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Anticoagulant Resistance in Rats and Mice in the UK – new data for August 2024 to July 2025

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Summary

1. DNA extraction and sequencing of rodent tissue samples was undertaken for the first time by scientists from Science and Advice for Scottish Agriculture (SASA) during the period August 2024 to July 2025.
2. The number of rodent tissue samples submitted to the CRRU resistance testing programme declined in the sampling period, as they had the previous year. A possible explanation for this is that interest in anticoagulant resistance among rodent pest management practitioners is declining, given the apparent ubiquitous nature of the phenomenon in rodents tested in the UK over recent years – in other words "why send in samples when we already know the results?" Other explanations are also plausible, such as uncertainty on the part of those who submit samples about when test results will be received caused by the batch-testing laboratory methodologies employed. CRRU will pursue efforts in the coming year to obtain more samples by engagement with practitioners in all sectors of UK rodent pest management and by providing clarity on the time-scales for reporting of results.
3. Scientists at SASA had established a direct procedure for the acquisition of tissue samples from pest management practitioners in Scotland and a substantial collection of frozen tissue samples had been obtained. A sub-sample of these was made available to the CRRU resistance testing programme and subsequently extracted and sequenced at SASA. Maps presented in previous reports in this series show a lack of samples from Scotland. Thus, the strong bias in the sample described in this report for tissue samples from Scotland goes some way towards correcting this disparity.
4. A total of 140 rodent tissue samples were received for DNA sequencing at the SASA laboratories during the period August 2024 to July 2025, comprising those from the CRRU scheme and those from SASA; a total of 127 Norway rat (*Rattus norvegicus*) samples and 13 mouse samples. Among the rat samples, five either did not yield DNA that could be sequenced at all or could not be sequenced in the areas of the genome where the five common rat resistance single nucleotide polymorphisms (SNPs) are found. This resulted in 122 viable Norway rat samples. Among the mouse samples, only four were of house mouse tissue and could be sequenced. Others were either tissue samples of house mouse that could not be sequenced, were submitted without necessary accompanying information or were from wood mice (*Apodemus sylvaticus*).
5. The SNP that was found most frequently among the Norway rat sample was L128Q, with 93 individuals carrying this mutation (this value includes hybrid resistant rats that also carried the Y139C SNP, see below). This reflects the very high preponderance of samples tested from Scotland where this SNP is commonly found. Among those that carried only the L128Q SNP, 75 were homozygous and 11 heterozygous. This situation, in which there is such a high proportion of homozygosity, is indicative of a long-established and embedded resistance focus. A single sample was received for the first time from Shetland and it too carried L128Q.
6. Seven individuals carried the L128Q SNP in combination with Y139C i.e. hybrid resistance, all from the south of Scotland and Northumberland.
7. The numbers of Norway rats carrying other mutations were as follows: Y139C, 15 (including the seven L128 hybrids); L120Q, three and Y139F, two. Among the L120Q rats, two were from the well-known focus in Hampshire and one from its periphery in Devon. The two Y138F rats were, unsurprisingly, from Kent.
8. A total of 16 Norway rats carrying the susceptible genome were recorded from widely separated areas in Scotland (12 individuals) and England (3 individuals), as well as one from Northern Ireland.

9. Only four viable house mouse samples were obtained and all were anticoagulant resistant. Three from Aberdeenshire (one homozygous L128S and two hybrid-resistant for the L128Q and Y139C SNPs) and one from Liverpool that was also homozygous L128S.
10. During the period 2009 and 2025 in which DNA resistance sequencing has been conducted, first at the University of Reading and APHA, and now at SASA, a total of 753 Norway rat and 144 house mouse tissue samples have been successfully sequenced; among these samples 75.1% of rats and 94.4% of mice carried one or more of the single nucleotide polymorphism known significantly to affect the efficacy of anticoagulant rodenticides. (Note. These results may not reflect the true frequency of resistance in the two species because samples are often sent by those experiencing difficulties in obtaining control of rodent infestations with anticoagulants.)
11. The new data presented here do not much enhance understanding of the distribution of resistance in rats and mice in England and Wales, because few samples were received from those territories. However, they added significantly to the information available on resistance in Norway rats in Scotland. It is apparent that the less-severe resistance SNP L128Q is prevalent and widespread in the areas of Scotland for which we have samples. The more severe Y139C SNP, against which the use of bromadiolone and difenacoum is not recommended, is increasingly found in the south of Scotland and in particular the Scottish borders. The discovery of seven individuals from that area with L128Q/Y139S hybrid resistance is a cause of concern as we have no definitive information on the efficacy of anticoagulants against hybrid resistant Norway rats (or mice for that matter).
12. The data presented here are supplied to the Rodenticide Resistance Action Committee of CropLife International in Brussels, which publishes on-line maps providing immediate access to the information via an informative interactive on-line platform that can now also be down-loaded onto mobile devices (https://rrac.info/index.html).

1. Introduction

The Campaign for Responsible Rodenticide Use (CRRU) UK is responsible for the co-ordination and operation of the UK Rodenticide Stewardship Regime (see https://www.thinkwildlife.org/about-crru-uk/). It is a requirement of the Health and Safety Executive (HSE) and the Government Oversight Group (GOG) that CRRU annually provides information on anticoagulant resistance among populations of Norway rats (*Rattus norvegicus*) and house mice (*Mus musculus*) in the UK (HSE, 2023). Reports are produced based on DNA sequencing and analysis of tissue samples submitted, mainly by professional pest control technicians and those conducting rodent control on agricultural holdings. The last in this sequence of reports was provided by CRRU[1] in 2024 and summarised all data available up to July 2024 (Buckle et al., 2024). The present report provides additional data on the incidence of anticoagulant resistance in rats and mice covering the period August 2024 to July 2025, and summarises previous work. This study is now the most comprehensive examination of anticoagulant resistance among pest rodents ever conducted.

The sampling period August 2023 to July 2024 showed a significant decline over previous years in the number of tissues samples submitted to the CRRU resistance testing programme (Buckle et al., 2024). That decline continued during the period covered in this report. Fortunately, separate efforts by SASA scientists had produced a substantial collection of rodent tissue samples from Scotland held in frozen storage at the SASA laboratories. These had been acquired by a proactive process of direct communication with pest management practitioners across Scotland. An agreement was reached between SASA and CRRU that some of these samples should be admitted to the CRRU programme, so that their DNA could be extracted and sequenced. The samples were chosen to cover as wide a geographical area as possible. A high proportion of the new data presented and mapped in this report is therefore from Scotland, which had been previously under-represented in University of Reading and CRRU sampling programmes (see the maps shown in Buckle et al., 2024).

Anticoagulant resistance is of interest to those who engage in rodent pest management in the UK because resistance significantly impacts the efficiency and cost of rodent control. It is also of interest to the CRRU-run UK Rodenticide Stewardship Regime and its Government Oversight Group. The purpose of the regime is to provide best practice guidance on the use of rodenticides in rodent pest management with an objective, among others, of reducing residues of anticoagulants in non-target wildlife (Buckle et al., 2017; HSE, 2023; CRRU, 2024). Residue monitoring in non-target animals has recently shown an increase in residues of the most potent 'resistance-breaking' second-generation anticoagulants (SGARs) (e.g. Ozaki et al., 2022; Ozaki et al., 2024; George et al., 2024; Campbell et al., 2024). This has probably been brought about, at least in part, by the apparent increasing incidence of the most severe resistance mutations among rats (i.e. L120Q, Y139C, Y139F), specific recommendations from the Rodenticide Resistance Action Group (RRAG) about which substances to use against highly resistant rodents, namely brodifacoum, difethialone and flocoumafen (Buckle et al., 2021 a and b) and, consequently, an increasing adoption by pest management practitioners of these requirements.

[1] Where the acronym CRRU is used in this report this refers to the Campaign for Responsible Rodenticide Use UK.

2. Materials and Methods

2.1 Origins of samples

Tissue samples analysed for genetical mutations in this programme were either submitted by pest control technicians, collected after trapping by staff of the Vertebrate Pests Unit (VPU) at the University of Reading or sent in by others involved in rodent pest management. In the 2024 to 2025 sample period this latter group was significantly increased through a process whereby SASA scientists had communication with pest management practitioners across Scotland to solicit tissue samples directly from them. The majority received from sites where technicians had experienced difficulties in obtaining effective control with anticoagulants, possibly because of resistance or, in the case of VPU sampling, were taken from the borders of known resistance areas in an attempt to identify their boundaries.

