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Anticoagulant Resistance in Rats and Mice in the UK – Summary Report with new data for 2021 and 2022

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Contents:

Summary

1. During the period 2009 and 2022, in which these DNA resistance surveys have been conducted, first at the University of Reading and now at the Animal and Plant Health Agency, a total of 489 Norway rat and 129 house mouse tissue samples have been examined and DNA has been extracted from them and sequenced. Among these samples we found that 77.9% of rats and 94.6% of mice carried one or more single nucleotide polymorphisms (SNPs) 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.
2. Norway rats in the UK carry five different resistance mutations that are known to have adverse consequences for the effectiveness of anticoagulants (Y128Q, Y139S, L120Q, Y139F and Y139C), and house mice carry three such mutations, (L128S, Y139C and the '*spretus* introgression'). The latter having been found for the first time in 2022 in three mice from Hertfordshire.
3. 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. Y139F is found mainly in Kent, East Sussex and Greater London. Y139C is ubiquitous, with no distinctive geographical central focus.
4. However, and 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, either disseminated by natural rodent movement or by human transportation systems. Although, there remains evidence of an area of remnant susceptibility in some counties of the Midlands and on the English north-east coast, but these areas are now increasingly infiltrated by resistance.
5. Fewer house mouse samples are obtained but these show that anticoagulant resistance is also widespread in this species. In this respect, 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.
6. As foci of resistance in both rats and mice spread and overlap there is increasing occurrence of 'hybrid resistance', in which individuals carry more than one different resistance SNP. We know little of the consequences of hybrid resistance on rodenticide efficacy but evidence is emerging from studies of house mice in France that hybrid resistance may render rodents less susceptible to anticoagulants than those that carry only one SNP.
7. 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 of great important to the objectives of the Campaign for Responsible Rodenticide Use (CRRU) and rodenticide stewardship, 3) reduce unnecessary and ineffective emissions of anticoagulants into wildlife and the wider environment.
8. The information presented here should be the subject of a concerted effort of dissemination in an attempt to prevent the purchase of certain rodenticides in areas where there is compelling evidence that their use would be ineffective.

1. Introduction

The use of DNA sequencing to determine the resistance status of rodents is now very well established (Pelz and Prescott, 2015; McGee et al., 2020). Recent studies in Ireland (Mooney et al., 2018), France (Damin-Parnik et al., 2020), Spain (Ruis-Lopez et al., 2022) Finland (Koiviso et al., 2021), Germany (Song et al., 2011) and New Zealand (Cowan et al., 2017) have examined single nucleotide polymorphisms (SNPs) that are associated with anticoagulant resistance in Norway rats (*Rattus norvegicus*), house mice (*Mus musculus*) and roof rats (*Rattus rattus*). However, knowledge of the presence of one or more SNPs, either in individual rodents or populations does not permit understanding of the severity of the resistance conferred by the mutation. For this, other studies are required, both from the laboratory and field (Baxter et al., 2022). Fortunately, due to the very long history of resistance research in the UK, both among rats and mice, we have much of the information needed to understand the consequences for rodent management practitioners of these different rat and mouse SNPs (Buckle, 2013; Pelz and Prescott, 2015; Prescott et al., 2017 and 2018; McGee et al., 2020).

The first national survey of anticoagulant resistance conducted anywhere in the world was in the UK for Norway rats (Greaves and Rennison, 1973). These workers were also able to record, from other laboratory and field studies, that many of the resistant infestations they found were phenotypically distinct. Re-examination of the information presented by these authors (Fig. 1) permits some interesting conclusions. Present on the 1973 map are resistance foci in Scotland, Wales and in three parts of England corresponding to where we know five different Norway rat resistance SNPs presently occur. We can be reasonably certain that the mapped foci in Scotland, on the Anglo-Welsh border, in Kent and in Berkshire (Fig. 1) were sites of early L128Q, Y139S, Y139F and L120Q resistant infestations respectively. The origin of Y139C in the UK is more difficult to discern among the other foci recorded by Greaves and Rennison (1973). However, the resistance they recorded in Gloucestershire is now at the centre of an extensive Y139C focus (Buckle et al., 2020). If these early observations indeed presaged the current biogeography of resistance in the UK, it would mean that the severe resistance SNPs L120Q, Y139C and Y139F, which confer resistance to some less potent second-generation anticoagulant rodenticides (SGARs), developed as the result of exposure to first generation anticoagulants, such as warfarin, and before the SGARs had been invented.

A previous report by Buckle et al. (2020) introduced the term ‘hybrid resistance’. This is where a single individual rodent carries two or more different resistance SNPs. An early report of house mice carrying two or more resistance mutations came from Germany involving the Y139C, L128S and ‘*spretus* introgression’ SNPs (Pelz et al., 2011). The term ‘double resistance’ has been also used to describe this phenomenon (Blažić et al., 2020), although the term would not be applicable if, as is possible hypothetically, rodents carry three or more SNPs. The occurrence of Norway rats with several combinations of the common resistance SNPs in the UK is the first for that species (Buckle et al., 2020).

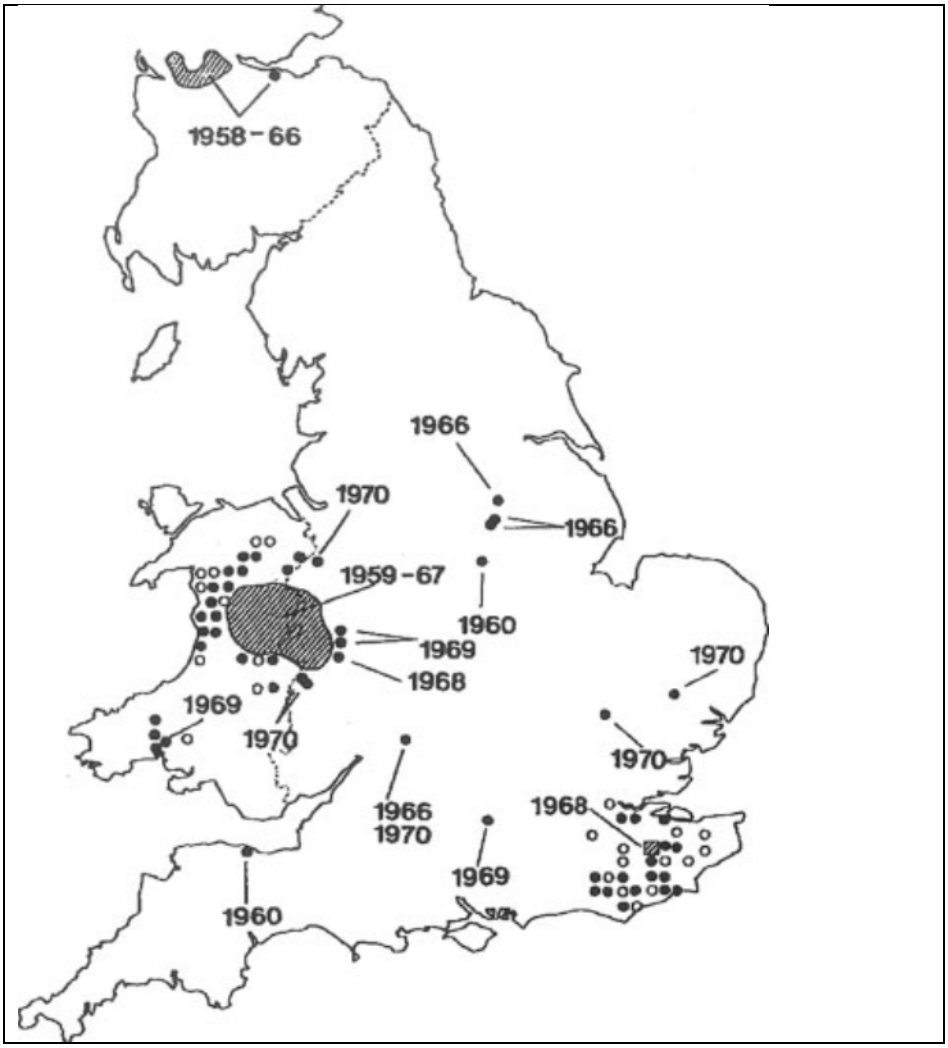
It is a requirement of the Health and Safety Executive (HSE) and the Government Oversight Group (GOG) that the Campaign for Responsible Rodenticide Use (CRRU) UK provides information on anticoagulant resistance among UK populations of Norway rats and house mice in the UK (HSE, 2019). Reports are produced 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 in 2020 and summarised all data available up to

February 2020, when the Vertebrate Pests Laboratory at the University of Reading closed (Buckle et al., 2020).

In order to continue this work, and to satisfy HSE/GOG requirements, CRRU UK entered into an annual contractual arrangement with the laboratory of the Animal and Plant Health Agency (APHA) to provide services of DNA sequencing and analysis. Validation work was done involving the two laboratories to facilitate hand-over. A further arrangement was made with the University of Reading to continue the work of mapping resistance locations using Global Positioning System (GPS) software. The data provided in this report cover two one-year contract periods; the first running from August 2020 to July 2021 and the second from August 2021 to July 2022. The report also summarises the previous work conducted at the University of Reading in the period 2009-2020.

The distribution of anticoagulant resistance in Norway rats and house mice in the UK is, of course, of significant interest and some concern to those who engage in the work of rodent pest management for the protection of 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).

Fig. 1. Map of resistance in UK Norway rats obtained as a result of surveys using feeding tests on rats removed from the field and into the laboratory. Filled circles – resistant samples. Open circles – non-resistant samples. Undated circles – samples tested in 1972. Greaves and Rennison (1973).



2. Materials and Methods

2.1 Origins of samples

The tissue samples analysed for genetical mutations were submitted by pest control technicians, were collected after trapping by staff of the Vertebrate Pests Unit (VPU) at the University of Reading and 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.

During 2019 and 2020 additional effort was expended in obtaining samples from areas of the UK from which samples had not previously been received. This was continued in the present sampling period. The maps presented in previous reports had shown that samples have not been obtained, for example, from a large area in the centre of the country, including many counties of the Midlands. This area is of particular interest because, from the very few samples that have been received, there appears to be a low incidence of anticoagulant resistance among Norway rats. Consequently, calls were put out in the magazines serving the UK professional pest control community asking for samples from these areas (see for example Jones and Talavera, 2019; https://www.thinkwildlife.org/free-tests-and-new-guide-tackle-spread-of-resistant-rats/ . These efforts have been rewarded with more samples obtained from areas not previously studied.

2.2 Methods of DNA analysis

As in the previous studies described above, 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 is shown in Table 1.

