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Anticoagulant Resistance in Rats and Mice in the UK – new data for August 2022 to July 2023

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Contents:

Summary

1. Further to earlier reports in this series, a total of 122 rodent tissue samples were received for DNA sequencing at the laboratories of the Animal and Plant Health Agency (APHA) during the period August 2022 to July 2023. Among these, 22 samples did not yield DNA material that could be sequenced. Of the remaining 100 samples, 95 were of Norway rat tissue and 5 were house mice.
2. Among the 95 Norway rats, 25 were wild type (i.e. fully susceptible) and 70 carried one or more or the well-known resistant mutations (i.e. Y139C, Y139S, Y139F, L128Q, L120Q). Thus, 73.7% of Norway rats were anticoagulant resistant. This frequency was similar to that found in previous studies of Norway rat resistance in the UK and is similar to the value for the entire 2009 to 2023 sample (see below).
3. The SNP that was found most frequently in the sample was Y139C, with 27 individuals carrying this mutation. Among these, 16 were heterozygous and 7 homozygous, with a further four hybrid resistant. The large number of Y139C-resistant rats continues a trend found in the previous sampling period (2021-22), in which the same mutation also predominated among Norway rats. The frequency of heterozygosity suggests that Y139C rats may be spreading into areas in which a degree of susceptibility remains. 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.
4. The numbers of Norway rats carrying the four other mutations were as follows: Y139S, one; Y139F, nine; L120Q, 17; L128Q, 20. Among these, the majority was found in the expected 'heartlands' of their respective foci, but one heterozygous L120Q animal was found as an extreme outlier in northern Derbyshire, the first record of any kind for the county, and one heterozygous Y139F individual was found in South Lancashire, near previous outlying records of this mutation.
5. Hybrid resistance was again found in the sample, with two rats from West Sussex carrying the L120Q and Y139C mutations and two from Lanarkshire carrying the L128Q and Y139C mutations.
6. Only five house mouse samples were obtained but all were found to be anticoagulant resistant. Three were homozygous for the L128S mutation, one homozygous for Y139C and one animal carried both mutations. It is the position of the Rodenticide Resistance Action Group (RRAG) that all UK house mouse infestations should be assumed to carry resistance and treatments should be conducted against them accordingly.
7. During the period 2009 and 2023, in which DNA resistance sequencing has been conducted, first at the University of Reading and now at APHA, a total of 584 Norway rat and 134 house mouse tissue samples have been examined, with DNA extracted and sequenced. Among these samples we found that 77.3% of rats and 94.8% 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.
8. Large 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 with increasing frequency in East Anglia and the far south-west. Y139F is found mainly in Kent, East Sussex and Greater London, but now with an established focus in the north-west.

9. 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 this sample in northern Derbyshire. These are disseminated either by natural rodent movement or by human transportation systems. Although, there remains evidence of areas of remnant susceptibility in some counties of the Midlands and on the north-east coast, these areas are now increasingly infiltrated by resistance.
10. 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 Campaign for Responsible Rodenticide Use (CRRU) UK and rodenticide stewardship,) reduce unnecessary and ineffective emissions of anticoagulants into wildlife and the wider environment.
11. This information is increasingly understood by those who use professional rodenticides, with the apparent consequence that quantities used of the most commonly resisted second-generation anticoagulants, bromadiolone and difenacoum, may be decreasing. However, the reverse of that coin is the more potent resistance-breaking active substances, brodifacoum, difethialone and flocoumafen, and in particular the former, are increasingly used against resistant rodents, resulting in a reduction in residues of bromadiolone and difenacoum in barn owls and an increase in residues of brodifacoum.
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 downloaded 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 (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, 2019). 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 2022 and summarised all data available up to July 2022 (Buckle et al., 2022). The present report provides further additional data on the incidence of anticoagulant resistance covering the period August 2022 to July 2023, and summarises previous work.

There have been significant developments in the detection, recording and interpretation of anticoagulant resistance in recent years. The use of DNA sequencing to provide definitive evidence of the presence or absence of resistance mutations, also known as single nucleotide polymorphisms (SNPs), in samples of rodent tissue is common across all recent published studies. The technique continues to provide new evidence of the incidence of anticoagulant resistance, both in Europe (e.g. Koiviso et al., 2021; Ruis-Lopez et al., 2022; Damin-Pernik et al., 2022; Buckle et al., 2022), and elsewhere (e.g. Rached et al. 2022; Sran et al., 2022).

Before such studies can be used to inform rodent pest management decisions, additional information is required on the ability of these mutations to confer resistance on rodents that carry them, in situations where anticoagulant rodenticide treatments are conducted effectively. Many researchers rely on previously published information from other sources to interpret the biological significance of the mutations they find (e.g. Mooney et al., 2018; Koiviso et al., 2021; Damin-Pernik et al., 2022; Ruis-Lopez et al., 2022). This approach is probably reliable when the information used is derived from the same or nearby territories, but may be less so when interpretations cross country boundaries or even continents. Fortunately, in the UK, we have good background information on anticoagulant efficacy, much of it from the field, to permit interpretation of DNA sequencing results for many of the mutations found (Buckle, 2013). The exception is the Y139C SNP in Norway rats, for which we rely on studies conducted mainly in Germany (Endepols et al., 2007; Endepols et al., 2012; Buckle et al., 2012a, b). It must also be borne in mind that not all resistance mechanisms are mediated by changes to the genetics of rat and mouse VKORC1 (Boitet et al., 2017; McGee et al, 2020).

A common theme in published resistance studies is that they are conducted at a point in time and only provide a 'snapshot' of the resistance situation in the area studied – usually the proportion of resistant animals found, their genetical composition and geographical incidence. By contrast, the strength of our study is that it is based on a fourteen-year data set and provides a comprehensive assessment of the distribution and severity of anticoagulant resistance in the UK.

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

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

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.

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.

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
NR	**Tyr**osine139**Ser**ine	Y139S†	Anglo-Welsh border
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
HM	**Tyr**osine139**Cys**teine	Y139C†	Reading
HM	**Leu**cine128**Ser**ine	L128S†	Cambridge

† 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 2.7 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 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 2023. In that period a total of 584 Norway rat tissue samples from around the UK have been studied using the DNA sequencing technique. Of these, 451 (77.3%) were found to possess one or most of the resistance mutations that are known to have a significant effect on anticoagulant rodenticide efficacy. The remaining 133 animals (22.8%) carried the wild type genome. Maps showing the geographical locations from which these samples were sent have been presented previously (Buckle et al., 2020a, 2022) and are also the main source of the UK mapping information available at the website of the 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 2022-2023 and frequency of resistance

Among the 95 samples (Table 2) that were capable of being sequenced in the period August 2022 to July 2023, a total of 70 (73.7%) were found to carry one of the five main Norway rat anticoagulant resistance mutations (Table 1). The remaining 22 animals (23.2%) carried the wild type genome. The proportion of resistant Norway rats in the sample did not differ from that found in previous surveys (i.e. 2020, 74.1%; 2021-2022, 74.1%).

Four animals each possessed two different resistance mutations, two were hybrid resistant for L128Q and Y139C and two for L120Q and Y139C. Both hybrid resistances had been found in previous surveys.

Among those rats that carried a single SNP, the severe Y139C mutation was the most common (38.6%, n=27) in the 2022-23 sample. Of those, 74.1% were heterozygous for the mutation, possibly indicating resistant animals becoming established in areas in which rats had previously been mostly susceptible. The numbers of resistant samples carrying L120Q, which had previously predominated, declined to 24.3% of the total. It is unlikely that this is because of a reduction in the incidence of this resistance but more likely because it is now so well known by practitioners that only one sample was received from the main focus in Berkshire and Hampshire.

