Current Biology 21, 1296–1301, August 9, 2011 ©2011 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2011.06.043

Report

Adaptive Introgression of Anticoagulant Rodent Poison Resistance by Hybridization between Old World Mice

Ying Song,[1] Stefan Endepols,[2] Nicole Klemann,[3] Dania Richter,[4] Franz-Rainer Matuschka,[4] Ching-Hua Shih,[1] Michael W. Nachman,[5] and Michael H. Kohn[1,*]
[1]Department of Ecology and Evolutionary Biology, Rice University, Houston, TX 77005, USA
[2]Environmental Science, Bayer CropScience AG, D-40789 Monheim, Germany
[3]D-48231 Warendorf, Germany
[4]Division of Pathology, Department of Parasitology, Charité–Universitätsmedizin, D-10117 Berlin, Germany
[5]Department of Ecology and Evolution, University of Arizona, Tucson, AZ 85721, USA

Summary

Polymorphisms 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 show that resistant house mice can also originate from selection on *vkorc1* polymorphisms acquired from the Algerian mouse (*M. spretus*) through introgressive hybridization. We report on a polymorphic introgressed genomic region in European *M. m. domesticus* that stems from *M. spretus*, spans >10 Mb on chromosome 7, and includes the molecular target of anticoagulants *vkorc1* [1–4]. 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 introgressed allele after the introduction of rodenticides in the 1950s, we found signatures of selection in patterns of variation in *M. m. domesticus*. Furthermore, we detected adaptive protein evolution of *vkorc1* in *M. spretus* (Ka/Ks = 1.54–1.93) resulting in radical amino acid substitutions that apparently cause anticoagulant tolerance in *M. spretus* as a pleiotropic effect. Thus, positive selection produced an adaptive, divergent, and pleiotropic *vkorc1* allele in the donor species, *M. spretus*, which crossed a species barrier and produced an adaptive polymorphic trait in the recipient species, *M. m. domesticus*.

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 have shown that genetic variations in *vkorc1* determine the physiological response of humans and rodents to warfarin [2, 3, 7]. Currently, at least 16 nonsynonymous single-nucleotide polymorphisms (SNPs) at ten positions in *vkorc1* have been confirmed by in vitro and/or in vivo studies to alter 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 a rodenticide in the 1950s, reports of resistant Norway rats (*Rattus norvegicus*) emerged between 1960 and 1969, followed by reports of resistant house mice (*Mus musculus* spp.) in 1964, roof rats (*R. rattus*) in 1972, and other rat species (e.g., *R. tiomanicus, R. r. diardii*, and *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 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 nonsynonymous SNPs at nine positions in *vkorc1* are now known (Figure 1A). Of these, nine were previously published [2, 3] and a novel one is reported here (Figure 1A). Foremost, however, we report here that in mice, at least four of ten nonsynonymous SNPs (40%) at four of nine positions (~45%) of *vkorc1* were introduced into the *M. m. domesticus* genome by adaptive introgressive hybridization with *M. spretus* (Figure 1A). We use the term "adaptive introgressive hybridization" [11] to describe the naturally occurring process that includes interspecific 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 introgressed effectively neutral alleles adaptive [11].

We studied patterns of *vkorc1* introgression between *M. spretus* and *M. m. domesticus* from across Western Europe (Figure 1B; see also Table S1 available online). *M. spretus* separated from *M. musculus* spp. ~1.5–3 million years ago [12]. The species are more strongly reproductively isolated than is predicted by Haldane's rule [13, 14], i.e., female offspring, in addition to all male 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 (Figure 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 (Figure 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 nonsynonymous SNPs and by ~1.24%–1.39% across the entire gene (Figure 1A; [16]). DNA sequence analysis of *vkorc1* of 106 *M. m. domesticus* revealed that only 59 of 106 mice (55.7%) carried