Table 1. VKORC1 mutations in Norway rats (NR) and House mouse (HM) in UK. From: Pelz *et al*., 2005; Rost *et al*., 2009; Prescott *et al*., 2010; Pelz and Prescott, 2015; Clarke and Prescott, 2015 unpublished report. Major resistance mutations with known practical consequences shown in bold.

Species	Mutation	Abbreviations	Where present
NR	**Leucine128Glutamine**	**L128Q**†	**Central Southern Scotland, Yorkshire, Lancashire**
NR	**Tyrosine139Serine**	**Y139S**†	**Anglo-Welsh border**
NR	**Leucine120Glutamine**	**L120Q**†	**Hampshire, Berkshire, Essex, Norfolk and elsewhere**
NR	**Tyrosine139Cysteine**	**Y139C**†	**Gloucestershire, Norfolk, Lincolnshire, Yorkshire, SW Scotland and elsewhere**
NR	**Tyrosine139Phenylalanine**	**Y139F**†	**Kent, Sussex, Norfolk, Suffolk**
NR	Argenine33Proline	R33P‡	Nottinghamshire
NR	Phenylalanin63Cysteine	F63C*	Cambridge/Essex
NR	Tyrosine39Asparagine	Y39N*	Cambridge/Essex
NR	Alanine26Threonine	A26T#	Cambridge/Essex
HM	**Tyrosine139Cysteine**	**Y139C**†	**Reading**
HM	**Leucine128Serine**	**L128S**†	**Cambridge**

† Known either from field experiments and/or field experience to have a significant practical effect on anticoagulant efficacy
‡ Known from laboratory experiments to confer warfarin resistance
* Shown in laboratory experiments to have a significant impact on protein function
# Unlikely to confer any significant degree of resistance

2.3 Methods for GIS maps

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. The maps available (see example for the UK at: http://guide.rrac.info/resistance-maps/united-kingdom/) use Google 'heatmap' 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

During the period of its operation from 2009 to February 2020, The Vertebrate Pests Laboratory (VPU) of the University of Reading extracted DNA from 330 Norway rat tissue samples from around the UK. Of these, 75 (22.7%) carried the wild type genome and the remainder (77.3%) carried one or more resistance SNPs. Maps showing the geographical locations from which these samples were sent have been presented previously (Buckle et al., 2020) 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, however, 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 2020-2022

The work of DNA extraction and sequencing began at the Animal and Plant Health Agency in August 2020. Laboratory protocols required that a new and secure system was developed for despatch of the samples by post to the laboratory by those who had collected them. Only information on those samples that could be successfully sequenced is provided in the following paragraphs.

Among the 85 samples (Table 2) that were capable of being sequenced in the period August 2020 to July 2021, a total of 63 (74.1%) were found to carry one of the five main Norway rat anticoagulant resistance mutations (Table 1). The remaining 22 animals (25.9%) carried the wild type genome. Four animals possessed two different resistance mutations, three were hybrid resistant for L128Q and Y139C and one for L120Q and Y139C. Among those rats that carried a single SNP, the severe L120Q mutation was the most common (34%, n=29) in the 2020-21 sample. Of those 58.6% were homozygous for the mutation, indicating a high degree of selection for that SNP among the rat populations, mainly in central southern England.

DNA was extracted and sequenced from 74 Norway rats in the period August 2021 to July 2022. Among these, 13 (17.6%) were wild type and 61 (82.4%) carried one or more resistance SNPs. The frequency of homozygosity among resistant Norway rats in this sample was 42.6%. None of the animals were hybrid resistant. There appeared to be a substantial increase in the number of rats sequenced that carried the L128Q mutation, with marked increase in the frequency of homozygosity (Table 2). There was a decrease in the numbers of recorded cases of L120Q in the 2021-22 sample, and in the frequency of susceptibility.

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	Year	Genotype		Totals
		Homozygous	Heterozygous	
L120Q	2020-21	17	12	29
	2021-22	8	7	15
L128Q	2020-21	6	0	6
	2021-22	15	10	25
Y139C	2020-21	0	16	16
	2021-22	4	13	17
Y139F	2020-21	0	2	2
	2021-22	0	3	3
Y139S	2020-21	0	1	1
	2021-22	0	1	1
totals (mutations)		50	65	115
L128Q and Y139C*	2020-21	0	1	1
	2021-22	0	0	0
L128Q and Y139S*	2020-21	0	3	3
	2021-22	0	0	0
totals (hybrid resistance)		0	4	4
Susceptible	2020-21	22	0	22
	2021-22	13	0	13
totals (susceptibles)		35	0	35

*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

The records given in this report, combined with those from the earlier studies conducted at the University of Reading (section 3.1), provide almost 500 individual observations of the occurrence of anticoagulant resistance in UK Norway rats. This is the largest study of its kind ever undertaken and provides, for the first time, an opportunity to present separate maps showing the

distribution of the five main Norway rat resistance SNPs (Annexes 1-5) and to examine the likely development of resistance foci in the UK over the last 40 years.

A preliminary qualification is necessary however. The collection of samples for the study is conducted in an *ad hoc* manner, without temporal continuity at any site. This means that finding resistance at a site does not mean that resistance had recently arrived there. Nor can it be said with certainly whether the resistance was transported to a particular site either by the natural movement of rodents, or by human agency, or if it started following a *de novo* mutation event. It is useful to know, however, that *de novo* mutations resulting in the resistance SNPs commonly found in the UK appear to be rare genetical events. For example, in spite of the proximity of the two countries, and many zoogeographical similarities, it is noteworthy that none of the five resistance SNPs commonly found in the UK Norway rats so far been found 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). Furthermore, until very 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* mutation events may be relatively rare and this phenomenon is therefore unlikely to be the main cause of the proliferation of anticoagulant resistance that we currently see in UK Norway rats.

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 mean that resistance is present but no sampling has yet been undertaken.

L128Q – Scottish Resistance (Annex 1)

The first UK Norway rat resistance focus, found in 1958, was that in the central belt of Scotland and described by Boyle (1960). Fig. 2 shows the present incidence of all L128Q records so far received. Rats carrying this mutation remain restricted to Scotland and the north of England. L128Q is a relatively weak resistance. All SGARs are fully effective against it and this may be why it has not spread more widely in the UK. Where it occurs, however, it is now found in geographical continuity with several of the other SNPs (Fig. 2) and is often in hybrid resistance with other SNPs (Fig.3).

Y139S - Welsh Resistance (Annex 2)

First discovered in 1959, just a year after the L128Q focus in Scotland, Y139S was the subject of intensive resistance research for more than 30 years. A government laboratory was established at the centre of the focus in Welshpool and the early field work on the efficacy of SGARs against anticoagulants was conducted against populations carrying this mutation. By the time of the publication of the 1973 survey (Greaves and Rennison, 1973), systematic records had been made of the distribution of this mutation and these showed that the focus then covered a very large area on both sides of the Anglo-Welsh border (Fig. 1). In Wales, resistant rats were found on farms westwards to the coast of Cardigan Bay.

It is unlikely that that the focus is smaller now that it was in 1973, in spite of the paucity of records from our DNA studies (Annex 2). This may be due to the fact that Y139S has limited effects on the efficacy of SGARs (Buckle et al., 2021b) and therefore does not cause significant control problems in this resistance focus. There are apparent outlier records in south Wales and Merseyside, but this could be accounted for by a radial spread from the original centre of the

focus over a half century. More interesting is the recent discovery of Y139S rats in North Yorkshire. Attention has been drawn to the fact that, for many years, the laboratories of the Food and Environment Research Agency at Sand Hutton held rats carrying this mutation.

Y139F – Kent Resistance (Annex 3)

A third resistance focus was initially discovered in Kent in the late 1960s. Surveys of infested farmsteads, supported by laboratory resistance testing, allowed the extent of the focus to be determined to include much of Kent and East Sussex. The focus was sufficiently defined to allow field testing of the, then, novel SGAR difenacoum to be conducted in the area (Rennison and Hadler, 1975). However, that was the last work that was done on this resistance until, almost 30 years later, researchers at the University of Reading received a report of the failure of bromadiolone to control an infestation of Norway rats at a poultry farm in Kent. Analysis of the DNA of rats from the farm confirmed the presence of the Y139F mutation (Prescott et al., 2010).

The known extent of the focus is shown in Fig. 4 and closely reflects that found in the Greaves and Rennison survey (Fig. 1). 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.

It is interesting to note that Y139F is the most common Norway rat resistance SNP across the Channel on the northern coast of France, and in Belgium and the Netherlands. It is tempting to suggest that either resistant rats moved from the UK to Europe, or *vice versa*, because of the very extensive transport links between those countries of the EU and the UK at the Straits of Dover.

As was clear from the field reports of field failure of bromadiolone that led to the rediscovery of this focus, Y139F is a severe resistance mutation that compromises the efficacy of the two less potent SGARs bromadiolone and difenacoum.

L120Q – Hampshire/Berkshire Resistance (Annex 4)

A resistance focus was discovered in 1969 in central southern England close to Basingstoke (Greaves and Rennison, 1973). Rats from the focus were taken to the government research laboratory at Tolworth and were the subject of extensive research, both in the laboratory and field for more than 30 years. It was quickly recognised that the resistance phenotype conferred the strongest form of resistance so far discovered. Field trials showed that the early SGARs, bromadiolone and difenacoum, were largely ineffective and even one of the most potent, brodifacoum, was less efficient than expected when used at a concentration of 25 ppm (Greaves et al., 1982).

Molecular work revealed that the SNP present in Hampshire was L120Q and the current extent of the focus is shown in Annex 4. This SNP is now prevalent across the whole of central southern England and it appears prudent to assume, when conducting pest control operations in the area, that Norway rats encountered in the counties of Somerset, Wiltshire, Berkshire, Hampshire, Surrey and West Sussex will carry this mutation. Outlying foci in Devon and Cornwall, across East Anglia and in Gloucestershire, are close enough to the main focus to be accounted for by radial spread over a period of more than 50 years. It is apparent that these are foci usually composed of heterozygous animals, suggesting a recent origin. While the new foci in the east and west of England may be explicable by natural dispersal, the focus found in West Yorkshire is perhaps far enough from the main focus to require different explanation.

Y139C – Gloucestershire Resistance (Annex 5)

Although it seems likely that the resistance focus found in Gloucestershire in 1966 was populated by rats carrying the Y139C mutation, the frequency of the mutation in early University of Reading DNA studies was relatively low (Buckle et al., 2020). Sufficiently low, indeed, to permit reports of each novel location as it was found. However, the frequency of this SNP appears to have substantially increased in the period 2020-2022, with thirty-nine new Y139C locations discovered among samples received in just in two years.