Table 2. The numbers of Norway rats tissue samples received and analysed 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	5	12	17
L128Q	13	7	20
Y139C	7	20	27
Y139F	6	3	9
Y139S	0	1	1
Totals (mutations)	31	43	74
L128Q and Y139C*	0	2	2
L120Q and Y139C*	0	2	2
Totals (hybrid resistance)	0	4	4
Susceptible	25	0	25
Total (susceptible)	25	0	25

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

3.3 Norway rats – geographical distribution of SNPs

Introduction

It is important again to provide information that sampling is not conducted systematically, but rather in an *ad hoc* manner and without temporal continuity at any site. Thus, discovery of resistance at a location does not mean that it recently arrived there. Nor can it be known whether resistance was transported either by the natural movement of rodents, by human agency, or initiated by a *de novo* mutation event. However, *de novo* mutations resulting in the resistance SNPs appear to be very rare events. For example, despite of the proximity of the two countries, and many zoogeographical similarities, none of the five resistance SNPs commonly found in the UK Norway rats has appeared in the Republic of Ireland (Mooney et al., 2018). Also, in spite of a half-decade of selection pressure caused by the intensive and wide-scale use of anticoagulants in Europe, only one SNP exists in Norway rat populations in both Germany and Denmark (Y139C). Also, until recently and in spite of numerous studies across Europe and its existence in the UK for more than 60 years, the Y139S SNP existed nowhere else but on the Anglo-Welsh border in the UK. These observations lead to the conclusion that *de novo* mutations are very rare genetical events and so unusual as to be able to be discounted as a significant cause of the proliferation of anticoagulant resistance we now see among Norway rats in the UK.

It is equally important to note than the absence of demonstrated resistance in Norway rats in any location shown in this report may, indeed, mean that no resistance exists there, but it may also mean that resistance is present but no sampling has yet been undertaken.

L128Q – Scottish Resistance (Annex 1)

Although not know as such at the time, L128Q in Scotland was the first resistance to anticoagulants found anywhere in the world (Boyle, 1960). Rats carrying this mutation remain restricted to Scotland and the north of England. L128Q is a relatively weak resistance and all SGARs remain effective against it. This may explain why it has not spread more widely in the UK as other SNPs have done (Fig 1. and Annex 1). Significant numbers of new records of this mutation (n = 20) were added to the map from the samples submitted in 2022-23 (Fig. 2), but none was outside the known focus. The large geographical area of the L128Q focus means that it is contiguous with several other spreading foci and, consequently, is now found in hybrid form with Y139S, Y139C and L129Q (Fig. 3).

Y139S - Welsh Resistance (Annex 2)

The Welsh Y139S resistance mutation was the subject of intensive research conducted in UK government laboratories over more than 30 years. Consequently, perhaps more is known about the biological effects of this mutation on anticoagulant efficacy than any other. It is, however, a relatively weak resistance, as both bromadiolone and difenacoum retain some efficacy against it. Therefore, studies of anticoagulant efficacy against Y139S should not be used to interpret the wider impacts of other SNPs on the efficacy of SGARs.

The same government research provided thorough understanding of the size of the Welsh Y139S focus in the 1970s and showed it covered a very large area on both sides of the Anglo-Welsh border in Central Wales, west across to Cardigan Bay and south into Carmarthenshire (Greaves and Rennison, 1973). It is most unlikely that the focus is smaller now than it was then despite the paucity of records from our DNA studies (Fig. 1 and Annex 2). The lack of samples recently submitted from the Y139S focus may be due to the fact that it is known that the mutation has limited effects on the efficacy of the most widely-used SGARs, bromadiolone and difenacoum (Buckle et al., 2007).

There are two areas of Y139S resistance apparently outside the original focus. One is in Merseyside, which may be accounted for by natural spread from the original focus to the south. Another is the recent discovery of Y138S rats in West and North Yorkshire, apparently now spreading almost up to the border of County Durham (Annex 2). This is far enough from the original focus to suggest that initiation was due to human agency.

A single rat carrying Y139S was present in the 2022-23 sample reported here. It was found in Carmarthenshire, from where there have been records both recently in our study and in the early survey of Greaves and Rennison (1972).

Y139F – Kent Resistance (Annex 3)

The 1970s survey disclosed a substantial resistance focus in Kent and East Sussex (Greaves and Rennison, 1973) but this went unremarked subsequently for almost forty years. Then, after a report of the failure of bromadiolone to control an infestation of Norway rats at a poultry unit in Kent, analysis of DNA from rats on the farm confirmed the presence of the Y139F mutation for the first time in the UK (Prescott et al., 2010). The known extent of the focus is shown in Fig. 1

and Annex 3, and closely reflects that found in the earlier Greaves and Rennison survey. However, there is now considerable spread to places as far apart as the Suffolk coast and East Lancashire. Central London now also seems to be a 'hot-spot' for Y139F Norway rat resistance. The high degree of homozygosity among the records from Kent and East Sussex suggests a very well-established and long-term focus.

Nine rats were found to carry Y139F among the 2022-23 samples (Fig. 2). The majority of these were found in London and Kent, within the long-established focus. However, one animal was found in southern Lancashire, far from the known focus. This record, added to several others found previously, suggests a growing focus of this highly resistant phenotype in north-west England, almost certainly translocated by human agency.

L120Q – Hampshire/Berkshire Resistance (Annex 4)

L120Q is the most severe form of anticoagulant resistance in Norway rats so far discovered (Buckle, 2013). A recently published paper describes development of the focus from the late 1960s to the present time, the efficacy of three SGARs on infestations with this mutation (or rather in the case of bromadiolone and difenacoum the lack of efficacy) and the potential impacts of L120Q on environmental exposure of wildlife to rodenticides (Buckle et al., 2020b).

The original focus of L120Q in central southern England was restricted to farmsteads around the towns of Reading, Andover, Winchester, Basingstoke, Newbury and Alton (Greaves and Rennison, 1973). However, previous reports from this project now show this SNP to be prevalent across the whole of central southern England. There are also increasing records from Devon, Cornwall and East Anglia, suggesting spread both to east and west (Fig 1 and Annex 4). The records from the 2022-23 sampling are mainly from outside the core focus, with new records from Derbyshire, Somerset, Dorset, Oxfordshire and Buckinghamshire (Fig.2).

Y139C – Gloucestershire Resistance (Annex 5)

The Y139C SNP was relatively infrequent in tail tissue samples in this study until the samples received in 2021-22 (Buckle et al., 2022). A total of 33 samples carried the Y139C SNP in that two-year period and the sample for August 2022 to July 2023 reported here added a further 27 (Table 1, Fig. 2). Records of this SNP are now very widely spread across England, Scotland and Wales (Fig. 1 and Annex.5).

The severity of this mutation, and the demonstrated lack of efficacy of both bromadiolone and difenacoum against it (Endepols et al., 2007; Buckle et al., 2012a), makes these findings potentially problematic for pest management practitioners over a very wide area of England and Wales (and Scotland if hybrid resistance is included) (Fig. 3). The continued unabated use of these two substances across the UK to control Norway rats may explain the proliferation of this SNP.

Norway rat - Consolidated findings 2009-2023

Maps showing the occurrence of the individual mutations (Annexes 1-5) are amalgamated and presented on the map shown at Fig.1. With almost 600 individual resistance locations shown, this is the largest study of Norway rat anticoagulant resistance ever undertaken.

With the exception of some isolated occurrence of L120Q and Y139C, Scotland and the north of England presently appear to remain largely free from the most severe resistance mutations,

although of course L128Q is widespread. This means that the two less potent SGARs, bromadiolone and difenacoum should retain good efficacy in those areas for the control of Norway rats.

Although there is a severe shortage of samples from Wales, it appears that the situation is similar there. It is probable that, in spite of a paucity of records Y139S from much of Wales, this mutation remains very widespread, but once again bromadiolone and difenacoum retain substantial efficacy against it, although the former may be may be somewhat less effective than the latter (Buckle et al., 2007). Isolated foci of Y139C and Y139F were also found in Wales and it will be interesting to see if future samples show these severe mutations becoming more prevalent.

The situation is quite different in the south of England, where the severe L120Q and Y139F mutations are both very frequent and widespread. It is interesting to note that although the L120Q and Y139F SNPs predominate in the south, the other severe resistance mutation Y139C is beginning to occur with greater frequency. Therefore, it would seem wise that practitioners anticipate that the Norway rat infestations contain one of these three severe mutations over a very large area of southern England. Although there is some susceptibility in the far south-west, there is also heterozygous occurrence of L120Q.

No samples of Norway rats from Buckinghamshire had been tested until a collection of nine were provided by a technician in November 2022. Unfortunately, one did not yield DNA that could be sequenced but the two severe mutations were identified, L120Q (n = 3) and Y139C (n = 1). All positives were heterozygous, showing susceptibility remains there but it is to be expected that resistance will now spread if resisted active substances continued to be used.

