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REPORT SERIES:
VPU/24/001

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Anticoagulant Resistance in Rats and Mice in the UK – new data for August 2023 to July 2024

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Summary

1. Further to earlier reports in this series, a total of 71 rodent tissue samples were received for DNA sequencing at the laboratories of the Animal and Plant Health Agency (APHA) during the period August 2023 to July 2024. Among these, 18 samples did not yield DNA material that could be sequenced. Of the remaining 53 samples, 47 were of Norway rat tissue and 6 were from house mice.
2. There was a substantial decrease in the number of tissue samples sent for analysis in the 2023-24 sampling year, and the target of 100 viable samples was not met. The reasons for this are uncertain, but it may be that the efforts of the Campaign for Responsible Rodenticide Use (CRRU) UK and the Rodenticide Resistance Action Group (RRAG) to disseminate information about the occurrence of resistance may deter sampling from areas where it is now known that anticoagulant resistance is prevalent. Greater effort in the advertisement of the value of these samples to resistance management is required.
3. There was a significant number of samples that could not be sequenced. Once again, the reasons cannot be determined with certainty. However, the most likely cause is the degradation of the DNA material in the samples prior to extraction and sequencing. This is a significant waste of CRRU resources and efforts are required to ensure that only very fresh samples are sent to the laboratory and they spend as little time as possible in transit.
4. Among the 47 Norway rats, 24 were wild type (i.e. fully susceptible) and 23 carried one or more of the well-known resistant mutations (i.e. Y139C, Y139S, Y139F, L128Q, L120Q, and combinations of them). Thus, 48.9% of Norway rats were anticoagulant resistant among the 2023-24 samples. This frequency is lower than that seen in previously reported studies from this project.
5. The SNP that was found most frequent in the sample was Y139C, with 10 individuals carrying this mutation. Among these, 6 were heterozygous and 4 homozygous, with another individual carrying this mutation in combination with L128Q. As in the years immediately preceding this study, Y139C-resistant rats were the most frequent resistant strain in the samples. The geographical distribution of the Y139C mutation in the UK has no central focus, unlike the other resistance mutations, and is found virtually anywhere in England south of a line joining the estuaries of the Mersey and Tees.
6. The numbers of Norway rats carrying the other mutations were as follows: Y139F, three; L120Q, three and L128Q, six. Among these, two L120Q animals were recorded on the western periphery of the known range of this mutation, but all Y139F and L128Q samples were found within their known ranges. No rats carrying the Y139S mutation were found in this sample.
7. Hybrid resistance was again found in the sample, with a single rat carrying both the L128Q and Y139C mutations recorded from East Yorkshire.
8. Norway rats carrying the fully susceptible genome were recorded from widely separated sites in England and Wales. Samples were also received for the first time from Northern Ireland and all proved to be from fully susceptible animals. It is known that anticoagulant resistance is not well-established on the island of Ireland.
9. Only six viable house mouse samples were obtained and all but one was found to be anticoagulant resistant. Three were homozygous for the L128S mutation, two homozygous for the Y139C mutation and one was fully susceptible. It is the position of the Rodenticide Resistance Action Group (RRAG) that, such is the prevalence of resistance among UK house mouse infestations, all should be assumed to carry resistance and treatments should be conducted against them accordingly.

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10. During the period 2009 and 2024, in which DNA resistance sequencing has been conducted, first at the University of Reading and now at APHA, a total of 631 Norway rat and 140 house mouse tissue samples have been examined, with DNA extracted and sequenced. Among these samples we found that 75.2% of rats and 94.4% of mice carried one or more single nucleotide polymorphisms which are known significantly to affect the efficacy of anticoagulant rodenticides. These results may not reflect the true frequency of resistance in the two species, however, because samples are generally sent by those experiencing difficulties in obtaining control of rodent infestations with anticoagulants.
11. Increasing numbers of samples permit the geographical distribution of resistance in Norway rats in the UK to be determined. L128Q is largely restricted to Scotland and the north of England. Y139S is found mainly in Wales, on the Anglo-Welsh border and in an expanding focus in North Yorkshire. L120Q is very widespread across central southern England, but is found with increasing frequency in East Anglia, the far south-west and elsewhere. Y139F is found mainly in Kent, East Sussex and Greater London, but now with established foci in the north-west and East Anglia.
12. Particularly with regard to the three most severe Norway rat mutations, namely L120Q, Y139F and Y139C, outlying resistant foci occur with increasing frequency almost anywhere in England, such as the one found in last year in northern Derbyshire. These are disseminated either by natural rodent movement or by human transportation systems.
13. Although, there remains evidence of areas of remnant susceptibility in some counties of the eastern and south Midlands and on the north-east coast, these areas are now increasingly infiltrated by resistance. Elsewhere, susceptible Norway rats coexist with resistant rats.
14. The maps of Norway rat and house mouse resistance foci presented in this report permit reasonably fine-grained advice to be given to rodenticide users about which interventions to use and which to avoid, following recommendations of the RRAG. Implementation of that advice would: 1) facilitate faster and more effective rodent control for the better protection of human and animal health, 2) prevent the increasing severity and spread of anticoagulant resistance, and 3) (and of great importance to the objectives of the CRRU UK and rodenticide stewardship,) reduce unnecessary and ineffective emissions of anticoagulants into wildlife and the wider environment.
15. 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 downloaded onto mobile devices (https://rrac.info/index.html).

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1. Introduction

The Campaign for Responsible Rodenticide Use (CRRU) UK is responsible for the co-ordination and operation of the UK Rodenticide Stewardship Regime (Buckle et al., 2017) and it is a requirement of the Health and Safety Executive (HSE), and the Government Oversight Group (GOG), that CRRU provides information on anticoagulant resistance among UK populations of Norway rats (*Rattus norvegicus*) and house mice (*Mus musculus*) in the UK (HSE, 2023). Reports are produced annually based on DNA sequencing and analysis of tissue samples submitted, mainly by professional pest control technicians. The last in this sequence of reports was provided by CRRU[1] in 2023 and summarised all data available up to July 2023 (Buckle et al., 2023). The present report provides additional data on the incidence of anticoagulant resistance in rats and mice covering the period August 2023 to July 2024, and summarises previous work.

Anticoagulant resistance in Norway rats and house mice in the UK is of interest to those who engage in rodent pest management for human and animal health and hygiene. Consequently, the Rodenticide Resistance Action Group of the UK, a voluntary panel of resistance experts from academia, government, industry and trade bodies, publishes guidance booklets for both house mice (Buckle et al., 2021a) and Norway rats (Buckle et al., 2021b). This guidance may have had a significant effect on the use of second-generation anticoagulant rodenticides (SGARs) in the UK revealed in another monitoring element of the stewardship regime in which barn owl livers are collected and tested for SGAR residues (Ozaki et al., 2022). These studies have revealed a significant recent reduction in both the incidence and concentration of residues of the commonly resisted SGARs bromadiolone and difenacoum and an increase in residues of the resistance-breaking brodifacoum and difethialone.

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

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2. Materials and Methods

2.1 Origins of samples

The tissue samples analysed for genetical mutations were either submitted by pest control technicians, were 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. Thus, samples were generally received from areas in which 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 is the same as in previous reports. Genetical material was obtained from the field in the form of either tail tip samples or fresh droppings. 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 Qiagen DNeasy tissue extraction kit following the manufacturer's recommendations (Qiagen Ltd., Crawley, West Sussex, UK). Briefly, a small quantity of tissue (approximately 3mm x 2mm x 2mm) was shaved from each tail using a sterile sharp razor blade, and then placed in a 1.5ml microtube. Pre-warmed extraction buffer ATL (180 µl) was added, followed by 20 µl of proteinase K. The mixture was vortexed and incubated at 55˚C on a rocking platform overnight (approx. 17 h). Genomic DNA was then purified and eluted from spin-purification columns in 80 µl of elution buffer and the quality and yield were assessed spectrophotometrically using a nano-drop instrument.

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 purified using the QIAquick PCR purification kit (Qiagen Ltd., Crawley, West Sussex, UK). Product samples (3.5µl) were then sequenced with BigDye version 3.1 terminator chemistry (ABI) on a 9700 ABI thermal cycler, and the terminated products were resolved on an ABI 3130XL capillary sequencer. The sequence trace files were visually analysed and any ambiguous bases were edited using the DNASTAR Lasergene software. The sequence alignments were compiled using ClustalW2.

