

# Gene duplication, protein evolution, and the origin of shrew venom

Jean K. Lightner

Evolutionists have suggested that gene duplication plays a major role in evolution. The popular level story is that once a gene is duplicated, one copy is free to mutate while the other can retain its original function. The naturalistic processes of ‘random’ mutation and natural selection are believed sufficient to allow for novel functions to arise in one copy. These can be preserved if they benefit the organism. Gene duplication is believed by some evolutionists to be a major force in the acquisition of novelty since the emergence of the putative universal common ancestor.

While gene duplications do occur, creationists have pointed out that they are overestimated within the evolutionary paradigm.<sup>1</sup> Perhaps the biggest problem with many assumed duplications is the fact that there are no plausible ‘intermediates’. The gene that supposedly arose via duplication is often essential in its function, leaving no way for organism to survive while it is waiting for the duplication and subsequent mutations to occur.<sup>2</sup> Also, it is clear that when gene duplications do occur, a variety of outcomes are possible in the duplicated gene.<sup>3</sup>

## Gene duplications in the creation model

It is important to understand the role of gene duplication within the creation model. Purdom discussed a putative gene duplication in yeast where a single gene which carries out two functions in one species appears to be duplicated in another species.<sup>4</sup> In the second species, each one of the duplicates carries out one of those functions. This phenomenon is known as subfunctionalization. In the Duplication-Degeneration-Complementation model, genes are duplicated and each of the genes is retained because each carries on part of the function of the original gene. This certainly fits within the creation model, although it would seem there should be some purpose to it if it occurs often.

Interestingly, the evolutionary researchers proposed that the gene duplication in yeast relieved an ‘adaptive conflict’.<sup>5</sup> After the duplication, each gene was free to change in an adaptive way, or become more specialized in one of the functions, which wouldn’t have been possible in the single ancestral gene. This seems to me to have teleological implications, making me suspicious that naturalistic processes alone can’t account for this, assuming the proposed changes are real. Purdom points out that the fitness differences in the yeast were very small, calling into question the ability of natural selection to fix such changes in a population.<sup>6</sup>

Liu examined the immunoglobulin heavy chain gene family to determine if gene duplication was a plausible explanation for genes within this family. He concluded that the gene family was irreducibly complex and could not have arisen by duplication. In contrast, the copy number variants present in humans could plausibly be accounted for by gene duplications.<sup>7</sup>

I examined gene duplications in organophosphorus-resistant blowflies<sup>8</sup> and leaf-eating monkeys.<sup>9</sup> In blowflies the resistant alleles in the *LcaE7* gene were duplicated, perhaps by unequal crossing over, allowing resistance to two different insecticides to be carried on a single chromosome. In the second case, gene duplication was followed by adaptive mutations in the pancreatic ribonuclease gene allowing for more efficient digestion in the reduced intestinal pH of leaf-eating monkeys. Given the details, it appears highly unlikely that natural selection can account for this non-random pattern in monkeys. Instead, these patterns seem to indicate that some impressive genomic programming was involved in the timing and placement of the duplications and at least some of the mutations.

## Shrew venom

While venom is more commonly associated with reptiles, it also exists in some mammalian species. The American short-tailed shrew (*Blarina brevicauda*) produces a serine protease toxin, BLTX, which has been isolated

	ins 1	ins 2	ins 3	ins 4
Baboon	NMSLLK~~~~~NHT~~~~RQADEDYSHDLM		QAALYHFSTFQ~~~CGGILV	CLASGWGSI~~~EPENFSYPDDLQC
Dog	NLSLLK~~~~~NHT~~~~RLPEEDYSHDIM		QAALYHYSKFQ~~~CGGVLV	CYASGWGSI~~~EPDKFIYPDDLQC
Pig	NLSLLK~~~~~NHT~~~~KADGKYSHDLM		QVAIYHYSSFQ~~~CGGVLV	CQASGWGSI~EPGPDDEFPPDEIQC
Blarinasin-1	NMRLKLLLSDELNDTYTYDEISLGADFSHDLM		QALL~TFTNGLDGVCGGVLV	CHASGWGSM~DPYSRNFPRGTGKLQC
Blarinasin-2	NMRLKLLLSDEMNDTYTYEIFPGADFSHDLM		QALL~TFTNGLDGVCGGVLV	CHASGWGSM~DPYSRNFPRGTGKLQC
BLTX	NMTLLNLLLSHKMNLTFYKTFGLGADFSHDLM		QALL~TFTRKHNSVCGGVLV	CHVSWGRTSQNYENSEFVLPEKLQC
	loop 1		loop 3	

