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Adaptive introgression of anticoagulant rodent poison resistance by hybridization between Old World mice

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Summary

It is known that evolution by selection on new or standing single nucleotide polymorphisms (SNPs) in the vitamin K 2,3-epoxide reductase subcomponent 1 (*vkorc1*) of house mice (*Mus musculus domesticus*) can cause resistance to anticoagulant rodenticides such as warfarin [1–3]. Here we report an introgression in European *M. m. domesticus* spanning as much as ~20.3 megabases (Mb) and including *vkorc1*, the molecular target of anticoagulants [1–4], that stems from hybridization with the Algerian mouse (*M. spretus*). We show that in the laboratory the homozygous complete *vkorc1* allele of *M. spretus* confers resistance when introgressed into *M. m. domesticus*. Consistent with selection on the introgression after the introduction of rodenticides in the 1950s we document historically adaptive population genetics of *vkorc1* in *M. m. domesticus*. Furthermore, we detected adaptive protein evolution of *vkorc1* in the *M. spretus* lineage (Ka/Ks=1.54–1.93) resulting in radical amino-acid substitutions that apparently have anticoagulant tolerance of *M. spretus* as pleiotropic effect. Thus, positive selection produced an adaptive, divergent and pleiotropic *vkorc1* allele in the donor species, *M. spretus*, which crossed a species barrier where it is expressed as adaptive trait in the recipient species, *M. m. domesticus*. Resistant house mice originated from selection on new or standing *vkorc1* polymorphisms and from selection on *vkorc1* polymorphisms acquired by adaptive introgressive hybridization.

Results and Discussion

Warfarin is used as a blood-thinning drug in medicine and as an anticoagulant rodenticide [5]. It inhibits the vitamin K epoxide reductase enzyme complex (*VKOR*) essential for vitamin K recycling and blood coagulation [6]. The vitamin K epoxide reductase subcomponent 1 (*vkorc1*) encodes the warfarin-sensitive component of *VKOR* [1, 4]. DNA sequence analyses showed that genetic variations in *vkorc1* determine the physiological response of humans and rodents to warfarin [2, 3, 7]. Currently at least 16 non-synonymous SNPs at 10 positions in *vkorc1* have been confirmed by *in vitro* and/or *in vivo* studies to alter

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blood clotting kinetics and/or *in vitro* VKOR activities in humans and rodents in response to exposure to anticoagulants [2]; additional SNPs in *vkorc1* await such experimental proof. A mere ~10 years after the inception of warfarin as rodenticide in the 1950s reports of resistant Norway rats (*Rattus norvegicus*) emerged between 1960–1969, followed by reports of resistant house mice (*M. musculus spp*.) in 1964, roof rats (*R. rattus*) in 1972, and other rat species (e.g. *R. tiomanicus*, *R. r. diardii*, *R. losea*) [3, 8–10]. Resistant rodent colonies have been discovered in Europe, the Americas, Asia, and Australia [8]. In response to such warfarin-resistant colonies other anticoagulant rodenticides were developed that target the VKOR, including coumatetralyl, bromadiolone, and difenacoum. However, resistance to these has also evolved in rats and mice. The degree, to which *vkorc1*-mediated resistance has convergently evolved in different rodent pest species, and in different populations within each species, illustrates how large natural rodent populations can respond to selection on novel and/or standing genetic variants.

In house mice (*M. musculus spp.*) ten non-synonymous SNPs at nine positions in *vkorc1* are now known (Fig. 1A). Of these, nine were previously published [2, 3] and a novel one is reported here (Fig. 1A). Foremost, however, here we report that in mice at least four of 10 (40%) non-synonymous SNPs at four of nine (~45%) positions of *vkorc1* were introduced into the *M. m. domesticus* genome by adaptive introgressive hybridization with *M. spretus* (Fig. 1A). We use the term adaptive introgressive hybridization [11] to describe the naturally occurring process that includes inter-specific mating (hybridization) followed by generations of backcrossing (introgression) and selection on introgressed alleles if these are expressed as advantageous traits at some point of their sojourn times. Changes in ecological settings, such as sudden rodenticide exposure, can render initially effectively neutral alleles adaptive [11].