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.

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Table 1. The main VKORC1 mutations in Norway rats (NR) and House mouse (HM) in UK mentioned in this report.

Species	**Mutation**	**Abbreviations**	**Where present**
NR	**Leu**cine128**Glu**tamine	L128Q†	Central Southern Scotland, Yorkshire, Lancashire, and elsewhere
NR	**Tyr**osine139**Ser**ine	Y139S†	Anglo-Welsh border, North Yorkshire
NR	**Leu**cine120**Glu**tamine	L120Q†	Hampshire, Berkshire, Essex, Norfolk and elsewhere
NR	**Tyr**osine139**Cys**teine	Y139C†	Gloucestershire, Norfolk, Lincolnshire, Yorkshire, SW Scotland and elsewhere
NR	**Tyr**osine139**Phe**nylalanine	Y139F†	Kent, Sussex, Norfolk, Suffolk and elsewhere
HM	**Tyr**osine139**Cys**teine	Y139C†	Many southern England
HM	**Leu**cine128**Ser**ine	L128S†	Ubiquitous

† Known either from field experiments and/or field experience to have a significant practical effect on anticoagulant efficacy

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 APHA and University of Reading) 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.

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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'.

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3. Results

3.1 Norway rats – historical records

This longitudinal study has operated at the University of Reading, and later at the laboratories of the Animal and Plant Health Agency, during the period 2009 to July 2024. In that period a total of 631 Norway rat tissue samples from around the UK have been studied using the DNA sequencing technique (Figure 1). Of these, 474 (75.2%) were found to possess one or more of the resistance mutations that are known to have a significant effect on anticoagulant rodenticide efficacy (Buckle, 2013). The remaining 157 animals (24.9%) carried the wild type genome. Maps showing the geographical locations from which these samples were sent have been presented previously (Prescott et. al., 2018; Buckle et al., 2020a, 2023) and are also 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.

3.2 Norway rats – records for 2023-2024 and frequency of resistance

Among the 47 samples (Table 2) that were capable of being sequenced in the period August 2023 to July 2024, a total of 23 (48.9%) were found to carry one of the five main Norway rat anticoagulant resistance mutations (Table 1). The remaining 24 animals (51.1%) carried the wild type genome (see Figure 2). The proportion of resistant Norway rats in the sample differed appreciably from that found in previous surveys (i.e. 2020, 74.1%; 2021-2022, 74.1%). The reasons for this are unknown but it is more likely to be the result of sampling bias than an actual decrease in the incidence of resistance among UK Norway rats.

A single animal found on the western outskirts of Kingston upon Hull possessed both the L128Q and Y139C mutations (Figures 2 and 3). Hybrid resistance had been found in previous surveys in many localities.

Among those rats that carried a single SNP, the severe Y139C mutation was the most common (21.4%, n=10) in the 2023-24 sample. The numbers of resistant samples carrying L120Q, which had previously predominated, declined to just 6.4% of those recorded (n = 3). It is likely that this is because this resistance is well known in central southern England and practitioners are no longer minded to submit samples. Two of the three L120Q records were towards the likely extreme western edge of the focus and may indicate continuing westward spread (Figure 2).

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

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Table 2. The numbers of Norway rats tissue samples received and analysed in 2023/24, 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	2	1	3
L128Q	4	2	6
Y139C	4	6	10
Y139F	2	1	3
Y139S	0	0	0
Totals (mutations)	12	10	22
L128Q and Y139C*	0	1	1
L120Q and Y139C*	0	0	0
Totals (hybrid resistance)	0	0	1
Susceptible Total (susceptible)	24	-	24

*These four animals were heterozygous for each of two the resistance mutations. Each of these mutations is also counted separately in the records above.

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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 is also included. Records for 2009-2024.

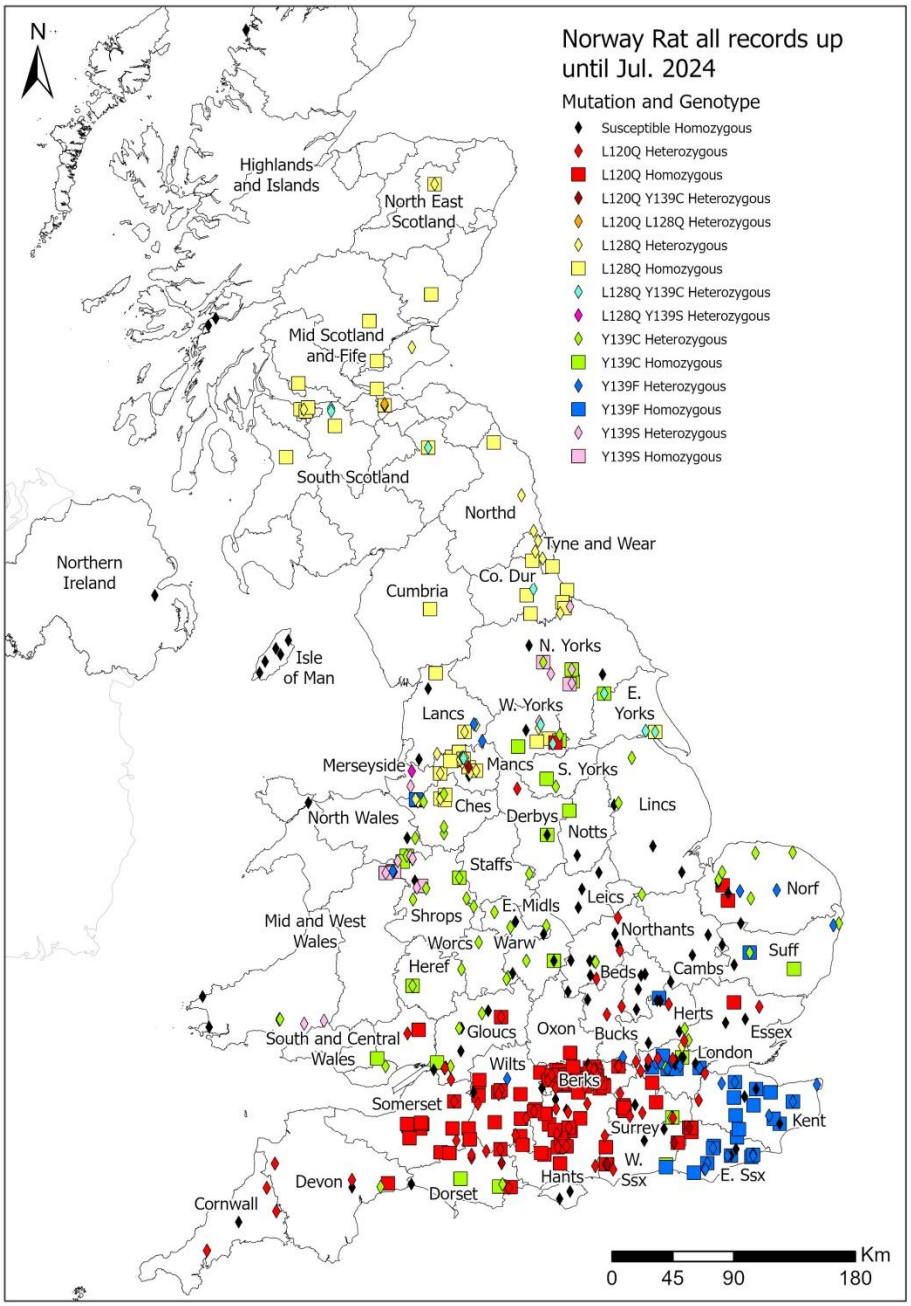

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Figure 2. July 2023.Geographical locations of all new Norway rat records for the period August 2023 to July 2024

