Developmental Selection of Mutations

Abstract. The direction of evolution may at times be determined by the internal selection of genotypes during development, rather than by the external selection of phenotypes. Biological theory and experiment have reached the point where those concerned with evolutionary problems or with nuclear and cellular organization should consider this possibility.

In studies of the genetic basis of the theory of evolution by natural selection there has been, until recently, little explicit consideration of the possibility that the internal developmental selection of mutations (in contrast to the environmental selection of developed organisms) may sometimes be an important factor in determining the direction of evolutionary change. Some degree of developmental selection of genotypes has occasionally been considered to be probable, and is indeed implicit in the conception of lethal mutations. But the fact that the internal developmental selection of genotypes, rather than the external adaptive selection of phenotypes, may at times be the primary factor determining the direction of evolutionary change has seldom been made explicit (*1*). Though environmental selection is always selection of the ultimate developmental effects of genes, the internal selection of genes and of their earlier effects is a distinct process which merits more consideration than it has yet been given. A line may be drawn between the two at the moment when the developing zygote faces a nonparental environment.

In any structural view of organisms point mutations unrelated to the structure of the organism, or other accidental structural rearrangements, which occur in a highly patterned chromosomal and other neighborhood, will necessarily be subject to a complex and progressive selective process in which the criterion is compatibility with the internal structure and processes of the system, in particular with chromosomal activities. It is widely agreed that in certain respects the genes act as unblending units, and in others as cooperative or "coadaptive" elements in an ordered gene complex which must satisfy certain over-all conditions if an adequately coordinated organism is to result, but little is known regarding these conditions.

The internal selective process may be roughly divided into two phases: (i) some mutations will not adequately conform, at their locus, to the specific ordered molecular structure characterizing the genic system and may therefore be physically or biochemically unstable and be at once eliminated; and (ii) some of those surviving this test will prove less capable (or incapable) of having their activity coordinated within the highly ordered processes of replication, activation, differentiative development, and prefunctional activity, and either they will be modified (by a return mutation or otherwise) in the course of these processes so that they do adequately conform, or, the resulting structures will damage the internal coordination and in severe cases arrest development. Mutations at any locus, to be nonlethal, must possess specific features which, though not yet understood, may play a part in determining the evolutionary process at certain times possibly equal to that of environmental adaptation. "Natural" selection, understood in the full sense, comprises two separable selective processes: developmental selection (where the criterion is internal organizational efficiency permitting continued growth), and environmental selection (dependent on adaptive success, permitting continued life and reproduction). Any phenotype which is adaptively successful must correspond to a genotype which has passed the internal selective process, but that is no reason for neglecting to consider the effects of internal selection, as far as knowledge permits. In much current writing on genetic and evolutionary theory terms such as "natural selection," "adaptive," "favorable," and so forth, are used in a manner which excludes internal developmental selection.

In the view presented here initially haphazard mutations are rapidly sifted, the particular organism choosing what is sufficiently compatible with its existing specific structure. The struggle for survival of mutations begins at the moment mutation occurs. Members of a viable species must be not only adaptively well adjusted, but internally well coordinated, and the latter property is tested first and may, in certain respects or during certain periods, be the more severe restriction on permissible mutations.

The effect of this internal organizational selection of mutations on evolutionary change has received relatively little analysis, probably because until recently it lay outside the scope of biological experiment (*2*) and theory (*3*). But if a prior selective process operates on the mutated genotype, in terms of its compatibility with the highly specific structural processes of development—and it is hard to see how this could fail to be the case on any structural theory of organisms accounting for their coordination—then many arguments concerning evolution by natural selection (gene stability, rates of variation, speciation, macroevolution, and so forth) may have to be reconsidered. The absence of direct evidence for such an effect does not justify its neglect by evolutionary theory, if it is a natural inference from any structural interpretation of organic processes.

The purpose of this note is to invite the attention of specialists to three questions: (i) What evidence exists for developmental selection and what light does the evidence throw on the criteria involved? (ii) What contribution can chemical or other theories of cellular or nuclear organization make to this issue? (iii) Under what circumstances may developmental selection play a decisive role in determining the direction of evolution?

