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Representing Genes: Classical Mapping Techniques and the Growth of Genetical Knowledge

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Classical genetics and its relation to molecular biology have been subject to extensive philosophical discussion. Most of the debate so far has been focused on the question of whether classical or "transmission" genetics has been reduced, in some sense, to molecular biology.[1] This question was motivated by a notion inherited from logical empiricist philosophy of science, namely that the growth of scientific knowledge consists in (derivational) reduction of statements from the precincts of an older (but mature) theory by those of a more advanced theory. However, no consensus has been reached on where exactly on a reduction-replacement scale[2] the case of genetics should be located. I do not wish to add another one to the long list of elaborate arguments advanced on this problem. Instead, I shall try to open a new perspective on the growth of genetical knowledge by examining the role of classical gene mapping techniques.

In my view, one of the major shortcomings in philosophical discussions of genetics so far has been an inadequate account of the theoretical content of classical genetics. Many authors appear to have assumed that this content is somehow exhausted by a number of laws or explanatory schemata about the transmission of alleles from one generation to the next.[3] Classical genetics may contain such schemata (and perhaps even laws), however, this is not the whole story.[4] I suggest that a substantial part of classical genetic theory consists in representations of genetic structures which were derived from genetic, cytological and physical maps.[5]

According to R. E. Kohler's fascinating historical account of the development of Drosophila as a model organism, genetic mapping was invented as a tool to classify the large number of mutants discovered in Morgan's lab.[6] This may have been one of the practical benefits of mapping and may even have facilitated its rapid adoption in Drosophila laboratories, however, I propose to maintain that mapping also provided theoretical knowledge on the structure and function of chromosomes and genes. First, genetic, cytological and physical maps featured in some of the major theoretical debates

about the spatial organization, inner structure and function of genes. Second, at several historical occasions, theoretical claims made on the basis of maps were confirmed later by the development of new mapping techniques, which may provide grounds for scientific realism in genetics (if such grounds can be had). Third, gene maps[7] exhibited an increase in resolution as genetics progressed, and were eventually linked to the molecular level.

In this paper, I try to substantiate these three claims by examining five historical episodes in which mapping played a central role: (I) The debate between Castle and the Morgan group on the linear arrangement of genes, (II) the development of cytological mapping of insect giant chromosomes, (III) the unsuccessful attempt to elucidate the inner structure of genes by mapping complex loci in Drosophila, (IV) Benzer's fine structure maps of bacteriophage T4, and (V) Yanofsky's demonstration of colinearity of fine structure maps with amino acid sequences in E. coli. Special attention will be given to linearity assumptions and to various interrelations existing between maps prepared by different techniques. A discussion of the criteria under which map structures can be viewed as representations of genetic structures and of some possible implications for realism in genetics concludes this essay (VI).

I

The oldest gene mapping technique consists in counting the frequency of crossing-over events between two genetic markers. T. H. Morgan first realized in 1911 that, on the assumption that genes are arranged linearly on the chromosomes, the frequency of crossing-over between them could be used as a measure for the linear distance separating them.[8] This idea, perhaps one of the single most important theoretical advances that led to modern genetics, was used by A. H. Sturtevant in 1913 to produce the first genetic map, showing that six sex-linked factors of Drosophila could be arranged in a linear order on the basis of recombination distances.[9] However, Sturtevant was rather cautious in his interpretation of this result: "Of course there is no knowing whether or not these distances as drawn represent the actual relative spatial distances apart of the factors"[10], he wrote. Concluding his paper, Sturtevant suggested: "These results … strongly indicate that the factors investigated are arranged in a linear series, at least mathematically".[11]

The harmless-sounding qualification "at least mathematically" was a precaution on Sturtevant's part against possible criticism that there were no strong grounds for interpreting the recombination frequencies as physical distances on the chromosome. For Sturtevant realized that the occurrence of double crossing-over between two loci to be mapped would lead to an underestimation of the separation between distant factors if compared with the sum of the distances of factors located in between, which is actually what he observed in his crosses. Thus, the evidence for the linear arrangement of genes, at this point, suffered from two defects: (1) There was no direct evidence that physical distance on the chromosome was the cause of the observed strength of linkage, (2) there were deviations for long recombination distances which had to be accounted for by what W. E. Castle later termed the "subsidiary hypothesis" of double crossing-over.

These weaknesses in the Morgan-Sturtevant theory of linkage were fully exploited by Castle in his "rat trap" model of the chromosome.[12] Castle used recombination frequencies which were not corrected for double crossing-over to construct a three-dimensional map for the Drosophila X-chromosome. This model was subsequently criticized by H. J. Muller.[13] Muller noted that Castle's model, too, was based on an auxiliary assumption, namely that double crossing over does not occur.[14] In addition, Muller provided an interesting empirical argument against Castle's model: He first pointed out that any three strongly linked factors formed a straight line or at least a highly degenerate triangle if arranged geometrically according to the crossover frequencies.[15] For less strongly linked factors the map distance appeared shorter if calculated directly from the frequency of crossing-over between them in comparison with the sum of the distances based on the closely linked factors lying in between. However, the deviation from a straight line (i.e., from additivity) thus obtained was itself a function of the map distance, which is what one would expect on the hypothesis of double crossing-over. In contrast, this regularity was inexplicable under Castle's theory. As Muller pointed out, from the three-dimensional model one would expect exactly the opposite: Three remote factors in a three-dimensional model of the chromosome are more likely to fall in a straight line than three factors located in each other's proximity.[16] Presumably, Castle submitted to Muller's arguments, for he accepted the linear interpretation and the double crossing-over hypothesis when he published a linkage map of the second chromosome of Drosophila one year later.[17]

I suggest that the debate between Muller and the Morgan group on the one, and Castle on the other side was a theoretical debate on how to interpret genetic map data.[18] The central issue of these debates was whether the linear genetic maps that can be constructed from crossing-over frequencies can be viewed as representations of a linear genetic structure, i.e., a linear arrangement of genes on the chromosomes. This question is theoretical in at least two senses: (1) Since genes are not directly observable entities, their spatial arrangement had to be inferred from experimental data. However, Castle's "rat trap model" showed that there was more than one way of doing this. Like in other experimental sciences, the interpretation of the experimental data has to rely on auxiliary hypotheses about the processes occurring in the experiment, leading to underdetermination of theory by the data. In this case, the auxiliary hypotheses included the hypothesis of double-crossing over, as well as the hypothesis of a more or less linear dependence of crossing-over frequency on physical separation. To settle the issue of which was the correct interpretation of the data, Muller had to adduce additional evidence and an "inference to the best explanation" argument: The regularities in the deviation from additivity are best explained by the assumption of a linear arrangement of genes and the occurrence of double-crossing over. Underdetermination, auxiliary hypotheses, "inference to the best explanation"-arguments etc. are characteristic for controversies on the theoretical interpretation of experimental data. (2) The knowledge of the spatial arrangements of genes on the chromosome has explanatory value. In combination with known cytological facts about the behavior of chromosomes in meiosis, the linear arrangement of genes explains a number of phenomena, such as independent segregation, linkage, crossing over etc. Hence, the interpreted genetic map should be viewed as a central part of genetical theory.