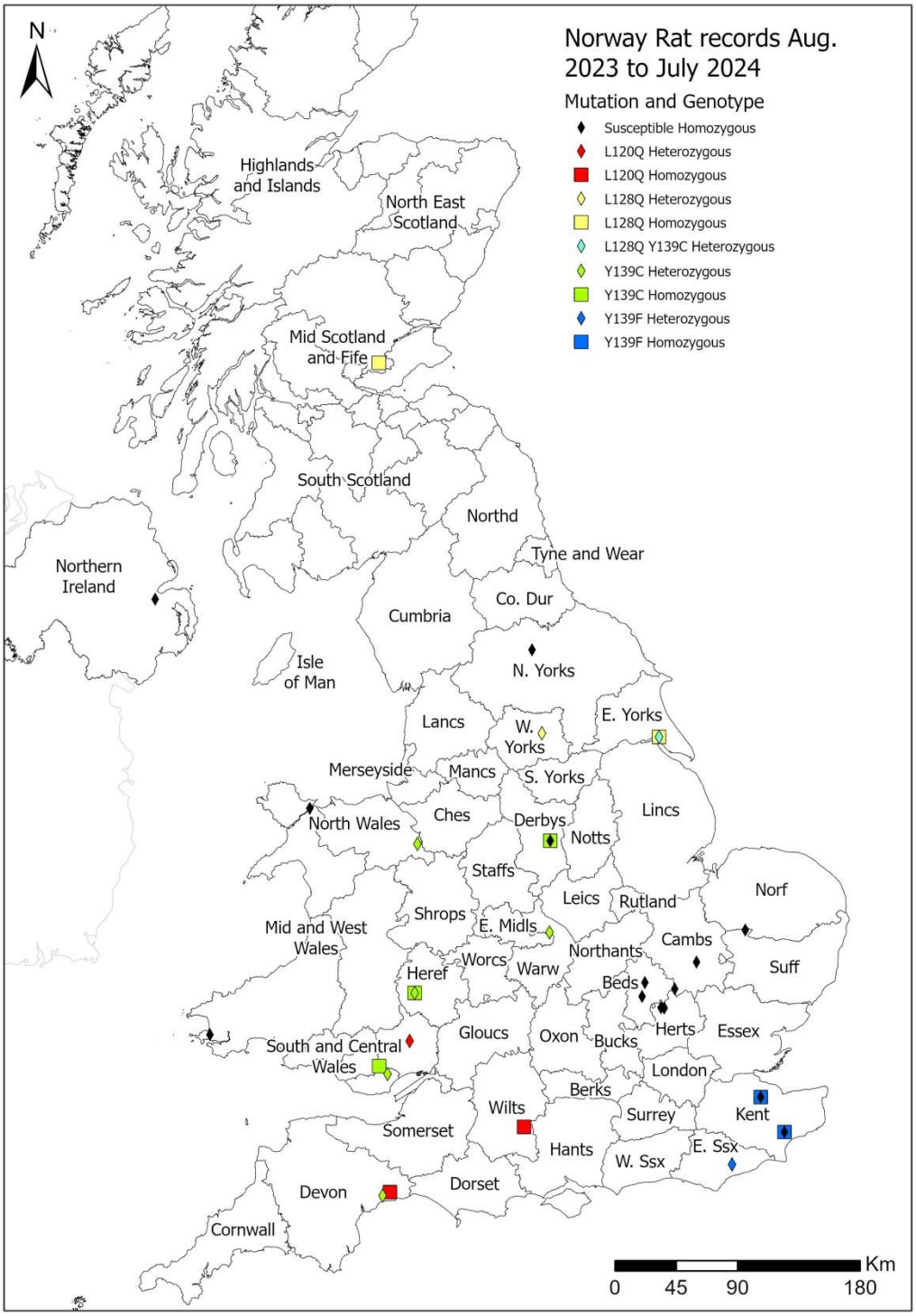

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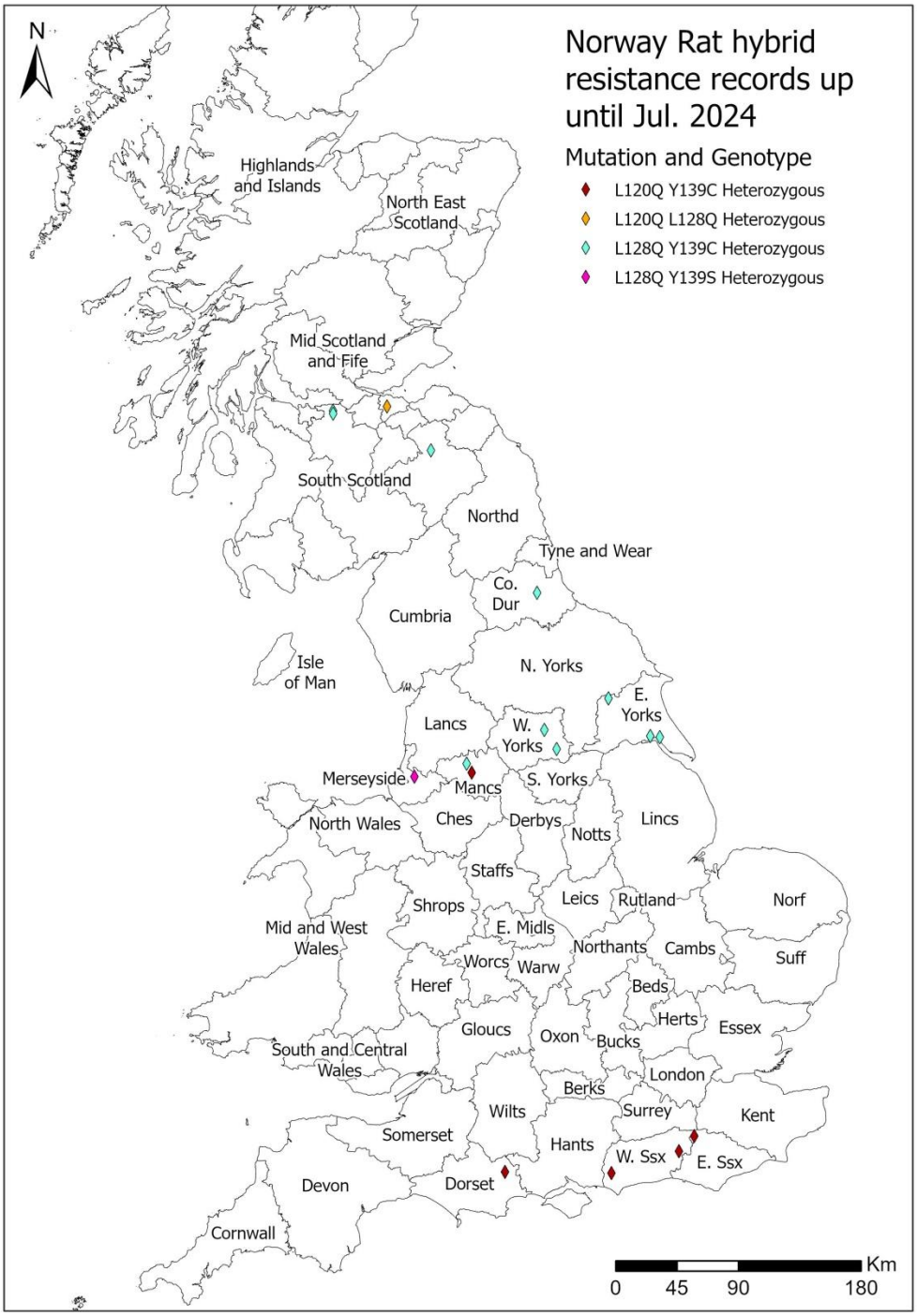
Fig. 3. Map showing all Norway rats found to carry two different anticoagulant resistance SNPs (i.e. hybrid resistance). Records for 2009 to 2024.



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3.3 House mice

Samples of house mouse tissue are received much less frequently that those of Norway rats and that continued to occur in our sample for 2023-24, with only six received. Various hypotheses were put forward to explain this in a previous report (Buckle et al., 2022) to which can be added another. The advice of the Rodenticide Resistance Action Group (Buckle et al., 2021a) is that practitioners should assume all house mice in the UK carry one or more resistance SNPs. This would provide a significant disincentive to submit samples if it is to be assumed that all mice are resistant. This is regrettable because information on the spread of the highly resistant hybrid Y139C/L128S mice is still very much needed.

Whatever the reason for these relatively small numbers, a total of 140 mouse tissue samples has now been received, and this is one of the largest resistance surveys of house mouse resistance ever conducted. In this survey, animals were found to carry both common UK mouse resistance SNPs, Y139C and L128S (see Table 1), with a small but significant number of mice that were hybrid resistant, carrying both mutations. These mice occur especially in London and were mentioned in more detail in the previous report, as was the newly-found *spretus* mutation (Buckle et al., 2022).

