Cross-species Gene Transfer; Implications for a New Theory of Evolution

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It has been established that genes can be transferred and expressed among procaryotes of different species. I am hypothesizing—and there is mounting evidence for this conclusion—that genes are transferred and expressed among all species, and that such exchange is facilitated by, and can help account for, the existence of the biological unities, from the uniform genetic code to the cross-species similarity of the stages of embryological development. If this idea is correct, the uniformity of the genetic code would allow organisms to decipher and use genes transposed from chromosomes of foreign species, and the shared sequence of embryological development within each phylum would allow the organism to integrate these genes, particularly when the genes affect complex morphological traits. The cross-species gene transfer model could help explain many observations which have puzzled evolutionists, such as rapid bursts in evolution and the widespread occurrence of parallelism in the fossil record.

Introduction

There are now a number of hints indicating that functional genes can naturally spread across species boundaries. The existence and mechanism for such transfer are well documented, especially in the prokaryotic world. In bacteria transfer of DNA is facilitated by plasmids, by phages or by direct ingestion. The genes can be established within the cell by a variety of means. If they are on a plasmid, they can be replicated autonomously, and if they are on a non-replicating molecule, they can be incorporated into the bacterial chromosome, either by general recombination or through the action of transposable elements. Transposable elements are genes which are capable of moving from one chromosome to another (Kleckner, 1981). If we then consider the fact that the transposable elements have the ability to pick up the normally stable chromosomal genes when they move (Guarente *et al.,* 1980) we can see how it is possible to transfer any gene among many different strains. The R-plasmids, with their associated antibiotic resistant transposons, provide the most dramatic example for cross-

species gene exchange. We may infer that bacteria may also incorporate genes from higher organisms. If we include DNA transformation, the ability of most cells to directly ingest large DNA molecules, and consider that the ultimate carrion eaters are bacteria, then it is clear that bacteria are continuously exposed to genes from throughout the living world. That some of these genes can be expressed there should be no doubt, as dozens of examples have now been recorded in the gene cloning projects (Struhl, Cameron & Davis, 1976; Talmadge, Kaufman & Gilbert, 1980). The possible significance of cross-species gene transfer in bacterial evolution has been discussed by Campbell (1981).

Does transfer of genes across species lines occur in the higher organisms? Certainly the mechanism for such transfer is there. Plants and animals are infected with viruses whose hosts range include many different species and it is a common observation that animal viruses can assemble into their particles genetic material from their host. In addition, eukaryotic cells carry transposable elements (McClintock, 1956; Symposium, 1981), apparently in greater abundance than in bacteria. Recently an animal virus carrying a chromosomal transposable element has been described (Miller & Miller, 1982). In fact there are already some indications of lateral gene transfer involving higher organisms. Buslinger, Rusconi & Birnstiel (1982) have reported that two distantly related sea urchins have histone genes that are nearly identical at the nucleotide sequence level. The authors interpret this as an example of cross-species gene transfer. There is a case of a symbiotic bacteria having the same protein product as does its eukaryotic host (Martin & Fridovich, 1981). Plant leghemoglobin seems to be very closely related to animal globins, possibly a result of transfer from animals to plants (Hyldig-Nielson *etaL,* 1982). The first example of cross-species gene transfer in mammals was the finding of closely related oncogenes from unrelated species (Benveniste & Todaro, 1974). Later Shilo & Weinberg (1981) found a highly conserved oncogene between arthopods and vertebrates. Singh, Purdom & Jones (1980) have found that a middle repetitive sequence from reptiles—a sequence that they suspect is a transposable element—cross hybridizes with sequences from fruit flies and mice. Possibly, what may turn out to be one of the most dramatic examples of a transposable element crossing species boundaries is the *P* factors that are found in *Drosophila melanogaster* (Engels, 1983). It appears as if the *P* factor has become established in the fly population only in the past 50 years.

A major question is posed by these cases: Are they isolated cases of no general significance, or are the phenomena sufficiently widespread that they might constitute an important evolutionary mechanism? There is one report, based on a comparison of mammalian ß-globin sequences, suggesting that

for certain sequences in genes, the rate of cross-species gene exchange is high enough to reduce the rate of fixation of neutral mutations appearing in a population (Syvanen, 1984). This raises the possibility that lateral gene transfer could play a large role in evolution.

