Patterns of change over time: organophosphorus resistance in the Australian sheep blowfly, Lucilia cuprina

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A deeper understanding of patterns of change in creatures over time is necessary to advance the creationary view of biology. While much research has been done at a molecular level with bacteria, there is a need to evaluate changes in sexually reproducing organisms. The development of insecticide resistance in insect populations has been studied in considerable detail. A literature review focusing on insecticide resistance in Lucilia cuprina, the Australian sheep blowfly, was conducted. While the development of resistance to malathion can be easily explained by natural selection, resistance to diazinon is not so easily explained. It is suggested here that diazinon resistance and multiple resistance from gene duplication may be the result of designed mechanisms that allow for adaptation in created life. It is pointed out that evolutionists are increasingly discussing genetic and metabolic systems within the context of computer programming. A deeper understanding of the underlying mechanisms involved in genetic changes may explain the considerable variation within created kinds (baramins) and the ability of these creatures to adapt to changing environments. In other words, we can gain a deeper understanding and appreciation for how God sustains his creation in a fallen world.

To further develop a creationist view of biology, it is necessary to more fully understand patterns of change within creatures and the likely role of these types of changes during history. Unfortunately, since evolution is sometimes defined as 'change through time', creationary apologists have sometimes responded with vague arguments that creatures don't really change much—an ambiguous and not necessarily biblical response. It is not so much the amount (very small genetic changes can result in large phenotypic changes) as the pattern of the changes that is important. The evolutionary model predicts an overall upward trend from chance processes to account for the origin and subsequent major restructuring of well integrated morphology and biochemical pathways. This trend should be obvious since this model claims to be able to account for the diversity of extant kingdoms and phyla. The biblical model may include providential changes (since God cares for his creation[1]) or degenerative changes (since the world was cursed as a result of mankind's rebellion[2]), but not the overall 'creative' changes by purely random processes that characterize the evolutionary model.

Considerable research has been done describing changes in different life forms. Much research has been done at the molecular level in bacteria since they are so convenient to study in the laboratory. It is interesting that researchers in this field who are not part of the creation or intelligent design movements have pointed out that many changes in the genetic code appear as a result of far more complex mechanisms than just random, chance processes.[3] For example, when bacteria are starved, directed mutations may occur to alleviate the stress. It is unclear if similar directed mutations occur in sexually reproducing life forms.[4] One issue is that there must be a mechanism for introducing these mutations into the germline.

Insects as models for studying adaptive genetic change in sexually reproducing organisms

Insects cause tremendous damage to crops and livestock. Numerous insecticides have been developed to control or eliminate these pests. Much to the dismay of those involved in agriculture, insect populations regularly develop resistance to insecticides. Due to its economic impact on agriculture, this resistance has been fairly well studied and provides a logical place to look for patterns of change in sexually reproducing animals. Emphasis will be placed here on a specific pest, *Lucilia cuprina*, the Australian sheep blowfly (figure 1).

There are several popular organophosphorus insecticides (OPs) used to control ectoparasites in sheep. These poisons target acetylcholinesterase, a product of the *Ace* gene. Normally this enzyme breaks down acetylcholine after it has been used to transmit nerve impulses (figure 2). The OPs inactivate acetylcholinesterase so it cannot break down acetylcholine. This results in a build up of acetylcholine at the nerve synapse and a hyperexcited central nervous system which kills the insect. While some insects (e.g. *Drosophila melanogaster*[5] and *Musca domestica*[6]) have developed OP resistance through mutations in the *Ace* gene, *L. cuprina* has not, despite the fact that it has a highly homologous gene in which 'All major structural and functional features of the protein are conserved.'[7] Further study indicates that the product of the *Ace* gene in *L. cuprina* does not interact as readily with OPs as does the product of another gene (*αE7*). The reverse situation occurs in *Drosophila*.

Malathion resistance and natural selection

Malathion is an OP often used to control lice in sheep. *L. cuprina* has developed resistance to this pesticide through

Figure 1. *Lucilia cuprina*, the Australian sheep blowfly, is an introduced pest that costs the Australian wool industry over $160 million a year. Eggs laid on living sheep hatch and the maggots eat through the animal's flesh in what is called flystrike.

a point mutation in the *LcαE7* gene which results in a Trp251Leu substitution. The *LcαE7* (sometimes known as *Rop*-1 or *Rmal*) gene normally produces a carboxylesterase, E3. The mutation decreases the carboxylesterase activity while improving the enzyme's ability to break down dimethyl OPs, particularly malathion.[8] In an attempt to determine if this variant was present prior to selection by OP use, pinned specimens were sampled. It was found that this particular mutation was fairly widespread prior to the introduction of OPs.[9] Thus, the development of resistant *L. cuprina* populations appears to be a classic case of natural selection. It is not that the data precludes the possibility of directed mutations playing a role, but such an explanation appears unnecessary.

