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Introduction

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Molecular genetics is a field in a state of explosive growth, and conceptually challenging empirical findings are an almost weekly occurrence. The pace of conceptual change in the last two decades makes it necessary to attend to current work in the field as well as to the history of the discipline. But beside the traditional methods available to historians and philosophers in biology like historical and conceptual analyses new methods borrowed from other fields, especially the social sciences, can also be employed. The introduction of empirical research methods makes it possible to evaluate competing accounts of the gene concept and its variants in a more rigorous and systematic way. There is widespread agreement amongst historians and philosophers of biology that the gene concept is not merely 'vague' or 'flexible', but rather that biologists in different fields conceive of the gene in one or more specific ways that reflect their research practice. The following contributions differ in which strategies they follow, and therefore in their totality give a pretty good state of the art of contemporary philosophy of molecular genetics. Where all papers converge is probably on their assessment that we are not looking for the right definition of the gene but for interesting interpretations how those new empirical findings can be incorporated in theories about the gene to elucidate what genes are, do, and are for in their course through development and evolution.

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Genes: Philosophical Analyses Put to the Test

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ABSTRACT - This paper describes one complete and one ongoing empirical study in which philosophical analyses of the concept of the gene were operationalized and tested against questionnaire data obtained from working biologists to determine whether and when biologists conceive genes in the ways suggested. These studies throw light on how different gene concepts contribute to biological research. Their aim is not to arrive at one or more correct 'definitions' of the gene, but rather to map out the variation in the gene concept and to explore its causes and its effects.

KEYWORDS: gene concept, conceptual change, gene expression, gene-D, gene-P, genomics

1. Introduction: Empirical Philosophy Tracks Shift in a Scientific Concept

This is a particularly exciting time to be studying molecular bioscience because of the extraordinary rate of change in basic concepts. Discoveries that would constitute a 'scientific revolution' in many disciplines are regular occurrences. The ways in which bioscientists conceptualize DNA and related molecules are thus not only fascinating in their own right, but also an important case study for the history and philosophy of science – a case study of conceptual change and its role in science.

Empirical science is a powerhouse of conceptual innovation. Scientists use and reuse their terminology in a way that Hans-Jörg Rheinberger has accurately characterized as 'exuberant' (Rheinberger 2000). Examples for this are the use of '*cis*' and '*trans*' without reference to the cis-trans test, or the use of the term 'exon' to refer to a stretch of DNA which is included in the mature mRNA transcript after various forms of post-transcriptional processing but is not translated into

protein. Both these usages contradict all but the most recent textbook definitions and, speaking from experience, seem irritating and wrongheaded to some biologists. Yet both usages are common in certain research communities.[1] For the practitioner concepts are tools that classify experience shaped by experimentalists to meet their specific needs and reshaped in the light of new empirical findings and as those needs change. This attitude is sometimes made explicit: '…we must sharpen our conceptual tools as best we can and have faith that in using them to untangle the complexity we shall see how to fashion better ones' (Hinde 1985, 990). The same attitude is implicit when scientists describe a statement as a 'definition' and yet regard it as hostage to future empirical findings, as they commonly do. If scientific concepts are evolving tools, it should not be the aim of philosophers of science to identify the one correct conception associated with a word or phrase, or even to eliminate slippage of meaning caused by the presence of several different conceptions in a single community. Historical analysis has shown how slippage of meaning was essential to the rapid progress of genetics in the first half of this century (Falk 1986; Rheinberger 2000; Falk 2000). History of science thus allows us to take what James G. Lennox has called a 'phylogenetic' approach to the study of science. Like the history of a biological species, the history of a concept allows the reconstruction of a transformation series and provides evidence about the selective pressures that drove change and diversification.[2] If history of science can become *conceptual phylogenetics*, philosophy of science, so we believe, can and should take on the corresponding role of *conceptual ecology*. In the research reported here we are trying to describe the current diversification of the gene concept to meet the increasingly diverse goals of workers in the increasingly diverse set of fields we call 'molecular bioscience'. At the very least, we hope to discern some species boundaries or incipient speciation events, at best to throw some light on the particular epistemic pressures that have caused scientists to explore one *conceptual niche* or another.

In the next section we give an overview of some historical and philosophical analyses of the gene concept. Section three summarizes the objective, design and results of a small-scale survey of molecular biologists at Sydney University (Stotz, Griffiths, and Knight 2004) which acted as a preliminary study for the ongoing project described

[1] For a similar example, see (Falk In Press).

[2] It remains to be determined whether and to what extent conceptual phylogenies mirror sociological descent relations amongst scientists.

in section four. The methodologically innovative element of this ongoing research is the integration of empirical research methods with the traditional philosophical task of analyzing key concepts and elucidating their role in scientific reasoning. With the help of a group of experts in fields ranging from history and philosophy of science, bioethics and science communication, to practitioners of molecular bioscience we have administered an online survey to a wide range of working biologists in order to explore the prevalence of different conceptions of the gene in various fields.

2. The Concept of the Gene

Since the term 'gene' was introduced a century has passed during which its meaning has been transformed almost beyond recognition (Johannsen 1909; Keller 2000). Despite the lack of a stable definition the gene has proven to be an enormously fruitful scientific tool, and in addition has become a cultural icon and the carrier of multiple hopes and promises within science and medicine. Throughout its history there has been a tension between the current conception of the gene and the latest empirical results. Following the general acceptance of the view that genes can be structurally identified as sections of chromosomes, the function of those chromosome segments evolved from determining a unit character, to determining an enzyme, to determining a polypeptide. This dialectical development of the gene concept can be interpreted as reflecting a desire to keep the structural and functional definitions of the gene focused on a single entity. When the best structural definition turns out to create units with indeterminate function, structure and function can be brought back into step by using a more proximal description of function: rather than a gene having an indeterminate effect on the phenotype, it has a determinate effect on one of the structural elements that contributes to the phenotype (Griffiths and Neumann-Held 1999; Kitcher 1984). This process of conceptual evolution seemed to reach a stable resting point with the 'Classical Molecular Gene Concept' (Neumann-Held 1999). The classical molecular gene has a clear functional identity as a segment of DNA that codes for a single polypeptide chain.[3] It has a clear structural identity as an open reading frame with adjacent promoter. This structure and this function are taken to be tightly associated with one another.

[3] Little violence is done to the classical conception by extending it to cover genes that determine the sequence of a functional RNA rather than a polypeptide.

The reality of genome structure today challenges the classical picture of the molecular gene in the same way that the reality of particle physics challenges the traditional picture of matter. The 'particles' of the quantum world can lack such apparently essential features as having mass or being in some particular place. In the same way, just about any of the normal expectations we have when we hear the word 'gene' is violated by some important class of DNA sequences. Physicists changed their concept of a particle in response to the strange world that quantum physics revealed. Just so, in the 'post-genomic' world bioscientists continue to talk about 'genes' but often mean something quite at odds with the picture of the gene found in introductory textbooks.

So what exactly are the problems that confront the classical molecular picture of a gene? Most of them arise from complications that have been added to the classical picture of gene expression. Classically, gene expression proceeds via the transcription of the continuous open reading frame of a classical molecular gene into a single messenger RNA and the translation of that mRNA into a polypeptide, both processes starting at a determinate point on the nucleic acid molecule which is being processed. An immediate complication arises in eukaryotes (fungi, plants and animals), since in eukaryote cells a primary or pre-messenger RNA is transcribed from the DNA sequence and the final mRNA transcript is derived from this by cutting out non-coding sequences (introns), and splicing together of the remaining coding sequences (exons). This alternative (*cis*-) splicing means that one classical gene can correspond to more than one polypeptide, often far more than one. The resultant 'one-many' relationship between stretches of DNA and gene products is further complicated in cases of 'overlapping genes' or 'gene sharing' (Burian, this volume). Genes are not lined up on a chromosome like pearls on a string, but instead one gene can start within another gene. The similarity of the products depends on the proportion of shared sequences, and on whether these shared sequences are read in the same frame. If transcription of the second gene does not begin at the beginning of a codon of the first gene, then the reading frame is altered and the two transcripts might share much of their sequence whilst coding for quite different products.[4] An even more surprising

[4] 'Frameshift' in reading English would turn 'The old man can run' into the meaningless' (T) heo ldm anc anr un'. In the genetic code, however, every sequence of three 'letters' (bases) is guaranteed to be a meaningful 'word' (codon). A good example of such a case is (Sharpless and DePinho 1999).

source of 'one-many' relationships arises from the discovery that the same DNA can be read in both directions. A single stranded DNA can only be read in one direction, from its 3' end to its 5' end, but the complimentary strand can also be read from *its* 3' end to *its* 5' end. The discovery of this process of 'antisense transcription' has demonstrated that the very same stretch of DNA can encode gene products as radically different as, in one case, a polypeptide and a functional RNA (Coelho *et al.* 2002).

The relationship between DNA and product can be 'many-one' as well as 'one-many'. For example, adjacent genes can be co-transcribed to produce a single 'fusion transcript'.[5] Or, in the phenomenon of *trans*-splicing, a final mRNA transcript is produced by splicing together from more than one independently transcribed pre mRNA. Like *cis*-splicing, *trans*-splicing can occur in alternate forms to make several products from the same collection of DNA elements (Finta and Zaphiropoulos 2000b; Pirrotta 2002). The 'many-many' relationship between DNA and gene products that has been revealed by the discovery of these processes leaves one critical feature of the classical conception untouched. The linear order of amino acids in the gene product still corresponds to the linear order of DNA bases in some set of sequences of DNA read in some frame or another. But this too turns out not to be a universal feature of gene expression. Exons can be repeated, they can be put together in a new order (exon scrambling) and they can be inverted in the final transcript (antisense *trans*-splicing), so that the linear order of codons no longer corresponds to that of bases in the DNA from which they are derived.[6] Moreover, the complete mRNA transcript can be edited one base at a time before translation. This process of 'mRNA editing' converts C bases into U bases, which can have such radical effects as truncating translation by introducing a novel stop codon. Last but not least, the recently discovered process of 'protein splicing' changes the final product once more, but in this case by splicing 'inteins' in and out of the actual polypeptide (Liu 2000).

In the face of these complications, the statement that a gene is a DNA segment which determines a polypeptide is an insufficient basis on which to answer such apparently simple questions as: 'How many genes are contained in a given genome?'; 'Where does one gene ends and

[5] See for example (Magrangeas *et al*. 1998; Finta and Zaphiropoulos 2000; Communi *et al.* 2001).
[6] For a range of examples see (Takahara *et al.* 2002; Caudevilla *et al.* 1998; Flouriot *et al.* 2002).

another begin?'; and 'To which gene does this particular segment of DNA belong?'. The recent literature both in the philosophy of biology and in biology itself contains many proposals that aim to answer these questions in a principled and useful manner. Our work is in part an attempt to test whether the claims made by these authors reflect how biologists in various fields have chosen to handle these issues.

Some Alternative Conceptions of the Gene

The geneticist and historian of genetics Raphael Falk distinguishes four ways in which genes might be conceived (Falk 2000). First, the gene can be understood abstractly as something which figures in certain calculations, as in population genetics and possibly in predictive medicine. This conception of the gene resembles what Lenny Moss has termed 'Gene-P' (see below) and, more generally, a 'top-down' approach to genes that identifies them via their effects. Second, genes can be primarily conceived as material, structural entities, perhaps consistently associated with certain functions. Seymour Benzer's interpretation of H.J. Muller's particulate gene falls into this category, which Falk thinks is a good candidate of the genetic engineer's ideal but which he doubts is viable as an approach to the whole genome and its significance. A third approach conceives genes as functional, biological entities, whose structural identity is secondary, allowing for multiple realizability of 'the same gene' in different DNA. The fourth approach conceives genes as generic operational entities, with the term 'gene' merely shorthand for whatever class of DNA elements is currently of interest. Falk thinks this pragmatic approach to the concept has been adopted by many molecular biologists.

The biologist Thomas Fogle has suggested that biologists use what he has termed a 'consensus gene' concept: a collection of flexibly applied parameters of features of well-defined genes (Fogle 2000). A gene is a sequence that has 'enough' of the features of the gene stereotype (e.g. has an RNA transcript, has a TATA box, contains an ORF, etc., etc.). Fogle argues that by combining structural and functional features in a single stereotype the consensus concept hides both the diversity of structure that can perform the same function and the diverse functional roles of the same structures. We would argue further that as is the case with stereotypes more generally, even when people have been exposed to cases that violate the stereotype, they tend to forget the problematic cases and revert to the stereotype in future work. It is perhaps for this reason that many of the forms of gene expression introduced in the last

section were initially treated as exceptions that happen either only in 'low' animals (prokaryotes; trypanosomes; nematodes), or in very rare instances (in certain cells at very particular developmental stages), or in organelle genomes (mitochondria; chloroplast).

One of the best known attempts to go beyond the classical conception of the gene is Kenneth C. Waters' 'fundamental' molecular gene concept. The fundamental element of the molecular gene concept according to Waters is the preservation of the linear sequence of the original DNA sequence in the product, however proximally or distally defined. As Rob D. Knight has usefully expressed it, the gene is the 'image in the DNA' of a gene product (personal communication). Within this guiding conception, the uses of the term 'gene' in molecular biology corresponds to research interests along a continuum of more or less distal stages of gene expression (Waters 1994; 2000). Waters' view expresses a critical insight into the conceptual structure of classical molecular biology, but it is unclear that the resulting conception of the gene is adequate to describe transcription events that include phenomenon such as mRNA editing or antisense *trans*-splicing (see above).

Another important recent analysis is that of Lenny Moss, who has argued that there are two distinct conceptions of gene in play in current scientific and clinical thought (Moss 2002). These are 'Gene-D' and 'Gene-P', each heir to one of two major historical schools of embryological thought, preformationism and epigenesis.[7] The Gene P conception treats genes as statistically valid predictors of phenotypes and abstracts away from the molecular nature of the DNA elements that underly these statistical patterns. In the simplest case, different genetic lesions that impair one or more of the functions of a DNA element to the same degree are treated as 'the' allele for the impaired phenotype(s). In contrast, the Gene D conception focuses on the intrinsic capacity of a given sequence to template for RNAs. Gene-D thus bears some resemblance to a suggested 'Contemporary Molecular Gene Concept' (Knight and Griffiths 1999) according to which a gene is a DNA sequence that is expressed as a particular range of molecular products across a range of cellular condition. In other words, one gene equals one molecular norm of reaction (see for similar conceptions Alberts *et al.* 2002; Falk 2001).

[7] For the idea that conceptions of the gene are fundamentally *embryological* in nature, see (Griesemer 2000)

Like Fogle, Moss thinks that current conceptions of the gene can hinder as well as facilitate research. Each of the two conceptions has a valuable role in the research contexts in which it arose, but their conflation into a single 'informational gene' whose intrinsic molecular nature is strongly linked to its ultimate phenotypic effect leads to a simplistic and unhelpful conception of genetic causation

3. How Scientists Conceptualize Genes: An Empirical Study

A survey of some of the analyses of the gene concept described in section two led us to advance some hypothesis about how genes are conceptualized differently in different fields of biology. In this section we summarize the objectives, methods and results of a questionnaire study of 81 biological scientists at the University of Sydney, Australia, in 2000 designed to test those hypotheses. We cannot give a detailed account of the study here for reasons of space. For full details see (Stotz, Griffiths, and Knight 2004) and supporting online materials cited therein. The results provide tentative support for our three hypotheses:

1. Hypothesis One: Molecular Versus Evolutionary Biologists. We expected molecular biologists to emphasize the investigation of the intrinsic, structural nature of the gene and to be reluctant to identify a gene only by its contributions to relatively distant levels of gene expression. Conversely, evolutionary biologists should be more interested in genes as markers of phenotypic effects and reluctant to treat two similar DNA sequences as the same gene when they lead to different outcomes for the larger system in which they are embedded.

2. Hypothesis Two: Developmental Versus Evolutionary Biologists. A second expectation was that developmental biologists would emphasize the intrinsic nature of the gene as a molecular object and contextual effects on gene expression, whereas evolutionary biologists would emphasize the predictive relationship between genes and phenotypes. Consequently, there should be stronger support for the informational concept of the gene from evolutionists.

3. Hypothesis Three: Molecular Versus Developmental Biologists. We expected developmental biologists to be less attracted to Moss's Gene-P and to the informational conception of the gene than (other) molecular biologists. We expected developmental biologists to be attracted to conceptions that emphasize contingency and context

dependency, such as Moss's Gene-D and various developmentally-oriented conceptions of the gene canvassed in the literature on evolutionary developmental biology.

In addition to these specific hypotheses, we saw this as an exploratory study and were interested in what the responses suggest about the general state of the gene concept in contemporary biology. We also examined the effects of age and gender.

The questionnaire had three sections, the first part designed to determine the subject's research field, the second asking them direct questions about the gene concept and the third asking them to apply the gene concept to specific cases. The first section of the questionnaire gathered data on the professional training, research experience and current research field of subjects, along with age and gender. The second section of the questionnaire contained direct questions about the definition of the gene, the function of the gene and the methodological value of the gene concept. The answer alternatives for each question were designed to capture the various conceptions of the gene discussed in the literature. We used a number of different formulations of each conception to avoid superficial effects, such as antipathy to particular words or phrases. The actual wordings of many of the answer alternatives were taken from the literature and from genomics websites.[8] Each question had an 'Other' alternative in which subjects could supply their own answer, but no useful data was obtained by this means. This section of the questionnaire contained both 'free choice' and 'forced choice' tasks. The former required subjects to indicate for each question all the answer alternatives to which they could agree. The latter required subjects to choose the single best answer amongst the alternatives offered. The third section of the questionnaire was based on the design of an informal study conducted by Rob D. Knight in New Zealand with 10 respondents. This section used 'indirect' questions that asked subjects to apply their conception of the gene, rather than to answer questions about it. Subjects were given twenty-two examples of specific ways in which two DNA sequences could differ from one another and asked whether, in each case, two such DNA sequences would be two copies of the same gene.

[8] An annotated version of the questionnaire indicating these sources is available in the online documents cited in Stotz, Griffiths and Knight 2004, documents which are located on the Philosophy of Science Association preprint server (http://philsci-archive.pitt.edu).

To test our hypotheses we identified those answers that, if the hypothesis were correct, should be more attractive to one group than another. For example, to test our hypothesis one using the free choice responses to section 2, we identified those answers to the five questions in section 2 that we expected would be more attractive to the molecular group than to the evolutionary group and, conversely, those answers that we expected would be more attractive to the evolutionary group than to the molecular group. We then tested for significant differences between the responses of the two groups in the predicted directions. As an example, the results for hypothesis three are given in Table 1.

1.6	D 50%, M 21%, ns	1.1	M 43%, D 14%, ns
2.4-7	D 60%, M 53%, ns	2.1	**M 41%, D 0%, .353/.041**
4.3	D 88%, M 75%, ns	2.2	M 72%, E 60%, ns
4.4v6	D 60%, M 55%, ns	4.1	M 73%, D 60%, ns
5.5	**D 40%, M 13%, .282/.048**	5.4	M 20%, D 0%, ns

TABLE 1. Test of hypothesis 3 with data from the free choice task. Left-hand column shows answer alternatives for which we predicted agreement by the developmental group (D), right-hand column those for which we predicted agreement by the molecular group (M). Result cells: the numbers behind the characters show percentage of yes answers among the respective group (D, M), the following fractions indicate strength (from 0 to 1) and significance (0 – 1) of association. Results marked ns were not significant (>10% or .100). **Bold** results indicate high significance (< 5% or .050), italic results show associations in the reverse direction to that predicted.

The purpose of the forced choice task was to reveal differences hidden by the free choice task, in which minimally acceptable options would not be distinguished from highly preferred options. Just as with the free choice task we predicted the answers that we expected from each group for each question. Because this was a forced choice task in which each subject chose only one option; in cases where more than one answer option seemed likely to be preferred by a particular group we coded a strong disjunction of these answers as a single answer. Table 2 shows the result of our grouping and recoding exercise for the forced choice answers according to hypothesis three.

Predictions for Developmental Group	Predictions for Molecular Group
1.3, 1.4, 1.6	1.1, 1.5
2.4, 2.5, 2.6, 2.7, 2.8	2.1, 2.2, 2.3
4.3, 4.4, 4.6	4.1
5.5, 5.7, 5.9	5.2, 5.3, 5.4, 5.6

TABLE 2. Grouped force-choice prediction for Hypothesis 3. The answer alternatives in each cell were combined by strong disjunction on the grounds of their expected appeal to one group.

Once again, we tested out hypotheses by looking for significant differences between our groups in the predicted directions. As an example, Figure 1 shows the results for hypothesis three using data from section 2, question 1.

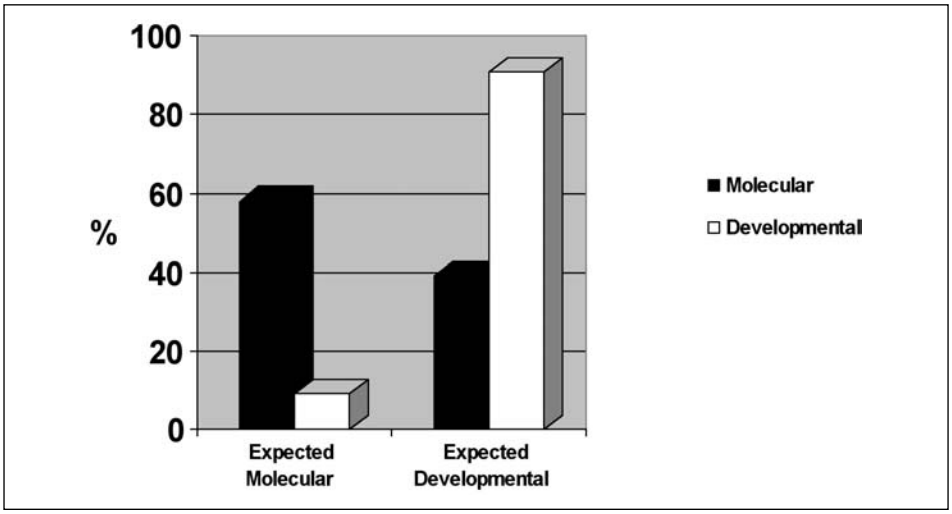

FIGURE 1. Results for Hypothesis 3 for the forced choice task on Section 2, Question 1 of the questionnaire. Paired columns show percentage of 'molecular' and 'developmental' respondents who actually gave the predicted answers for molecular and for developmental respondents. Association 0.430, significance 0.011.

The results from the analysis of the free and the forced choice task of section 2 of the questionnaire did not confirm our hypotheses one

and two, both involving evolutionary biologists as the comparison group. The results from this section, in which we asked direct questions, suggest that biologists with an evolutionary focus in their research do not conceptualize genes in terms of their phenotypic effects in any way that distinguishes them from biologists with a purely molecular or a developmental research focus. A very different picture emerged, however, from responses to the *indirect* questions in Section 3, in which the pattern of answers showed the evolutionary group responding significantly more strongly to changes in distal function than the molecular group.

Overall, the results provided tentative support for our three hypotheses. Hypothesis three seems most strongly supported. Biologists whose research focus is in developmental biology seem to conceptualize genes in a distinctive way, a way that appears to reflect their use of the gene concept to investigate the complex, developmental pathways through which genes are expressed. Hypotheses one and two, which suggest, in broad terms, that biologists whose research focus is in evolutionary biology, conceptualize genes primarily via their effects on phenotypes, are supported in some tests but not others. The fact that the hypotheses are supported when indirect questions are used, but not when direct questions are used, led us to advance an intriguing further hypothesis. We proposed that these biologists may have an explicit belief that genes are molecular entities and should be defined and investigated at that level, whilst deploying in their actual thinking about genetic problems a conception of the gene that abstracts away from differences at the molecular level and focuses on phenotypic effects. We hope to investigate this hypothesis in future research.

Our general results for the whole subject population were consistent with Fogle's suggestion that the classical molecular gene concept continues to function as something like a stereotype for biologists, despite the many cases in which that conception does not give a principled answer to the question of whether a particular sequence is a gene (Fogle 2000). Given the extensive psychological literature on prototype-based categorisation and on the reasoning processes it supports, this also suggests productive lines of future inquiry. Given the small number of subjects in this study and the simple criteria used to group them, we were encouraged by the ability of the study to discern differences between the groups. In ongoing research in the United States (see next section) we have been able to increase the number of subjects by half an order of magnitude (from

81 to 500) by recruiting subjects via the mailing lists of scientific societies and have used more sensitive measures to define our groups.

4. The Representing Genes Project[9]

Our earlier results indicate the importance of distinguishing between explicit and implicit ideas about the gene. In our ongoing research we asked subjects to engage in tasks such as ranking research proposals, or annotating transcription events, which were designed to reveal their implicit understanding. These tasks have the added advantage of providing numerical in addition to categorical data, allowing a wider range of statistical procedures to be employed. The study is not yet complete, and here we will only sketch its design and discuss its potential significance.

The research instrument was the outcome of a workshop at which we and our collaborators[10] agreed on a range of research questions we wanted to see addressed and on strategies for operationalizating those questions. This resulted in an instrument with four sections, three of which will be briefly summarized here. These were a task in 'abstract sequence annotation', a 'review' of competing proposals to research complex genetic diseases, and a set of questions designed to distinguish biologists working in different fields for comparison with one another. The fourth section asked some qualitative (open-ended) questions about the biologists' understanding of and attitude towards some prevalent genetic metaphors, including 'genetic information', 'gene for', 'genetic program', and 'developmental program'.

4.1 Task in 'Abstract Sequence Annotation'

We showed in section two that the classical molecular gene concept leaves open many decisions researchers have to make when annotating genomic sequences. In the post-genomic world it is not at all obvious which sequences to count as genes. The simplest product of a genome 'annotation' is an overall figure for the number of genes in a genome, such

[9] Project website: http://www.pitt.edu/~kstotz/genes/genes.html

[10] The following people have actively collaborated with us on the Representing Genes Project: Richard Burian, Sharyn Clough, Raphael Falk, Thomas Fogle, Scott Gilbert, James Griesemer, Jonathan Kaplan, Evelyn Fox Keller, Rob Knight, Brendan Larson, Lenny Moss, Hans-Jörg Rheinberger, Jason Scott Robert, Sahotra Sarkar, Kenneth Schaffner, Kenneth Waters, and from the University of Pittsburgh Ingo Brigandt, Megan Delahanty, James Lennox, Alan Love, Sandy Mitchell, Robert Olby, Lisa Parker, Jeff Schwartz, and James Tabery.

as the strikingly low figures produced in the immediate aftermath of the sequencing of the human genome, ranging from a conservative 26 000 to a generous 40 000. But simple gene counts disguise the highly problematic nature of most initial annotations. In our current state of knowledge many different approaches to identifying genes are defensible, and different research groups often produce only partially overlapping lists. Celera Genomics and the public Human Genome Consortium, for example, counted in their drafts a similar *number* of potential genes, but significantly often these were not *the same* potential genes (Hogenesch *et al.* 2001).

The decisions made in annotating a sequence or a transcription event reveals how the respondent is conceptualizing genes and other genetic elements. We set out to extract some of the information implicit in these decisions by asking which factors distinguish cases that are treated as one gene with several products from those that are treated as multiple genes. This part of the survey contains graphical representations of fourteen known transcription events, two of which will be discussed in detail below.[11] There are several potential 'axes of difference' that separate the cases from each other and which might influence the decision whether to recognise one or more than one gene. The cases were chosen to allow as far as possible pairwise comparisons with respect to just one potential axis of difference at a time. We give two examples here to indicate the nature of the questionnaire materials and to anchor the ensuing discussion of the framework in which we intend to analyse this data.