2.2 Methods of DNA analysis

The following description of methods used at SASA differs slightly from those used in previous reports. Genetic material was obtained from the field in the form of tail tip samples. Where possible, samples were placed in tubes containing 80% alcohol and then stored at -20°C as quickly as possible. Some unfrozen samples were shipped to the laboratory using a courier service, surface mail or by hand delivery, and were frozen on receipt.

Genomic DNA was extracted using the MagMAX™ DNA Multi Sample Kit following the manufacturer's recommendations for 'Isolation of genomic DNA from mouse tails' (ThermoFisher Scientific, UK). Briefly, approximately 1cm of the tail tip was placed in a 2 mL tube with 184 µL PK buffer and 16 µL Proteinase K (100mg.mL). Tubes were incubated overnight at 55 ˚C without shaking. Genomic DNA was purified using either a KingFisher™ Flex Purification System or KingFisher™ Duo Prime Purification System (ThermoFisher Scientific, UK) depending on the number of samples being processed.

The three exons of the VKORC1 gene, designated 1, 2 and 3, were amplified by PCR following the methodology of Rost et al. (2004). PCR products were cleaned by digestion with ExoSAP (Exonuclease I and shrimp alkaline phosphatase). Product samples (2 µL) were then sequenced with BigDye version 3.1 terminator chemistry (ThermoFisher Scientific, UK) on a VeritiPro™ Thermal Cycler (ThermoFisher Scientific, UK), and the terminated products were resolved on a SeqStudio™ Flex Genetic Analyzer capillary sequencer. The sequence trace files were visually analysed and any ambiguous bases were edited using the DNASTAR Lasergene 17 software. The sequence alignments were compiled using ClustalW and compared to reference sequences HM181983 (rat) and GQ905709 (mouse).

A list of the VKORC1 mutations found in Norway rats and house mice in the UK known to have a significant detrimental effect on the efficacy of anticoagulant rodenticides is given in Table 1.

Table 1. The main VKORC1 single nucleotide polymorphisms (SNPs) in Norway rats (NR) and House mouse (HM) in UK mentioned in this report that are known either from field experiments and/or field experience to have a significant practical effect on anticoagulant efficacy. Abbreviated from Buckle 2013.

Species	Single nucleotide polymorphism	Abbreviation*
NR	**Leu**cine128**Glu**tamine	L128Q
NR	**Tyr**osine139**Ser**ine	Y139S
NR	**Leu**cine120**Glu**tamine	L120Q
NR	**Tyr**osine139**Cys**teine	Y139C
NR	**Tyr**osine139**Phe**nylalanine	Y139F
HM	**Tyr**osine139**Cys**teine	Y139C
HM	**Leu**cine128**Ser**ine	L128S

* Standard abbreviations are used throughout the report.

2.3 Methods for GIS maps

Once again, the following account is similar to that given in previous reports. Data were collated in Microsoft Excel spreadsheets (by the University of Reading, APHA and latterly SASA) documenting all the processed samples for Norway rats and house mice from which DNA could be extracted and sequenced. Data from APHA for each year ran from August to the following July. Each annual spreadsheet contained the following information:

- Location of samples (in most cases this was a postcode and occasionally a description such as the local town) plus the county.
- The date samples were received for processing.
- Number (count) of samples received from each location on a date.
- Information on the mutation and genotypes identified by exon.

The postcode information (or relevant locational descriptor) was converted to a British National Grid coordinate (easting and northings) to enable mapping. In some cases locational information was not provided and these points were not mapped.

ArcGIS Pro 2.9 was used to map each of the locational points and its relevant information from the spreadsheet.

Symbology: identifying the mutation and genotype was assigned (colours and symbols) using the following order of dominance where different resistances, and therefore different symbols, from the same location caused symbols to be superimposed on the maps:

Brown rats: Strongest = L120Q > Y139S > Y139F > Y139C > L128Q = Weakest
House Mouse: Strongest = L128S Y139C > L128S > Y139C = Weakest

Maps were presented at a UK scale using Ordnance Survey county and area boundary outlines and exported as a high resolution jpeg files for use in the report.

2.4 Rodenticide Resistance Action Committee (RRAC) interactive global resistance map

The results from this study are provided to the Brussels-based RRAC of CropLife International (http://www.rrac.info/). The results are collated with those obtained from other global studies and presented in an interactive form on the RRAC web-site and lately available through applications (apps) on hand-held devices. The maps (see example for the UK at: http://guide.rrac.info/resistance-maps/united-kingdom/) use Google 'heat map' technology to ascribe different weightings to records depending on the numbers of positive samples and the frequencies of their closest neighbours. Users of the maps are able to scroll in to find their own location, that of the nearest confirmed incidence of anticoagulant resistance, the mutation of that record and to obtain advice about the correct use of anticoagulants in the area. It is anticipated that this scheme will help pest control practitioners to make informed choices about which anticoagulant active substance to use and will support a 'competent workforce'.

3. Results

3.1 Norway rats – historical records

This study has operated at the University of Reading, and later at the laboratories of the Animal and Plant Health Agency and Science and Advice for Scottish Agriculture, during the period 2009 to July 2025. In that time a total of 753 Norway rat tissue samples from around the UK have been analysed using the DNA sequencing technique. Their geographical locations are shown in Figure 1. Of these, 580 (77.0%) were found to possess one or more of the resistance mutations that are known to have a significant effect on anticoagulant rodenticide efficacy (Table 1). The remaining 173 animals (23.0%) carried the wild type (i.e. anticoagulant susceptible) genome.

Maps showing the geographical locations from which these samples were sent have been published previously (e.g. Buckle et al., 2024) and are the main source of the UK mapping information available at the website of the international Rodenticide Resistance Action Committee (https://guide.rrac.info/resistance-maps.html). It is important to keep in mind that these samples are generally submitted by those having difficulty in obtaining effective control of rat infestations with anticoagulants and may not reflect the true frequency of resistance in the UK Norway rat population as a whole. The records accumulated during the sixteen years of the project reflect the resistance present at the time the samples were taken and may not reflect the current situation at the sampling site. However, there is little evidence, either published or anecdotal, to show that resistance recedes once it is established.

3.2 Norway rats – records for 2024-2025 and frequency of resistance

Among the 122 samples (Table 2) that were capable of being sequenced in the period August 2023 to July 2024, a total of 106 (86.9%) were found to carry one of the five main Norway rat anticoagulant resistance mutations (Table 1). The remaining 16 animals (13.1%) carried the wild type genome. The proportion of resistant Norway rats in the sample was higher than that found in previous surveys (see Buckle et al., 2024). The apparent reason for this is that a high proportion of rats sampled in 2024-25 came from Scotland where there is a very high prevalence of the L128Q SNP.

The sample provides a more comprehensive understanding of the status of anticoagulant resistance in Scotland than available before (Fig. 2). It is apparent that the L128Q SNP, previously called ‘Scottish resistance’ is exceedingly prevalent in the Central Belt, which is where about 70% of the Scottish population lives, and more widely across all of the eastern parts of the country. It was also found in the single sample obtained from Mainland Shetland. In spite of these new records, much of Scotland remains poorly sampled and it is therefore impossible to say if this situation occurs more widely.

Also of interest in the samples obtained from Scotland were the occurrence of a single homozygous Y139C animal and seven others that possessed both the L128Q and Y139C SNPs, each in heterozygous form. Many of these were from the Borders region, with a second focus in Lanarkshire. A lack of samples from between these two foci means we cannot presently be certain these foci are separate or contiguous (Figs. 2 and 3). The source of this Scottish focus of Y139C is uncertain but it is interesting to note that the nearest area where this resistance is prevalent is more than 150 km away in North Yorkshire. Transport links between these areas are, of course, frequent and abundant.

Among the samples received from other parts of the country, the Y139C SNP was the most common, as has been the case in recent years. Records from East Yorkshire and Cambridgeshire are close to previous foci of this SNP, and not surprising, but its appearance in this sample on the Devon-Cornwall border extends the occurrence of this severe resistance considerably to the west. Similarly, findings of Y139F in Kent and L120Q in Hampshire were to be expected but the record of a homozygous L120Q individual in Devon supports previous findings from that area that the UK's most severe resistance is now well established in the West Country (Figs. 1 and 2). If resistance development there follows the pattern seen some years ago in central southern England, it will quickly become the case that the majority of Norway rats in the south-west will carry the most severe form of anticoagulant resistance.

A single sample received from Langport in Somerset was found to carry the L128Q SNP. This is by a very wide margin the most southerly occurrence of this resistance type (Fig. 1.).

It is reassuring that the only sample received from Northern Ireland was fully susceptible, as was the case for a larger sample tested in the last sampling period (Buckle et al., 2024). In Scotland, susceptible rats appeared to be more common in the far north and west than elsewhere, possibly because the very low human population density means little pest control is conducted there.