A collection of six samples were received from the Isle of Man. All were found to be wild type and therefore fully susceptible to anticoagulants. Interestingly, this reflects the situation found on the island of Ireland (Mooney et al., 2018).

London appears to be a ‘hot-spot’ of Y139F resistance, with heterozygous L120Q animals also appearing there.

Many of the counties of the Midlands and eastern England are very sparsely sampled, but for several years past the majority of the samples received from there were wild type (i.e. susceptible). However, in recent surveys, and particularly over the last four years, there is an increasing infiltration of heterozygous Y139F, L120Q and particularly Y139C animals. It therefore appears that the few substantial remnant areas of susceptibility in the English Midlands may not remain much longer.

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 2009-2023.

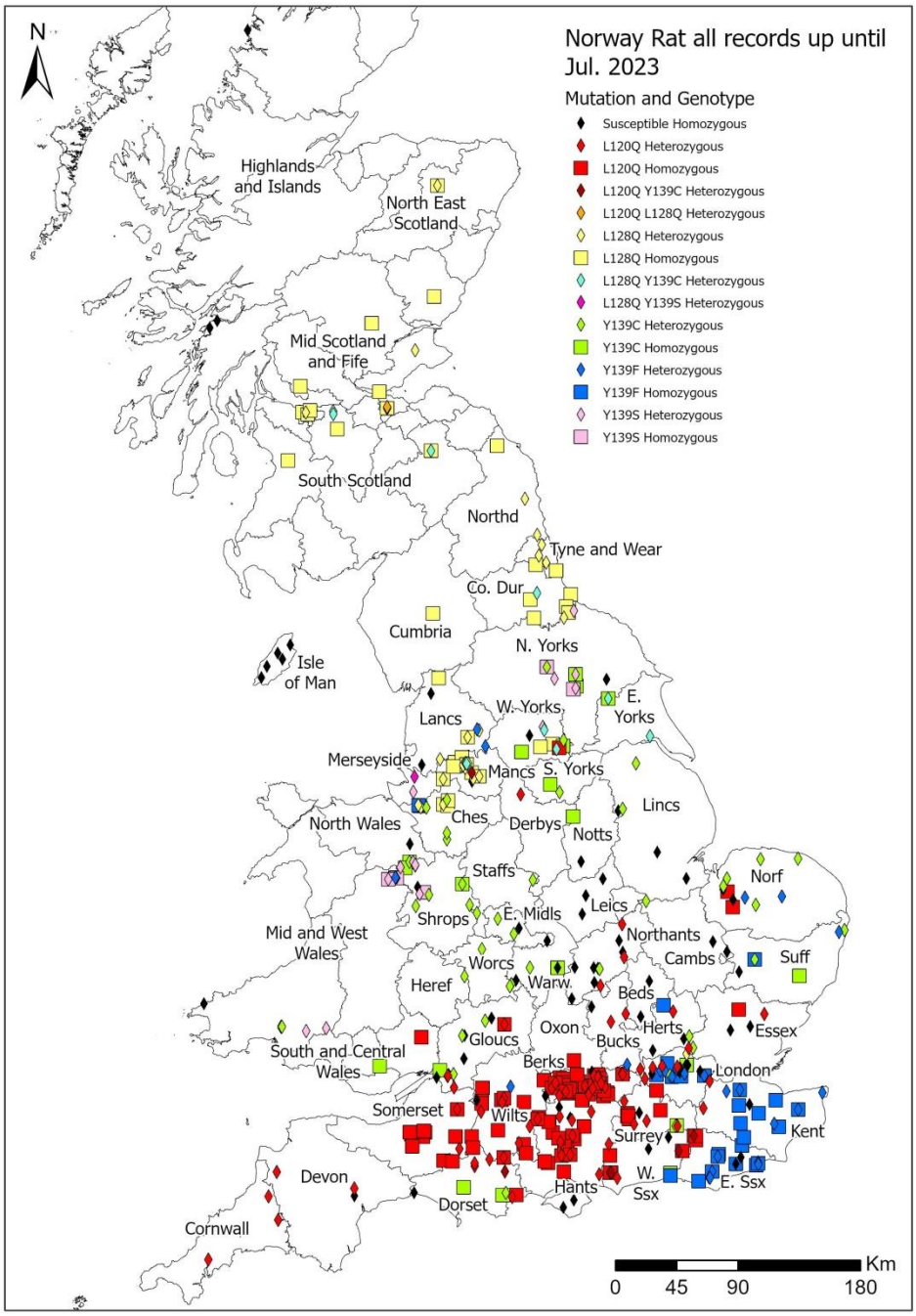


Figure 2. Geographical locations of all new Norway rat records for the period August 2022 to July 2023.