Example 1. Overlapping Genes with Shared Sequences in Alternative Reading Frames

The first example involves a primary RNA transcript that is processed into two mRNA transcripts by alternative splicing, and thereby gives rise to two structurally divergent protein products (Figure 2). Both proteins play important, though different roles in cell growth. The two transcripts differ in their first coding exons (1 or 2) but share the coding sequences of the remaining exons (3 and 4). However, the presence of the different first exon (1 or 2) in the two cases results in exons 3 and 4 being read in alternative reading frames (ARF) in the two transcripts. As a consequence, there is hardly any amino acid identity between the resulting proteins.[12]

[11] For a more detailed description of 6 of the 14 cases see (Stotz and Bostanci In press). The entire questionnaire with all 14 cases is available at the Representing Genes website: http://www.pitt.edu/~kstotz/genes/genes.html

[12] See the human *INK4A/ARF* tumor suppressor region (Sharpless and DePinho 1999).

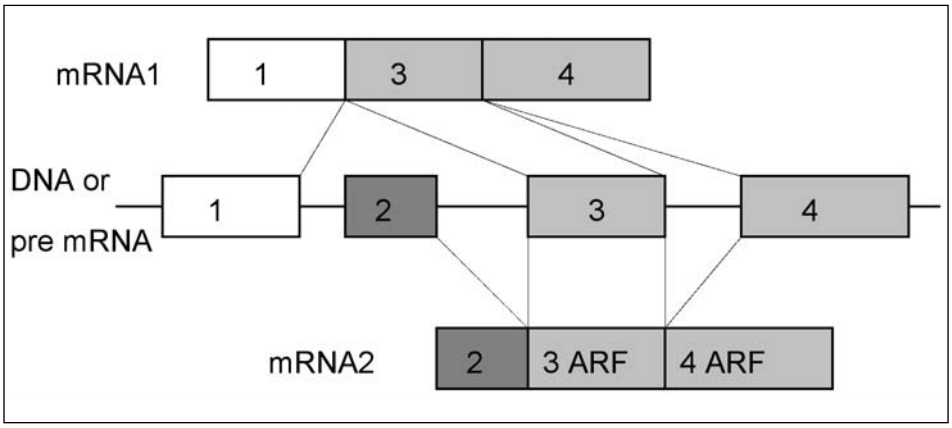

FIGURE 2. Overlapping genes with shared sequences in alternative reading frames

Biologists may label such cases of alternative splicing either as one or as several genes, and they may endorse a given annotation with different levels of confidence. The questions asked about the case were designed to capture those decisions (Table 3).

Question:				
a. Would you describe this case as one in which one or more than one gene is involved in generating the final transcript/s and/or the polypeptide/s that result from the process described?				
Clearly only ☐ one gene	Probably only ☐ one gene	Unclear ☐	Probably more than ☐ one gene	Clearly more than ☐ one gene
b. How appropriate are the following descriptions of this case?				
One gene: 1 to 4	appropriate ☐	neutral ☐	inappropriate ☐	
Two genes: 1+3+4 and 2 to 4	appropriate ☐	neutral ☐	inappropriate ☐	
Three genes: 1 to 4; 1+3+4 and 2 to 4	appropriate ☐	neutral ☐	inappropriate ☐	
Other: ……..				
c. Are there any other specific names you would use for any of the regions of the sequence in this case?				
d. If the case description does not provide you with the information you need to reply, please indicate what else you would need to know.				

TABLE 3. Follow-up questions concerning example 1.

Example 2. Overlapping Genes Without Shared Coding Sequences

Alternative splicing creates two mature mRNAs from a single pre-mRNA (Figure 3). The mature mRNAs share a noncoding exon as a common translation start site. However, the entire coding region of the first transcript is found within the first intron of the second transcript. The two transcripts consequently have no overlapping coding sequences and encode structurally unrelated proteins.[13]

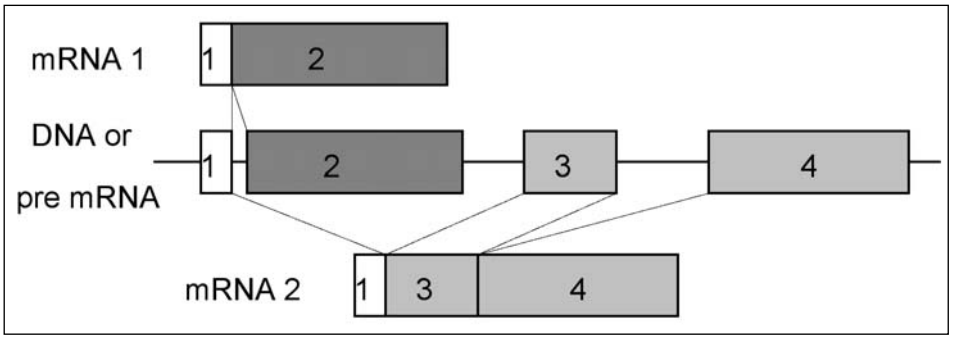

FIGURE 3. Overlapping genes without shared coding sequences

In contrast to the previous question, which involves overlapping genes with a significant amount of shared coding sequence, albeit in a different reading frame, this event highlights the possibility of overlapping genes that only share a non-coding sequence. In both cases the two products result from a single primary transcript.

We aim to identify axes of difference between cases such as the two above which seem to influence preferences in annotating the cases, either in the whole group of subjects or in subgroups with particular research interests. A first axis of difference is the *number of promoters* involved in the transcription of the DNA segments in question. Possession of a separate promoter is a standard criterion used in real-life genome annotation. A second axis of difference is whether the DNA elements in question have a known biological *function in the gene expression process*, even if they are alone not able to code for a product (e.g. non-coding region, regulatory binding site, etc). Without

[13] Example: the DNA complex *IP259/Dub80* in *D. melanogaster* (Mottus *et al.* 1997; Blumenthal 1998).

such a function DNA elements are often dismissed as 'junk DNA' or 'pseudo-genes'. Whether the DNA elements involved in a transcription event are able to *function independently* of one another is a third axis of difference. Can they code for a product without working together with the other DNA element present in this specific transcription event? This seems likely to affect whether the elements are treated as several cooperating genes or as parts of a single gene. A fourth potential axis of difference is the *relative position* or distance of these elements to each other in the genome. Are they *cis* or *trans* located,[14] are they in the same or distal chromosomal region or at least at the same chromosome, and are they transcribed in the same direction? A fifth consideration that might influence the decision between alternative splicing of one gene and overlapping sequences of two genes is the amount of shared sequence, whether the shared sequence is a coding sequence, and whether it is read in an alternative reading frame. We treat facts of this kind as contributing to a general axis of *sequence similarity*. The two cases given as examples above differ along this last axis.

Other potential axes of difference concern the *coding relationship* between DNA and product. Given the importance normally accorded to the template capacity of genes one obvious axis of difference is whether the *linear order* of nucleotides in the open reading frame is preserved in the linear sequence of amino acids in the gene product. Another point that has been the focus of much discussion of the gene concept is the *numerical relation* between DNA segments and 'gene products'. This relation may be one-to-many, many-to-one or many-to-many, as outlined in Section 2. If the ratio is either one-to-many or many-to-one another question follows: how *proximal or distal* from the DNA sequence is the initial branching point in the process of DNA expression? Last but not least one might want to know about the *functionality of the final product*. This can be hard to answer, as sometimes the result of a known transcription event is a polypeptide or RNA structure with unknown function. In this case it helps, for example, to show that the particular case of gene expression is regulated by the cell (shows a typical spatial or temporal distribution), or otherwise shows biological activity, in order to distinguish a case of functional gene expression from so-called 'transcriptional noise'.

[14] In one current usage, *cis*-elements are transcribed as part of a single unprocessed mRNA whereas *trans*-elements are transcribed separately and united at some stage of post-transcriptional processing (*trans*-splicing).

4.2 Review of Research Strategies for Complex Genetic Diseases

A second part of the questionnaire asked subjects to undertake a minimal version of another real-world task, in this case refereeing research proposals for a funding agency. This section was designed to document the use of a top-down gene concept, the first option in Falk's list of potential conceptions of the gene and one whose importance has been suggested elsewhere in the literature. We also hoped to find indications of two conceptual schemes corresponding to Moss's Gene D and Gene P.

We approached these issues by constructing four different research strategies to investigate the molecular bases of certain 'genetic' diseases. Each strategy was intended to appear compelling in the light of a particular conception of the gene. The nature of the genetic disease was recognized as another factor that might affect the assessment of research strategies. Two axes of difference were expected to influence responses: physiological versus behavioral/psychological, and human versus animal. We therefore chose to look at four types of complex genetic pathology:

- Human behavioral: Novelty Seeking
- Human psycho-physical: Frontotemporal dementia (similar to but not as well-known as Alzheimers)
- Human physiological: Malignant Hypothermia
- Animal physiological: Porcine Stress Syndrome (a.k.a. Porcine Malignant Hypothermia)

As an example we will summarize our treatment of Frontotemporal dementia (FTD). The initial question (Table 4) was followed by a short description of the genetic disease highlighting some of the scientific knowledge to date including probable genes and possible epigenetic factors affecting the onset and course of the disease (Table 5).

Question
You have been asked to referee proposals for research on the genetic basis of FTD. Assess the following proposals, assuming that all the lead investigators have equally impressive track-records, institutional backing, and so forth. Please remember, as you would when faced with a real proposal, that resources are limited.

TABLE 4. Question asked in the questionnaire to the case example FTD.

Case: Frontotemporal Dementia
Frontotemporal dementia (FTD) is a common, non-Alzheimers form of neurodegenerative disease. FTD displays considerable genetic heterogeneity. In some families it is associated with several alleles of the tau gene on chromosome 17, while in others it is associated with a less well-characterized polymorphism on chromosome 3. At least one allele of the APOE gene on chromosome 21 is a risk factor for FTD in individuals who lack both the chromosome 17 and chromosome 3 variants associated with the disease.

TABLE 5. Description of the case FTD.

This information was followed by short descriptions of four research strategies which were presented in random order. Respondents were asked to assess the potential value of pursuing each of the strategies on a five-point scale similar to that used by some real funding agencies.

The first strategy was based on the assumption that increased understanding of the genetic basis of a disease requires identifying a stable association between a specific mutation and a specific phenotype (Table 6). The existence of multiple independent correlations in complex genetic diseases threatens the viability of this approach and one strategy to recover a simpler mutation-disease relationship is to divide the original phenotype into subtypes, each corresponding to its own genotype ('splitting the trait'). This approach to the role of genes in disease would suggest that a researcher is employing a top-down conception of what genes are, or something like Moss's Gene-P conception.

Strategy 1.
Team A presumes cases correlated with variation at different loci will reveal phenotypic differences on further investigation ('splitting the trait'). The team proposes to refine the diagnostic criteria for FTD by identifying differences in age of onset, course of the disease and other symptomatic factors and to use these refined phenotypes to further investigate the genetic basis of the disease. The expectation is that these refined phenotypes will prove more genetically uniform.

TABLE 6. First research proposal (research strategy) to be assessed by the respondents of the questionnaire.

A weaker top-down or Gene-P conception might give up on the idea that a full-fledged phenotypic disease can be directly and unambiguously linked to a mutation and choose instead to bring the phenotype closer to the genotype by substituting some biochemical or cellular state (endophenotype) for an organism level phenotype (Table 7).

Strategy 2.
Team B suggests that the phenotype defined by clinical presentation or by the neuropathology associated with the full-blown disorder is unsuitable for genetic analysis. The team proposes to characterize a number of intermediate phenotypes (endophenotypes), using physiological challenges, biochemical assays and physiological measures to obtain primary indicators of disease pathology. These endophenotypes would form the basis of further investigations of the genetic basis of the disease. The expectation is that the endophenotypes will prove more genetically uniform.

TABLE 7. Second research proposal to be assessed by the respondents of the questionnaire.

The third strategy reflects the idea that genes primarily represent sources of multi-potent gene products which interact with many other such products in a biochemical pathway (Table 8). This is consistent with a Gene-D conception because the gene is primarily identified via its biochemical template capacity and, conversely, the disease phenotype is not associated directly with the presence of one or more genes, but instead with a biochemical pathway to which different genes can contribute on different occasions.

Strategy 3.
Team C proposes to locate a common pathway to which variations at different loci can contribute in different sub-populations of affected individuals. They plan to combine microarray and other gene expression data with protein annotation and new advances in the understanding of protein-protein interactions to identify the role of the several polymorphisms in the developmental pathway leading to FTD, using a series of transgenic mice. The expectation is that the disease can only be properly understood at the genetic level by understanding how multiple genes interact.

TABLE 8. Third research proposal to be assessed by the respondents of the questionnaire.

The last strategy emphasizes the potential importance of epigenetic factors that confer specific significance on a gene that otherwise has no privileged link to a specific phenotype (Table 9). This is a full-blown Gene-D conception, one that would emphasize, for example, the fact that oncogenic mutations may be found in non-cancerous cells of breast (sometime the other breast) of women with cancerous cells that have the same mutation. The strategy embodies the belief that genotype-phenotype links are held in place by a rich context of other developmental factors.

Strategy 4.
Team D proposes studying asymptomatic individuals with known mutations and symptomatic individuals with no known mutations, so as to identify epigenetic factors affecting the development of FTD, including maternal effects, environmental factors (such as disease states, head trauma in earlier life), variation in inflammatory and immune responses, and modifier genes. The expectation is that FTD can be explained as a developmental phenomenon, with known risk factors making a contribution to this developmental outcome in a suitable context.

TABLE 9. Fourth research proposal to be assessed by the respondents of the questionnaire.

4.3 Kinds of Biologists: Independent Variable

In our earlier survey we identified different groups of biologists in the simplest possible way, by asking subjects about the disciplines in which they had been educated and in which they presently worked. In our ongoing study we have chosen to study a series of potentially subtler indicators. In each case, subjects were presented with a list of options and also given the opportunity to provide 'Other' responses. We asked the following questions:

- In which scientific journals would they most like to see their work published?
- Which scientific journals do they make most effort to read?
- Which professional societies do they belong to?
- Which levels of biological organization do they see as the focus of their research?
- How would they describe their own field of research?
- Which laboratory and other techniques do they use?
- Which model organisms do they use?

– Do they see their work as part of medical science?
– Do they see their work as fundamentally comparative?

In addition to their value in analysing responses to the other parts of the survey, we anticipate that the analysis of correlations between these several indicators will provide new insights into the structure of contemporary biology, similar to those obtained in the new discipline of 'knowledge domain mapping' (Shiffrin and Börner 2004).

5. Conclusion

Philosophical, historical and experimental research on conceptualizations of the gene and of other DNA elements, and on related ideas about heredity and development are important because these concepts play roles both in scientific discourse and in a much larger set of overlapping discourses in bioethics and public policy, in popular science and, ultimately, in contemporary understanding of what it is to be human. One might thus expect that different representations of the same or similar genomic elements, as a consequence of different conceptualizations of the elements and their action, may result in significantly different understandings of these biological processes or 'genes' on the part of wider audiences. The work presented in this paper may therefore lead not only to a better understanding of how various gene concepts contribute to biological research, but also to a better understanding of how they figure in the dissemination of genomic knowledge to other audiences. The study of conceptual change and conceptual diversity in genomics is thus relevant to the work of bioethicists, medical sociologists, and science communicators, as well as to philosophers and historians of biology

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What Concept Analysis in Philosophy of Science Should Be (and Why Competing Philosophical Analyses of Gene Concepts Cannot Be Tested by Polling Scientists)

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ABSTRACT - What should philosophers of science accomplish when they analyze scientific concepts and interpret scientific knowledge? What is concept analysis if it is not a description of the way scientists actually think? I investigate these questions by using Hans Reichenbach's account of the descriptive, critical, and advisory tasks of philosophy of science to examine Karola Stotz and Paul Griffiths' idea that poll-based methodologies can test philosophical analyses of scientific concepts. Using Reichenbach's account as a point of departure, I argue that philosophy of science should identify and clarify epistemic virtues and describe scientific knowledge in relation to these virtues. The role of concept analysis is to articulate scientific concepts in ways that help reveal epistemic virtues and limitations of particular sciences. This means an analysis of the gene concept(s) should help clarify the explanatory power and limitations of gene-based explanations, and should help account for the investigative utility and biases of gene-centered sciences. I argue that a philosophical analysis of gene concept(s) that helps achieve these critical aims should not be rejected on the basis of poll-based studies even if such studies could show that professional biologists don't actually use gene terminology in precise ways corresponding to the philosophical analysis.

KEYWORDS: concept analysis, epistemic virtue, gene concept, metaphilosophy, Reichenbach, scientific concept

1. Introduction

Karola Stotz and Paul Griffiths' Representing Genes Project (RGP) raises questions about what philosophers are trying to accomplish when they analyze scientific concepts (see Stotz and Griffiths 2004 and in this volume for a general description of the overall project). A central motivation behind the project was to put to the test (or 'evaluate') competing philosophical accounts of the gene concept.[1] Though

[1] Stotz and Griffiths state the goal of their early RGP-type research: 'Our aim in this study was to evaluate some of the competing accounts of the gene concept in a more rigorous and systematic way'(Stotz and Griffiths in press).

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philosophers might resist the idea that a philosophical analysis could be subjected to a poll-based test, the question is: 'why not?'. As Griffiths has asked, if the RGP study revealed that scientists don't actually think of genes in the way set out by a philosophical account, then what value could the account possibly have? What is concept analysis if it is not a description of the way scientists think? I investigate these questions by contrasting the poll-based methodology of the RGP with the critically-oriented project of rational reconstruction set out by Hans Reichenbach (1938). Using Reichenbach's account of the tasks of epistemology *as a point of departure*, I will propose that philosophy of science should seek to provide a critical understanding of scientific knowledge that goes far beyond the kinds of descriptions that could be tested by poll-based studies.

I begin by assuming that an RGP-type, poll-based study revealed that the way biologists actually use gene terminology does not closely correspond to a philosophical analysis of the gene concept(s). Making this assumption requires assuming that a number of practical difficulties confronting the RGP can be solved, an assumption that may be unrealistic for reasons I briefly discuss. Next, I review Reichenbach's account of epistemology to explore what conclusions could appropriately be drawn if a philosophical analysis failed a poll-based test. Reichenbach identified three kinds of tasks for epistemology: descriptive, critical, and advisory. I use Reichenbach's account to show that the descriptive task is not purely descriptive; it involves a critical dimension that poll-based studies of term usage are not designed to handle. Hence, even if an analysis of the gene concept failed an RGP-type test, that is, even if a poll-based study revealed that the way biologists actually use gene terminology does not closely correspond to the philosophical analysis of the gene concept(s), the analysis might nevertheless provide the best description of the gene concept at play in biology.

I use Reichenbach's account of the critical and advisory tasks of epistemology to develop and defend the idea that an important aim in philosophy of science is to construct interpretations of scientific knowledge. Reichenbach acknowledged this aim when he said the inclination behind the critical and advisory tasks was to acquire 'valid thinking'. I suggest that philosophers of science should aim to develop an interpretation of concrete scientific knowledge with respect to epistemic virtues such as truth, explanatory power and precision, and predictive success. The goal should be to develop accounts of particular sciences that (1) show what epistemic virtues are realized by the science (and the extent to which these virtues are realized) *and* (2)

reveal a science's limitations with respect to these and perhaps other epistemic virtues. Another way to express this goal is to say that philosophy of science should identify and clarify epistemic virtues and describe scientific knowledge in relation to these virtues.

Moving beyond Reichenbach's position, I will not assume that there is a fixed set of epistemic virtues. Some virtues are valued more by scientists in some areas of science than by scientists in other areas. In fact, scientists in different periods or contexts of the same discipline may (appropriately) emphasize different epistemic virtues. I will go further and argue that the epistemic virtues that matter to those of us outside professional science are not necessarily exactly the same virtues valued by professional scientists conducting research in the relevant discipline. Some epistemic virtues, such as epistemic success are valuable to all of us who care about science or the impact of science in society. But other epistemic virtues, such as conceptual precision, may matter to those of us who are concerned about policies based on misunderstandings of science even if the virtues are not prized by scientists in the discipline who are focused on conducting research and promoting their scientific work.

According to this account of philosophy of science, the role of concept analysis is to articulate scientific concepts in ways that will help reveal epistemic virtues and limitations of the relevant sciences. This means an account of the gene concept(s) should help clarify the explanatory power and limitations of gene-based explanations, and should help account for the investigative utility of the chief methods of gene-centered sciences. I argue that a philosophical account of gene concepts that helps achieve these critical aims should not be rejected on the basis of a poll-based study even if the study could show that professional biologists didn't actually use gene terminology in the precise ways set out by the philosophical account.

My primary aim in this paper is to answer questions about what philosophers of science should try to accomplish when they analyze scientific concepts and interpret scientific knowledge. The claim that poll-based methodologies can test a philosophical analysis of scientific concepts provides a useful foil for examining these questions. My quarrel is with this claim about testing, not with the overall project of the RGP. In fact, I agree with Stotz and Griffiths' contention that poll-based studies such as the RGP could provide information about how terms such as *gene* are used differently by individuals in different disciplinary contexts. I agree that this kind of information would elude individual philosophers appealing to linguistic intuitions. And I acknowledge that this kind of information could be valuable for the

critical analysis of scientific concepts. But Stotz and Griffith's idea that information gathered by poll-based studies about actual patterns of term usage could provide a test between competing philosophical analyses of the corresponding concepts suggests that they have a different conception of the philosophical project of scientific concept analysis than I do. My aim is to examine what the philosophical project of scientific concept analysis should be and what it has to contribute to the critical understanding of scientific knowledge.

2. Practical Difficulties for Using Poll-Based Research to Test Accounts of Gene Concepts

There are a number of practical difficulties for using a questionnaire-based study to test competing accounts of the gene concept(s). Many difficulties arise from the fact that it is not easy to draw connections between an account of the gene concept(s) and patterns of term usage recorded on questionnaires. First of all, some philosophical analyses of the gene concept(s) claim there are at least two distinct concepts playing important roles in contemporary genetics (e.g. Waters 1994 and Moss 2003).[2] One difficulty for testing a pluralist account is that the testing project needs to separate tests of one concept identified by the pluralist analysis from tests of the other concepts identified by the same analysis. This is especially difficult given the possibility that one and the same biologist can use one gene concept in certain investigative stages or explanatory contexts and another gene concept in other stages or contexts. The testing project needs to distinguish different stages and contexts so that accounts of gene concepts are tested with respect to the situations in which they allegedly apply.

Another complication is that different biologists might use different concepts. Some analyses (pluralist or monist) are targeted on research geneticists/molecular biologists, others on evolutionary biologists. The aim isn't necessarily to account for the way all biologists, or even all research biologists, conceive of genes. Any testing project needs to be careful to separate tests of one group of scientists from tests of another.

[2] According to my 1994 analysis, one concept, derived from the Morgan school of classical genetics, conceives of genes simply as units in the chromosome that are difference makers. This classical concept does not presuppose what genes are made of or what genes do. It only holds to the *difference principle* that differences in genes cause phenotypic differences in particular genetic and environmental contexts. Another gene concept, conceived at the molecular level, on the other hand, does attribute a kind of physical structure and function to genes.

In addition, testing may be uninformative unless it 'catches biologists in the act'. Asking biologists abstract questions about the gene concept such as the question 'are introns part of a gene or not?' provide conflicting and misleading results.[3] Answers to such questions on a poll would not necessarily reflect how biologists actually employ terms. The challenge is to find ways to write a questionnaire that sets up appropriate contexts and invites subjects (separated into appropriate groupings) to employ genetic terminology. Then one might determine whether the patterns of usage among target biologists in different contexts corresponds to the patterns suggested by particular analyses of the concept.

Stotz and Griffiths are well aware of these kinds of practical issues as well as a host of other difficulties. In fact, they argue that an advantage of their questionnaire-based approach is that it can gather a diverse body of relevant data that is more informative than the linguistic intuitions of any single philosopher (Stotz, Griffiths, and Knight in press). On this basis, they argue that their approach provides the best way to evaluate competing philosophical accounts of the gene concept. Griffiths and Stotz are working with a group of consultants to sort out the difficulties such as the ones mentioned above.[4] Although the practical difficulties are daunting, I will assume for the rest of this paper that Stotz and Griffiths overcome the difficulties and obtain results that confirm or disconfirm claims that patterns of linguistic usage of an appropriate target group of biologists in appropriate contexts correspond to particular philosophical analyses of the gene concept(s).

Now we can ask, what if actual patterns of term usage do not correspond to the patterns suggested (or 'predicted') by an account of the gene concept(s)? Does this mean the account is mistaken? Professor Griffiths once cheerfully remarked that in such a case, he wouldn't know what the philosophical account would be good for. I think this question raises interesting meta-questions about the nature and purposes of concept analysis in philosophy of science. What is our philosophical aim, if not to clarify the concepts actually in play in science. And what could it mean for a concept to be in play if biologists' usage of the corresponding term does not closely correspond to the concept?

It is tempting to answer that concept analysis in philosophy of science is aimed at something deeper than accounting for linguistic

[3] I base this claim on extensive personal experience.

[4] The fact that they sought to include skeptics, including me, in their group attests to the integrity of their endeavor.

behavior (the use of terms). But what? To answer this question, I turn to Reichenbach's account of the three tasks of epistemology.

3. Reichenbach's First Task for Epistemology: the Descriptive Task

Reichenbach begins his account of the tasks of epistemology with the premise that the subject matter of epistemology, knowledge, is a 'concrete thing'. It might surprise contemporary critics of 'positivism' that Reichenbach took knowledge to be a sociological phenomenon:

> Knowledge, therefore, is a very concrete thing; and the examination into its properties means studying the features of a sociological phenomenon. (Reichenbach 1938, 3)

The first task for epistemology, according to Reichenbach's account, is to describe knowledge 'as it really is'. This does not mean that Reichenbach thought epistemologists should describe the sociological phenomenon in all its complexity, and he used two distinctions to carve out the special domain that epistemology describes.

The first distinction Reichenbach invoked to delimit what part of the sociological phenomenon should be described by epistemologists was between 'internal relations' and 'external relations':

> [the descriptive] task concerns the internal structure of knowledge and not the external features that appear to an observer who takes no notice of its content. (Reichenbach 1938, 4)

Reichenbach formulated this distinction as if it were based on a difference between kinds of observations. But this distinction rests crucially on what he meant by 'content'. It is tempting to say that by 'content' he must have meant ideas, and that he was claiming that the descriptive task of epistemology is to describe relations among ideas, not relations among concrete entities such as people, instruments, and institutions. But drawing the distinction this way seems to contradict his premise that knowledge is a concrete thing. Furthermore, Reichenbach claimed that astronomers' interest in music was 'external' (and presumably he would have viewed their ideas about music external as well). In the end, it seems that Reichenbach didn't offer an account of the internal/external distinction; he simply assumed such a distinction exists.

Regardless of whether Reichenbach's internal/external distinction is meaningful, it appears that some sort of distinction is needed. Even if we think of scientific knowledge as a complex phenomenon that includes

cognitive, material, and social components and their relations, it seems that certain aspects of the scientific enterprise are not relevant to describing the knowledge. The difficulty is distinguishing which elements are relevant and which are not. This is made all the more difficult by recent works in philosophy of science (e.g. Longino's 2001 work on social epistemology) and history of science (e.g. Galison's 2003 work on clocks), which show that many elements that seemed 'external' turned out to be relevant for understanding the kinds of things epistemologists have traditionally been interested in understanding (e.g. objectivity and investigation). Perhaps the best position to take is that there is no a priori, general distinction. Distinguishing what is relevant for understanding scientific knowledge can be determined only locally, only with respect to particular epistemological questions, and only by investigating actual science. In any case, the descriptive task of epistemology involves describing science as it is, but not necessarily in all its complexity.

The second distinction Reichenbach drew to set out the boundaries for the descriptive task of epistemology is the difference between describing how ideas are logically connected and describing the psychological processes that connect them. He pointed out that the psychological operations are 'vague, fluctuating processes' that 'almost never keep to the ways prescribed by logic' (Reichenbach 1938, 5). This creates a problem for epistemology since philosophers are interested in examining how ideas are related, in identifying hidden assumptions, and in examining lines of reasoning. For example, when philosophers analyze concepts, they presume the principle of non-contradiction. One reason for favoring the idea that there are at least two gene concepts is that no single gene concept is consistent with the range of ways 'the' concept is appropriately used in biological explanations. Hence, the principle of non-contradiction leads one to suppose that there may be more than one gene concept. Reichenbach pointed out that it would be 'a vain attempt to construct a theory of knowledge which is at the same time logically complete and in strict correspondence with the psychological processes of thought' (Reichenbach 1938, 5). So, what are philosophers describing if not actual thought processes? Reichenbach's answer was that epistemologists are describing how the thinking 'ought to occur'. Constructivists might appreciate Reichenbach's account of the description process.

