Reconstituting the Phenomena

1. Introduction: Biochemical Genetics

In the last two chapters we have illustrated the use of localization and decomposition in developing models of complex systems. In all the cases we have discussed, the development of explanatory models was significantly constrained by lower-level theories as well as systemic behavior. These constraints varied in relative strength. In Chapter 6 the initial models of brain function incorporated simple decomposability. Linguistic functions on the one hand, and memory functions on the other, were assumed to be functionally independent and discretely localized. However, research did not limit itself to simple localization. The initial localizationist models were followed by an attempt to reveal underlying physical mechanisms. Decomposability nonetheless was retained, as providing at least a first-order approximation of systemic function. Both higher-level, behavioral, constraints and lower-level, physical, constraints were significant in the development of the resulting models of system behavior and organization. In these cases the lower-level constraints on the resulting model were largely correlational and empirical. Though important, they were relatively weak. The primary constraints on the structure of the resulting model were imposed by the higher level. Cognitive models of memory function, or of linguistic processing, were projected top-down onto the physical level.

In Chapter 7 we turned to research into fermentation. There was once more an initial commitment to localization and decomposition, but it eventually resulted in a model emphasizing integration and organization of physical components rather than decomposition. Here again there was initially a commitment to discrete physical units realizing distinct higher-level functions. Single enzymes were supposed to mediate complex biochemical activities; however, the organization revealed by research into fermentation eventually showed that the biochemical mechanisms provided an interactive and integrated system, rather than one that was simply or nearly decomposable. Once again there were higher-level constraints on the models. Fermentation, including the formation of lactic acid and alcohol, was the overall process to be explained, and it was important that a model embody biologically realistic processes. Moreover, there were important empirical constraints. We emphasized, particularly,

cases discussed in Chapter 6. They imposed strict limitations on the actual structure of the resulting explanatory models, by limiting biochemical processes to simple chemical reactions or sequences of chemical reactions. As a result of the attempt to satisfy the constraints simultaneously, linearity was abandoned, and with it, even near decomposability.

The case that will be at the focus of the current chapter, biochemical genetics, has substantial parallels to the cases in both of the previous chapters. Two traditions converged in influencing the development of biochemical genetics; they provide, respectively, the higher- and lower-level constraints on the development of the field. The first tradition consisted of classical Mendelian investigations into the structure and organization of the genetic material. With the "rediscovery" of Mendel's experimental results at the beginning of the twentieth century,[1] there was a commitment to a particulate model of inheritance, with segregation, dominance, and independent assortment. There was also a commitment to "autonomy" in the expression of genes. Jointly, these Mendelian principles were tantamount to simple decomposability. Deviations from these principles were noticed almost immediately, and they provided the basis for a rich characterization of the phenomena and mechanisms of inheritance in the hands of the Morgan school. As the research program developed, it became clear that simple decomposability could not be maintained. As we will see in the following section, with this recognition it also became apparent that some of the central phenomena revealed in the Mendelian program could not be explained in Mendelian terms, but required a lower-level explanation.

The second tradition influencing biochemical genetics was research into biochemical pathways and their significance for development. While this research included work in physiological chemistry, it focused on work connecting genetic differences and development. This method incorporated assumptions paralleling those affecting research into fermentation. In particular, it required that the basic processes involved in developmental models be known chemical reactions; that intermediaries in metabolic processes be independently isolable; and, initially at least, that there be linear organization. As a result this method imposed strong physical constraints on the resulting models.

The synthesis of these two traditions in the development of biochemical genetics placed strong independent constraints on the resulting genetic models from both levels; models of gene action had to reflect realistic biochemical processes and explain the phenomena uncovered by the Morgan school. As in the case of research into fermentation, satisfying these

result was a reconceptualization of the problem, which led to a new understanding of the phenomena to be explained—a conception requiring that the phenotype be understood in biochemical terms. This *reconstitution of the phenomena* reinstituted decomposability and localization.

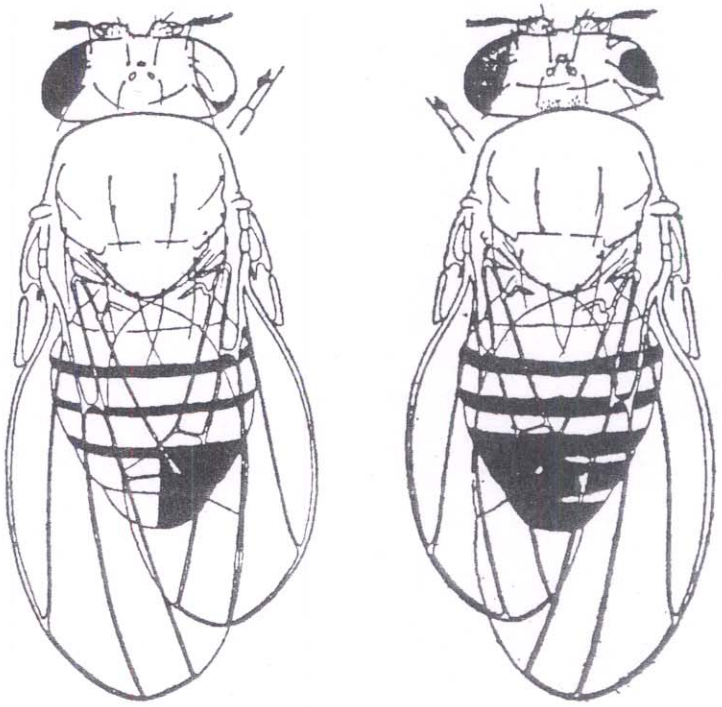
2. Classical Genetics

In their simplest textbook form, the crucial experimental results attributed to Mendel, and supposedly rediscovered in the early twentieth century, are segregation and independent assortment: alternative alleles maintain their independence through sexual crossings, and genes are distributed to sexual gametes in a random mixture.[2] If true these principles would insure that genes are discrete genetic units. Neither of these results—commonly termed "Mendel's Laws"—commits us to the functional, or physiological, independence of the genes. In particular, neither demands that there be single genes sufficient for the production of unique traits, or that genes affect only single traits. That is, genetic independence as observed in transmission does not guarantee that the relation between genes and observable phenotypes will be simple or straightforward. As far as these principles are concerned, there may be many genes affecting each trait, and their influence may be complex and context sensitive (cf. Morgan et al. 1915, ch. 9).