Separate maps for each of the resistance SNPs and for susceptible Norway rats are given in Annexes 1-6.

Table 2. The numbers of Norway rats tissue samples received and analysed in 2024/25, and their status of resistance or susceptibility. (See Table 1 for further explanations of the different resistance mutations.)

Resistance status	**Genotype**		**Totals**
	Homozygous	**Heterozygous**	
L120Q	3	0	3
L128Q	75	11	86
Y139C	5	3	8
Y139F	2	0	2
L128Q and Y139C*	0	7	7
Total resistant	85	21	106
Susceptible	16	-	16
Total animals tested			122

*These seven hybrid-resistant animals were heterozygous for each of two the resistance mutations.

Fig. 1. Consolidated map showing all Norway rats found to carry an anticoagulant resistance SNP, both in homozygous and heterozygous form, for any of the five main resistance mutations found in that species, and for combinations of them (i.e. hybrid resistance). Data on susceptible individuals are also included. Records for 2009-2025.

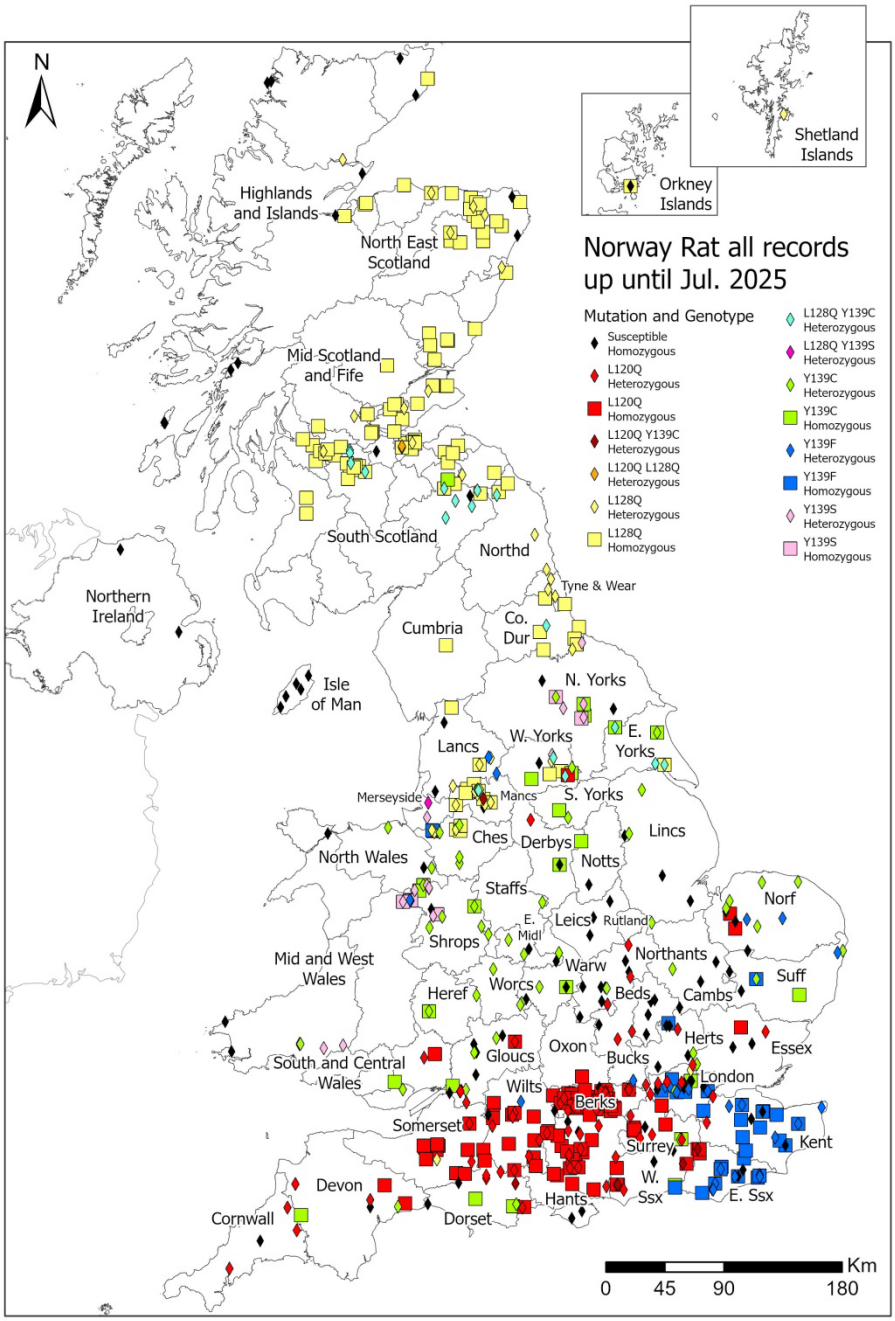


Figure 2. Geographical locations of all new Norway rat records for the period August 2024 to July 2025