We studied patterns of *vkorc1* introgression between *M. spretus* and *M. m. domesticus* from across Western Europe (Fig. 1B; c.f. Table S1). *M. spretus* separated from *M. musculus spp.* ~1.5 to 3 million years ago [12]. The species are more strongly reproductively isolated than is predicted by Haldane's Rule [13, 14], i.e. in addition to all male offspring female offspring also can be sterile depending on the direction of the cross, and the two species tend to remain ecologically and behaviorally separated even when allopatric [14]. These species are partially sympatric and can hybridize in Africa and Europe [15], but elsewhere *M. m. domesticus* is allopatric (Fig. 1B).

We found that *M. m. domesticus* from Spain and Germany carry the complete or partial *vkorc1* allele of *M. spretus* (*vkorc1*^{spr}). Heterozygous individuals and intragenic recombinants occur (Fig. 1A), which we also designated as *vkorc1*^{spr} to reflect that these contain sequences derived from *M. spretus*. The *vkorc1* of *M. m. domesticus* (*vkorc1*^{dom}) differs from *vkorc1*^{spr} by at least four non-synonymous SNPs and by ~1.24% –1.39% across the entire gene (Fig. 1A and [16]). DNA sequence analysis of *vkorc1* of 106 *M. m. domesticus* revealed that only 59/106 (55.7%) mice carry *vkorc1* genotypes identifiable as comprised solely out of *M. m. domesticus* (*vkorc1*^{dom}) sequences (genotypes 1–6; Fig. 1). However, 43/106 (40.6%) mice carry *vkorc1* genotypes that across the entire length of *vkorc1* (N=10; genotypes 14–20), or parts of it (N=33; genotypes 8–13), correspond to the allele of *M. spretus*, all designated as *vkorc1*^{spr} (Fig. 1). Four (3.8%) similar genotypes could not be assigned with confidence across the entire length of the gene to either species (pooled as genotype 7; Fig. 1).

In our sample from Spain only 2/29 (6.9%) mice carry pure *vkorc1*^{dom} (genotype 1); all others (27/29 or 93.1%) carry *vkorc1*^{spr} genotypes that across the entire (N=6, genotypes 14–20) or parts of *vkorc1* (N=21; genotypes 8–10) correspond to the *M. spretus* allele (Fig. 1). In Germany, 4/50 (8%) mice carry *vkorc1*^{spr} genotype 20 that matches up over the entire length with the *M. spretus* allele, and 12/50 (24%) samples carry *vkorc1*^{spr} (genotypes 8,

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11–13) that match up over parts of the *vkorc1* allele of *M. spretus*. Thus, in the area where *M. m. domesticus* and *M. spretus* could hybridize the vast majority of house mice carry *vkorc1*^{spr}, and even in the distant Germany where house mice are allopatric, 32% of mice carry *vkorc1*^{spr}.