*Correspondence: hmkohn@rice.edu

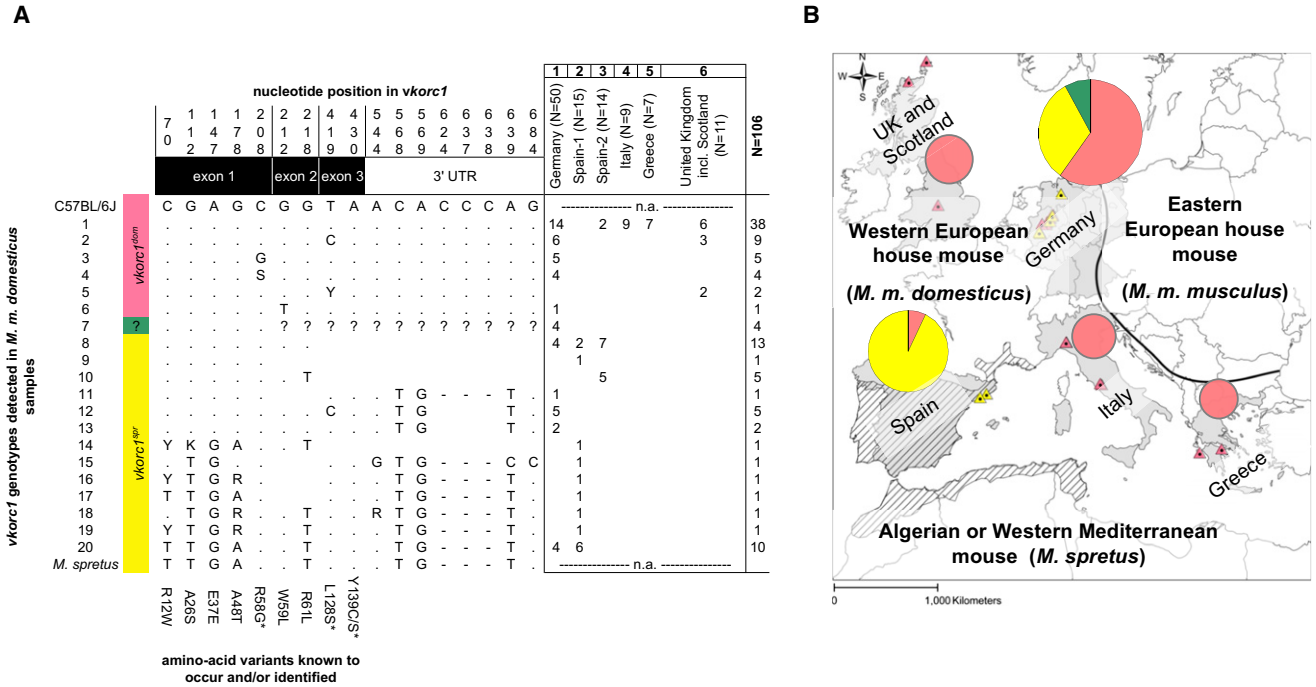

Figure 1. Polymorphisms in *vkorc1* of European House Mice and Their Geographic Distributions

(A) Variable positions defining 20 *vkorc1* genotypes identified in the transcribed sequences of 106 Western European house mice (*Mus musculus domesticus*) (no variants were detected in the 5′ untranslated region). DNA Sanger sequencing and alignments to *vkorc1* of the Algerian mouse (*M. spretus*) were performed. 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* are shown at 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 noncoding 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 in form of heterozygosity and/or intragenic recombination in any part of the coding and noncoding 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′ portion of the gene were pooled as genotype 7 (green). 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 noncoding sequences [16]); question marks depict missing information that could not be assigned to either species based on flanking sequences. The standard nucleotide ambiguity and one-letter amino acid codes apply. Countries and genotype counts are shown at right (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] (we did not detect Y139C/S variants). The newly discovered W59L SNP affects a position in *vkorc1* known to alter warfarin tolerance in form of a W59R nonsynonymous 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 (*Mus musculus* spp.) 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 the form of heterozygosity and/or intragenic recombination (all *vkorc1*^{spr}, yellow, genotypes 8–20 in A), and ambiguous genotypes (genotype 7, green). Countries sampled in this study are shaded in gray. Sampling locations (some overlapping as a result of proximity) are shown as triangles (pink, *vkorc1*^{spr} absent; yellow, *vkorc1*^{spr} present).

See Table S1 for sample information and Table S2 for PCR and sequencing primers.

vkorc1 genotypes identifiable as comprised solely of *M. m. domesticus* (*vkorc1*^{dom}) sequences (genotypes 1–6; Figure 1). However, 43 of 106 mice (40.6%) carried *vkorc1* genotypes that correspond across the entire length of *vkorc1* (n = 10; genotypes 14–20) or parts of it (n = 33; genotypes 8–13) to the allele of *M. spretus*, all designated as *vkorc1*^{spr} (Figure 1). Four similar genotypes (3.8%) could not be assigned with confidence across the entire length of the gene to either species (pooled as genotype 7; Figure 1).