There is no obvious explanation for the apparent rapid acceleration of the spread of this resistance mutation. However, it is apparent in the map shown at Annex 5 that Y139C can now be found almost anywhere in England and Wales. 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., 2013), makes these new findings potentially problematic for pest management practitioners over a very wide area of England and Wales.

Norway rat - Consolidated findings 2009 to 2022

Maps showing the occurrence of the individual mutations (Annexes 1-5) are amalgamated and presented on the map shown at Fig.2. With almost 500 individual resistance locations shown, the map has become somewhat congested. However, some general conclusions can be made.

With the exception of some isolated occurrence of L120Q and Y139C, Scotland and the north of England presently appear to be 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 in that country. 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 this mutation, although bromadiolone may be somewhat less effective (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 to be more widespread.

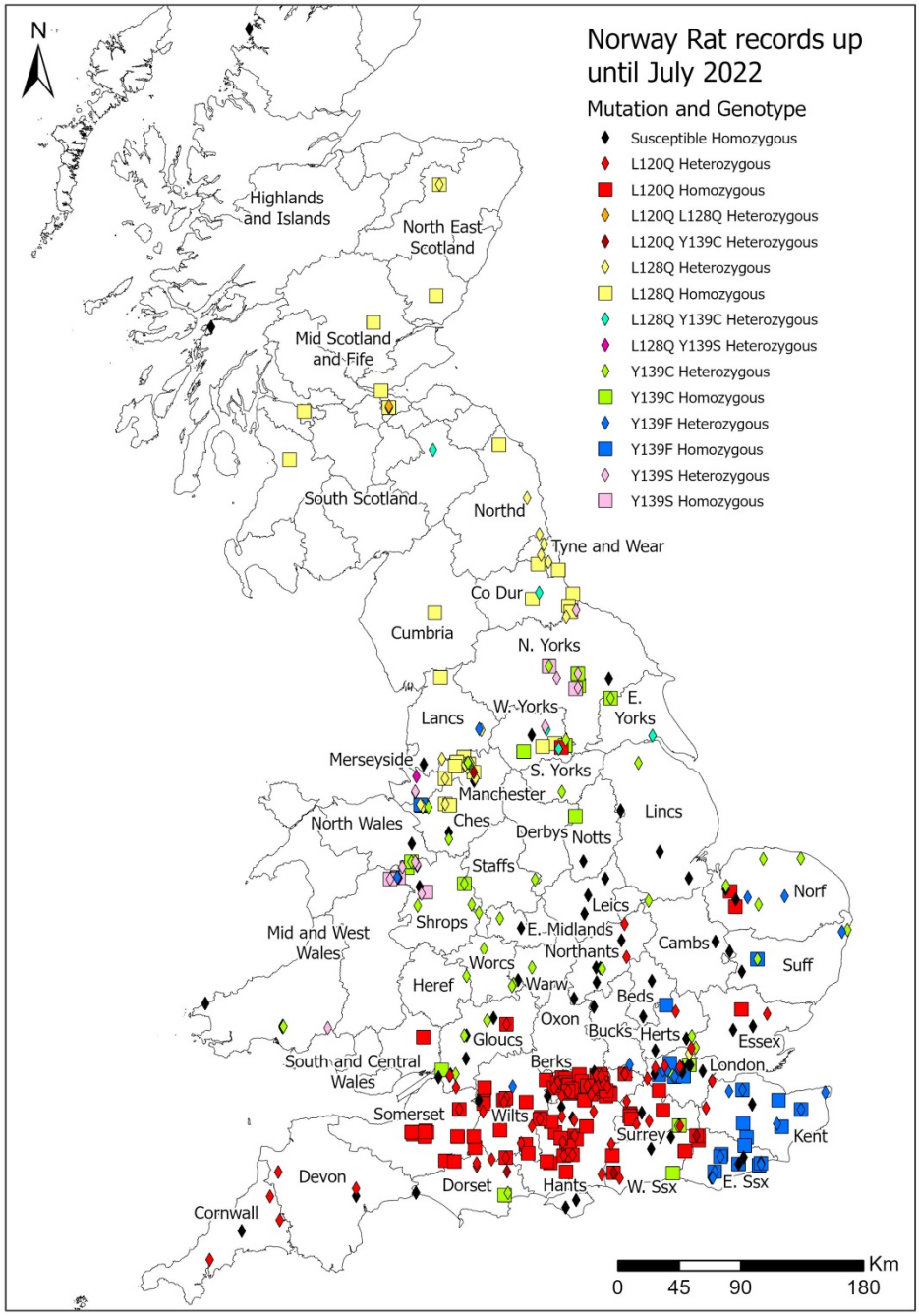
The situation is quite different in the south of England, where the severe L120Q and Y139F mutations are both very frequent and widespread. It is interesting to note that although the L120Q and Y139F SNPs predominate in the south, the other severe resistance mutation Y139C is beginning to occur with 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, indicating more recent spread into the counties of Devon and Cornwall not seen in earlier surveys.

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 found to be wild type (i.e. susceptible). However, in recent surveys, and particularly over the last two years, there is an

increasing infiltration of heterozygous Y138C, Y139F and L120Q animals. It therefore appears that some substantial remnant areas of susceptibility may not continue for much longer.

Fig. 2. 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 for 2009 to 2022.



Norway rats – Hybrid Resistance

The first Norway rat with a genome that contained two different resistance SNPs was found just outside Edinburgh in 2017. The animal was heterozygous for both L128Q and L120Q mutations. The former was to have been expected, because L128Q is commonly found in the area, but the latter was not anticipated as the nearest L120Q focus was, at the time, almost 300 miles away in Norfolk.

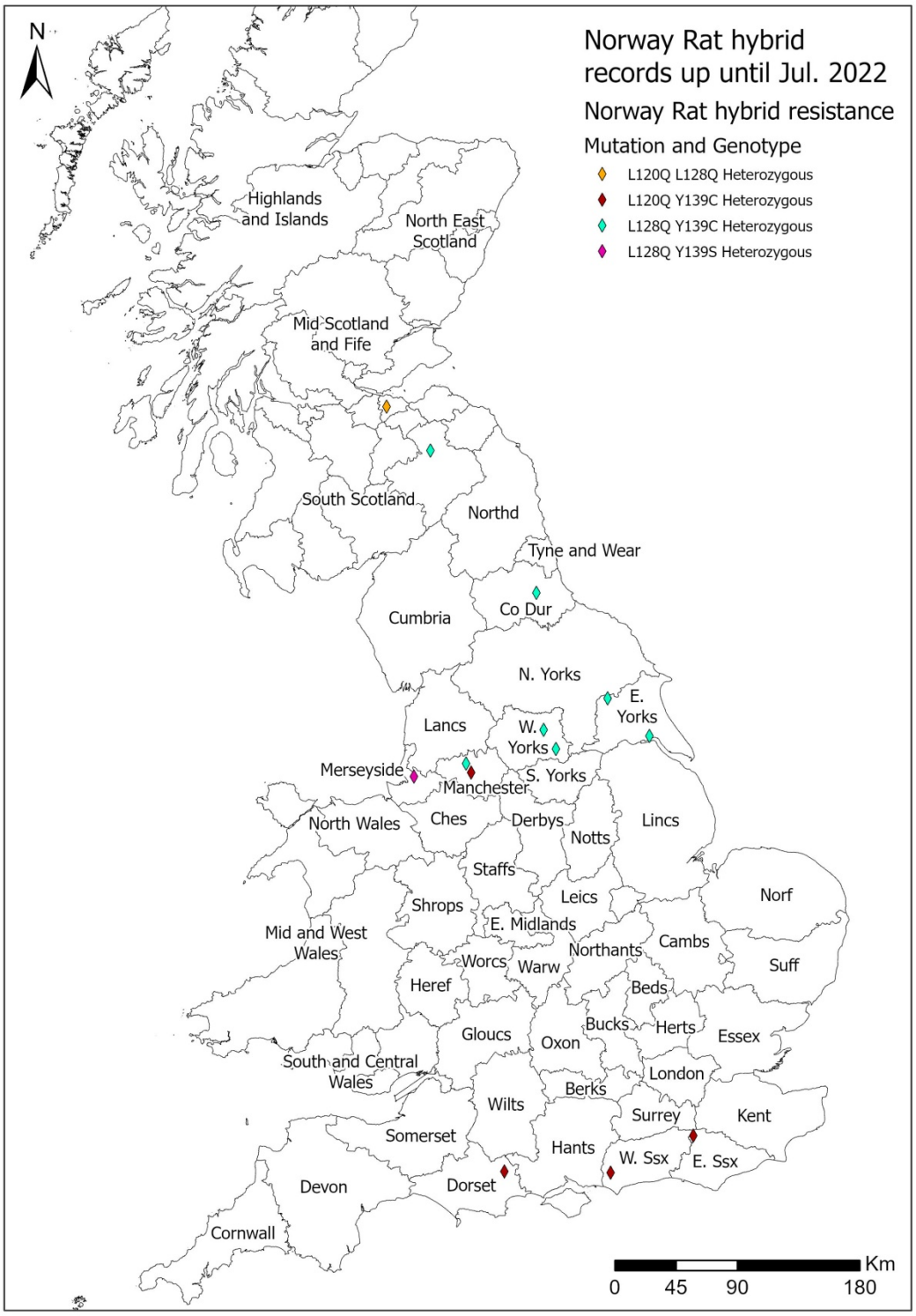
No other hybrid mutants were found until eight were discovered in the relatively small sample collected in 2020 (Buckle et al., 2020). Among these were animals carrying three new mutation combinations. The L120Q/Y139C combination was found in animals from the very widely separated counties of Dorset, East Sussex and East Lancashire. L128Q/Y139C animals were found in East Lancashire, both West and East Yorkshire and County Durham. Finally, a single L128Q/Y139S animal was found in Merseyside.

Further occurrence of hybrid resistance is seen in the sample from 2020-2021, in which one animal carried both the L128Q and Y139C mutations and three were L128Q/Y139S hybrid resistant. It is interesting to note that, although the L128Q SNP is restricted in distribution to Scotland and the north of England, it is frequent in cases of hybrid resistance there.

The distribution of hybrid resistant Norway rats in the UK is shown in Figure 3. These findings have identified four different hybrid resistances occurring in UK Norway rats, out of 10 different possible combinations. Those found are as follows: L128Q/L120Q, L128Q/Y139C, L128Q/Y139S and L120Q/Y139C. Of these L128Q/Y139S appears to be presently the most frequent and widespread. Only the Y139F SNP has not been found in hybrid form and this is somewhat surprising given the large interface in south-eastern England of this SNP and the L120Q mutation (Fig. 2). Unlike the situation in house mice (see below), no Norway rats have so far been found with a homozygous genome for one SNP and in combination with a second.