Historically, though, the experimental paradigm *was* taken by advocates of Mendelism to indicate that, as a general rule, characters developed autonomously. It is not possible to understand classical genetics without understanding the role this assumption played in its experimental paradigm, the breeding experiments with *Drosophila melanogaster*. The assumption is nicely illustrated in Morgan and Bridges's (1919) extended examination of gynandromorphs—mosaic *Drosophila* with some structures that are characteristically male and some characteristically female. Commonly these hybrid gynandromorphs will be male on one side of the body and female on the other and will retain, bilaterally, the sex-linked characteristics of their parents. Figure 8.1, redrawn from Morgan and Bridges (ibid.), depicts hybrid individuals that are bilaterally distinguished. In one case the fly is male on the right; in the other, on the left (for details consult the accompanying text). Morgan and Bridges observe:

> A striking fact in regard to these gynandromorphs is that the male and female parts and their sex-linked characters are strictly self-determining, each developing according to its own constitution. No matter how large or how small a region

tively impervious to influences from other cells in the same organism, no



a b

Figure 8.1. Two Gynandromorphs of *Drosophila*. In these cases of bilateral differentiation, one gynandromorph (a) exhibits male characteristics on the right and female on the left, while the other (b) exhibits male characteristics on the left and female on the right. Male characteristics result from a haploid constitution; female, from a diploid constitution. The right side of gynandromorph (a) is male (note the coloring of the abdomen), with a characteristically smaller size and shorter wings. The white eye results from an elimination of one X chromosome, allowing white to be expressed. The left eye is wild type, resulting from the combination of two chromosomes, coding for white and sable. The right side of gynandromorph (b) is female, with longer wings and a larger size (again, notice the color of the abdomen). The left eye is vermilion and of normal size, resulting from a single chromosome. The right side carries the dominant genes for wild-type (red) eye color and a bar configuration. (Redrawn from Morgan and Bridges 1919, p. 36.)

> There is considerable correlation between the gene as a block within which crossing over does not occur and the gene as an apparent physiological unit. Multiple alleles in general affect the same characters and frequently seem to differ only in the degree of effect. . . . On the other hand, there is little or no tendency for genes that are close together in the linkage system to be similar in effect. . . . In general the effect of replacing one allele by another is as independent of replacement in neighboring loci as in any other parts of the genetic system. The translocation of a gene, whether type or mutant, to another region has no apparent effect on its physiological activity. (1941, pp. 490–91)

Even within a single cell there is minimal interaction between genes. Wright concludes that the gene is an "unbreakable physical and physiological unit" (ibid. p. 491). This is direct localization: The genetic system underlying the expression of biological traits is simply decomposable, and each trait is under distinct genetic control. These distinct characters are, in turn, localized in different regions of the chromosomes.

Neither independent assortment nor autonomy were to stand uncompromised for long after their rediscovery. It was only a matter of months until the purity of Mendel's laws was tarnished by laboratory results. In fact, it would be nothing of an exaggeration to say that deviations from the abstract principles were most important for the development of Mendelian genetics, and that these deviations naturally led to problems that required a synthesis of Mendelian and developmental genetics. This synthesis would be realized fully only in the middle decades of this century.

Carl Correns (1864–1933), one of the "rediscoverers" of Mendel's laws, took note of both incomplete dominance and linkage in the very year he published his first paper on Mendelism. In hybridization experiments with *Hyoscyamus*, Correns found that the first-generation hybrids showed an intermediate color rather than that of either parent, a discovery that challenged dominance:

> The rule [of dominance] can only be applied to a certain number of cases, for the present only to those in which one member of the character pair dominates, and for the most part probably only to racial hybrids. That all pairs [in] all hybrids follow it is quite out of the question. (1900, p. 167; quoted in Olby 1985)

Correns (1903) concluded that the explanation of segregating characters and the phenomenon of dominance should finally be explained in terms of chemical processes. Thirty years later Sewall Wright made the point most clearly: "It is clear that dominance has to do with the physiology of the organism and has nothing to do with the mechanism of transmission" (1934, p. 24; cf. Bateson 1909 and Olby 1971).

synthesize it with the chromosome theory (cf. Allen 1978, pp. 125ff.). The identification of genes with discrete segments of chromosomes required that linkage, or "coupling," of traits be common, though it did not appear to be the case.[3] Moreover, Morgan's training as an embryologist left him ill-disposed toward preformed characters in the germplasm—a view he regarded as a revival of preformationism. In 1910, though, Morgan observed a white-eyed mutant male among his *Drosophila*. After crossbreeding the white-eyed male with a normal, red-eyed female, the first generation was uniformly red-eyed. Matings between siblings produced the three to one ratio that would be predicted on Mendelian grounds, assuming that the white-eye is a simple recessive. This confirmation of segregation, however, was not what most struck Morgan. He noticed that *all* the white-eyed offspring were male, though the offspring were equally divided between male and female. The overall ratio was two red-eyed females to one red-eyed male to one white-eyed male. Assuming a chromosomal basis for sex determination, this "sex-limited" inheritance could be explained if the Mendelian factors for eye color were localized on the chromosomes determining sex. As Morgan found more mutants, and two more with sex-limited inheritance, his skepticism of the chromosome theory vanished.

Under the leadership of Morgan, C. B. Bridges, H. J. Muller, and A. H. Sturtevant, the *Drosophila* labs confirmed that genes do not assort independently and are not irresolvably linked. There is significant linkage between traits, but it is incomplete; though traits are separable, as shown by the fact that in a small proportion of offspring they are not transmitted together, that proportion is far less than a simple aggregative model satisfying independent assortment would require. The real work of the Morgan group then began. The researchers set about systematically to map the distances between genes on the assumption that variations from independent assortment indicated the linear distance between linked genes on the chromosome: the greater the distance of two genes on a chromosomal map, the more frequent the recombination between them. An additive model displaced an aggregative one.

During these early years, as we have said, there were investigators who held out for a simple model with minimal interaction between genes; that is, for autonomy. Two lines of research showed that the assumption of autonomy could not be sustained; both came from A. H. Sturtevant (1891–1970), then working in Morgan's lab. The first arose from work with gynandromorphs and provided a demonstration of interaction between distinct cells in development. The second, commonly referred to as *posi-*

action effects depending on simple spatial contiguity on the chromosome. As a result of these findings, the hopes for a simple, autonomous model of gene action were crushed.

Sturtevant (1920) showed that eye color in *Drosophila* could be altered when under the influence of other tissues. Working with a gynandromorph whose head showed paternal characters and whose inherent genetic constitution would require that it have vermilion (v) eyes, Sturtevant observed that it in fact developed a wild-type color (v +). The color of the eyes deviated systematically from what would be predicted on the basis of the gynandromorph's intrinsic constitution, and it did so in the direction of the maternal constitution. Since he knew eye color was a sex-linked trait, Sturtevant concluded that the nonvermilion color was not autonomously determined by the genetic makeup of the tissues constituting the eye, but was under the influence of the wild-type body tissue. He insightfully speculated that this was due to some *diffusable substances*. For developmental purposes it was clear that autonomy was compromised.