Another question is whether the Morgan group's interpretation of the experimental data and the associated theory of the arrangement of genes was correct. Underdetermination has been used as an argument against scientific realism, or for methodological holism.[19] I therefore proceed to to discuss an episode from the history of genetic mapping which provided independent evidence for the linaer arrangement of genes.

II

Before 1933, recombination mapping was the only intra-chromosomal mapping technique available. Cytology before that time was limited to crude observations of whole chromosomes or some very conspicuous chromosomal features such as the famous knob on chromosome 9 of some strains of Zea mays.[20] Although this "crude" cytology was important for initial support for the chromosome theory[21] and for establishing the correspondence between cytological crossing over and genetic recombination, no intra-chromosomal cytological mapping was possible. This changed in the 1930s with T. S. Painter's technique of staining giant chromosomes prepared by squashing larval salivary glands.[22] After staining, such preparations exhibit fingerprint-like banding patterns which are unique for each chromosomal region. Translocations, duplications, inversions and deletions are readily identified by comparing the banding patterns between salivary gland preparations obtained from different strains of insects. By using various chromosomal rearrangements, Painter was able to assign some loci from the Drosophila melanogaster recombination map a position on the giant chromosome. Thus, for the first time, it was possible to compare two maps prepared with different methods. As Painter pointed out in his short communication in Science (1933): "Geneticists will be interested … in the close correspondence between the cytological and crossover maps."

The fact that Painter's first salivary chromosome map as well as the more detailed map published shortly afterwards by C.B. Bridges[23] exhibited colinearity of the recombination map and the cytological map (to the extent of the number of loci that were mapped by both methods) is remarkable, given the debates on the linear arrangement of genes that went on 15 years before.[24] The sense of "colinearity" employed here needs some elaboration: First, the distances on the recombination map and those on the cytological map are not fully proportional. This would only be true if the probability of crossing over was the same along the whole chromosome, which is not the case. But the linear order of loci on the two maps was the same.[25] There was therefore a one-one mapping from one map to the other. In other words, the two maps were isomorphic. Second, the physical distances determined from cytological mapping of salivary chromosomes cannot be expected to correspond to the physical separation of the loci in meiotic metaphase chromosomes (where crossing-over takes place), as the latter represent a different state of the chromosomes. Therefore, three possible relationships of recombination and cytological maps need to be distinguished: (1) absolute physical separation, (2) relative physical separation, and (3) linear ordering. Genetic and cytological maps can only be expected to correspond with respect to (3) and approximately with respect to (2). But all that is required for there to be an isomorphism between the maps is (3).[26]

This isomorphism would be a striking coincidence if recombination- and cytological maps were experimental artifacts. However, in order to draw any conclusions from this coincidence, it has to be established that the two methods are independent.

How are genetic loci located on salivary chromosomes? Painter listed three basic techniques, which all make use of the "synapsis" of salivary chromosomes isolated from heterozygous larvae: (1) If one sister chromatid contains an inversion, this will lead to readily visible handle-like structures in the salivary gland preparations.[27] Comparison of such synapses from heterozygotes with regular salivary chromosomes allowed Painter to localize the breakage points of inversions such as delta 49. The knowledge of which loci are affected by the chromosomal rearrangement allows inferences as to their location to the left or right of the breakage points. (2) Larvae heterozygous for deletions will show asymmetrical loops in salivary chromosome preparations. Knowing which loci are affected by the deletion, one can locate them in the looped region of the synapsis.[28] (3) Translocations where whole pieces of chromosomes have moved to a different chromosome can be identified by the distinct banding patterns as well as by the structures they generate in heterozygotes. Again, one has then only to know which loci are affected by the translocation to locate them on the salivary chromosome. Using these techniques, Painter and later Bridges generated fairly detailed cytological maps of the known genetic loci on the four Drosophila melanogaster chromosomes.

What theoretical presuppositions are required in order to prepare these cytological maps? I suggest that the following knowledge is necessary for interpreting the cytological data: (1) Knowledge concerning the constancy of salivary chromosomes. They form the same "landmarks" in each larva of a given genotype.[29] (2) Knowledge about the nature of synapsis. In heterozygotes, synapsis may be incomplete due to chromosomal rearrangements and deletions. Incomplete synapsis is visible under the microscope and leads to distinct structures (handles in inversions, loops in deletions, appendages in translocations). (3) Knowledge about the phenotypic differences caused by the genes to be mapped, such that specific mutants can be identified. (4) Correlations between certain phenotypes and certain chromosomal structures as seen under the microscope. For instance, Painter's associate Mackensen found two deletion mutants displaying the vermillion phenotype. The deletions were readily identified on salivary chromosome preparations and they slightly overlapped. This allowed Mackensen to locate the vermillion locus to the region of overlap of the two deletions.

Obviously, if a large amount of data on the same loci in different rearrangements is analyzed, the cytological map will be overdetermined. The localities of the genes as determined by different deletions, inversions and translocations had better turn out the same. To my knowledge, none of the Drosophila-workers involved in salivary mapping faced any substantial difficulties in this respect. Because of overdetermination, the coherence of the cytological map for a given chromosome constitutes a first important test of the soundness of this method (the same is true, obviously, for recombination mapping and for all other mapping techniques).

Two points need to be emphasized: First, no interpreted data from recombination mapping are required to prepare the cytological map.[30] Second, cytological mapping

could be done, in principle, without knowledge of the correlation between recombination frequency and physical separation in meiotic metaphase chromosomes, the mechanism of crossing over (chiasmata), the correction factors for double crossing over etc. These are the theoretical presuppositions that inform the interpretation of data from crossing experiments used for recombination mapping, as was explained in the last section. Hence, recombination mapping and cytological mapping critically depends on genetic theory, however, it involves different parts of that theory, which seem to be independent in the sense that the truth or falsity of one set of theoretical propositions does not entail that of the other set.[31] For instance, even if crossover frequencies were not causally related to the physical separation of loci (i.e., if the Morgan-Sturtevant theory of linkage was false), this would not affect the interpretation of cytological data. Hence, the colinearity of the two maps is a coincidence which is not explained by the fact that they both depend on the same theory. It is better explained by the hypothesis that the map isomorphism is generated by the existence of an objectively existing structure corresponding to the map representations.

