

Averting Sense for Nonsense in Horizontal Gene Transfer

*What do most scientists do when faced with findings that threaten to topple the ruling paradigm? They describe the findings in detail, fail to interpret them correctly, and avoid discussing their practical implications, dismissing incriminating evidence. They try desperately to paper over the cracks of the crumbling edifice and engage in rampant speculations. **Dr. Mae-Wan Ho** challenges these scientists to tell the truth, to themselves and to the public.*

Horizontal gene transfer – the transfer of genes across distinct species including those in different kingdoms – goes counter to modern genetics and to the theory of evolution. In the case of evolution, both the general theory of evolution - that different organisms descended with modification from earlier common ancestors - and the special neo-Darwinian theory – that organisms evolve by the natural selection of random genetic mutations – are under threat.

In 1859, the year that Charles Darwin's *Origin of Species* took Victorian Britain by storm, Gregor Mendel in Brno, also published his work, which, according to Darwin's followers would have supplied the mechanism of heredity that was lacking from Darwin's theory. It took more than 40 years to the beginning of the last century before Mendel's work was rediscovered to inaugurate the search for the material basis of heredity. As everyone knows, that search culminated in the triumphant discovery of the DNA double-helix.

The significance of DNA for neo-Darwinism is encapsulated in Francis Crick's statement of the 'Central Dogma' of molecular biology: that 'genetic information' flows in one direction only from DNA to RNA to protein, and never in reverse. The Central Dogma lends support to the most hard line genetic determinist assumptions of neo-Darwinism [1, 2]:

- Genes determine characters in a straightforward, additive way: one gene-one protein, and by implication, one character. Environmental influence, if any, can be neatly separated from the genetic.
- Genes and genomes are stable, and except for rare, *random* mutations, are passed on unchanged to the next generation.
- Genes and genomes cannot be changed *directly* in response to the environment.
- Acquired characters are not inherited.

The first assumption has been severely criticised since modern genetics began. Many biologists including Richard Goldsmith and later, C.H. Waddington have pointed to the large influence that the environment has on development, and that the effects of genes and the environment cannot be disentangled [3, 4]. But discoveries of the complex gene-protein relationship and the fluidity of the genome within the past twenty years have really blown that assumption apart [1, 2, 5].

Horizontal gene transfer – a key process of the fluid genome - makes neo-Darwinism untenable in perhaps the most explicit fashion. It means that genes and genomes are *not* stable, that 'characters', encoded by genes, *can* be acquired under certain environmental conditions, and can be inherited. This momentous significance is not even hinted at, in a recently published volume [6] replete with detailed examples of horizontal gene transfer within and across every living kingdom.

One chapter – the shortest in the volume – promises to spell out the relevance of horizontal gene transfer to the release of genetically modified organisms. Syvanen, one of the two co-editors, recalls how, when GM crops were first approved, the US Food and Drug Administration responded to fears of the spread of antibiotic resistance genes by denying this could take place. He himself, however, was just then trying to convince his colleagues that horizontal gene transfer between life's kingdoms was a natural process.

“If these speculations were true, it would make more sense to *defend the transgenic crop industry* (italics mine) by arguing that gene transfer is a natural phenomena [sic] than by arguing that it does not occur.” He continues, “As is well-documented throughout this book, we can infer numerous cases cross-species gene transfers from the whole genome sequencing studies. However, this does not necessarily mean that the frequency of transfer is high enough for gene transfer on the farm may to occur [sic].”

Actually, an important chapter by Russell Doolittle warns against the over-estimation of horizontal gene transfer in our evolutionary past. When the human genome was first announced, the sequencers claimed that more than 200 genes in the human genome were transferred from bacteria. Doolittle dismissed every one of these. Sure enough, these ‘bacterial’ genes soon turned up in the genomes of ‘lowly’ animals, such as the nematode and slime mould [7].

Doolittle points out that horizontal gene transfer is not so very frequent in our evolutionary past that it changes the general theory of evolution, ie, descent with modification, with each species keeping largely genetically distinct, only occasionally exchanging genes horizontally.

But genetic engineering is something else. It sets out to overcome the genetic barriers between species and to *enhance* horizontal gene transfer. One main concern is the transfer of antibiotic resistance marker genes to bacteria that cause diseases.

Syvanen claims to have screened for antibiotic resistance marker genes in feces of mice fed transgenic plant material, and found none. But these results were unpublished and non-peer reviewed, and presumably should be dismissed in the same way that he despatches the study reported by a German scientist who found antibiotic resistance marker genes in transgenic pollen transferred to the yeast and bacteria living in the gut of bee larvae [8].

Syvanen cites a report claiming no gene transfer to bacteria growing on transgenic vegetables, and fails to mention that actually, the report [9] documented high frequencies of gene transfer in the laboratory, *which were reduced down to almost zero by arbitrary assumptions of ‘natural conditions’*. (I have dealt with this and other cases of mis-citing and misinterpretation of evidence on horizontal gene transfer elsewhere [10].)