I feel that there is now sufficient evidence that cross-species gene transfer exists to render it fruitful to speculate about its possible evolutionary consequences. In light of the recent examples of cross-species gene exchange, I have re-examined the broad question of why so many biological processes are uniform among distantly related species. It is currently believed that these biological similarities or unities result from the descent of various organisms from common ancestors and that functional constraint preserves certain ancestral processes. I will offer an alternative explanation to this prevailing view.

Biological Unities

WHY THE GENETIC CODE IS UNIFORM

The uniformity of the genetic code is usually explained by either one of two models. The stereochemical model postulates that there are some inherent chemical interactions between the triplet nucleotides and their amino acids which dictates the code. The second, and more generally accepted theory, is that of the frozen accident (Crick, 1968). According to this theory today's code results from the descent of life from a single interbreeding population in which one arbitrary code had become established. I would like to explore the alternative possibility that today's genetic code is the result of a continuous selective pressure for uniformity *per se*.

Central to both the stereochemical and frozen accident theories is the implicit assumption that code altering tRNA mutations would be lethal. In the case of the stereochemical model, this is true by definition, and in the case of the frozen accident model, it follows from the observation that there is no significant divergence from the genetic code. But discoveries made during the 1960's showed that such mutations can be viable. The nonsense suppressors seen in bacteria and yeast are tRNA mutations that clearly represent divergence of the code. We can imagine yet more radical divergences following, once a nonsense suppressor has occurred. If selective pressure for a suppressor remained in effect for a long enough time the usual outcome would be reversion of the suppressed gene and loss of the suppressor. However, occasionally, the organism could possibly gradually change its codon usage such that the original nonsense suppressor would no longer be a recessive lethal mutation. In fact, some of the exceptions to the uniform code (such as those seen in mitochondria) seem to involve a

change of nonsense codons to sense codons. A second kind of drift that could also be anticipated is the simple loss of some of the degenerate codons. For certain amino acids it is well known that the use of some codons is preferred, while others are used infrequently (Haridson *et al.,* 1979). If we infer drift in codon usage frequency from this fact, then it becomes a puzzle why, in some lineages, certain codons are not lost altogether by simple drift into a zero boundary. Any lineage that altered its code would obtain one beneficial side effect in that it would become immune to broad host-range viruses whose primary reservoir was a lineage with a different genetic code. Thus we can see there could be some-selective pressure *for* drift in the code. The absence of such drift, then, implies the existence of an ongoing selective pressure that maintains the unified code.

I will argue, as Norman Anderson (1970) first suggested, that transfer of genes across species boundaries is the basis for the selective pressure that maintains the unified genetic code. If horizontal exchange of genes in fact occurs, and since the genetic code is uniform, it follows that each species has available for evolutionary diversification any foreign genes it may encounter as well as those it carries in its own germ line. If the rate of gene exchange is high compared with the rate of evolution, then we can view living organisms as being continuously exposed to foreign genes that may fortuitously enter their germ line. These may become part of the lineage by means of natural selection, should the gene offer an adaptive trait, or by random fixation. Any evolutionary line that began to experiment with a new genetic code would isolate itself from this great gene network and consequently slow its rate of evolution. These lineages would more likely become extinct. This, then is the selective pressure for a uniform code.

One might argue that the genetic code would have diverged among those species that evolve slowly. I suggest that this is unlikely, for the reason that "slowly evolving" refers only to the rate of evolution for certain morphological features (those preserved in fossils). We can infer nothing about the evolution of simpler biochemical traits, such as digestive ability or toxin resistance. By the criteria of the fossil record, the most slowly evolving of all life is the rod-shaped bacteria. But, we know that transfer of foreign genes that carry selective traits is important in bacterial evolution (e.g. drug resistance genes). Such transfers would appear to be sufficient to maintain a uniform code, even when morphology remains constant.

IMPORTANCE OF SIMILAR EMBRYOLOGICAL DEVELOPMENT

The importance of cross-species gene transfer for survival among bacteria is quite evident and it seems reasonable that it is important for their

evolution. In the following sections I will consider how an evolutionary theory incorporating cross-species gene transfer is consistent with observations in the evolution of higher organisms—namely those observations based on the fossil record. If cross-species gene transfer contributes to evolution at this level, then it means that complex morphological traits are affected by such a process. Whereas it is relatively easy to see how a simple biochemical trait, such as resistance to a toxic chemical, could be transferred from one lineage to another, it is more difficult to envisage how morphologic traits derived from foreign genes could become integrated into the complex morphology of a new host.