> [Epistemologists] construct thinking processes in a way in which they ought to occur if they are to be ranged in a consistent system; or to construct justifiable sets of operations which can be intercalated between the starting-point and the issue of thought-processes, replacing the real intermediate links. (Reichenbach 1938, 5)

He called the result of such work, a 'rational reconstruction'.[5]

Reichenbach's idea of 'rational reconstruction' has been unfairly dismissed. It has often been mistaken as a license for making up one's history of science (or account of contemporary science) in order to falsely substantiate one's favored model of scientific rationality. It is true that Reichenbach held that 'Many false objections and misunderstandings of modern epistemology have their source in not separating these two tasks [the psychologists' from the epistemologists']; it will, therefore, never be permissible objection to an epistemological construction that actual thinking does not conform to it' (Reichenbach 1938, 6). And admittedly, some philosophers might have invoked Reichenbach's term to take such license (e.g. Lakatos). But this is not what Reichenbach had in mind. He held that a rational reconstruction must correspond to actual thought processes and had to satisfy the *postulate of correspondence*:

> In being set before the rational reconstruction, we have the feeling that only now do we understand what we think; and we admit that the rational reconstruction expresses what we mean, properly speaking. (Reichenbach 1938, 6)

The idea was that rational reconstructions must represent scientists' thoughts so well that they would agree that the reconstruction accurately described their knowledge in a clear and complete way. Lakatos' notorious histories did not satisfy this constraint.

Reichenbach used 'we' in his formulation of the postulate of correspondence (and throughout related passages) to include both philosopher and scientist as if their thinking (and reasoning) are the same. This assumption is problematic and we should therefore distinguish between the thinking of scientists and the thinking of philosophers of science. This gives rise to two versions of the postulate of correspondence. One identifies a condition of accuracy (or what might be called a 'truth condition'), the other an evidential criterion. The accuracy condition of the postulate of correspondence could be specified as follows:

> **The accuracy condition for rational reconstruction:** In being set before the rational reconstruction, the target scientists would have the feeling that only now

[5] Reichenbach assumed that the framework for epistemology is logic. He distinguished the psychological project of describing how ideas are *psychologically* interconnected with the epistemological project of describing how they are *logically* connected. Reichenbach said rational reconstructions are 'a logical substitute' for the real (i.e. psychological) processes. But his basic point can be taken more abstractly by thinking of rational reconstructions as 'critical' substitutes of actual thinking processes. One can take logical rigor to be an important ideal, but not the only ideal, for constructing critical descriptions of the contents of science.

> did they understand what they thought; and they would admit that the reconstruction expressed what they meant, properly speaking.

Or more briefly:

> The rational reconstruction expresses what target scientists mean, properly speaking.

The evidential criterion, that is the methodological principle used for determining whether the truth condition is satisfied, might be articulated as follows:

> **The evidential criterion for rational reconstruction:** After internalizing the thinking processes of target scientists, and after considering the rational reconstruction, the epistemologist would recognize that the reconstruction expresses what the scientists mean, properly speaking.

This evidential criterion is inadequate. As formulated here, the criterion suggests a quick, intuitive judgment when in fact determining whether a reconstruction provides an accurate description of scientific ideas and methods 'properly speaking' involves careful scrutiny (as I explain below).

It is important to note that Reichenbach acknowledged that the descriptive task of epistemology is not purely descriptive; he recognized that the task necessarily includes a *critical* element. The epistemologist is invoking standards of logic to determine what belongs in the description of knowledge (i.e. in the rational reconstruction). It is worth pointing out that this is true of historians' descriptions as well. Historians must apply standards of logic or relevance to represent the knowledge of the past. Otherwise, they could not identify assumptions and biases, connections among ideas, or internal tensions within a scientific view. We all assume certain standards of logic or relevance to provide coherent accounts of knowledge.

Reichenbach remarked that 'rational reconstruction is bound to factual knowledge in the same way that the exposition of a theory is bound to the actual thoughts of its author' (Reichenbach 1938, 7). In fact, an exposition of a theory is a rational reconstruction. Hence, although historians and philosophers of science have been quick to dismiss 'rational reconstructions' it is difficult to articulate a critical approach to analyzing the ideas of science that does not include the endeavor Reichenbach had in mind when he introducted the phrase. Any historian, sociologist, anthropologist or philosopher who has identified an unstated assumption, pointed out an inconsistency, or

revealed a systematic bias in a scientist's thinking has been involved in what Reichenbach called 'rational reconstruction'.

4. How Does Polling Scientists Relate to the Descriptive Task of Epistemology?

Of the three tasks for epistemology identified by Reichenbach, RGP is most closely related to the descriptive task, and it is convenient to discuss this relation before moving on to the critical and advisory tasks. A stated aim of RGP is to 'test' philosophers' accounts of the conceptual element(s) of biological knowledge associated with the term gene (Stotz and Griffiths 2004 and in this volume, and Stotz, Griffiths, and Knight forthcoming). This suggests that the task of describing the gene concept were purely descriptive, that is, as if the task entailed describing actual thinking processes. But Reichenbach's account shows that the philosophers' descriptive task is not purely descriptive. It contains a critical element because philosophers of science aim to construct a logically coherent account (at least) whereas the actual psychological processes are logically incomplete, vague, and fluctuating. The descriptive task of epistemology involves a trade-off between psychological accuracy and logical coherence (I will argue in section 6 that the tradeoff is between psychological accuracy and a host of epistemic virtues, not just logical coherence).

As explained in the previous section, Reichenbach's postulate of correspondence identified a condition that must be met for the purpose of accuracy. Epistemological descriptions may depart from the way scientists actually talk (in order to heed epistemological standards such as consistency and logical completeness), but the descriptions must satisfy the following condition: scientists would agree that the accounts accurately describe what they mean, 'properly speaking'. The poll-based methodology of the RGP, however, invokes an evidential criterion that is not necessarily aligned with this condition of accuracy. RGP checks for accuracy by determining whether the epistemologists' descriptions match the linguistic behavior of scientists, as observed by examining scientists' answers to questionnaires. Of course one might design a questionnaire to explicitly ask scientists whether a philosophical account of the gene concept captures what they mean 'properly speaking', but this would entail presenting a lengthy and in-depth account of each philosophical account to be tested. The strategy of RGP is to cut to the chase by using a short answer questionnaire to determine how scientists actually employ the gene concept in particular situations and what

features scientists attribute to genes. The RGP is not set up to poll how scientists judge competing philosophical accounts; rather, its questionnaires are designed to determine how scientists actually think about genes. But as Reichenbach pointed out, actual thinking processes are typically vague and incomplete.

The misalignment between the evidential criterion of poll-based methodology of the RGP and the descriptive standard (or truth condition) proposed by Reichenbach can be explored by considering the possibility that an account of the gene concept fails an RGP-type test but satisfies Reichenbach's postulate of correspondence. It is not difficult to imagine such a situation. Suppose a philosopher sets out to describe the gene concept associated with an area of biological inquiry and explanation. She finds, as Reichenbach would expect, that discussions employing the term *gene* partially reflect the vague and fluctuating nature of psychological processes. But she succeeds in constructing a coherent description of the underlying concept that eliminates contradictions and helps us better understand explanations invoking the term. Further, suppose that when biologists are provided a complete account of her description of the gene concept, including how her account resolves contradictions and clarifies the explanatory and investigative import of the concept, scientists agree that she has indeed captured what they mean 'properly speaking'.

Now suppose that the scientists are confronted with evidence, based on the questionnaire methodology of the RGP, that their use of the term 'gene' contradicted the account given by the epistemologist. Would the questionnaire-based evidence, which 'contradicts' the philosopher's account of the concept, force scientists to admit that the epistemological account failed to describe accurately what they actually meant, 'properly speaking'? Of course not. After reviewing their responses to the questionnaire, scientists might decide that their responses reflected sloppiness in their linguistic behavior or sloppiness in their thinking (perhaps even patterns of sloppiness). One might expect scientists to say that their linguistic behavior, as observed in their responses to short questions, should not be accepted uncritically, that their linguistic behavior or responsive thinking does not necessarily reflect accurately the finest points of their knowledge. And regardless of what scientists would say, this should certainly be the position of a philosopher, one whose aim is to develop a *critical* understanding of scientific knowledge.

When philosophers of science seek to describe knowledge as it is, they should aim to understand how the pieces (including conceptual elements) logically fit together insofar as they do fit together, to clarify

how the concrete explanations work insofar as they work, and to explain what makes the research program productive insofar as it is productive. This may require developing a critical understanding that is at odds with details of linguistic behavior as reflected in scientists' responses to short answer questionnaires. This brings us to one of the questions motivating this paper. If an account of the gene concept failed an RGP-type test, that is if the account failed to predict patterns of term usage revealed by a poll-based study, then what could the account possibly be good for? Part of the answer is that the account could nevertheless provide a basis for critically understanding how concrete gene-based explanations work and why gene-centered research is so productive. The empirical criterion imposed by the RGP is not the right criterion for judging the adequacy of a description of the gene concept for aims of philosophy of science such as the aim of understanding the strengths and limitations of gene-based explanations.

In saying that the empirical criterion of the RGP is not the right evidential criterion for the descriptive task of philosophy of science, I don't mean to suggest that the RGP is irrelevant to epistemology's descriptive task. Examining patterns of term usage might help reveal important information. For example, one of the aims of RGP is to determine whether different groups of biologists use the term *gene* differently from one another (Stotz and Griffiths 2004). If the project succeeds in establishing clear differences in patterns of usage, philosophers of science would be wise to investigate whether this reflects differences in knowledge. Furthermore, using a questionnaire to identify what features biologists readily attribute to genes might help reveal unwarranted biases in thinking. So my claim isn't that the results of an RGP-type study are useless. Much might be learned through an RGP. But it is important to understand that the poll-based methodology of the RGP cannot test competing epistemological descriptions of the gene concept(s) because epistemological description involves a trade-off between psychological and linguistic accuracy on the one hand and epistemic completeness (e.g. completeness with respect to accounting for the explanatory power, investigative utility as well as the explanatory and investigative limitations of the knowledge) on the other.

One might try to argue for an intermediate position. On the one hand, one might grant Reichenbach's point that historians and philosophers descriptions of scientific thinking are not purely descriptive and accept my argument that a poll-based study cannot provide the grounds for deciding between competing philosophical descriptions. On the other hand, one might argue that pure

description of thinking is nevertheless possible. Putting the two hands together, one might advance the idea that purely descriptive accounts describe how scientists *actually think* and that philosophical accounts describe how they *ought to think*. The poll-based methodology of the RPG would allegedly shed light on the philosophical accounts by indicating which of the competing accounts match how scientists actually think. Such an intermediate position, however, would not take Reichenbach's point about description to heart. He suggested that actual thinking is too 'vague' and too 'fluctuating' to be described without imposing at least standards of logic. He thought the only way to describe thinking 'as it is' entails invoking critical standards. In fact, discussions about how to construct the polling instruments for the RPG invoked ideals of logic, explanatory coherence, and so on. Perhaps it will one day be possible to use models from cognitive science to construct purely descriptive framework for describing thinking. But the idea that it is possible to use a polling-based methodology and non-critical frameworks available today to establish a purely descriptive, description of scientists' thinking is dubious.

I have considered the possibility in which an account of the gene concept satisfies Reichenbach's postulate of correspondence and fails as poll-based test. But it is also possible that an account of the gene concept that passes a poll-based test could fail to satisfy Reichenbach's postulate of correspondence.

	satisfies postulate of correspondence	*does not satisfy postulate of correspondence*
fails poll-based test	*possible*	*all too likely*
passes poll-based test	*possible*	*possible*

poll-based test: test of the descriptive adequacy of an account of the gene concept based on scientists' responses to short answer questionnaires.

postulate of correspondence: an account of the gene expresses what target scientists mean, 'properly speaking' (and would be endorsed by scientists as describing what they meant, 'properly speaking').

That is, the results of a questionnaire might reveal patterns of linguistic usage that are consistent with a particular account of the gene concept and yet scientists might object to the model on the grounds that it does not capture what they actually mean, 'properly speaking'. Scientists might acknowledge that a descriptive account of the gene concept is consistent with the way they have used the term *gene*, but nevertheless argue that their use of the term is misleading and doesn't precisely reflect what they actually mean. Scientists might insist that to truly understand the underlying concept you need to understand the role it plays in concrete explanations or the way it helps motivate a research program. Perhaps a concrete example will help make this possibility clearer. Suppose, for example, that a minimalist account of one of the gene concepts, such as my account of a gene concept invoked at the molecular level (see Waters 2000), passes a poll-based test. The concept is minimalist in the sense that it contains only what is necessary to substantiate concrete gene-based explanations and explain the fruitfulness of gene-centered investigations; it does not include references to 'developmental units' or 'information'.

Scientists might concede that a minimalist account of the gene concept is consistent with what they say in the context of the questionnaire (hence that it passes an RGP-type test), but insist that it doesn't capture the depth of their conception of the gene because it does not conceive of genes as developmental units and doesn't represent genes as information containing entities. The philosopher of science might disagree with the scientists, but their disagreement would have to be based, not on the results of a poll, but on critical considerations. The issue is how to understand the knowledge critically, or how to understand the knowledge 'properly speaking'. This does not reduce to a question of how we should account for or predict term usage. That is, the issue depends not just on how scientists think, but also on the nature of the ideals of proper thinking. I will argue in section 6 that critical considerations concerning proper thinking are broader than those considered by Reichenbach or most contemporary philosophers of science. I will also propose that scientists do not have final say on the ideals of proper thinking and that therefore, contra Reichenbach, scientists do not have the final say about how scientific knowledge should be understood by those of us outside the scientific community. Hence, I will reject Reichenbach's postulate of correspondence as a condition of descriptive accuracy (or as a truth condition for the reconstruction of scientific knowledge). But I am getting ahead of myself. It is time

to consider Reichenbach's account of the critical and advisory tasks of epistemology.

5. Reichenbach's Second and Third Tasks for Epistemology: the Critical and Advisory Tasks

The second task of epistemology, according to Reichenbach, is critical. It involves analyzing the system of knowledge and judging it with respect to its 'validity' and reliability, not simply describing it. He noted that part of this task is already partially performed in rational reconstruction. Although he believed that the critical task is intertwined with the descriptive task, he thought it was important to distinguish between the two. The descriptive task involves a trade off between psychological accuracy and critical validity. The aim was to provide as logically complete an exposition as possible while satisfying the postulate of correspondence. Descriptions satisfying the postulate of correspondence are (at least typically) logically incomplete. The critical task, according to Reichenbach, is to render the account logically complete. Reichenbach suggested that the descriptive and critical tasks represent different tendencies in epistemology. The 'descriptive tendency' is to 'remain in correspondence with actual thinking'; the 'critical tendency' is to 'obtain valid thinking'.

Many of the critical tasks identified by Reichenbach are familiar, such as evaluating the warrant of particular scientific beliefs. But Reichenbach also discussed less familiar tasks and one of them is particularly relevant to this paper's investigation of the nature and aims of concept analysis. Reichenbach said that an important critical task is to identify elements of knowledge that are not required to preserve truth, such as elements that are included on the basis of a convention.

> That there are certain elements of knowledge, however, which are not governed by the idea of truth, but which are due to volitional resolutions, and though highly influencing the makeup of the whole system of knowledge, do not touch its truth-character, is less known to philosophical investigators. (Reichenbach 1938, 9)

Reichenbach distinguished the *critical task* of identifying the logical status of decisions to include elements as volitional decisions from the *descriptive task* of specifying the decision scientists made to include

them. He further distinguished between two classes of volitional decisions. Some volitional decisions, 'conventional decisions', are between 'equivalent' conceptions and he said that these decisions do not influence the content of knowledge. Examples of conventional decisions include choices between different systems of measurement. Other volitional decisions, however, lead to divergent systems of knowledge. Reichenbach said that such decisions 'stand at the very entrance of science' where we must ask questions such as 'what is the purpose of scientific inquiry' (Reichenbach 1938, 10). Reichenbach called decisions leading to non-equivalent systems of knowledge 'volitional bifurcations'.

Reichenbach stressed that an important task of epistemology is to identify elements whose presence in knowledge are contingent on volitional decisions. He said that epistemology also includes the critical task of identifying interconnections between such elements and entailments among decisions to include them. That is, the critical task includes pointing out that 'If you choose this decision, then you are obliged to agree to this statement, or to this other decision'(Reichenbach 1938, 17). This is a critical task (rather than a descriptive one) because the relations among possible decisions depends on logic background knowledge, not on the decisions scientists actually made.

Reichenbach said the third task for philosophy is advisory, namely to make proposals concerning volitional decisions and to implement the proposals in expositions of related subjects. Presumably, the result of attending to the advisory task might be an account ('exposition') of knowledge that incorporates different volitional choices from those actually made by the scientists. Reichenbach was, as the above quotation illustrates, careful to emphasize that epistemologists should not insist that their proposals be accepted because the proposals were not determinations of truth. Rather, the proposals were suggestions to the effect that making certain volitional choices would have certain advantages. The critical task of epistemology does include determinations of truth, such as determinations about the logical connection between statements, the logical status of decisions to include particular elements, the entailment relations among volitional decisions, and so forth. In learning about these matters of fact, philosophers might discover advantages of making certain volitional choices and hence make proposals based on these discoveries and use these proposals in their epistemological accounts of the knowledge.

> The concrete task of scientific investigation may put aside the exigencies of logical analysis; the man of science does not always regard the demands of the

> philosopher. It happens, therefore, that the decisions presupposed by positive science are not clarified. In such a case, it will be the task of epistemology to suggest a proposal concerning a decision; . . . We may point out the advantages of our proposed decision, and we may use it in our own expositions of related subjects; but never can we demand agreement to our proposal in the sense that we can demand it for statements which have been proven to be true. (Reichenbach 1938, 13)

This is what Reichenbach meant by the 'advisory task'.[6]

The identification of volitional decisions is relevant to the task of analyzing gene concepts because the choice of gene concepts, in fact, even the choice to include a gene concept, is a volitional decision. This point needs to be clarified. The choice to exclude particular gene concepts is not always volitional. Some gene concepts should not be adopted because attempts to apply them would presuppose falsehoods about the inner workings of organisms. So, for instance, choosing a gene concept that included the idea that individual genes autonomously carry out individuated functions at the gross phenotypic level would not be a volitional decision in Reichenbach's sense. Application of this concept would contradict what biologists have learned about development and hence touch upon the 'truth character' of the knowledge. Therefore, a decision to include such a concept would not be volitional (in Reichenbach's sense) and epistemologists could have no warrant for advising inclusion of such a concept. Epistemologists' have an advisory role only when the decision would not affect the truth-character of the science.

Although the decision to incorporate a one-gene / one-gross-phenotypic-function gene concept would affect the truth-character of biology, other decisions about gene concepts could be made without affecting truth character of the science and hence would be volitional. For example, biologists might, as Evelyn Keller (2000) and Tom Fogle (2000) recommend, dispense with gene concepts altogether. Biologists could reconstruct gene-centered explanations in terms of DNA units such as promoters, transcription regions, termination sequences, and so on. Choosing to generalize by identifying some elements of DNA

[6] Reichenbach was so cautious about treading on the turf of scientists that he suggested that the advisory task could be avoided if epistemologists simply pointed the advantages and disadvantages of alternative volitional decisions and refrained from making explicit recommendations. Presumably this would mean that epistemologists could not use proposals in their reconstructions of knowledge. Hence, although he presented this option as if it would not affect the substance of epistemology, it would constrain epistemologists' quest for valid knowledge by restricting them to the volitional choices made by scientists.

as genes is a volitional decision. Furthermore, there are alternative ways to conceive of genes consistent with what biologists have learned about genetics. Biologists could choose different gene concepts without affecting the truth-character of their science (provided that they adapted their explanations accordingly). For instance, biologists could choose to use only the classical gene concept, a concept that does not attribute a physical structure or biological function to genes (see Waters 1994). Alternatively, they could use only the molecular gene concept, which does attribute a physical structure and biological function to genes but does not associate genes with gross phenotypic traits (see Waters 2000). Or they could choose to use only a restricted molecular gene concept that applies only to genes for unprocessed RNA molecules.[7] The decision to use both the classical gene concept and an unrestricted molecular gene concept is a volitional decision in the sense that biologists could choose to dispense with gene concepts entirely, or they could choose to use only one of these concepts, or they could choose to use a restricted version of the molecular gene concept, without affecting the truth character of their science (provided that they adapted their explanations accordingly).

Identifying the volitional status of the decision to use a particular gene concept (rather than to use another or to use none at all) is a critical task of epistemology. It is part of the task of identifying a range of options and determining advantages and disadvantages of the options within this range. Reichenbach did not say that epistemologists should identify only options actually considered by scientists. In the tendency to pursue valid science, epistemologists are free to identify and explore the advantages and disadvantages of any option that would not affect the truth character of science. But he cautioned that since this is not a decision about truth, epistemologists should not insist that scientists adopt one, rather than another option. The advisory task of epistemology is limited, according to Reichenbach, to making suggestions based on the critical analysis of what might be called the volitional decision space.

The volitional decision space (my phrase, not Reichenbach's) is bounded by the restriction that any choice within the space does not

[7] In previous publications, I have presented a very general gene concept that can be applied at the molecular level. This concept, 'the molecular gene concept' can be applied to genes for unprocessed RNA molecules (these would include introns as well as exons), genes for processed RNA molecules (these would not include introns), and genes for polypeptides (these would also not include introns). In the face of the incredible complexities of RNA processing in higher Eukaryotes (especially humans), biologists might decide to focus on DNA segments that determine the linear sequences of unprocessed RNA molecules. That is they might choose a *restricted* molecular gene concept.

directly affect the truth character of the science. Some philosophers might be tempted to raise a Quinean objection by arguing that when it comes to decisions about concepts, either all options fall within the volitional decision space or no options do. If scientists are allowed to reconstruct the rest of science, then all options are open because one could preserve truth if one was willing to make changes in other concepts as well. One could, the argument might go, adopt a one-gene/one-gross-phenotypic-function concept of a gene if one was willing to re-conceptualize gross, phenotype, and function. If one tries to rule out such a move by prohibiting re-conceptualization and insisting that a decision between two alternative concepts is only volitional when no re-conceptualization is required, then only the most trivial decisions would be volitional. For instance, if one ruled out re-conceptualization, then one could argue that the decision between the unrestricted molecular gene concept and a molecular gene concept restricted to genes for unprocessed RNA transcripts is not volitional because adopting one rather than the other concept requires making adjustments in how one articulates particular explanations. Hence, it might appear that either no choices about concepts or all choices about concepts are volitional.

We can neutralize this objection by specifying that the choice is not simply between two versions of a concept, but between two versions of a concept plus any additional conceptual modifications that will need to be made to preserve truth. Hence, the full identification of a choice needs to include a specification of any additional adjustments that will need to be made in order to preserve the truth character of the science. Adopting the one-gene/one gross-phenotypic-function concept is not itself within the volitional decision space. But the option of adopting such a concept while at the same time making whole-scale changes to the conceptual landscape of biology might be. Although I disagree with Fogle's and Keller's proposals to drop the gene concept, their works illustrate the appropriate philosophical methodology. When Fogle (2000) and Keller (2000) identify the choice of dispensing with gene concepts entirely, they offer suggestions about how other concepts can be used to preserve the truth-character of explanations and descriptions that now include the term 'gene'. Provided we keep in mind that the choices include not just a choice of a particular concept, but also the decisions entailed by it (so as to preserve truth), the volitional decision space is indeed bound by the restriction that options within the space must preserve truth.

The critical task of analyzing of the volitional decision space includes exploring the advantages and disadvantages of the options.

Reichenbach didn't have a lot to say about what sort of advantages and disadvantages he had in mind,[8] but I propose that the task is to examine the options in terms of epistemic virtues such as explanatory power, predictive success, investigative utility, clarity, simplicity, and so on. For example, I've argued elsewhere that one of the advantages of employing an unrestricted molecular gene concept in molecular contexts is that it reveals a uniformity of causality that is exploited by scientists in concrete explanations and experimental investigations (e.g. Waters forthcoming). The disadvantage of abandoning the gene concept altogether is that it would obscure a causal uniformity that underpins a broad range of explanations in genetics as well as the chief investigative methods in the science. Generalizing about this uniformity by means of the gene concept helps us understand gene-centered sciences such as genetics. Whether the molecular gene concept should be included in our account of the knowledge is not a question of simple truth or falsity.

We can have truth and avoid falsehoods with or without the molecular gene concept. The question about whether the concept should be included in our account of the knowledge of genetics, molecular biology, and genomics (for instance) is also not a question about how biologists think, even properly speaking. It is a question about how to construct an account of the knowledge of genetics, molecular biology, and genomics that helps us understand how the sciences work (and don't work) with respect to epistemic virtues that we value. It is a question of whether the concept helps us understand the epistemic virtues and limitations of the science such as the science's explanatory power and range, the science's predictive successes and failures, the science's investigative strategies, and the science's investigative reach.

6. What Concept Analysis Should Be (Moving Beyond Reichenbach's Epistemology)

Two questions are raised by the idea that an important aim of philosophy of science is to analyze scientific concepts in order to construct an interpretation of the corresponding knowledge that will

[8] Reichenbach's example of Euclidian geometry might be suggestive of the kinds of advantages and disadvantages he had in mind. He pointed out that a decision to include Euclidean geometry in modern physics might lead to 'the occurrence of strange forces, "universal forces", which distort all bodies to the same extent, and may lead to even greater inconveniences concerning the continuous character of causality' (p. 14).

help *us* understand the epistemic virtues and limitations of the science. The first question concerns the '*us*' in the above formulation. Is the epistemological interpretation aimed at providing an understanding in relation to epistemic ideals of scientists engaged in the basic research or in relation to the epistemic ideals that matter to a broader community? The second question, which I will deal with much more briefly, concerns the breadth of what we're trying to interpret and understand. Is the relevant sense of scientific knowledge limited to the knowledge of professional scientists, or does it also include the broader system of knowledge that extends to the use and transmission of scientific ideas beyond the laboratories of research scientists?

6A. Is Philosophy of Science Aimed at Providing an Understanding of Scientific Knowledge in Relation to Epistemic Ideals of the Scientists Engaged in the Basic Research, or Does it Aim at Understanding Scientific Knowledge in Relation to Epistemic Ideals That Matter to a Broader Community?

The question 'for *whom* are we seeking an epistemological interpretation of scientific knowledge?' is important because epistemology is a value-laden enterprise. The aim of epistemology is not limited to providing a purely descriptive description of actual thinking, or even to describe how scientists would prefer to have their thinking described (the standard set by Reichenbach's postulate of correspondence). The aim should be to describe scientific thought and activities with respect to epistemic values. And this raises the question, with respect to *whose* epistemic values?

It may appear that it is the epistemic values of scientists themselves that matter. This is what Reichenbach seemed to be assuming. His criterion for descriptive success, the postulate of correspondence, involved epistemic values, as his phrase 'properly speaking' attests. If my interpretation of the 'we' in the postulate of correspondence is correct (section 3), the postulate gives scientists final say on whether the rational reconstruction succeeds in clarifying the knowledge, properly speaking. Hence it is the scientists' epistemic values, their sense of properly speaking, that ultimately count in Reichenbach's scheme. His assumption that it is the scientists' epistemic values that matter is perhaps more evident in the way he parsed tasks between the scientist and epistemologist. His account of the critical task of epistemology involved examining the advantages and disadvantages of

making certain volitional choices, but left the decision for making those choices to scientists. This indicates that Reichenbach assumed that scientists were the ultimate arbiters of epistemic values.