The episode presented in this section appears to be one where genetics has made progress in the sense that a new technique both confirmed and expanded the theoretical knowledge about the spatial arrangement of genes. The basis for this was the existence of an isomorphism between different kinds of maps, as I have tried to demonstrate. In the next section, I shall discuss a case where no such isomorphism could be found, which led to the rejection of a particular interpretation of genetic maps.

III

As I have shown so far, geneticists had amassed a robust body of knowledge bearing on the arrangement of genes on chromosomes by the early 1930s. It was therefore natural to assume that the techniques of genetic mapping could be used to elucidate the nature of the genes themselves. As we know now, such knowledge would eventually not come from studies in Drosophila, but from progress in the genetic analysis of microorganisms, as well as from biochemical studies of nucleic acids and protein synthesis. Nevertheless, it is worth to have a look at the unsuccessful attacks on the gene launched from the Drosophila labs.

In well-behaved genetic loci, the possession of two recessive alleles by a genotype which shows the wild phenotype indicates that the two alleles reside at different loci, i.e., they represent different genes. The genetic defects carried by the two alleles complement, since each allele has a dominant partner which prevents the loss of function. If two recessive alleles produce, in a heterozygote, a mutant phenotype, this usually indicates that they are alleles of the same gene. In Drosophila, however, some loci showed a more complex kind of behavior. For instance, N. P. Dubinin analyzed a series of alleles at the achaete-scute locus, which affects bristle numbers on different parts of the adult fly body.[32] In the homozygous condition, each of these mutant alleles produces a characteristic bristle pattern. In heterozygotes, the following regularity was disclosed: Different scute alleles consistently produced a phenotype in which the phenotypic manifestations common to both alleles were expressed, whereas the traits with respect to which the two alleles showed different manifestations were in the wild-type condition. Hence, these alleles were non-allelic according to some traits (those traits that were normal in the heterozygote) and allelic for some other traits ac-

cording (those traits showing the mutant phenotype). These “pseudo-alleles” could be arranged in a series ranging from alleles being almost fully non-allelomorphic with respect to each other (i.e. the heterozygous flies were almost normal) to alleles which seemed to be allelomorphs in that the heterozygous showed the full mutant phenotype. Because of this discrete array of degrees of allelomorphism, this phenomenon was termed “step-allelomorphism” and characterizes what was later termed “complex loci.”

The interpretation given to the phenomenon of step-allelomorphism by Dubinin was the following:

“The complete reversion to wild type of a certain portion of characters of scute transgenation in corresponding compounds with other scute allelomorphs gives the idea that the corresponding parts of the transgenes are in a heterozygous condition in these compounds. This suggests the hypothesis that a partial non-allelomorphism is dependent on some amount of non-coincidence among the corresponding allelomorphs.”[33]

(The terms “transgene” and “transgenation” refer to mutant genes.) The interpretation given by Dubinin was straightforward: Non-allelomorphism usually indicated the existence of two independent genes, which may also be separable by crossing over, if they are not located too closely to each other. Allelism, in contrast, meant that one was dealing with a single gene. Hence, the idea that in step-allelomorphs there may be something like allelism between parts of a single gene was quite natural. These gene parts would determine different traits in the fly. If two different alleles came together, those traits would revert to wild-type whose determining gene parts - called “centres” - were not shared by the two alleles. In contrast, those traits determined by centres shared by both alleles would remain in the mutant condition. The fact that the step-alleles could be arranged in a linear series suggested that the “centres” were arranged linearly within the gene: “In connection with the linear disposition of these centres, the non-correspondence of the characters of two transgenes is a result of their different localisation inside the basigene” (ibid.).

Dubinin found that the recessive lethal achaete^{2} was partially allelic to scute^{3}, i.e., it reverted, in the heterozygous compound with this allele, some of the mutant traits produced by scute^{3}.[34] Similar cases of partial allelism involving other alleles of the scute-achaete series are described by Dubinin in the same paper, and he was able to produce a linear scheme for the arrangement of his “centres.” However, Dubinin’s model of the achaete-scute-locus quickly encountered difficulties, because some alleles could not be accomodated in the linear scheme.[35]

Step-allelism is basically what is called complementation today, and it can be used to construct “complementation maps” by ordering the alleles in a series of arrays that overlap according to the degree of expression of the mutant phenotype.[36] Complementation later became the basis of S. Benzer “cis-trans” test (see below). However, in contrast to Benzer, Dubinin and his colleagues were unable to separate their mutational sites by crossing-over and, hence, to produce a functional map of the genetic regions in question. The reason for this is that Benzer worked with microorganisms, where - due to the very large number of individuals present in the experimental cul-

tures - rare crossing-over events between closely adjacent markers can be detected. In Drosophila, the resolution of recombination maps is limited by the number of flies that can be analyzed in test-crosses.

A fresh attempt to attack the nature of genes by studying complex loci had to await the 1950s - the decade of Watson and Crick and of most remarkable advances in fine-structure mapping of genes by using micro-organisms. Although complex loci did not lead to any insights into gene structure, for my argument I shall need the glorious failure of trying to establish a correspondence between complementation and recombination maps in Drosophila.

E. A. Carlson studied the complex locus dumpy, the first allele of which (truncate wings) was found as early as 1910 by Morgan. Whereas all previous attempts to separate the mutational sites defined by step-alleles through recombination were unsuccessful, Carlson obtained recombinants and prepared a recombination map for 15 markers belonging to the dumpy series.[37] The question was whether the map distances between the members of the dumpy series would show any correlation to the strength of complementation between the alleles. A theoretical model that could account for such a correlation might, for instance, explain the allelic interaction in the heterozygotes in terms of the physical distance between two mutational sites.[38]

But there was no such correlation: "The relation between the mutants is not based on a phenotypic or obvious physiological gradient of ordered subloci, but appears, rather, to be scrambled".[39] Hence, a model of linearly arranged "subgenes" or "subloci" which would interact according to their spatial separation in the chromosome was not supported by the data.

Carlson felt strongly compelled to compare his complementation analysis of complex loci in Drosophila with Benzer's elegant functional maps of the rII region in bacteriophage T4.[40] Although phage has quite a different genetic system, a kind of genetic analysis resembling classical Mendelian hybridization is possible in these organisms. Phage genomes can be observed in a "heterozygous" condition by infecting E. coli cells with two different strains of phage. As is well known, Benzer performed such double infections with various mutants in the rII region of T4, which fail to produce lysis plaques on plated E. coli K cells. In the infective cycle, phage DNA can undergo what microbial geneticists call "general genetic recombination" (as opposed to site-specific and "illegitimate" recombination) at homologous sites on the genome. Like in Drosophila, the frequency of such recombination events can be used to calculate linear maps of mutational sites on the phage genome. In addition, Benzer devised his famous "cis-trans" test which provides a basis for grouping mutational sites into functional regions on the genome which Benzer termed "cistrons." Why was this not possible with complex loci in Drosophila?