In our sample from Spain, only 2 of 29 mice (6.9%) carried pure *vkorc1*^{dom} (genotypes 1–6); all others (27 of 29, or 93.1%) carried *vkorc1*^{spr} genotypes that correspond across the entire length of *vkorc1* (n = 6; genotypes 14–20) or parts of it (n = 21; genotypes 8–10) to the *M. spretus* allele (Figure 1). In our sample from Germany, 4 of 50 mice (8%) carried *vkorc1*^{spr} genotype 20, which matches over the entire length with the *M. spretus* allele, and 12 of 50 samples (24%) carried *vkorc1*^{spr} (genotypes 8 and 11–13), which matches over parts of the *vkorc1* allele of *M. spretus*. Thus, in the area where *M. m. domesticus* and *M. spretus* could hybridize, the 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 were merely observing 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 ten *M. m. domesticus* from Germany and Spain, as well as one *M. spretus* (Figure 2A; see also Table S2). These sequence comparisons distinguished ten genome profiles (I–X; Figure 2B) and delineated a large region of chromosome 7 where *M. spretus* sequence variants could be detected in some *M. m. domesticus*, i.e., sequence similarity between

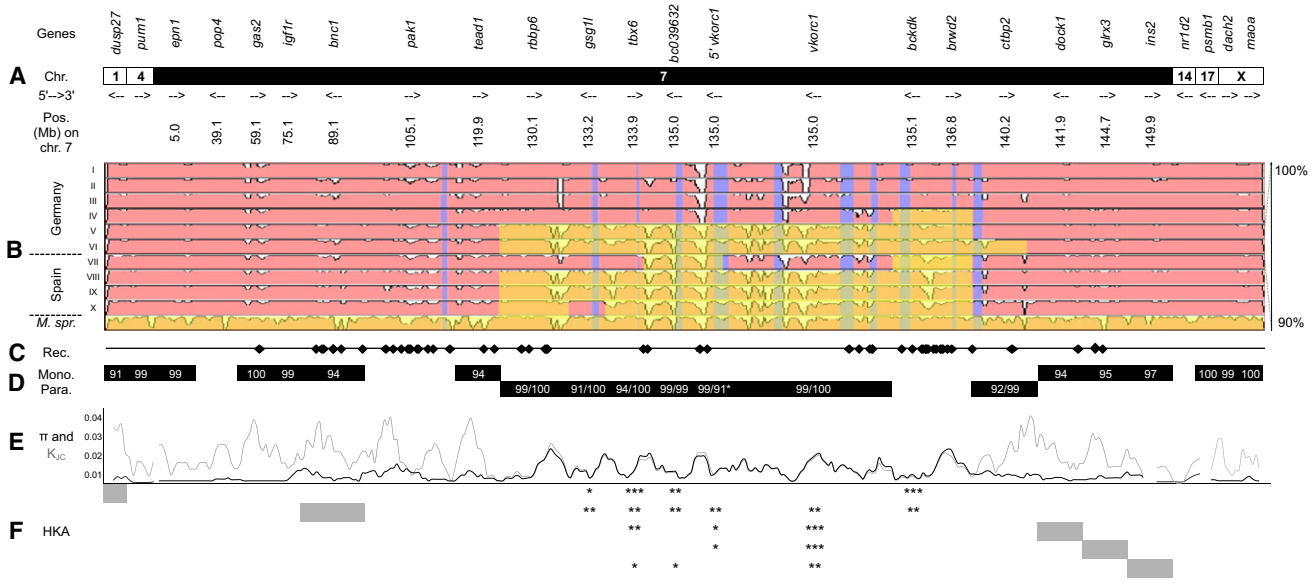

Figure 2. Genome Profiling of Ten *M. m. domesticus* from Germany and Spain

(A) Coverage of genes, their transcript orientation, and their chromosomal positions (in megabases) (see Table S2 for gene and PCR/sequencing primer information).

(B) VISTA plot depicting pairwise DNA sequence similarity scores (y axes, right, scaled between 90% and 100%) between C57BL/6J and six *M. m. domesticus* from Germany (genome profiles I–VI) and four *M. m. domesticus* from Spain (genome profiles VII–X). Exons are shown in purple; the coloring scheme is as in Figure 1 indicating, at a coarse resolution, regions comprised of predominantly *M. m. domesticus* sequences (pink) and *M. spretus* (*M. spr.*) 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 Figure S1B.