The consequences of hybrid resistance for the efficacy of anticoagulants used against Norway rats in the UK will be discussed elsewhere in this report.

Fig. 3. Map showing all Norway rats found to carry two different anticoagulant resistance SNPs (i.e. hybrid resistance). Data for 2009 to 2022.



3.4 House mice – historical records

Samples of house mouse tissue are received much less frequently that those of Norway rats. This is somewhat surprising given the fact that resistance is more prevalent in the former species. The reasons are uncertain but may be because house mice are less frequently encountered, although this seems unlikely. There is also a general perception that mice are less serious pests than are rats, and therefore a failure to control a rat infestation may provide greater incentive to submit tissue samples for resistance testing. Another possible explanation is that pest control practitioners more frequently use the most potent anticoagulant substances, probably most often brodifacoum, which are effective against the mouse resistance SNPs found in the UK. This in turn may be because mouse infestations are usually indoors and these products can therefore be used more extensively against them, with less risk to wildlife and the environment.

Whatever the reason for the relatively small numbers, only a total of 93 mouse tissue samples was received during the period 2009 to 2020 (Buckle at al., 2020). 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. Maps of the distribution of resistance show that the Y139C mutation is largely restricted to the south-east of England, while L128S is more ubiquitous. Within the sample 93 individuals, 87 house mice carried one or both SNPs, giving a very high frequency of resistance among UK house mice of 93.5%. This frequency of resistance has led the Rodenticide Resistance Action Group to make the recommendation that those who use anticoagulant products against house mice in the UK should assume that all infestations are resistant (Buckle et al., 2021a).

3.5 House mice – Records for 2020-2022

Seventeen house mouse tissue samples were received in the period August 2020 to July 2021. However, five of these came from a single correspondent in Devon and seven from one in Suffolk. Other than these, single samples were received from Edinburgh, Lancashire, London, Somerset and Essex. The frequency of resistance in the samples received was 100%. Seven mice carried the L128S mutation and four carried Y139C. Surprisingly, no fewer than six (35.3%), two from Suffolk and four from Devon, carried both mutations. It may have been that difficulty in getting control of the infestations that contained these hybrid resistance animals caused the correspondents to send samples. Indeed, the samples from Devon came with an anecdotal report that the potent SGAR difethialone had failed to provide control of the infestation containing hybrid resistant individuals.

The sample from August 2021 to July 2022 contained tissue from 19 house mice and none were susceptible. A total of 12 mice carried the L128S SNP alone, six carried only the Y139C SNP and one was hybrid resistant for both.

Again, two correspondents provided multiple samples; four from Midlothian and three from Hertfordshire. All of the Midlothian mice were homozygous L128S resistant, indicating a long-established resistant population. The mice from Hertfordshire were even more interesting however. All carried what has become known as the '*spretus* introgression'. This is the translocation of a series of four linked mutations (R12W/A26S/A48T/R61L) that had, at some time in the past, transferred by interbreeding from the Iberian mouse species, *Mus spretus*, into *Mus musculus* (Song et al., 2011). Mice carrying this introgression are now found in Germany (Pelz et al., 2011) and are prevalent in Spain (Song et al., 2011; Ruis-Lopez, 2022). This is the first occurrence of the *spretus* introgression in house mice in the UK. Two of the *spretus* mice

were heterozygous for the introgression; one of these was also heterozygous for L128S and the other homozygous. The third mouse was homozygous for the introgression and wild type for Y139C and L128S. Hybrid *spretus*/L128S resistant mice also occurred among the samples of Pelz at al. (2011) in Germany. The facility in which these mice were taken was a large warehouse receiving, among other things, bulk shipments of bird seed. It seems likely that these *spretus* mice arrived from Europe with these imported products, although this remains speculation.

3.6 House Mice – geographical distribution of SNPs

Introduction

Early laboratory work on resistance in house mice was conducted with colonies established using animals taken from the vicinity of Cambridge (Rowe and Redfern, 1965). It was subsequently found that these mice carried the L128S mutation (Pelz et al., 2005). Much later, mice were found near Reading which appeared to be anticoagulant-resistant, but showed a higher degree of resistance than those known previously in the UK (Prescott, 1996). Once again, a laboratory stock was established for resistance studies and these animals were later found to carry the Y139C SNP (Pelz et al., 2005). These are the two major house mouse resistance mutations found across Europe (Pelz and Prescott, 2015) and, until the recent discovery of mice with the *spretus* introgression in Hertfordshire, were the only ones found in the UK.

L128S – Cambridge Resistance (Annex 6)

Resistance in UK house mice was first found near Cambridge and it soon became obvious that resistance was very widespread (Rowe and Redfern, 1965). Mice from Cambridge were used to found the first laboratory strains on which much early house mouse resistance research was conducted (e.g. Hadler, Redfern and Rowe, 1974) and it was later established that the strain carried the L128S SNP (Pelz et al., 2007).

Since DNA testing began in 2009, we have slowly established a picture of the distribution of the L128S SNP and it is apparent from the map that this mutation is present across England (Annex 6). However, its absence from any particular area in not necessarily evidenced by our data, merely the fact that we have limited samples from very large areas and, in particular, Scotland, Wales and the West Country.

Because of the work in London of a member of RRAG, Adrian Meyer, we have received a substantial number of samples from that city. These show the presence of L128S in London and the high frequency of homozygosity among the samples indicates that the resistant populations are well established.

Y139C – Reading Resistance (Annex 7)

For some time it was apparent that the Y139C mutation was restricted to the south and south-east of England; with again a high degree of resistance and homozygosity in samples from London. More recently, however, homozygous Y139C individuals have been found on the south-west coast of Scotland and a single heterozygous animal was found in Derbyshire. The lack of samples from other areas makes it impossible, therefore, to draw any firm conclusions about the geographical distribution of Y139C-resistant house mice.

The* 'spretus *introgression' (Annex 8)

Annex 8 shows the position of the first occurrence in the UK of this series of four linked mutations known as the '*spretus* introgression' from a warehouse in Hertfordshire.

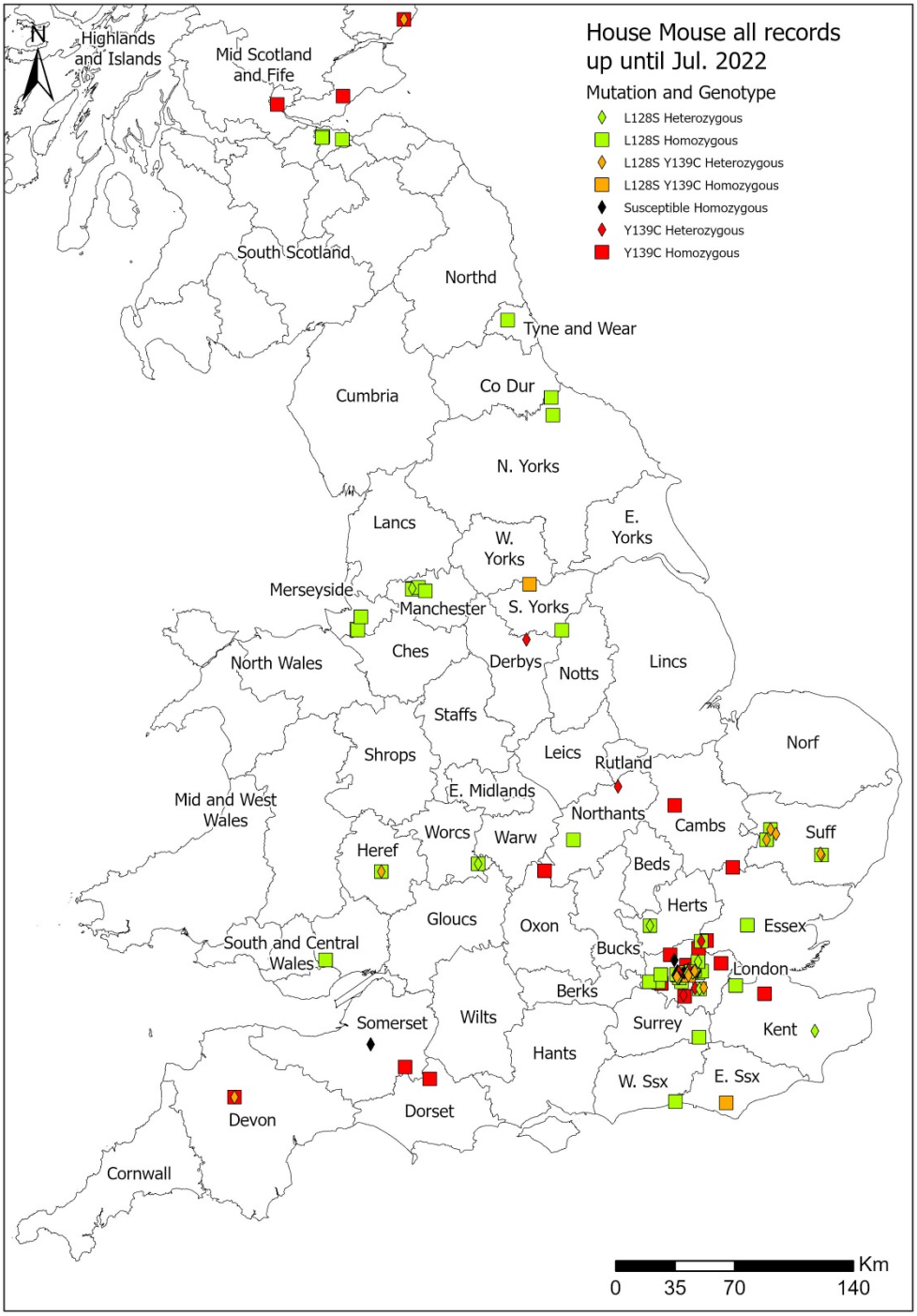
House mice - Consolidated findings 2009 to 2022

A total of 129 house mouse tissue samples have been sequenced for DNA resistance mutations during the period 2009-2022. Among these, 122 (94.6%) were found to carry one or more resistance SNP. The geographical distribution of these records is shown in Fig. 4. The frequency of resistance among UK house mice has resulted in the RRAG recommendation that practitioners should assume at the outset of treatments that all UK house mouse infestations are anticoagulant resistant (Buckle et al., 2020a).

House mice – hybrid resistance

The widespread distribution of the two main SNPs, L128S and Y139C, has led to frequent hybridisation and the appearance of 'hybrid'-resistant individuals (Fig. 4). A relatively large number of samples have been received from the London area thanks to liaison work between the pest control service departments of some of the London boroughs. Analysis of these samples indicates that the two SNPs L128S and Y139C are present in London, with many homozygotes, as well as hybrids of the two. Some of the hybrids found in London are homozygous for L128S and heterozygous for Y139C. Recent work has shown that homozygous Y139C mice are more resistant to anticoagulants than heterozygotes (Baxter et al., 2022). Furthermore, anecdotal evidence from a respected source has indicated that some mice in London are difficult to control with the advanced SGAR difethialone. This may be exacerbated by the fact that baits containing this substance are sold only at the concentration of 25 ppm.