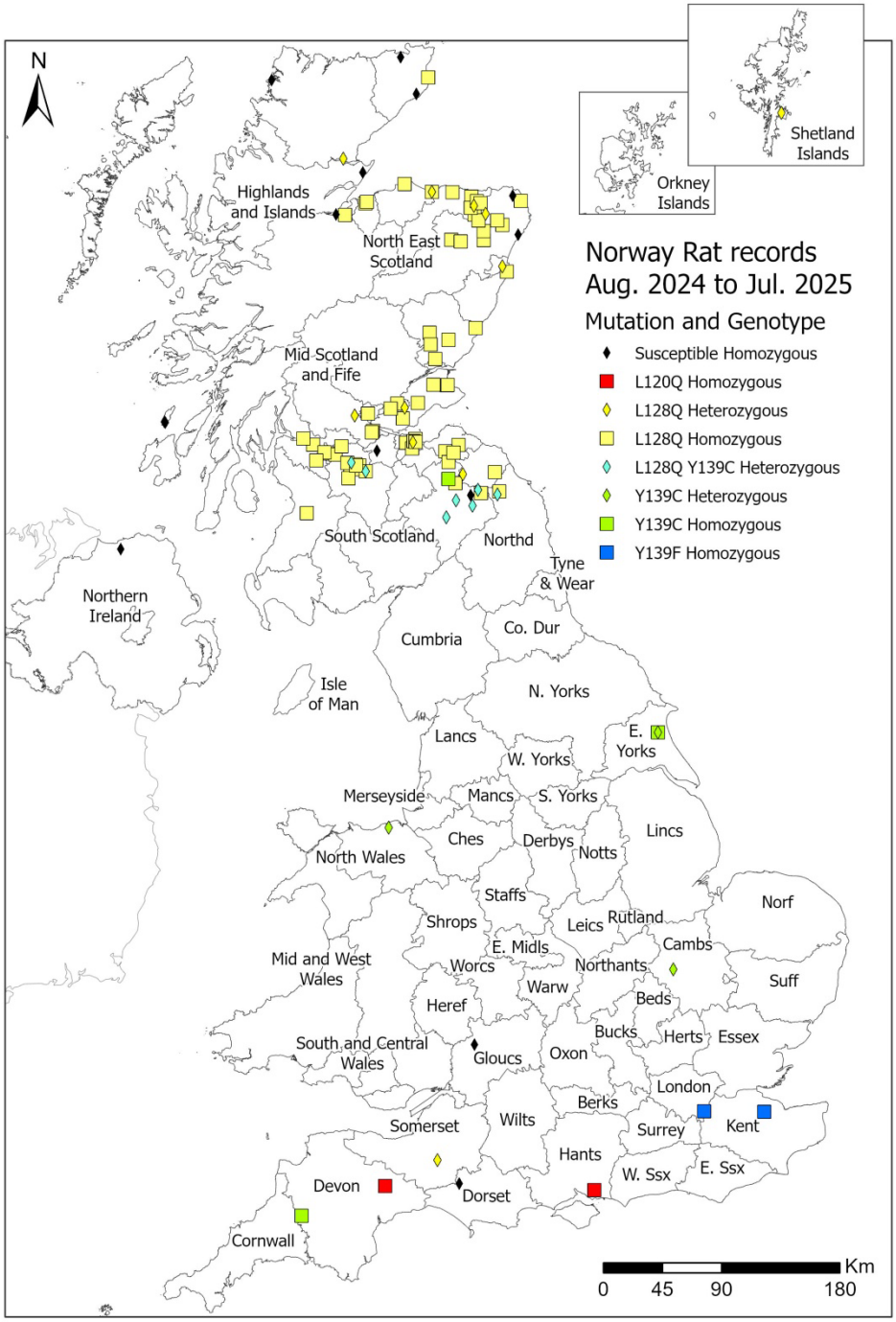
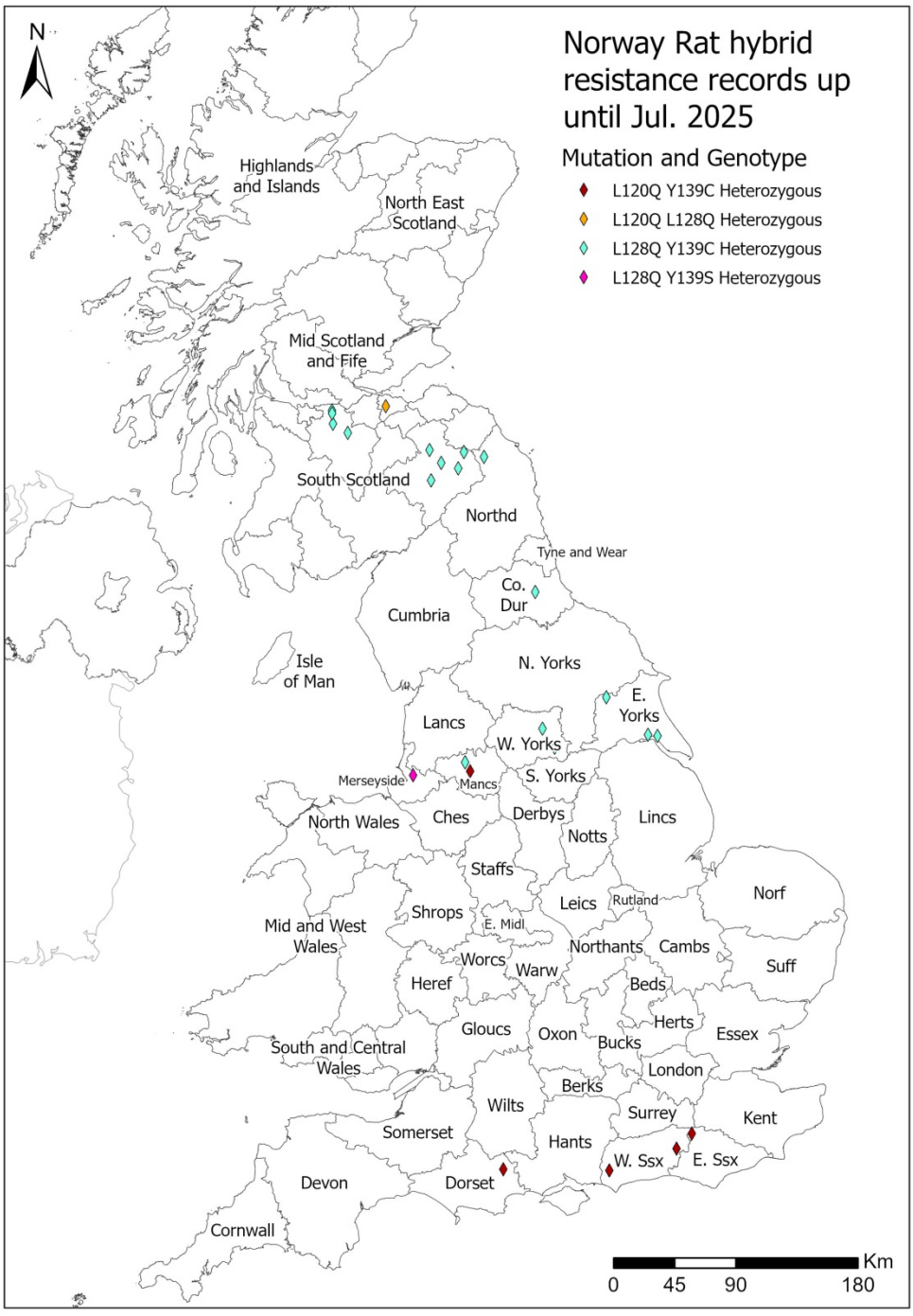


Fig. 3. Map showing all Norway rats found to carry two different anticoagulant resistance SNPs (i.e. hybrid resistance). Records for 2009 to 2025. SNP records shown on this map are not included in the maps for the relevant individual SNPs. This is particularly important regarding the distribution of Y139C in southern Scotland.

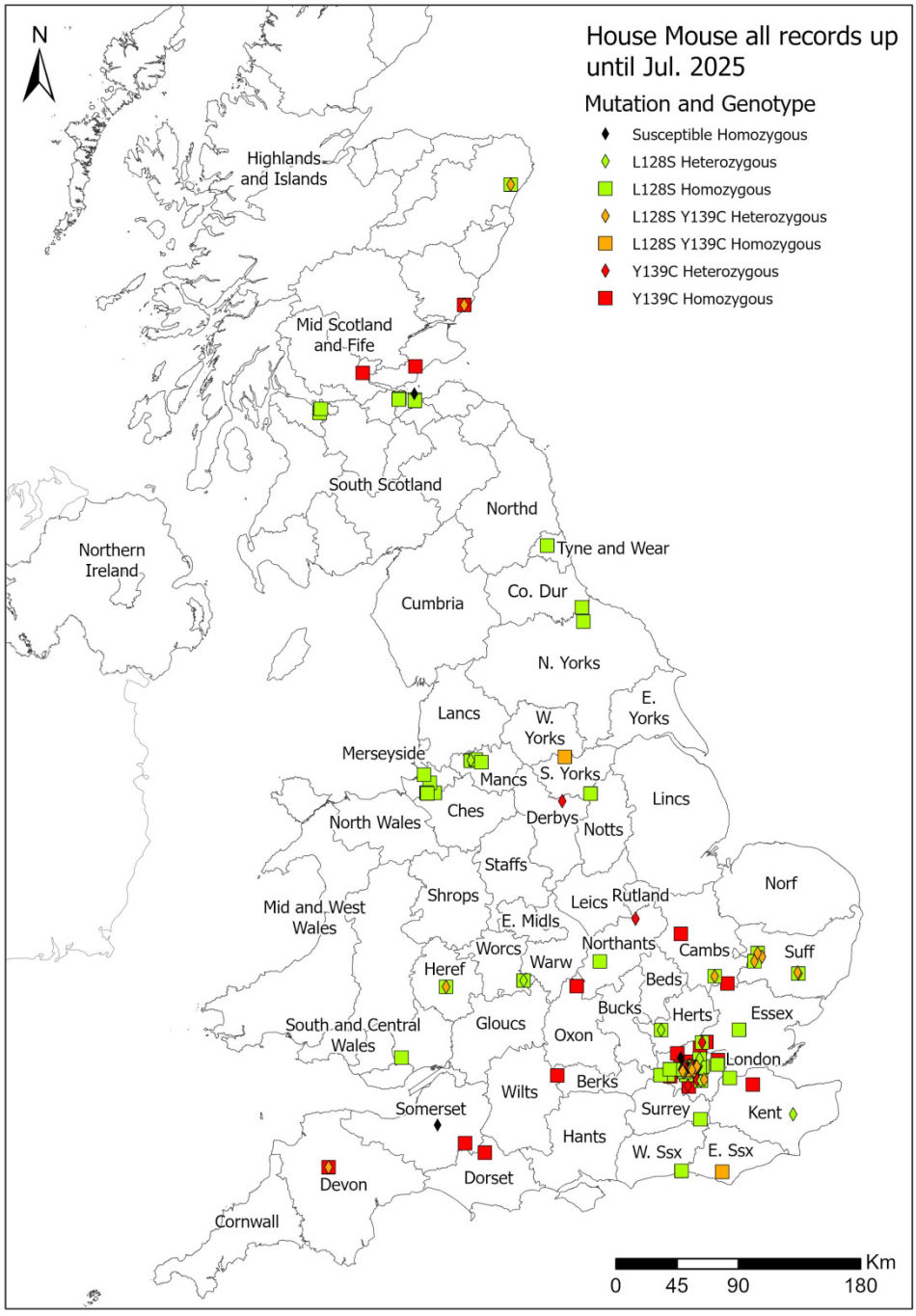


3.3 House mice

Once again, only a small number of house mouse tissue samples were received and only four of them provided DNA that could be extracted and sequenced. All were resistant. Among three samples received from the Elon area of Aberdeenshire, two animals were hybrid resistant, carrying the L128S and Y139C SNPs each in the heterozygous state. The third mouse was homozygous L128S resistant. The other house mouse received from Liverpool was also homozygous L128S resistant. When these samples are added to those previously recorded they bring the total of house mouse tissue samples sequenced to 144 with a frequency of resistance of 94.4%. These new records are shown in Figure 4 together with all previous house mouse records.