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References and Notes

1. Some relevant references are: H. T. Pledge, *Science Since 1500* (Harper, New York, 1959), p. 226 ("the gene-complex is an 'environment' for mutations in the organism"); I. I. Schmalhausen, *Factors in Evolution* (Blakiston, New York, 1949) (The idea of developmental selection appears to be used, though not made fully explicit); C. H. Waddington, *Strategy of the Genes* (Macmillan, New York, 1957), pp. 65–66 (The possibility of selection acting directly on the mutated genes is considered but dismissed); T. Dobzhansky, *Genetics and the Origin of Species* (Columbia Univ. Press, New York, 1937) (Evolutionary paths may conceivably diverge owing to genetic control of the *directions*, as well as the *rates*, of mutation); J. B. S. Haldane, in *Darwin's Biological Work*, P. R. Bell, Ed. (Cambridge Univ. Press, Cambridge, 1959), p. 147 (Haldane says that if certain mutations "interrupt some important developmental process . . . the possibilities of evolution open to a species depend not so much on its genes and their mutability, as on its developmental processes"); ———, *J. Genet.* **56**, 11 (1958); J. H. Woodger, in *The Axiomatic Method*, L. Henkin *et al.*, Eds. (Humanities, New York, 1959), p. 427 (Woodger stresses the importance of random development, as well as random union of the gametes, in obtaining the Mendelian ratios).
2. One of the first observational indications of the internal selection of mutations was reported by Lima-de-Faria [*Chromosoma* **5**, 1 (1952); see p. 53]. See also C. H. Waddington, *Strategy of the Genes*, p. 66, on mutator genes.
3. The possibility of coordinated chromosomal action, which is connected with the present argument, has been discussed by Schmalhausen in *Factors in Evolution*, by R. B. Goldschmidt in *Theoretical Genetics* (Univ. of Calif. Press, Berkeley, 1955), p. 184–186, and 487, and by Waddington in *Strategy of the Genes*. But conceptions such as "autoregulation," "canalization of development," "systemic mutations," and so forth, might be better defined by explicit consideration of developmental selection.

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Atropine-like Actions of Muscarine Isomers

Abstract. Molecular pharmacology of muscarine isomers has been studied in the rat intestine and frog heart. The significance of the peculiar finding of atropine-like action of some of the isomers is discussed.

The activity of a drug is mainly characterized by affinity and intrinsic activity (*1*). The intrinsic activity, representing the ability of a drug to produce an effect, is also a measure of the

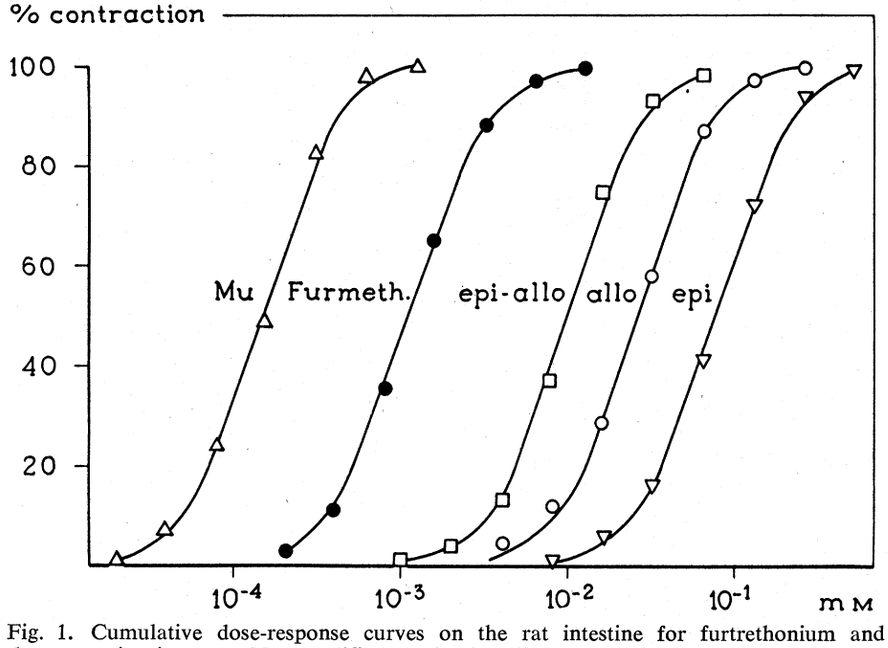

Fig. 1. Cumulative dose-response curves on the rat intestine for furtrethonium and the muscarine isomers. Note a difference in the affinity as a difference in the place on the contraction axis and an identical intrinsic activity as an equal maximal height.

ratio between agonistic and competitive antagonistic actions.

Postganglionic parasympathetic stimulants, parasympathomimetics, or muscarinics have a high intrinsic activity, whereas postganglionic depressants, parasympatholytics, or atropine-like drugs have a very low intrinsic activity. Minor changes in the molecular structure of parasympathomimetic drugs result in considerable changes in both the affinity and the intrinsic activity (*2*). For instance, substitution of a propyl group for the methyl group in the hydrofuran ring of muscarine causes a change from parasympathomimetic to parasympatholytic action. DL-Propyl-demethylmuscarine (*3*) thus is a purely competitive antagonist of a parasympathomimetic such as furtrethonium (*4*).