Carlson explains: "Phage cistron is too simple, as it stands, to account for the dumpy series"[41], the reason being that "the presence of allelism, complementation, and pseudo-allelism in the same region makes the cistron a more complex unit to define on a rigid cis-trans functional test".[42] Indeed, the case was much simpler in the phage studies: All mutational sites could be separated by recombination and thus mapped.

Recombination mapping was much less tedious with phage, as very large numbers of individual phage genomes could be handled in the laboratory, thus yielding highly reliable recombination frequency data.[43] At the dumpy locus, not all markers were separable by recombination and the accuracy of the recombination map was limited by the number of flies that could be handled in crossing experiments.

But most importantly, there was no allelic interaction between cistrons in the rII region, which, in retrospect, makes one wonder why Carlson was tempted to compare his data to Benzer's. The phage cistrons are functionally independent: Mutations mapping in the same cistron produced no (or very little) lysis in trans and extensive lysis in cis.[44] Mutations located in different cistrons showed lysis both in cis and trans. In contrast, dumpy pseudo-alleles showed a vast range of different phenotypes in trans, i.e., in the heterozygous state, ranging from almost full complementation through intermediate phenotypes where the phenotypic effects not shared by both alleles complemented, to cases where most of the mutant traits, in these cases shared by both alleles, were expressed. Finally, the extent of complementation between two pseudo-alleles showed no correlation to their linear distance as read from the recombination map. It was thus impossible to group the dumpy mutations into functional units or cistrons.

In 1960, Carlson surprisingly found that the complementation data from the dumpy locus could be represented in a circular complementation map.[45] This was an entirely formal procedure with no intended physical interpretation: "It is, of course, unlikely that these non-linear maps have any physical correspondence to gene structure".[46] Carlson used the circular complementation maps only to disprove the then widespread belief, which was a direct consequence of Benzer's success, that there has to be a colinearity between complementation and recombination maps:

"It seems […] that the alterations in the gene molecule and the functional disturbances produced in its final product are more complex than can be accounted for on the assumption of mere colinearity between genetic recombination and genetic complementation."[47]

Whatever these "functional disturbances" were, they prevented a simple analysis of complex loci into cistrons.[48]

This brings us back to the issue of representation and interpretation of map data. Any such data can be represented in some kind of a geometrical pattern. How simple that pattern turns out depends on the data. The question is always - in genetic mapping and probably elsewhere - whether that geometrical pattern represents some objectively existing structure. In the case of mapping whole chromosomes, the linear interpretation of the map data was decisively confirmed because the same linear sequence of genes was found in an isomorphic fashion in a different map prepared with different methods. No such relation could be found between recombination maps and complementation maps at complex loci. The geometrical patterns generated by complementation mapping at complex loci thus failed to be confirmed and were dropped from genetical theory.

IV

In the last section, I have discussed some mapping experiments which - though ingeniously designed - failed to reveal any insights into the nature of individual genes. But the question remains to what extent classical genetic methods, including phage genetics, helped to elucidate gene structure. The studies by Benzer already mentioned certainly need to be examined in this respect, but also a number of related experiments which marked the transition from classical transmission genetics to the molecular era.

Benzer's fine structure map of a genetic region in bacteriophage led to a number of important insights: First they showed that a number of mutable sites in the rII region of T4 can be assigned to two functionally separable units or cistrons. Second, it was possible to construct a recombination map displaying, at least in some regions, the well-known property of additivity[49] of recombination distances, suggesting - like in Sturtevant's first map of a Drosophila chromosome - a linear arrangement of mutational sites. It was already widely accepted, in 1955, that phage chromosomes are a linear duplex fiber of nucleic acid, the "genetic information" residing in the sequence of the four constituent nucleotides. All Benzer had to assume to interpret his maps as representing the physical structure of genes was (1) that the T4 genome was a single DNA molecule and (2) that the recombination frequency between two sites is approximately proportional to their physical separation on the DNA molecule, which seems reasonable; however, especially the second assumption is problematic on strict methodological standards, since there was no independent evidence justifying it (see below).

Although it was Benzer's declared aim to "extend genetic studies to the molecular (nucleotide) level"[50], this is what he did not achieve in 1955. For instance, his data did not allow him to decide whether there were genuine point mutations, i.e., single nucleotide substitutions among the mutants analyzed.[51] In addition, Benzer was able to narrow down the "recon" or unit of recombination to "the order of one dozen nucleotide pairs," but there was no knowing whether this was due to the experimental limit of resolution or due to the nature of the recombination process.

Most importantly, Benzer had demonstrated a certain relation between the "complementation map" and the recombination map. This notion requires some elaboration, since Benzer's pair-wise complementation of genetic markers did not produce a "map" in an obvious sense. It only allowed the assignment of genetic markers to complementation groups, which did not form a series in the case Benzer was studying.[52] Therefore, the result of a complementation test is a matrix lacking any dimension that could be interpreted as some spatial structure. But there exists an interesting topological relation between the actual matrices that Benzer obtained and the linear recombination map (see Fig. 1). The fact that Benzer's complementation groups could be embedded in the linear genetic map - which has an obvious physical interpretation, namely the string-like DNA molecule - means that Benzer provided the first evidence that genes/cistrons, represented by two distinct regions on the genetic map, are linear structures. For if the complementation analysis had produced a structure which can only be embedded in a circular or some even more complex structure, and yet there were grounds to believe that the genetic map has a linear physical interpretation, Benzer would have had to conclude that something strange was going on in the complementation test. The cistrons could in this case not considered to be segments of a lin-

ear structure. This, as we have seen, was Carlson's fate when he was trying to extend Benzer's analysis to complex loci. As I have tried to explain, the reason for this was that Carlson was dealing with inter-allelic complementation, whereas Benzer observed intra-allelic complementation.

Furthermore, Benzer suggested that "each segment might control the production of a specific polypeptide chain, the two chains later being combined to form an enzyme".[53] Although Benzer did not use the word "gene" a single time in the 1955 paper, on the assumption of the "one gene-one polypeptide" hypothesis it follows that he has located the spatial boundaries of two "genes" on the recombination map. If the recombination map could be translated into a physical map of DNA, Benzer would have been the first to have delimited a gene as a molecular entity.

However, as already indicated, there was no independent evidence to confirm that Benzer's recombination maps represented physical distances on a DNA molecule. Second, it was not known how exactly a DNA segment was related to its functional product, a polypeptide chain.[54] In fact, it was not even certain whether the genetic determinants of proteins were contiguous on the DNA molecule.[55] A number of independent mapping experiments done in the 1960s provided additional information relevant to these issues.