To examine whether we observe hybridization between *M. spretus* and *M. m. domesticus* (e.g. as in [15]) or, specifically *vkorc1* introgression, we partially sequenced 18 nuclear genes on chromosome 7, including *vkorc1* and its 5′ region, and 6 nuclear genes on five other chromosomes from 10 *M. m. domesticus* from Germany and Spain, as well as *M. spretus* (Fig. 2A; c.f. Table S2). These sequence comparisons distinguish 10 genome profiles (I–X; Fig. 2B) and delineate ~20.3 Mbs of chromosome 7 where *M. spretus* sequence variants can be detected in some *M. m. domesticus*, i.e. sequence similarity between both species is much higher, or sequences are identical, than is for genes on other chromosomes or genes more distantly linked to *vkorc1*. Recombination, including intragenic recombination (e.g. in *vkorc1*; c.f. Fig. 1A), has taken place throughout the region that carries *M. spretus* variants (Fig. 2C). However, high levels of linkage disequilibrium remain (Fig. S1A and B). Analysis of introgressed and recombining nuclear sequences using phylogenetic methods [17] in our case are expected to result in gene genealogies where *M. m. domesticus* should be paraphyletic with respect to *M. spretus* (i.e. the *M. spretus* sequence is nested within *M. m. domesticus* sequences) or are poorly resolved. For *vkorc1* and for genes closely linked to *vkorc1* reconstructed gene genealogies indeed were paraphyletic or poorly resolved (Fig. 2D, c.f. Fig S1C and D). In contrast, analysis of nearly all other distantly linked or unlinked genes, including mtDNA D-loop, *dusp27 and maoa*, identified *M. m. domesticus* as being monophyletic, i.e. the sequence from *M. spretus* was the sister lineage to a group containing all *M. m. domesticus* sequences, and thus, that all mice sampled are *M. m. domesticus*, whether or not they carry *vkorc1*^{spr} (Fig. 2D, c.f. Fig S1C and D). Finally, when genome profiles I–X were analyzed for their divergence to polymorphism ratios by applying Hudson-Kreitman-Aguade (HKA) tests to sliding windows taken from mouse chromosome 7 and reference loci then the putatively introgressed region displayed significant deficiencies of divergence relative to polymorphism, which is consistent with divergent *M. spretus* variants now segregating as polymorphisms in *M. m. domesticus* (Fig. 2E and F) [17]. No such deficiencies were observed when only putatively pure *M. m. domesticus* genome profiles (I–III) were analyzed (not shown).

These observations show that *vkorc1*^{spr} has entered *M. m. domesticus* as part of an introgression on chromosome 7 by hybridization with *M. spretus*. However, consistent with theories that put hybrid genotypes at a selective disadvantage [18, 19], previous studies have shown that hybrid genotypes are confined to the area of sympatry [15]. In contrast, our observed spread of *vkorc1*^{spr} beyond the area of sympatry to the area of allopatry in Germany is the first of a number of compelling indicators for the adaptive value of at least one of the *vkorc1*^{spr} alleles. Notably, the presence and spread of co-introgressed *M. spretus* variants linked to *vkorc1* that currently are segregating as polymorphisms in *M. m. domesticus* shows that even though such variants may be detrimental [20], the benefits of carrying of *vkorc1*^{spr} appear to outweigh any such adverse linkage effects.

An adaptive value of *vkorc1*^{spr} is conceivable if it is assumed that at least one of its alleles is expressed as anticoagulant rodenticide resistance trait in *M. m. domesticus*. We hypothesize this mechanistic connection because of the well-known biochemical action and molecular targets of anticoagulants: the protein complex VKOR and the gene *vkorc1* [1–4 and references given therein, and c.f. e.g. supplementary references 34–38]. Moreover, a pest control officer whom we work with provided us with anecdotal reports of his difficulties to control a population of *M. m. domesticus* in the township of Hamm, Germany, by means of bromadiolone. Our DNA sequence analysis of mice sampled from this population confirmed

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that they carry the homozygous complete *vkorc1*^{spr} (genotype 20; Fig. 1A) while having genome profile VI (Fig. 2B), i.e. are *M. m. domesticus*. Mice from this location were brought to the laboratory for resistance testing (Table 1). We found that in this genetic background *vkorc1*^{spr} lowers mortality to the anticoagulant rodenticides coumatetralyl or bromadiolone to 20% or 9%, respectively. In contrast, *M. m. domesticus* carrying wildtype *vkorc1*^{dom} (genotype 1; Fig. 1A; genome profile I) displayed mortality rates of 84–100% to coumatetraly or 85% to bromadiolone. Moreover, 20% of *M. m. domesticus* carrying complete *vkorc1*^{spr} survived difenacoum trials, whereas all wildtype *vkorc1*^{dom} succumbed. These differences observed in the laboratory likely translate into considerable selection coefficients in the wild, and thus, support our assumed adaptive value of some *vkorc1*^{spr} alleles, foremost complete homozygous *vkorc1*^{spr}. Further testing of complete and partial *vkorc1*^{spr} in various genetic backgrounds of mice will be useful for further elucidating these genotype-phenotype connections. However, it is reasonable to postulate that our data showing association of *vkorc1*^{spr} with higher survival to anticoagulant exposure captures a significant part of this genetic response.