The answer to the problem of the transfer of morphological traits may be found in another of the biological unities—the observation that large groups of species share similar sequences of embryological development. This phenomenon is known as von Baer's law of development: Differentiation proceeds from general embryological forms to more specialized adult forms (Gould, l977a). And these general embryological forms can be nearly indistinguishable among distantly related species. The existence of highly similar embryological pathways between two species might enable them to exchange traits that affect morphology. If a gene affecting morphology moves from one into the other's germ line, it might then be expressed within an embryological program similar to that from which it came, at least through the earliest stages of development. Integration of the trait into the more specialized adult could then proceed from this common program with the determination of size, innervation and such being controlled by the global and hormonal developmental patterns for that species.

REINTERPRETATION OF THE EVOLUTIONARY SIGNIFICANCE OF VON BAER'S LAW

The phylogenetic significance of von Baer's law has been debated for over a century. The 19th century theory that ontogeny recapitulates phylogeny originally was created in order to explain unified ontogenies. Today it is still generally accepted that the embryonic stages do reflect in some way the evolutionary changes in the descent of modern organisms. However, no longer explained is why the same embryonic stages of ancestors are preserved in so many disparate descendants. The currently accepted explanation for uniform ontogenies is that the mechanical-chemical constraints on passing from a single cell to a complex organism require an organism to pass through certain common stages. This position is summarized by de Beer; "there is apparently a natural order in which things can be done: a necessitation which affects all ontogenies alike. Repetition of such

(ontogenic) sequences is evidence, therefore, not of any influence of phylogeny on ontogeny, but of the limitation of physico-chemical possibilities in the transition from the simple to the more complex" (de Beer, I95Ia). A good example for this is the appearance of the notochord in the embryos of vertebrates; it appears to serve as a scaffold upon which the final backbone is assembled. However, examples of common embryonic stages that simply atrophy and give rise to no specialized organ are more difficult to explain in this way. Gill slits in the development of all vertebrates is the example most frequently referred to. The question can still be asked: Are there other types of selection preserving these common stages of embryonic growth during evolution?

I am proposing an alternative to the theory of physico-chemical limitations. I suggest that the selective pressure that maintains similar ontogenies is the same as that which maintains the uniform genetic code. The phylogenetic importance for similar ontogenies, then, lies not in its reflection of ancestral development, but in its present function as a facilitator of the integration of foreign traits. This would represent a new dimension in evolution that provides a versatility not previously suspected. For any given lineage, natural selection could be acting indirectly through "cousin" lineages. This allows natural selection to act on juvenile and even embryonic features that can be completely incipient and have no adaptive significance to the species that bears them.

General Considerations

So far, I have argued that if cross-species gene exchange is an important factor in evolution, then it might provide the explanation for certain biological unities of biochemistry, gene expression and embryology. In doing this I have introduced the possibility that there is a general pull in the direction of interspecies unity which acts in opposition to the recognized pull in the direction of species specialization and differentiation. In the following sections I will discuss certain general evolutionary concepts, and how cross-species gene exchange might alter our perspectives on these questions.

Parallelism

Parallelism, or convergent evolution, designates the phenomena of two independent evolutionary lineages appearing with a similar trait—the trait being absent from any common ancestral form. The commonly accepted mechanism for this phenomena is implied in either name; i.e. the trait evolved independently in the different lineages. Obviously, cross-species gene transfer will yield the same result, but the trait need only evolve in one of the lineages and then spread into other lineages.