A. D. Kaiser used bacteriophage λ to correlate points of breakage in mechanically treated DNA molecules to the recombination map.[56] If a solution of DNA is stirred vigorously, hydrodynamic forces can break DNA molecules in half. With phage λ DNA, the fragments produced by this procedure retain their biological activity if co-infected with a helper phage. Kaiser mapped points of breakage in sheared phage DNA by using several genetic markers on the sheared DNA and on the helper phage (mechanical breakage of the DNA molecule destroys the linkage between markers located to the left and right of the point of breakage, respectively). Remarkably, Kaiser found the physical breakage map prepared by this method to be colinear with the recombination map.

This experiment is interesting for the following reason: Kaiser was working with phenol-extracted DNA, i.e., pure DNA. The physical integrity of the DNA fragments produced by shearing was established by careful sedimentation studies using an ultracentrifuge. Hence, Kaiser was dealing with the phage chromosome as a molecular entity. With his co-infection experiments he established the position of genetic markers on this physical entity. The recombination map, in contrast, is based on the biological process of crossing-over, which is assisted by enzymes of the bacterial host which were not yet known. Hence, the two maps for which a relation of colinearity was established were based on physically distinct processes and can therefore be considered to be independent methods in the sense discussed in the context of cytological mapping (section II). Again, the correspondence of two maps prepared by different procedures confirmed their interpretation as a representation of a linear genetic structure. Benzer's assumption that recombination between two markers is roughly proportional to their physical separation on the DNA molecule was thus retrospectively justified.

V

The final mapping experiment I wish to discuss is the study by C. Yanofsky demonstrating the colinearity of gene structure and protein structure.[57] Yanofsky and his co-workers started from the already well-substantiated hypothesis that genes specify the structure of proteins. This hypothesis was supported, among other evidence, by G.W. Beadle's and E.L. Tatum's famous study[58] of mutations affecting the metabolism of Neurospora and by the first studies on the mechanism of protein synthesis which eventually lead to the "cracking" of the genetic code and to the elucidation of the role of various kinds of ribonucleic acid in the biogenesis of proteins; now generally recognized as part of the "molecular revolution" in biology.[59] However, when Yanofsky and his co-workers took on their investigation of tryptophane auxotrophs in E. coli, there was no direct evidence for a colinear relationship between the sequence of nucleotides in DNA and the sequence of amino acids in a protein. For the purpose of the present study, Yanofsky's experiments are interesting because they provide a link between classical genetic mapping methods and molecular biology.

Yanofsky's group prepared a detailed genetic map of the fine structure of the tryptophane A gene in E. coli. Mutations in this gene lead to a deficiency of active molecules of the A protein of tryptophane synthetase, a very complex aggregate of proteins enabling the cell to produce its own supply of the amino acid tryptophane. Mutations in this gene are detected by a strain's inability to grow on a medium lacking tryptophane, whereas it grows perfectly well on a medium containing this amino acid. 16 such mutations were mapped by Yanofsky et al. by using general phage transduction. This method uses the ability of some bacteriophages to transfer pieces of the host bacterial chromosome to a new host. For instance, if trp^- cells are infected with phage produced by a trp^+ strain, some phage particles may contain an intact trp^+ gene that was excised from the host chromosome and got inserted into the phage genome. trp^- cells which are infected by such a phage particle may undergo homologous recombination between the trp^+ copy carried by the phage genome and the defective copy in host chromosome, resulting in a trp^+ phenotype. Alternatively, two different trp^--mutants can be "crossed" that way, and the frequency of recombination can be used to estimate the distance between the two mutations, like in Mendelian crosses in higher organisms. Yanofsky et al. carried out a whole series of crossing experiments between various trp^- mutants, including also deletion mutants. They were able to arrange the mutational sites in a linear order by combining the results of various test crosses.

Amino acid substitutions in protein A were determined for all the mutants by chemical primary structure determination. Strikingly, the positions of amino acid replacements were in the same linear order as the corresponding mutations in the recombination map. The ratio of genetic map distance and peptide residue distance varied between 0.01 and 0.05 with most of the values being approximately 0.02. That this ratio be exactly the same for all mutants was not to be expected, since the recombination data already indicated that recombination frequency is not fully constant along the gene. Nevertheless, Yanofsky et al. concluded: "It would appear […] that distances on the genetic map are representative of distances between amino acid residues in the corresponding protein".[60]

The colinearity of genetic maps and amino acid sequence was an important piece in the emerging picture of the molecular biology of the gene. The study by Yanofsky and co-workers provided the first direct evidence that what a gene qua a linear arrangement of mutable sites does is to specify the linear order of amino acids in a protein. I wish to emphasize, in particular, that the gene concept invoked here is not a molecular one.[61] Although Yanofsky et al. accepted the "correspondence of the genetic map with the sequence of blocks of nucleotides in DNA"[62], this presupposition was not critical for their experiment. The gene, for them, was a functional unit on the genetic map, pretty much in the sense of Benzer's "cistron." It was the gene in this sense that was shown to "encode" a protein's primary structure, to use the now widespread terminology. Although Benzer's gene concept might be different at least intensionally from the "classical" concept of the Morgan school, it was still not a molecular concept. Like the "classical" concept, it was based on genetic mapping. However, the subsequent elucidation of the genetic code and the concomitant insights into the mechanism of protein synthesis fully confirmed the thesis of the colinearity of gene and protein structure (in prokaryotes).

With this additional knowledge, the following relation was established (see Fig. 2): The sides of this triangle represent relations of colinearity. Side a was established by the study by Yanofsky et al. just discussed. Side b was established a few years later by the discovery of the mechanisms of protein synthesis (the theory of protein synthesis clearly entails that nucleotide sequence and amino acid sequences are colinear).[63] Side c is supported, for instance, by Kaiser's physical breakage map of phage chromosomes discussed above. The interesting thing about this triangle is that genetic maps are part of it. It suggests a rather intimate relation between the classical genetic map and the "molecularized" gene.[64] In addition, this triangle finally establishes what has been suspected by some geneticists as early as the 1930s (e.g., Dubinin, see above), namely that genetic information is linear all the way down to the molecular level. The classical gene map was finally shown to be a representation of a DNA molecule.

The triangle also provides an argument that fine structure maps prepared by measuring intragenic recombination frequencies represent something real. Taken by itself, all the fine structure map does is to arrange a number of mutable sites in an imagined space which is theoretically assumed to be the gene (i.e., the cistron). The protein sequence is entirely independent of the fine structure map. It is deduced from experimental data whose interpretation requires nothing but theories from organic chemistry. Hence, it would be a stunning coincidence if the fine structure map did not represent a mind-independently existing structure and yet fit into the colinearity triangle. The triangle demonstrates that what the fine structure map represents are mutational sites on a linear stretch of nucleotides which can cause differences in the amino acid sequence of a protein.