**Figure 1.** Amino acid sequence alignment of kallikrein-1 enzymes from baboon (Q28773.1\*), dog (NP\_001003262.1) and pig (NP\_001001911.1), with blarinasin-1 (Q5FBW2.1), blarinasin-2 (Q5FBW1.1) and the blarina toxin, BLTX (Q76B45.1), from *Blarina brevicauda*. Regulatory loops and insertions (ins) are indicated after Aminetzach *et al.*, ref. 11.

\* Accession number

from its salivary glands.<sup>10</sup> Two homologs of BLTX are also present in this shrew: a non-toxic blarinasin-1, and an uncharacterized blarinasin-2. All three proteins show the greatest sequence similarity to mammalian glandular kallikrein-1 serine proteases (figure 1). A recent paper provides an intriguing scenario that could account for the origin of venom in this shrew and some divergent species.<sup>11</sup>

Relative to other mammalian kallikrein-1 sequences (from 12 species of 6 different orders), *B. brevicauda* carries four short in-frame insertions. Each of these insertions falls within one of the first three of five regulatory loops of the protein. The first loop, where two of the insertions fall, contacts the substrate and contributes to the specificity and rate of catalysis of the enzyme. The other loops likely regulate enzyme activity as well.

### Shrew gene duplication

If BLTX and its homologs were derived from a kallikrein-1 gene, then three insertions appear to have occurred prior to an initial gene duplication (figure 2). Even if the genes did not derive from a kallikrein-1 gene and these inferred insertions were part of the original creation, it appears an in-frame (3 base pair) insertion occurred

to lead to the formation of BLTX.<sup>12</sup> A subsequent duplication in the other gene would explain the existence of blarinasin-1 and blarinasin-2. This scenario should allow for a viable animal, thus making it plausible.

### Non-random mutations and toxin formation

The insertions alone do not appear to account for the toxic properties of BLTX. Comparison of BLTX with its homologs revealed a high number of non-synonymous substitutions that resulted in extensive sequence evolution within the regulatory loops. These changes affect both the physicochemical and structural properties of BLTX.

Similar to the findings in the pancreatic ribonuclease gene of leaf-eating monkeys, amino acid substitutions are strongly directional. These changes have resulted in a hydrophobic loop 1 in BLTX compared to the hydrophilic loop 1 in blarinasin-1 and -2. Also, loop 2 in BLTX is strongly hydrophilic in contrast to the hydrophobic nature of this loop in both blarinasins. In both these loops, the BLTX is more positively charged.

Simulations suggest that these changes affect the structure of the BLTX molecule. The hydrophobic nature of the first loop results in a

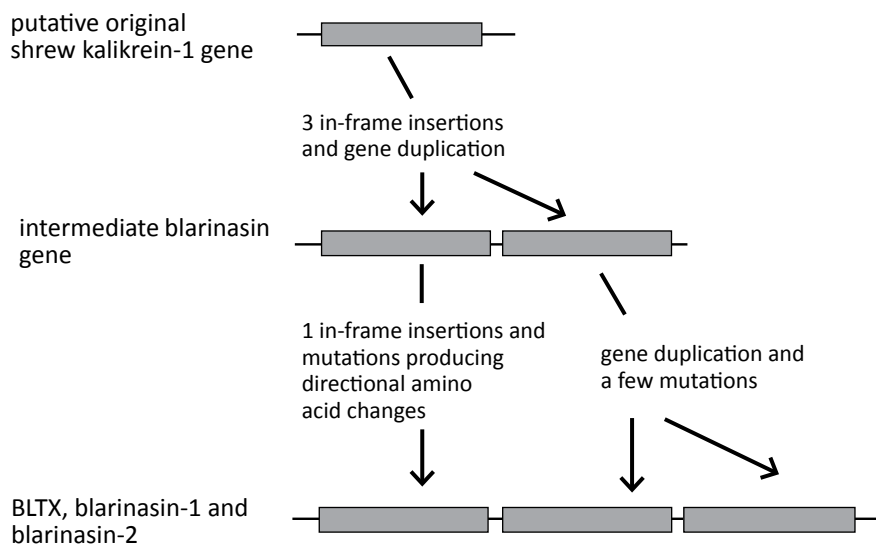
more compressed conformation which exposes the active site. This could contribute to increased catalysis, which is believed to be the cause of BLTX toxicity. In addition, BLTX is considerably more flexible, perhaps due to its increased length and polarity. Finally, the alteration of the charge distribution around the active site may also affect catalysis.