Sturtevant also uncovered significant interaction between genes within the cell. Working with a variant form of *Drosophila* (Bar) which exhibited a reduction in the number of facets in the eye, Sturtevant found yet another variant (Double Bar) which suffered an even more extreme reduction in the number of facets (see Figure 8.2.). Sturtevant and Morgan confirmed that this extreme variant was the result of unequal crossing-over; that is, recombination brought genes normally on *homologous* chromosomes into adjacent positions on a *single* chromosome, and the contiguity of the genes caused the accentuated reduction in the eye. Sturtevant (1925) saw that this position effect could not be accommodated without interaction of a nonadditive nature between genes. It was not only the relative dose of a gene that mattered, but their relative placement on the chromosome. Sturtevant and Beadle (1939) were later to suggest that the correct explanation of this too would be found at the chemical level. Garland Allen comments on the importance of Sturtevant's discovery:

> The idea of position effect was a notable departure from the pure Mendelian view that genes are independent units and thus not influenced by other genes associated with them. . . . The concept of position effect, like that of modifier genes, introduced into the hereditary process a greater degree of complexity than the older notion, derived from some of the early Mendelians, that saw genes as rigidly determined "characterlets." Now, even *position* was seen as affecting the phenotypic expression of a gene. (1978, p. 241)

Allen is certainly right that position effect is a notable departure from the "pure Mendelian view," though the acknowledgment of diffusable sub-

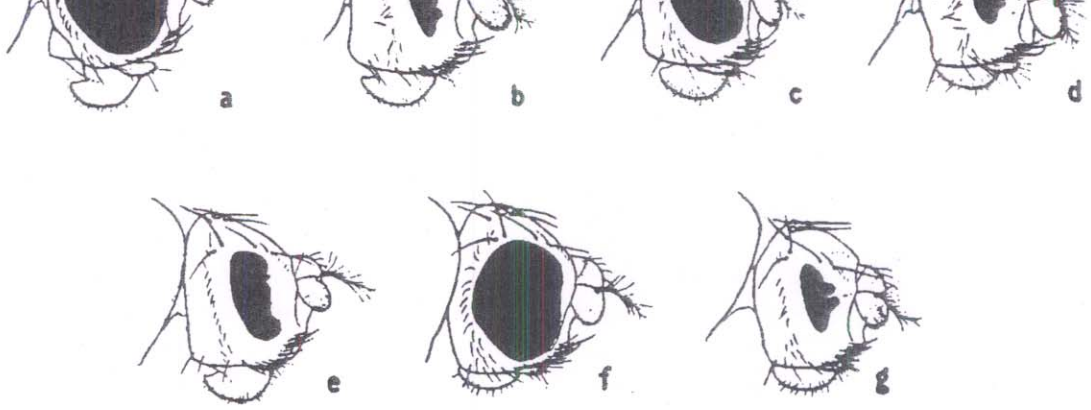


Figure 8.2. Position Effect in the Eye Structure of *Drosophila melanogaster*. The relative area reflects differences in the number of eye facets, seen from the side: (a) Normal, wild type female, (b) Homozygous bar female, (c) Heterozygous bar female, (d) Double bar male, (e) Homozygous infrabar female, (f) Heterozygous infrabar female, (g) Double infrabar male. The bar and double bar forms result from unequal crossing over and are not true mutations. (Redrawn from Sturtevant and Beadle 1939)

stances had already compromised any hope of autonomy in phenotypic expression. Even at this point, and in the absence of a satisfying explanation of the position effect or the nature of diffusable substances, it was clear that interaction between genes discredited autonomy. There simply was no properly *genetic* explanation of this interaction.

The recognition of interaction between genes thus came in a variety of forms. There were many genes affecting each trait, there was important interaction between genes, and there were epigenetic mechanisms at work in controlling development. While speaking before the Royal Society in 1922, Morgan acknowledged the point as clearly as anyone could.

> These ultimate units [genes] are not necessarily to be thought of simply as the representatives of each part of the organism, for every part of the organism must result from the activity of a large number of elementary units. . . .
>
> The evidence has given us a glimpse at least of processes that are so orderly and so simple as to suggest that they are not far removed from physical changes and the order of magnitude of the materials is so small as to suggest that its component parts may come within the range of molecular phenomena. If so, we may be well on the road to the promised land where biological results may be treated as physical and chemical events. (cited in Carlson, 1966, p. 85)

The breeding program of the Morgan school was successful in unraveling the hereditary mechanisms, but it proved relatively ineffective in reveal-

Sturtevant's diffusable substances. The next task for genetics was to investigate the structure of genetic control. This meant treating it as a matter of chemical action.

3. Developmental Genetics

Even as the Morgan group was revealing the mechanisms of inheritance, and forging the basis for a Mendelian triumph, there were figures such as Lucien Cuénot (1866–1951), A. E. Garrod (1857–1936), J.B.S. Haldane (1892–1964), and Sewall Wright (1897–1990) who were concentrating on the role that the hereditary materials played in controlling biochemical reactions in the cell. Though research into the biochemical basis of genetic control fell into relative neglect from roughly 1910 through 1935, it was subsequently revived in the landmark work of George Beadle and Boris Ephrussi (1936, 1937).[4] This work revealed a complexity in the biochemical control of development that had remained obscured within the program of classical genetics.

The work of Garrod and Cuénot, as well as much that preceded and followed it, employed assumptions paralleling those generally assumed in biochemistry and discussed in the previous chapter. In particular it was assumed that the basic reactions regulated (whether directly or indirectly) by genes should correspond to known chemical reactions of relatively simple sorts. Ideally these would take the form of enzyme-mediated reactions. This was a natural assumption for Mendelian genetics. For example, differences in pigmentation naturally led to explanations in terms of the presence or absence of biosynthetic enzymes. As Robert Olby said, "An association between the facts of Mendelism and those of biochemical individuality and metabolism was seen by the early Mendelians as natural" (1974, p. 124). It was further assumed that though the reactions controlling development would be complex, the isolation of intermediaries would provide evidence concerning the specific sequence of reactions. They would therefore provide indirect evidence concerning the genes that exercised control over these reactions.

These assumptions and their implications for Mendelian research can be illustrated in two cases. The first concerns work by Archibald Garrod in the first decade of the twentieth century on the genetic basis of metabolic deficiencies. Garrod's work is especially useful in revealing the importance of these two constraints on biochemical genetics during the period and the significance of biochemical constraints and methodology. The second case focuses on work conducted by Boris Ephrussi and George

tion 4.