Earlier work has detected selective sweeps at the warfarin-resistance locus (*Rw*), which is now known to correspond to *vkorc1* [1, 4], in wild rat populations [21]. Here, we detected such selective sweeps associated with *vkorc1*^{spr} in populations of *M. m. musculus* from Spain, which is an additional observation of this study that is consistent with an adaptive value of the introgression. Specifically, we sequenced two Spanish populations (Spain 1 and 2; Fig. 1) of *M. m. domesticus* carrying complete or partial *vkorc1*^{spr} for *tead1* and *dock1* flanking the introgression (c.f. Fig. 2A). Populations from Spain were analyzed because any selective sweeps should have occurred in the area of sympatry first. We detected genetic hitchhiking for *tead1* in both populations (Fay and Wu's normalized H_n =−3.02 and −1.82, p=0.014 and 0.047, respectively). Likely due to recombination, which was more frequent on the 3′ ends of *vkorc1* and of the introgression compared to the 5′ ends (Fig. 2C and Fig. S1B), for *dock1* a sweep was only supported for one population (H_n= −3.03, p=0.042). Thus, regardless of whether the populations presently carry complete (Spain-1) or partial *vkorc1*^{spr} (Spain-2), the introgression of at least one *vkorc1*^{spr} allele appears to have been of at least temporally adaptive value in the recent past.

We modeled selective sweeps at *vkorc1* to investigate whether their timing could be considered consistent with the timing of the introduction of rodenticides in the 1950s. We conducted a composite-likelihood analysis of simulated incomplete sweeps using algorithms implemented in *ssw* and *clics* [22, 23]. We analyzed 30 inferred *vkorc1* haplotypes of mice from population Spain-1. As the adaptive amino acid changes in *vkorc1*^{spr} we considered R12W, A26S and A48T (c.f. Fig. 1B). Simulations provided maximum likelihood estimates of $\alpha = 2Ns = 5.6–6.6\times10^3$, where N is the effective population size and the selection coefficient $s = 0.28–0.33$.

We used the expression ~2ln(2N)/*s* to calculate that the sweep took place ~61–71 generations ago, which, assuming a generation time of 0.2–0.3 years for mice, corresponds to ~13–22 years. We obtained a more recent timing of the selective sweep (25–36 generations or 5–11 years ago) when α was obtained by bootstrapping ($\alpha = 1.13–1.61\times10^4$; $s = 0.56–0.81$). These estimates are consistent with a recent selective sweep when rodenticides were already in use. Notably, it would require broader geographic sampling of *M. m domesticus* and *M. spretus* to clarify whether the introgression has multiple origins, which is a possible explanation for polymorphisms seen within the introgression, and to better describe the timing, geographic spread and population genetics of complete, partial and recombinant *vkorc1*^{spr}. Our study explains the presence of *trans*-species polymorphisms in *M. spretus* and *M. m. domesticus* by adaptive introgressive hybridization. Other studies have detected possible hybrids in the area of sympatry between *M. spretus* and *M. m. domesticus*

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[15] or showed rare much more ancient *trans*-species polymorphisms; some of these seemingly being maintained by balancing selection [24, 25].