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). Data for 2009 to 2022.



4. Discussion

The 489 Norway rat and 129 house mouse tissue samples that underwent DNA extraction and sequencing for this study at the University of Reading and APHA laboratories makes this the largest survey of anticoagulant resistance ever conducted. As stated previously, the frequency of resistance revealed in this survey, which was 77.9% for Norway rats and 94.6% for house mice, is not necessarily a reflection of the true incidence of resistance in Norway rat and house mouse populations in the UK. It does, however, indicate levels of resistance among infestations in which pest control practitioners were currently experiencing control problems. Similarly, the techniques used in this study do not tell us when resistance arrived in the locations where we find it, but merely confirm it to be present when the samples were collected. There is no evidence however, either from the UK or elsewhere, that resistance recedes once it has taken hold.

For the first time, the large numbers of Norway rat samples obtained permit a reasonably comprehensive assessment of the UK disposition of the five main resistance SNPs (Annexes 1-5). The early work of Greaves and Rennison (1973), using quite different survey techniques, plotted the outset of some of these resistance foci and several, such as those in Kent and East Sussex, central Scotland and on the Anglo-Welsh border, that were already widely disseminated (Fig. 1). We now know that these foci were populated by Norway rats carrying the Y139F, L128Q and Y139S mutations respectively. All of these resistances are still present in the UK, although their evolution in the following years has been somewhat different.

The spread of resistance can take place via several different mechanisms. Firstly, novel mutation events may occur in the genomes of individual rodents during (now) well-understood genetic processes (see Pelz and Prescott, 2015; McGee et al., 2020). If these mutations confer an evolutionary advantage, as is the case with anticoagulant resistance during the widespread application of resisted anticoagulants, individuals carrying the mutations will predominate in populations over susceptible individuals because of that advantage. However, although, evidently, many resistance SNPs were already present in UK rat and mouse populations in the early 1960s (Greaves and Rennison, 1973), it seems likely from their comparative rarity in other countries that these mutation events are relatively infrequent.

A second mechanism of resistance spread is, of course, driven by the natural mobility of individual rodents. The spread of the Welsh resistance focus was studied intensively and the speed of resistance spread by this means was calculated to be about 4.8 km/year (Greaves and Rennison, 1973). Of course, resistant rodents entering receiving populations only thrive when, once again, resisted anticoagulants are used against them and their resistance is therefore a selective benefit. They are at a disadvantage in the absence of anticoagulant use because several resistance mutations confer pleiotropic costs on animals that carry them (Pelz and Prescott, 2015). This cost is principally a greater requirement for dietary vitamin K (McGee et al., 2020). However, given the very long period of time between the Greaves and Rennison study and our own, it is apparent that any resistance mutation might have appeared almost anywhere in the UK by this mechanism alone. Nevertheless, our maps (Annexes 1-8) also show in some cases very long distances between the edge of a known, and probably expanding, resistance focus and the establishment of a new and isolated occurrence of resistance. This then suggests a third mechanism – that of spread facilitated by human agency, most likely unintentional transportation. This mechanism would be more likely to explain new house mouse resistance foci than novel rat foci because the much smaller species is more easily concealed and overlooked. The present distributions of the Norway rat and house mouse resistance mutations are each the result of one or more of these mechanisms. The appearance for the first time of the *spretus* introgression adds yet

another resistance SNP to the extensive list of them occurring in UK Norway rats and house mice.

The appearance of hybrid resistance, first found in Norway rats in Scotland in 2017, signalled the occurrence of a phenomenon that was to be expected given the extensive and expanding UK resistance foci shown in the maps in this report. Resistance foci, at first discrete, expand, as first seen with Y139S in Wales, and then coalesce when their margins overlap. Individuals carrying two different resistance mutations meet and interbreed. The resultant recombination of their genomes in their offspring inevitably means that some of the young carry both resistance SNPs borne by their parents. The term 'hybrid resistance' has been used to describe this process (Buckle et al., 2020).

This is now happening more widely in the UK in both Norway rats (Fig. 3) and house mice (Fig. 4). Among the ten possible combinations of the five main rat resistance mutations, four are now found (section 3.4). Some of these are found in expected areas where it is known that the two mutations exist. Others are entirely unanticipated, such as the L128Q/L120Q found in Scotland, where the nearest known L120Q focus is several hundreds of miles away. This may, of course, be explained by the occurrence of unknown foci in the areas in between.

Of course, only one hybrid is anticipated in UK house mice and that is Y139C/L128S. This is now found in widely separated parts of the UK. London appears to be a 'hot spot' for this hybrid and this is known because of the large numbers of samples received from some London boroughs. If the occurrence of house mice carrying the *spretus* introgression in the UK in 2022 was surprising, even more unexpected was that one of the first two *spretus* introgression mice found in Hertfordshire was a hybrid also with homozygous L128S. These hybrids, however, appeared frequently in the samples from Germany collected by Pelz et al. (2011).

The consequences of hybrid resistance for the efficacy of anticoagulant rodenticides are 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; McGee et al., 2020). 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; McGee et al., 2020). This work was done using novel *in vitro* resistance testing techniques and the authors claim that their findings are consistent with other, better-known resistance testing technologies, such as laboratory feeding tests and blood clotting response tests. However, more work is needed to confirm that this initial assertion is robust. There are, however, anecdotal reports, made to the RRAG by trusted and experienced technicians, of apparent failures of baits containing difethialone against house mouse infestations in Devon and London known to contain Y139C/L128S hybrids.

Although more work is required to confirm these field observations, the resistance specialists of the RRAG have devised some precautionary advice for those practicing rodent pest management in the UK who find that the infestations they are treating contain hybrid resistant animals (Annex 9). This advice involves mainly avoiding the use of both first-generation anticoagulants and the lower potency, and more frequently resisted, second-generation anticoagulants bromadiolone and difenacoum, using baits that carry the maximum available concentration of the active substances and using non-anticoagulant rodenticides and non-chemical methods.

The 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 all those who use rodenticides in the UK. The use of anticoagulants against rodent populations that are resistant to them has three very important adverse consequences: 1) the speed of removal of treated infestations is reduced, with obvious 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 remain, and 3) resistant rodents survive for long periods after unsuccessful treatments carrying high body burdens of persistent anticoagulants, which may be taken subsequently by non-target predators and scavengers. This latter consequence is of particular importance to the work of CRRU UK. It is for these reasons that it is essential that an intensive and concerted effort is made 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), to disseminate resistance management advice to all rodenticide users and to avoid the sale of resisted substances in those areas where it is known, with a high degree of confidence, that rodents resistant to them predominate.

5. Acknowledgements

The authors wish to acknowledge with grateful thanks the work of the former staff and students of the Vertebrate Pests Unit, the University of Reading, including Emily Coan, Clare Jones, David Rymer and Montse Talavera, who coordinated and publicised the sampling operation, established and conducted laboratory DNA extraction and sequencing, and interpreted, recorded and reported the results of these studies over the period 2009 to 2020.