Archibald Garrod and Alkaptonuria

The study of alkaptonuria, a harmless condition marked by a blackening of urine on exposure to air, provides us with a useful starting point.[5] As Olby (1974) points out, it was not altogether clear in the period prior to Garrod's work just how useful the study of alkaptonuria would be in investigating normal metabolic pathways. This is because the most plausible chemical changes in deriving alkapton—or homogentisic acid, the darkening substance—involves changes that did not meet the assumption that metabolic reactions must be limited to known chemical transformations. The chemical structure of alkapton was discovered in 1891 by M. Wolkow and E. Baumann. Wolkow and Baumann noticed that the excretion of alkapton was significantly increased if tyrosine was fed to patients, and they concluded that alkapton must be a result of the oxidation of tyrosine. As depicted in figure 8.3, the reaction required a migration of the hydroxyl group as well as an oxidation. Because the former was a reaction not known at the time, Wolkow and Baumann concluded that it was not a simple chemical reaction and therefore attributed the alteration to the activity of microorganisms in the gut. Wolkow and Baumann remained remarkably unconcerned over *how* the microorganisms accomplished the transformation of tyrosine into alkapton; the important conclusion was that the transformation was *not* a consequence of standard metabolic action, but an anomalous effect due to the influence of invading organisms.

By the time Garrod undertook his studies, biochemists had come to accept side-chain migrations of the sort the model of Wolkow and Baumann required, and so they were acceptable as chemical reactions. Garrod confirmed Wolkow and Baumann's conclusion that homogentisic acid was the cause of alkaptonuria. He had been working with a child who had exhibited the condition since 1898, and by the spring of 1901 a second child who also had alkaptonuria was born to the family. After some questioning Garrod found that the parents were first cousins. This set the stage for a Mendelian interpretation of the disease. Bateson and Saunders (1902) pointed out that such marriages would be most likely to reveal rare and recessive characters. Garrod happily embraced the explanation:

> The mode of incidence of alkaptonuria finds a ready explanation if the anomaly in question be regarded as a rare recessive character in the Mendelian sense. Mendel's law asserts that as regards two mutually exclusive characters, one of

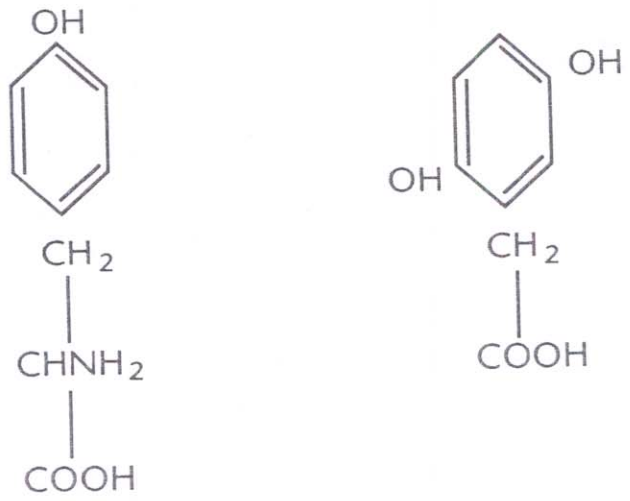


Figure 8.3. A Structural Model for the Derivation of Alkapton from Tyrosine. Wolkow and Baumann (1891) proposed that alkapton (Homogentisic Acid) was derived from tyrosine. This required the migration of a hydroxyl group. Because this was, at the time, a structural change that was unacceptable from a chemical standpoint, it was therefore ruled out.

> which tends to be dominant and the other recessive, cross-bred individuals will tend to manifest the dominant character, but when they interbreed the offspring of the hybrids will exhibit one or other of the original characters. (1908, p. 5)

Garrod acknowledged that the actual proportions found in studies of families with alkaptonurics was not what would be required by Mendelian principles, but he maintained nonetheless that it was a Mendelian recessive, primarily because "albinism, which so closely resembles it in its mode of incidence in man, behaves as a recessive character in the experimental breeding of animals" (ibid., p. 6).

What is important for our purposes is not just that Garrod thought alkaptonuria was a recessive character, but the way he integrated this with a biochemical picture of metabolism. Appealing explicitly to Bernard, Garrod said,

> The view is daily gaining ground that each successive step in the building up and breaking down, not merely of proteins, carbohydrates, and fats in general, but even of individual fractions of proteins and of individual sugars, is the work of special enzymes set apart for each particular purpose. (Ibid., pp. 1–2).

ates should be excreted. Abnormal excretions should therefore provide direct evidence concerning metabolic intermediaries, and indirect evidence concerning the metabolic processes themselves. If it is assumed that the presence or absence of an enzyme is controlled by Mendelian factors, then there is a natural explanation for the "inborn errors of metabolism" such as alkaptonuria in terms of specific genes controlling enzymatic reactions. That is precisely what Garrod proposed.

Garrod's proposal clearly embodies both assumptions noted above. To begin with, it assumes that the explanation of alkaptonuria must be carried out at a chemical level, appealing only to reactions of a known character. Enzyme action is assumed to be specific and limited. Moreover, once an enzyme system is blocked, Garrod assumes that the reaction it mediates is unlikely to be accomplished by an alternative mechanism: "If the conception of metabolism in compartments, under the influence of enzymes, be a correct one, it is unlikely, *a priori*, that alternative paths are provided which may be followed when for any reason the normal paths are blocked" (1908, p. 2). Secondly, once an enzyme is blocked, Garrod assumes metabolic intermediaries will be detected in excretions. Alkaptonuria must be seen "as an arrest rather than as a perversion of metabolism" (ibid., p. 217). It is essential to Garrod's view of the metabolic pathway that neither tyrosine nor homogentisic acid be normally excreted and so have to be broken down in the more common cases. He conjectures, therefore, that the benzene ring is split by an enzyme that is simply absent in the congenital alkaptonuric. Genes regulate metabolism by controlling these enzymatic reactions; when the dominant gene is absent, so is the enzyme.[6] Lack of the enzyme blocks the normal process, resulting in intermediaries which are ultimately excreted. This is a natural experiment in which the metabolic processes are interrupted, yet it says very little about the complexity of metabolism and catabolism. It is in fact consistent with an assumption that the process is linear, degrading more complex proteins in a stepwise fashion. Garrod seems to have entertained nothing else.