The *vkorc1* gene is evolutionarily conserved from invertebrates to mammals [1]. It was therefore unexpected to find evidence for positive selection on *vkorc1* in *M. spretus*, and notably, that this adaptive evolution involved radical amino-acid substitutions at conserved positions in the VKORC1 protein: one position conserved between human/rodents and Anopheles (R61L), two positions in the transmembrane domain conserved between human/rodents and chicken (R12W and A26S), and one position conserved between human and rodents (A48T) (c.f. Fig. 1 and Ref. [1]). Our analysis of interspecific protein sequence evolution, Ka/Ks, between *M. spretus* and *M. m. domesticus* identified *vkorc1* as one of the fastest evolving *M. spretus* transcripts sequenced so far (Ka/Ks=1.54–1.93; Fig. 3A). This high evolutionary rate Ka/Ks>1 was predominantly seen between *M. musculus spp.* and *M. spretus*, i.e. after the split between mice from rats (Fig. 3B). The mapping of nucleotide substitutions on the phylogeny of mice and *R. norvegicus* constructed based on the full *vkorc1* protein coding sequence pinpointed this evolutionary rate acceleration to the *M. spretus* lineage exclusively, where we observed an excess of 4 non-synonymous substitutions (Tajima's relative rate test[26], p=0.045; Fig. 3C), but not of silent substitutions (p=0.317).

The adaptive molecular evolution of *vkorc1* in *M. spretus* has led to the fixation of amino acids that confer anticoagulant resistance in the genomic background of house mice. Interestingly, *M. spretus* is also highly tolerant to rodenticides; in the sole study published, 4/7 (57%) mice tested succumbed to bromadiolone, and 0/9, 0/10, and 0/7 succumbed to difenacoum, chlorphacinon, and coumatetralyl, respectively [27]. One hypothesis to explain the adaptive evolution of *vkorc1* in *M. spretus* implicates adaptation to a granivorous vitamin K-deficient diet [28]. The tolerance of *M. spretus* to rodenticides could thus be a pleiotropic effect of a physiological adaptation unrelated to rodenticide selection. Other granivorous rodents, including Shaw's gerbil (*Meriones shawi*), the Egyptian spiny mouse (*Acomys cahirinus*), and the golden hamster (*Mesocricetus auratus*), display similar high levels of tolerance to rodenticides despite being naive to the poisons [28]. However, which of the amino acid changes in $vkorc1^{spr}$ mediate resistance is not known, and *in vitro*, individually these amino-acid changes appear to have no protective effect on *vkorc1* in the presence of warfarin [1]. Thus, epistatic interactions between sites within *vkorc1*, or between sites elsewhere in the genome, would need to be invoked to explain resistance observed *in vivo* in *M. m. domesticus* and *M. spretus*. Nevertheless, *vkorc1* clearly has undergone adaptive molecular evolution in *M. spretus* since it separated from other *Mus* lineages, and the introgression of complete $vkorc1^{spr}$ appears to have transferred this tolerance to house mice, although differences in the resistance phenotype due to the genomic background are to be expected.

Conclusion

Our study illustrates that an adaptive trait can convergently evolve by selection on new or standing genetic polymorphisms as well as by adaptive introgressive hybridization between species, with these processes eventually becoming connected through the establishment of recombinant genotypes. Interestingly, human-mediated dispersal likely was a factor in this horizontal transfer of rodenticide resistance between *M. m. domesticus* and *M. spretus*, because until the spread of human agriculture enabled the dispersal of house mice the species were allopatric [29]. Moreover, a selection regime altered by humans by introducing rodenticide appears to have driven the adaptive introgressive hybridization between the two species by locally and temporarily elevating the fitness of hybrids over that of the

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rodenticide susceptible parental species, at least over that of *M. m. domesticus* carrying wildtype *vkorc1*^{dom}.

Supplementary Material

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Highlights

- European house mice (*M. musculus domesticus*) are polymorphic for an introgression from the Algerian mouse (*Mus spretus*) that includes the molecular target of anticoagulant rodenticides (*vkorc1*).
- The *vkorc1* allele of *M. spretus* can cause anticoagulant resistance when introgressed into *M. m. domesticus*.
- *M. musculus domesticus* has evolved anticoagulant rodenticide resistance by selection on alleles that evolved from new or standing polymorphisms in *vkorc1* and by selection on a divergent pleiotropic *vkorc1* allele acquired by inter-specific mating with *M. spretus*.