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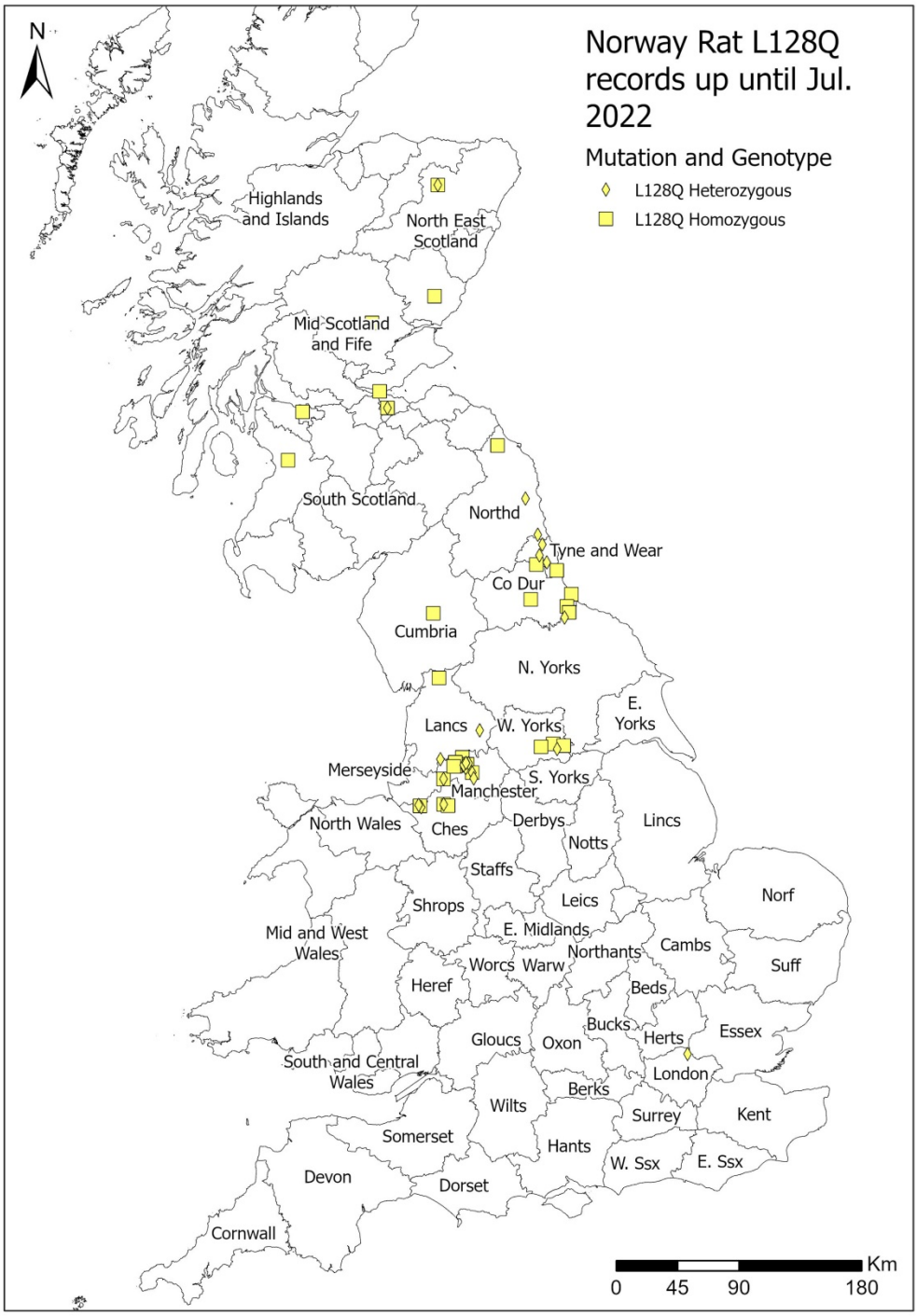
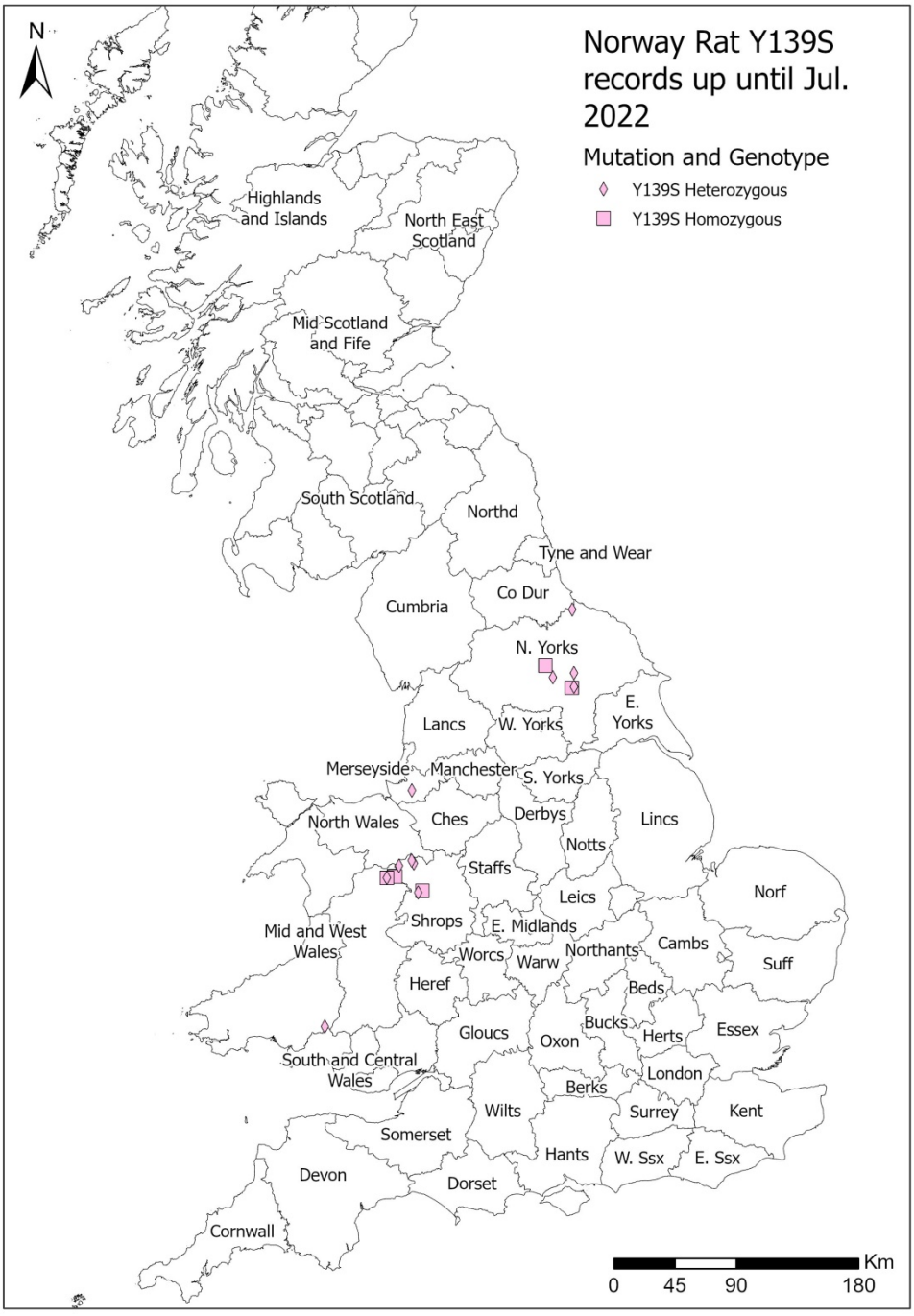
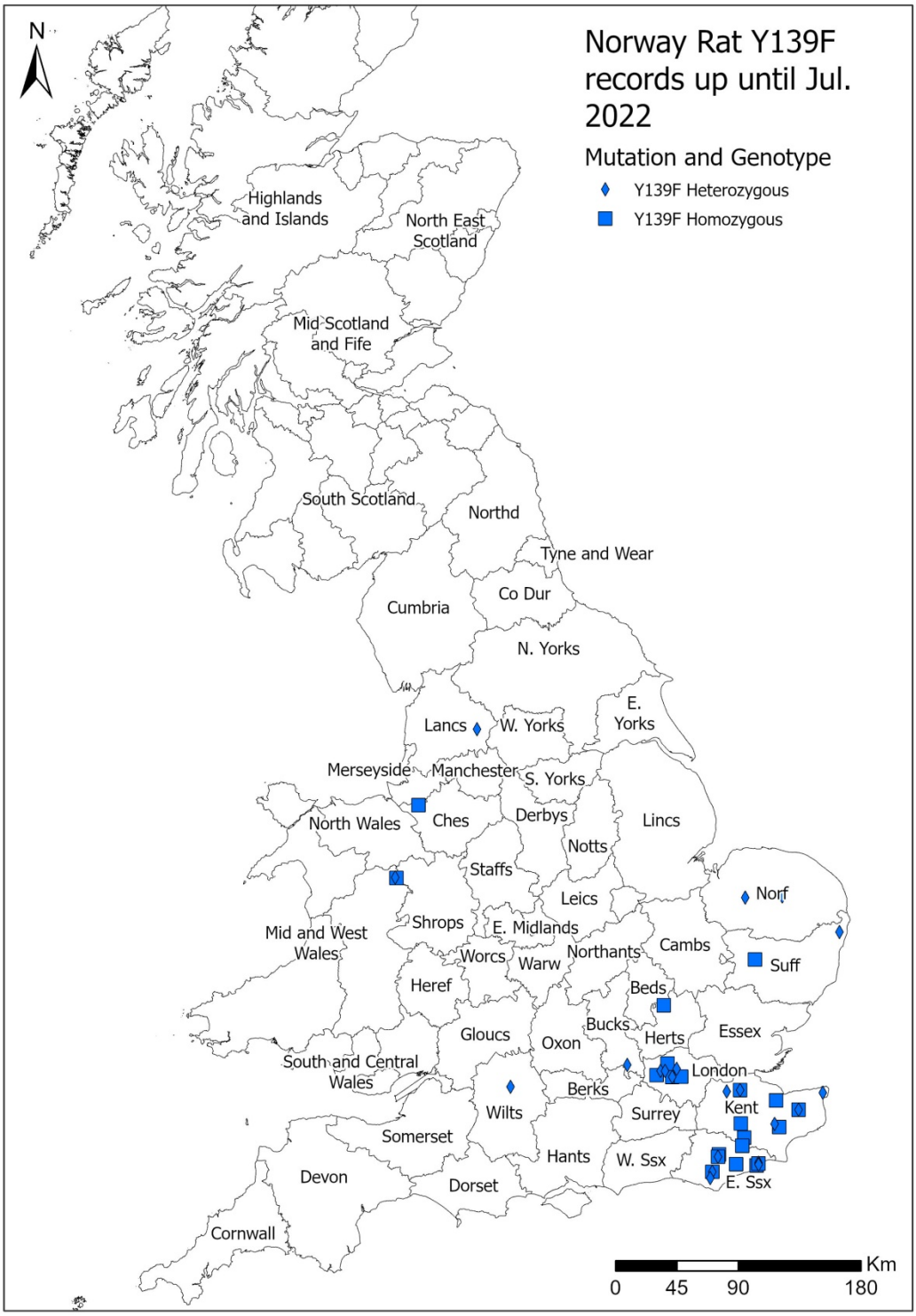
Annex 1. Map showing the geographical locations of Norway rat tissue samples submitted for analysis up to July 2022 which carried the L128Q mutation.



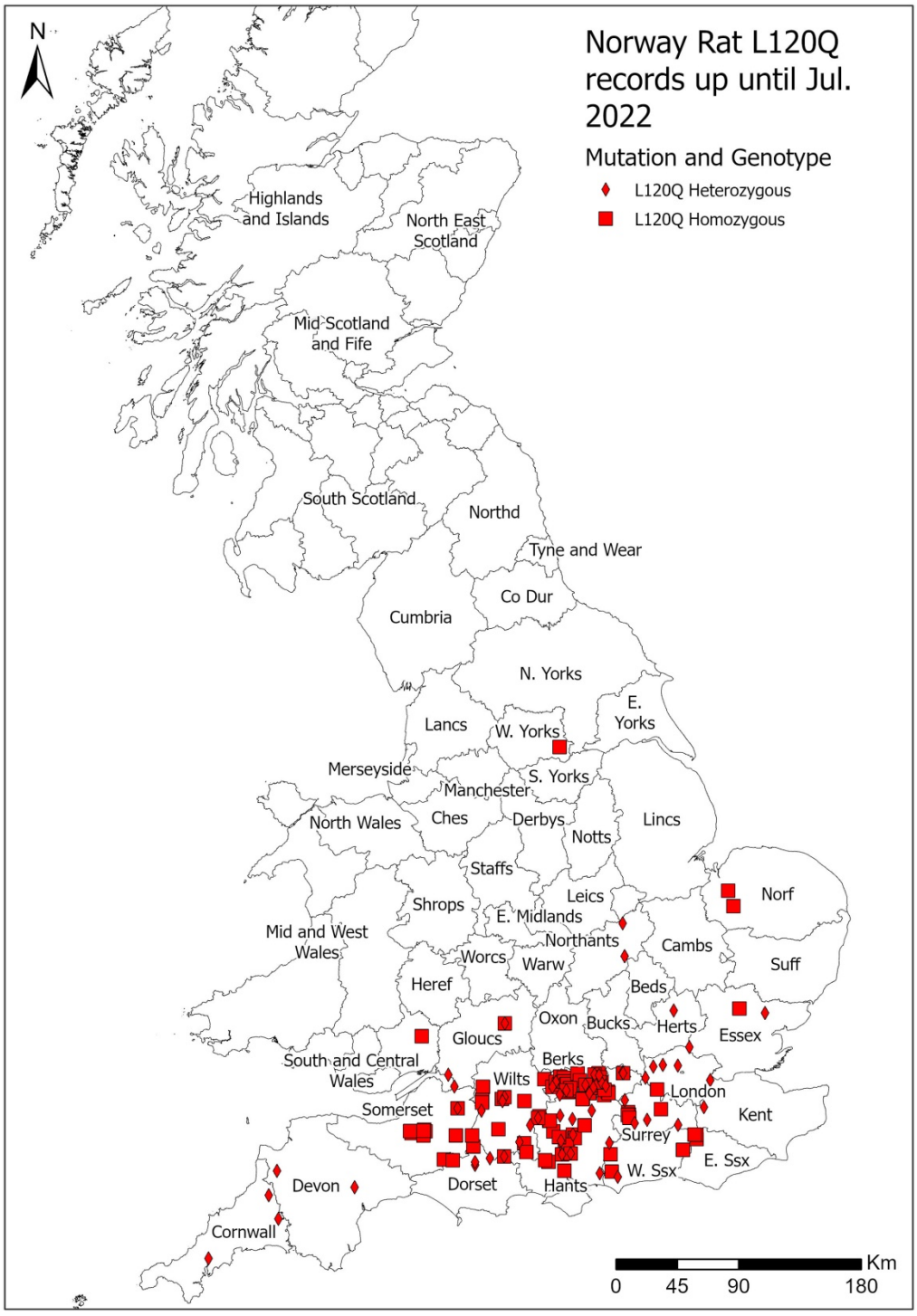
Annex 2. Map showing the geographical locations of Norway rat tissue samples submitted for analysis up to July 2022 which carried the Y139S mutation.