(D) Gene genealogies of *M. m. domesticus* identified as monophyletic (Mono.) or paraphyletic (Para.) with respect to *M. spretus* using 90% support for nodes as cutoff (Figures S1C and S1D). 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*). Asterisk indicates significance for *vkorc1* 5′ region taken from genealogy constructed using C57BL/6J as outgroup.

(E) Plot of polymorphism (expected hereozygosity; π) in *M. m. domesticus* relative to divergence (Jukes Cantor corrected K) to *M. spretus*.

(F) Asterisks mark significance (at α = 0.05, 0.01, and 0.001) of rejection of Hudson-Kreitman-Aguade (HKA) testing performed on select nonrecombining segments representing reference genes (gray boxes; see A for gene identifiers).

both species was much higher, or sequences were identical, than for genes on other chromosomes or genes more distantly linked to *vkorc1*. Recombination, including intragenic recombination (e.g., in *vkorc1*; Figure 1A), has taken place throughout the region that carries *M. spretus* variants (Figure 2C). However, high levels of linkage disequilibrium remain (Figures S1A and S1B). Analysis of introgressed and recombining nuclear sequences using phylogenetic methods [17] in our case would be 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 were indeed paraphyletic or poorly resolved (Figure 2D; Figures S1C and S1D). At a bootstrap significance cutoff of $\geq$90%, the introgression ranged from at least between *rbbp6* and *ctbp2* (~10.2 Mb). 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 were *M. m. domesticus*, whether or not they carried *vkorc1*^{spr} (Figure 2D; Figures S1C and S1D). Finally, when genome profiles I–X were analyzed for their divergence to polymorphism ratios by applying Hudson-Kreitman-Aguade tests to sliding windows taken from mouse chromosome 7 and reference loci, 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* (Figures 2E and 2F) [17]. No such deficiencies were observed when only putatively pure *M. m. domesticus* genome profiles (I–III) were analyzed (data 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 cointrogressed *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 an anticoagulant rodenticide resistance trait in *M. m. domesticus*. We

Table 1. Mortality of *M. m. domesticus* Tested during No-Choice Feeding Trials with Broken Wheat Bait Containing One of Three Rodenticides

Strains/*vkorc1* Genotypes of *M. m. domesticus*	Sex	375 ppm Coumatetralyl		50 ppm Bromadiolone		50 ppm Difenacoum	
		Mort.	Cons.	Mort.	Cons.	Mort.	Cons.
1: homozygous *vkorc1*	M	9/9	14.2	–	–	–	–
genotype 1 (*vkorc1*^{dom})	F	10/10	18.0	–	–	–	–
2: homozygous *vkorc1*	M	9/10	7.0	9/10	12.3	10/10	4.9[a]
genotype 1 (*vkorc1*^{dom})	F	7/9	11.1	8/10	12.2	10/10	4.0[a]
3: homozygous *vkorc1*	M	4/10	11.4	2/11	16.4	9/10	10.9
genotype 20 (*vkorc1*^{spr})	F	0/10	7.5	0/11	10.1	7/10	11.2

Strain 1 was *Cd1*/J, strain 2 was a wild-derived *M. m. domesticus* strain from the city of Leverkusen, Germany, and strain 3 was a wild-derived *M. m. domesticus* strain from the township of Hamm, Germany. Strains 1 and 2 were maintained by S.E. in the laboratory of F.-R.M. The following abbreviations are used: ppm, parts per million anticoagulant in bait; Mort., mortality as observed throughout a 14-day period following bait feeding; Cons., average consumption of bait (in grams) per mouse.

[a] Choice trial with broken wheat as alternative food; mortality during choice trials is lower than during no-choice feeding trials, so choice trials were used to identify mildly tolerant strains.