Separate maps showing the distributions of the two house mouse resistance SNPs are provided in Annexes 7 and 8.

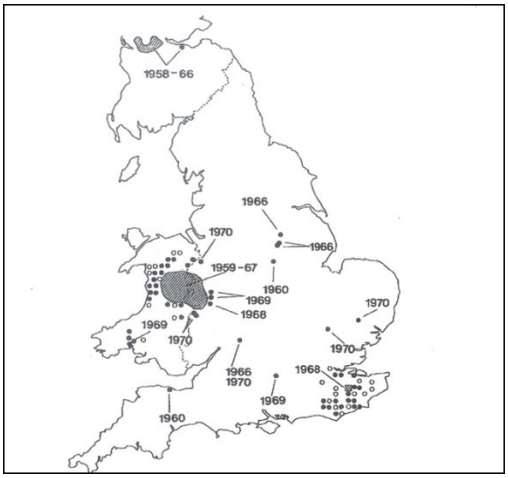
Fig. 4. Consolidated map showing all house mice found to carry an anticoagulant resistance SNP, both in homozygous and heterozygous form, for any of the three resistance mutations found in that species, and for combinations of them (i.e. hybrid resistance). Records for 2009 to 2025. (The Hertfordshire focus of the spretus introgression is obscured by other overlaying resistance records at the same site.)



4. Discussion

The sample of Norway rats acquired by SASA scientists, and processed through the CRRU monitoring scheme, has increased our understanding of the occurrence of anticoagulant resistance in Scotland. It is apparent in the areas sampled that L128Q resistance is prevalent (Fig. 2), with a very high incidence of homozygous rats (approximately 87% of the total) and few susceptible rats. This is indicative of a long-established resistance focus that has undergone prolonged selection for the resistance mutation caused by the ineffective use of anticoagulants, and demonstrates a higher frequency of anticoagulant resistance in Scotland than in the rest of the UK. This is hardly surprising since the very first incidence of anticoagulant resistance discovered was found in Scotland in 1958 (Fig. 5). Boyle (1960) reported that rats taken on a farm in the 'west of Scotland' were found to be resistant to warfarin and diphacinone, first in the field and later when tested in the laboratory. When DNA resistance testing became available it was found that this resistance was conferred by the L128Q SNP (Pelz et al., 2007). Further testing in the surrounding areas in the years following 1960 (Fig. 5) showed an extensive resistance focus across much of the Central Belt of Scotland (Greaves and Rennison, 1973).

Figure 5. Sites of anticoagulant resistance in the UK from surveys conducted during the years 1959 to 1970. Filled symbols show where resistant Norway rats were found, open symbols where resistance was not found. From Greaves and Rennison (1973).



The new data on the L128Q resistance focus presented here permits the observation that, in Scotland, a very high proportion of Norway rat infestations down the entire the east side of the country and in the Central Belt would be expected to contain mostly resistant individuals (Figs. 1 and 2). Previous reports in this series have also shown that, since the original discovery in 1958, the focus has now spread to include most of England north of a line taken from the Humber to the Dee estuaries (Fig. 1 and Annex 1).

An extreme L128Q outlier was found at a site in Langport in Somerset, approximately 230 km from the nearest L128Q infestation known previously. It is not possible to say with certainty if this occurrence is due to long-distance transportation or to a *de novo* mutation event. However, we believe it is more likely to be the former because the latter are thought to be extremely rare (Buckle et al., 2024) and it may be relevant to note that the site where the resistant rat was found

is only a few miles from the M5 motorway, which connects the south-west of England to the north-west.

The L128Q mutation confers a relatively low level of resistance to animals that possess it compared to other SNPs, such as L120Q, Y139F and Y139C (Buckle, 2013). Consequently, the UK RRAG states that all five authorised second-generation anticoagulants may be expected to be effective against it (Buckle et al., 2021b), as well as, of course, the non-anticoagulant active substances. The very high current prevalence of the mutation among rats in Scotland, across much of northern England, appears to show that the use of these substances over a period of almost 50 years has done little to curtail the incidence and spread of L128Q.

Recent surveys have found an increasing occurrence of the severe Y139C genotype in many areas of the UK (Buckle et al., 2024); with a strong focus apparently developing in Yorkshire (Fig. 1 and Annex 5). The coexistence of Y139C and L128Q in that area would lead to an expectation of hybridisation and, indeed, many L128Q/Y139C hybrid-resistant rats have now been found across northern England, with an individual furthest north in County Durham (Fig. 1). The English counties of Northumberland and Cumbria are very sparsely sampled and the situation in them remains largely unknown. However, the occurrence of a single homozygous resistant Y139C individual among the 2024-25 samples found near to Galashiels indicates that this SNP may be established in the Scottish borders. The occurrence of several L128Q/Y139C hybrids in the sample indicates that the Y139C SNP may be more common in Scotland than our current distribution data indicates. The occurrence of L128Q/Y139C hybrid-resistant rats in Scotland, and elsewhere, is a cause for concern because we have no definitive information, either from the laboratory or field, about the efficacy of anticoagulants against hybrid-resistant rats. It may therefore be prudent only to use the most potent anticoagulants and non-anticoagulant substances against them.

Too few samples were received from the other parts of the UK to permit more useful narrative than was published in an earlier report (Buckle et al., 2024) (and see Fig.1), although the appearance of the single heterozygous L128Q rat in this sample in Somerset is noteworthy (see above).

As was the case in the previous sampling period, the number of tissue samples submitted into the CRRU resistance testing programme in the period August 2024 to July 2025 was substantially below the target number of 100 samples. Continuing difficulty in providing sufficient incentive for pest control practitioners in the UK to submit samples is the cause of concern, as it may limit the ability of CRRU, and its collaborators SASA and the University of Reading, to continue a viable UK resistance testing programme. Samples of house mouse tissue are received even less frequently than those of Norway rats and this occurred once more in our 2024-25 samples, with only four viable mouse tissue samples received.

The resistance monitoring programme is a requirement set by HSE and GOG to permit the continued authorisation and use of the SGARs in the UK (HSE, 2023). The principal objective of the work is to populate maps with information from sampling points were resistance testing of Norway rats and house mice has been conducted. The maps are published annually in reports such as this, which enter the public domain on the website of CRRU UK (https://www.thinkwildlife.org/), and through the interactive maps provided by the Rodenticide Resistance Action Committee of CropLife International (https://rrac.info/index.html). Pest control practitioners are, therefore, able to establish the proximity of any site on which they are working to a known resistance focus. Knowledge of the type of resistance present provides

information on the most effective active substances to use against resistant rodent infestations (Buckle et al., 2021a and b).

Pest management practitioners would undoubtedly prefer immediate resistance information about a site on which they are working. However, batch processes are used in the SASA laboratory (as previously at APHA) to make the extraction and sequencing of DNA tissue samples cost-effective, and hence samples are collected and stored until enough are available to fill a batch. This makes it impossible for the CRRU project to offer an immediate 'reactive' service to provide practitioners with 'real time' resistance information. Such a service, involving testing individual samples as they arrive at the laboratory, would be unrealistically expensive. It is important that CRRU either provides clarity to those who submit samples or establishes new ways of working that would provide more immediate resistance information to those who submit samples, albeit at a much greater cost.

It is valuable to reiterate here, once again, that the continued use of anticoagulants against rodent populations that are resistant to them has important adverse consequences: 1) the speed of removal of treated infestations is reduced, with consequent risks to human and animal health, 2) resistance is both further spread and its severity increased when susceptible rodents are removed from infestations but resistant ones are left, and 3) resistant rodents survive for long periods after unsuccessful treatments carrying high body burdens of persistent anticoagulants until their natural deaths and meanwhile may be taken by non-target predators and scavengers (Buckle et al., 2020). It therefore continues to be important to publicise the resistance distribution maps in this report, and the interactive versions found at the RRAC website (https://guide.rrac.info/resistance-maps.html), and to disseminate resistance management advice to avoid the sale and use of resisted substances in areas where resistant rodents are now known predominantly to occur.

5. Acknowledgements

During the period covered by this report the work of DNA extraction from rodent tissue and sequencing was conducted for the first time at the laboratories of Science and Advice for Scottish Agriculture. The professionalism of the SASA scientists who expertly managed the transition from APHA to SASA is greatly appreciated. Grateful thanks are also due to SASA staff members who worked with those engaged in rodent pest management to obtain the rodent tissue samples that have greatly increased our understanding of anticoagulant resistance in Scotland.

The authors wish to express sincere appreciation to all those who submitted rodent tissue samples for DNA analysis to permit these resistance maps to be produced. It is quite obvious that without them this study would not be possible.