Garrod's investigation of the inborn errors of metabolism preceded those of the Morgan school and fit largely within a more medical context with a different research agenda. According to the program of the Morgan school, phenotypic traits were explained in terms of localized genes, and the problems surrounding gene expression were relegated to a secondary status. The focus was on what the statistical distribution of traits within breeding populations could reveal about chromosomal structure and the localization of genes. Garrod's work, by contrast, focused on how genes were expressed; the inborn errors of metabolism were of interest pre-

both normal and pathological development lay in the genes, proximate control was attributed to enzymes. Biochemical pathways were thus interposed between phenotypic traits and genes, and the presence or absence of an enzyme was what received explanation in Mendelian terms. The details of chromosomal structure were of secondary importance.[7]

Genetic Control of Development

A similar emphasis on gene expression and development, with parallel assumptions, can be seen in subsequent work on genetic control carried out in the mid-1930s by Boris Ephrussi and George Beadle.[8] The nonautonomous control of eye color in *Drosophila* discovered by Sturtevant promised a direct route to understanding gene action and the underlying means of control (for useful reviews, see Beadle 1945, pp. 33 ff.; Ephrussi 1942). It was known from the crossbreeding of mutant forms that the red eye of normal, or wild-type, *Drosophila* was the result of two components: a red pigment and a brown pigment. Either could be inhibited by mutations. The formation of brown pigment could be blocked (to varying degrees) by mutations at at least four loci, leaving the red pigment and a bright red eye. The red pigment also could be absent, and its formation inhibited by mutations, leaving only brown pigment and a brown eye. Finally, both pigments could be blocked, leaving no pigment and a white eye. The recognition of nonautonomous control for eye color inspired transplant studies by Beadle and Ephrussi (1936, 1937) in which the imaginal disks from the eyes of mutant larvae of *Drosophila melanogaster* were implanted into the abdomens of normal larvae, and vice versa. The resulting eye color of the implanted disk would be influenced both by the intrinsic genetic constitution of the eye disk and by the surrounding tissue. Their work showed that the color deviated systematically from what would be predicted on the basis of the genetic composition of the larvae alone.

As Table 8.1 indicates, v imaginal disks transplanted into v+ larvae developed a color appropriate to v+, and v+ disks implanted into v larvae maintained a v+ color. Parallel results were also seen with a second mutant color, cinnabar (**cn**). These results were consistent with the view that v and **cn** were simple recessives, and the wild-type v+ a Mendelian dominant trait. Normal development followed if either the imaginal disk or the host tissue contained v+ genes. The most significant cases were the reciprocal transplants of v and **cn**. A v disk implanted on a **cn** larvae developed v+ color. Conversely, however, a **cn** disk implanted on a v larvae developed a **cn** color. These results were incompatible with an interpreta-

Vermilion (**v**)	Cinnabar (**cn**)	Wild Type (**v+**)
Cinnabar (**cn**)	Vermilion (**v**)	Cinnabar (**cn**)
Cinnabar (**cn**)	Wild Type (**v+**)	Wild Type (**v+**)
Wild Type (**v+**)	Cinnabar (**cn**)	Wild Type (**v+**)
Wild Type (**v+**)	Vermilion (**v**)	Wild Type (**v+**)

Table 8.1. Summary of Transplantation Experiments by Beadle and Ephrussi (1936, 1937). It is particularly important that an implanted **cn** disk develops a **cn** phenotype under the influence of a **v** body type, but that an implanted **v** developed into a **v+** phenotype under the influence of a **cn** body type. This implies that the relevant genes control sequential reactions in development, rather than being allelic variants, or providing independent contributions.

tion treating **v** and **cn** as allelic variants or as providing independent contributions. They could not be accommodated without positing a sequential, or linear, organization. Beadle and Ephrussi concluded that there were at least two intermediaries implicated in the formation of pigment in a normal eye and that they occupied sequential positions in the metabolic chain. Ephrussi wrote in 1942:

> There are two different substances, one responsible for the change from vermilion to wild type and the other for the change from cinnabar to wild type. The wild type lymph contains both these substances. The lymph of the mutant cinnabar contains only one of them, namely the substance responsible for the change from vermilion to wild type. The mutant vermilion contains none of these substances. . . . [The] two substances are formed in the course of a single chain of reactions, of which the **v+** substance represents the first and the **cn+** the second link: ⇒ **v+** ⇒ **cn+**. (pp. 329–30)

There were at least two diffusable substances, rather than one, and they were organized sequentially. The **v** mutant failed to carry out the first reaction and therefore could not synthesize the second of the diffusable substances. As a host the **v** mutant could not supplement the **cn** implant, which also could not carry out the second transformation. The **cn** mutant could perform the first reaction, but not the second. As a host it therefore could carry out the first step in the reaction and could "repair" the eye color of the **v** implant. In 1939 Beadle and Ephrussi concluded that the blockage in reactions was due to a lack of specific enzymes (cf. Olby 1974, p. 141).

regulated chemical reactions involved and that the intermediaries would accumulate when subsequent reactions were blocked. Whether or not this was a reliable interpretation of Beadle and Ephrussi's earlier assumptions, their work did reveal a structure in the synthesis of pigments and indicated that genetic action needed to be conceptualized at a more discriminating level. Eventually Beadle and Tatum (1941a) came to portray the synthesis of brown eye pigment as in Figure 8.4.

As with Garrod's work, Beadle and Ephrussi's model manifests the three assumptions involved in biochemical research. It incorporates only chemical reactions mediated by specific enzymes; genes are conceived as acting to control specific reactions, either because they are enzymes or because they control enzymes. It assumes that disruption of these meta-