Paedomorphosis

Paedomorphosis is the result of a change in developmental timing whereby adult descendants assume many of the traits of ancestral juveniles. The importance of paedomorphosis to evolution was initially recognized to be the simplification of form, or the "escape from specialization" (Hardy, 1954; Gould, l977b). With cross-species transfer of morphological traits, paedomorphosis takes on an additional importance. Assume that a horizon-ally transferred trait becomes fixed in a population. Even though advantage to a lineage may be the result of the gene transfer, we can imagine instances where the newly acquired trait may be in disadvantageous competition with previous specialized traits. In these cases, there may be pressure upon this lineage for subsequent changes to accommodate the trait. One way this could be accomplished is by simplification and removal of those competing specialized functions; i.e. by paedomorphosis. One aspect of paedomorphosis previously thought to be incidental is the tendency to converge morphology of different lineages. However, this factor would make paedomorphosis particularly useful in accommodating a laterally transferred trait since the trait would now occupy a form closer to the morphology in which that trait originally evolved. Figure 1 illustrates how both cross-
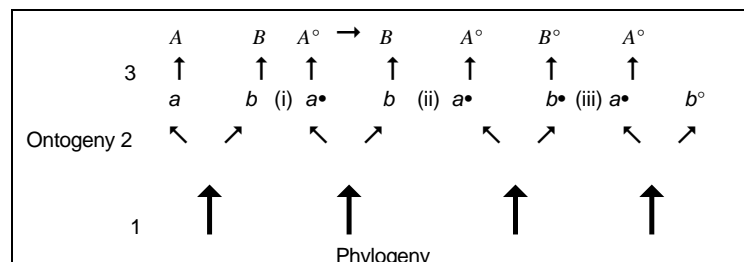

FIG. I. Parallel evolution involving cross-species gene transfer and paedomorphosis. Two lineages share ontogenic stages through (1) but then their development diverges through (2) and (3) to give the distinct adult morphologies A and B. (i) A new trait° evolves through natural selection in lineage A. (ii) The trait° confers survival value to B and after a lateral gene exchange B° arises (iii). The trait° is not well adapted for B and after paedomorphosis the new adult, b° arises. The juvenile form of °, as indicated by •, is pushed back earlier into ontogeny in the development of b°. As is apparent from the diagram, b is fewer steps removed from A in developmental steps than is B from A.

species transfer of a trait followed by subsequent paedomorphosis would tend to converge morphology of two different lineages. It should be stressed, I am not suggesting that the gene transfer is inducing paedomorphosis; only that should a foreign gene be transferred, then this may establish a phenotype upon which selective pressure towards paedomorphosis may occur.

RATES OF EVOLUTION

On the basis of the fossil record it is quite apparent that evolutionary rate fluctuates. This has been recognized for many years (Simpson, 1944; Haldane, 1954) and has gained recent prominence in the punctuated equilibrium hypothesis of Gould & Eldredge (1977). Central to these ideas is that new species appear suddenly, and the traditional view of gradual change is probably incorrect. Mayr (1954) has presented a plausible mechanism for how new species may suddenly appear in the fossil record, based upon his analysis of coadaptation of polymorphic genes and founder populations.

Cross-species gene exchange, if an important factor in evolution, could have significant influence on fluctuating rates, as was first noted by Reanny (1976). This follows since the rate of evolution for a given lineage should be influenced by the number of closely-related lineages. For example, the larger the number of closely-related lineages occurring alongside any given lineage, the larger the gene pool and the more morphological alternatives available. These lineages should have a faster evolutionary rate than do isolated lineages. For example, consider a number of closely related lineages that are involved in a multi-step process of creating a particularly advantageous system. If sufficiently successful, new lines will be formed (usually by branching, occasionally by convergence) the consequence of which will be to increase the rate of evolution. In general, when the rate of an event is increased by the success of that event, a very rapid increase can result. If this were a chemical reaction, we would call it an explosion, which is the appropriate metaphor that has been used to describe those periods of rapid speciation. Given that biological systems are inherently limited, these periods must be brief. Whatever the constraints on expansion may be, cross-species gene transfer provides a rational basis for large fluctuations in evolutionary rates. The other side of this argument would explain why extinction of entire families is common. If too many species within a family become extinct for whatever reasons, then the rate of evolution of the survivors would be reduced. These lineages would most likely become extinct or rarely, if they survived, become frozen into their original form as living fossils.

Macroevolutionary Trends

I have presented in general terms how evolutionary change could be affected by lateral gene transfer. In this section some patterns that have emerged from paleontology will be reviewed that, I believe, can be more easily understood from the basis of lateral gene transfer. The pattern I have

chosen to explore was initially stressed by advocates of the theory of program-evolution.