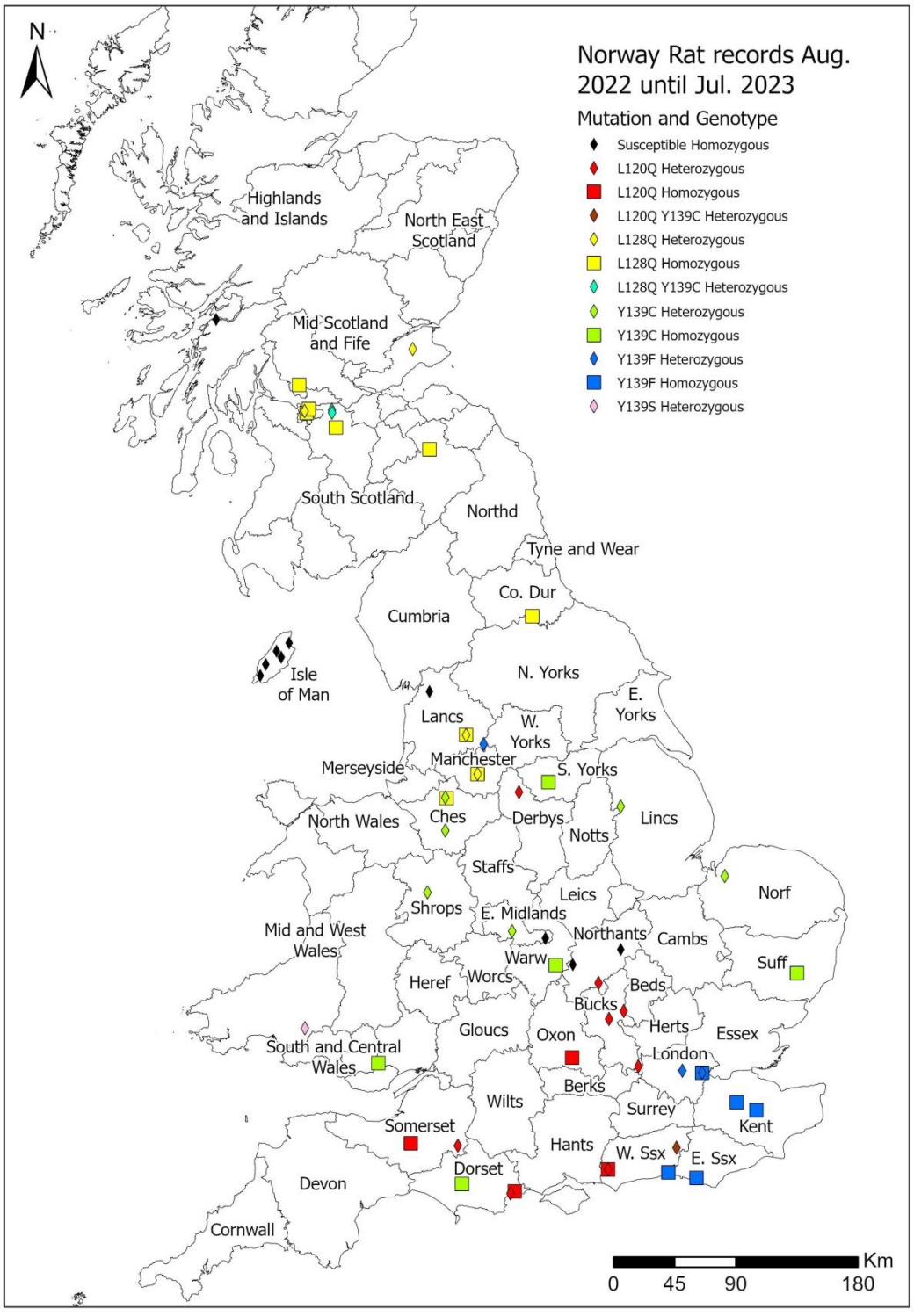
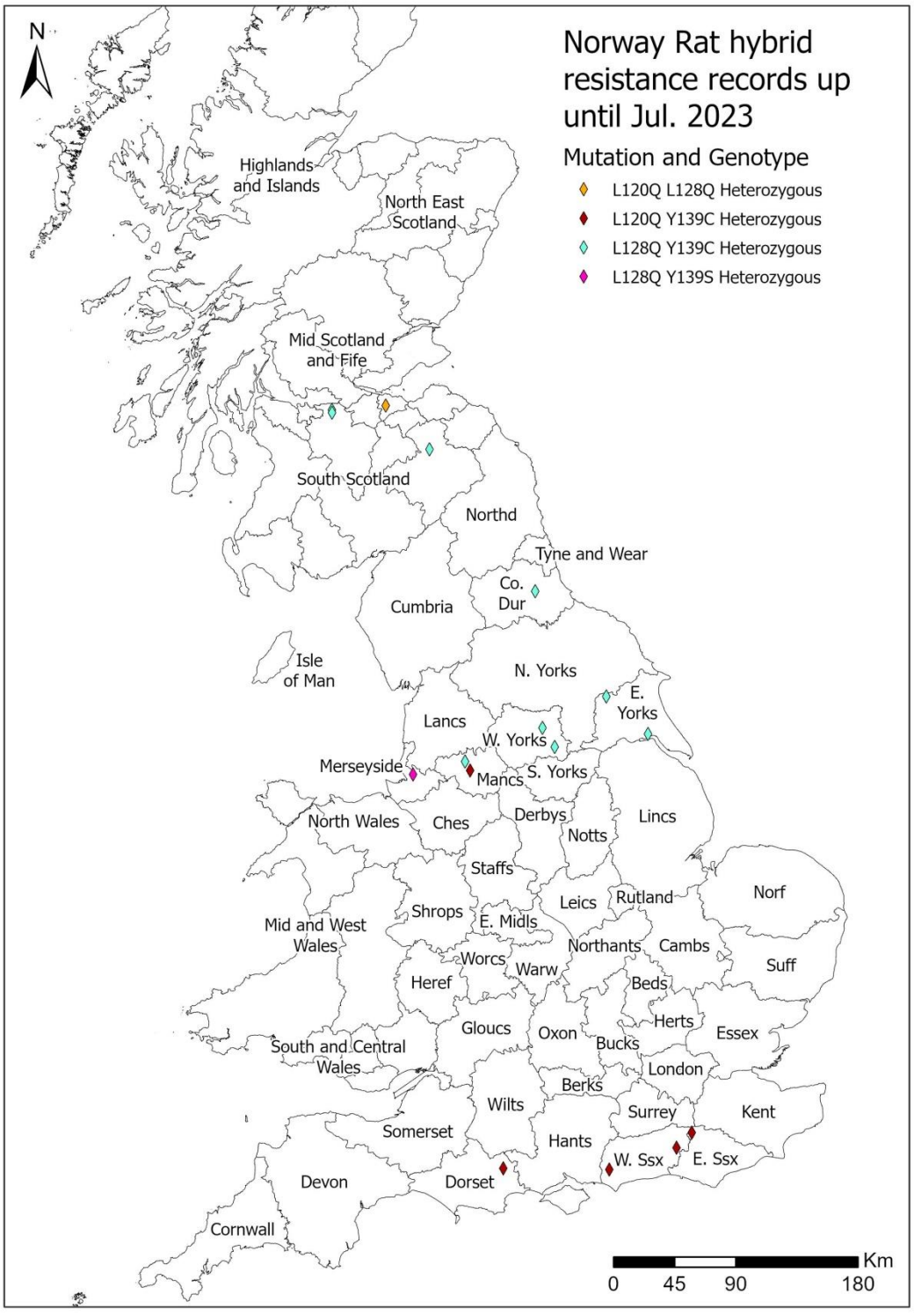


Fig. 3. Map showing all Norway rats found to carry two different anticoagulant resistance SNPs (i.e. hybrid resistance). Records for 2009 to 2023.



Norway rats – Hybrid Resistance

Hybrid resistance, that is Norway rats carrying more than one resistance SNP, was found once again in four individuals in 2022-2023. Two animals from Lanarkshire carried the L128Q and Y139C mutations and two from West Sussex carried L120Q and Y139C (Fig. 3). The records for West Sussex were from an area near Redhill not far from where this hybrid had been found previously and where rats carrying each mutation separately are to be found (Fig.1), suggesting that hybrid resistance will become, or has already become established there.

The two hybrid resistant records from Wishaw, Lanarkshire are more interesting. The rats carrying hybrid resistance were from infestations about 2 km apart, suggesting a substantial focus. L128Q is long-established in the area and has been since the initial discovery of resistance in Scotland more than 60 years ago. It is therefore unsurprising to find hybrids carrying this mutation in that locality. However, the other mutation carried, Y139C, has not been found before in any Norway rats in Scotland (other than a single animal also hybrid L128Q/Y139C from the Scottish Borders). The nearest confirmed Y139C individual in our samples is from North Yorkshire (Fig. 1). Therefore, it seems likely that either Y139C infestations are present more widely in the far north of England and southern Scotland, but remain unrecorded because of the scarcity of samples, or Y139C animals have been translocated to these areas to hybridise with L128Q rats. The importance of this finding is that up to now, with only L128Q resistance found, and this being susceptible both to bromadiolone and difenacoum, these hybrid resistant rats may no longer respond as expected to applications of these commonly-used substances.

It remains that we know little of the practical implications of hybrid resistance for the use of anticoagulants. More research is required and there are promising initial studies from researchers in France on novel, and rapid, *in vitro* assays which reveal resistance ratios for various anticoagulant active compounds and different resistance SNPs, including hybrid resistance (Goulois et al., 2017).

3.4 House mice

Samples of house mouse tissue are received much less frequently that those of Norway rats and that continued in our sample for 2022-23, with only five 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 may be correctly 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 the relatively small numbers, a total of 134 mouse tissue samples were received during the period 2009 to 2023. Among these were found animals carrying both common UK mouse resistance SNPs, Y139C and L128S (see Table 1; Pelz and Prescott, 2015) and there was also a small but significant number of mice that were hybrid resistant, carrying both mutations. These mice occurred especially in London and were mentioned in more detail in the previous report, as was the newly-found *spretus* mutation (Buckle et al., 2022).