While scientists will certainly make their own decisions about volitional choices according to what they value, it is not obvious that scientific knowledge should be understood only with respect to the epistemic values of the practicing scientists. Some philosophers and historians have persuasively argued that scientists appropriately adopt different epistemic ideals in different disciplinary contexts. I wish to go a step further and argue that the set of epistemic ideals favored by practicing scientists for understanding an area of science and the set favored by outsiders for understanding the same area of science might appropriately differ. Scientific knowledge, even scientific knowledge within a particular disciplinary context, can be understood with respect to a variety of epistemic virtues and an important task for philosophy of science is to articulate what those virtues could be and why they might be valued, either by practicing scientists or by people outside professional science. While philosophers should be cautious about speaking for others, we (philosophers) should take a critical stance (not necessarily negative, but critical) towards what research scientists publicly claim to be *the* epistemic ideals of scientific knowledge. There may be differences between the epistemic values important for pursuing or promoting scientific research and the epistemic values important for understanding scientific knowledge in ways that will improve the impact that science has in society. Contemporary genetics provides an example where practicing scientists might prefer to reject an epistemic virtue that is nevertheless an important ideal for those of us concerned with the impact of their science in society.

Geneticists use the term 'gene' in sloppy ways. Leading textbooks provide definitions that contradict one another, textbooks use the term in conflicting ways that violate the definitions provided in their own glossaries, and geneticists often apply the terms in *apparently* conflicting ways (e.g. sometimes they will include introns in molecular specifications of a gene and sometimes they will exclude them). Asking pointed questions about the gene concept (e.g whether control regions or introns are part of the gene) typically leads geneticists to admit that their underlying concept(s) is (are) not clear or precise. But they often defend their loose, metaphorical talk about genes on the grounds that it provides flexibility for conducting research. I've often been told that being forced to adopt clear definitions would have impeded the advance of their science. Imposing philosophers' standards of clarity, they argue, would require geneticists to be too

rigid in their thinking. I find it difficult to argue with the proven success of muddling through.

Although muddling through with loose concepts seems to have provided geneticists with freedom to advance their investigations, it has also contributed to widespread misunderstandings about genetics. These misunderstandings have often led towards exaggerations of the roles genes play in development, inflationary accounts of what can be explained in terms of genes, and unjustified conclusions about genetic constraints that will thwart efforts to improve lives or society by changing environments. The 'philosophical' standards of clarity that geneticists sometimes resist, and perhaps rightly in the local context of their investigations, are important in the context of a society inclined to exaggerate the role of genes and the limitations genes impose on individuals and society. If we wish to understand precisely what gene-based explanations can explain and how they work, if we wish to understand why gene-centered investigations have been so productive, and if we wish to demarcate the current limitations of gene-centered perspectives, then we should value the epistemic virtues of clarity and precision regardless of whether the scientists producing the knowledge share this value.

I am not claiming that scientists don't value the virtues of clarity and explanatory precision. I am simply making the point that those of us outside of genetics have our own interests for understanding the scientific knowledge with respect to these values independently of whether the values serve the purposes of research geneticists. Philosophers have been too quick to assume that there is a universal set of epistemic values appropriate to all scientists in all contexts and that these are the values with respect to which scientific knowledge should be understood by everyone regardless of how the science tends to be used or abused in society. I propose below that there is no given set of epistemic virtues and that one of the roles of philosophy of science is to articulate different virtues and to show how particular virtues might be relevant to different local contexts including contexts which involve what might be called the consumption, rather than production, of scientific knowledge.

6B. A Proposal Concerning Whose Values Are Relevant to Philosophical Interpretation

I propose that an important role for philosophers of science is to provide independent judgments about a variety of epistemic virtues and

to interpret scientific knowledge with respect to epistemic ideals that are important with regard to the impact as well as the practice of science. Philosophers should be open to the possibility that the importance of some epistemic virtues could vary from context to context. This means that virtues highly valued by scientists practicing in some disciplines might not be very important to scientists practicing in other disciplines. This also means that some virtues might be more important to scientists in the context of laboratory practice and other virtues might be more important to a broader group of people in contexts where science impacts our understanding of human possibilities or public policy. I will take it for granted, however, that the epistemic virtues of truth and empirical success will be valued by anyone interested in science as an epistemic enterprise. Scientists will always be an important source of information about the value of science, but philosophers should offer society independent judgments on the epistemic virtues and limitations of current scientific knowledge.

Although the idea that philosophers do not need to reconstruct the knowledge of a scientific discipline in terms of the epistemic ideals adopted by scientists in the discipline may seem radical, it is consistent with many mainstream works in philosophy of science. For example, in debates about scientific realism, philosophers are not arguing about how scientists actually understand their knowledge. They are arguing about how the knowledge ought to be understood or what parts of the knowledge ought to be literally believed. Many anti-realists acknowledge that scientists are often realists and some anti-realists, such as van Fraassen, even admit that the productivity of science might depend on scientists' mistaken ideas about which of their scientific claims should be believed to be true. What we should value in the end, according to van Fraassen's position, is empirical success, regardless of what the scientists who produced the knowledge actually value (or say they value).

Reichenbach's conservatism about accepting the epistemic values of scientists is not necessarily the default position in epistemology. Many philosophers have assumed that part of the task of epistemology is to identify what is epistemically valuable. If I am parting from the orthodoxy of philosophy of science, I do so only in rejecting the assumption that epistemic values are necessarily universal. I am willing to accept the possibility that what is epistemically valuable in the context of the laboratory might differ from what is epistemically valuable in the context of a public policy debate.

Another way in which this proposal relates to mainstream ideas is that philosophers and other non-scientists do not feel compelled to

give scientists final authority over values in science when it involves ethical values. For example, society is not reluctant to make independent judgments about the ethical value of scientific practice when it comes to the treatment of human subjects. Few people would argue that the final arbiters of the ethical values legitimately at issue in the use of human subjects is the group of professional scientists conducting research on human subjects. In describing the ethical virtues and limitations in particular scientific practices, we do not assume that the ethical ideals of the practicing scientists are the right ideals for judging the ethics of their science. A poll of scientists' views on the ethics involved in experimenting on human subjects would provide useful information, but it would not provide a basis for directly evaluating competing philosophical accounts of the morality of using human subjects for scientific research. I am suggesting a similar stance with respect to epistemology. I do not believe we need to give scientists the final say on what is epistemically valuable about their work.

It is important to emphasize the special responsibility entailed by parting with scientists about the knowledge science provides because many outsiders have been shamelessly irresponsible. I am thinking, for example, of individuals who claim that science has provided evidence for the claim that human life begins at conception or that science has not provided compelling evidence that smoking causes lung cancer. I realize that the position I'm advancing here might be abused by outsiders who want to advance particular values such as the value some creationists place on believing their own interpretations of the book of Genesis. But I think the alternative of adopting whatever epistemic values scientists take to be important is also risky (i.e. philosophers should be restricted by Reichenbach's postulate of correspondence). The kind of work philosophers of science have conducted on the creation/evolution controversy is important. But I believe that their kind of research will be most effective if they provide independent judgments about basic epistemic standards. In fact, I believe the best treatments on the creation/evolution issue, including Michael Ruse's (2003) and Robert Pennock's (2003), do advance epistemic standards that are more defensible than the naïve falsificationist standard offered by some practicing evolutionary biologists. I think many evolutionary biologists would largely agree, for example, with Pennock's or Ruse's interpretation of their knowledge, but my point is that even if scientists clung to a different interpretation of the knowledge, couched with respect to different epistemic values (e.g. preference for 'falsifiable' theories), others

would have something important to learn from seeing evolutionary biology in light of the epistemic values articulated by responsible philosophers of science such as Ruse or Pennock. This does not mean that Ruse or Pennock should tell evolutionary biologists how to theorize, but it does imply that philosophers of science can make independent judgments about how to understand the knowledge gained through scientific investigation.

6C. What *Breadth* of the Enterprise of Science Should Be Considered in a Philosophical Interpretation of Scientific Knowledge?

The second question raised by the idea that an important aim of philosophy of science is to describe and interpret knowledge in relation to epistemic ideals concerns the breadth of the science we're trying to interpret. Traditionally, philosophers have construed scientific knowledge narrowly, in terms of the ideas, methods or practices of research scientists. But might philosophers of science extend their attention to how these ideas, methods, and practices are played out in society, including the realms of public intellectual discourse, public policy, education, and private enterprise?

6D. A Proposal Concerning the Breadth of the Scientific Enterprise

I propose that philosophy of science should be open to interpretations of scientific knowledge that take into account how the knowledge is played out in larger society as well as in the confines of professional science. This does not mean that philosophers of science must always have the wider scope in mind, but it means that philosophers might relate scientific ideas, methods, and practices to epistemic virtues relevant to realms broader than the realm of professional, research science.

7. Conclusions Concerning Poll-Based Studies

I have argued that a general aim of philosophy of science is to identify and clarify epistemic ideals and to describe scientific knowledge in relation to these ideals. Concept analysis is important because interpreting bodies of scientific knowledge in ways that reveal their explanatory power and limitations (see Waters 2000) and

in ways that enable one to distinguish between their explanatory range and investigative reach (see Waters 2004) requires making choices about how to articulate central scientific concepts. Some choices can obscure the epistemic character of a science while other choices can make the epistemic virtues and limitations more salient. Constructing epistemologically illuminating analyses of scientific concepts requires learning a great deal about the content and practice of science. There are a number of approaches that philosophers employ or draw upon to learn about the content and practice of science: examining contemporary research literature and textbooks; observing how scientists' discuss their work in settings ranging from lab meetings to public conferences; interviewing scientists; investigating the history of the relevant science; and employing methods of social sciences. Now we can ask, how can questionnaire-based studies, such as the RGP, fit into the project of articulating scientific concepts in ways that reveal the virtues and limitations of concrete scientific knowledge.

The questionnaire-based methodology of the RGP is aimed towards understanding how scientists themselves think about concepts or use terms. *If* successful, the RGP could help reveal how different scientists use gene terminology and how they think about genes. And as Griffiths and Stotz have been emphasizing, poll-based studies might help reveal differences in the way scientists from different disciplines think about genes and differences in how individual scientists think about genes in different contexts. This could help the philosophical project in at least two ways. First, it could help establish which volitional decisions scientists have actually made. In the case of those accounts in which philosophers have argued that there are a plurality of legitimate underlying gene concepts, a poll-based study could help sort out the contexts when scientists invoke one or another of the plurality of gene concepts that have been identified by philosophers. In fact, the questionnaire for the RGP was designed in part to determine whether and in what contexts scientists actually use the different kinds of gene concepts articulated by philosophers. This is an important goal and my criticisms do not apply here. What the poll-based study could not do, however, is evaluate the scientists' volitional decisions with respect to the philosophical aim of making salient the epistemic strengths and limitations of the overall body of scientific knowledge. This is a critical task that cannot be carried out by trying to read off the thinking of scientists. But a poll-based study could provide useful information for this critical task and this is the second way the RGP could contribute to the concept analysis. *If* polls

indicated, for example, that biologists working on eukaryotes conceive of genes much differently than biologists working on prokaryotes, then philosophers might search for reasons that would favor the different volitional choices being made in the different contexts. Given the possibility that there are a plurality of gene concepts, poll-based studies aimed towards uncovering differences in the way biologists think about genes could provide enormously useful information. This is a motivation legitimately emphasized by Stotz and Griffiths.

While entertaining the important contributions that poll-based studies might make to the project of concept analysis, it is important to keep a couple of points in mind. First, *if* poll-based studies such as the RGP are successful, they will compliment, but not supersede the approaches philosophers already employ or draw upon to learn about the content and practice of science (see above). Second, the '*if's* in the above phrases warrant emphasis. It is possible that scientists muddle through in ways that will resist obtaining clear results from a poll-based, RGP-type study. It's possible that scientists always muddle through, or perhaps they muddle through in most contexts, except when thinking about narrow research problems related to their own specific research projects. It's also possible that getting a clear handle on the plurality of ways that different scientists use gene concepts in different contexts will elude polling-based studies because of practical difficulties. These difficulties include constructing context sensitive questionnaires, getting suitable sample sizes, and identifying disciplinary boundaries of the scientists polled. The practical difficulties for poll-based studies are daunting.

Relating the poll-based methodology of the RGP to philosophical concept analysis is complicated because the aims of philosophy of science are not simply descriptive, and even when they are largely descriptive, they are not purely descriptive. *If* successful, the RGP could provide useful information for concept analysis that might not otherwise be obtainable. But the notion that the RGP could provide a test or evaluation of competing philosophical accounts of gene concepts is mistaken. Articulating gene concepts in ways that will help, for instance, clarify what can and cannot be explained in terms of genes, goes beyond merely describing how scientists actually use the term *gene*. The philosophical project involves critical considerations that could possibly reveal that what scientists say about the gene concept and how they employ gene terminology, while heuristically well-suited to advancing scientific inquiry and rhetorically suited to promoting their science, is inflationary and hence not useful for

pinpointing the true epistemic strengths and weaknesses of their gene-centered knowledge.

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Molecular Epigenesis, Molecular Pleiotropy, and Molecular Gene Definitions[1]

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ABSTRACT - Recent work on gene concepts has been influenced by recognition of the extent to which RNA transcripts from a given DNA sequence yield different products in different cellular environments. These transcripts are altered in many ways and yield many products based, somehow, on the sequence of nucleotides in the DNA. I focus on alternative splicing of RNA transcripts (which often yields distinct proteins from the same raw transcript) and on 'gene sharing', in which a single gene produces distinct proteins with the exact same amino acid sequence. These are instances of *molecular pleiotropy*, in which distinct molecules are derived from a single putative gene. In such cases the cellular and external environments play major roles in determining which protein is produced. Where there is molecular pleiotropy, alternative gene concepts are naturally deployed; *molecular epigenesis* (revision of sequence-based information by altering molecular conformations or by action of non-informational molecules) plays a major role in orderly development. These results show that gene concepts in molecular biology do, and should, have both structural and functional components. They also show the need for a plurality of gene concepts and reveal fundamental difficulties in stabilizing gene concepts solely by reference to nucleotide sequence.

KEYWORDS: alternative splicing, gene concepts, gene identification, gene sharing, genotype-phenotype mapping, molecular epigenesis, molecular pleiotropy

> 'Genes are not autonomous entities. Rather, they interact with other genes and gene products to make pathways and networks.'
> (Gilbert 2003, 691)

Introduction

There has been considerable debate recently about the status of molecular concepts of the gene (e.g. Beurton, Falk and Rheinberger 2000; Falk 1986, 1995, 2001; Griffiths 2002; Griffiths and Neumann-Held 1999; Hall 2001; Morange 2001; Moss 2003; Neumann-Held

[1] The original version of this paper was presented at the International Society for History, Philosophy, and Social Studies of Biology in a symposium organized by Karola Stotz entitled 'Representing Genes: Testing Competing Philosophical Analyses of the Gene Concept in Contemporary Molecular Biology'. I thank Dr. Stotz, my fellow symposiasts, and the audience for constructive comments. Thanks also to Scott Gilbert for helpful discussions and to Rafi Falk, Joram Piatigorsky, Bob Richardson, and Karola Stotz for helpful comments on a late draft.

2001; Portin 2002; Snyder and Gerstein 2003; Waters 2000). A good number of authorities hold that no exact molecular definition of the gene or molecular criteria for delimiting genes can serve the needs of molecular biology in general, let alone the various disciplines with which molecular biology is allied.[2] I share this view because I am committed to the idea that the criteria used to identify or delimit genes usually combine structural features of the genetic material with phenotypic or functional effects of those materials across cellular or organismal generations (The genetic material has an impact on what is inherited, and genes cannot be properly understood independently of this impact). This view has the consequence that the structure of the relevant nucleic acids alone is not sufficient for specifying or delimiting genes, a position that, I think, is now fairly widely held.[3] If this view is correct, scientists are often clear about what they mean when, in context, they talk about particular genes and they have fairly intuitive and natural ways of communicating across disciplinary barriers without confusion. But it is nonetheless not possible to specify *the* structure of genes in terms of nucleic acid sequence alone. Furthermore, attention to the phenotypes and functions in terms of which genes are delimited helps to explain some of the ways in which scientists and popularizers fall into traps when they conflate, for example, evolutionary gene concepts with traditional molecular gene concepts referring to segments of DNA that, supposedly at least, contain the information specifying the amino acid sequence of a polypeptide as produced on the ribosomes.

If delimitation of genes depends on identifying phenotypes or functions of interest, then it is necessary to achieve a clear understanding of how phenotypes and the relevant biological contexts are delimited before one can hope to work out a determinate account of gene structure. Phenotype delimitation depends, at least in part, on the problems, traits, or functions of interest to different scientists and different disciplines and on the available background knowledge. Furthermore, if genotypes and phenotypes are to be brought into

[2] 'Today the gene is not *the* material unit or *the* instrumental unit of inheritance, but rather *a* unit, *a* segment that corresponds to *a* unit-function as defined by the individual experimentalist's needs' (Falk 1986, 169). See also Rheinberger (2000).

[3] Lenny Moss's term 'gene D' purports to treat nucleic acid sequences independent of their functions. If gene D is intended to refer to any arbitrary nucleotide sequence, fair enough. But if it really is meant to pick out a nucleotide sequence that is, in some sense, available as a developmental resource (which seems to be how he uses the term at least some of the time), then I think that there are some issues to work out about whether even Moss's use of the concept of a gene D is completely free of commitment to functional criteria. For more on this topic see the appendix to Burian (2005 b).

relation with each other, phenotype delimitation also turns on the specifics of the biology involved – and those specifics are typically extremely complex. In this paper, using only examples from eukaryotes, I deal mainly with a subset of molecular phenotypes connected to synthesis of polypeptide chains and the roles those chains play in different contexts (including genes for transcription factors). This leaves aside major cases of interest, such as genes for ribosomal and transfer RNAs, many genes for regulatory controls of gene expression (though not genes for transcription factors), and many genes delimited in work on development, evolution, or medical genetics. There are plenty of biological complexities in the restricted group of cases of interest here, including, for example, alternative splicing of sequence-identical RNAs different physiological circumstances in the nuclei of cells of different cell types, and trans-splicing of materials from different 'raw' transcripts, yielding one mature mRNA.[4] These phenomena, and many more (which add endless complexity to the full story), are described in great detail in up-to-date textbooks of molecular biology or genetics. Yet more complexity arises from additional processing that occurs after an mRNA has been exported from the nucleus. This includes post-translational splitting of polypeptide chains, splicing of polypeptide chains from different sources, and alteration of the conformation and composition of chains. Yet further, chaperoning and the blocking of chaperoning (Rutherford and Lindquist 1998) affect the conformation of polypeptide chains, enable such chains to perform different functions in different physiological circumstances (Li and Lindquist 2000), and mark some cells containing chains with 'aberrant' conformation for destruction. But we have enough on our plate without considering these additional complexities in any detail.

Like many others, I have argued (Burian 1985, 1993a, 1993b, 1995, 1997, 2000) that biochemists, evolutionists, developmental biologists, molecular biologists of various stripes, pharmacogeneticists, regulatory geneticists, etc. deploy different means of delimiting genes that often do not map well on one another. One reason for this is that the scientists in question study different phenotypes and functions by different experimental modalities (see also Falk 2000; Rheinberger 2000). And, again like many others (e.g., Fogle 2000; Griffiths and

[4] For a technical description of some of the many types of alternative splicing found in the human genome and some of the tools used to study alternative splicing, see Croft, Schandorff *et al.* (2000). A related website, http://isis.bit.uq.edu.au/a_splicers.html, supplies a fuller account of the many variant classes of splicing.

Neumann-Held 1999; Moss 2003; Neumann-Held 2001; Portin 2002), I have argued that the complex and tangled pathways by means of which polypeptide chains are manufactured already make a clear general structural definition of genes impossible.

Complexities on the path from DNA to Polypeptide Chains

In this section I explore a new line of argument to support the claim that a clear general structural definition of genes in terms of nucleotide sequences alone cannot handle the complex and tangled pathways by means of which polypeptide chains are manufactured. My starting point is drawn from the epigraph of this paper: 'Genes are not autonomous entities. Rather, they interact with other genes and gene products to make pathways and networks' (Gilbert 2003, 691). This quotation serves as a marker for the clear-cut victory of epigenesis in developmental biology. There are, importantly, different, albeit interrelated, senses of epigenesis here,[5] one rather vague, but belonging to a deep tradition, another explicitly molecular. The traditional meaning may be formulated roughly as follows: epigenetic processes, which are required for development, are not determined by the contents of the fertilized egg alone, but are due to interactions among genes, gene products, and contingent cellular and environmental features that affect the determination, differentiation, or formation of cells, tissues, and organs, or specify the identities of cells or of major features (e.g., secondary sexual characters) of organisms.[6] Very often such changes (for example, changes in brain development or secondary sexual characters) are not reversible, but are highly canalized in Waddington's sense.[7]

In one of the recently-elaborated molecular senses of the term, epigenetic changes are changes of DNA or DNA packaging (such as methylation of DNA and alteration of histone or chromatin structure) that are stabilized in cells or cell lineages and that systematically affect

[5] For a more detailed and refined account of four senses of 'epigenesis' and related terms, see Müller and Olsson (2003). For a volume that presents a major review of historical and contemporary issues about epigenesis, see Van Speybroeck, Van de Vijver, and de Waele (2002).

[6] Wilhelm Roux (1885, 427) wrote that 'If…development occurs essentially by interaction between many or all parts [of the fertilized egg], the fertilized egg needs to consist of only a few different parts, which by mutual interactions gradually generate a great complexity. Development in this case essentially is *production of complexity, epigenesis* in our sense' (as translated in Sander 1991, 3). Granting that the interactions of the parts depend also on environmental inputs and conditions, this is a useful statement, revealing the roots of the molecular meanings of *epigenesis* in the traditional concept.

[7] This usage conforms to Waddington's introduction of the term 'epigenetics' in Waddington (1940). It traces back, of course, to the much older embryological notion that new structures in an embryo develop from an originally undifferentiated mass of living matter.

gene expression *without any change in nucleotide sequence*. Such changes are, in principle reversible, although they often have irreversible effects on the development of a particular organism or lineage of organisms.[8] Molecular epigenesis seems firmly established at this point. Even for the clearest examples of molecular genes such as those traditionally thought to specify polypeptide sequence, epigenetic change ensures that nucleotide sequence alone is not, in general, sufficient to predict whether a polypeptide product will be produced or, if it is, what the resulting sequence of amino acids will be.

The complexities already listed, such as alternative splicing, systematic silencing of DNA by methylation and various modifications of histones, have thoroughly disrupted the notion that the DNA encodes information or contains a program that can be read out in any simple way. A cellular context is required for DNA to function, and different cellular contexts extract different information from the same DNA sequence. Furthermore, the pathways and networks into which nucleic acids and their products enter are multi-leveled and are replete with feedback loops that cross multiple levels. Yet further, the physiological and nutritional states of cells, exogenous signals from the extracellular matrix, other cells, or the external environment (such as heatshock[9] or endocrine disruption[10]) alter the networks and can have stable molecularly epigenetic effects with dramatic lifelong consequences for the organism's morphology or physiology (elaborated in one direction by Newman and Müller 2000). Signal transduction modules work as packages, but what they do – what gets transduced to what by a given signal transduction module – is affected by evanescent signals, physiological states, chromatin packaging, timing, temperature, integration of the signal into a variety of larger modules, and much else. Thus the networks have components of strikingly different sorts, and their behavior is affected by intra-cellular, extra-cellular, and external environmental conditions. Nonetheless, *in context*, they determine when a particular segment of

[8] This articulation draws on Reik and Dean (2002) as well as Müller and Olsson (2003). See Jablonka and Lamb (1995) for an important early review of molecular epigenesis and Newman and Müller (2000) for an important article, which helped me think through this paper, that sets the narrow sense of epigenesis into a wider conceptual context and into a discussion of morphogenesis, character origination, and evolutionary change. Note the connection between the importance of timing of epigenetic change and Waddington's well-known concept of canalization: at appropriate stages, a small change, however triggered, may send an organism down a different development channel or pathway than would otherwise have been expected. As is argued in Newman and Müller (2000), epigenetic changes of this sort may be an important means of achieving developmental and evolutionary innovations.

[9] See, for example, Rutherford and Lindquist (1998); Wagner, Chiu, and Hansen (1999).

[10] For a classic exposition of permanent organismal effects of transient exposure to particular endocrine disruptors, see Vom Saal, Cooke *et al.* (1998).

DNA is transcribed, where the raw transcript of that segment begins and ends, how it is spliced – in short, what is made of it even at the level of nuclear RNA. To repeat a key point: although most network components are found within the cell, some are external to the cell (for example, hormones and other active compounds circulated within the body) and others come from outside the organism (for example, ambient temperature, the circadian light cycle, and exogenous endocrine disruptors taken in by the mother). And thanks to the importance of timing, large developmental effects may result from epigenetic changes such as those due to seasonal changes in timing of ecological events or some form of external stimulus or input.

All of this fulfills (in then-unimaginable ways) an old pre-Mendelian vision that haunted the dialectic between genetics and embryology for much of the last century.[11] Here, for instance, is an articulation by Hans Driesch in 1894:

> Insofar as it carries a nucleus, every cell, during ontogenesis, carries the totality of all primordia; insofar as it contains a specific cytoplasmic cell body, it is specifically enabled by this to respond to specific effects only... When nuclear material is activated, then, under its guidance, the cytoplasm of the cell that had first influenced the nucleus is in turn itself changed, and thus the basis is established for a new elementary process, which itself is not only a result but also a cause (Driesch 1894, my translation).

Molecular Epigenesis and Molecular Pleiotropy

I now explore a more radical consequence of the way in which this old vision has been filled in, drawing specifically on what we have learned about the difficulty of getting from DNA sequence to amino acid sequence in eukaryotes. I begin with what seems to be a simple terminological point. As we will see, it clears away a crucial expository difficulty. The use of databases containing nucleotide sequences is well established. As part of this process, a particular use of gene concepts is codified on the basis of which one can identify various genes and count the number of genes in a given genome. This usage is important and legitimate, but, as I will argue, it employs an impoverished gene concept that cannot serve many of the purposes that gene concepts are supposed to serve. One symptom of the impoverishment of the sequence-based notion of a gene underlying these sorts of gene counts

[11] For references and an elaboration of this point, see Burian (2005a).

is that there are a good number of instances in the literature of scientists who agree that they are talking about the same gene in this sequence-based sense and disagree about whether the gene in question ('really') is a gene or a pseudo-gene. I argue below that we need to work with a plurality of gene concepts and that many legitimate gene concepts would recognize multiple genes within the particular genes picked out by use of the databases. Accordingly, I shall speak of genes as identified by sequence data alone as 'nominal genes'. A good way of parsing the conclusion of my argument is that nominal genes are a useful device for ensuring that our discourse is anchored in nucleotide sequences, but that nominal genes do not, and probably can not, pick out all, only, or exactly the genes that are intended in many other parts of genetic work.

The argument rests on the recognition that there is a trade-off at the molecular level between the criteria for identifying genes in various contexts and the extent to which genes are considered pleiotropic. (The restriction to the molecular level means that to count as pleiotropic a given gene must make more than one molecular product – or, to use a more slippery phrase, make products with more than one molecular phenotype – in different contexts.) My account of how genes ought to be identified is controversial and deserves wider examination than is feasible here. At root, the problem is this: when a nucleotide sequence is considered as a gene, i.e., as a functional entity, its identity *as a gene* is sufficiently sensitive to cellular-context, network embedment, and delimitation of functions that, in typical cases, we should think of the identity of the gene as context-dependent. For clarity I will use two moderately familiar examples to sharpen the point, focusing on molecular pleiotropy in order to illustrate what I mean by multiple contexts.

Take a standard and fairly typical nominal gene in vertebrates, say, the rat α-tropomyosin gene (see Figure 1).[12] The DNA contained in this gene, however its exact boundaries are drawn, includes nine exons and has three distinct terminal repeating units. The RNA transcripts of this nominal gene are known to be spliced in at least seven distinct ways, regulated by various promotors and enhancers that respond to signals found in different physiological, tissue, and cellular contexts. Here is a brief synopsis of the alternative splicing involved.