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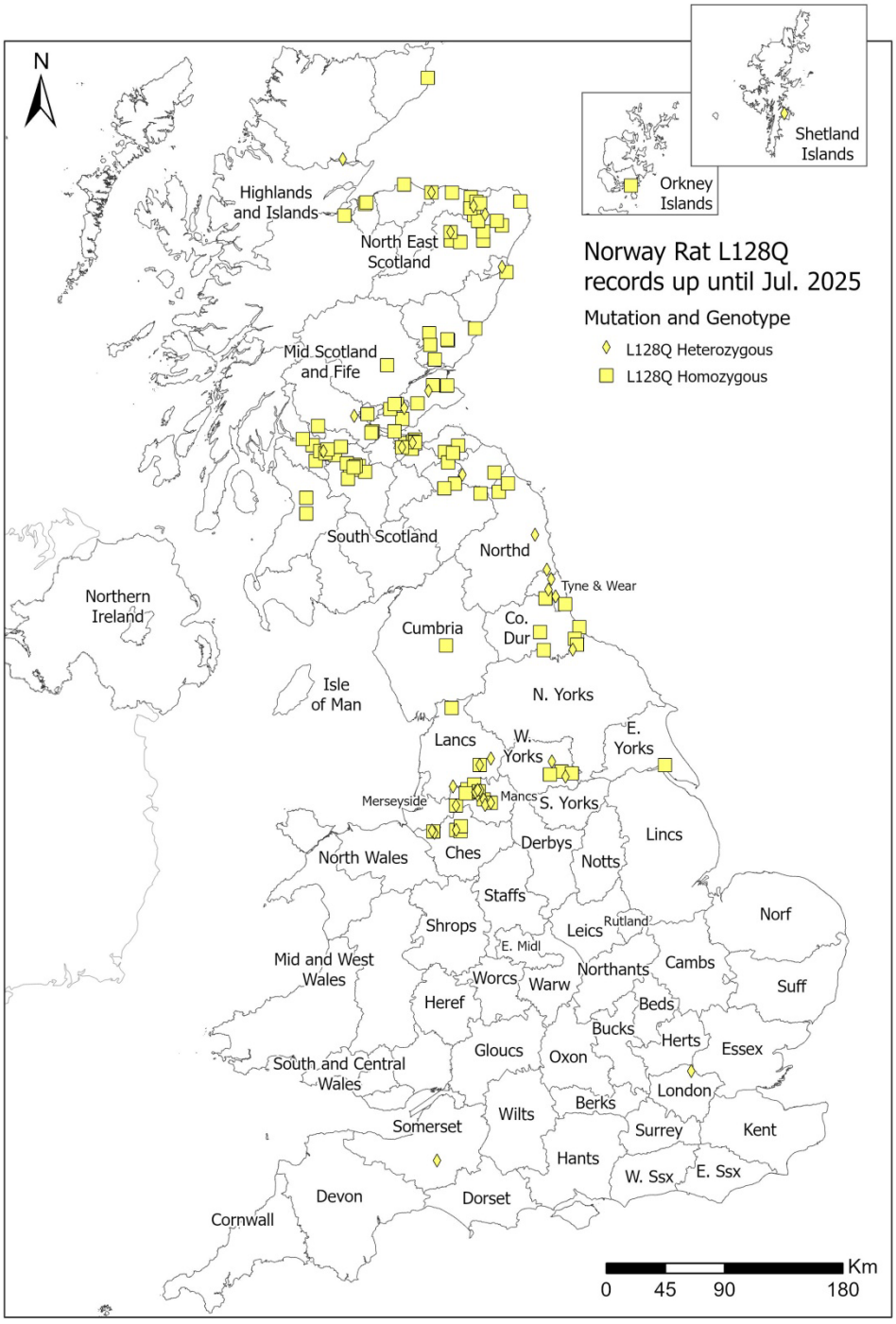
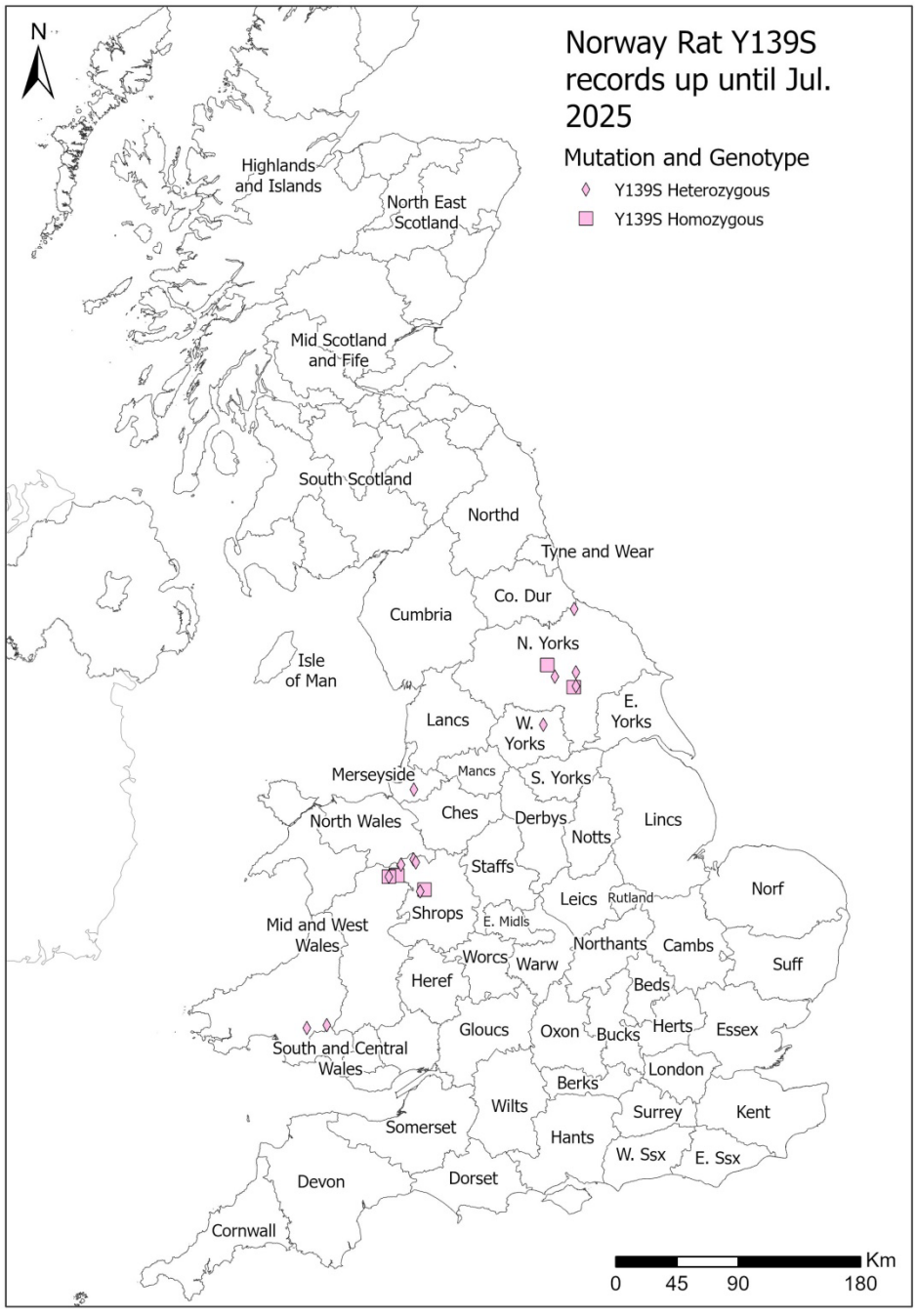
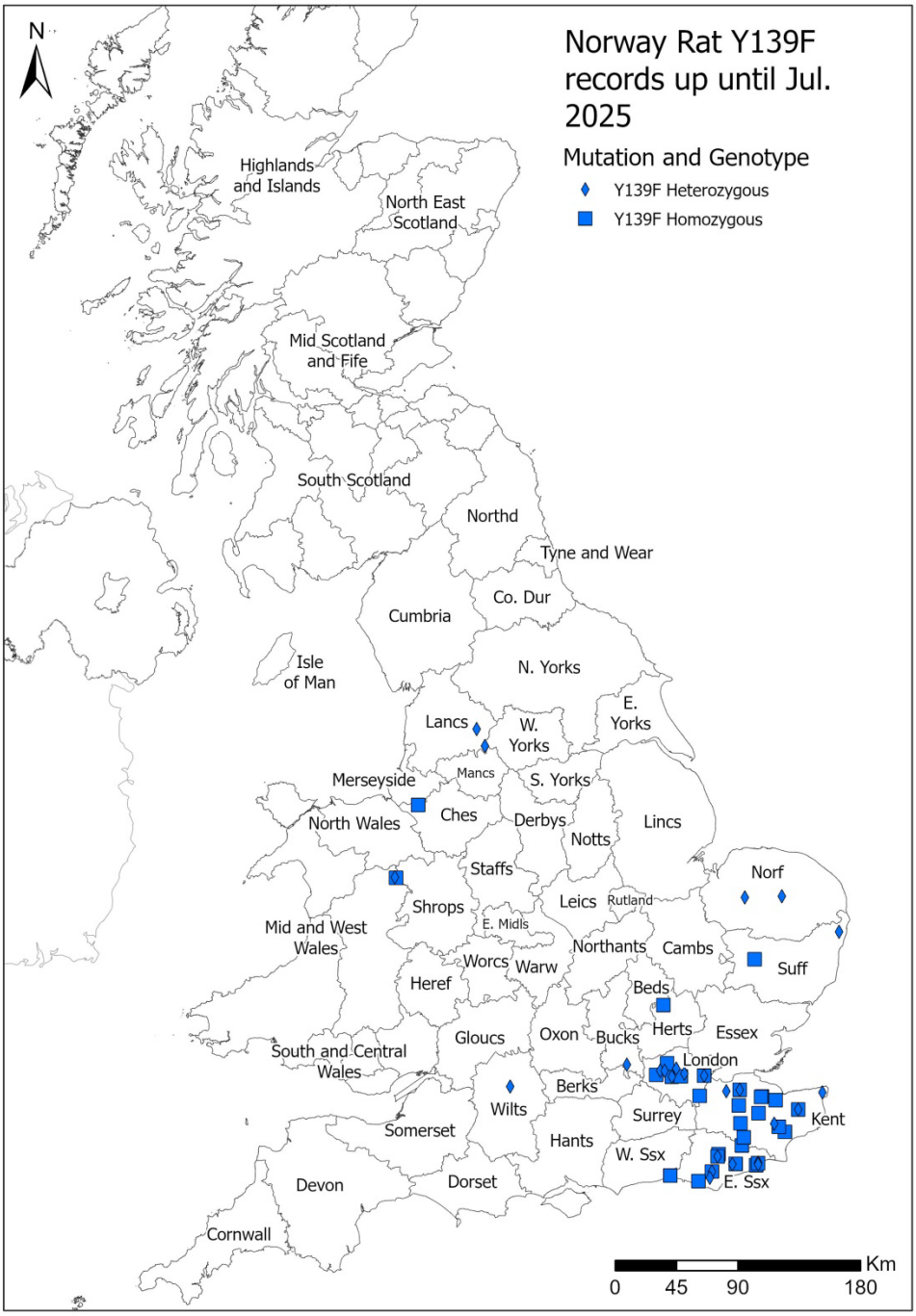
Annex 1. Map showing the geographical locations of Norway rat tissue samples submitted for analysis up to July 2025 which carried the L128Q mutation.