Maps of the distribution of house mouse resistance shows that the Y139C mutation is largely restricted to the south-east of England, although there have been findings of this mutation among mice in Scotland, while L128S is more ubiquitous (Figure 4). Within the total sample of 140 individuals, 132 house mice carried one or both common SNPs, giving a very high frequency of resistance among UK house mice of 94.4%. This frequency of resistance has led the Rodenticide Resistance Action Group to make the recommendation (mentioned above) that those who use anticoagulants against house mice should assume all infestations to be resistant.

Six house mouse tissue samples were received in the period August 2023 to July 2024 and among them five samples were homozygous-resistant. Three mice carried the L128S SNP in homozygous form, one each from Edinburgh, Liverpool and Dagenham in Greater London. Two house mice carried the Y139C mutation in homozygous form, both from Willesden in Greater London.

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

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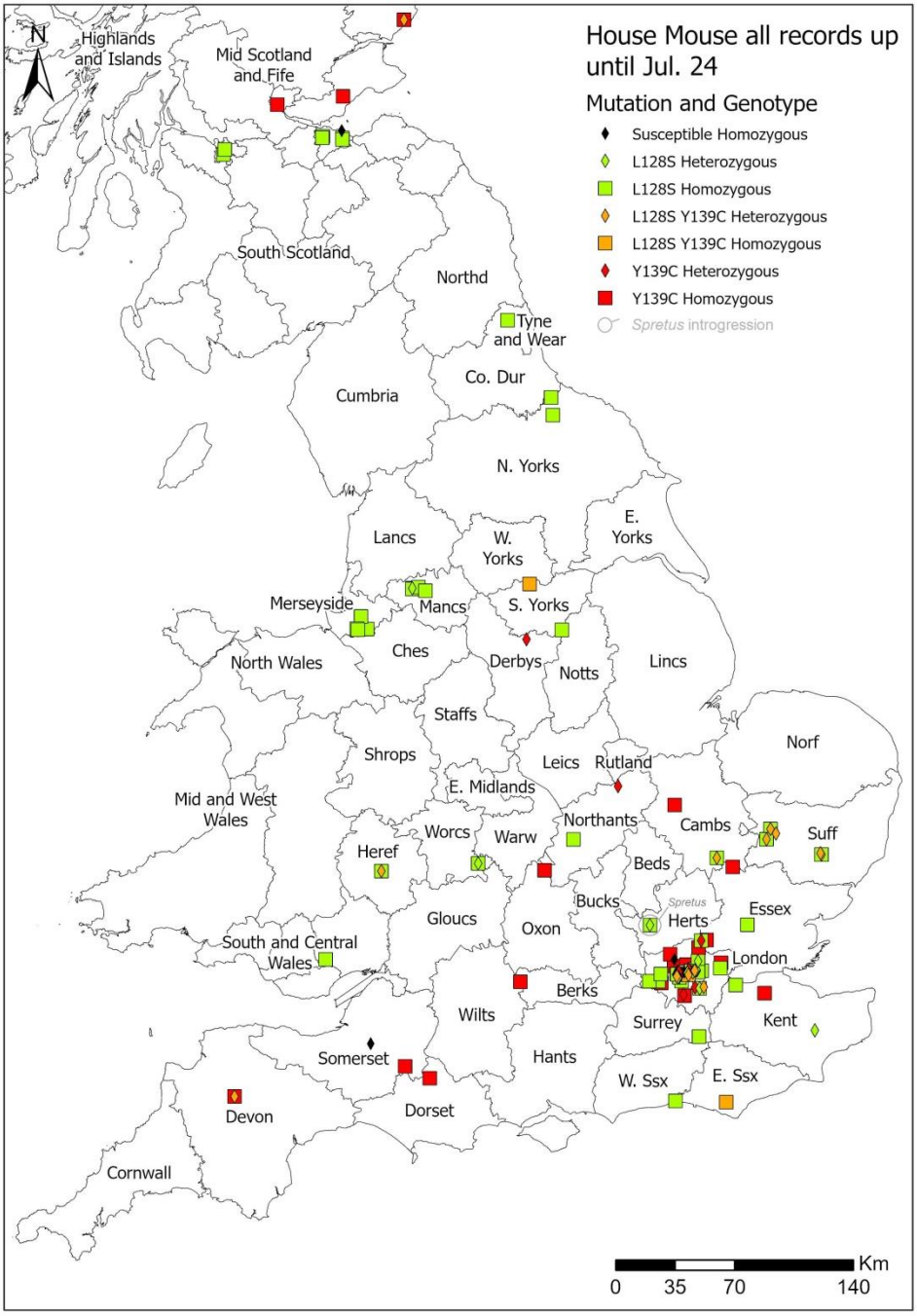
Fig. 4. Consolidated map showing all house mice found to carry an anticoagulant resistance SNP, both in homozygous and heterozygous form, for any of the three resistance mutations found in that species, and for combinations of them (i.e. hybrid resistance). Records for 2009 to 2024. (The Hertfordshire focus of the spretus introgression is obscured by other overlaying resistance records at the same site.)



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4. Discussion

The results of resistance testing, conducted in the period August 2023 to July 2024 reported here, support previously published findings on the geographical distribution and incidence of anticoagulant resistance in UK Norway rat and house mouse populations (e.g. Prescott et al., 2018; Buckle et al., 2023). Although the frequency of resistance in the small sample of house mice reported here (83.3%, n = 6) is broadly in line with that found in previous studies, the frequency of resistance in Norway rats was somewhat lower than seen before; only 23 out of 47 (48.9%) were resistant.

Before going on to discuss the incidence and distribution of resistance in UK Norway rats and house mice it is useful to consider two general aspects of the present survey. The annual target tissue sample size is 100, although it has been recognised that more samples would be valuable. Only 71 samples, 57 of Norway rats and 14 of house mice, were received in the period August 2023 to July 2024 and this is the lowest total in recent years. This substantial decrease in the numbers of samples submitted is something that must be addressed if we are to continue to expand our knowledge of anticoagulant resistance in the UK. Of course, samples from areas from which they have not been previously received would be the most useful. Figure 1 shows these are in the north of England, across the whole of Scotland, much of the eastern side of England, the far south-west of England and all of Wales. Nevertheless, all samples are useful, especially from areas where there may be an interface between two spreading foci harbouring different resistance mutations. Such samples would provide useful information on the spread of hybrid resistance. A second consideration is that, among the 71 samples received, 15 could not be sequenced. This was particularly apparent among mice; eight of the 14 samples received did not yield viable DNA. It is also important to address this, either by improvement if feasible in the extraction of DNA or by a request to those who submit samples only to send fresh samples and to ensure that their transportation to the laboratory is expedited. The combination of these two factors resulted in only 56 new DNA records.

Because of the relatively small number of tissue samples from which DNA could be extracted, limited additional information has been added to our understanding of the incidence and geographical distribution of anticoagulant resistance among UK Norway rats and house mice.

The continuing proliferation of the Y139C mutation in Norway rats in recent sampling periods is readily apparent and in 2023-24 the Y139C mutation was, once again, the most commonly found. A total of 70 Norway rats tested positive for this SNP in samples received in the period 2021 to July 2024, and it is apparent from the map showing the distribution of this mutation across England and Wales that it might now be expected to be found almost anywhere (Annex 5). No certain cause can be attributed to the apparent spread of Y139C other than the fact that it is one of the most severe mutations in the UK and, generally, the continuing use of resisted substances against resistant rats will, of course, result in the spread of resistance (Greaves, 1994).

The distribution of L120Q in central-southern England is well-established and few samples are now received from the centre of the focus. This may be because this phenomenon is so well publicised, and therefore so well known, that practitioners are no longer motivated to submit samples and because active substances are used that are fully effective against L120Q rats. However, samples are still received from areas where users are probably encountering this severe resistance for the first time. Thus, in the current sampling period, tissue samples from L120Q-resistant rats were received from Exeter and Caerphilly.

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The latest survey confirms the very widespread occurrence of anticoagulant resistance in UK Norway rat and house mouse infestations (Figures 1 and 3). Among Norway rats, the most severe resistance SNPs, L120Q, Y139C and Y139F, are particularly abundant in southern parts of England. Indeed, the most severe SNP, L120Q, is prevalent across the entire area of central-southern England. Given existing advice from RRAG about the use of SGARs against these resistances (Buckle et al., 2021a), practitioners choosing to employ biocides against Norway rats in an area south of a line from the Thames to the Severn estuaries, would be prudent only to use either non-anticoagulants, such as cholecalciferol, or the effective SGAR substances brodifacoum, difethialone and flocoumafen.