[12] This synopsis derives from Figure 5.28 of Gilbert (2003), which is the source of Figure 1; the basic information stems from Breitbart, Andreadis, and Nadal-Ginard (1987).

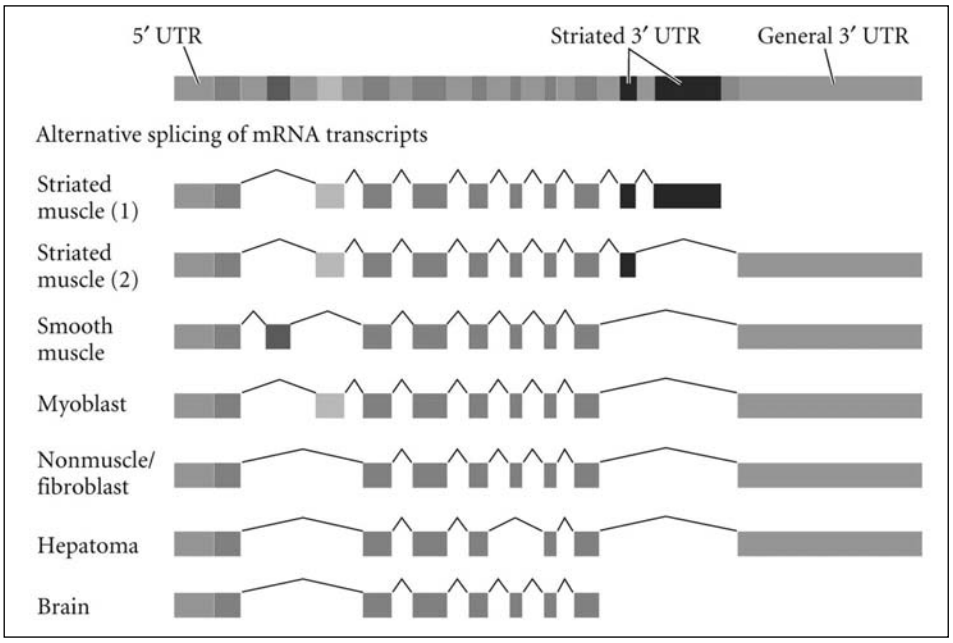

FIGURE 1. Alternative RNA splicing to form a family of rat α-tropomyosin proteins. The DNA sequence is represented at the top. Thin lines represent sequences that become introns and are spliced out in forming mature mRNA. Redrawn from Gilbert (2000, fig. 5.28), after Breitbart, Andreadis, and Nadal-Ginard (1987).

Most of the exons are included in all seven of the common variants of the mRNA produced from this gene. However, the second exon is included in only one of those mRNAs, an mRNA whose protein product is a component of smooth muscle tissue. The third exon is included in only three of those mRNAs (all distinct from the one that includes the second exon). Two of these mRNAs encode products normally occurring in striated muscle and the third in myoblasts. The mRNAs whose products occur in striated muscle have distinct terminal repeating sections, unique to striated muscle. With one exception, all of the other mRNAs use only the so-called general terminal repeating section. The mRNA expressed in myoblasts has the same exons as the striated muscle mRNA, but neither of the terminal repeats distinctive of mRNAs expressed in striated muscle cells. The remaining three common mRNAs include neither the second nor the third exon. The mRNA that yields a product standardly found in nonmuscle fibroblasts is identical to the mRNA found in myoblasts and in smooth muscle except that it includes neither the second nor the third exon. The remaining two mRNAs are produced in hepatomas

(i.e., a particular kind of liver cancer) and brain cells respectively. The hepatoma mRNA is like non-muscle fibroblast mRNA except that it deletes the seventh exon (and nothing else) and the mRNA expressed in brain cells is like non-muscle fibroblast mRNA except that it deletes only the terminal repeat unit.

Thus, of the nine exons, 1, 4, 5, 6, 8, and 9 are expressed in all of the α-tropomyosin proteins, 7 is expressed in all but the hepatoma protein, and the terminal repeats (which do not ultimately appear in protein) are part of what distinguishes how the products are processed and used in the cells in which they occur. At least four of the proteins produced from these mRNAs have distinct amino acid sequences. If genes are identified by the polypeptide sequences they produce, 'the' α-tropomyosin gene should count as at least four genes, producing four products in the same family of proteins. To count it as a single gene is to count it as molecularly pleiotropic, i.e., as yielding distinct molecules in distinct locations. This is not a trivially semantic point; the system is tightly segulated in such a way that the potential impact of mutations in particular exons differs from exon to exon. The differences in the range of cell types in which different exons are expressed places limits on the range of tissues initially affected by a mutation and on the specific direct effects of different variants of the gene product. Indeed, since the second exon is expressed only in smooth muscle cells and the third exon is expressed only in striated muscle cells and myoblasts, mutations in one of these exons should have medically quite distinct classes of effects from mutations in the other. This *might*, in practice, prove to be important for understanding differences between, and potential treatments for, different muscle-wasting diseases and thus is a potentially serious ground for handling the matter in terms of distinct genes. Parallel claims seem justified in most cases in which highly regulated alternative splicing in different cellular or tissue contexts causes nominal genes to produce distinct proteins. The number of cases of this sort is quite large: a standard textbook claim is that approximately 40% of human (nominal) genes are alternatively spliced in different contexts!

The fact that the same raw RNA transcript yields different molecules in different cellular contexts is the result of specific (molecular) regulatory controls that greatly alter the probability of alternative ways of splicing the RNA transcript from one context to another. Almost always, the resultant mRNA is, therefore, context-specific. The result is a form of molecular epigenesis: the different molecular environments encountered by the nominal gene alter the ways in which the DNA sequence is processed (or block it from being

processed), thus producing a product with a different amino acid sequence (or no product). If we knew enough of the contextual details, we could predict the probability of one vs. another of the amino acid sequences being produced. But we could not do so solely from knowledge of the nominal gene; we would need to know the details of the molecular environment. We could also make such predictions from cruder information, not about the molecules, but about the cellular context. If a cell's fate has already been determined, if it has been determined to be, say, a myoblast precursor, by far the most likely α-tropomyosin family member to be generated is the one that includes introns 1, 3, 4, 5, 6, 7, 8, and 9, and it will hook up with other proteins in a way that is typical for myoblasts and not for striated muscle. Thus if we wish to take amino acid sequence or protein product as a molecular phenotype, the nominal gene from which it is produced is, in general, not sufficient to predict the phenotype. *Molecular epigenesis is thus responsible for the molecular pleiotropy of the nominal gene and for the insufficiency of complete DNA sequence information for predicting which product(s) of that gene will be produced in which contexts.*

Narrow Cases of Molecular Pleiotropy

A second class of examples strengthens the idea that it is sensible to think in terms of trade-offs between molecular pleiotropy and criteria for identifying genes. It illustrates how powerfully cellular context determines the function of a polypeptide sequence and the highly distinctive uses to which a single polypeptide product can be put. The issue involves what Joram Piatigorsky (one of the leading experimentalists studying lens crystallins) and his colleagues call 'gene sharing' (reviewed in Piatigorsky 1998; Piatigorsky 2003).[13] I don't yet know enough about the actual biology (which is quite difficult) to answer some obvious questions, but I hope that the importance of the questions *will* be obvious. Gene sharing occurs when 'two distinct protein phenotypes are produced by the same transcriptional unit' (Piatigorsky and Wistow 1991, 1078). In nearly all vertebrates, and many invertebrates, lens crystallins are composed largely of proteins that belong to one or another family of proteins (in the sense in which

[13] The phenomenon of gene sharing, also known as 'moonlighting proteins' turns out to be quite widespread, with many cases now known that do not involve lens crystallins. For a recent review, which I discovered while writing the penultimate draft of this article, see Jeffery (2003).

the α-tropomyosins are a family of proteins). Typically (at least in vertebrates), the lens is composed of several lens crystallins, some of them produced at distinct developmental stages. In invertebrates the proteins belong mainly to families of metabolic enzymes; in vertebrates many crystallins belong to small heat shock protein families and the rest mainly to such metabolic enzyme families as lactate dehydrogenase or cytoplasmic aldehyde dehydrogenase, enzymes produced in the liver. In some species, two or more nominal genes, evolutionary duplicates belonging to the same gene family, are involved. But in many cases organisms of a given species have only one copy of a gene from the family in question. In such cases, there are normally no amino acid sequence differences between the enzyme produced from the gene (e.g., in the liver) and the corresponding lens crystallin in the eye.

In the most extreme cases of gene sharing, the amino acid sequences of two proteins are identical, but the proteins are distinct. This is what I mean by narrow molecular pleiotropy: two distinct proteins (e.g., an enzyme and a lens crystalline) with identical amino acid sequences are derived from one nominal gene. In vertebrates, a significant percent (10%-50%) of the lens crystallins are commonly produced by this kind of gene sharing (Piatigorsky 2003; Piatigorsky and Wistow 1991). The enzymes that share genes with a lens crystallin have been found to be quite diverse; gene sharing of this sort occurs in a wide variety of protein families. Many instances of this sort involve taxon-specific gene sharing, which is to say that in each taxon a distinctive enzyme and a lens crystallin share a gene. The same phenomenon is found in invertebrates, although it is not yet as well studied.[14]

Gene sharing is helpful for the present argument because it provides clear illustrations of a way in which identification of genes is, in practice, dependent upon the functions or phenotypes considered and the specific criteria employed for identifying genes. Assume (as is almost certain) that the conformations of, say, lactate dehydrogenase

[14] Jeffery makes a point about gene sharing parallel to one I made earlier about α-tropomyosin. When a specific polypeptide chain has multiple functions in distinct locations, 'knowing one function of a protein, for example its enzymatic activity, might not fully describe the function of a protein in the cell or organism'. She goes on to add that this impacts rational drug design because 'correcting only one function of a multifunctional protein might not be sufficient to effectively treat a disease' (Jeffery 2003, 33). I add that one could allocate the two functions to one gene or allocate the distinct functions (e.g. for drug discovery purposes) to 'the gene for protein$_1$' and 'the gene for protein$_2$', both contained in the same stretch of DNA and yielding the same polypeptide sequence serving distinct functions in different locations. Which strategy is appropriate might well depend on the task and tools at hand or on the phenotypes under consideration. The differences between a change in protein conformation or function and a change in amino acid sequence is obviously important here, but its relevance to the 'protein phenotype' (Piatigorsky and Wistow's term) and to the delimitation of genes may not be as straightforward as it at first appears.

and the corresponding lens crystallin produced by the same nominal gene are different.[15] The proteins in question are, thus, easily distinguished in spite of having the same amino acid sequence. Furthermore, before the 1950s, even if it had been shown that the amino acid formulae of the enzyme and crystalline were identical, they would have counted as distinct proteins. For it was only in the 1950s (with the work of Sanger and others) that amino acid *sequence* was recognized as a key to identifying proteins. Well into the 1950s (until the work of Sanger and others on amino acid sequences was widely appreciated) the orthodox view was that genes (or cytoplasmic derivatives of genes) serve as templates and that a single substrate could yield several distinct proteins by this template mechanism. Thus, at the time, it was expected that distinct proteins would have the same biochemical formula; such a finding in a particular case would not have led geneticists or biochemists to suppose that the same gene produced the two proteins. If, by some lucky chance, there were appropriate mutations available so that a 1:1 correlation between a mutation in the enzyme and the lens crystallin were observed, that would raise the question whether, surprisingly, the same gene made (or controlled) both proteins and/or whether the proteins were distinct. The chromosomal theory of the gene, but not the pre-chromosomal theories of Bateson or Johannsen (see Burian 2000), would provide a clear-cut test to help resolve this question – the linkage test. If it the two mutations could not be separated in linkage tests, the chromosomal theory would indicate, at least provisionally, that just one gene was involved. But it is quite difficult (using classical techniques) to carry out linkage experiments sufficiently powerful to distinguish very closely linked genes, as is illustrated by the classical example of Sturtevant's inability to separate *eosin* and *white* in Drosophila in an experiment using 150,000 flies (Burian 1985, 32-33; Carlson 1996, 64 and chap. 8).

For this reason, Mendelian genetics would have faced conflicting criteria for delimiting the genes in question in cases of gene sharing. The existence of two distinct proteins in different locations would suggest (insofar as it was thought that genes somehow determine protein structure) that there were two different genes, one producing (for example) a particular lens crystallin, the other lactate dehydrogenase. On the other hand, if the materials were available to carry out a linkage test,

[15] As I understand it, lens crystallins must be very nearly linear and positioned orthogonally to the nearest surface of the lens if light is to be transmitted through the lens to the retina. In order to facilitate the specific reactions that they do, enzymes require specifically shaped pockets. They thus almost certainly have a different conformation than the sequence-similar lens crystallins.

that test would have suggested that a single gene at a single chromosomal location determined the two proteins. Bringing things up to date, one might argue that in cases in which the exact same transcript is produced from a single nominal gene, molecular genetics trumps Mendelian genetics. The identity of the polypeptide sequence, built by standard processes from the same DNA but utilized differently in different cellular contexts, provides a powerful argument for saying that we have one gene, with one product, deployed differently in different contexts. I would argue, to the contrary, that whether we count this as one gene or two can still be couched in terms of how we divide up phenotypes – by amino acid sequence or by functional protein. And it is also probable (although I do not know whether a clear example has been found) that there are instances in which two distinct nominal genes from the same gene family produce sequence-identical polypeptides, for in many species gene duplication and corresponding specialization of function has occurred.

Furthermore, even when only one nominal gene is involved, it is not always clear whether all of the polypeptide sequences of the 'different' products of a given gene have the identical amino acid sequence. It will be very difficult to rule out the possibility that different polypeptide products might result from alternative splicing or other differences in processing. A *prima facie* candidate of this sort is an interesting lens crystallin studied by Piatigorsky's group (Piatigorsky, Norman *et al.* 2001). This is the J3-crystallin of the eye of a particular species of jellyfish (*Tripedalia cystophora*), which appears to be produced from a unique nominal gene (with seven exons) in this species. The gene belongs to an evolutionarily widespread and diverse family of genes that produce proteins called saposins. In *T. cystophora*, the J3-crystallin contains amino acids corresponding only to exons 2-4 of the gene and is thought to be produced by cleaving the amino acids corresponding to these three exons from a longer polypeptide in the eye. The gene is also expressed elsewhere in the jellyfish, e.g., in the tentacles. The literature I have found so far does not resolve the question whether the same three exons (only) are expressed in the tentacles or where else these or other exons from this gene are expressed.[16]

The general point, however, is established, whatever the outcome of

[16] In response to a draft version of this paper, Joram Piatigorsky kindly informed me (pers. commun.) that his laboratory has not yet been able to firmly establish the structure of the J3 crystallin mRNA, whether the crystallin is in fact produced (as is anticipated) by cleavage of a larger polypeptide, or whether the product of the nominal gene is different in the different locations in which the gene is expressed. An additional question that arises in this case is whether some other tag, such as a different leader or terminal sequence, is employed as a marker that determines the way in which the transcript or the subsequently-produced polypeptide chain is processed.

this case. There are many subtle ways in which 'the same' gene yields subtly different molecules in different contexts. The cases of pleiotropy, as Karola Stotz pointed out to me, form a rough gradient of increasingly dramatic pleiotropy. Gene sharing is the mildest form of pleiotropy, at least when the distinct products share a common amino acid sequence. Pleiotropy is more clearly established where there are distinct products with different amino acid sequences. Other cases, not discussed here, are yet more dramatic. Among the examples are instances of overlapping genes, including instances in which a single transcription unit yields very different polypeptide chains because of RNA editing or ribosomal frameshifting (Alberts *et al.*, 2002, 438). Epigenetic factors of very diverse sorts act at every level, even at very narrow molecular levels.

For present purposes, important as it is to understand the different mechanisms of producing distinct proteins, the precise basis for the differences in protein phenotypes produced from a specific RNA transcript does not matter much. Even where we have a clear account of the causes of the protein phenotype differences in particular cases, the problem of molecular pleiotropy remains important. The issue as to whether to count the proteins in question as the products of one pleiotropic gene or of two distinct genes located in the same initial segment of DNA depends on how we classify the molecular phenotypes and what weight we give to the common source of the nucleotide sequences that yield systematically different products in different contexts. This claim does not depend on whether or not there is a difference in the amino acid sequences of the 'different' proteins in the different locations, nor on how the systematic differences in different contexts were brought about.

Semantic issues like this will often arise where there is significant molecular pleiotropy. This is true even in the rather standard case of rat α-tropomyosins. It is an open question whether the seven α-tropomyosins, each produced in specific tissues, are best considered to be produced by one gene or by distinct genes. For some purposes it may be simpler or more sensible to delimit these genes one way, and for other purposes to delimit them in other ways. If this 'easy' case is unpersuasive because the terminology of 'the' α-tropomyosin gene is deeply entrenched, there are plenty of hard cases in which no easy resolution is feasible. The possibility of divergent ways of delimiting genes arises whenever (as is common!) a nominal gene produces molecularly distinct products that perform different functions.

For the sake of clarity, perhaps I should add that I am not yet

arguing about downstream effects, at least not directly. Changes in striated muscle α-tropomyosin can have effects in virtually every part of the body. Thus any mutation that affects the functioning of this protein will have (conventional) pleiotropic effects. Rather, my case is built on *molecular* pleiotropy, the production of distinct molecular products based on the nucleotide sequences found within a putatively single gene (or distinct protein conformations of a particular polypeptide sequence built by readout from the same sequence of nucleotides). The difficulty arises *because the concept of a gene is at least partly delimited in terms of function*. The need to parse the functions of concern opens up the need to introduce conceptual alternatives at just this juncture.

These considerations feed back onto an evaluation of the status of nominal genes. Note that the actual procedures for counting genes covertly take structures into account *because they have known effects connected to gene expression*. We cannot treat *any* particular nucleotide sequence as a gene. Can there be any genes that are one nucleotide long? Three nucleotides long? That consist of a group of 300 repeats near the middle of a highly repetitive short sequence? Criteria that are strictly intrinsic to the DNA, i.e., that are based on structural features of nucleotide sequences alone and do not take into account (perhaps tacitly!) how those features of DNA affect the organism in various contexts offer virtually no prospect of providing widely usable criteria for delimiting genes. For example, requiring that a gene begin with a specific sort of sequence tag, e.g., an open reading frame ('ORF'), does not work. Not only is this structural feature not present in all nominal genes,[17] but ORFs are tacitly chosen as a significant structural feature because they have significant impact on the probability that transcription might be initiated near them in appropriate intracellular molecular contexts. Indeed, while there is no single correct way to delimit genes, the gene concept will lose all value if there are no principles by means of which to answer when to count two stretches of DNA as belonging to different genes and when to count them as belonging to no gene at all. In general, those principles depend (often tacitly) on the phenotypes and functions under study.

[17] There are many instances known in which multiple nominal genes are expressed in one initial raw transcript, starting from one ORF. In many cases the raw transcript is separated into three (or more) separate transcripts or polypeptide chains at some later stage of the processes of transcription, editing, translation, and post-translational processing. The division into three separate genes in such cases is based on knowledge not of the structural features of the DNA downstream from the ORF, but on knowledge of subsequent events and, often, of the existence of the corresponding genetic material in other organisms in separate nominal genes with separate ORFs.

The nucleotide sequences of nominal genes sometimes match closely and sometimes match poorly with those that are relevant to the phenotypes in question. For practical purposes, the boundaries of the nucleotide sequences relevant to a given function must often be revised or contextualized after considering the contextually relevant molecular and supra-molecular matters that determine when and where which portions of the nucleotide sequence become causally relevant to the phenotypes of concern. For reasons like these, the semantic issue as to whether to count a stretch of DNA as containing multiple genes or containing one gene with highly pleiotropic effects will not be easily resolved. Indeed, no *general* or *all-purpose* resolution to this issue is available. The issue is best treated as a pragmatic one, to be answered for the convenience of the parties who are interested in it and need to communicate about it *according to what particular phenotypes or functions are of focal interest in their discussion, their experiments, or their disciplines*.

Conclusion

This brief examination of two illustrative examples (rat α-tropomyosin and the various enzyme and heat shock protein genes that produce lens crystallins by gene sharing) establishes that molecular pleiotropy raises serious problems for gene delimitation. In these examples, what looks like pleiotropy can often be equally well interpreted as activation of distinct genes even though those genes happen to pass through a stage in which they yield sequence-identical raw transcripts. There are, after all, a good number of other cases of overlapping genes at a single locus, such as the viral genes that can be read out properly from frameshifted starting points and the cases in which the introns for one gene contain exons for another gene or material that is converted into small nuclear RNAs and other distinctive functional products. In our cases, the molecular networks that control gene expression have triggered the transcription of the DNA from which, in specifically different cellular circumstances, distinctly different proteins are eventually derived. In the α-tropomyosin case, the cellular conditions in question cause the raw transcript produced from the nominal α-tropomyosin gene to yield different polypeptide strings in spite of starting from 'the same' – i.e., sequence-identical – raw transcript. But, for *some* purposes, isn't that to say that the DNA in question in *these* cells functions as one gene, and in *those* cells as another? And should – or shouldn't – the same claim apply to the nominal gene that produces a lens crystallin in the

eye and lactate dehydrogenase in the liver? If the phenotype of concern is the amino acid sequence, it shouldn't, at least not unless it is produced by starting from two numerically distinct nominal genes. But if the phenotype is the protein, the claim that the genes are distinct is equally supportable.

The stability of the distinct contexts – myoblast vs. smooth muscle cells, liver vs. lenticular cells, etc. – is exactly the sort of stability that is needed to determine what will be produced from a given RNA transcript, and hence from the DNA sequence that, *in that context*, produces *that* raw transcript.[18] And the stability of context arises because of the stability of *epigenetic* developmental processes. This, then, constitutes an argument that the concept of a gene that specifies a polypeptide sequence is incomplete without specification of the cellular context (or the relevant features of the cellular context). Thus, it is not sufficient to list the relevant nucleotide sequence of the DNA that is mirrored in the mRNA that is actually translated into an amino acid sequence or to list the nucleotide sequence containing all of the relevant exons and associated introns. We are forced to admit that one nominal gene yelds distinct products because of the impact of non-sequence based information or causes. We are also forced to admit that, in general, we cannot asses what polypeptide chain or protein product will be produced from nucleotide sequence alone. Thus, even such limited phenotypes as polypeptide sequence or protein product cannot be specified solely by information about nucleotide sequences. This means that insofar as we wish to develop analyses of genes as units that determine amino acid sequences or protein formation, we need to interpret genes with greater latitude than is provided by the nominal genes from which gene counts are obtained. Putting the point more generally, no system of specifying or delimiting genes by reference only to nucleotide sequences (with no further information about context) can satisfactorily specify the gene(s) that yield a particular protein.

Perhaps this may be viewed as old news. I think it is not. By getting this close to molecular detail, we have seen why a DNA nucleotide sequence does not genuinely specify the molecules that are or can be produced from that DNA. These considerations help explain why, even at the early step of specifying proteins, the correlation between genotype and phenotype cannot be nailed down neatly and, *a forteriori*, why the genome does not specify the organism. They explain

[18] Note also that a given nominal gene often produces primary transcripts of different lengths in different physiological circumstances.

why and how there is a trade-off between molecular pleiotropy and gene identity and why identification of the phenotypes of concern is critical for identification of genes. But once phenotypes are specified in distinctive ways, we are required to recognize a plurality of gene concepts, even when the phenotypic distinctions of concern are as limited as the distinction between the amino acid sequence produced and the protein produced. Nucleotide sequence similarity plays an enormous practical role in identifying the various distinct embodiments of particular genes, such as 'the' α-tropomyosin gene (or genes) in various organisms. But without enormously deep and interesting evolutionary, functional, and phenotypic stories backing up our choices, the sequence similarities do not suffice to reidentify these distinct sequences as being instances of the same gene. Furthermore, we must recognize that even amino acid sequences of polypeptide products are sometimes misleading. Consider, for example, the cases in which multiple proteins, separated after translation, are produced from one transcript derived from one ORF, but are produced in related organisms from separate transcripts that start at different ORFs i.e., that belong to distinct (nominal) genes. The point, of course, is not to insist that there is one correct answer as to how to determine the number of genes involved in such cases. Rather, given the state of present knowledge and present means of individuating genes, there is no single correct answer about the number of genes involved. This is no great hindrance to clear communication. We simply have to be clear how we are building the unavoidable context-dependence into our gene concept.

Covertly or overtly, but unavoidably, the principles on which genes are identified and individuated are context dependent. To recur to the epigraph from Scott Gilbert, the sense in which genes are not autonomous entities is very strong indeed. Exactly what gene we are dealing with depends on the context and networks involved and how we take account of them. And those contexts and networks extend beyond the organism into the external environment. Given this, the muddiness of gene concepts, frustrating to many philosophers, but celebrated by a few, is inevitable. If this strong version of context dependence is correct, the continuing evolution of gene terminology is not going to stabilize at some new orthodoxy based on a strictly intrinsic characterization of genes. At the same time, this contextualist stance highlights the usefulness of gene concepts and the possibility of retaining reasonably good control of cross-disciplinary and cross-contextual use of gene terminology. As a bonus, it undermines the genetic determinism still found in the rhetoric of the human genome

program and still deployed by many geneticists. I hope, therefore, that I have taken a small step toward convincing readers who were puzzled by ongoing controversies over gene concepts that those concepts can be fairly readily understood once one recognizes their strong context dependence. Such understanding will help us straighten out the most serious difficulties that arise when people talk about genes across contexts. Handled with care, these difficulties, too, can be overcome.

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Commentary on Stotz and Griffiths, Burian, and Waters: Genes, Concepts, DST Implications, and the Possibility of Prototypes

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1. Introduction

As Stotz and Griffiths write at the beginning of their paper in this special issue, 'this is a particularly exciting time to be studying molecular bioscience because of the extraordinary rate of change in basic concepts' (Stotz 2004). Other papers in this issue offer representative examples of this ferment in our basic concepts, with a primary focus on the concept of the gene. The biological, and biomedical, sciences have clearly entered a period of 'rational exuberance' in the ways that variant fundamental ideas in genetics are developed and deployed.[1] As I see it, there are three engines driving this exuberant diversification of concepts: (1) attempts to link various levels of aggregation, observation, experimentation, and theorizing, (2) the extraordinary financial support and investigator efforts that molecular biomedical science has witnessed in the past 50 years since Watson and Crick's discovery of the double helical structure of DNA in 1953, and, (3) the stunning array of powerful new experimental techniques and instruments developed in this time period.[2]

In the mid-1950s, shortly after Watson and Crick's understanding of the gene as related to a DNA sequence appeared, Seymour Benzer found he had to reconceptualize the classical notion of the gene and

[1] This phrase amalgamates a comment by Hans-Jörg Rheinberger with an earlier one, albeit modified and in a very different context, of US Federal Reserve Chairman Alan Greenspan.
[2] Breaking out this third point explicitly was suggested by Dick Burian.

propose corrected notions in order to keep neoclassical genetics consistent with the emerging molecular genetics (Benzer 1957). Benzer's new notions of muton, recon, and especially cistron, had philosophical implications for classical philosophical models of reduction (Schaffner 1967, 1968), a debate that has continued through various peregrinations and vagaries until the present day (see (Schaffner 2002). But this was not only a philosophical debate that Benzer's early efforts launched, since periodically molecular geneticists themselves felt the tensions between classical notions of their discipline and new experimental findings. Illustrative of this was an editorial in the April 14, 1971 issue of *Nature New Biology*[3] entitled 'A Gene by Any Other Name' (Nature 1971). This editorial began with the following two sentences: 'What is a gene? The answer to this apparently simple question seems to be of increasing uncertainty.' That 1971 editorial then noted emerging problems involving different genetic and biochemical perspectives on the gene, and the implications of recent discoveries in regulatory genetics and the operon model that motivated this initial question. The pace of new discoveries then rapidly quickened, including those of so-called 'junk' DNA, gene-splicing, and more recently extensively pleiotropic DNA findings, reviewed by Falk, Downes, and Burian in this issue. These discoveries have only served to increase our perplexities as to 'What is a gene?'