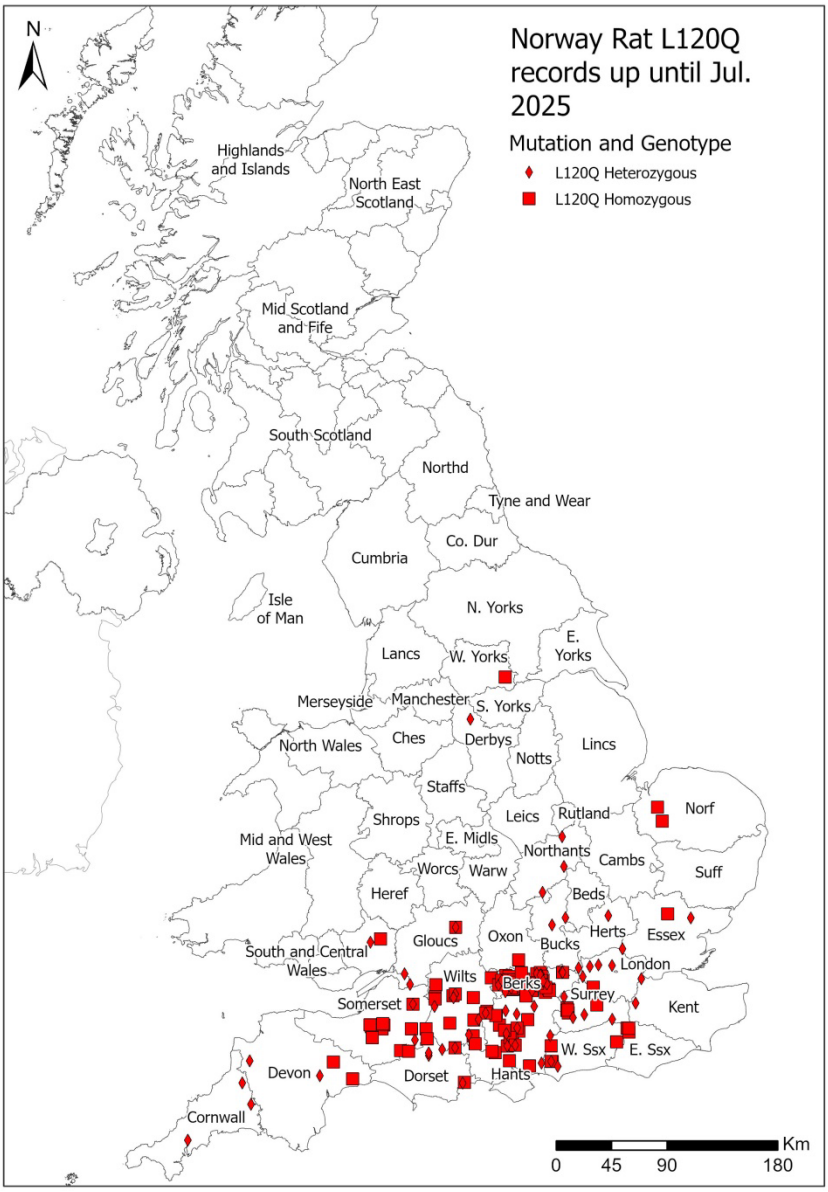
Annex 2. Map showing the geographical locations of Norway rat tissue samples submitted for analysis up to July 2025 which carried the Y139S mutation.



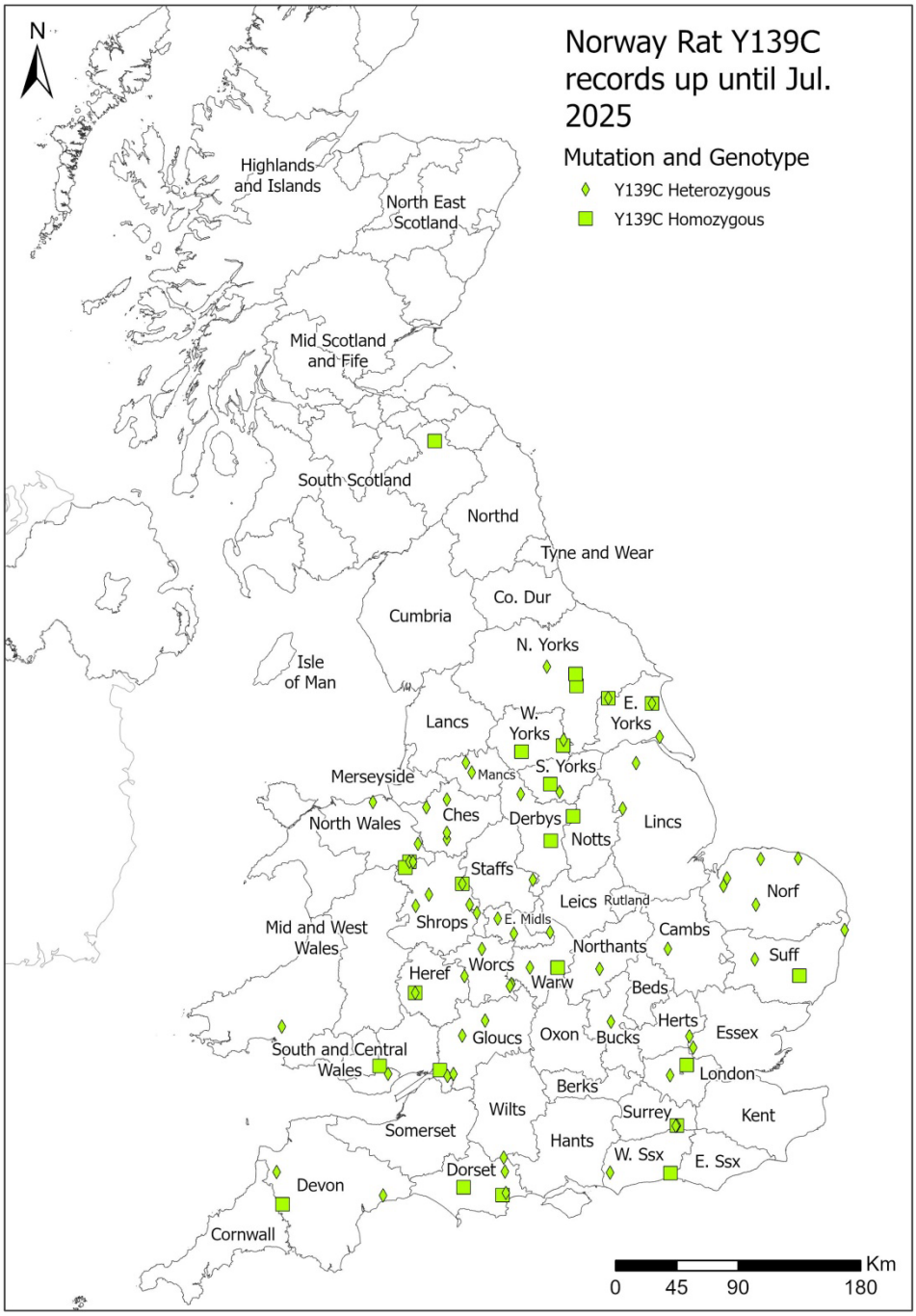
Annex 3. Map showing the geographical locations of Norway rat tissue samples submitted for analysis up to July 2025 which carried the Y139F mutation.