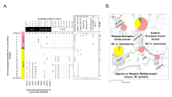

Figure 1.

A) Shown are the variable positions defining 20 *vkorc1* genotypes identified in the transcribed sequences of 106 Western European house mice (*M. musculus domesticus*) (no variants were detected in 5′ UTR). DNA Sanger-sequencing and alignments to *vkorc1* of the Algerian mouse (*M. spretus*) were done. Intron sequences were determined whenever possible (i.e. not obscured by insertion/deletion polymorphisms) and used to infer whether ambiguous transcript sequences were of *M. m. domesticus* origin or were *M. spretus*. *M. m. domesticus* C57BL/6J and *M. spretus* shown on the top and bottom, respectively. We define genotypes as $vkorc1^{dom}$ if no traces of any *M. spretus* polymorphisms could be detected in the coding and non-coding regions of the gene (genotypes 1–6). The *vkorc1* genotypes that correspond to the *vkorc1* sequence of *M. spretus* fully (genotype 20), or contain any discernible *M. spretus* variants either in form of heterozygosity and/or intragenic recombination in any part of the coding and non-coding portion of the gene (genotypes 8–19) are defined as $vkorc1^{spr}$. Ambiguous genotypes that contain *vkorc1* of *M. m. domesticus* (exons 1–2) but could not be assigned to either species in the 3 prime portion of the gene (green, pooled as genotype 7). Dots depict nucleotide states identical to C57BL/6J. Hyphens depict insertions/deletions. Empty fields depict missing information (but confirmed as either $vkorc1^{dom}$ or $vkorc1^{spr}$ based on flanking non-coding sequences [16]), and '?' depict missing information that could not be assigned to either species based on flanking sequences. The standard nucleotide ambiguity and amino acid one-letter codes apply. Countries and genotype counts are shown in the right panel (n.a.–not applicable). Populations Spain-1 and -2 are listed separately because we analyzed these in more detail (see text). Asterisks mark those amino-acid variants and positions present in *M. musculus* and known to affect warfarin tolerance in rodents or humans [2, 3] (Y139C/S variants were not detected by us). The newly discovered W59L SNP affects a position in *vkorc1* known to alter warfarin tolerance in form of a W59R non-synonymous SNP in *Rattus norvegicus* [2].

B) Distribution of *vkorc1* genotypes in Western European *M. m. domesticus*. The hatched area depicts the native range of *M. spretus* [12]. The house mouse has become a cosmopolitan species and now is occurring across the entire area depicted and beyond [29]. Pie charts show the frequencies of pure *vkorc1* of *M. m. domesticus* origin ($vkorc1^{dom}$) (pink, genotypes 1–6 in A), genotypes that correspond to the complete *M. spretus vkorc1* allele or share parts of it in form of heterozygosity and/or intragenic recombination (all $vkorc1^{spr}$, yellow, genotypes 8–20 in A). Countries sampled are shaded in grey. Sampling locations (some overlapping due to proximity) are shown as triangles (pink–$vkorc1^{spr}$ absent, yellow - $vkorc1^{spr}$ present). C.f. Table S1 for sample information and Table S2 for PCR and sequencing primers.

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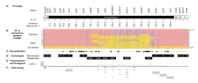

Figure 2.