My comments will begin with a discussion of the paper by Stotz and Griffiths, which introduce us to the two projects that led to the papers comprising this special issue. I then turn to Burian's paper (Burian 2004), and conclude with some observations about Water's alternative approach to studying the concept of the gene (Waters 2004).

2. Empirical Studies of Foundational Concepts

Stotz and Griffiths recount two empirically-based projects of theirs that have sought to understand how biologists utilize their concepts of the gene. In the beginning of their paper, they sketch the background of recent attempts by a number of biologists and philosophers to analyze the changing notions of the gene, primarily looking at the late

[3] *Nature New Biology* was an early 1970s premature attempt by *Nature*'s publishers to spin off additional journals under the *Nature* umbrella, and after a couple of years, it, and its companion *Nature New Physics*, were reabsorbed back into *Nature* proper. The mid-1990s, however, saw another effort at a spin-off of additional *Nature* journals, such as *Nature Genetics*, which have been extraordinary successful.

1990s-early 2000s literature, including the proposals of Falk, Moss, Fogle, and Waters. This, they write, led them to suspect that molecular, developmental, and evolutionary biologists were working with different concepts of the gene – suspicions they believed could be put to an empirical test using questionnaires designed around the three hypotheses they delineate in their paper. The numbers obtained for this project were fairly small (N = 81), but did lead to some statistically significant results that supported some of these hypotheses, particularly the notion that developmental biologists held to a distinctive concept of the gene. Evolutionary biologists' views were more difficult to assess, but indirect answers suggested that this group focuses more on a resultant phenotype of a gene than on the molecular DNA of the gene. Overall, Stotz and Griffiths think that their scientists polled in the first project work with a classical molecular Foglean stereotype or prototype, which does *not* yield determinate answers to whether any *particular sequence* is a gene or more than one gene.

These results were the backdrop for the larger and ongoing 'Representing Genes Project' (hereafter RGP) that is discussed in the remainder of Stotz and Griffiths' paper. Results from the largely completed empirical phase of the project are still in the process of being digested and analyzed, so it would be premature to try to assess it generally. Nonetheless some very interesting instruments have been developed to probe biologists' genetic conceptions in more nuanced ways than in the earlier and simpler project. Two examples of problematic overlapping genes are presented in some detail by Stotz and Griffiths, along with important questions that may indicate how genes are defined and individuated. Another instrument attempts to see whether something like Falk's top-down gene notion and Moss' Gene D and Gene P concepts might be at work in geneticists' assessment of four proposed research projects, each with four different research strategies. (As I understand it, scientists only saw two projects each, though they were provided with all four of the strategies for pursuing the projects.) The first strategy examined splitting the trait to deal with complex effects, and the second suggested searching for endophenotypes, which might give stronger and less confusing genetic signals. The third strategy proposed that a common biochemical pathway focus might account better for the variation that may be observed in affected sub-populations, and, finally, strategy 4 took an epigenetic approach, and compared asymptomatic individuals with known mutations and symptomatic individuals with no known mutations.

These are rich and complex tools with which to attempt to tease apart the conceptual structures that geneticists employ in conducting and analyzing their research. We may see support for top down notions, or Gene D, Gene P, or for some other notions of the gene that are still in the process of emerging. Absent the final analysis, it is difficult to assess this second project, but I want to share two general caveats with the readers of this commentary. These caveats can also serve as a framework within which to comment on the accompanying papers of Burian and Waters.

3. Caveat I: The Revenge of the DST?

Burian's paper in this issue examines issues of molecular pleiotropy and molecular epigenesis. Pleiotropy has been of interest to geneticists for nearly a hundred years, and was defined by Plate in 1910 as multiplicity of phenotypic characteristics dependent on the same 'unit of inheritance' (Plate 1910). But as Pyeritz notes in his excellent historical review of the concept (Pyeritz 1989), its importance has 'waxed and waned' since those early years of the last century, and probably reached its nadir in the 1940s with the advent of Beadle and Tatum's 'one-gene-one enzyme' hypothesis. However, in the midst of discoveries in the 1980s of multifunctional proteins, alternative splicing, and overlapping coding sequences, Pyeritz and other medical geneticists saw new value in the pleiotropic concept as a possible tool to assist in clarifying the basis of medical syndromes and other complexities in medical genetics. Though his paper had a largely historical thrust, at the conclusion of it Pyeritz presented a series of late 1980s molecular explanations of genuine pleiotropy, as well as some mechanisms that would broaden the scope of pleiotropy.

Burian in his paper uses a current understanding of pleiotropy to support his thesis that a simple structural (DNA sequence) characterization of a gene will not suffice to define the gene in any univocal way. As I read Burian, his argument often turns on integrating pleiotropy with a notion of molecular epigenesis, in which cellular (and possibly organism positional) context differentially determines what happens to the product of any given DNA sequence. In perhaps his strongest case, 'two distinct protein phenotypes are produced by the same transcriptional [also translational (?)] unit' (Burian 2004, quoting Piatigorsky and Wistow 1991). The specific example is one amino acid sequence that has invariant primary structure, but which becomes (presumably) two different tertiary

structures with radically different functions when found in two different locales (lens crystallin in the eye and an enzyme, lactate dehydrogenase, in the liver). This, and his other example of α-tropomyosin, are used to argue that the notion of a gene only makes sense if we also know the 'context' in which the gene operates, and that a plurality of gene concepts is needed which reveal 'fundamental difficulties in stabilizing gene concepts solely by reference to nucleotide sequence'.

But as Burian's epigraph from Scott Gilbert indicates, a context refers to networks and pathways and other genes, and presumably gene products such as proteins, which can in turn regulate the genes, both in terms of picking them out and modulating their expressions. This is similar to a vision that Lee Hood's systems group has arrived at through extensive research on yeast using data obtained from DNA microarrays, quantitative proteomics, and databases of known physical interactions. They have shown in the galactose metabolism process in yeast that an account of what genes are doing, as determined by microarray experiments, will give a very partial and misleading picture of yeast cell and other organism's mechanisms, unless protein-protein interactions and protein-DNA interactions are provided. (Ideker *et al.* 2001). Such a vision is strongly similar to developmental systems theory (DST) – to which Griffiths is a major contributor – that argues *against* the primacy and centrality of DNA and genes, and for parity of other molecular entities in accounting for key trajectories in biological processes (see Griffiths' [1998] comments on Schaffner [1998])

Thus, what we seem to have in Burian's paper is a confirmation of a DST-like approach which, if it turns out to be widespread among biologists, and even if implicit, may make the results of the RGP extraordinarily difficult to interpret, as the project moves from this data gathering phase to its analytical phases. This is so because the fundamental unit of analysis in genetics may no longer be the concept of the gene, but the concept of the DNA embedded in a reticulate network (see Fogle 2000). As I will suggest in the following section of these comments, the gene concept, unlike DNA, may not turn out to anything like a 'natural kind'. Thus there may be no univocal simple coherent notion(s) of the gene, and the concept may depend critically on the context and circumstances and interests of the investigators at that time, in that system, and for that inquiry. This, however negative it seems, would *still* be a most interesting and important result of the RGP – whether it was anticipated or unanticipated by the PI.

4. Caveat II: The Problem of Rationally Reconstructing Not-Quite-Natural Kinds – a Need for Identifying Prototypes?

In his article in this issue, Ken Waters argues that the RGP cannot fully succeed in its attempt to determine which of several philosophically-based candidate gene concepts are actually being used by which types of biologists. Partly this is because the questionnaire and interactive poll-based instruments may not be nuanced enough to detect different concepts in use in subtly different contexts. Waters offers a different, but complementary, approach to elucidate the nature of basic scientific concepts such as the gene. Waters has himself put forward a definition of the gene, which in the present paper is only referred to in passing but is more extensively referenced in the paper by Stotz and Griffiths. But to get the best grasp on such fundamental concepts, Waters urges we follow and develop Hans Reichenbach's approach to *rational reconstruction* in science.[4] Waters' account of Reichenbach's stages is clear and useful, but I suspect it may not work without further contextualization that Waters provides elsewhere, but which may not be evident in the present paper (more on Waters' related work in a moment).

Part of my concerns here arise from the fact that Reichenbach's grounding was in physics and not in biology. The strengths of Reichenbach's own efforts at rational reconstructions were all in scientific domains such as relativity theory, including space and time, in quantum mechanics, and also in mathematics (specifically in probability theory). Several of his analyses included axiomatizations of the theories which he was analyzing, e.g., in relativity (Reichenbach 1969).[5] Conceptual analysis along Reichenbachean lines can be a very powerful and scientifically important tool, and it is not only done by philosophers. Pre-Reichenbach, Ernst Mach's operational definition of inertial mass provided the science of mechanics its first clear account of that then hoary notion, and Mach's subsequent similar analyses of temperature in thermodynamics seems to have stimulated Einstein to develop his own conceptual analysis of simultaneity as a preface to his special theory of relativity. And Einstein's conceptual re-analysis has to be counted as one of the most significant in the history of science.

[4] A similar approach to conceptual clarification was pursued by Rudolph Carnap whom Waters does not discuss. See for example the introductory chapter in (Carnap 1950). Carnap also attributes this type of inquiry to both Kant and Husserl.

[5] Even this powerful axiomatization had problems with it – see my review of Reichenbach's analysis in (Schaffner 1970).

(For references to Mach and Einstein, see Schaffner 1974) These are all stunning examples of the value of conceptual analysis.[6]

But in my view, biology (and biology's applications in medicine) is a science of a different kind than is physics and (at least some parts of) chemistry. In some deep sense, biology is a science that does not deal with 'natural kinds', or at least with natural kinds of the same relatively simple type as we find in classical particle physics, and in chemistry in the periodic chart. That a gene in its increasingly reticulate aspects may not be a natural kind has been suggested by Gayon (2000). Hull has also discussed this issue in biology more generally (Hull 1988, 78-86), and a rough but adequate definition for the purposes of the present paper is given by Bechtel of natural kinds as 'sets of objects which figure in scientific laws and have defining conditions' (Bechtel 1988, 57), where defining conditions are a collection of necessary and sufficient statements. Given the paucity of laws and axiomatized theories in biology, we might think that traditional natural kinds might not be found in this discipline.[7]

There is an alternative view of biology that preserves its scientific status, and also permits the use of counterfactuality in causal generalizations within biology. In my view, the fundamental concepts in biology are better captured by prototypes that are usually termed models or mechanisms. Such prototypes permit the existence of exceptions in similar though not identical models, and rely heavily on similarity relations. Each model can be represented as a collection of causal generalizations, but the generalizations typically have a narrow scope of application. Collections of such models, which often contain overlapping more specific models, are biology's surrogate for theories. A well-known example is the operon model for genetic regulation. This prototype view is an issue that I have written about extensively elsewhere (see Schaffner 1980, and also Schaffner 1993a, esp. chapter 3) and a commentary is not the place to summarize it.

But I think it is appropriate to suggest that fundamental concepts in such a science with a prototype structure may not have the precision for which the current RGP nor Waters' approach seemed

[6] I also think that scientists themselves perform an implicit kind of staged Reichenbachean analysis when they write what are called 'review articles' for some subdomain of their science. In these, they often scrutinize and assess concepts by employing various standards akin to the epistemic virtues that Waters discusses. Waters seems to agree with this (personal communication), though I could not find an explicit discussion of review articles in his paper.

[7] I write 'paucity' here and not absence, since the Nobel laureate MacFarlane Burnet once found it important to present an informal axiomatization of his clonal selection theory of immunology, and the Jacquard axiomatization of Sewall Wright's population genetics was at one point in time canonical. For details of these two examples see my (Schaffner 1993a, 40-41, 222-223 and 341).

prima facie to be searching, and which may be requisite for both the RGP and for a Reichenbachean-like rational reconstruction. My initial impression in reading Waters' paper was that by adopting Reichenbach's approach, what he might be seeking would be a rational reconstruction that would eventuate in one, or possibly two, conceptual explications of the gene concept. I think this is a fair inference, based on Waters' early writings on the gene concept, though he now seems to take what I interpret as a different tack. This different tack is clearly evident in Waters' insightful analysis of causal regularities in biology (Waters 1998), which develops a nuanced non-essentialist metaphysics of biological kinds. When an extended Reichenbachean rational reconstructive approach is explicitly embedded in this kind of nonessentialist context, it may well be suited to capture this prototype-based vision of a science. However, my caveat language seems to be appropriate here, since readers do need to be directed to this kind of a nonessentialist context since it is not fully evident in the paper. In a sense, Waters paper may be too procedural and methodological in its present form – it is in fact part of a larger research program and within which it needs to be located – to provide the reader with indications that he is pointing them toward this different vision. I understand that an earlier and much longer version of Waters' paper did provide additional substantive application of the extended Reichenbachean methodology that is no longer present. But a good substitute for that missing application is to urge readers to read the present paper in tandem with his (Waters 1998) essay.

In a similar way, perhaps the RGP needs to be extended and partially re-oriented so that it can identify the prototypes with which different individual and groups of geneticists are operating. Interestingly, the results of the first early project of Stotz and Griffiths seem to have been best interpreted using Fogle's stereotype of a classical molecular gene (Stotz and Griffiths 2004, 10). Furthermore, Stotz and Griffiths write that there is an 'extensive psychological literature on prototype-based categorisation and on the reasoning processes it supports.' (Stotz and Griffiths 2004, 16) And even though they add that this also suggests productive lines of future inquiry for the use of prototype-oriented approaches to conceptual structure, this apparently (so far) has not been pursued in RGP. Such a prototype-oriented direction, however, for reasons mentioned in the two caveats above, may be where the RGP, and possibly rational reconstructions, need to go next.

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Alternative Splicing, the Gene Concept, and Evolution

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ABSTRACT - Alternative splicing allows for the production of many gene products from a single coding sequence. I introduce the concept of alternative splicing via some examples. I then discuss some current hypotheses about the explanatory role of alternative splicing, including the claim that splicing is a significant contributor to the difference in complexity between the human genome and proteosome. Hypotheses such as these bring into question our working concepts of the gene. I examine several gene concepts introduced to cope with processes such as alternative splicing. Next I introduce some hypotheses about the evolution of mechanisms alternative splicing in higher organisms. I conclude that attention to alternative splicing reveals that we adopt an attitude that developmental theorizing must inform evolutionary theorizing and vice versa.

KEYWORDS: alternative splicing; evolution; gene

1. Introduction

Alternative splicing is one of a number of post-transcriptional controls known to operate between the transcription of DNA and the ultimate production of proteins in the cell. Recent estimates (catalogued in Modrek and Lee 2002, 14) place the number of human genes that are alternatively spliced between 22% and 59% of our genetic complement. Work on alternative splicing, as well as work on other post-transcriptional controls, introduces new questions about both the gene concept itself and about evolution. I briefly address both these issues here. First, I introduce alternative splicing via a few illustrative examples. I then discuss relations between alternative splicing and organismal complexity. This relation is illustrated by comparing an organism's genomic complexity with the complexity of its proteosome. This discussion leads us to question the referent for the term 'gene'. In the following section I examine two recent definitions of the gene introduced to deal with related difficulties to the ones introduced here: Lenny Moss' (Moss 2001; Moss 2003) Gene-D and Eva Neuman-Held's (Neumann-Held 2001) Process Molecular Gene (PMG). I defend a modified version of Moss' Gene-D as a device to

help understand the referent of the term 'gene' in much contemporary molecular biology. Finally, I turn to the evolution of alternative splicing. How we approach the evolution of alternative splicing should be connected to our overall approach to understanding evolution. I argue for an approach to the evolution of alternative splicing that shares input from both developmental and evolutionary theorizing.

2. Alternative Splicing, Splice Variants and Some Examples

RNA splicing is known to occur in a huge range of organisms. The existence of the process is familiar to all molecular biologists and an outline of the process is presented in all introductory texts in molecular biology. Primary transcript RNA molecules in the nucleus of eukaryotes contain on average 6000 nucleotides while mature m-RNA molecules contain on average 500 nucleotides. The main process responsible for this reduction in nucleotide number is splicing, the removal of introns from the transcribed RNA (often called precursor-mRNA or pre-mRNA). What remains after this process is an mRNA strand that only contains the RNA version of the code from the exons in the original DNA sequence. (Figure 1) The outcome of this process can be modified by alternative splicing. Alternative splicing involves the production of a mature mRNA molecule that contains a selection

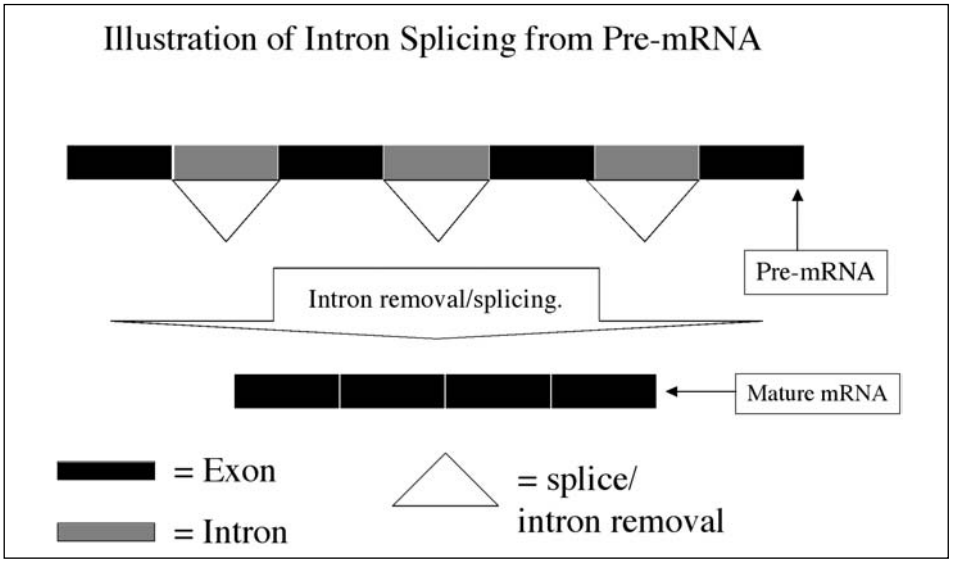

FIGURE 1

of the available exons present in the pre-mRNA molecule. Splice variants are the results of this process and are the different sequences of mature mRNA produced from an identical strand of pre-mRNA. (Figures 2 & 3) Important examples of splice variants include both

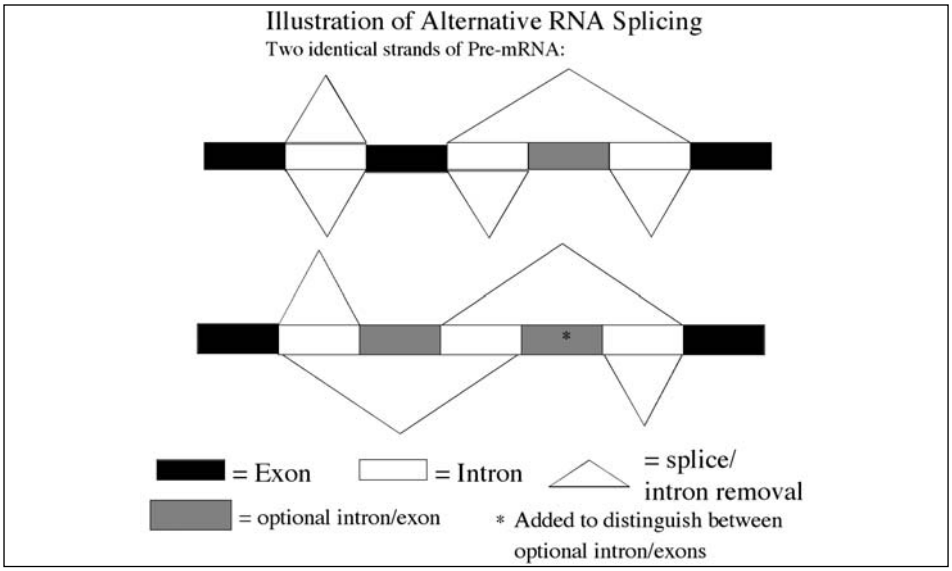

FIGURE 2

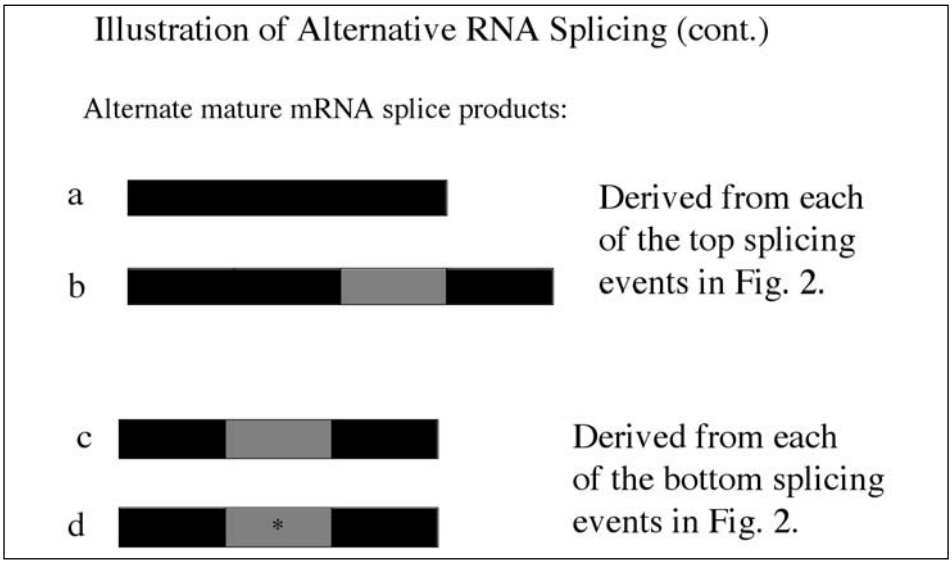

FIGURE 3

those that code for variants on a protein (protein isoforms) leading to a limited change in function and those that code for completely different proteins and hence different subsequent biological functions. (This latter form of post-transcriptional control is also called 'gene switching' or 'gene sharing' or explained in terms of 'overlapping genes' [Alberts *et al.* 2002, 438]).[1]

Alternative splicing is found in many organisms, including humans as I mentioned in the introduction. Here I mention just a few examples of alternative splicing.

Perhaps the most well known and most cited example of alternative splicing (especially in textbooks) (see e.g. Li and Graur 1991; Alberts *et al.* 2002) is the process that leads to sex determination in *Drosophila m.* (reviewed in Baker 1989) (Figure 4). Sex determination in *Drosophila m.* is primarily controlled by the ratio of X chromosomes relative to sets of

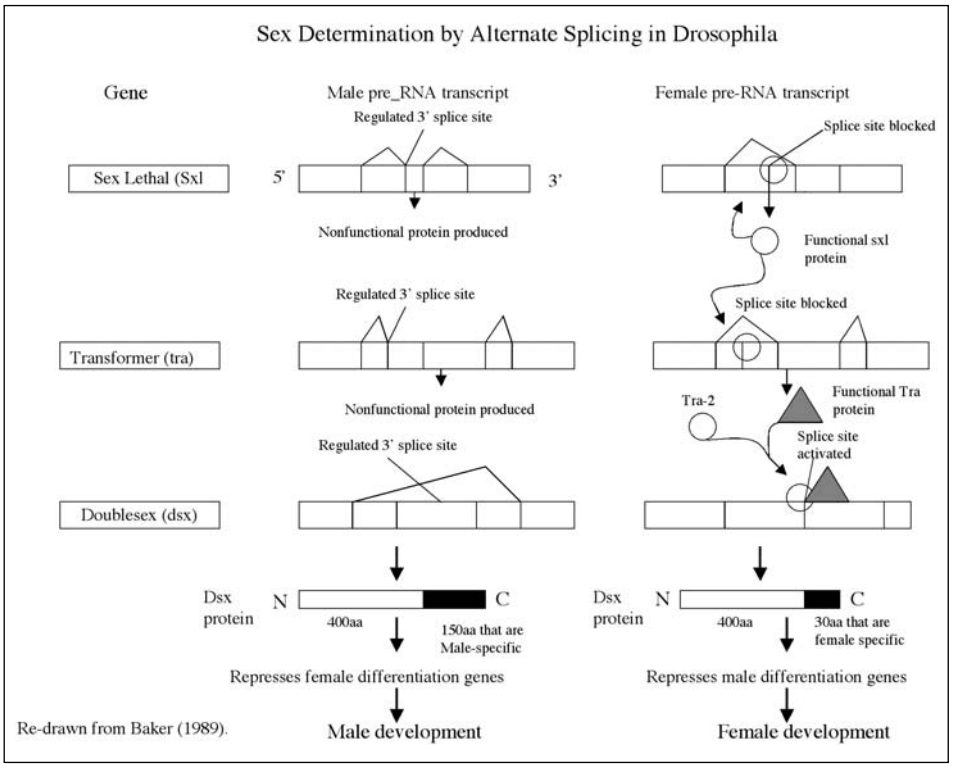

FIGURE 4

[1] Burian (this volume) discusses these cases among others.

autosomes, the X:A ratio (Baker 1989). Flies with a ratio of 1 are female, while those with a ratio of 0.5 are male. Whether the embryonic fly becomes a male or female is determined by a series of RNA splicing events initiated by these ratios. The male development pathway is the 'default' pathway. The X:A ratio of 1 triggers an alternate pathway leading to female sex characteristics. Let's focus on this pathway. The connection between the X:A ratio of 1 and the initiation of the pathway, the blocking of the splice site leading to the production of functional Sxl protein (seen at the top of the right hand column of Figure 4.) appears to be regulated by both maternal and zygotic gene products. Sxl is the first of two splicing regulatory proteins; one that blocks a splice and the other that activates a splice. The effect of these alternate splicings is the production of the female specific form of the protein Dsx. (The process for each sex is illustrated in Figure 4.) Sex determination in *Drosophila m.* is apparently not determined for by a specific DNA sequence but rather by alternative splicing regulators.

Work on various species of Drosophila has revealed another striking example of alternative splicing: the alternative splicing of the RNA transcripts of the Drosophila DSCAM gene. DSCAM proteins help direct growth of cells in the Drosophila nervous system. The pre-mRNA transcript of DSCAM contains 115 exons and each mature mRNA contains twenty four exons and four of these are each selected from four groups (of 12, 48, 33 and 2) of the original 115 (Figure 5).

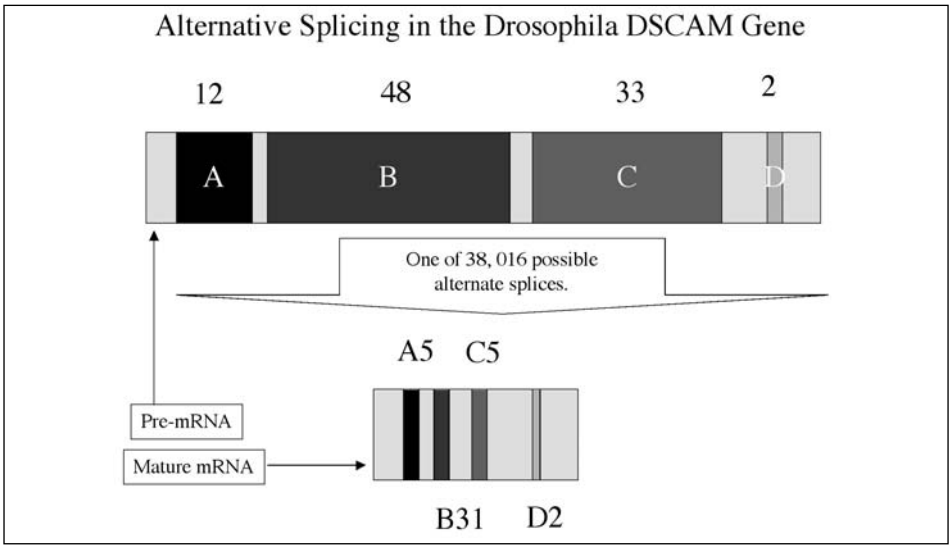

FIGURE 5

The remaining 20 exons in the pre-mRNA are always in the mature mRNA. The combinatorics here allow for 38,016 possible splice variants. Each of the variant DSCAM proteins has a similar structure, so there is not as much contrast between the outcomes of the alternative splicings as there is in the sex determination case but none of the proteins could be produced without alternative splicing occurring (adapted from Alberts *et al.* 2002).