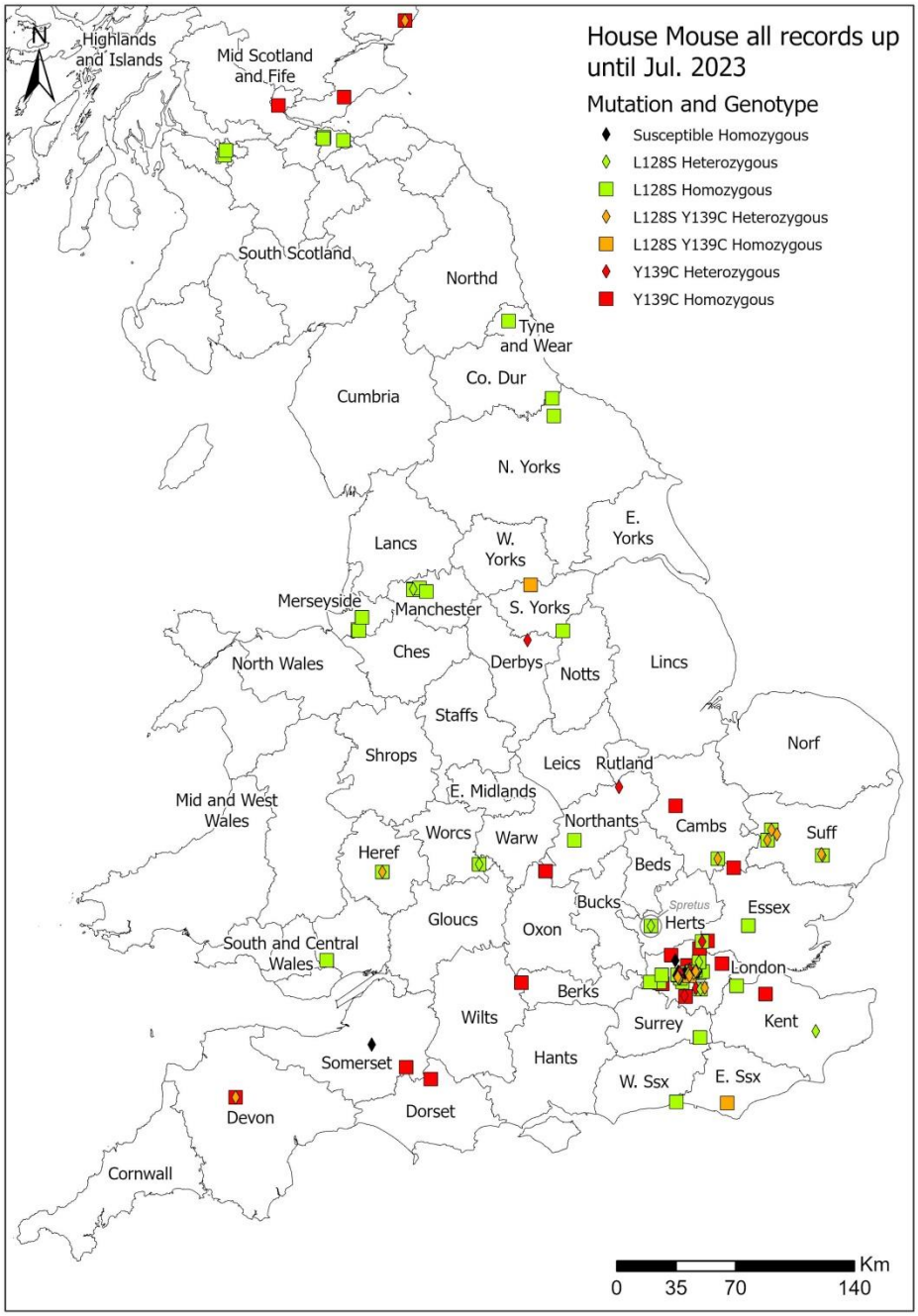
Maps of the distribution of house mouse resistance shows that the Y139C mutation is largely restricted to the south-east of England, although there have been recent findings of this mutation among mice in Scotland, while L128S is more ubiquitous (Figure 4). Within the total sample of 134 individuals, 127 house mice carried one or both SNPs, giving a very high frequency of

resistance among UK house mice of 94.8%. 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 (Buckle et al., 2021a).

Five house mouse tissue samples were received in the period August 2022 to July 2023 and all were resistant (Table 2). Three mice carried the L128S SNP in homozygous form, one from Glasgow, one from Lanarkshire and, unsurprisingly, one from the original site of this resistance, Cambridge. One animal taken from the original Y139C focus in Reading was homozygous for this mutation and another from the same infestation as the previous Cambridge animal carried both the Y139C and L128S SNPs in hybrid resistance.

Separate maps showing the distributions of the two house mouse resistance SNPs are provided in Annexes 6 and 7.

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 2023. (The Hertfordshire focus of the *spretus* introgression is obscured by other overlaying resistance records at the same site.)



4. Discussion

The results of resistance testing, conducted in the period August 2022 to July 2023 reported here, support previously published findings on the overall incidence of anticoagulant resistance in UK Norway rat and house mouse populations and their geographical distributions (Buckle et al., 2022). In particular, the frequency of resistance was found to be similar to that found in earlier samples for both rats and mice (i.e. about 75% and 95% respectively). There were, however, some new observations that are worthy of further consideration.

The first is the apparent proliferation of the Y139C mutation in Norway rats in recent sampling periods. A total of 60 Norway rats tested positive for this SNP in samples received in the period 2021 to July 2023. This mutation in known from other studies to confer resistance to two SGARs, bromadiolone and difenacoum, commonly-used in the UK against Norway rats (Buckle et al., 2012. a, b; Endepols et al., 2012). The persistent use of resisted substances against resistant rats will, of course, result in the spread of resistance (Greaves, 1994). These observations cannot be said with certainty to be due to recent colonisations by Y139C resistant rats of areas that previously contained mainly susceptible animals. However, technicians operating in those areas had not previously submitted samples for resistance testing, presumably because they had not encountered treatment failures. The fact that they have recently done so supports an assumption that the occurrence of resistance is also recent.

Several counties of the Midlands, including Derbyshire, Nottinghamshire, Leicestershire and Northamptonshire are extremely poorly represented in Norway rat samples we receive for resistance testing (see also Buckle et al. 2022). Again we may assume this is because treatment failure was infrequent and the few samples that have been tested from these counties were found to be anticoagulant-susceptible. However, resistance is now increasingly found. A case in point is the occurrence of L120Q in a sample received from the Hope valley in Derbyshire. This is the first sample ever received from the county and the fact is significant that the individual in question carried the SNP found more commonly in southern central England (Fig. 2, Annex 4). L120Q has now been found elsewhere in these Midland counties and there has also been an increasing occurrence of Y139C. All this leads to the possible conclusion that one of the few remaining strongholds of anticoagulant susceptibility in England is increasingly infiltrated by severe resistance mutations (Fig. 2). To remove highly-resistant rats from areas where substantial anticoagulant susceptibility remains, RRAG guidelines recommend the occasional use of the most potent SGARs or non-anticoagulant substances.

The nearby counties of Oxfordshire, Buckinghamshire, Hertfordshire and Bedfordshire are also poorly represented in samples received during the project; probably for the reason mentioned above. In the case of the first, this is particularly surprising because it shares a long border with Berkshire, where the density of records of L120Q is extraordinarily high (Fig. 1). However, we received rat tissue from Oxfordshire for the first time among the 2022-23 samples; this from the south of the county near Didcot (Fig. 2). Unsurprisingly the sample was found to be homozygous L120Q, possibly indicating an established focus there. Samples were also received from Buckinghamshire and, among the very few so far received from that county, L120Q was found for the first time. No samples were received from Hertfordshire and Bedfordshire and the former appears to be one of the few southern counties in which resistance has not yet been found. Neighbouring counties, once mostly susceptible, now harbour Y139C, L129Q and Y139F rats and it seems unlikely that this situation will long remain.

The finding of hybrid resistance in Norway rats in Scotland in 2017 (Buckle et al., 2022) presaged the wider occurrence of this phenomenon that was to be expected given the extensive, and expanding, UK resistance foci revealed by this project. It is now being found more widely in the UK in both Norway rats (Fig. 3) and house mice (Fig. 4). However, the occurrence of hybrid resistance remains very uncommon, particularly in Norway rats, so the finding of L128Q/Y139C hybrid resistance at two different localities in the Lanarkshire town of Wishaw was surprising. This is particularly the case given the distance between there and the nearest known site of Y139C far to the south (Fig 1).

The consequences of hybrid resistance for the efficacy of anticoagulant rodenticides remain largely unknown. All UK resistance studies, over a period of more than 50 years, have been on rodents thought to carry only a single mutation (Buckle, 2013). A reasonable hypothesis is that hybrid resistant animals may be more resistant than those that carry only one resistance mutation. Research work has begun in French laboratories on hybrid resistance in house mice and initial findings appear to support this hypothesis (Goulois et al, 2017). This work was done using novel *in vitro* techniques and the authors claim that their findings are consistent with other, better-known resistance testing technologies, such as laboratory feeding and blood clotting response tests. However, more work is needed to confirm that this initial assertion is robust and similar work needs to be done with hybrid resistant rats.

The latest survey confirms the very widespread occurrence of anticoagulant resistance in UK Norway rat and house mouse infestations. It also confirms the widespread, although much less common, occurrence of hybrid resistance. It is essential that this information, and its practical consequences, are widely disseminated and fully understood by those involved with rodenticides in the UK. The use of anticoagulants against rodent populations that are resistant to them has three 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 at al., 2020). It is therefore 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 to occur.