At the other end of the country, although sampling is much less intensive, the less severe SNPs, L128Q and Y139S appear to predominate in both northern England and Scotland. Thus, practitioners working in an area north of a line joining the Humber and Mersey estuaries might expect all SGARs to be effective and the principle of the risk hierarchy (CRRU, 2024) would lead them to use mainly bromadiolone and difenacoum, although vigilance is needed because more severe SNPs are certainly increasingly present.

The regions between those two boundaries appear either to be dominated by the severe SNP, Y139C, or to harbour mainly fully susceptible Norway rats. Once again, RRAG guidance would require the use of brodifacoum, difethialone and flocoumafen among the SGAR substances. Although samples are scarce, susceptibility seems to predominate in some counties of the eastern and southern Midlands and in Lincolnshire. However, once again, vigilance is needed because there is increasing infiltration of the L120Q and Y139F SNPs from the south and east of England.

For the first time, a sample of four Norway rat tissues was available for extraction and sequencing, submitted from Belfast. All of the animals were fully anticoagulant-susceptible and this supports previous published records of the relative lack of anticoagulant resistance among Norway rats on the island of Ireland (Mooney et al., 2018).

It is valuable to reiterate here that the continued use of anticoagulants against rodent populations that are resistant to them has three 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. These may be taken subsequently 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.

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5. Acknowledgements

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.

It is with regret that we acknowledge that, because of other pressures on its resources, it is no longer possible to continue this work at the laboratories of the Animal and Plant Health Agency. Colleagues at The University of Reading and CRRU UK wish to express their grateful thanks to the APHA staff members who have contributed so much to this work over the last five years.

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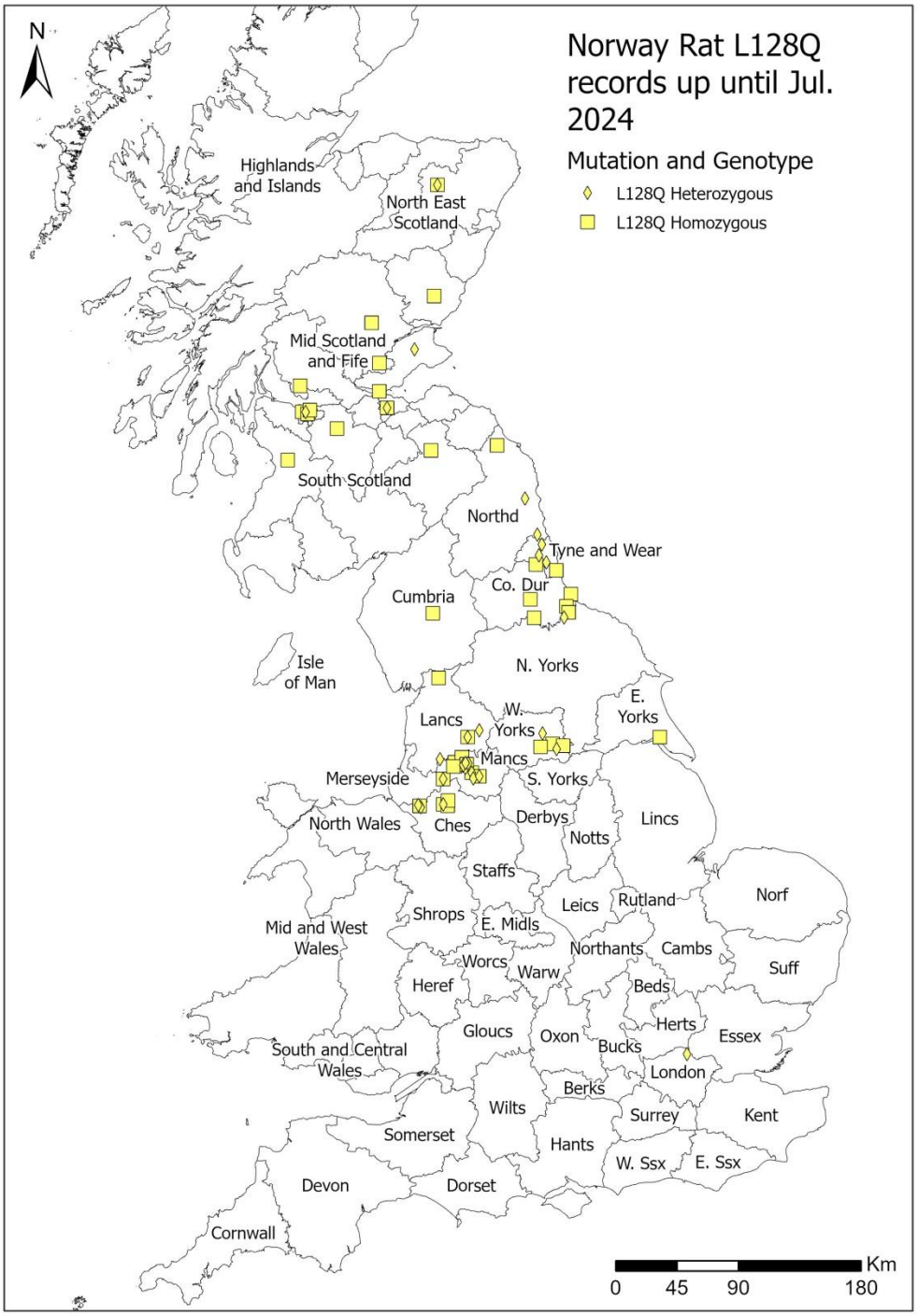
Annex 1. Map showing the geographical locations of Norway rat tissue samples submitted for analysis up to July 2024 which carried the L128Q mutation.



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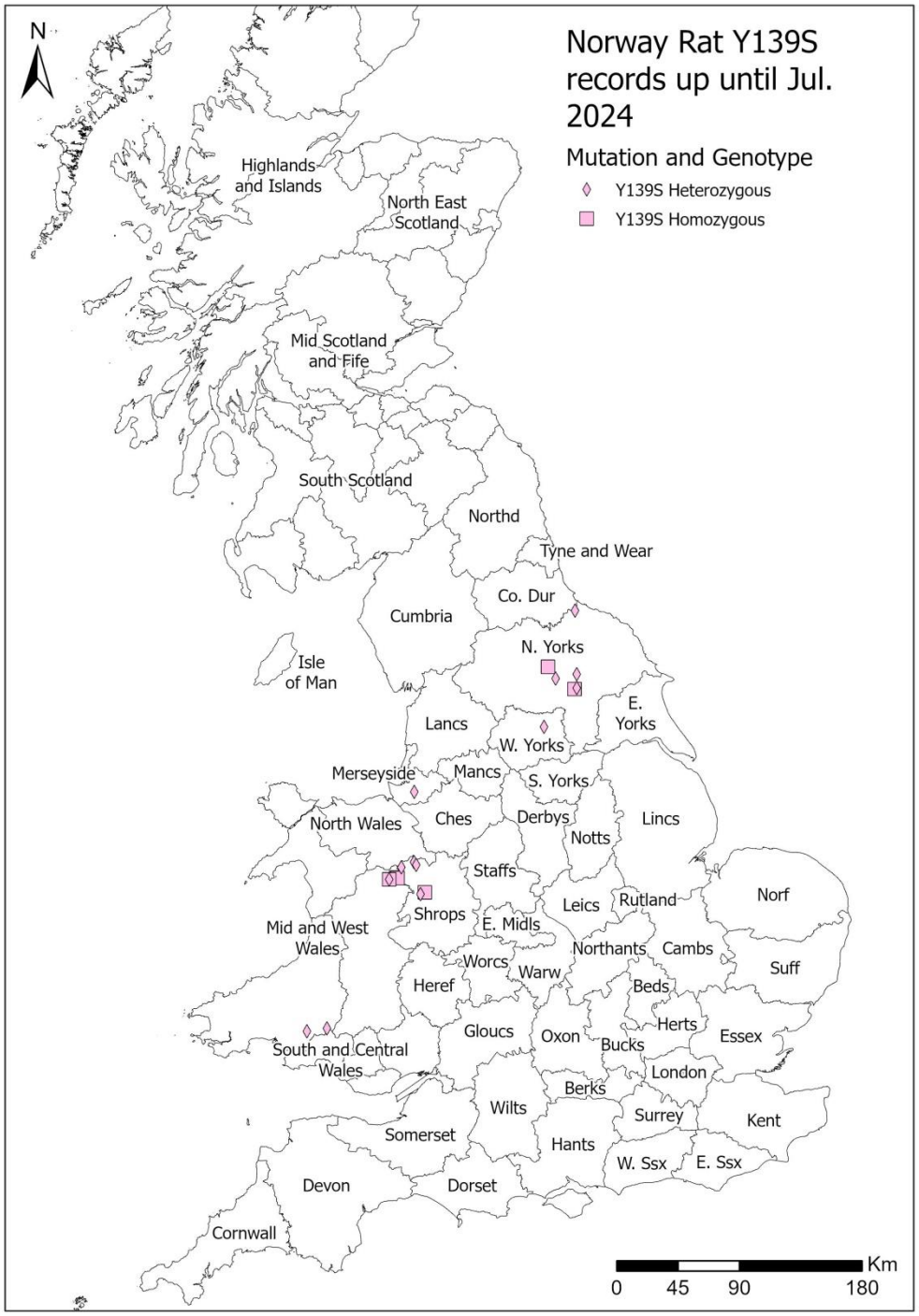
Annex 2. Map showing the geographical locations of Norway rat tissue samples submitted for analysis up to July 2024 which carried the Y139S mutation.