Moss discusses a similar example presented by the human NCAM gene. The human NCAM gene has 19 exons but Moss points out 'there are no NCAM proteins that are composed of the protein domains encoded for by all 19 exons' (Moss 2003, 186). The alternate NCAM proteins are produced in a similar way to the alternate DSCAM proteins in Drosophila: each sequence of mature mRNA producing an NCAM protein is a splice variant.

There are many more examples of alternative splicing in the literature and doubtless many more will be appearing in the human molecular literature if the estimates from the bio-informatics work cited above are correct.

3. Alternative Splicing and Organismal Complexity

One of the more surprising findings to come out of the human genome project is the figure proposed for the number of human genes. While our chromosome complement contains a relatively large number of DNA base pairs, about 3 billion (nothing compared to a lot of plants and a few sharks), recent estimates put our number of genes at around 30,000. (For comparison, Drosophila appear to have in the region of 15,000 genes.) The reason that the proposed number of genes is surprising is that if each gene coded for only one protein, we would come in well under our protein complement, what is known as our proteosome. The number of proteins an organism can produce is a rough guide to the organism's overall complexity. (I leave out any further discussion of the concept of complexity in this paper.) Our proteosome is enormous and is roughly five times that of Drosophila.

Alternative splicing provides one explanation for disparity between our gene count and our protein count. As we have seen, alternative splicing can produce many proteins from one pre-mRNA transcript. Several additional explanations of this disparity have been proposed but most of these additional explanations should be understood as complementary and not competing. From now on I focus on

alternative splicing as an explanation for the gene to protein disparity but I am not proposing that this is a sufficient explanation. Molecular biology is beginning to provide us with numerous complementary explanations of this phenomenon.

Two classes of questions confront us when we propose alternative splicing as an explanation for the disparity between gene number and protein number. The first has to do with just exactly what the term 'gene' refers to and the second has to do with the evolution of alternative splicing machinery. I deal with each in turn.

4. Alternative Splicing and the Gene Concept

What Sterelny and Griffiths (1999) call the 'classic molecular gene concept', that a gene is 'a stretch of DNA that codes for a single polypeptide chain' (1999, 132) does not seem to help us make sense of the predicament outlined above. Molecular biologists talk about the number of genes in an organism and the number of proteins an organism can produce and point out that these numbers are different. Hence we need to be able to distinguish a gene from its RNA splice variants and their corresponding proteins. While many molecular biologists use a similar gene concept to Sterelny and Griffiths' classical molecular gene concept in some contexts, they explicitly invoke alternative gene concepts in others. Alberts *et al.* (2002), in the fourth edition of their text, tackle worries about the gene concept head on. They say that 'the discovery of split genes and introns in the late 1970's could be readily accommodated in the original definition of a gene, provided that a single polypeptide chain was specified by the RNA from any one transcribed DNA sequence. But it is now clear that many DNA sequences in higher eukaryotic cells can produce a set of distinct (but related) proteins by means of alternative RNA splicing' and go on to ask 'How then is a gene to be defined?' (2002, 438). Let's look at a few suggestions.

There are two distinct types of gene definition that have arisen in response to worries such as the one articulated here: gene concepts that locate the referent for the term gene in the DNA complement of the cell (DNA sequence gene concepts) and gene concepts that disperse the referent of the term gene over varying parts of the cellular machinery (inclusive or wide gene concepts). I present these two first and then briefly discuss definitions of another (intermediary) type (pragmatic gene concepts).

First type: DNA sequence gene concepts.

Alberts *et al.* (2002) answer their own question with a proposal to 'count as a gene any DNA sequence that is transcribed as a single unit and encodes one set of closely related polypeptide chains (protein isoforms)' (438). They go on to say that 'this definition of a gene also accommodates those DNA sequences that encode protein variants produced by post-transcriptional processes other than RNA splicing' (438). This definition allows us to talk about genes that lead to the production of a large number of proteins. A similar approach is adopted by Moss but his starting point is different.

Moss distinguishes two types of genes referred to in biological literature, gene-P and gene-D. Gene-P stands for phenotype or predictor gene and is 'defined in its relationship to a phenotype […] when one speaks of a gene in the sense of Gene-P, one simply speaks *as if* it causes the phenotype' (Moss 2001, 87). This is the definition that best captures the usage of the term gene in medical genetics and some parts of population genetics but does not adequately capture the use of the term in molecular biology. Moss proposes gene-D, standing for developmental resource gene, for this purpose: 'Gene-D is defined by its molecular sequence […] to be a Gene-D is to be a transcriptional unit (extending from start to stop codons) within which are contained molecular template resources' (Moss 2001, 88). My take on Moss' definition is that he intends genes-D to be DNA sequences and therefore his definition is consistent with Alberts *et al.*'s. This is supported by Moss' discussion of the NCAM genes where he says that the gene-D for NCAM contains 19 modular units or exons and that it is a 'resource for making a protein' (Moss 2003, 186). He also puts the discussion of genomic vs. proteosome complexity in these terms: 'The human genome has twice the number of Genes-D as that of the fly or worm' (Moss 2003, 187). Again, Moss' gene-D allows us to talk about genes and their multiple protein products. An advantage of Moss' definition over Alberts *et al.*'s is that it comes with a handy label: gene-D. But more importantly Moss' definition is more inclusive than Alberts' as gene-D applies to both DNA strands that are transcribed into polypeptides and DNA strands that produce r-RNA and t-RNA molecules and no polypeptides.

Second type: inclusive or wide gene concepts.

Neuman-Held (2001) proposes a definition of the gene that is also developed in response to problems for the gene concept generated by complexities of molecular development. She says that her goal is to establish a gene concept that applies to '*developmental processes* on

those *molecular* levels of interactions, which have to do with *DNA* and end with the synthesis of *linear polypeptide* chains' (75; her italics). She proposes and defends the process molecular gene concept (PMG): 'PMG […] allows for inclusion of not only DNA, but also non-DNA located entities, thereby integrating into the gene concept those relevant entities that are necessary for the functional specification of the DNA sequences involved' (80). This is a wide gene concept. The concept tries to capture the idea that the gene produces the relevant protein. Given that numerous cellular processes are involved in producing protein from a DNA strand, Neumann-Held includes them in the referent of the term 'gene'. This approach solves some problems, for example there could be one PMG for each polypeptide chain, but still makes it hard for us to understand what molecular biologists are saying when they say that the human genome only contains 30,000 genes. The human genome (unless it also is redefined in PMG terms) contains no PMG's.

Intermediary types: Pragmatically derived gene concepts.

In a much cited passage discussing the gene concept Philip Kitcher says 'it is hard to see what would be lost by dropping talk of genes from molecular biology and simply discussing the properties of various interesting regions of nucleic acid' (Kitcher 1992, 130). The implied definition of a gene here is that a gene is any region of interesting nucleic acid. Interesting regions of nucleic acid include all the DNA strands accounted for by gene-D but also much of the machinery Neumann-Held wants to include in PMG as much of that machinery is RNA. Kitcher's motivation for proposing this move is that cataloguing the uses of the term 'gene' leads us to a far too hazy and ambiguous concept. The two alternative gene definitions considered above work by dividing the reference of the term 'gene' and introducing new terms for the partitioned referent. This approach seems more promising than abandoning the term 'gene' altogether. Aside from the fact that abandoning the term gene would require a super-Orwellian effort at re-writing molecular biology.

A related pragmatic approach to the term 'gene' is proposed by Sterelny and Griffiths (1999) when they say: 'molecular biologists do not seem to use the term *gene* as a name of a specific molecular structure. Rather, it is used as a floating label whose reference is fixed by the local contexts of use. Molecular biologists often seem to use *genes* to mean 'sequences of the sort(s) that are of interest in the process I am working on'. Their rich background of shared assumptions make this usage perfectly satisfactory' (1999, 133). (Their

proposal is close to Waters' gene concept (Waters 1994).[2] At this point their proposal is intermediary between gene-D and PMG. Later on in their discussion they seem to come closer to a gene-D concept for molecular biology: 'The concepts of classic genetics, most notably *gene* itself, continue to play a role in molecular biology, although perhaps as little more than shorthand for the various DNA sequences and collections of interacting DNA sequences used in molecular biological explanations of organisms and their traits' (148).

The gene concept that best accounts for the use of the term gene in molecular biology practice is going to have an element of stipulation to it. The issues generated by focusing on alternative splicing and other post-transcriptional controls lead me to proposing gene-D, restricted to DNA, as the best definition of the term 'gene' of those considered here. Closer examination of other molecular developmental processes, or consideration of alternative gene concepts, may require a revision of this position.

5. The Evolution of Alternative Splicing: Two Contrasting Perspectives

My discussion so far has made no reference to evolution. The background to the discussion of the definition of the term 'gene' presented here resides entirely in molecular developmental biology: the articulation of the processes involved in the production of proteins in cells. Many would argue that the relevant constraints on the gene concept come from articulating its explanatory role in evolutionary biology and not developmental biology. Maynard Smith, for example, argues that the evolutionary gene concept should be imported into developmental biology and that this would be an instructive and useful move for developmental biology (see e.g. Maynard Smith 1998). So why the emphasis on molecular developmental biology here?

Here are a few brief general responses: First, alternative splicing has been proposed as an explanation of the existence of higher order diversity (see discussion in Brett *et al*. 2002). This presents discussion of alternative splicing in an evolutionary context. If we ask how higher orders of complexity arose in higher organisms, one answer could be by alternative splicing. Hence explaining how these organisms evolved

[2] Waters has developed a more complex and inclusive gene concept since his 1994 paper. The criticisms in my paper do not target an important component of Waters' newer gene concept: his technical definition of the molecular gene. My arguments are directed at pragmatic gene concepts, which owe a lot to Waters' earlier paper. Assessing whether my arguments apply to Waters' mature gene concept is a subject for a different paper.

requires invoking the cellular processes involved in alternative splicing. Second, a process like alternative splicing is important for evolutionary theorists to focus on because it is just one, of many, processes that lead to the production of proteins in cells. If any of the systems for controlling these processes are heritable in ways that parallel and accompany DNA transmission, then these systems have evolutionary significance. My view is that to confront these evolutionary questions we need to pay careful attention to theoretical developments in molecular developmental biology and work towards an account of evolution that is consistent with these findings. So I resist Maynard Smith's proposal, not by reversing the direction of his proposal and suggesting that evolutionary biology must import concepts from developmental biology, but by recommending theoretical influence in both directions. This suggestion is consistent with the goal of evolutionary developmental biology as defended by Hall who suggests that evolutionary developmental biology is 'a synthesis of evolution and development with emergent properties not found from analysis of development or evolution alone' (Hall 2000, 177-178).

I now look at some specific suggestions about the evolution of alternative splicing. There are two positions in the discussion of the evolution of alternative splicing or evolution resulting from new alternative splicing events. The first emphasizes change as a result of mutations in DNA sequences and the second emphasizes change as a result of changes in RNA and other splice controlling mechanisms. Proponents of both perspectives agree that the production of new splice variants leads to greater diversity of phenotypes.

Li and Gruar (1991) represent the first perspective. They argue that 'the evolution of alternative splicing requires that an alternative splice junction be created de novo. Since splicing signals are usually 5-10 nucleotides long, it is possible that such splice sites are created with an appreciable frequency by mutation' (160). They discuss one example of this process, the b^+-Thalassemia gene. Unfortunately, this is not the best illustration of their point as possession of the mutation is lethal. The general principle of their idea is grounded in the distinction between weak and strong splice sites. The b^+-Thalassemia mutation creates a strong splice site that leads the cell to always produce the deleterious protein. The production of a weak splice site will provide an opportunity for the cell to produce both the original protein and the new one, hence giving the cell the potential to produce a new protein with perhaps a new function. Alberts *et al.*

(2002) add that there is an interplay between weak and strong splice sites in pre-mRNA. If a strong splice site is blocked (as in the Drosophila sex determination example above) a weak splice site may be exposed to produce a different splicing pattern. But this added explanation exposes a weakness in Li and Gruar's perspective: whatever novelty is produced is not a result simply of a mutation in the DNA, the gene-D, but also the result of the differential effects of regulatory proteins and RNA machinery.

Alberts *et al.* (2002) defend an alternative perspective. They argue that the 'RNA-splicing cascade is an ancient control device, left over from a stage of evolution where RNA was the predominant biological molecule and controls of gene expression had to be based almost entirely on RNA-RNA interactions' (Alberts 1994, 456; Alberts *et al.* 2002, 439). As a result they emphasize an examination of the processes that lead to changes in these regulatory structures. Now of course this could amount to the suggestion that we look back to the DNA but if various regulatory structures involved in RNA splicing are inherited independently from DNA transmission, then their proposal is different than and supplementary to Li and Gruar's.

Moss (2001; 2003), Gerhardt and Kirshner (1997) and several proponents of developmental systems theory and its variants (see e.g. Jablonka 2001) hold out for this latter perspective. Moss' approach is illustrative, his view is that evolution is not achieved by the elaboration of a master code script in DNA (e.g. simply by mutation and selection) but rather 'by the fragmentation of the functional resources of the cell into many modular units whose linkages to one another have become contingent' (Moss 2003, 188-189). Exploitation of various combinations of these modular units in varying ways leads to the production of novel proteins and structures. Moss supports the emphasis of Alberts *et al.* in approaching the evolutionary problem via looking at the inheritance of mechanisms other than DNA sequences that guide splicing and other post-transcriptional processes.

My sense is that neither perspective on the evolution of alternative splicing should overwhelm the other. If we adopt the gene-D account, then it seems consistent to say that mutations in genes-D provide new opportunities for developmental processes. We can say this without ruling out investigation into the inheritance and variation over time in developmental regulatory systems outside the DNA.

6. Conclusion

Looking at the process of alternative splicing provides an opportunity to examine both the gene concept and our views about what perspective to emphasize when explaining the evolution of cellular processes. I have argued that a slightly modified version of Moss' gene-D best fits the concept of gene invoked in discussions of alternative splicing. I have also argued that explaining the evolution of cellular processes requires adopting (at least) two perspectives on evolution. This move requires adopting an attitude that developmental theorizing must inform evolutionary theorizing and vice versa.

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Long Live the Genome! So Should the Gene

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ABSTRACT - Developments in the sequencing of whole genomes and in simultaneously surveying many thousands of transcription and translation products of specific cells have ushered in a conceptual revolution in genetics that rationally introduces top-down, holistic analyses. This emphasized the futility of attempts to reduce genes to structurally discrete entities along the genome, and the need to return to Johannsen's definition of a gene as 'something' that refers to an *invariant entity of inheritance and development*. We may view genes either as generic terms for units of inheritance whose referents are pragmatic ad hoc and context-dependent, or as (epistemologically) representing entities of cell functions. It is cellular functions that determine the structural referents along the DNA. Structures that happened to secure specific functions that were essential for or conducive to the survival of cells were selected for.

With natural selection being the etiological background of genes as functions, genes obtain again their theoretical role as intervening variables, abstractive variables that purely 'summarize' characters. The importance of DNA sequences is that of all possible phenotypes these are the most basic ones from which we can read off the genotype directly.

KEYWORDS: gene; genome; functional gene; top-down causation; development and evolution

> Placing genes in context is one of the major unresolved problems in biology. …the gene's home, context, and locus of operation is the cell. (Hall 2001, 226)[1]

There can be little doubt that the HGP and the wall-to-wall sequencing of the genomes of an increasing number of species has brought about a radical change in genetic research far beyond the expectations, not to say the gloomy predictions of many of us, myself included. It must be admitted that to a large extent this transformation has been driven by technological and computational achievements; the project engendered methods and technologies that allowed handling

[1] Hall actually distinguishes explicitly between the *concept* of the gene and the *phenotype* of the gene: 'What components and processes lie between the inherited genotype (including the phenotype of the gene) and phenotypes?' (Hall 2003, 220).

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and thinking in terms of many gigabases, rather than few kilobases of nucleotides. Most significantly: experimentalists were required to think in terms of wholes as such, rather than along the conventional genetic conception of an increasing number of discrete units: one gene, two, three, … up to *n* distinct genes. Such a conceptual revolution in our thinking prompted authors like Evelyn Fox Keller to call the incoming twenty-first century 'the century of the genome', in contrast to the twentieth, 'the century of the gene' (Keller 2000).

The genomic revolution is not the first technology- or methodology-driven conceptual revolution, and it should not be difficult to show that, typically, such revolutions brought to an end long periods of conceptual instability and disputes. I suggest that the new science of genomics may similarly stabilize the concept of the gene after nearly a century of constructive unrest. More specifically, I wish to claim that:

a. The efforts to identify unique structural entities for the functionally defined genes that, following the Watson-Crick model of DNA, culminated in the notion of the *cistron* as a sequence of nucleotides that corresponds to a specified sequence of amino-acids, and in the Central Dogma of a one-way information flow from polynucleotides to polypeptides, increasingly failed.
b. The exhaustion of the reductionist methodology and the failure of the reduction of heredity to genetics encouraged functional thinking, including attention to top-down evolutionary causation and the return of developmental considerations.
c. The specific structures that functionally-defined genes assume were conditioned by the historical constraints and by insights with regard to the evolutionary pressures of cellular and organismic needs and opportunities.
d. The analysis of the genome as a complex interactive phenotype restores the role of genes as functionally meaningful units of reference, by and large, irrespective of their structural organization.

The concept of the gene was born in tension at the outbreak of the twentieth century. Although the gene's identification depended on functional tests, definitions of genes ranged from abstract conceptions to those of material discrete entities (or 'atoms') of matter. With the establishment of the Watson and Crick structural model of DNA as that corresponding to the demands of genetic material, it seemed that the dialectic tension was resolved in favor of the material concept of the gene as a discrete and specific sequence of nucleotides, collinear with the sequence of amino-acids in the polypeptide coded by that gene (the 'Classical Molecular Gene').

This notion culminated in 1958 with Crick's Central Dogma, according to which genes are repositories of protein-coding sequences

(except for those that specify infrastructural RNAs) (Crick 1958). It has been generally assumed that canonical genes code for proteins, and that proteins fulfill not only most structural and catalytic but also most regulatory functions, in all cells (see Crick & Lawrence 1975; also Mattick 2003). As W. Wayt Gibbs pointed out:

> No great wonder, then, that many biologists (and journalists) have taken the central dogma to imply that, with very few exceptions, a DNA sequence qualifies as a gene only if it can produce a protein.
> Typically when people say that the human genome contains 27,000 genes or so, they are referring to genes that code for proteins, … there is no clear correspondence between the complexity of a species and the number of [canonical] genes in its genome. (Gibbs 2003, 47)

An early indication of the exhaustion of the notion of genes as 'causal atoms' of discrete DNA structures of heredity was Jacob and Monod's model of genetic regulation. They opened their 1961, *Journal of Molecular Biology* paper with an explicit statement of a gene being a discrete structure with a unique function: 'According to its most widely accepted modern connotation, the word 'gene' designates a DNA molecule whose specific self-replicating structure … become[s] translated into the specific structure of a polypeptide chain' (Jacob & Monod 1961, 318). Yet, they went on to provide a view of a functional integration of such molecules into the 'operon' that is regulated directly by an RNA molecule rather than by a protein molecule (in this last point it turned out that they were wrong). In the specific case of β-galactosidase, the functional integration of the operon is reflected in integration at the structural level – but this need not be the case, see, e.g., the arginine operon in *E. coli* that maps at multiple loci.

The Pandora Box was, however, opened wide a couple of years later with the discovery that most of the nuclear DNA contents of higher organisms does not code for proteins (Britten & Kohne 1968). Protein-coding chunks account for less than 2% of the DNA in human chromosomes!

During the decades that followed, it became increasingly clear that there is no one, 'objective' functionally-independent way to define a material canonical gene. Various criteria have been used to identify genes in the monotonous DNA sequences: 1. The obvious way to find protein-coding genes was through identifying large ORFs (open reading frames) of uninterrupted codons of nucleotides translatable to amino-acids. Other criteria included: 2. Specific features of ORFs, such as 'codon biases,' i.e., non-random representation of codons in given sequences. 3. Conservation of the sequences among species as

an indication to their importance. 4. Transcription, an RNA or protein product of the sequence suggesting that it has some functional role. 5. Inactivation of a sequence, whether by mutation or other interference, indicating functional entity-coherence of the sequence. However, the application of none of these criteria is straightforward, and the numbers of annotated genes of a species are considerably revised over time and laboratory site (Snyder & Gerstein 2003; Hild *et al.* 2003). Eventually, different research groups each defined their own 'gene' concept. Viewed from above, the 'gene' became rather a generic term, like 'chair' or 'table'.

In short, as a precise referring concept it seemed that the gene had reached its end.

When Russell Gray announced in 1992 'The death of the gene' he annunciated a wide spread mood that wished 'to dislodge the gene from the privileged site it has occupied in our accounts of development and evolution' (Gray 1992). Yet, I claim that the rumors of the death of the gene were premature. Gray suggested that his 'de-centering of the gene … should not be seen as an argument for the return of the organism, the cytoplasm, the environment, or any other developmental resource'. Notwithstanding, precisely the emergence of the 'genome' closely traced the return of 'the organism, the cytoplasm, the environment, or any other developmental resource', and with it I expect also the re-entry of *the gene in its original role*, as an entity of heredity *defined* by its functional role(s), rather than one *defining* a specific structural entity. The genome provides a structural setting for the functional gene. Rephrasing Beurton: A gene is a genome's way of making a trait (or function) (Beurton 2000, 296).[2]

Introducing the Genome

The term 'genome' is not a newcomer. Rieger, Michaelis and Green, in their *Glossary of Genetics and Cytogenetics* attribute the term **Genome** to H. Winkler in 1920: 'in eukaryotes, the basic (monoploid) chromosome set of an organism, consisting of a species-specific number of linkage groups' (Rieger, Michaelis, & Green 1976). However, a significant change was introduced in the 5th, 1991 edition

[2] Beurton, however, commented (personally) that although such a rephrasing may be true, he sees no way how to make this statement operational or at least theoretically operational (if there is such a thing) – while to say something like '"A gene is a population's way of adding some increment unto itself" (in terms of evolution) seems to me at least "theoretically operational"'.

of the *Glossary* (Rieger, Michaelis, & Green 1991). In 1976 the text continues saying: 'hence the sum total of its genes'. In 1991 it says: 'and the genes contained in it'. Whereas in the earlier edition the authors refer to the genome as *composed* of genes, in the later edition they do not commit themselves to the nature of the genes *contained* in the genome.

Lewontin stresses the type-token distinction between genome and genotype with its component genes: 'The actual physical set of inherited genes, … make up the *genome* of an individual, and it is the description of this genome that determines the genotype of which the individual is a token' (Lewontin 1992, 139). The genome is the token, the *material* that happens to provide for the genes as *type* or *genotype*.

Similarly, Whitherly, Perry, and Leja (2001) define a genome as the DNA contained in an organism or a cell: 'A genome is the complete collection of genetic information, including the genes and the extra DNA used to package the DNA.' Whereas 'genotype is the genetic identity of an individual that does not show as outward characteristics'.[3]

Thus, in spite of some confusion the genome refers to the hereditary *material*, namely to the (usually monoploid) totality of DNA. Genotype and gene refer to the *notion* of inheritance and its units. Put differently, genes and genotype maintain their role in Johannsen's definition as 'something' that refers to an *invariant entity of inheritance and development*, whereas genome takes its position as the material realization of these abstractions, with no commitment to the existence of any entities, other than that of being composed of a sequence of base pairs.

Reductionism and Its Discontents in Genetics Research

In contrast to de Vries, who was an outright ontological reductionist, both Mendel and Johannsen, conceived of discrete factors of inheritance in terms of methodological reductionism: Mendel selected those traits that segregate as discrete entities, while Johannsen worked with quantitative, continuous variables so that genes were for him just those entities of the genotype that Mendelize. In the years that followed, genetics became the paradigmatic case of reductionism in the life sciences. It guided genetic research all the way

[3] Note that **gene** is defined by Witherly *et al.* (2001) in more equivocal terms as: 'The functional and physical unit of heredity, which carries information from one generation to the next; a segment of DNA, composed of a transcribed region and a regulatory sequence that make transcription possible.'

from the methodological, or 'abstract hierarchical reduction' to 'strong, physical reduction' (Sarkar 1998).

> Reductionism refers rather to a particular way of viewing the relations between the underlying particles (genes) and the objects (organisms) which are in some sense composed of, or controlled by these particles (Beurton 2000, 289).

To the exclusion of few, like Richard Goldschmidt (and to some extent also Lewis J. Stadler, who was an operationalist as propounded by P.W. Bridgman), geneticists strove to reduce genes to instrumental, possibly physico-chemical entities. But to no avail. Arguably, the last heroic efforts for such ontological reductionism of Mendelian to molecular genetics were, at the experimental level those of Seymour Benzer, defining the 'cistron' as a segment of DNA which codes for a specific gene product (Benzer 1957), and at the philosophical level that of Ken Schaffner, providing reduction functions of classic Mendelian terms to molecular DNA sequences (Schaffner 1976).

To successfully analyze and understand a phenomenon we must define our variables at the appropriate level of complexity. The notion that an organism's morphological phenotype was determined by its genotype was increasingly contested. Proposals that the correlation of an organism's form with its genotype, rather than being a *defining condition* of morphological evolution, is a *highly derived property* implied that other causal determinants of morphogenesis have been active over the course of evolution besides differential selection of a population's gene pool as requires by the neo-Darwinian notion (see, e.g., Newman & Müller 2000, 304).

Yet, in spite of the increasing consciousness that it is the structure and function of the whole cell, even the whole organism, which endow living systems with their specific properties, there were no adequate methodological tools to allow concepts of embryology, especially notions such as evolutionary necessity in face of historic constraints, to become effective in terms of molecular developmental biology. In the words of Günther Wagner, there were no tools to turn 'evolution of development' into 'developmental evolution' (Wagner 2000). Gould's (1977) *Ontology and Phylogeny* amply emphasized the need for such a breakthrough.[4]

[4] Gould, however, puts the emphasis on gradual change, while other students of evolution, like John Maynard Smith and Eörs Szathmáry (1995) attributed a greater role to major evolutionary innovations.

Things seemed to have dramatically changed with the introduction of sequencing techniques, first for polypeptides and eventually for polynucleotides, and especially with the introduction of sequencing of whole genomes and of the simultaneous surveying of micro-arrays of many thousands of transcription and translation products of specific cells.

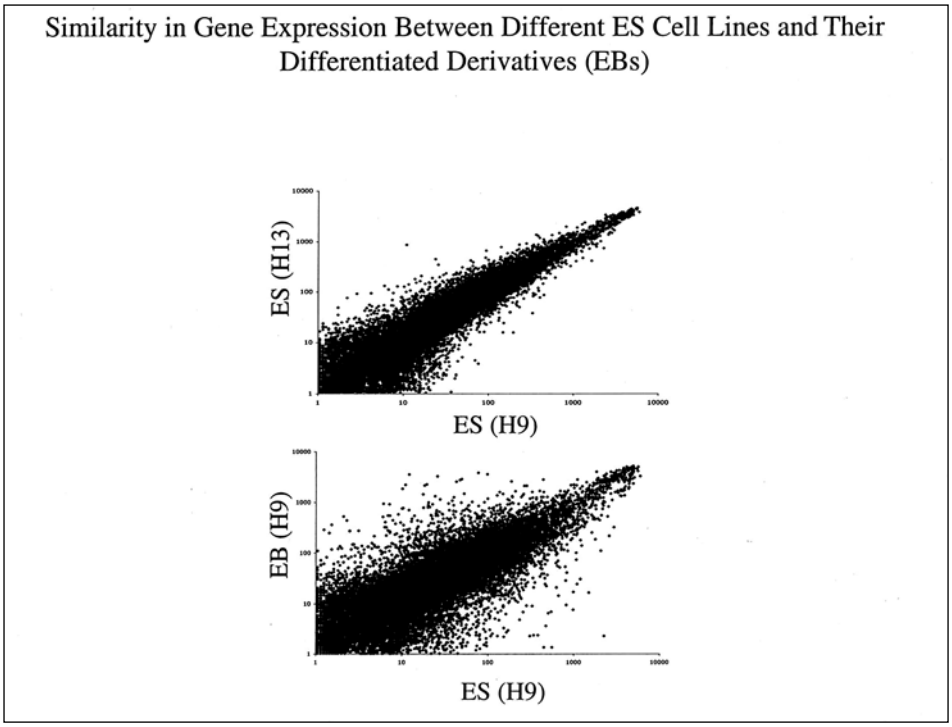

Fig. 1. RNA micro-arrays: There are 22,000 dots on each graph, each dot represents one gene. The X and Y values give the abundance of RNA produced by the genes in two cells of the same line and culture conditions (above) or two cells of the same line, one undifferentiated the other differentiated (below). Genes are defined by those stretches of DNA that produce mRNA. Courtesy: Nissim Benvenisty, Department of Genetics, The Hebrew University of Jerusalem.