For the reasons given in the previous paragraph, dissemination of information on the incidence of resistance and advice on resistance management is highly desirable (see Buckle 2020, a, b). If the advice is heeded, this may lead to a reduction in the use of the widely resisted first-generation anticoagulants (although none are currently authorised) and the less potent SGARs bromadiolone and difenacoum. Very little information is available to us on quantities of anticoagulant active substances sold and used in the UK to permit understanding of patterns of rodenticide use. Some valuable data is regularly available from Scotland (e.g. Reay, et al., 2020) but rodenticide use there may be affected by the low apparent occurrence of anticoagulant resistance in Norway rats (Fig.1) and the fact that the most common Scottish resistance mutation, L128Q, is susceptible to bromadiolone and difenacoum (Buckle et al., 2020 a). However, the frequency and concentrations of SGAR residues found in livers of barn owls may provide insight into what appears to be happening more widely across the UK (Ozaki et al., 2022). When CRRU initially took up responsibility for funding the barn owl liver residue study, bromadiolone and difenacoum residues predominated at roughly similar levels, both in terms of frequency and concentration. This is clearly seen in what has become known as the ‘baseline data set’ (Shore et al., 2014). The

frequency of brodifacoum residues was considerably less. This situation continued, with small and usually statistically insignificant variation, throughout the early years of the UK Rodenticide Stewardship Regime from 2016 (Buckle et al., 2017). However, more recently, residues of bromadiolone and difenacoum in barn owl livers have declined significantly and those of brodifacoum and difethialone have increased (Ozaki et al., 2022). The causes of these changes are complex but it is likely that the increased use of the potent SGAR resistance-breakers for resistance management of both Norway rats and house mice has probably played a significant part.

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.

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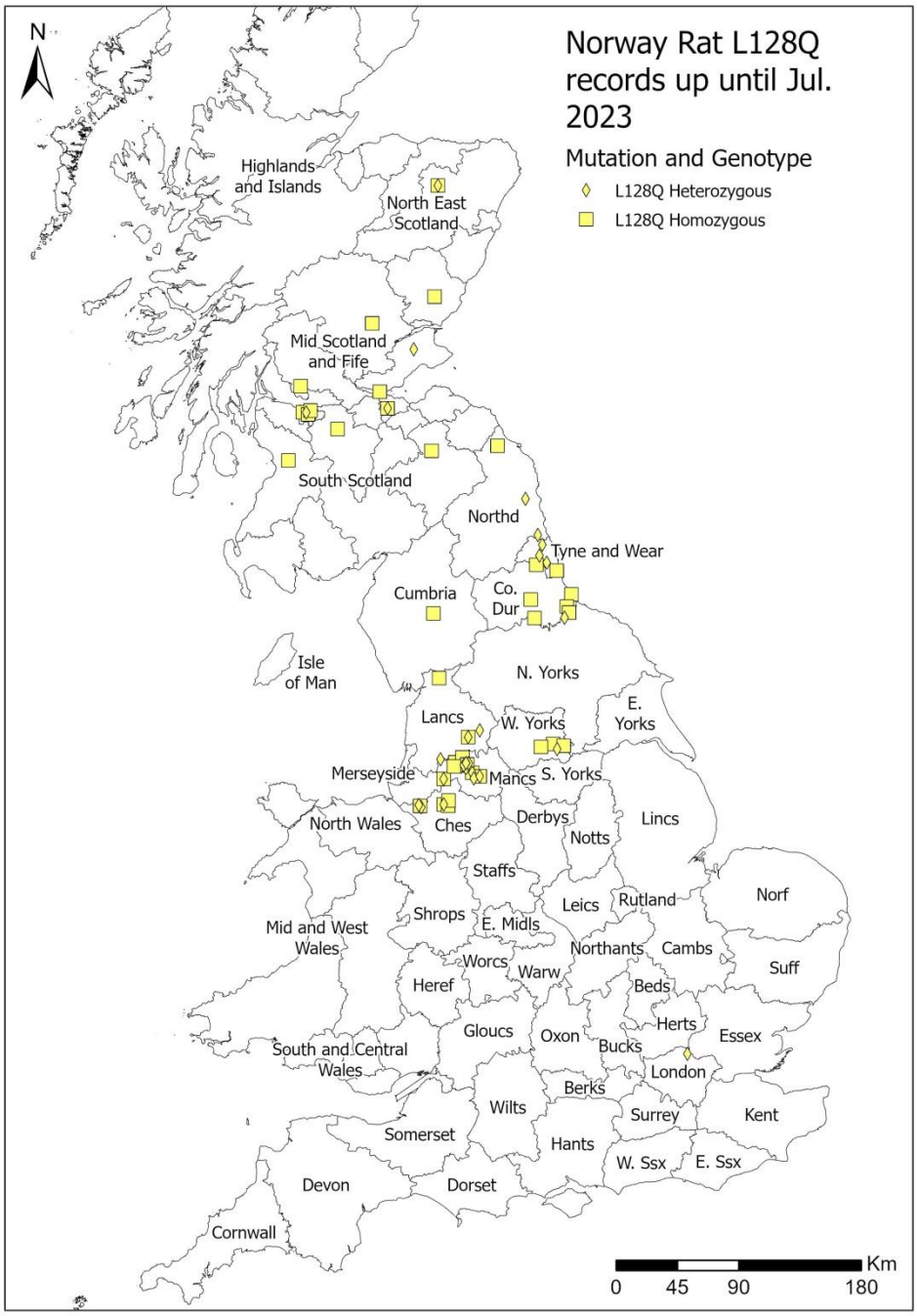
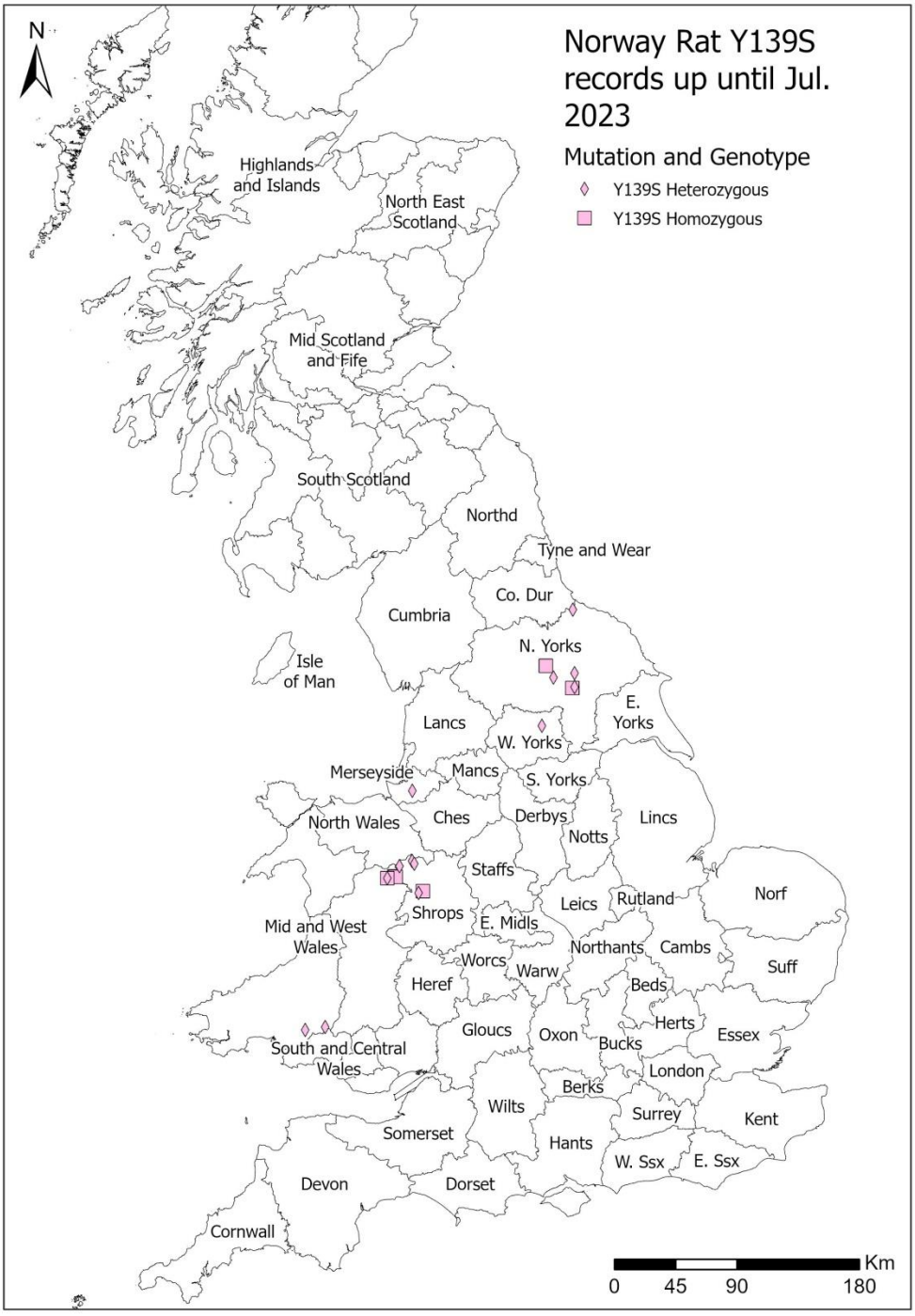
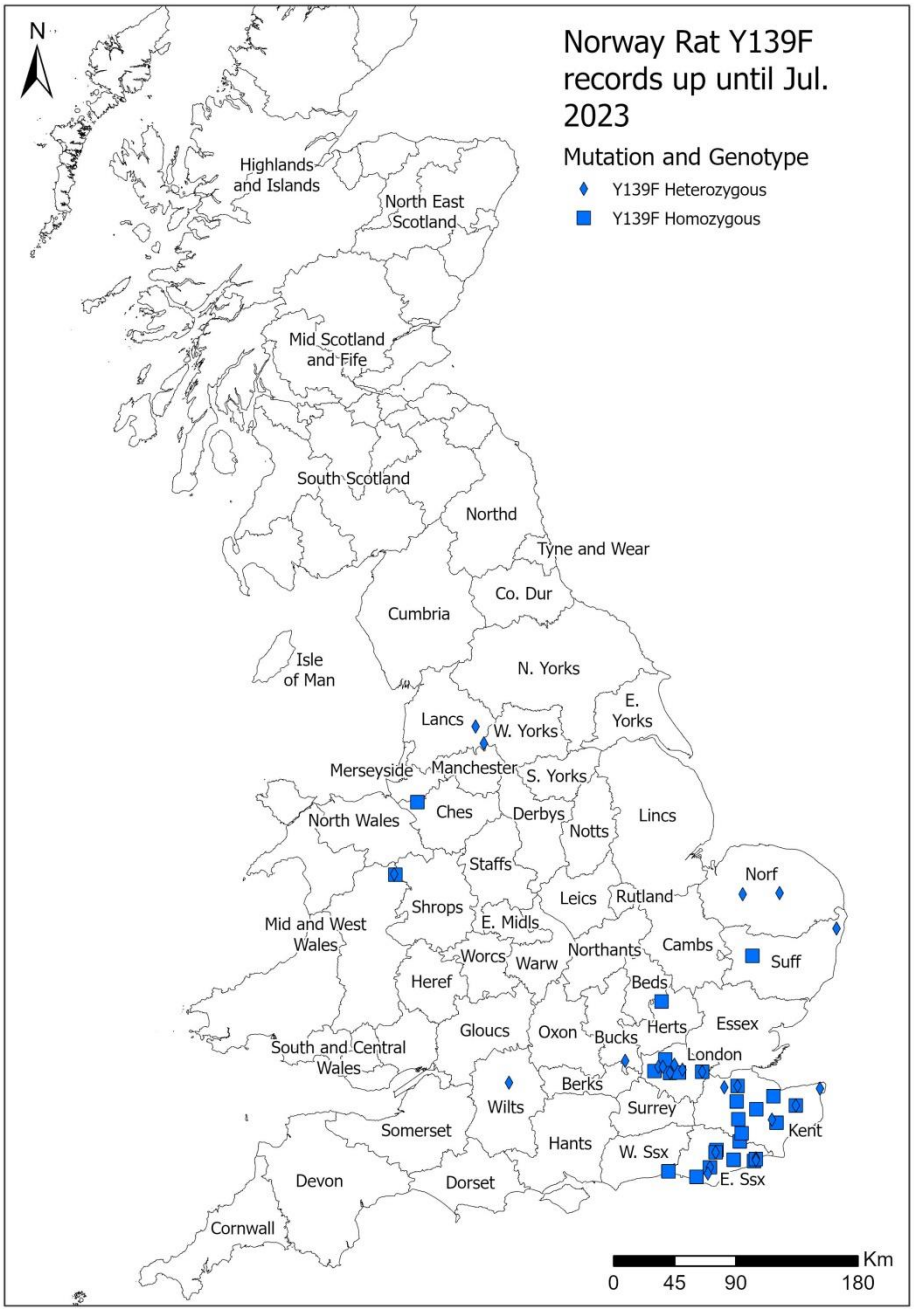
Annex 1. Map showing the geographical locations of Norway rat tissue samples submitted for analysis up to July 2023 which carried the L128Q mutation.