Obviously, there are many molecular facts known today which complicate this simple picture. First, there are mutational sites which do not cause amino acid substitutions. Mutations in a cis-acting regulatory element, for instance, may be expressed phenotypically by altering the regulatory element's binding affinity for trans-acting factors controlling transcription. Second, it is known today that not all genes are spatially contiguous. Eukaryotic genes, in particular, frequently contain intervening sequences

or “introns” which do not code for protein. Hence, the colinearity of nucleotide sequence and protein sequences is restricted to parts of the gene only, namely its exons. A gene may even encode different polypeptides via alternative splicing. These facts occur at a level of detail which are clearly beyond the reach of classical mapping methods. Gene structure and function are much more accessible today by the powerful tools of recombinant DNA technology, which I did not discuss because I wanted to examine the role of classical mapping methods. However, as should be evident from the historical episodes discussed in this section, genetic mapping played an important role in the “molecular revolution.”

VI

I have tried to show in the preceding sections how classical gene mapping techniques produced a series of representations of genetic structures of increasing resolution, i.e., at an increasingly smaller scale of chromosomal organization. There are many more classical mapping experiments which could not be discussed here for lack of space. To mention just one additional example, the circular structure of the E. coli chromosome, which can be observed in electron micrographs as well as by molecular techniques today, was predicted from genetic mapping experiments alone. However, the historical episodes discussed here should suffice to establish the following conclusions: (1) Gene maps are a central part of classical genetic theory.[65] On their basis, it was shown, for instance, that eukaryotic genes are arranged linearly on the chromosome, that complementation series did not represent the physical structure of complex loci, and that prokaryotic genes are linear structures (i.e., segments of DNA) which encode the amino acid sequence of proteins. Many more genetic structures and processes were revealed by classical mapping techniques which could not be discussed here, e.g., the existence of gene duplications or of geographical chromosomal variation, which had momentous implications for evolutionary theory.[66] (2) There exist various relations between different kinds of maps: the isomorphism of recombination- and cytological maps of whole eukaryotic chromosomes, an embedding relation between prokaryotic fine structure maps and the complementation matrix, a colinearity relation between genetic and physical map in phage λ, and another colinearity relation of fine structure map and amino acid substitutions in the E.coli trp gene, which was at the same time predicted by the theory of protein synthesis. Eukaryotic complementation maps from complex loci are not related in any systematic way to some other representation structure. (3) Through the mapping experiments discussed in IV and V, genetic maps were shown to be functional representations of DNA molecules. The representation is functional because it does not represent structural-molecular features, but sites that can genetically affect the organism’s phenotype if they are altered. I suggest that this fact constitutes one of the most significant inter-theoretic relations between classical and molecular genetics; whether or not this relation should be viewed in terms of “reduction” I shall not discuss here.

The correspondence relations between different kinds of maps that I have described may have some interesting methodological implications. As I have already indicated (section II), the independent confirmation of the linear arrangement of genes by cytological mapping could be used to argue for the reality of the structures represented in the corresponding maps. A possible strategy for such an argument is the following: If the corresponding maps did not represent some real structure, the fact that they are

isomorphic (in the sense outlined) would be a striking coincidence. This coincidence is best explained by the assumption that there is a common cause for the outcome of the independent mapping experiments, namely the existence of a linear sequence of genes on each chromosome.[67] Whether or not one endorses such arguments for realism, cytological mapping clearly provided independent empirical evidence for the theory of the linear arrangement of genes.

The cases discussed in sections III - V are more difficult to interpret. As I have pointed out, Dubinin's linear model of the arrangement of pseudo-alleles at complex loci (section III) was an attempt to extend mapping and complementation analysis from whole chromosomes into individual genes.[68] The linear model collapsed because of alleles that did not fit into it. Several decades later, Carlson's circular map of a similar complex locus nicely systematized the experimental data, however, it was not plausible as a representation of a genetic structure. The main reasons were, first, that no independent evidence existed for circular structures within genes. Second, such structures could not be matched with the linear DNA molecule (in contrast to Benzer's map), which was widely accepted as the carrier of genetic information come the 1960s. Hence, at this point, molecular biology already constrained genetic theory, although the two domains were not yet as closely knit together as they are today. Later, molecular biology was able to explain some cases of inter-allelic (i.e., intragenic) complementation, and why it failed as an attempt to elucidate the structure of genes.

In contrast, Benzer's fine structure map of a region of the bacteriophage T4 genome admitted of a simple linear interpretation. In addition, these maps could be divided into two functional subregions or "cistrons", which defined two linear segments on the genetic map - presumably two genes.[69] In sections IV and V, I have demonstrated that Benzer's studies were supplemented by two important findings: First, genetic maps were shown to be colinear with DNA molecules by physical mapping of a phage genome, strongly suggesting that the cistrons (or genes) are segments of DNA. Second, through the demonstration of colinearity of fine structure maps and protein sequence, cistrons/genes were shown to specify the primary structure of proteins. This was a remarkable result, given that molecular biological theory predicted exactly that from what was already known at that time on the mechanism of protein synthesis ("DNA makes RNA makes protein"). Thus, again, there was a correspondence of results from genetic mapping experiments and an independent body of evidence.[70] If the genetic map did not faithfully represent important aspects of gene structure, the fact that it fits into independently supported theories of molecular biology would be most unexpected. Thus, a "miracle" argument for scientific realism[71] can be applied to gene maps.

A comparison of the unsuccessful mapping experiments (section III) with the successful ones (sections I, II, IV and V) is also interesting for the issue of scientific realism: Why was the idea that map representations of complex loci represent gene structure given up, while the linkage maps of large chromosomal regions and the prokaryotic fine structure maps are still viewed as faithful representations? The reason, I suggest, is that in the successful cases there was independent support, both theoretical and experimental, for the assumption that the maps represent some real structure. This sup-

port consisted in the fact that these maps could be correlated with other maps which were prepared by different methods (e.g., cytological maps) and with predictions from independent theories (e.g., from molecular biology). No such support existed for the map representations of complex loci. For scientists, independent evidence for the existence of some theoretical entity or not directly observable structure makes all the difference in the world.