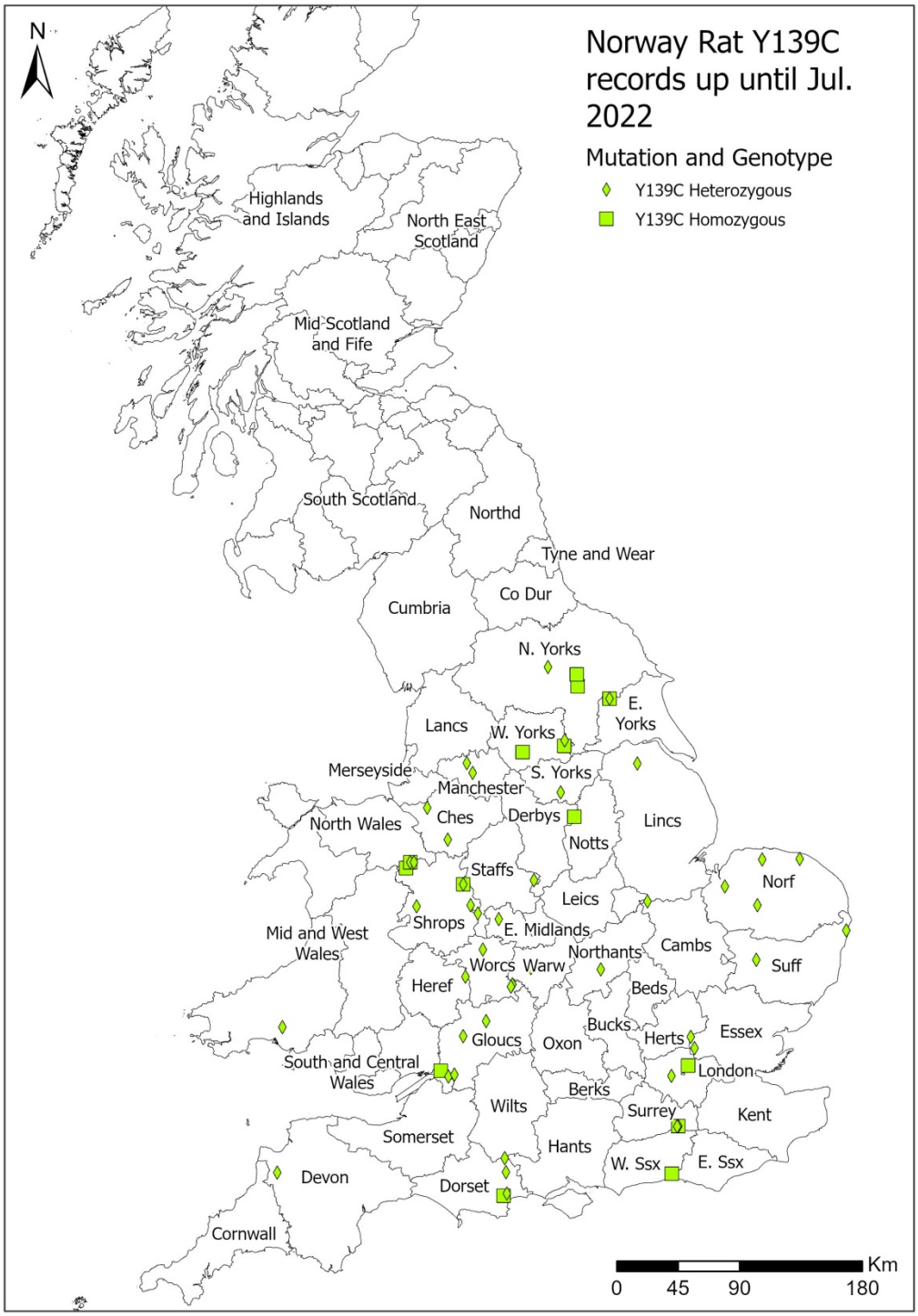
Annex 3. Map showing the geographical locations of Norway rat tissue samples submitted for analysis up to July 2022 which carried the Y139F mutation.



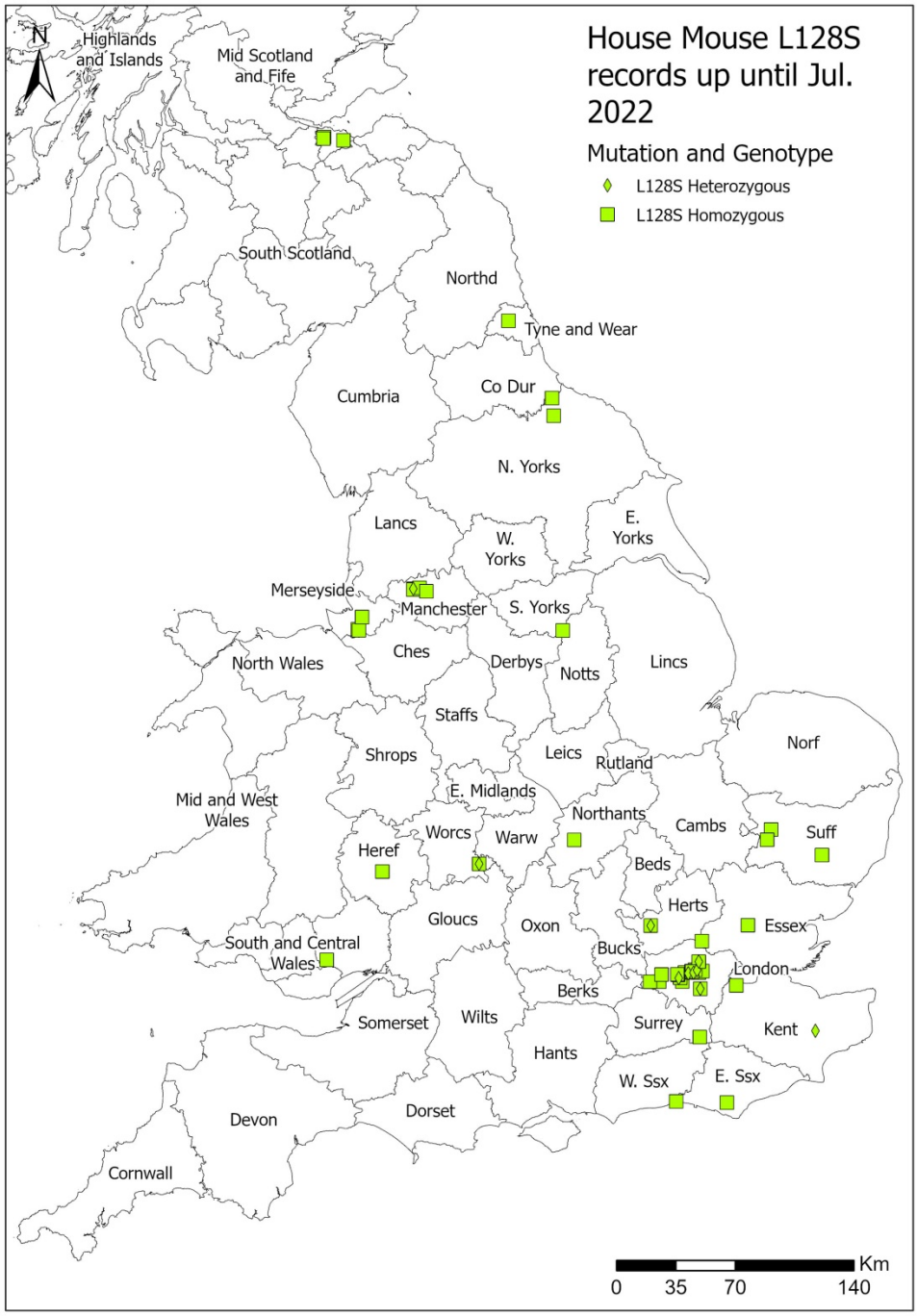
Annex 4. Map showing the geographical locations of Norway rat tissue samples submitted for analysis up to July 2022 which carried the L120Q mutation.