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 therein; see also Supplemental Experimental Procedures). Moreover, a pest control officer with whom we work provided us with anecdotal reports of difficulties in controlling 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 that they carried the homozygous complete *vkorc1*^{spr} (genotype 20; Figure 1A) while having genome profile VI (Figure 2B), i.e., they were *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} lowered mortality to the anticoagulant rodenticides coumatetralyl and bromadiolone to 20% and 9%, respectively. In contrast, *M. m. domesticus* carrying wild-type *vkorc1*^{dom} (genotype 1; Figure 1A; genome profile I) displayed mortality rates of 84%–100% to coumatetralyl and 85% to bromadiolone. Moreover, 20% of *M. m. domesticus* carrying complete *vkorc1*^{spr} survived difenacoum trials, whereas all wild-type *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 a signature of positive selection associated with *vkorc1*^{spr} in populations of *M. m. musculus* from Spain, which is an additional observation of this study consistent with an adaptive value of the introgressed allele. Specifically, we sequenced two Spanish populations (Spain 1 and 2; Figure 1) of *M. m. domesticus* carrying complete or partial *vkorc1*^{spr} for *tead1* and *dock1* flanking the introgression (Figure 2A). Populations from Spain were analyzed because any selective sweeps should have occurred in the area of sympatry first. We detected a skew in the distribution of allele frequencies (i.e., genetic hitchhiking due to a selective sweep) 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 as a result of recombination, which was more frequent on the 3′ ends compared to the 5′ ends of *vkorc1* and more frequent on the 3′ ends of the introgression in general (Figure 2C; Figure S1B), for *dock1* this pattern 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 (Spain 2) *vkorc1*^{spr}, 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 (Figure 1B). Simulations provided maximum-likelihood estimates of $\alpha = 2Ns = 5.6–6.6 \times 10^3$, where N is the effective population size and the selection coefficient $s = 0.28–0.33$. We used the expression $\sim 2\ln(2N)/s$ 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 ($\alpha = 1.13–1.61 \times 10^4$; $s = 0.56–0.81$). 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 transspecies 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* [15] or showed rare, much more ancient transspecies 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

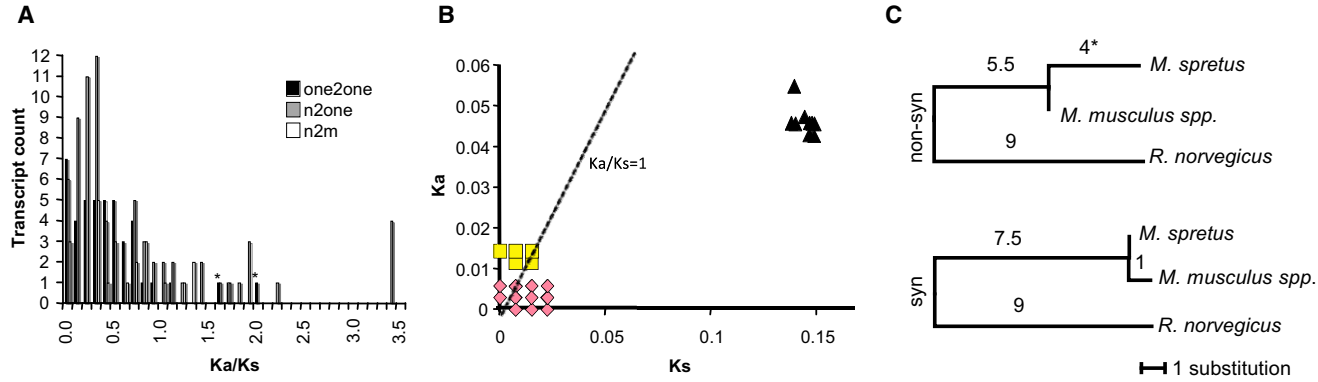

Figure 3. Adaptive Evolution of *vkorc1* in the *M. spretus* Lineage

(A) Plot of Ka/Ks between 184 *M. m. domesticus* and *M. spretus* gene transcripts. To reflect our confidence in orthology, transcripts are grouped in order of decreasing confidence in orthology as one2one, one 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 indicated with an asterisk.

(B) Plot of Ks versus Ka of *vkorc1* between *R. norvegicus*, *M. spretus*, and *M. musculus* spp. (*M. m. domesticus*, *M. m. musculus*, *M. m. castaneus*, and *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 nonsynonymous and 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. The asterisk indicates significant excess of nonsynonymous substitutions (n = 4), as determined using Tajima's relative rate test.