The program-evolutionists pointed out the widespread occurrence of parallelism in the invertebrate fossil record and the correlation of this event with given speciation bursts and, occasionally, paedomorphosis. Program-evolution was not widely accepted in its time and has been largely ignored since then, because it maintained that there was a directionality to evolution that was somehow "programmed" into lineages (Lang, 1923). The idea was accused of being teleological and was non-Darwinian. However, the theory was based on a rather intriguing pattern in the fossil record and consequently attracted the backing of a number of paleontologists.

Bulman (1933), a program evolutionist, reviews the history of the grap-tolites, a simple colonial animal that lived during the paleozoic. He recorded that the graptolites went through about seven speciation-extinction cycles before they finally died out. Not only were several of the speciation "bursts" characterized by specific parallelisms, but frequently the progression (through time) of some of the lineages worked in the direction of simplification of overall morphology and were said to be "regressive" or "degenerate". These simplifications are probably paedomorphic and, in fact, deBeer (1951 b) uses Bulman's descriptions as one of his examples of paedomorphosis.

The widespread occurrence of parallelism and high frequency of paedomorphosis had been noted earlier by Lang (1923) in the speciation-extinction cycles in ammonites and polyzoas. He showed that each cycle was characterized by its own parallelisms and that at the beginning of each cycle, overall morphology in the whole class returned to a form similar to the form existing at the beginning of the previous cycle (this must be paedomorphosis) with the allowance that "each cycle in evolution is no mere indefinite repetition of identical stages; it is essentially a progress, and a new period is a repetition of an earlier period *in a new aspect* or *upon a higher plane"* (Lang's italics) (Lang, 1921).

Trends (and their attendant speciation-extinction cycles) thus make up what is called the theory of program-evolution, but no reasonable explanations were ever provided to explain these patterns. Both Lang and Bulman discount natural selection and the latter refers "to some internal factor" within lineages that "direct" the observed trends.

Cross-species gene transfer provides an explanation of the observed trends that advocates of program-evolution lacked; an explanation, however, that restores natural selection as a major driving force. For example, one of the consequences of evolution occurring among a group connected by lateral gene transfer is that parallelism of traits should occur frequently during

speciation bursts. In such cases evolution would appear "directed" in that independent lineages would be moving towards one another through the mechanisms of sharing traits and paedomorphosis (see Fig. 1). It is easy to see how the program-evolutionists, in the absence of the knowledge of transposon and viral mechanisms, could have resorted to a "non-Darwinian" theory to explain the perplexing pattern of their data.

Conclusion

Genetic engineers have transferred active genes from one species to another, with relative ease. This work has proved the feasibility of expressing foreign genes which give rise to functional proteins. I have described a few probable cases of naturally occurring cross-species gene transfer in eukaryotes. Yet to be demonstrated is the ability to transfer complex morphological features between distantly related species from higher animals.

The major suggestion of this paper is that the extent of lateral gene transfer is much greater than generally suspected. If so, then this leads to a specific prediction about the rate of the molecular clock (Wilson *et aL,* 1977). Kimura predicts that the rate of a molecular clock based on neutral mutations is equal to the rate at which those neutral mutations arise in the respective populations (Kimura, 1968; King & Jukes, 1969). If cross-species gene exchange is widespread, then it follows that the rate of neutral mutations occurring within any given population is necessarily greater than the measured molecular clock (Syvanen, 1984). This prediction must wait for the appropriate population studies where the mutation rates at the relevant neutral loci are determined.

A theory incorporating cross-species gene transfer could not have been proposed much earlier than this decade for the simple reason no mechanism for such an event was known. Over the last decade studies of the broad host range transforming viruses coupled with the growing appreciation of genetic instability (as evidenced by the transposons and other mobile elements) has clearly established possible mechanisms for lateral gene transfer. The picture of the mechanism of lateral gene exchange among higher plants and animals is incomplete, and I have had to infer some of the mechanism by analogy with the bacterial work. The possibility that bacterial plasmids and transposons may provide a useful paradigm for evolution has been explored previously (Anderson, 1966, 1970; Reanney, 1976). It has also been widely suspected that mobile elements-play an important role in the chromosome rearrangements that seem to characterize speciation events. In this paper I have extended these earlier suggestions and have proposed a more general evolutionary role for these moveable genetic elements.