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Notes and References

[1]E.g., Balzer, W. and Dawe, C. M. (1986) 'Structure and Comparison of Genetic Theories: (1) Classical Genetics', British Journal for the Philosophy of Science **37**, 55-69; Balzer, W. and Dawe, C. M. (1986) 'Structure and Comparison of Genetic Theories: (2) The Reduction of Character-Factor Genetics to Molecular Genetics', British Journal for the Philosophy of Science **37**, 177-191; Hull, D. (1974) Philosophy of Biological Science (Englewood Cliffs: Prentice Hall); Kitcher, P. (1984) '1953 and All That. A Tale of Two Sciences', Philosophical Review **93**, 335-373; Ruse, M. (1976) 'Reduction in Genetics', in PSA 1974 (Dordrecht/Boston: Reidel), pp. 633-651; Schaffner, K. F. (1993) Discovery and Explanation in Biology and Medicine (Chicago: University of Chicago Press); Waters, C. K. (1990) 'Why the Anti-Reductionist Consensus Won't Survive the Case of Classical Mendelian Genetics', in PSA 1990, Vol. 1 (East Lansing: Philosophy of Science Association), pp. 125-139; Waters, C. K. (1994) 'Genes Made Molecular', Philosophy of Science **61**, 163-185.

[2]see Schaffner (1993), pp. 427-432

[3]see Balzer and Dave (1986); Hull (1974); Ruse (1976); and particularly Kitcher, P. (1989) 'Explanatory Unification and the Causal Structure of the World', in P. Kitcher and W. C. Salmon (eds), Scientific Explanation. Minnesota Studies in the Philosophy of Science, Vol. XIII (Minneapolis: University of Minnesota Press), pp. 410-505.

[4]C. K. Waters argues that the classical knowledge of regularities of gene transmission should be viewed as a set of investigative tools for the elucidation of the nature of chromosomes and genes, rather than as the core of a finished theory (C. K. Waters, 'What Was the Body of Knowledge Called Classical Genetics?', unpublished ms., dated 7/9/96).

[5]For some excellent studies on the early developments in genetic mapping see Darden, L. (1991) Theory Change in Science: Strategies from Mendelian Genetics (Oxford: Oxford University Press); Wimsatt, W. C. (1987) 'False Models as Means to Truer Theories', in M. H. Nitecki and A. Hoffman (eds), Neutral Models in Biology (Oxford: Oxford University Press), pp. 23-55; Wimsatt, W. C. (1992) 'Golden Generalities and Co-opted Anomalies: Haldane vs. Muller and the Drosophila Group on the Theory and Practice of Linkage Mapping', in S. Sarkar (ed), The Founders of Evolutionary Genetics: A Centenary Reappraisal (Dordrecht: Kluwer Academic), pp. 107-166.

[6]Kohler, R. E. (1994) Lords of the Fly. Drosophila Genetics and the Experimental Life (Chicago: Chicago University Press), pp. 54-58.

[7]I use the term "gene map" for all kinds of maps, because "genetic map" has a technical meaning in genetics (it denotes recombination maps).

[8]Morgan, T. H. (1911) 'Random Segregation versus Coupling in Mendelian Inheritance', Science **34**, 384.

[9]Sturtevant, A. H. (1913) 'The Linear Arrangement of Six Sex-Linked Factors in Drosophila, as Shown by Their Mode of Association', Journal of Experimental Zoology **14**, 43-59.

[10]ibid., p. 49

[11]ibid. p. 58

[12]Castle, W. E. (1919) 'Are Genes Linear or Non-linear in Arrangement?', Proceedings of the National Academy of Science **5**, 500-506; see also Carlson, E. A. (1966) The Gene. A Critical History (Philadelphia: Saunders), pp. 79-81; Darden (1991), pp. 153-157; Wimsatt (1987).

[13]Muller, H. J. (1920) 'Are the Factors of Heredity Arranged in a Line?', American Naturalist **54**, 97-121.

[14]ibid., p. 99

[15]ibid., p. 103

[16]ibid., p. 104

[17]Castle, W. E. (1920) 'Model of the Linkage System of Eleven Second Chromosome Genes of Drosophila', Proceedings of the National Academy of Science USA **6**, 25-32.

[18]That map distance is a theoretical concept was also noted by Wimsatt (1987), p. 36.

[19]See Duhem, P. (1954) The Aim and Structure of Physical Theory (New York: Atheneum), p. 185ff.; Quine, W. (1953) 'Two Dogmas of Empiricism', in From A Logical Point of View (Cambridge Mass.: Harvard University Press), pp. 20-46.

[20]Creighton, H. B. and McClintock, B. (1931) 'A Correlation of Cytological and Genetical Crossing Over in Zea mays', Proceedings of the National Academy of Science **17**, 492-497.

[21]Bridges, C. B. (1916) 'Non-Disjunction as Proof of the Chromosome Theory of Heredity', Genetics **1**, 1-52, 107-163.

[22]Painter, T. S. (1933) 'A New Method for the Study of Chromosome Arrangements and the Plotting of Chromosome Maps', Science **78**, 585-586; Painter, T. S. (1934) 'Salivary Chromosomes and the Attack on the Gene', Journal of Heredity **25**, 464-476.

[23]Bridges, C. B. (1935) 'Salivary Chromosome Maps', Journal of Heredity **26**, 60-64.

[24]Note that the salivary chromosome mapping technique also spectacularly confirmed Sturtevant's prediction that the mutant Bar represents a tandem duplication of a gene. Sturtevant inferred this from recombination data alone, but the duplication was later shown by Bridges to be visible in salivary chromosome preparations. See Sturtevant, A. H. (1925) 'The Effects of Unequal Crossing Over at the Bar Locus in Drosophila', Genetics **10**, 117-147; Bridges, C. B. (1936) 'The Bar 'Gene' a Duplication', Science **83**, 210-211.

[25]see Wimsatt (1987), p. 36f.

[26]Isomorphism is a purely structural relation, which only requires a one-one mapping between two structures. Such a function exists between two linear orderings as they can be found on genetic and cytological maps.

[27]Painter (1934), p. 469

[28]ibid., p. 470

[29]see Painter (1934), p. 465

[30]Tom Fogle pointed out to me that Painter and co-workers in some cases relied on linkage relations to assign loci to chromosomal rearrangements, particularly when mapping translocations. In these cases, the two maps are not strictly independent. However, one could still argue that it would be surprising that the whole approach of relating recombination and cytological maps worked for these cases, if one or both of the maps were artifacts.

[31]See Culp, S. (1995) 'Objectivity in Experimental Inquiry: Breaking Data-Technique Circles', Philosophy of Science **62**, 430-450.

[32]Dubinin, N. P. (1932) 'Stepallelomorphism in Drosophila melanogaster-The Allelomorphs achaete^{2}-scute^{10}, achaete^{1}-scute^{11}, and achaete^{3}-scute^{13}', Journal of Genetics **25**, 163-181.

[33]ibid., p. 164

[34]ibid., p. 171

[35]Carlson (1966), p. 148

[36]ibid., p. 146

[37]Carlson, E. A. (1959) 'Allelism, Pseudoallelism, and Complementation at the Dumpy Locus in D. melanogaster', Genetics **44**, 347-373.