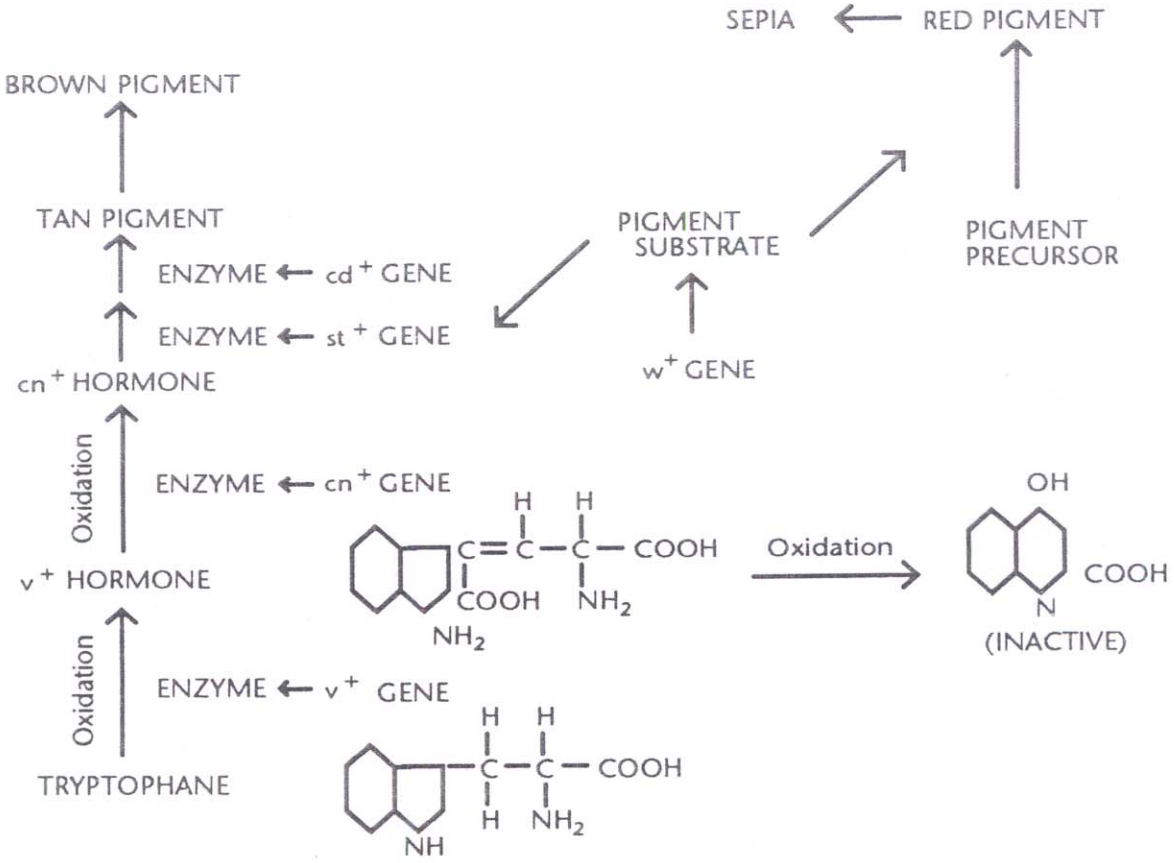


Figure 8.4. The Synthesis of Eye Pigment in *Drosophila*. Notice, in particular, that the transformations explicitly represented in the structure diagrams are simple oxidations and that the overall organization is linear. As in Beadle and Tatum's earlier representation of the process, inactivation of the v+ or cn+ genes would block the formation of brown pigment, leaving red pigment in the eye. (Beadle and Tatum 1914a, p. 114.)

pigment contributing to the normal red eyes. The organization the model uses is linear, and because of the linear organization the disruption of a gene will affect only what is subsequent to it in the synthesis of the pigment. Again, this is near decomposability. As with Garrod's work, the decomposition depends on an understanding of the biochemical processes and not simply on distributions of phenotypic traits.

4. One Gene/One Enzyme

In light of the work from the Morgan school and developmental genetics, the general strategy of Beadle and Tatum's classic experimental work on *Neurospora* is reasonably straightforward. However, despite its being a natural extension of a classical paradigm, it was to have revolutionary consequences. Beadle and Tatum recognized the limitations of the classical paradigm; in particular, they saw that the methodology almost inevitably left them unable to see anything but genes affecting superficial characters. This is because the methodology of Mendelian genetics committed it to beginning with the character and working backward to determine the sequence of reactions. The phenotype and statistical patterns of inheritance were the only acknowledged constraints. In practice this imposed several limitations. Breeding experiments could reveal genetic factors only when there were alternative alleles, and then only if those alleles had detectable phenotypic effects. This meant that alleles with small effects would be likely to escape detection, and, correspondingly, that those with the largest effects would likely be lethal. Since terminal reactions were those most prone to modification by nonlethal mutants, this also meant that the classical paradigm would naturally be led to focus on genes controlling these terminal reactions. The underlying complexity of developmental processes, and their genetic control, was going to elude the grasp of classical work.

The conjunction of Mendelian genetics with research on biochemical genetics restructured the problem into one also constrained by the nature of the biochemical processes underlying phenotypic expression. This required an experimental methodology and a model system that could avoid the problems facing Mendelian genetics. For this Beadle and Tatum drew on the earlier work with Ephrussi. They set about to investigate the nutritional requirements of *Neurospora*, recognizing that genes must control the biosynthetic processes in nutrition and, further, that these processes would exhibit considerable complexity at the genetic level. Their goal was to uncover the underlying genetic organization by investigating different

explained. This is what we call *reconstituting the phenomena*.

Beadle and Tatum retained the constraints characteristic of work in biochemistry and of the earlier work in the developmental genetics of *Drosophila* (cf. Tatum 1959, p. 1712). They also maintained the assumption that genes control biochemical processes through specific enzymes:

> From the standpoint of physiological genetics, the development and functioning of an organism consist essentially of an integrated system of chemical reactions controlled in some manner by genes. It is entirely tenable to suppose that these genes which are themselves a part of the system, control or regulate specific reactions in the system either by acting directly as enzymes or by determining the specificities of enzymes." (1941b, p. 499)

Genes are specialized in their action, and their heterocatalytic products will be equally specialized. As Beadle put it four years later:

> Each nucleus of those organisms sufficiently advanced in the evolutionary scale to have nuclei contains many thousands of genes. . . . Each of these thousands of gene types has, in general a unique specificity. This means that a given enzyme will usually have its final specificity set by one and only one gene. (1945, p. 19)

Specificity of enzymatic activity is methodologically important for reasons we have already seen in conjunction with Garrod's work. Specificity suggests that there will be no alternative pathways to mediate effects once a gene is inactivated. This makes it possible, in principle at least, to detect the consequences of such inactivation. It also makes enzymes a natural unit in terms of which to understand genetic action.

Beadle and Tatum also retain the assumptions that biosynthesis must occur via a series of sequential steps and that disruption at any given step should inhibit the overall effect. Mutants affecting metabolic functions should therefore be detectable, provided that the deleterious effects are initially overcome. We have already seen that these assumptions were present in the work on pigmentation, where the damaging effects of the mutant genes is what allowed them to be detected.

As we have noted, Mendelian methods were limited to dealing with naturally occurring, nonlethal variations. Beadle and Tatum's approach was designed to overcome these limitations. With this in mind, *Neurospora* was an ideal organism to use in their research. To begin with, it has simple nutritional requirements: it can be grown on a medium containing a carbon source (sugars, starch, etc.), a nitrogen source, inorganic salts, and biotin (a B vitamin); it can also be grown with more complex supplementation. This means that normally it is capable of synthesizing most of

netically identical individuals whose nutritional requirements can be evaluated; sexual reproduction enables researchers to carry out Mendelian crosses on individuals resulting from asexual reproduction. Finally, the organism is haploid. This means that there is no masking of genetic effects by dominance relations; a mutant gene will inevitably be expressed, and a gene inactivated by mutation will have the effect of disabling any pathway of which it is a constituent.

Beadle and Tatum induced mutations using X-rays.[9] The goal of this inhibitory study was to bring about gene mutations, with the hope that those mutations would affect the traits under examination. As Beadle explained, the "inactivation of specific genes is equivalent to the chemical poisoning of specific enzymes, with the important difference that genes are highly specific, whereas enzyme poisons are often discouragingly nonspecific" (1945, p. 61). Allowing meiosis to take place, Beadle and Tatum were able to obtain a host of genetically homogeneous spores. After initially growing them on an amply supplemented medium, until a suitably large population was available, they transferred samples to a medium having the minimum amount of supplements sufficient for growth of nonmutant strains. Any strains that did not grow on the minimal medium had to be mutant forms incapable of synthesizing some substance that the normal strains did synthesize. By transferring these mutant strains to a variety of media with different supplements, it was possible to isolate the substances the mutant strains were unable to synthesize.