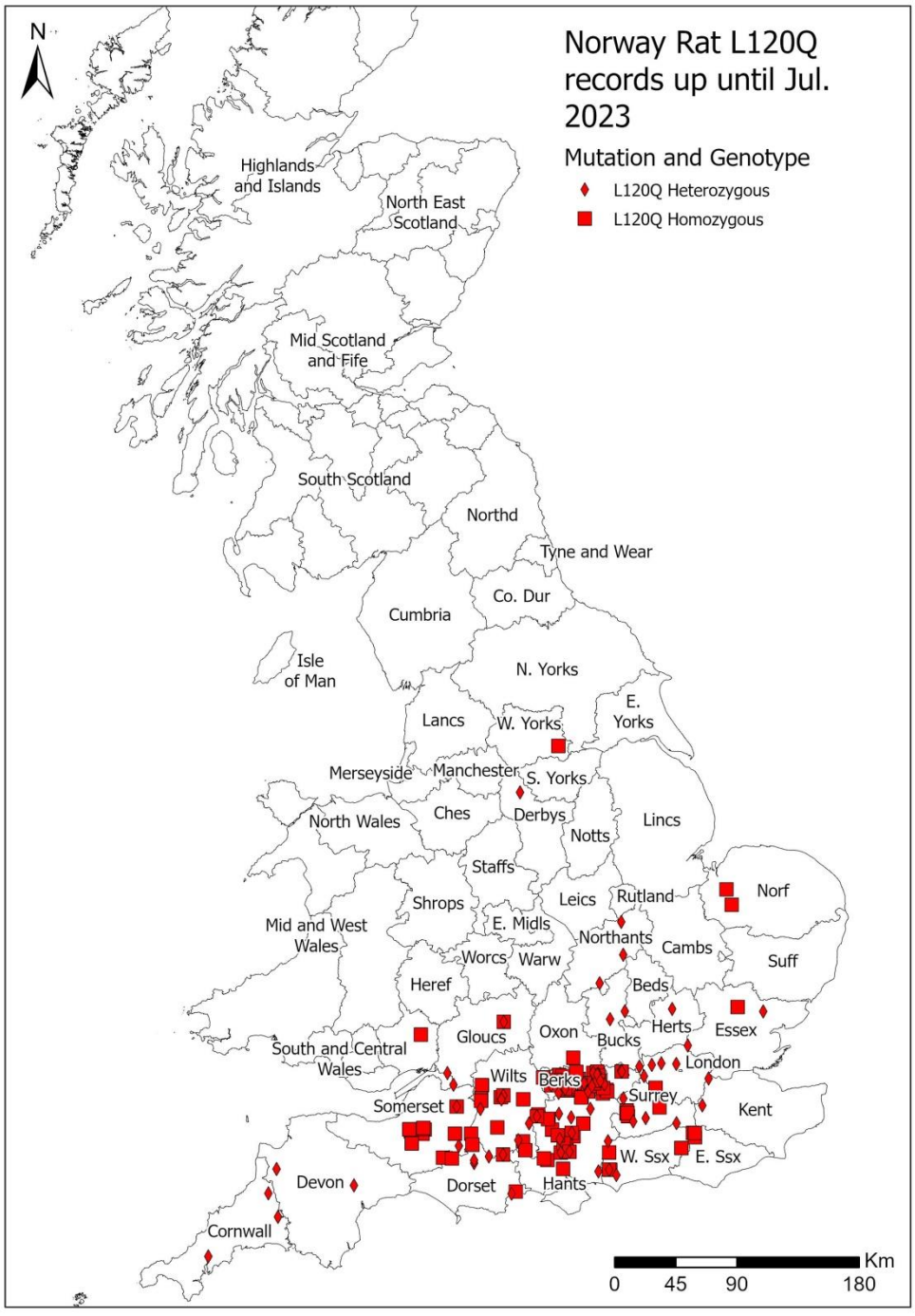
Annex 2. Map showing the geographical locations of Norway rat tissue samples submitted for analysis up to July 2023 which carried the Y139S mutation.