REFERENCES

ANDERSON, E. C. (1966). *Nature* **209**, 637.
ANDERSON, N. (1970). *Nature* **227**, 1346.
BENVENISTE, R. E. & **TODARO, G. J.** (1974). *Nature* **252**, 456.
BUSLINGER, M., RUSCONI, S. & **BIRNSTIEL, M. L.** (1982). *EMBO 1.* **1, 27.**
BULMAN, 0. M. B. (1933). *Biol Rev.* (Cambridge Phil. Soc.) **8,** 311.
CAMPBELL, A. (1981). *Ann. Rev. Genet.* **15,** 55.
CRICK, R. (1968). *J. Mol. Blol.* **38**, 367.
DEBEER, G. R. (1951). *Embryos and ancestors.* (a) p. 102, (b) p.66. Oxford: Oxford University Press.
ENGELS, W. R. (1983). *Ann. Rev. Genet.* **17,** 315.
GOULD, S. J. (1977). *Ontogeny and Phylogeny.* **(a) pp. 52-63; (b) Part II.** Cambridge: Belknap Press.
GOULD, S. J. & ELDREDGE, N. (1977). *Paleobiology* **3**, 115.
GUARENTES, L., ISBERG, R., SYVANEN, M. & SILHAVY, T. (1980). *J. Mol. Biol.* **141,** 235.
HALDANE, J. B. S. (1954). **In:** *Evolution as a Process* **(Huxley, J. *et al* eds.), pp.** 109. **London: George,** Allen & Unwin Ltd.
HARDY, A. C. (1954). **In:** *Evolution as a Process* **(Huxley, *J. et al* eds.), pp. 122.** London: George, Allen & Unwin Ltd.
HARRIDSON, R., BUTLER, E., LACEY, E., MANIATIS, T., ROSENTHAL, T. *&* EFSTRADIATIS, A. (1979). *Cell* **18,** 1285.
HYLDIG-NIELSON, J*., JENSEN, E., PALUDAN, K., WIBURG, R., GARRETT, R., JORGENSEN, P. & MARCHER, K. (1982). *Nucleic Acids Research* **10**, 689.
KING, J. L. & JUKES, T. H. (1969). *Science* **164**, **788.**
KLECKNER, N. (1981). *Ann. **Rev.** Genet.* **15,** 341.
KIMURA, M. (1968)**.** *Nature* **217,** 624.
LANG, W. D. (1921)**.** *Catalogue of Cretacious Bryozoa,* British Museum (Natural History) **3,** part 1: XIV-XVIII.
LANG, W. D. (1923). *Proc. Geol. Assoc*. **34,** 120.
MARTIN, J. P. & FRIDOVICH, I. (1981). *J. Biol. Chem.* **256**, 6080.
MAYR, E. (1954). In: *Evolution as a Process* **(Huxley, J. *et al.*** eds.), pp. 157. London: George, Allen & Unwin Ltd.
MCCLI NTOCK, B. (1956)**.** *Cold Spring Harbor Symp. Quant. Biol.* **21,** 197.
MILLER, D. W. & MILLER, L. K. (1982). *Nature* **299**, 562.
REANNEY, D. (1976). *Bacteriol. Rev.* **40,** 552.
SHILO, B. & WEINBERG, R. (1981). *Proc. Nat!. Acad. Sci. U.S.A.* **78**, 6789.
SIMPSON, G. G. (l944). *Tempo and Mode in Evolution.* New York: Columbia Univ. Press.
SINGH, L., PURDOM, I. & JONES, K. (1980). *CSH Symp. Quant. Biol.* **45**, 805.
STRUHL, K., CAMERON, J. & DAVIS, R. (1976). *Proc. Nat!. Acad. Sci. U.S.A.* **73**, 1471.
SYVANEN, M. (1984). *J. theoret. Biol.* **107**, 685.
SYMPOSIUM (1981). *Cold Spring Harbor Symp. Quant. Biol.* **45,** number 2.
TALMADGE, R., KAUFMAN, J. & GILBERT, W. (1980). *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3988.
WILSON, A. C., CARLSON, S. & WHITE, T. (1977). *Ann. Rev. Biochem.* **46**, 573.