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) (Figure 1; [1]). Our analysis of the rate of interspecific evolution leading to the amino acid sequence differences (Ka) relative to the corresponding rate at synonymous positions (Ks) between *M. spretus* and *M. m. domesticus* identified *vkorc1* as one of the fastest-evolving *M. spretus* transcripts sequenced thus far (Ka/Ks = 1.54–1.93; Figure 3A). This high evolutionary rate of Ka/Ks > 1 was predominantly seen between *M. musculus spp.* and *M. spretus*, i.e., after mice and rats split (Figure 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 four nonsynonymous substitutions (Tajima's relative rate test [26], p = 0.045; Figure 3C). No such excess was observed for silent substitutions (p = 0.317).

The adaptive molecular evolution of *vkorc1* in *M. spretus* appears to have 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 of 7 (57%) mice tested succumbed to bromadiolone, and 0 of 9, 0 of 10, and 0 of 7 succumbed to difenacoum, chlorphacinone, 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 jird (*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, these amino acid changes appear individually 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 the 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.

Conclusions

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 was likely 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 rodenticide-susceptible parental species, at least over that of *M. m. domesticus* carrying wild-type $vkorc1^{dom}$.

Accession Numbers

Sequence data described herein have been deposited at GenBank with the accession numbers GQ905681–GQ905750, GQ905756–GQ905769, GQ905772–GQ905862, and HM026946–HM027481.

Supplemental Information

Supplemental Information includes one figure, four tables, and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.cub.2011.06.043.

Acknowledgments

We thank Hans-Joachim Pelz for passing on mice to us via the Federal Research Centre for Cultivated Plants, Julius Kühn-Institut, Vertebrate Research (Münster, Germany). We thank pest control officer Jürgen Kruczweski from BioTec-Klute for work in the field; Shuwei Li for Perl scripts; and Joan Strassmann, David Queller, Volker Rudolf, Valerie Huguet, Ken Whitney, Jerry Coyne, and Mohamed Noor for comments on the manuscript. This work was funded partially by National Institutes of Health grant R01-HL091007-01A1 to M.H.K., Rodenticide Resistance Action Committee funds to M.H.K., and Bayer Environmental Science to F.-R.M. and N.K. We thank the anonymous reviewers and journal editors for helpful comments.

Received: March 24, 2011
Revised: May 19, 2011
Accepted: June 16, 2011
Published online: July 21, 2011