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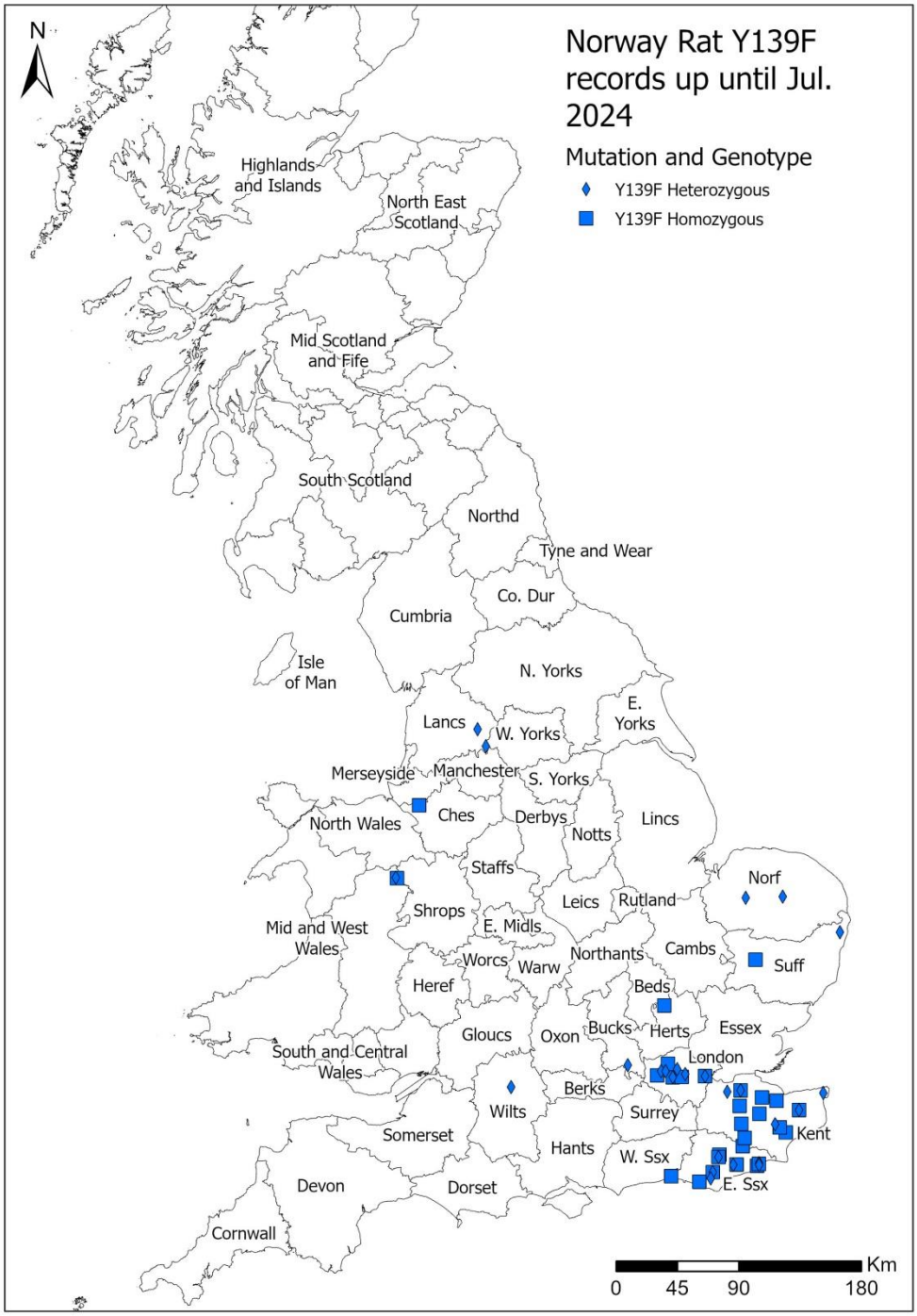
Annex 3. Map showing the geographical locations of Norway rat tissue samples submitted for analysis up to July 2024 which carried the Y139F mutation.



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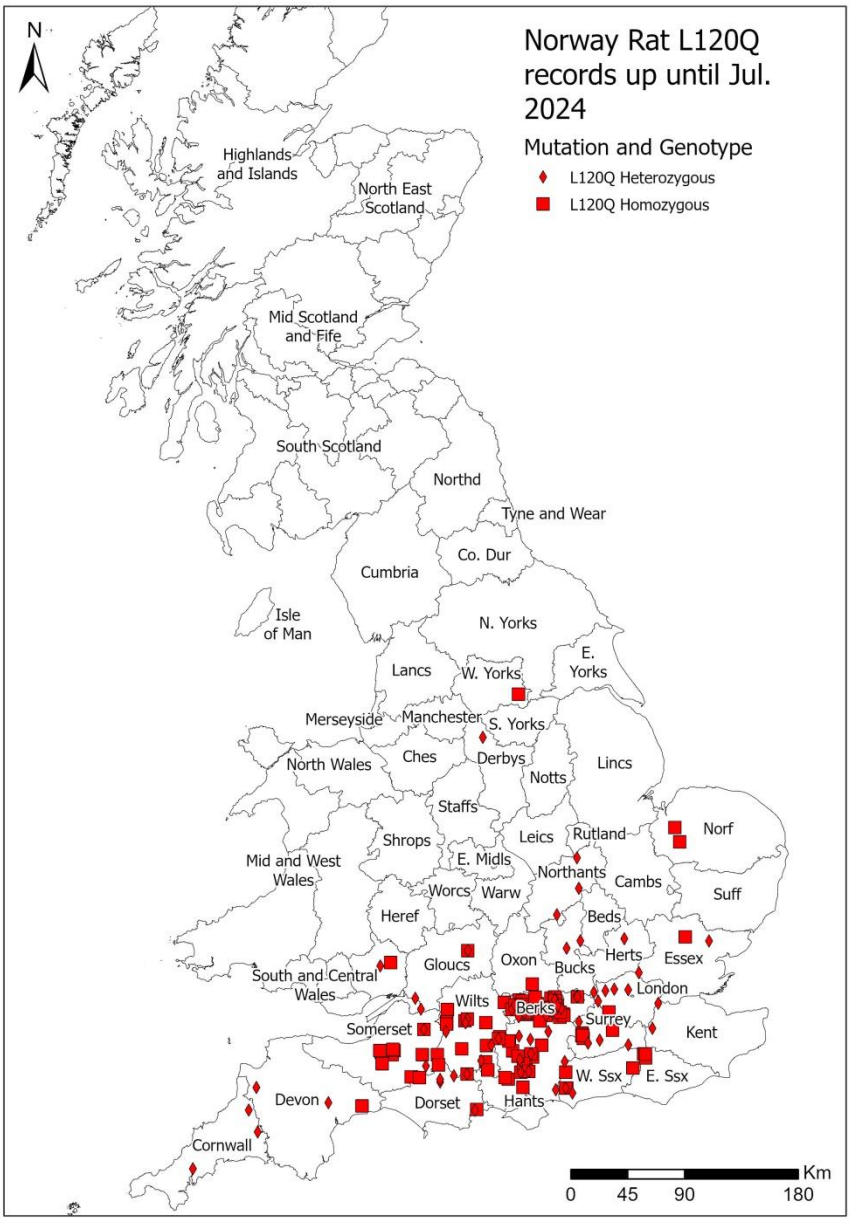
Annex 4. Map showing the geographical locations of Norway rat tissue samples submitted for analysis up to July 2024 which carried the L120Q mutation.