[38]ibid., p. 353

[39]ibid.

[40]Benzer, S. (1955) 'Fine Structure of a Genetic Region in Bacteriophage', Proceedings of the National Academy of Science USA **41**, 344-354.

[41]Carlson (1959), p. 364

[42]ibid., p. 367f

[43]Furthermore, extremely rare recombination events are detectable by plating phage on the non-permissive host K-12, which does not allow any mutant phage to grow and thus frees the investigator from having to search a large number of plaques for recombinants.

[44]"Cis" and "trans" refer to whether the two mutations are located on the same phage (=cis) or on different phages (=trans). "Lysis" means that the phage produces plaques on a bacterial lawn and is therefore active.

[45]Carlson, E. A. (1961) 'Limitations of Geometrical Models for Complementation Mapping of Allelic Series', Nature **191**, 788-790.

[46]ibid., p. 789

[47]ibid., p. 790

[48]The first attempt to explain such functional disturbances was Crick's and Orgel's theory of inter-allelic complementation which suggested that under some circumstances (namely protein-protein interactions) trans-complementation would occur between alleles of the same gene; see Crick, F. H. C. and Orgel, L. (1964) 'The Theory of Inter-Allelic Complementation', Journal of Molecular Biology **8**, 161-165. Although there are probably many other molecular mechanisms of inter-allelic complementation than Crick's and Orgel's simple model, there was an important lesson built into it: Complementation between Benzerian cistrons and inter-allelic complementation were now likely to be two entirely different phenomena. Benzer's system was simply not a case of inter-allelic complementation; it was more similar to two simple, independent Mendelian loci with multiple alleles at each locus. The Drosophila-geneticists seem to have mistaken cistrons as prokaryotic analogues for their "sub-genes" whereas, actually, cistrons are genes.

[49]Some recombination distances failed to show significant additivity, which Benzer explained with the hypothesis that the corresponding mutations extended over a certain length of the genome, production of wild types by recombination requiring crossing-over between these lengths.

[50]Benzer, op. cit., p. 345

[51]The main reason for this was that Benzer had selected his mutants to be immune to reversion and this probably enriched his experimental stocks with double point mutations, deletions and insertions.

[52]In contrast, step-alleles can be arranged in ordered series, see Carlson (1966), p. 146.

[53]Benzer, op. cit., p. 353

[54]Benzer wrote: "If one assumes that each segment has the "function" of specifying the sequence of amino acids in a polypeptide chain, then the specification of each individual amino acid can as well be considered a unitary function. It would seem feasible, with this system, to extend genetic studies even to the level of the latter functional elements" (op. cit., pp. 353-54). These "functional elements" where discovered later and are now known as "codons'.

[55]Today we know that they are not always contiguous; many eukaryotic genes contain introns which have no coding function.

[56]Kaiser, A. D. (1962) 'The Production of Phage Chromosome Fragments and Their Capacity for Genetic Transfer', Journal of Molecular Biology **4**, 275-287.

[57]Yanofsky, C., et al. (1964) 'On the Colinearity of Gene Structure and Protein Structure', Proceedings of the National Academy of Science USA **51**, 266-272.

[58]Beadle, G. W. and Tatum, E. L. (1941) 'Genetic Control of Biochemical Reactions in Neurospora', Proceedings of the National Academy of Science **27**, 499-506.

[59]Judson, H. F. (1979) The Eighth Day of Creation. Makers of the Revolution in Biology (New York: Simon and Schuster).

[60]Yanofsky et al., op. cit., p. 271

[61]In contrast, most authors on reduction in genetics seem to have assumed that molecular biology is based on a structural-molecular gene concept.

[62]Yanofsky et al., op. cit., p. 266

[63]Note that this colinearity relation will only hold within exons in eukaryotic genes. The experiments discussed here were done on a prokaryotic organism (E. coli).

[64]see Waters (1994)

[65]Maps could perhaps be viewed as theoretical models in R.N. Giere's sense; see Giere, R.N. (1988) Explaining Science: A Cognitive Approach (Chicago: University of Chicago Press).

[66]Weber, M. (forthcoming) Die Architektur der Synthese: Entstehung und Philosophie der modernen Evolutionstheorie (Berlin: Walter de Gruyter), Chpt. 4.

[67]W.C. Salmon has proposed such an argument to defend realism concerning the existence of atoms and molecules; see Salmon, W. C. (1984) Scientific Explanation and the Causal Structure of the World (Princeton: Princeton University Press). B.C. Van Fraassen has criticized this kind of argument mainly on the basis of quantum mechanical considerations; see Van Fraassen, B. C. (1980) The Scientific Image (Oxford: Clarendon Press). However, the kind of causal non-locality he has in mind seems of no relevance in the case of genetics. Instead of appealing to common causes, realism of genetic maps could also be defended by using Wimsatt's concept of robustness; see Wimsatt, W.C. (1981) 'Robustness, Reliability, and Overdetermination', in M.B. Brewer and B.E. Collins (eds), Scientific Inquiry and the Social Sciences (San Francisco: Jossey-Bass), pp. 124-163. Wimsatt (1987) makes some suggestions along those lines.

[68]There were several other such attempts, see Carlson (1966), p. 156ff.

[69]Benzer avoided the term "gene", probably because of the controversies surrounding the gene concept in the 1940s and 50s. See Stadler, L. J. (1954) 'The Gene', Science **120**, 811-819 for a penetrating analysis of the main issues in these debates. Although I cannot provide a detailed argument here, I suggest that Benzer's concept of cistron was not fundamentally different from the classical gene concept defined by the Morgan school (compare Waters 1994).

[70]The relationship between DNA, RNA and protein featuring in molecular theory was supported, e.g., by experiments on protein synthesis in cell-free systems (see Judson, op. cit., Chpt. 8).

[71]For a defense of this argument, see Boyd, R. N. (1983) 'On the Current Status of the Issue of Scientific Realism', Erkenntnis **19**, 45-90; for criticism see Carrier, M. (1991) 'What is Wrong With the Miracle Argument?', Studies in the History and Philosophy of Science **22**, 23-36. The "miracle" argument purports to (ampliatively) infer the existence of unobservable entities by an "inference to the best explanation": The assumption that theoretical entities featuring in a theory exist is the best explanation for the theory's empirical success. The main problem with this argument is the existence of historically successful theories which nevertheless suffered reference failure. However, it can be argued that an empirically successful theory must have got some-

thing right; see Carrier, M. (1993) ‘What is Right With the Miracle Argument: Establishing a Taxonomy of Natural Kinds’, Studies in History and Philosophy of Science **24**, 391-409; or Kitcher, P. (1993) The Advancement of Science. Science Without Legend, Objectivity Without Illusions (Oxford: Oxford University Press), especially Chpt. 5.