Diazinon resistance

Diazinon is an OP that is used to directly control the sheep blowfly. Resistance to this OP is associated with a separate point mutation in the *LcαE7* gene that results in a Gly137Asp substitution. In this case the carboxylesterase activity is abolished and a new OP hydrolase activity is conferred on the enzyme, making it more effective against diethyl OPs such as diazinon.[10] Initially this was associated with significant asymmetry and fitness costs in the absence of the insecticide. Eventually, a mutation appeared in a modifier gene which ameliorated these deleterious effects.[11] Since diazinon is used widely in sheep producing countries such as Australia, this mutation is present in the majority of *L. cuprina* sampled in these areas. However, this polymorphism has not been detected in any of the pinned specimens collected prior to OP use.[9]

The development of diazinon resistance has been cited as evidence for evolution.[12] Clearly this research has advanced our understanding of the molecular mechanisms of adaptation, but it sheds no light on the origin of molecular systems. Genetic changes which result in a shift of an enzyme's substrate hardly explain the origin of the gene for the enzyme.[13]

There are still many unanswered questions. For example, it could be argued that diazinon resistance was present in the population prior to the use of this OP, but was not detected due to low frequency in the population and the small sample size of pinned specimens. However, if this is true, it seems odd that natural selection would not have effectively eliminated it given the significant fitness costs associated with the loss of carboxylesterase activity. Conversely, it could also be argued that both the appearance of the resistant mutation and of the subsequent modifier mutation were the result of directed mutations resulting from the selection pressure. Interestingly, the same mutation conferring diazinon resistance has been found in a sister species, *L. sericata*,[9] and in the housefly *M. domestica*.[14] This has been interpreted as 'suggesting convergent evolution around a finite set of resistance options.'[9] Evolutionists have yet to provide credible explanation of how molecular systems that putatively originated by random, chance processes come equipped with 'options' that allow for adaptation. It appears that evolutionists generally accept that this mutation arose independently in separate species. The fortuitous timing of the appearance of this mutation that corresponds with OP use suggests something more than just random processes at work to allow for such dramatic adaptation.

Gene duplications

No variants have been found where both mutations occur together within the same gene. Moreover, it is predicted that if both mutations existed within a single gene, it would not confer effective resistance against both these OPs. This is because effective malathion resistance appears to require the presence of some carboxylesterase activity, and the mutation which confers diazinon resistance abolishes this.[15] However some isogenic strains of *L. cuprina* are resistant to these two OPs as a result of gene duplication. Intriguingly, three different gene duplications were identified and each involved a resistant form of the gene. No gene duplications were identified with any of the various susceptible alleles.[8] This suggests that gene duplication may be a designed adaptive mechanism, rather than just an accidental occurrence as the standard evolutionary paradigm predicts.

Recently, there have been articles in the scientific literature that seem to confirm this idea. For example, in humans differences in the copy number of genes are a significant source of variation.[16] Researchers examining gene duplications in fungal genomes concluded,

> '… that gene duplication and loss is highly constrained by the functional properties and interacting partners of genes. In particular,

stress-related genes exhibit many duplications and losses, whereas growth-related genes show selection against such changes. … By characterizing the functional fate of duplicate genes we show that duplicated genes rarely diverge with respect to biochemical function, but typically diverge with respect to regulatory control. Surprisingly, paralogous modules of genes rarely arise, even after whole-genome duplication. Rather, gene duplication may drive the modularization of functional networks through specialization, thereby disentangling cellular systems.'[17]

Laboratory development of resistance

At least one study has been done attempting to develop strains resistant to diazinon in the laboratory. Some of the blowfly males were mutagenized using ethyl methanesulfonate (EMS). When both susceptible and mutagenized strains were selected with a diazinon concentration that kills 100% of susceptible flies (0.0004% w/v), the LC_{100}, no susceptible flies survived. Some of the mutagenized flies survived and appeared indistinguishable from natural populations carrying the *LcαE7* resistant allele. In contrast, when susceptible and mutagenized strains were selected on low doses of diazinon (0.0001% w/v), there was no significant difference in the responses between strains. The insect populations developed a low level, polygenic resistance. The specific genes involved varied with each trial. However, none of these insects survived a challenge of diazinon at the LC_{100} concentration which discriminates between susceptible flies and heterozygotes for the *LcαE7* resistant allele.[18]

It is intriguing that mutagenesis resulted in *LcαE7* resistant phenotypes with selection above the LC_{100}, but not in selection significantly below this concentration. Perhaps some resistant insects were generated in both cases, but the selection with low levels of diazinon did not favour them significantly enough for that genotype to remain in the population. Alternatively, perhaps EMS did not directly generate the resistant allele, but instead affected the genetic stability which resulted in the resistant phenotype when significant pressure was applied.