New methods for handling simultaneously a very large number of variables, whether DNA sequences and other cellular products, were developed. These allowed, or enhanced, a complementary approach to the time-honored reductionist approach to science, where attention was paid to one factor at a time, while neutralizing or randomizing as far as possible all other factors contributing to the observed

phenomena. It considered holistic or comprehensive effects, yet remained conscious of these effects beings decomposable to discrete specific factors. Whereas in molecular biology the correlations are *defined* by the functions of the components, in the system-theoretical concept of the organism the correlations *follow* from the functions required from the components. This profound change that genomics introduced into the life sciences did not happen overnight: The integration of embryology – classically a holistic science – and reductionist genetics into developmental biology in the last three decades of the twentieth century could hardly have occurred without such a conceptual change. Although many premolecular developmental terms, like induction, polarity, fields of self-organization or of inhibition, found at least partial explanation in molecular analysis, other complex notions of developmental biology, like 'developmental constraint', could not be reduced to molecular mechanisms (see, e.g., Kirschner, Gerhart & Mitchison 2000). The techniques of genetic engineering and those developed specifically for the HGP finally provided the tools that allowed a genomic approach that could place holistic thinking in the centre of the consensus. This was, however, a different 'holism' from the classical one. It did not rely or imply 'supernatural' forces or concepts, such as vitalism, *Lebenskraft*, or entelechy, beyond those of physics and chemistry. It rather accepted the basic physical 'laws of nature', only that it could now view them in their totality, in their complex integrative perspective. Genomics did not dispose of the gene concept; though as a rule, it sublimed the concern with individual genes.

Genes Are Functional Rather Than Material Entities

Richard Burian (1995) suggested that there are two possibilities for gene concepts. Practicing experimental biologists chose as a rule a generic concept of an entity of inheritance,[5] whereas it remained for the more philosophically minded biologists, and historians and philosophers of science to cope with the incumbent well defined reference to a specific material entity.[6]

Although Amundson and Lauder (1994, 452) courageously attack the inclinations of philosophers for conceptual analyses that are

[5] Science is realized and constitutes in practices. 'The practices in which the sciences are grounded engender epistemic objects' (Rheinberger 2000, 200). The gene is an epistemic object (Moss 2003, 64).

[6] 'Peter Beurton offers what may read like a heroic last chance attempt to reconstitute the unified gene' (Moss 2003, 65).

dissociated from scientific practices, it is in this later capacity that I wish here to explore genes as units of inheritance whose referents are entities of cell function, rather than specific material entities.

The coordinated performance of a large yet *specific* number of functions is essential for and conducive to the survival of cells (and of whole organisms). According to the Darwinian conception, structures that *happened* to secure such functions were selected for and are maintained. Nearly forty years ago Dick Lewontin praised Frank Stahl for his attempt to write about the mechanics of inheritance in a way that takes into account molecular genetics.

> However, Stahl, in common with many enthusiasts, has mistaken the tail for the dog. . . . That is, except in a trivial sense, the laws of genetics are not the result of the structure of DNA, but rather DNA has been chosen by natural selection from among an immense variety of molecules precisely because it fits the requirements of an evolved genetic system. DNA is only the tactic adopted in the course of working out an evolutionary strategy. That is why some organisms can get on without it. (Lewontin 1964, 566)

Likewise, it is cellular functions that determine the structural referents along the extant DNA. In so far as what we refer to as genes assume specific structures, these are the historically constrained consequences of the evolutionary pressures of cellular and organismic functions. The genome serves as the long-term information storage organelle of each living cell (Shapiro 2002b, 113).

Function, in terms of 'in order to' is a teleological formulation of reverse causation. But, as emphasized by Larry Wright (1973), it may merely signify that something it stands for is natural (not preprogrammed, yet not random), being of general relevance rather than a one-time haphazard relation. Thus, 'functional explanations are in some sense etiological, concerning the causal background of the phenomenon under consideration'. The etiological, causal background of genes is natural selection (*selected effect*, SE account of function in the terminology of Amundson and Lauder 1994). Whatever sequence of DNA available that may provide the functions (even if not 'perfect' in retrospect) would do.

Peter Godfrey-Smith has developed this into the 'modern history theory of functions', according to which 'functions are dispositions and powers which explain the recent maintenance of a trait in a selective context'. He recognizes that this involves substantial biological commitments. '[P]erhaps many traits around now are not around because of things they have been doing.' – Take birds' feathers, for example, which presumably were originally selected as

functioning in body-temperature regulation rather than for their more recent function in flight. Godfrey-Smith admits that 'many modern-historical function statements will be false'. But, 'there is no avoiding risks of this sort' (Godfrey-Smith 1994). Returning to Lewontin's assertion, it is functions (traits) that determine genes.

Which Functions Qualify?

The assignment of genes for functions occurs not only at the etiological level, but also at the epistemological empirical discourse: We assign specific genes to functions or traits, implicitly or explicitly on a *ceteris paribus* rule. Like Mendel, we choose to follow (or define) traits and environmental conditions, which make conspicuous quantal phenotypic differences in the cell or the organism. Alternatively, we try to provide experimental conditions, such that variability in environmental and organismal conditions is kept to the minimum, except for the one trait that is targeted. The level of the quantal traits chosen has been changing with the vicissitudes of research opportunities: Morphological traits such as eye color have been replaced by specific enzymes, and later by specific stretches of DNA (e.g., RFLPs), polymorphic DNA sites (e.g., microsattelites, SNPs), or specific RNA transcripts. Analogous methods were developed also for the analysis of the different levels of continuously varying physiological or behavioral traits.

The great misfortune of genetic thinking has been the uncritical transformation of the term 'genes for' from an operational concept in a context of an organism as an integrative system into a causal deterministic concept with an old-fashioned teleological taint.

> The use of drastic gene mutations as the primary tool of investigation is a form of reinforcing practice that further convinces the biologist that any variation that is observed among organisms must be the result of genetic differences. This reinforcement then carries over into biological theory in general. (Lewontin 2000, 15)

For many years fellow biologists indignantly criticized my claim that rather than sticking to a notion of the genotype as referring to the confrontation of *nature versus nurture*, the genotype should be considered as just one of the environmental inputs to the phenotype – self-organization and developmental constraints being just some factors that shaped evolutionary history – a notion that Paul Griffiths has now formulated into the powerful 'parity thesis' (Griffiths 2001).

Once one considers the complexity of the cell, such contrived empirical deductions as 'the gene for trait X' would only rarely indicate a meaningful straightforward causal relationship. Godfrey-Smith (2000) proposes that a good approximation to such a relatively straightforward causal chain may be that of the 'genetic coding' *sensu stricto*, which cells perform when putting together complex protein molecules with DNA and mRNA. The template used in making a protein is not the protein itself.[7] Digital information transfer from DNA to RNA and it's processing, to polypeptide is eventually non-trivially converted into that of proteins' 3-D analogue information.[8]

Although even this 'straightforward causal chain' is *ceteris paribus* – constancy of intra-cellular environment, chaperons etc. are assumed – it would appear as if it might justify assigning to ORF-coding-sequences a specific ontological status as the structural corollaries of the concept of 'gene': A gene is 'for' the construction of a protein. Such an assignment is (almost) identical to Benzer's concept of 'cistron'.

Furthermore, Godfrey-Smith notes that any attempt to extend the concept of genetic coding to farther off morphological or functional traits that may be described by *entire causal paths* in which genes are involved, has 'no empirical basis and makes no contribution to our understanding'.

> There are good reasons for claiming that proteins are *made by being coded for*, and hence that a specific gene *codes for* a particular protein. But once we consider the complex traits of whole organisms, … none of these traits are coded for by the genes. …
> To make this claim is not to deny that at least some causal relations are transitive, and so to deny that genes can causally affect complex traits of whole organisms. … [G]enes can have a causal role which extends beyond the production of proteins, but proteins are all a gene can code for. (Godfrey-Smith 2000, 35)[9]

[7] 1. The template used in making a protein is not the same protein itself. There is a non-trivial rule of specificity linking the two elements that are chemically different; 2. The specification of proteins by these templates is combinatorically structured; and 3. The rule linking base triplets with amino acids is largely 'arbitrary', meaning that nothing about the *chemistry* of a particular amino-acid is responsible for its corresponding to a particular base triplet.

[8] The molecular approach in analyzing morphogenetic processes has been extremely successful in referring to the DNA sequences as the basic parameter. However, on the next level of analysis, that of proteins, although in principle a correspondence between nucleotides and amino acids operates, the properties of proteins give rise to highly ordered processes that cannot be derived from DNA sequences.

[9] Once one accepts this interpretation of Godfrey-Smith, indeed the protein molecule, or more precisely, the translated polypeptide chain is the only real phenotypic expression of a gene. It is immanently different from any other morphological, physiological or behavioral phenotypic marker.

But even this limited notion of bottom-up genetics, where the biopolymere sequence or structure provides enough information for function, as derived from classical information theory, is increasingly challenged. In a note to *Nature*, Jack Szostak called attention to 'the recent deluge of phylogenetic sequence data' that 'provides thousands of examples of related but different sequences encoding essentially identical structures and functions. [Still m]ore radical are examples of both RNA and protein molecules with entirely different structures but similar biochemical functions.' Thus, he concludes 'A new measure of information – functional information – is required to account for all possible sequences that could potentially carry out an equivalent biochemical function, independent of the structure or mechanism used' (Szostak 2003).

Once the conception started to move toward the genomic 'holistic' or top-down perspective, the dominance of the structural notion of the genes as discrete causal entities could be overcome. The DNA was now conceived as a means to functions, not merely as that of coding proteins. The notion 'superfluous' or 'junk' DNA of the era of the Central Dogma assigned to the non-coding sequences made way to a fresh approach. Snyder and Gerstein call explicitly for a revised 'Defining genes in the genomics era':

> With the advent of recombinant DNA and gene cloning, it became possible to combine the assignment of a gene to a specific segment of DNA and the production of a gene product. Although it was originally presumed that the final product was a protein, the discovery that RNA has structural, catalytic, and even regulatory properties made it evident that the end product could be a nucleic acid. Thus, we now define a gene in molecular terms as 'a complete chromosomal segment responsible for making a functional product'. (Snyder & Gerstein 2003, 258)

These authors conclude their paper suggesting that '[o]ne solution for annotating genes in sequenced genomes may be to return to the original definition of a gene – a sequence encoding a functional product – and use functional genomics to identify them' (Snyder & Gerstein 2003, 260).

Indeed, '[j]ournals and conferences have been buzzing with new evidence that contradicts conventional notions' of genes. It turns out that many of the 'non-coding' sequence of DNA have been preserved mostly intact through evolution, and a large number are transcribed into a variety of RNAs that perform a wide range of functions. 'Some scientists now suspect that much of what makes one person, and one species, different from the next are varieties in the gems hidden

within our "junk" DNA' (Gibbs 2003). Finta and Zaphiropoulos (2001) explicitly stated that 'Our perception of genes as well-defined DNA segments within a vast excess on nonfunctional sequences may not necessarily be true'. And Gibbs (2003) quoted John S. Mattick (2003) saying that '[t]he failure to recognize the full implications of … the possibility that the intervening noncoding sequences may be transmitting parallel information in the form of RNA molecules – may well go down as one of the biggest mistakes in the history of molecular biology'. It was the practicing molecular biologists who were led to 'the re-definition of a gene as a "transcription unit" or "a complete chromosomal segment responsible for making a functional product"' (Mattick 2003, 933).

DNA Sequences May Become Genes

A couple of years ago Peter Beurton published his ideas of a unitary concept of the gene in terms of the evolution of DNA sequences. Beurton defined the gene 'as the genetic underpinning of the smallest possible difference in adaptation that may be detectable by natural selection' (Beurton 2000, 286). Although he is concerned with DNA structures that once looked like particulate genes and now turn out to be 'scattered across parts of the genome with no hard-and-fast boundaries', he notes that the adaptive differences that build genes are *genomic*, not *genic* (Beurton 2000, 302). Beurton's notion of 'nature in and of herself turns DNA strings into discrete and well-established entities which deserve such a name' was criticized.[10] However, in envisioning genes as the outcome of downward causation of *the clever genome* (Beurton 2000, 296), I suggest that he may have, in a sense, anticipated the renewed role of the gene concept in the era of the genome.

Considering recent developments in the conception of the genome organization in evolution, which James Shapiro denoted as 'The Twenty-First Century View of Evolution' (Shapiro 2002a; Shapiro 2002b), we must take notice of the *evolution of the evolutionary process*

[10] Paul E. Griffiths (2002). Note, however, that 1. Downward causation is not Dawkins' notion; 2. Shapiro's Natural Genetic Engineering allows stability of collections; 3. Conflation of Gene P and Gene D may be a virtue; 4. Conceptual anti-reductionism does not exclude methodological reductionism. Moss (2003). Concerning 'the diachronic mind's eye perspective of the evolutionist [that] look[s] into the genome of an organism and sees what the synchronic view of the molecular biologist cannot' – see the argument in Godfrey-Smith (1994): The crucial phenotypic trait is *fitness*. Genome diversity through reshuffling provides for condensation into units associated with discrete traits.

in which it is 'complexity [that] permits sophisticated information processing' (Shapiro 2002a, 745), in the sense that cells are constantly evaluating internal and external signals and adjusting their activities, and carry out their computations by a process of molecular interactions. 'Genomes integrate into cellular information processing because they are organized as computational storage organelles' (Shapiro 2002a, 746). Once such an integrative system has been established, evolutionary change that had started as conventional evolution of random walk through adaptive space – Shapiro suggests – 'occurs largely by a process of Natural Genetic Engineering' (Shapiro 2002a, 746). That is, new functions arise mainly by *systemic genomic organization*, i.e., by cut-and-splice rearrangement of genetic modules, whether these are transposons, centromeres or telomeres, to a large extent as direct *response to a specific strain* of whole genomic systems.

It is the *sameness of reproduction rate* by which these DNA variations begin to meet the standards of being one single gene (Beurton 2000, 299). However, contrary to Dawkins (Dawkins 1976), as a rule genes do not reproduce 'in the interest' of their own, but rather 'in the interest' of organisms and their features. They are *slavish* rather than *selfish*.

* * *

Wilhelm Johannsen defined the phenotype as 'the observable properties (structural and functional) of an organism, produced by the interaction between the organism's genetic potential (its genotype) and the environment in which it finds itself'. The genome and its DNA sequences are nowadays certainly observable structural properties of an organism, produced by the interaction between the organism's genetic potential and the environment in which it has been finding itself. This genome may be parsed into functionally specified genes, for example by considering the mRNA sequences produced in given cells.

Genes in the age of the genome obtain again their functional role as intervening variables, abstractive variables that purely 'summarize' characters (Falk 1986). The importance of DNA sequences is that of all possible phenotypes these are the most basic ones from which we can read off the genotype directly (Griffiths, Gelbart, Miller, & Lewontin 1999, 576).

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Commentary on Falk and Downes

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Introduction

Stephen Downes and Raphael Falk have offered their contributions to the question of how best to think about the meaning or meanings of 'the gene'. Clearly, this is a question that can and must be approached in a variety of ways. Downes has presented a paper that approaches this question by way of looking at the phenomenon of alternative gene splicing and its evolutionary implications. What does alternative gene splicing tell us about what we mean by the word 'gene' and reciprocally what concept of the gene best facilitates our understanding of the possible evolution of alternative gene splicing? Raphael Falk has addressed this question by offering a conceptually challenging attempt to reconstitute a unified understanding of the gene in terms of what he refers to as an 'intervening variable'. By taking the gene as an intervening variable, Falk claims that what the genomic era has ushered in is not the end of the gene concept but a new phase of an otherwise continuous conceptual history that has served to productively mediate an always-present tension between functional and material specification. For Falk there is not many, nor even two, genes but only one and there is no sense in talking about genes as such if they do not stand in some relationship to functional specification.

The way I will want to proceed in commenting on these papers is by bringing them together. Specifically, I will want to look at Falk's argument for how to understand the meaning of the gene concept using Downes's characterization of the multi-spliceable gene-transcript source as our exemplar for a molecular gene, i.e., a 'Gene-D', in the age of

genomics. In the light of Falk's analysis, we can ask if the Gene-D/Gene-P distinction, that Downes endorses, will dissemble into the unified concept (with a continuous history as an intervening variable) that Falk seeks. First I'll take a moment to further clarify the Gene-P/Gene-D terminology that has already been introduced by Downes.

Falk has done much in the past to elucidate the thinking of Wilhelm Johannsen and his important place in the history of genetics (Falk 1991, 1995), but there is an aspect of Johannsen's concept of the (classical) gene that Falk has perhaps taken less account of. Consider the following quote (Johannsen 1923, 138):

> When we regard Mendelian 'pairs', Aa, Bb and so on, it is in most cases a *normal* reaction (character) that is the 'allele' to an *abnormal*. Yellow ripe peas is normal, the green is an expression for imperfect ripeness as can easily be proven experimentally, e.g., by etherization… The rich material from the American *Drosophila*-researches of Morgan's school has supplied many cases of multiple allelisms — most of all of them being different 'abnormalities' compared with the characters of the normal wild fly. . . To my mind the main question in regard to these units is this: Are experimentally demonstrated units anything more than expressions for local deviations from the original ('normal') constitutional state in the chromosomes?
> Is the whole of Mendelism perhaps nothing but an establishment of very many chromosomal irregularities, disturbances or diseases of enormously practical and theoretical importance but without deeper value for an understanding of the 'normal' constitution of natural biotypes?

To understand 'the gene' in terms of abnormal allelic variations that result in gross phenotypic transformations that have important medical or economical significance but do not 'get at' the core of the species-typic phenotype is indeed to use the concept of the gene as a kind of intervening variable, but a very specific kind of intervening variable. For Johannsen it was certainly *not* the case that 'a gene is a genome's way of making a trait' ([Beurton 2000, 296], quoted by Falk). Johannsen couldn't have been more clear in holding that a gene taken alone is *powerless*, that the phenotype is the result of the interaction of the genotype *as a whole* in the context of an environment. This sense of a gene as that which stands in a predictive relationship to some phenotypic outcome, but that is not thought of as a constitutive functional determinant of this outcome, is captured in the idea of a Gene-P (see Moss 2003). Genes-P, such as cystic fibrosis genes, and Marfan Syndrome genes, and breast cancer genes (BRCA1 or BRCA2), i.e., genes for phenotypes, serve as 'intervening variables' for elucidating the complex dynamics associated with phenotypic

expressions patterns that have been 'tweeked' by the absence of a certain 'normal' sequence (in one of any number of different ways), but they simply do not warrant the idea that the genome can be decomposed down to functionally (and thereby selectively) individuated units. Alternatively, one can now take the 'matter' of the genome and attempt to parse it in some way, but to do so one must bracket the idea that it will parse according to discrete phenotypic-functional specifications. Widespread contemporary practice in cell and molecular biology has been to designate stretches of DNA, that provide the templates from which mRNA transcripts are derived, as 'genes' for those transcripts (and any possible downstream RNA or protein derivatives as well). This is the sense of 'a gene' which is captured in the idea of a 'Gene-D' (Moss 2003). Inasmuch as the phenotypic consequence of any Gene-D is deeply context dependant, Gene-D is radically indeterminate with respect to phenotypic outcome.

Genome projects, as Fogle (2000) has described, use a 'flexible toolkit' in attempting to enumerate genes (Gene-D). The toolkit includes characteristics, such as the presence of an upstream promoter, or evidence of template role in producing an mRNA, which as individual criteria are neither necessary nor sufficient for identification of a gene, but which all told provide pragmatically useful criteria. As a flexible concept specified at the sequence level, Gene-D also plays the part of an intervening variable, but this time it is the relationship to some as yet contingent, underdetermined phenotypic outcome that needs to be empirically fleshed out, with the nature of the phenotypic ramifications apt to vary along with features of the particular cellular, tissue, developmental and/or environmental context.

In his attempt to forge a unified concept of the gene, Falk uses the idea of the genome as his point of departure and distinguishes it from the genotype. The genome for Falk is the matter without the form. It is the material substrate that can't be parsed into a genotype, he wants to claim, without functional specification. Falk is also playing with the idea of using the genome as a kind of phenotype on the model of classical genetics where the genome would then provide the window onto the underlying invisible entities that constitute the genotype. But Falk can't have it both ways. The genome can't simultaneously be the material substratum that is directly transmitted between generations and the phenotype without transgressing the most fundamental meaning that these terms have possessed since Johannsen coined them. The very point was to distinguish that which is chemically transmitted and relatively invariant from that which is a highly contextually-

contingent developmental achievement of the next generation. The meaning of technical words can surely and appropriately evolve over time but as of the present it as yet makes no sense to speak of the genome as a phenotype, so lets proceed on the basis of understanding the genome as, in the first instance, Falk's unspecified matter.

Falk attempt to reconstitute a unified, integral gene concept turns on two moves. First Falk hopes to bring the phenotype-function relationship to bear on molecular genes (Genes-D) by making the function in question be function *at the cell level*. So the first move is changing the relevant locus of 'function' from that of the whole organism to that of the cellular level. The next move is to borrow Peter Beurton's idea that evolution imprints functional specification, i.e., that of the genotype, onto the matter of the genome through natural selection. Genes are in this sense, according to Beurton's (2000) novel twist, evolutionary outputs rather than evolutionary inputs. Eschewing the idea that the genotype can ever be understood in purely structural terms, Falk turns away from the idea that a gene need consist of some stretch of continuous bases, even if progressive consolidation into such is, as Beurton seems to think, the larger evolutionary trend. But the real fulcrum of evolutionary, and thus functional, specification from Beurton's angle is consistent selective pressure toward some set of bases however they may be dispersed or distributed. The genotype, of which any particular genome is but a token, is an on-going evolutionary achievement, the impress of form onto genomic matter.

Falk, we've said, wants to redeem the idea of a continuous history of a single gene concept, as a particular kind of intervening variable, that turns on its identification with phenotypic function. As the ship of the gene has sailed into the sea of the genome the venue may have changed but the mission, for Falk, has persevered. In a footnote (10), Falk suggests that 'conflation of Gene-P and Gene-D may be a virtue' and clearly he means to reject any such analysis of the gene concept into separate and independent meanings. What I will argue is that with the first of Falk's moves, that of relocating the locus of function to that of the cell, Falk banishes Gene-P from his framework (and with it the entire sense of 'the gene' as it is used in certain disciplinary frameworks such as clinical genetics) and so can't assimilate it into his unified concept. However, with his second move Falk brings back Gene-P, albeit now in disguise and smuggled in through the back door, as the hidden motivating presupposition behind his (and

Beurton's) attempt to reconstitute the molecular gene as a functionally discrete unit. So my claim will be that not only has Falk failed to eliminate the Gene-P/Gene-D distinction, he has ultimately fallen prey to an other than virtuous temptation toward conflation.

Falk wants us to conceive of the structure of the genome, i.e., the impress of the genotype onto the matter of the genome, as functionally specified because natural selection designed it and if natural selection designed it then by force of etiological definition it is functionally specified. In other words if certain sequences are being uniformly selected for over time then it must be because they have adaptive value and therefore they must be serving a specific adaptive function – but does this all really follow? Downes has given us a picture of the coding units of the genome on the model of a Gene-D concept. Now if Downes's genes, such as Drosophila Sxl, Drosophila DSCAM, and the mammalian NCAM, with their multi-splicing potential, can be identified with Falk's functionally specified genotypic units then is it the case that Falk has succeeded in doing what I've argued can't be done which is to identify Gene-P with Gene-D? As pertains to the first move: Falk has switched his frame of reference for what counts as a gene for a phenotype from that which tracks an organismic trait to that which performs a function within the cell. Now there is no intrinsic problem with doing this but in so doing one leaves out that entire domain of what I've referred to as Gene-P. Within the confines of the cell there is no gene for blue eyes, no gene for cystic fibrosis, no gene for Marfan syndrome, etc. These identifications are realized only at the whole organism level as complex phenotypic outputs (although of course they can then be tracked back to the cell and translated into Gene-D language at which point they take on a different meaning and type of specificity [Moss 2003]). So in making this move Gene-P slips out of Falk's conceptual reach and thus can't be assimilated into his cell-level model. As to the second move, Falk (following Beurton) helps himself to the assumption that the parsing of the genome in evolution will result in discrete functional (if not structural) units at the cellular level by drawing upon a kind of aprioristic logic and it's here where Downes's more empirically oriented approach can help to illustrate the problem. The evolutionary trend toward more variability in splicing – toward more proteomic bang for the genomic buck – is exactly a move away from anything like a discrete functional correlation, even at the cellular level, with regard to coding sequence. I've given an illustration of this with the example of the NCAM gene that Downes

referred to. At different stages of development and in different tissues, the NCAM gene – a Gene-D, provides the resources for the synthesis of proteins that in some cases help to hold adjacent cells together, but in other cases, cause them to stay apart. What function it is performing, and whether the NCAM protein is performing in an aberrant or salutary fashion is not determined at the level of the sequence itself but only in and by the wider developmental and physiological context. The NCAM gene is naught but a resource in itself, the consequence of which is indeterminate even at the cellular level. Evolution has resulted in genomes that provide resources for organisms and this is what the Gene-D concept means to capture. But a genetic resource is not tantamount to a fixed determination for a phenotypic trait. There is no reason to assume that evolution will result in genomic units with fixed phenotypic consequences unless one takes it that the 'invisible hand of natural selection' somehow already has Gene-P in mind. What many lines of molecular-level research suggest is that what has been selected for in metazoan evolution is the enhanced developmental *capacity* of the whole organism. A proliferation of mRNA splicing and thus protein isoform possibilities does not reflect further functional specification of so much as a single nucleotide, let alone a series of same dispersed or otherwise, quite to the contrary. What one sees in Downes' examples are a diversification and proliferation of potential functions associated with the very same nucleotides. It is the developing organism that realizes the enhanced capacity, as increased sensitivity and/or flexibility. Functional specification remains indeterminate at the level of Gene-D. This perspective holds good in relation to the latest findings from work in functional genomics. When one attempts to define the function of molecular genes (Genes-D) on a genome-wide basis what is found is a probabilistic relationship of any gene to networks of other genes that may then be associated with some function. 'These probabilistic connections arise in part from our experimental uncertainties in connecting genes together but may also reflect the stochastic nature of protein function, with finite copies of proteins forced to participate between alternative tasks in a cell. 'What a gene does' is defined by where it resides in the network and the probabilistic paths that link it to features' (Fraser & Marcotte 2004). Likewise, it is assumed that all or most genes are pleiotropic with respect to function, so from the genomic angle of view, any particular gene is multiply indeterminate with respect to function, i.e., *which* function and *whether or not* it does so. For all but the most orthodox this shouldn't wrangle sensibilities. Organisms survive and

flourish on the basis of their overall capacities. Gene-P and Gene-D can still play the role of intervening variables, but just in interestingly different ways. Where Gene-P discloses propensities within the complex dynamics of the organismic system to respond in certain predictable ways to a kind of perturbation, Gene-D constitutes a flexible resource whose functional role within any network of interactants can only be appreciated *after the fact*.

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