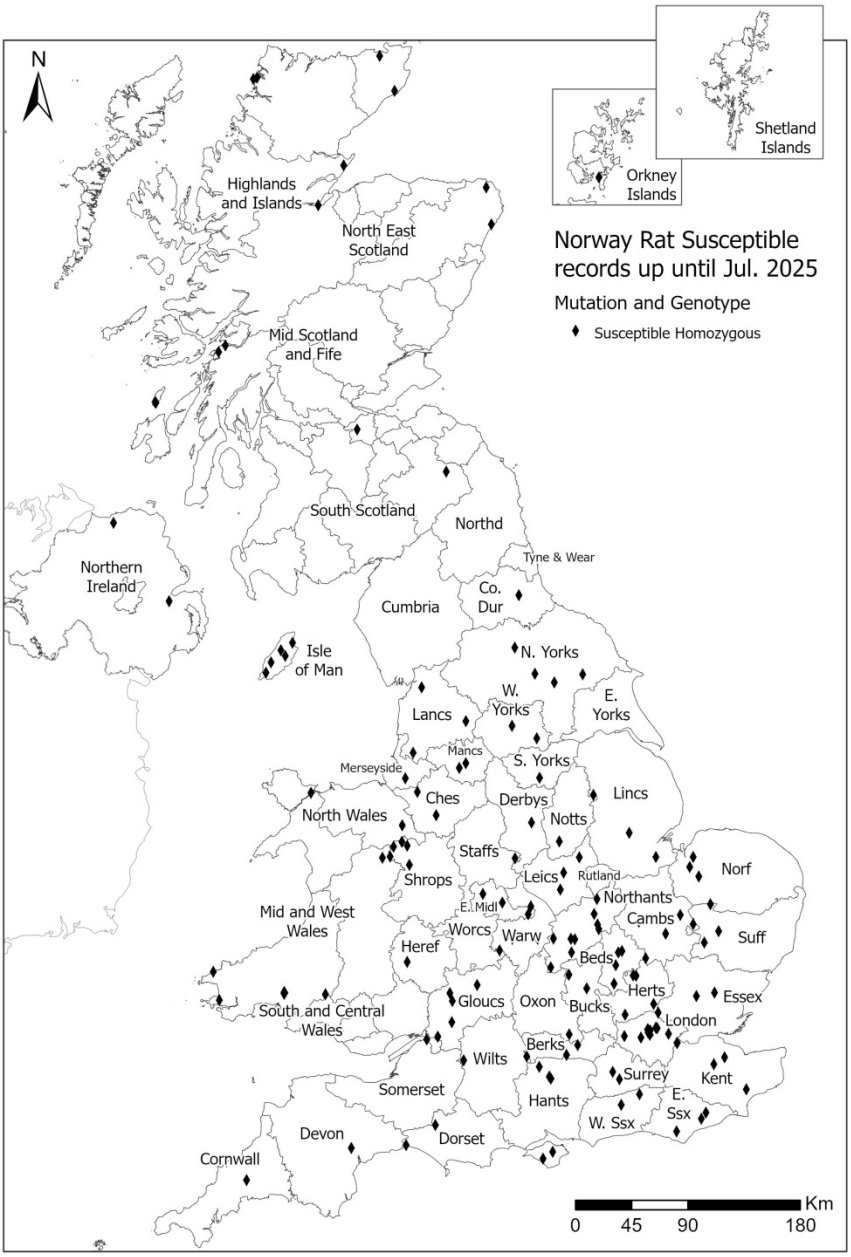
Annex 4. Map showing the geographical locations of Norway rat tissue samples submitted for analysis up to July 2025 which carried the L120Q mutation.



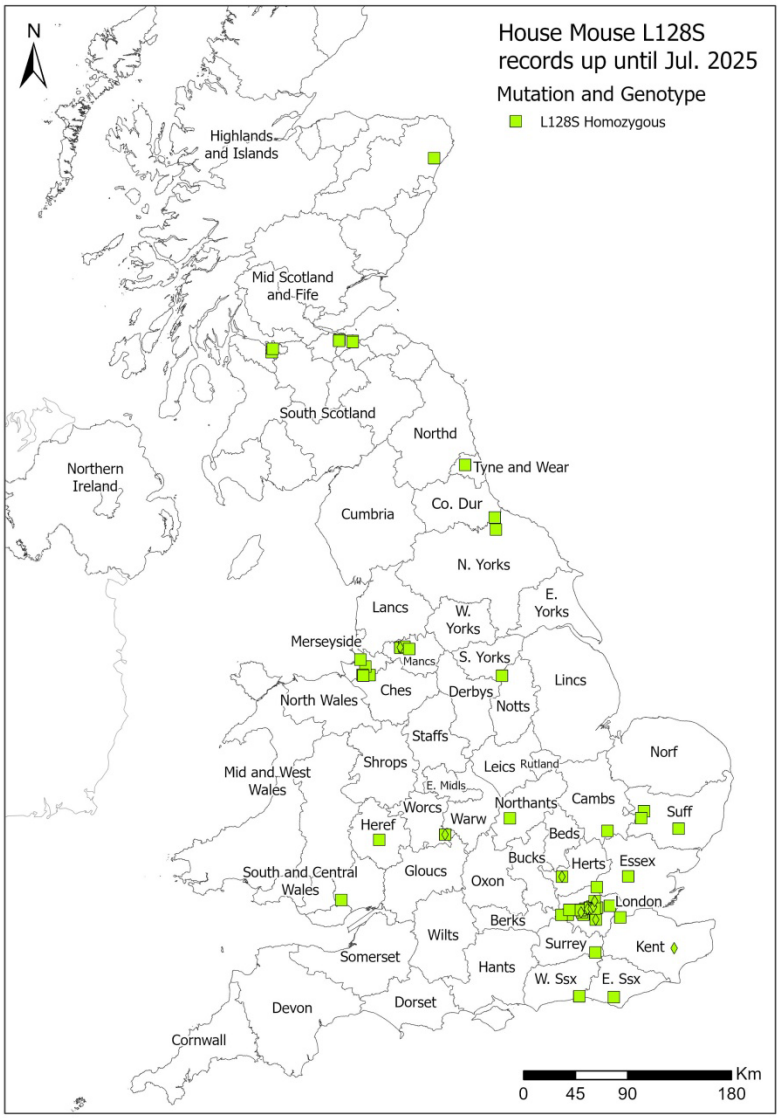
Annex 5. Map showing the geographical locations of Norway rat tissue samples submitted for analysis up to July 2025 which carried the Y139C mutation.



Annex 6. Map showing the geographical locations of Norway rat tissue samples submitted for analysis up to July 2025 which carried the fully susceptible genome.



Annex 7. Map showing the geographical locations of house mouse tissue samples submitted for analysis up to July 2025 which carried the L128S mutation.



Annex 8. Map showing the geographical locations of house mouse tissue samples submitted for analysis up to July 2025which carried the Y139C mutation.