Syvanen tells us that experiments demonstrating gene transfer with purified DNA from transgenic plants is due to “high frequency recombination”, as though we need not worry. He does not explain that such high frequency recombination arises from sequence homology (similarity in DNA base sequence), and as transgenic DNA is highly mosaic, it would have homologies to a wide variety of bacteria and viruses, and is hence optimised, in that respect alone, for horizontal gene transfer.

In a similar vein, he also dismisses findings that transgenic DNA fed to mice ended up transferred to the mice’s cells. He failed to cite a key field monitoring experiment that found plant transgenic DNA transferred to bacteria in batch cultures from the soil [11].

That is all the more striking when the same author and others in the volume then indulge in rampant speculations on how horizontal gene transfer could explain away one of the classic difficulties with neo-Darwinian theory – parallel evolution occurring in independent lineages. “We simply have not identified those genes responsible for the morphologies that characterise the major geological periods,” Syvanen admits. Nonetheless, he is not deterred from presenting “a series of more speculative pieces that describe the explanatory power of a theory of horizontal gene transfer as it may affect a general theory of evolution.”

I was one of the many critics of neo-Darwinism who, following D’Arcy Thompson and Allen Turing, have argued how forms can only be understood in terms of generic dynamic processes that *generate* the forms [12-16]. How else can I not but see those

mindless acts of speculation as evidence of the intellectual decline symptomatic of the degenerate research programme that's neo-Darwinian biology?

“The only possible explanation for parallel evolution,” it is stated in one chapter, “is similarity in selective force operating on the genetically isolated populations.” The idea that there may be other more appropriate explanations had apparently never crossed their mind.

Let me now describe a much more reasonable scenario based on empirical evidence presented in this book; and there are several others left as exercises for the many authors themselves.

Ferguson and Heinemann, in their chapter “Recent history of trans-kingdom conjugation”, describe evidence that should raise serious concerns over the release of GM crops, although the authors, characteristically, failed to address the issue.

Agrobacterium tumefaciens, the soil bacterium that causes crown gall disease, has been developed as a major gene transfer vector for making transgenic plants. Foreign genes are typically spliced into T-DNA - part of a plasmid called Ti (tumour-inducing) - that is integrated into plant genome; that much was known, at least since 1980.

But closer analyses reveal that the process whereby *Agrobacterium* injects T-DNA into plant cells strongly resembles *conjugation*, ie, mating between bacterial cells.

Conjugation, mediated by bacterial plasmids, requires a sequence called the origin of transfer (*oriT*) on the DNA that is transferred. All other functions (called *tra*) can be supplied from unlinked sources, referred to as ‘trans-acting functions’. Thus, ‘disabled’ plasmids, with no trans-acting functions, can nevertheless be transferred by ‘helper’ plasmids. And that is the basis of a complicated vector system devised, based on *Agrobacterium* T-DNA, for creating numerous transgenic plants.

But it soon transpired that the left and right borders of the T-DNA have characteristics of *oriT*, and can be replaced by it. Furthermore, the disarmed T-DNA, lacking the trans-acting functions (virulence genes), can be helped by similar genes belonging to many other pathogenic bacteria. It seems that the trans-kingdom gene transfer of *Agrobacterium* and the conjugative systems of bacteria are both involved in transporting macromolecules, not just DNA but also protein.

That means transgenic plants created by T-DNA vector system have a ready route for horizontal gene escape, via *Agrobacterium*, helped by the ordinary conjugative mechanisms of many other bacteria that cause diseases.

In fact, this possibility was raised in a 1997 report of a UK Government-sponsored study [17] showing that it was extremely difficult to get rid of the *Agrobacterium* used in the vector system after transformation. Treatment with an armoury of antibiotics and repeated subculture over 13 months failed to get rid of it. Furthermore, 12.5% of the *Agrobacterium* remaining still contained the binary vector (T-DNA and helper plasmid), and *were hence fully capable of transforming other plants*.

Several other observations make gene escape via *Agrobacterium* even more likely. *Agrobacterium* not only transfers genes into plant cells, the possibility of *retrotransfer* of DNA from plant cell to *Agrobacterium* is raised in Kado's Chapter, “Horizontal transmission of genes by *Agrobacterium* Species”. High rates of gene transfer are known to be associated with the plant root system, the rhizosphere [18], where conjugative activities are most likely. There, *Agrobacterium* could multiply and transfer transgenic DNA to other bacteria, as well as to the next crop planted in the same soil.

Finally, *Agrobacterium* attaches to and genetically transforms several types of human cells [19]. The researchers found that in stably transformed HeLa cells, the integration of T-DNA occurred at the right border, exactly as would happen when it is

being transferred into a plant cell genome. This suggests that *Agrobacterium* transforms human cells by a mechanism similar to that which it uses for transforming plants cells.

When will these and other scientists advising our government regulators dare to tell the truth about horizontal gene transfer to themselves and to the public?

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