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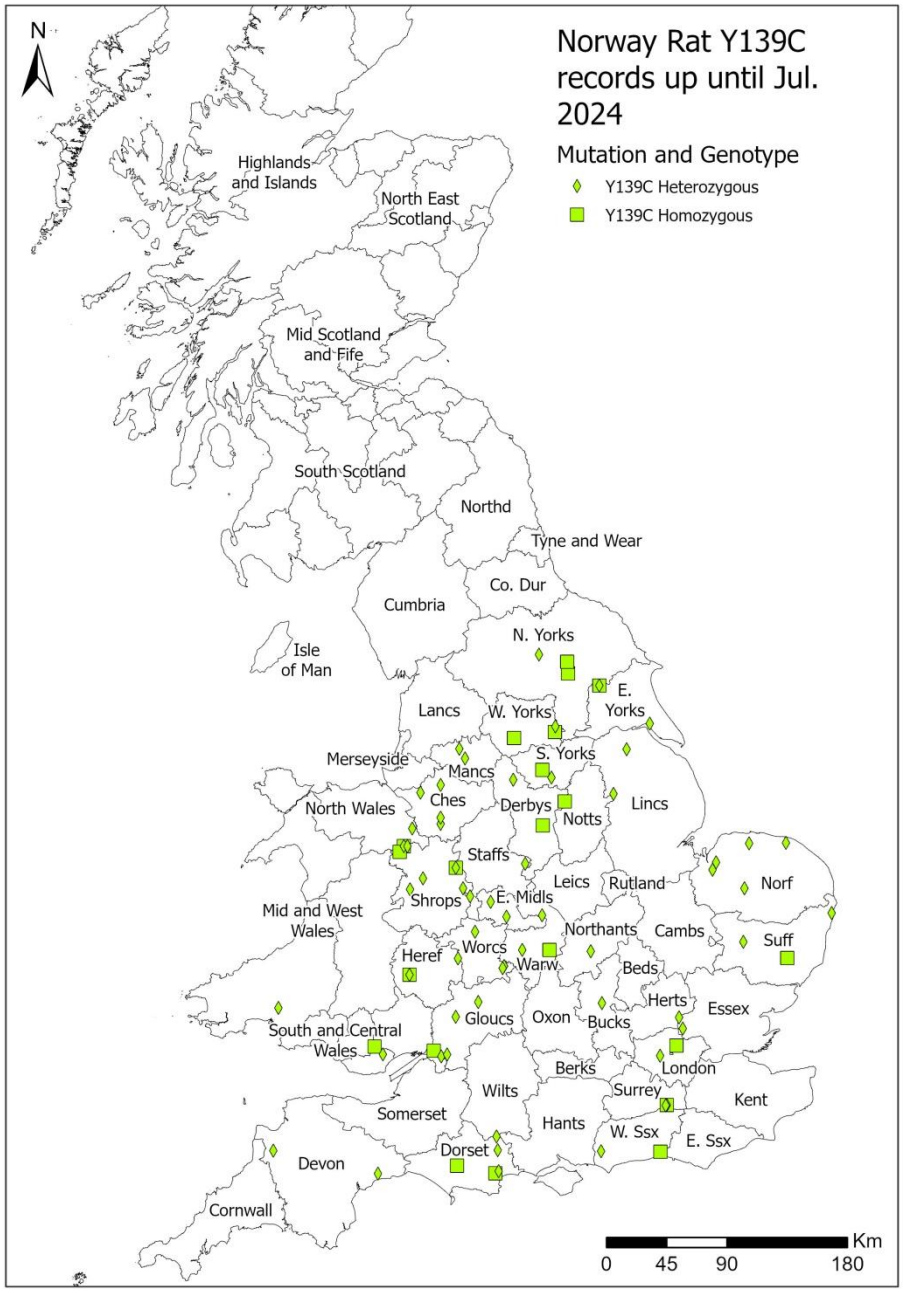
Annex 5. Map showing the geographical locations of Norway rat tissue samples submitted for analysis up to July 2024 which carried the Y139C mutation.



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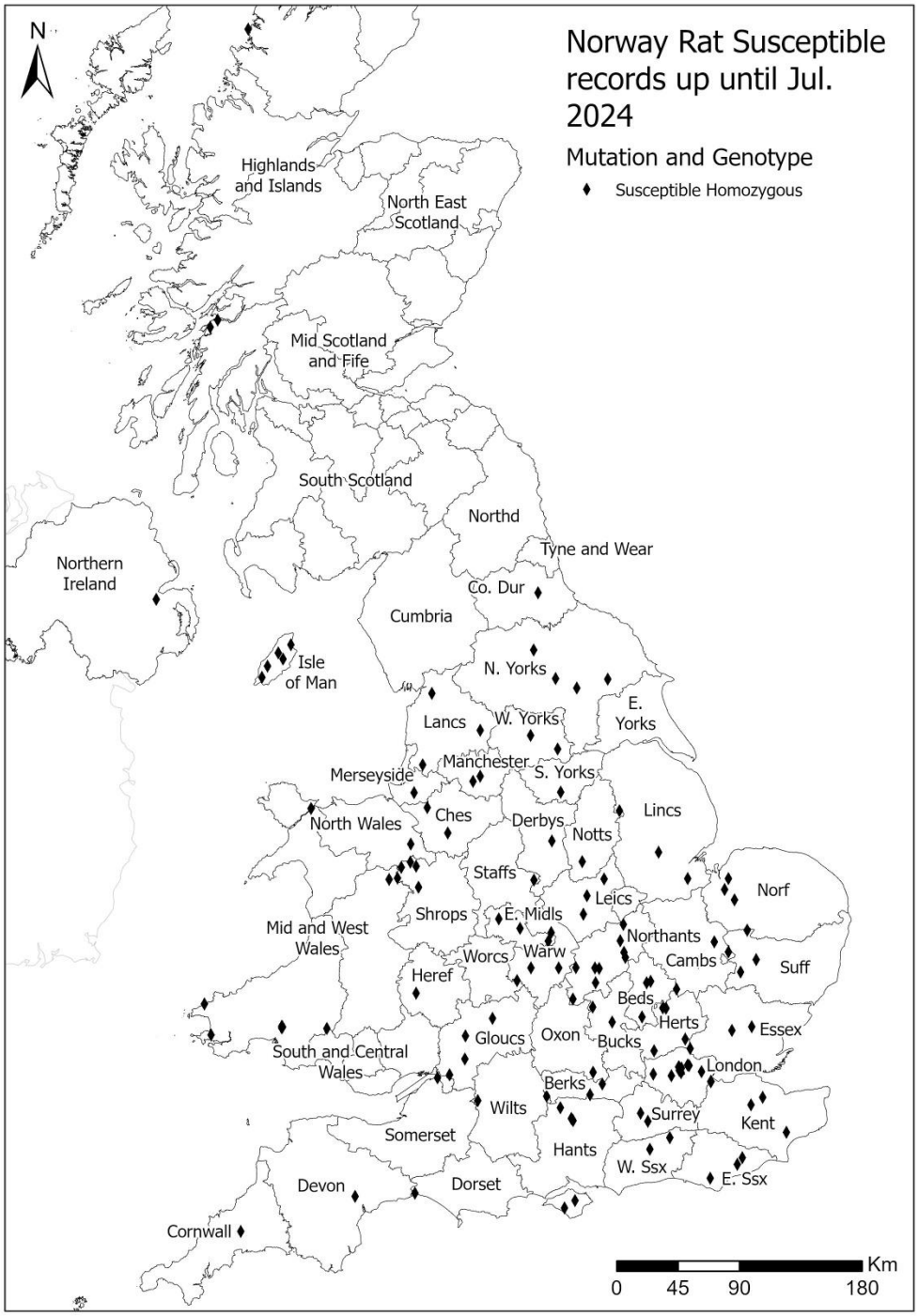
Annex 6. Map showing the geographical locations of Norway rat tissue samples submitted for analysis up to July 2024 which carried the fully susceptible genome.



