Evolution of molecular resistance to snake venom α-neurotoxins in vertebrates

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Chapter 6. Thesis Summary and General Discussion

We have examined sequences from the ligand-binding domain of the nicotinic acetyl choline receptor (nAChR) in 148 vertebrate species. We are in interested in this receptor because the α-neurotoxins of many venomous snakes binds to this receptor in its location at the neuromuscular junction in all vertebrates. Furthermore, some animals have evolved resistance to snake venoms and show modifications in the ligand binding domain of the nAChR which inhibit the binding of snake α-neurotoxins. Our analysis has shown that numerous vertebrate species, most of which were not previously known to possess α-neurotoxin resistance, do actually contain resistance-related modifications. These modifications are present in most of the taxa in our dataset, with the unexpected exclusion of the birds. It was particularly surprising to us that the snake-specialist predatory birds *Circaetus pectoralis* (black-chested snake eagle) and *Sagittarius serpentarius* (secretary bird) did not possess resistance modifications. There were also relatively few resistance-related mutations within the mammals. By contrast, there were multiple convergent evolutions of the well-characterised N-glycosylation motif within the squamate reptiles—particularly the snakes. We also identified a number of sites under positive selection, such as mutations to the proline subsite. Future functional testing will be needed to validate that these modifications do indeed confer resistance. To provide functional confirmation that resistance-related modifications do indeed reduce susceptibility to toxins, we used developmental bioassays. These assays showed that two species possessing resistance-related modifications of the nAChR (stickleback and bearded dragon) were less susceptible to the toxic effects of cobra venom than two species that lacked such modifications (zebrafish and chicken). In summary, we demonstrate that the range of mechanisms along with the

phylogenetic distribution of resistance to snake α-neurotoxin appears to be more extensive than was previously appreciated. It also shows strong evidence of the convergent evolution of the same resistance mutations in independent linages. Our findings also support the notion that the mutations we have identified in this thesis may represent adaptive change in response to selective pressures exerted by α-neurotoxic snake venoms in an evolutionary arms race. Thus, we conclude that the evolutionary arms race between predator and prey appears to be a pervasive feature of the trophic interactions surrounding venomous snakes, which is shaping the molecular evolution of the nAChR in the vertebrates.