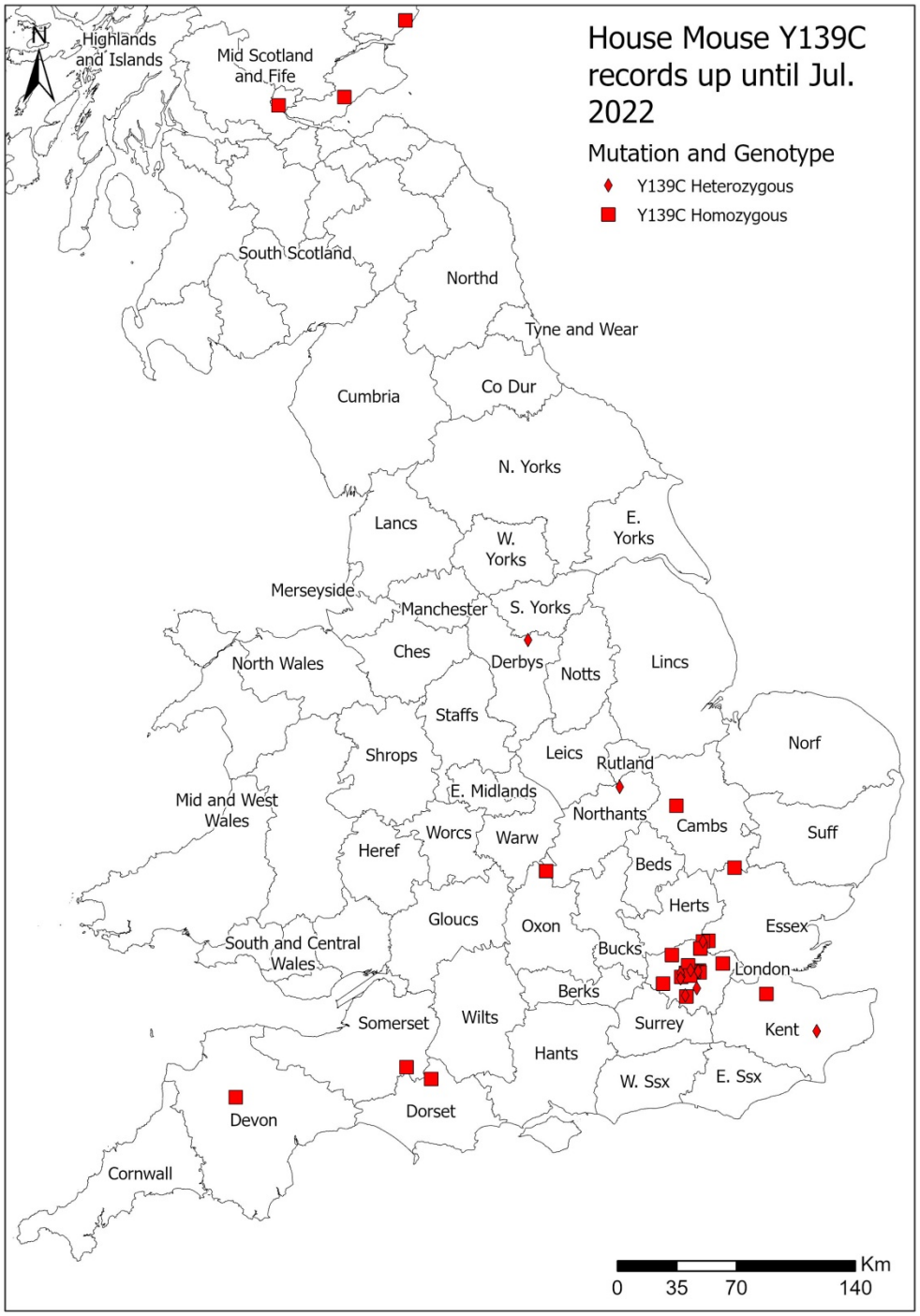
Annex 5. Map showing the geographical locations of Norway rat tissue samples submitted for analysis up to July 2022 which carried the Y139C mutation.



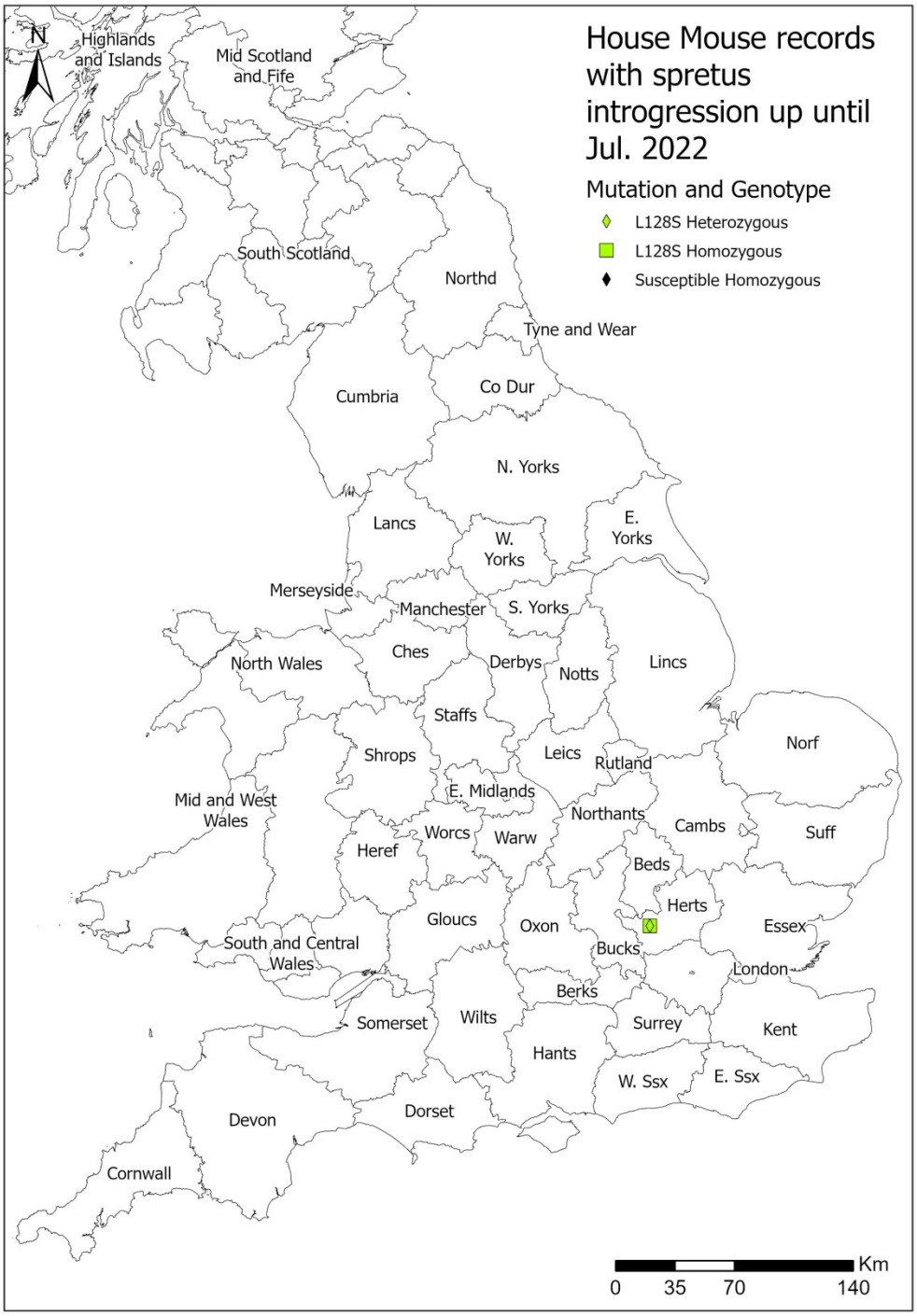
Annex 6. Map showing the geographical locations of house mouse tissue samples submitted for analysis up to July 2022 which carried the L128S mutation.



Annex 7. Map showing the geographical locations of house mouse tissue samples submitted for analysis up to July 2022 which carried the Y139C mutation.



Annex 8. Map showing the geographical location of house mouse tissue samples submitted for analysis up to July 2022 which carried the 'spretus' introgression. 3 records were identified from one site in Hertfordshire and include L128S homozygous, L128S heterozygous and 1 susceptible record.



Annex 9. Precautionary advice to those pest control practitioners who find that the infestations they are treating contain hybrid resistant rats and mice. Source Rodenticide Resistance Action Group.

Hybrid Anticoagulant Rodenticide Resistance

Hybrid resistance occurs when individual rodents, each possessing different anticoagulant resistance mutations, interbreed and produce offspring that carry mutations conferred by both parents.

Hybrid resistance is found with increasing frequency in the UK, in both Norway rats and house mice, in surveys of resistance conducted for the Campaign for Responsible Rodenticide Use (CRRU) UK by the Animal and Plant Health Agency (APHA).

Hybrid resistance is important because our knowledge of the severity and practical implications of anticoagulant resistance in the UK, developed over many decades, is based on studies of animals that carry only one mutation. Therefore, what we say about the impacts of hybrid resistance on practical rodent control must be based on carefully considered assumption and limited research done in other European countries.

The advice now provided by the Rodenticide Resistance Action Group is based on informed estimates of the likely impacts of hybrid resistance. It may change as field experience is gained and further studies are carried out.

UK Rodenticide Resistance Action Group

Advice for hybrid resistant house mice

- If you have mice at a site that carry two (or more) resistance mutations it is reasonable to assume that they are highly resistant to anticoagulants.
- If the '*spretus*' introgression is present, there is no evidence that, either on its own or in hybrid form with other mutations, it is more problematic than the more common mutations Y139C and L128S. Introgression is the transfer of genetic material from one species into another, in this case from *Mus spretus* into *Mus musculus*.
- If you use anticoagulants against house mice in the UK ALWAYS use an SGAR and NOT an FGAR.
- Among SGARs, choose products that contain either brodifacoum, difethialone or flocoumafen. These substances consistently have the lowest resistance factors and therefore highest potential control efficacy.
- Using bromadiolone and difenacoum products will exacerbate house mouse resistance problems.
- ALWAYS use baits with the maximum available concentration of the active substance – this is generally 50 parts per million (ppm) **for professional users**.
- Note that all products containing difethialone are available only at a concentration of 25 ppm. Therefore, even if difethialone is equally as effective as the other two substances (and there is some evidence it is not), mice will need to eat twice as much bait to achieve the same effect.
- Always consider the use of control methods that do not employ anticoagulants. These include the following:
    - Products containing the active substance cholecalciferol,
    - Products containing the active substance alphachloralose,
    - Physical methods, such a traps and glue boards (where available **and legally permitted**),
    - These products and methods have the very important advantage that they are equally effective against both resistant and susceptible mice. Therefore, they do not select in favour of resistance and cannot make resistance worse.

Advice for hybrid resistant Norway rats

- If you have rats at a site that carry two (or more) resistance mutations it is reasonable to assume that they are highly resistant to anticoagulants.
- If one of the resistances detected is Y139C, Y139F or L120Q, and you intend to use an SGAR, choose products that contain either brodifacoum, difethialone or flocoumafen. These substances consistently have the lowest resistance factors and therefore highest potential control efficacy.
- Using bromadiolone and difenacoum products against Norway rats with these three resistances will exacerbate resistance.
- ALWAYS use baits with the maximum available concentration of the active substance – this is generally 50 parts per million (ppm) **for professional users**.
- Note that all products containing difethialone are available only at a concentration of 25 ppm. Therefore, even if difethialone is equally as effective as the other two substances, rats will need to eat twice as much bait to achieve the same effect.
- Always consider the use of control methods that do not employ anticoagulants. These include the following:
    - Products containing the active substance cholecalciferol,
    - Physical methods, such a traps and glue boards (where available **and legally permitted**),
    - These products and methods have the very important advantage that they are equally effective against both resistant and susceptible rats. Therefore, they do not select in favour of resistance and cannot make resistance worse.