Adaptive introgressive hybridization with the Algerian mouse (*Mus spretus*) promoted the evolution of anticoagulant rodenticide resistance in European house mice (*M. musculus domesticus*)

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Abstract

Adaptive introgressive hybridization refers to the natural transfer of genes after interspecific mating between species, and subsequent expression of these genes in the recipient species as a new trait that confers selective advantages. Conceptually, the process harnesses immense potential to explain rapid evolution of traits. However, the process requires that reproductive isolation be overcome. Here we present a case of the adaptive introgression of anticoagulant rodenticide resistance from the Algerian mouse (*Mus spretus* Lataste, 1883) to the Western European house mouse (*M. musculus domesticus* Linnaeus, 1758). These once allopatric species have come into secondary geographic contact and hybridize occasionally. However, only for an approximately ~20.3 megabase-sized genomic fragment, carrying the vitamin K 2,3-epoxide reductase subunit 1 gene (*vkorc1*) of *M. spretus* (*vkorc1*^{spr}), has hybridization resulted in introgression into the *M. m. domesticus* genome.

This *vkorc1*^{spr} allele carries amino-acid substitutions, conferring resistance to anticoagulants, has evolved under positive selection in *M. spretus* (Ka/Ks=1.54-1.93) and displays adaptive population genetic dynamics in *M. m. domesticus* populations. Since its natural inception <60 years ago, this novel form of pesticide-resistant mice has spread far into the range of *M. m. domesticus*. Other *vkorc1* resistance alleles now known to occur in European house mice originated either by *de novo* mutation or from standing genetic variants. Recombinants between these and introgressed alleles are now emerging, but the role of such novel alleles in resistance has yet to be established. Our snapshot of the ongoing adaptive introgression of *vkorc1*^{spr} illustrates how hybridization, coinciding with strong selection with anticoagulants, resulted the breakdown of reproductive barriers between *M. spretus* and house mice. Pest control should anticipate the possibility of horizontal gene transfer between closely related rodent species as a mechanisms leading to rodenticide resistance.

Keywords: genetic introgression, hybridization, vitamin K epoxide reductase subcomponent 1, *vkorc1*

Introduction

Anticoagulant rodenticides inhibit the vitamin K 2,3-epoxide reductase complex and thereby block blood coagulation, such that susceptible house mice (*M. m. domesticus*) consuming the bait succumb to haemorrhage. Anticoagulant rodenticides, notably warfarin, which have been in use since 1950, locally have lost their effectiveness. Single point mutations in the vitamin K epoxide reductase subcomplex 1 (*vkorc1*) gene are known to form the basis of this resistance in mice, as well as in Norway rats (*Rattus norvegicus* Berkenhout, 1769) (Li et al., 2004; Pelz et al., 2005; Rost et al., 2004, 2009). Warfarin resistance is a classic mammalian case of microevolution by mutation that can be observed directly in the field. Newer rodenticides, including bromadiolone, were developed to counteract such resistance. Here we report on a population level survey in Europe that discovered that, in addition to point mutations in *vkorc1*, anticoagulant resistance in European house mice has evolved within the last 60 years by genetic introgression of *vkorc1* from *M. spretus* (*vkorc1*^{spr}) into the genome of house mice. We characterize the *vkorc1*^{spr} introgression, show that it can mediate resistance, and we report on its selective advantage among field populations of house mice.

Materials and methods

To determine the genetic mutations, we conducted DNA sequencing of the *vkorc1* gene in >100 mice from Europe following methods as described in Song et al. (2008). We determined the amount of genetic material in house mice derived from *M. spretus* by conducting a scan of chromosome 7 and sequencing 18 genes, including the *vkorc1* gene and its 5' region. We conducted population genetics tests to infer the selection history of the introgression. Finally, we conducted molecular evolutionary analyses of the *vkorc1* gene in mice. In order to assess the susceptibility of mice to anticoagulants, feeding trials were conducted with wild-derived strains of house mice from Germany carrying the complete version of *vkorc1*^{spr}. The testing protocol followed OPP 1.204 (US EPA, 1991) standards and was conducted under the German equivalent of IACUC (Institutional Animal Care and Use in Research) protocols.

Results

We showed that genetic variants have been introgressed from *M. spretus* into *M. m. domesticus* over ~20.3 megabases (Mb) on chromosome 7, including *vkorc1*. The introgressed *vkorc1*^{spr} (and recombinants thereof) has high frequency (>80%) in areas where *M. spretus* and house mice occur sympatrically (e.g. Spain), and remains abundant in areas where only house mice occur (e.g. Germany, ~33%). The *vkorc1*^{spr} was not detected in mice from England, Scotland, Italy and Greece. House mice carrying the complete *vkorc1*^{spr} displayed reduced susceptibility to three anticoagulant rodenticides when compared to house mice carrying the wild-type copy of the gene (mortality rates to coumatetralyl and bromadiolone were 20% and 9%, respectively). In contrast, *M. m. domesticus* carrying wild-type *vkorc* displayed mortality rates of 84-100% to coumatetraly and 85% to bromadiolone. Finally, 20% of *M. m. domesticus* carrying complete *vkorc1*^{spr} survived difenacoum trials, whereas all *M. m. domesticus* with wild-type *vkorc1* died when fed on difenacoum bait.

The *vkorc1* is one of the fastest-evolving genes in the *M. spretus* genome, and we showed that this rapid evolution took place after *M. spretus* had split from its congeneric species of *Mus*. A Ka/Ks ratio >1 indicates that this rapid evolution was due to positive selection on amino acids in the *M. spretus* lineage.

Discussion

Adaptive protein evolution of *vkorc1* in the *M. spretus* lineage was driven by unknown ecological factors, but the combination of amino-acids in *vkorc1*^{spr} protect *M. spretus* against anticoagulants (Baeumler and Asran, 1987), i.e. is have resistance as a pleiotropic effect. Horizontal transfer of *vkorc1*^{spr} to house mice transfers this trait. Apparently, low levels of inter-specific gene flow (through fertile female hybrid offspring) between *M. spretus* and house mice has been sufficient to enable the transfer of the anticoagulant rodenticide resistance gene during the past 60 years since the introduction of anticoagulant rodenticides.

The geographic origin of the introgression likely is in Southwestern Europe or in Northern Africa, where *M. spretus* is sympatric and on occasion apparently forms hybrids with *M. musculus* (Orth et al., 2002). Two independent evolutionary trajectories, both involving the *vkorc1* gene, have led to the adaptation to selection pressure applied by anticoagulant rodenticides in house mice: point mutation and adaptive introgressive hybridization.

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