### A repeating pattern

It has been noted that similar proteins can be recruited as toxins in divergent taxa.<sup>13</sup> The kallikrein-related serine protease GTX from the Mexican bearded lizard (*Heloderma horridum*), which is thought to possess toxicity due to increased catalysis as in BLTX, was examined for structural similarities to BLTX.<sup>14</sup> Similar to BLTX, a large in-frame insertion was identified adjacent to loop 1. When compared to twelve other lizard kallikrein-related proteins, this insertion was found to be unique. Loop 1 is also largely hydrophobic and positively charged, which appear to be unique features of BLTX and GTX. Similar conformational changes appear to be shared by these two proteins as well. The authors, who are presumably evolutionists, make the remarkable comment that the evidence “suggests that adaptation may be highly predictable”.<sup>15</sup> This is rather shocking since evolution, which depends on chance mutations for the origin of novelty, is supposed to lack predictability.<sup>16</sup>

### Consistency with the creation model

The creation model differs from the evolutionary one in that there is an omniscient Creator. With the detailed genetic information that has become available, it is clear that some genetic changes are the result of designed mechanisms. Thus the types of changes suggested in this scenario, gene duplication and non-random changes, are fully consistent with the creation model as long as genetic changes are not assumed to



**Figure 2.** Proposed development of the blarinasins and BLTX from a kallikrein precursor via insertions, gene duplication and mutations. These steps include plausible within-kind genetic changes that should allow for viable creatures.

be confined to naturalistic processes such as ‘random mutation and natural selection’.

Second, the creation model posits that the initial creation was very good. Venom either did not exist, or was otherwise prevented from causing harm. The observation that the same proteins have been recruited for venom across diverse taxa, along with the similarities between BLTX and GTX, suggest that various designed mechanisms have allowed for the origin of toxins in a cursed world. A deeper understanding of these patterns will help advance the creation model and possibly shed further light on strategies for adaptation.

More information would be helpful in exploring this hypothesis further. Unlike the studies involving blowflies and leaf-eating monkeys, intrabaraminic comparisons were not made with the shrews. Instead it was assumed that the original gene was similar to that of other mammals which came from different orders. While this assumption appears reasonable, that doesn’t make it correct. Also, more detailed analysis of enzymatic activity of the proteins and their various physiological roles could prove enlightening. Since shrews are ‘unclean animals’ which brought a limited amount of genetic variability through the Flood, more detailed intrabaraminic comparisons may allow for stronger conclusions. To do this well, baraminologic work needs to be done on shrews to identify the probable limits of the baramin.

### Predictable evolution?

A hallmark of neo-Darwinian evolution is that it is an undirected process where ‘random’ mutations and natural selection are major forces shaping genetic changes. Evolution should be chaotic in nature as opposed to following similar pathways. The parallel nature of toxin formation would not be expected, especially in such divergent taxa, even with similar selective pressure. Thus, the notion of predictable evolution should be an

oxymoron. In contrast, if creatures were created according to their kinds with the ability to make adaptive genetic changes, repeating patterns would seem more likely. Such patterns would be a reflection of similar design in genes that allow for changes and similar design in the mechanisms by which the changes are made. None of the examples discussed here show evidence of increasing the complexity of the genome by building well-integrated biological pathways. Instead, at least some of the changes are useful in allowing for adaptation to various environmental conditions. Such adaptation is necessary in a biblical worldview where God has expressed his desire that life fill the earth.<sup>17</sup>

Gene duplications require a pre-existing gene and mechanisms by which duplication can take place.<sup>18</sup> The fortuitous timing of some duplications, particularly those in blowflies and leaf eating monkeys, suggests cellular control over the timing and placement of these duplications. The gene must be constructed in such a way that potentially useful changes can be made and mechanisms must exist to facilitate appropriate changes. More extensive research on gene duplications and protein evolution is necessary to establish its importance in intrabaraminic diversification and adaptation.

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