A study attempting to induce particular mutations in bacteria found that low exposure times to radiation that killed roughly half the population failed to produce the desired mutants. As the exposure time increased killing 93% of the population, some mutants were found. Further increasing the time until there was a 96–99% mortality left only the desired mutants.[19] In both the bacteria and diazinon resistance in blowflies, the mutations are costly in terms of loss of normal function. Thus from a creationary viewpoint, it is not surprising that these changes are generally resisted. The example in bacteria suggests that selecting diazinon mutants might be most effective just below the LC_{100}. It would be interesting to see if the mutation can be induced in susceptible flies under these circumstances without the aid of EMS. In any case, there are many questions waiting to be answered to gain a deeper understanding of how, when and why these changes occur.

Evolving ideas of evolutionists

The neo-Darwinian view of random mutations driving variability is increasingly seen by evolutionists as inadequate to account for observational data. Recent theories have been advanced including natural genetic engineering[3] and facilitated variation.[20] Both these views encourage an understanding of genetic and metabolic systems within organisms in terms of computer programming. The properties of modularity, reusability and robustness presented in the theory of facilitated variation correlate with well thought out, good design patterns in computer engineering.[21] These and several other properties are combined in a way which allows for genetic variation and adaptation.

'These special properties reduce the number of genetic changes needed for phenotypic change, increase the number of targets for regulatory change, reduce lethality, and increase genetic variation retained in the population. Although the core processes are constrained in their own change of function, they deconstrain regulatory change.'[22]

The authors assume a naturalistic explanation for the origin of these properties; they never attempt to explain their origin. Nevertheless, many of these concepts may prove

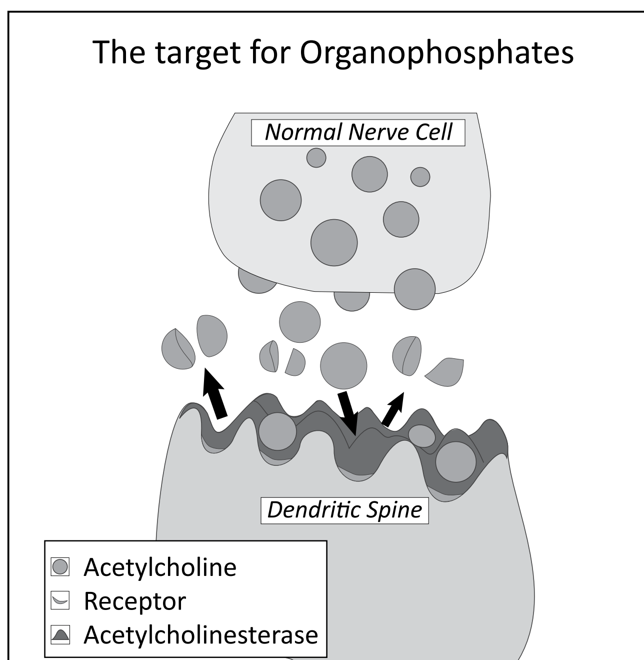

Figure 2. Acetylcholine is used to transmit nerve impulses. Acetylcholinesterase normally breaks down acetylcholine so the signal doesn't last indefinitely. Organophosphates bind acetylcholinesterase so it is unavailable resulting in a hyperexcited nervous system and, if the dose is high enough, death.

useful for creationists to explain the remarkable variation that occurs within created kinds (baramins) and the ability of creatures to adapt to changing environments.

Conclusions

Although the study of the development of insecticide resistance is often considered a topic in evolutionary biology, this type of research is essential for understanding the types of changes which occur in living things. The information derived from observations in this area are critical to further development of our understanding of the world God created and ways in which He sustains it while in its present fallen condition.

It is fascinating that evolutionists are increasingly describing living things in terms of programming. Since man was created in God's image[23] and has become somewhat proficient at programming, it should seem only logical that God himself is a highly proficient programmer. The notion that genetic changes are always from chance processes should be rejected by creationists. Instead, evaluation of conditions surrounding the appearance of particular changes can help elucidate what underlying mechanisms may be involved. This will provide more insight into how God sustains his creation in a fallen world. Scientific research continues to reveal the amazing complexity and design of creatures as well as their astounding ability to overcome immense environmental challenges; facts inconsistent with naïve naturalistic explanations of the origin of life. This area holds great promise as a fertile field for creationary researchers.

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