The initial studies by Beadle and Tatum (1941b) uncovered three mutant forms. All grew on the complete medium, none on the minimal medium. Each in turn could be grown on some medium supplemented in one way or another. One grew with the addition of vitamin B6, another with vitamin B1, and a third with paraaminobenzoic acid. Beadle and Tatum concluded that all three were single-gene mutants; they subsequently obtained a variety of other strains that could not grow without supplementation by some specific vitamin or amino acid. They concluded that the assumption that genes control enzymatic reactions must be correct: "A single gene may be considered to be concerned with the primary control of a single specific chemical reaction" (Tatum and Beadle, 1942, p. 240). As a consequence, "the gene and enzyme specificities are of the same order" (Beadle and Tatum, 1941b, pp. 499–500).[10]

Although initially the biochemical reactions were assumed to constitute a linear chain, linearity was eventually compromised here just as in the understanding of cell metabolism. This can be simply illustrated in the

by either arginine or citrulline; yet others by arginine, citrulline, or ornithine. On the face of it a simple two-step process was all that was needed, as illustrated in Figure 8.5. If the second reaction was blocked, then supplementation by arginine was required; if the first, then either citrulline or arginine sufficed. If the synthesis of ornithine was impeded and the two remaining reactions remained intact, then supplementation by any one of the three was sufficient.

However, the situation was more complex than this would suggest. Arginine was subsequently degraded into ornithine and urea, and the urea further broken down into carbon dioxide and ammonia. As a consequence, the overall reaction was better represented as a cycle (see Figure 8.6).

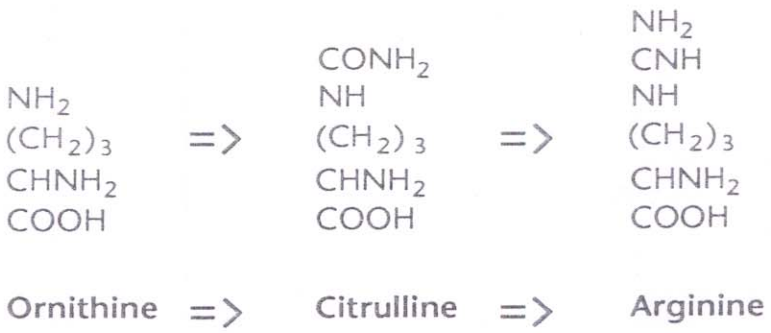


Figure 8.5. The Derivation of Arginine from Ornithine. Each step involves a relatively simple alteration of structure and preserves linearity.

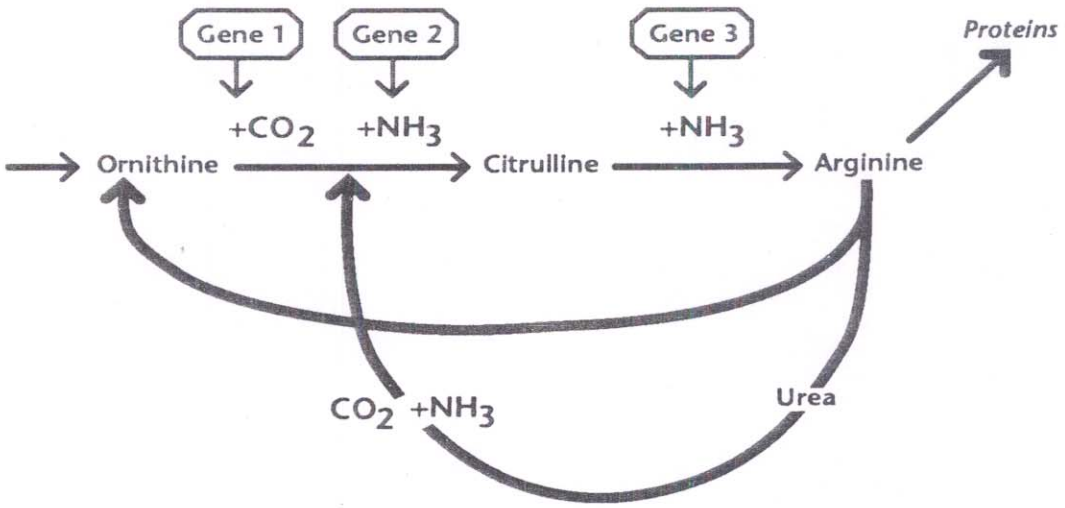


Figure 8.6. A Cyclic Representation. Once again the overall reaction can be better represented as cyclic; the byproduct of one reaction is a substrate in others that occur earlier in the process.

pathways under the control of a constellation of genes. Localization and decomposition appeared once again to have been compromised.

5. Conclusion: Reconstituting the Phenomena

Once again interaction and organization were critical. The simple localization of the Morgan school, projected on the basis of phenotypic patterns, did *not* take us to Morgan's "promised land where biological results may be treated as physical and chemical events." It *did*, however, spawn a program of research that gave us an understanding of the inherent complexity of the underlying biochemical processes. Direct localization was eventually foresworn. In part this was a simple consequence of the fact that phenotypic traits were products of many genes in a complex organization. The complexity of gene action was clear even in the *Drosophila* work itself. Though mutations would often have an additive effect, this was not always so. There were modifiers that would have no effect by themselves, but would affect the expression of other mutants. There were other interactions between genes based on location, and even effects between genes in different parts of the body. Moreover, distinct genotypes (such as scarlet, vermilion, and the double recessives) were almost indistinguishable phenotypically. Sewall Wright observed:

> There is usually a simple one to one relation between gene and the substances responsible for immunological specificity. The suggestion is that these substances or at least the active group . . . is a direct product of the gene. . . . In general, however, the relation between gene and such substances as the animal and plant pigments and excretion products is less direct. Many genes, often acting apparently in sequence, are necessary for a particular product. (1941, p. 514)

Autonomy in gene action turned out to be the exception rather than the rule. Decomposition into independent traits failed.

The abandonment of the simple localizationism of the Morgan school was also, in part, the consequence of the integration of the Mendelian program with an independent line of research derived from biochemistry. The breeding program of the Morgan school left a variety of effects, such as dominance and the position effect, which clearly needed explaining but could not be explained in the terms of Mendelian genetics. Some account of these phenomena was needed, and it was natural to look to biochemistry to unravel the mechanisms. The result was an increasingly complex

> The reaction chains connecting primary gene action and observed effects on morphological characters must be longer, more ramifying and more heterogeneous than where effects are on intracellular products. Even more indirect are the relations of genes to modes of behavior of the organism as a whole, although there are cases in which there is simple mendelian heredity. (1941, p. 521)

Not only are there generally multiple factors affecting characters, as even Mendelism allowed, but the relations of genes to the characters they produce is "indirect" and "heterogeneous." Gene expression involves interactions and dependencies beyond the reach of Mendelism.

