539	0.336
INRA169	2019-2020	118	4	0.262	0.412	0.181	-0.829	0.266	0.508	0.145	-1.696	0.090	0.576	0.114	-2.744	0.020
INRA087	2019-2020	118	7	0.612	0.611	0.139	0.006	0.413	0.693	0.092	-0.883	0.176	0.767	0.054	-2.895	0.016
INRA201	2019-2020	118	2	0.421	0.182	0.170	1.409	0.162	0.209	0.175	1.207	0.214	0.212	0.165	1.268	0.177
INRA140	2019-2020	118	3	0.398	0.306	0.182	0.501	0.385	0.386	0.171	0.065	0.439	0.454	0.134	-0.421	0.286
INRA119	2019-2020	118	3	0.571	0.311	0.184	1.409	0.076	0.384	0.168	1.108	0.120	0.455	0.138	0.838	0.190
INRA102	2019-2020	118	3	0.066	0.321	0.182	-1.392	0.116	0.381	0.167	-1.884	0.050	0.457	0.132	-2.954	0.012
SOL8	2019-2020	118	4	0.494	0.418	0.177	0.429	0.417	0.492	0.157	0.018	0.408	0.579	0.106	-0.803	0.184
SAT3	2019-2020	118	4	0.641	0.418	0.179	1.245	0.096	0.504	0.150	0.913	0.183	0.583	0.106	0.546	0.352
SAT8	2019-2020	118	2	0.295	0.182	0.166	0.679	0.287	0.211	0.169	0.495	0.345	0.223	0.167	0.428	0.357
SAT7	2019-2020	118	4	0.567	0.409	0.180	0.881	0.222	0.507	0.146	0.416	0.409	0.587	0.108	-0.180	0.342