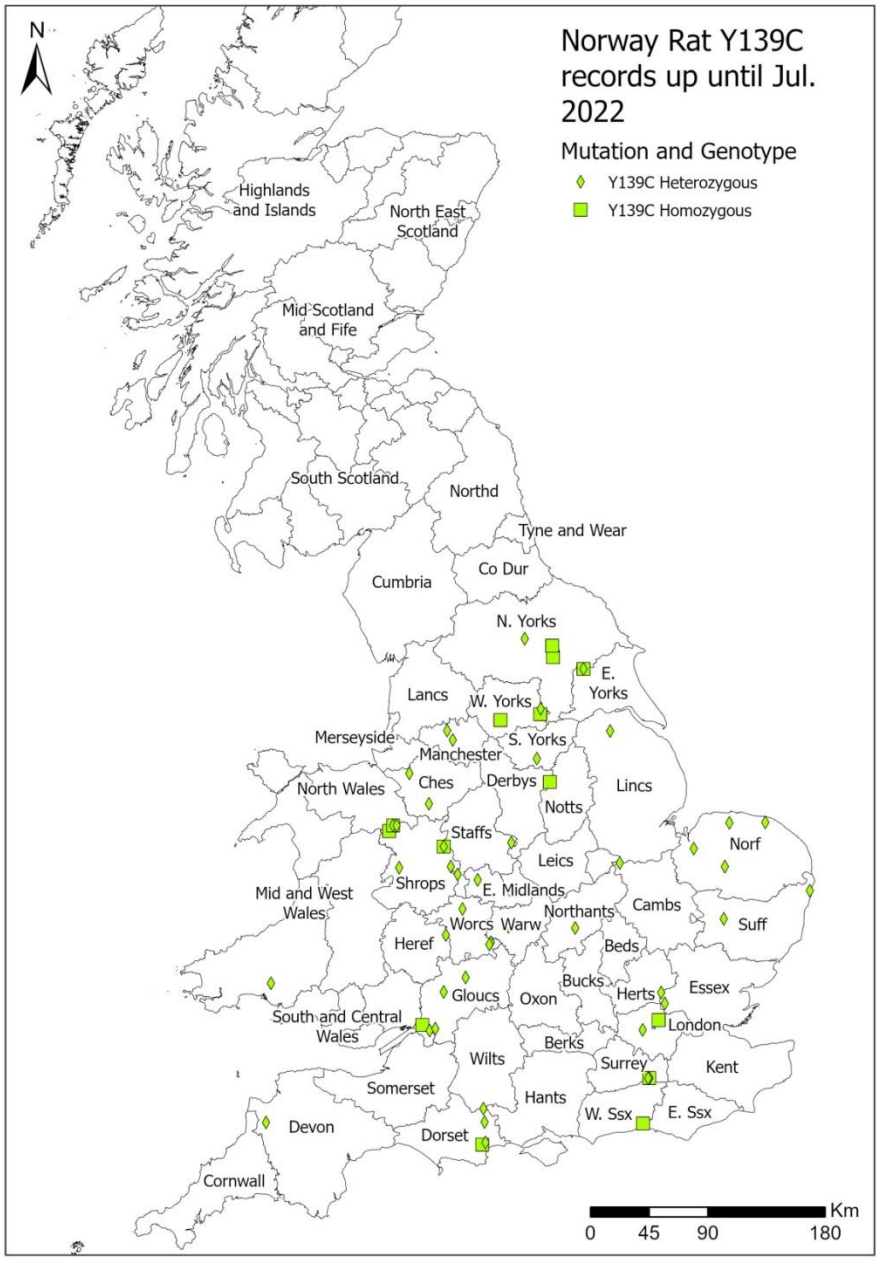
Annex 3. Map showing the geographical locations of Norway rat tissue samples submitted for analysis up to July 2023 which carried the Y139F mutation.



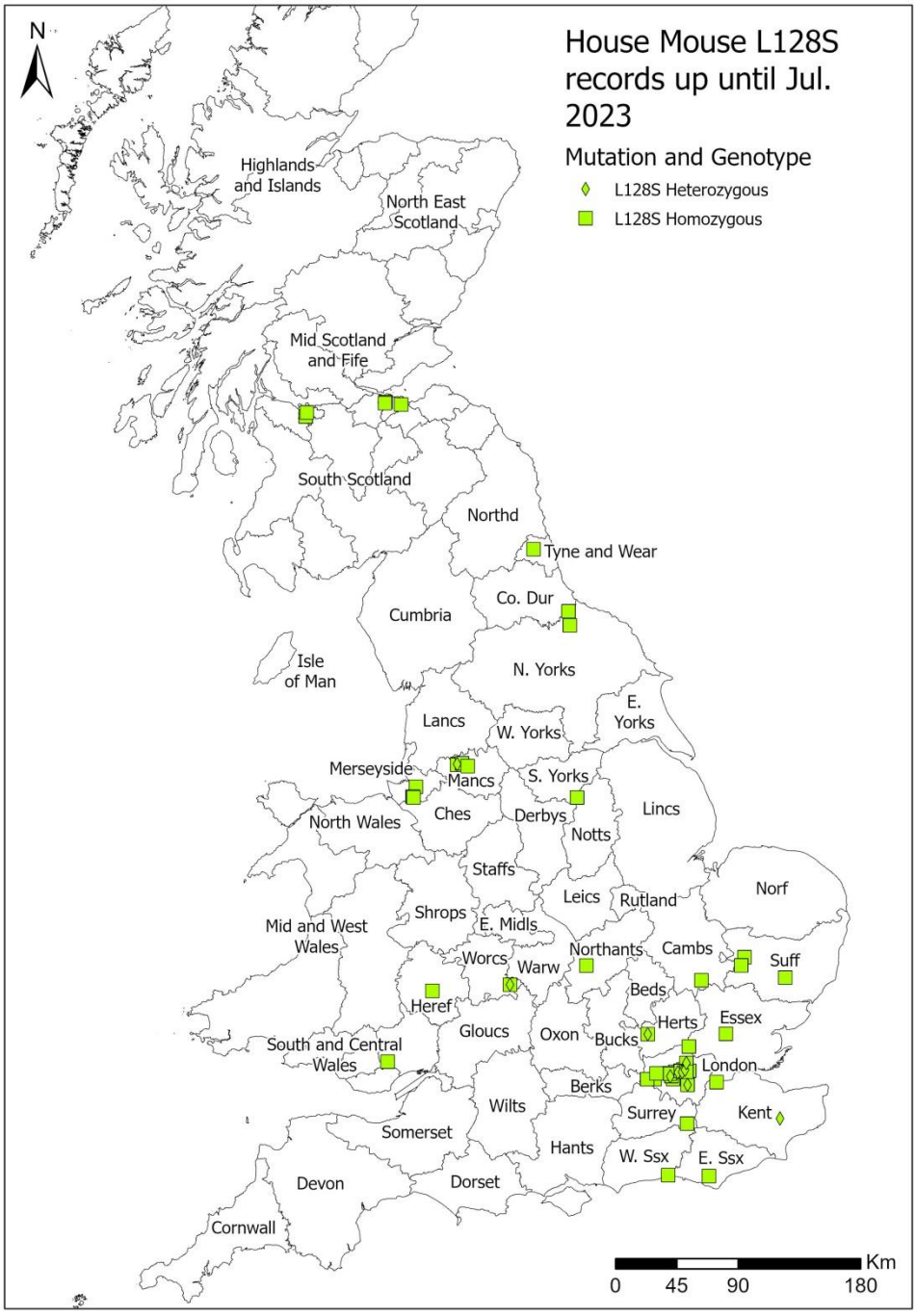
Annex 4. Map showing the geographical locations of Norway rat tissue samples submitted for analysis up to July 2023 which carried the L120Q mutation.



Annex 5. Map showing the geographical locations of Norway rat tissue samples submitted for analysis up to July 2022 which carried the Y139C mutation.



Annex 6. Map showing the geographical locations of house mouse tissue samples submitted for analysis up to July 2023 which carried the L128S mutation.



Annex 7. Map showing the geographical locations of house mouse tissue samples submitted for analysis up to July 2023 which carried the Y139C mutation.