The result of the integration of Mendelian and biochemical genetics was not simply a recognition of a more complex organization where previously a decomposable or a nearly decomposable system had been proposed. Rather, the phenomena of genetics were reconsitituted at the level of biochemistry. Joshua Lederberg explicitly suggested this as the moral:

> Experimental genetics is reaching its full powers in coalescence with biochemistry: In principle, each phenotype should eventually be denoted as an exact sequence of amino acids in protein and the genotype as a corresponding sequence of nucleotides in DNA. (1960, p. 269)

Lederberg's proposal was more radical than might first appear. What traditionally counted as a Mendelian trait, the macroscopically observable phenotypic trait, would be abandoned as the central Mendelian unit, with a shift of the entire analysis to a lower level. The one gene–one enzyme model told us that the level of specificity at which we must understand gene action was at the level of chemistry. In the face of a new vision of the mechanisms, Mendelism's one gene–one *trait* emphasis could be retained by embracing a one gene–one *enzyme* model. We simply had to reconceptualize the relevant traits at a lower level of analysis. Traits too needed to be identified and individuated at the level of enzymes, and the classical phenotype resolved into a complex of traits at that lower level. It was no longer eye colors, but the enzymes that produced them, which become the proper unit for a Mendelian analysis. The observable macroscopic traits that the Morgan school placed at center stage were dissolved under the stronger resolution of biochemical genetics.

Lederberg's suggestion had the merit of simplicity, and in many respects the practice of biology has followed that lead. In some regards the suggestion should appear suspect. We naturally assume that we understand antecedently *what* is to be explained, and an *adequate* explanation is one that conforms to the phenomena. Philosophical predilections to the

and models we develop.

Reconsitution of the phenomena does not compromise localization and decomposition, or the search for genetic mechanisms, but validates them. It is here that we see the real power of a fully developed mechanistic explanation. A mechanistic approach is not limited to explaining phenomena that are taken as simply "given," but can mandate a revision of the way the phenomena are to be conceptualized and what are given epistemic priority. In the case proposed by Lederberg the reconceptualization of the phenomena accompanies a shift to a lower level (Figure 8.7). Genes are thought of as specific in their action and as acting in relative independence of other genes. There is independence at the level of the enzymes they produce. Conceived in terms of the observable phenotypes, genes have a complex role in development and metabolism, and a complex organization. Simplification followed only on understanding the phenotype differently. A characterization of the phenomena in terms of observable traits was replaced by one couched in terms of biochemical products.[11] This was still localization and decomposition, but this time the reconstitution of the phenomena with a shift to a lower level allowed us to retain localization and decomposition in the face of complex organization.

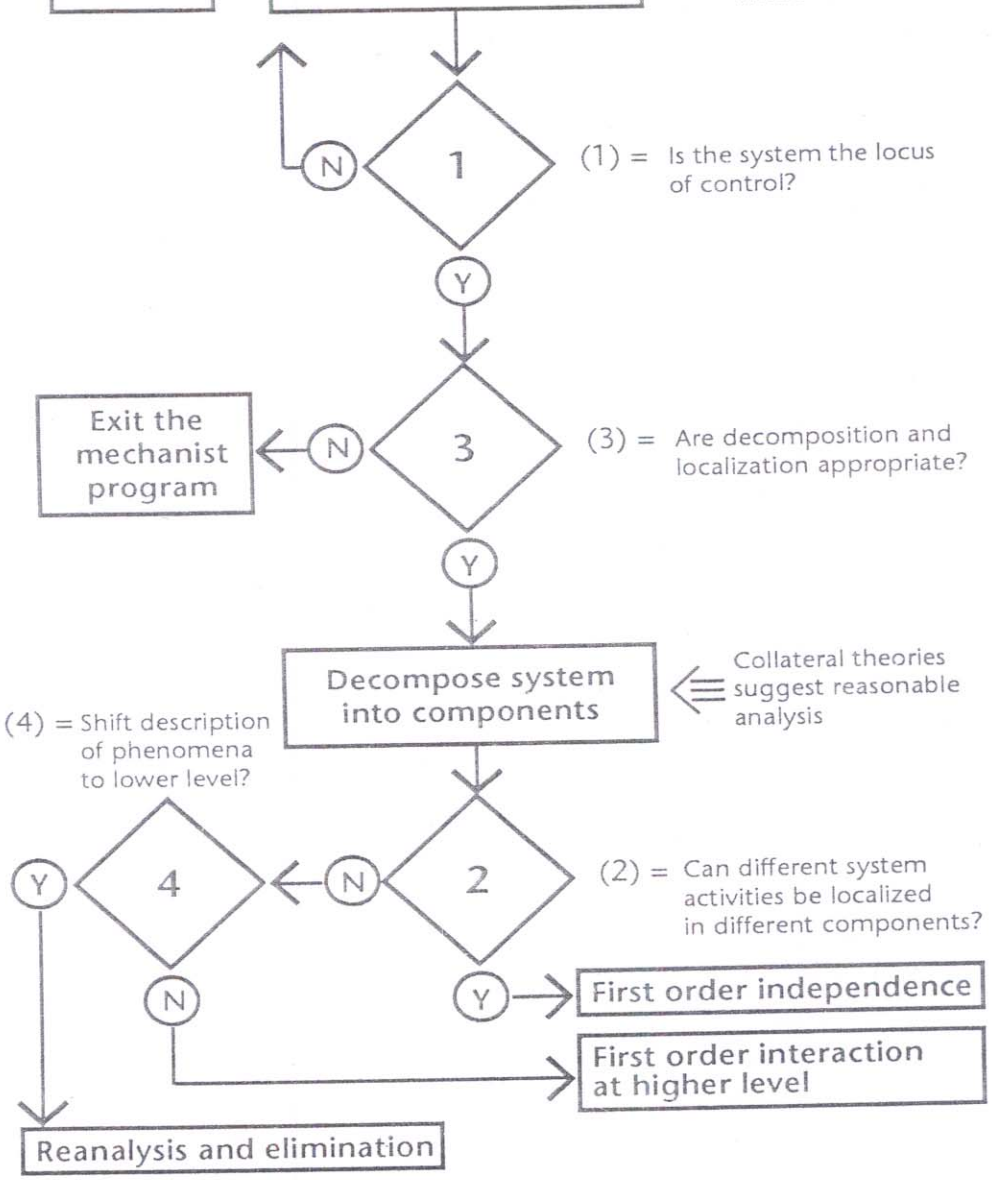


Figure 8.7. A Third Outcome of Decomposition and Localization. An emphasis on organization and interaction can appear to compromise decomposition and localization altogether, as constituent processes are strongly interdependent and organization is critical. However, one possible response, which we call reconstituting the phenomena, leads to a reconceptualization of the phenomena to be explained in terms of the underlying lower-level mechanisms.