Genome profiling of 10 *M. m. domesticus* from Germany and Spain. **A.** Coverage of genes, their transcript orientation and chromosomal physical positions (in megabases; Mb) (c.f. Table S2 for gene and PCR/sequencing primer information). **B.** VISTA plot depicting pairwise DNA sequence similarity scores (Y-axes, right, scaled between 90–100%) between C57BL/6J and 6 *M. m. domesticus* from Germany with genomic profiles I–VI and 4 from Spain (genome profiles VII–X). Exons are shown in purple, the coloring scheme is as in Figs. 1–2 indicating, at a coarse resolution; regions comprised out of predominantly *M. m. domesticus* sequences (pink) and *M. spretus* sequences (yellow). **C.** Minimum number of recombination events (black diamonds) within chromosome 7 among *M. m. domesticus* (excluding *M. spretus* and C57BL/6J). See also the analysis of linkage disequilibrium in Fig. S1B. **D.** Gene genealogies of *M. m. domesticus* identified as monophyletic or paraphyletic with respect to *M. spretus*, using 90% support for nodes as cutoff (c.f. Figure S1C and D). Significance of topologies is given in percent bootstrap values supporting monophyly of *M. m. domesticus* samples (top) or both clusters in paraphyletic topologies (bottom; first number *M. m. domesticus*, second number *M. spretus*). **E.** Plot of polymorphism in *M. m. domesticus* relative to divergence to *M. spretus*. **F.** Asterisks mark significance (at α=0.05, 0.01, and 0.001) of rejection of Hudson-Kreitman-Aquade (HKA) testing done on select non-recombining segments representing reference genes (grey shaded boxes; c.f. A for gene identifiers).

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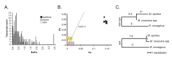

Figure 3.

Adaptive evolution of *vkorc1* in the *M. spretus* lineage. **A.** Plot of Ka/Ks between *M. m. domesticus* and *M. spretus* 184 gene transcripts. To reflect our confidence in orthology transcripts are grouped in order of decreasing confidence in orthology as one2one - one Inparanoid hit in each species; n2one - one hit in one species but many hits in the other; n2m - multiple hits in both species. The positions of *vkorc1* (minimum and maximum Ka/Ks) in this distribution are shown as *. **B.** Plot of Ks versus Ka of *vkorc1* between *R. norvegicus* and *M. spretus* and *M. musculus spp.* (*M. m. domesticus, M. m. musculus, M. m. castaneus, M. m. molossinus*) (black triangles), between members of *M. musculus spp.* (pink diamonds), and between *M. musculus spp.* and *M. spretus* (yellow squares). The dashed line depicts Ka=Ks expected under selective neutrality. **C.** Mapping of non-synonymous substitutions on the *vkorc1* neighbor-joining phylogeny of *M. musculus spp.*, *M. spretus*, and *R. norvegicus*. Numbers above branches indicate the average number of nucleotide substitutions. A significant excess of non-synonymous substitutions (n=4), as determined using Tajima's relative rate test, is indicated by*

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Table 1

Mortality of *M. m. domesticus* tested during no choice feeding trails with broken wheat bait containing one of three rodenticides

Strains/vkorc1 genotypes of *M. m. domesticus*[1]	Sex	375ppm coumatetralyl[2]		50ppm bromadiolone[2]		50ppm difenacoum[2]	
		Mort.[3]	Cons.[3]	Mort.[3]	Cons.[3]	Mort.[3]	Cons.[3]
1. Homozygous *vkorc1* genotype 1 (*vkorc1*^{dom})	M	9/9	14.2	-	-	-	-
	F	10/10	18.0	-	-	-	-
2. Homozygous *vkorc1* genotype 1 (*vkorc1*^{dom})	M	9/10	7.0	9/10	12.3	10/10	4.9*
	F	7/9	11.1	8/10	12.2	10/10	4.0*
3. Homozygous *vkorc1* genotype 20 (*vkorc1*^{spr})	M	4/10	11.4	2/11	16.4	9/10	10.9
	F	0/10	7.5	0/11	10.1	7/10	11.2

[1] Strains maintained by S.E. in the laboratory of FRH: 1. *Cd1*/J; 2. wild-derived *M. m. domesticus* strain from the city of Leverkusen, Germany; 3. Wild-derived (*M. m. domesticus* from the township of Hamm, Germany

[2] ppm–parts per million anticoagulant in bait

[3] Mort.–Mortality as observed throughout a 14-day period following bait feeding; Cons. – Average consumption of bait (in grams) per mouse

* Choice trial with broken wheat as alternative food, mortality during choice trials is lower than during no choice feeding trials, and are applied to identify mildly tolerant strains.

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