References

1. Rost, S., Fregin, A., Ivaskevicius, V., Conzelmann, E., Hörtnagel, K., Pelz, H.J., Lappegard, K., Seifried, E., Scharrer, I., Tuddenham, E.G.D., et al. (2004). Mutations in *VKORC1* cause warfarin resistance and multiple coagulation factor deficiency type 2. Nature *427*, 537–541.
2. Rost, S., Pelz, H.J., Menzel, S., MacNicoll, A.D., Leon, V., Song, K.J., Jakel, T., Oldenburg, J., and Muller, C.R. (2009). Novel mutations in the *VKORC1* gene of wild rats and mice–a response to 50 years of selection pressure by warfarin? BMC Genet. *10*, 4.
3. Pelz, H.J., Rost, S., Hünerberg, M., Fregin, A., Heiberg, A.C., Baert, K., MacNicoll, A.D., Prescott, C.V., Walker, A.S., Oldenburg, J., and Müller, C.R. (2005). The genetic basis of resistance to anticoagulants in rodents. Genetics *170*, 1839–1847.
4. Li, T., Chang, C.Y., Jin, D.Y., Lin, P.J., Khvorova, A., and Stafford, D.W. (2004). Identification of the gene for vitamin K epoxide reductase. Nature *427*, 541–544.
5. Scully, M.F. (2002). Warfarin therapy: Rat poison and the prevention of thrombosis. Biochemist *24*, 15–17.
6. Sadler, J.E. (2004). Medicine: K is for koagulation. Nature *427*, 493–494.
7. Gage, B.F. (2006). Pharmacogenetics-based coumarin therapy. Hematology (Am. Soc. Hematol. Educ. Program) *2006*, 467–473.
8. Jackson, W.B., and Ashton, D. (1986). Case histories of anticoagulant resistance. In Pesticide Resistance: Strategies and Tactics for Management, Committee on Strategies for the Management of Pesticide Resistant Pest Populations, National Research Council (Washington, DC: National Academies Press), pp. 355–369.
9. Ishizuka, M., Tanikawa, T., Tanaka, K.D., Heewon, M., Okajima, F., Sakamoto, K.Q., and Fujita, S. (2008). Pesticide resistance in wild mammals—mechanisms of anticoagulant resistance in wild rodents. J. Toxicol. Sci. *33*, 283–291.
10. Wang, J., Feng, Z., Yao, D., Sui, J., Zhong, W., Li, M., and Dai, J. (2008). Warfarin resistance in *Rattus losea* in Guangdong Province, China. Pestic. Biochem. Physiol. *91*, 90–95.
11. Arnold, M.L. (2004). Transfer and origin of adaptations through natural hybridization: Were Anderson and Stebbins right? Plant Cell *16*, 562–570.
12. Palomo, L.J., Justo, E.R., and Vargas, J.M. (2009). *Mus spretus* (Rodentia: Muridae). Mamm. Species *840*, 1–10.
13. Pelz, H.J., and Niethammer, J. (1978). Kreuzungsversuche zwischen Labor-Hausmäusen und Mus spretus aus Portugal. In Sonderdruck Zeitschrift für Säugetierkunde, *Volume 43* (Hamburg, Germany: Paul Parey), pp. 302–304.
14. Dejager, L., Libert, C., and Montagutelli, X. (2009). Thirty years of *Mus spretus*: A promising future. Trends Genet. *25*, 234–241.
15. Orth, A., Belkhir, K., Britton-Davidian, J., Boursot, P., Benazzou, T., and Bonhomme, F. (2002). [Natural hybridization between 2 sympatric species of mice, *Mus musculus domesticus L.* and *Mus spretus Lataste*]. C. R. Biol. *325*, 89–97.
16. Song, Y., Vera, N., and Kohn, M.H. (2008). Vitamin K epoxide reductase complex subunit 1 (*Vkorc1*) haplotype diversity in mouse priority strains. BMC Res. Notes *1*, 125.
17. Kliman, R.M., Andolfatto, P., Coyne, J.A., Depaulis, F., Kreitman, M., Berry, A.J., McCarter, J., Wakeley, J., and Hey, J. (2000). The population genetics of the origin and divergence of the *Drosophila simulans* complex species. Genetics *156*, 1913–1931.
18. Barton, N.H. (2001). The role of hybridization in evolution. Mol. Ecol. *10*, 551–568.
19. Teeter, K.C., Payseur, B.A., Harris, L.W., Bakewell, M.A., Thibodeau, L.M., O'Brien, J.E., Krenz, J.G., Sans-Fuentes, M.A., Nachman, M.W., and Tucker, P.K. (2008). Genome-wide patterns of gene flow across a house mouse hybrid zone. Genome Res. *18*, 67–76.
20. Whitney, K.D., Randell, R.A., and Rieseberg, L.H. (2006). Adaptive introgression of herbivore resistance traits in the weedy sunflower *Helianthus annuus*. Am. Nat. *167*, 794–807.
21. Kohn, M.H., Pelz, H.J., and Wayne, R.K. (2000). Natural selection mapping of the warfarin-resistance gene. Proc. Natl. Acad. Sci. USA *97*, 7911–7915.
22. Kim, Y., and Stephan, W. (2002). Detecting a local signature of genetic hitchhiking along a recombining chromosome. Genetics *160*, 765–777.
23. Meiklejohn, C.D., Kim, Y., Hartl, D.L., and Parsch, J. (2004). Identification of a locus under complex positive selection in *Drosophila simulans* by haplotype mapping and composite-likelihood estimation. Genetics *168*, 265–279.
24. Greene-Till, R., Zhao, Y., and Hardies, S.C. (2000). Gene flow of unique sequences between *Mus musculus* domesticus and *Mus spretus*. Mamm. Genome *11*, 225–230.
25. Johnsen, J.M., Teschke, M., Pavlidis, P., McGee, B.M., Tautz, D., Ginsburg, D., and Baines, J.F. (2009). Selection on *cis*-regulatory variation at *B4galnt2* and its influence on von Willebrand factor in house mice. Mol. Biol. Evol. *26*, 567–578.
26. Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics *123*, 585–595.
27. Baeumler, W., and Asran, A.A. (1987). Susceptibility of house mice (*Mus musculus*) of different origins to anticoagulants. Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz *60*, 1–6.
28. MacNicoll, A.E. (1993). Anticoagulant rodenticides–tolerance and resistance. Phytoparasitica *21*, 185–188.
29. Boursot, P., Auffray, J.C., Britton-Davidian, J., and Bonhomme, F. (1993). The evolution of house mice. Annu. Rev. Ecol. Syst. *24*, 119–152.