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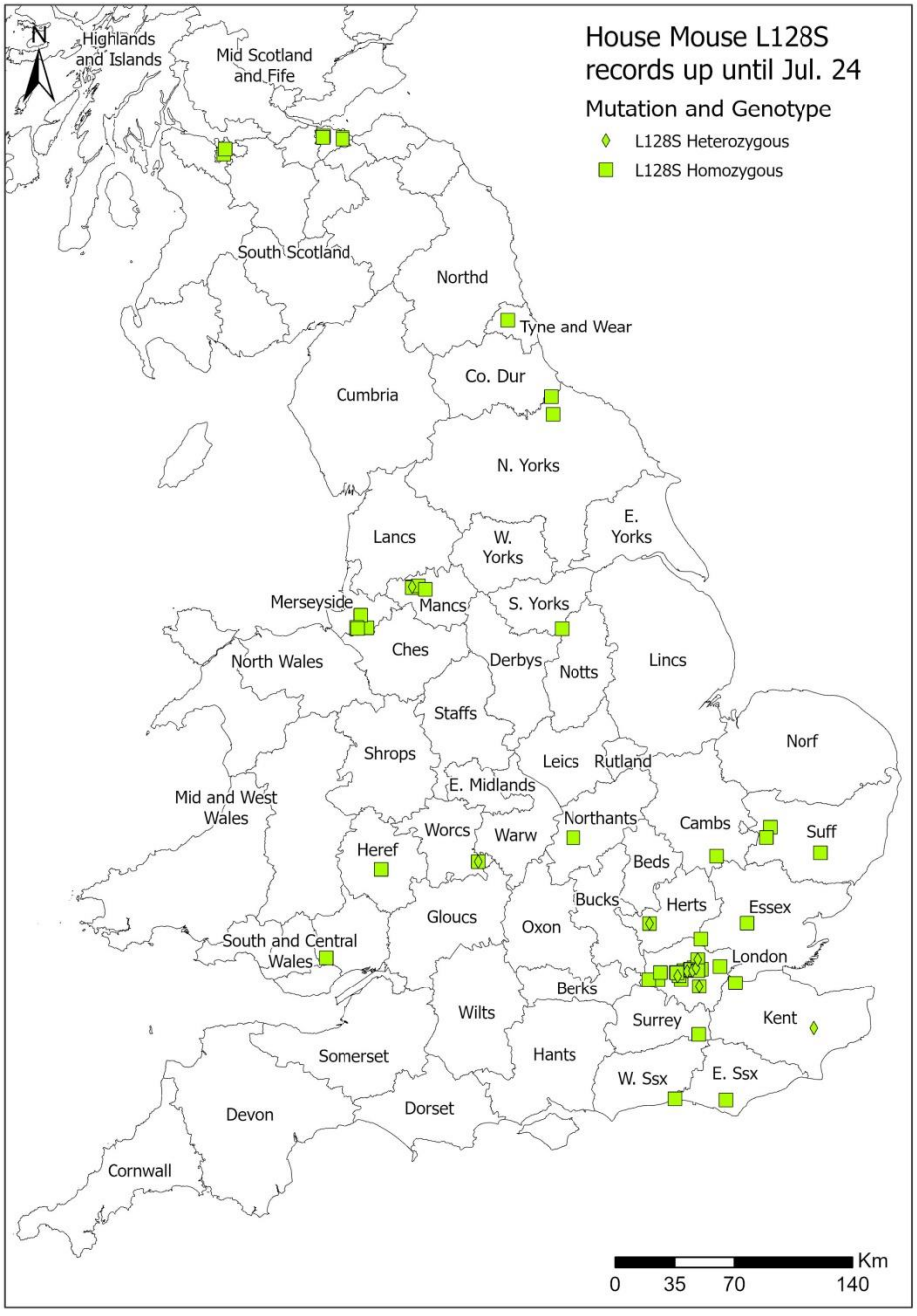
Annex 7. Map showing the geographical locations of house mouse tissue samples submitted for analysis up to July 2024 which carried the L128S mutation.



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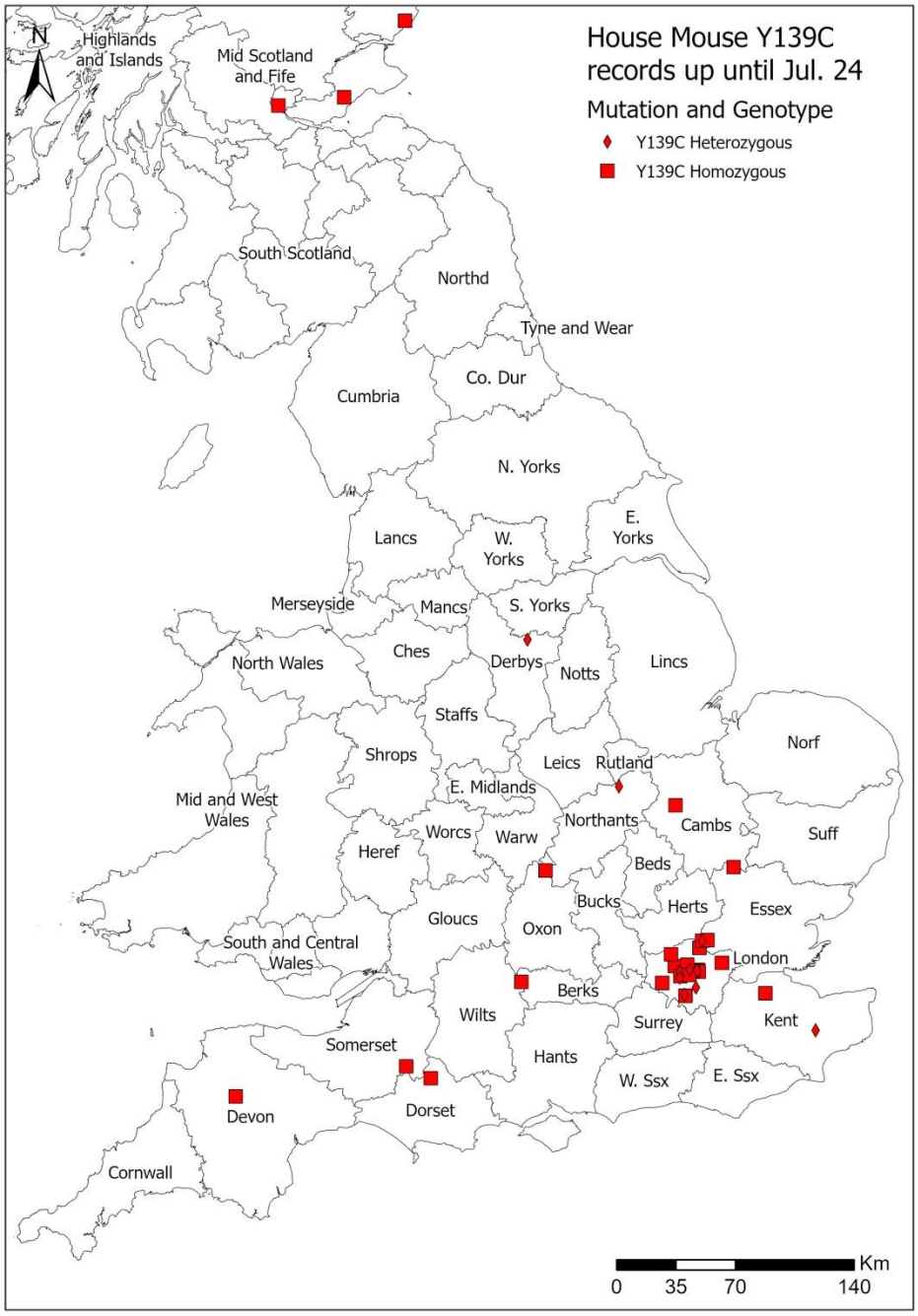
Annex 8. Map showing the geographical locations of house mouse tissue samples submitted for analysis up to July 20234which carried the Y139C mutation.



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