It has been clearly demonstrated that optical isomers of parasympathetic drugs differ largely in their affinity for the receptors (*5*). It is to be expected also that differences in the intrinsic activity will be found for stereoisomers, even to the extent that for certain parasympathetic drugs isomers will be found which exert atropine-like actions. In this respect it seemed worth while to investigate the stereoisomers (*6*) of DL-muscarine, which have been found to differ in potency (*7*).

DL-Muscarine appeared to be the most potent parasympathomimetic of the four possible racemates (*8*).

From the cumulative dose-response curves (Fig. 1) it may be seen that these isomers all behave as pure parasympathomimetics on the rat intestine. They have equal intrinsic activities (Table 1). Consequently, the difference in potency must be attributed to differences in their binding capacity with respect to the receptor.

The intrinsic activity, however, depends not only on the molecular structure of the drug but also on the molecular structure of the receptor. Consequently, the intrinsic activity of parasympathetic drugs may vary for different species and organs. It is known that the activity is generally found to be lower in the frog heart than in the intestine (*9*). For instance, the action of pilocarpine is largely parasympathomimetic on the intestine but almost purely atropine-like on the frog heart (*10*).

Indeed, it could be established that not all four isomers act upon the frog heart as pure parasympathomimetics, but that atropine-like actions become apparent in DL-epimuscarine and DL-allomuscarine. DL-Epimuscarine is able to antagonize the action of acetylcholine on the heart (Fig. 2, left). These atropine-like actions of epimuscarine can be overcome by increasing the concentration of acetylcholine, while epimuscarine also protects the heart against high doses of acetylcholine (Fig. 2, left). These experiments suggest a competitive inhibition of the effects of acetylcholine by DL-epimuscarine, indicating that the latter is a true parasympatholytic drug. The intrinsic activity consequently is very low (Table 1). DL-Allomuscarine is

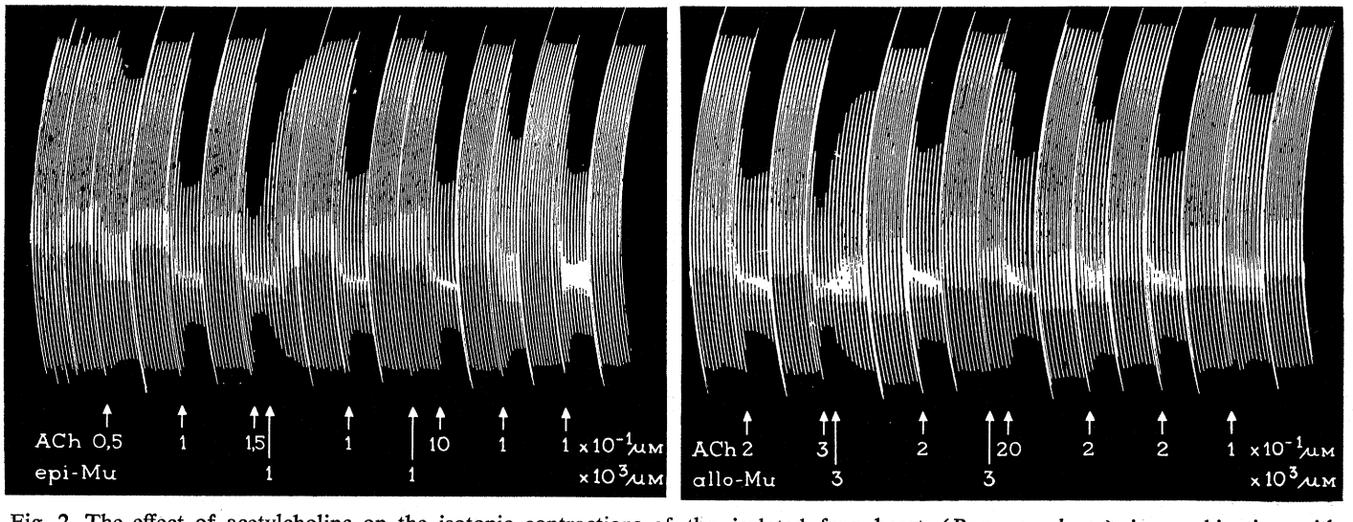

Fig. 2. The effect of acetylcholine on the isotonic contractions of the isolated frog heart (*Rana esculenta*) in combination with (left) epimuscarine and (right) allomuscarine. Note that epimuscarine is practically a pure competitive antagonist while allomuscarine is partly agonistic and partly competitively antagonistic.

Table 1. Intrinsic activities (α) and affinities (pD_2 or pA_2 values). Ach, acetylcholine; DL-allo-mu, DL-allomuscarine; DL-e/al-mu, DL-epiallomuscarine; DL-epi-mu, DL-epimuscarine; DL-mu, DL-muscarine; DL-pr-mu, DL-propyldemethylmuscarine; H fur, furtrethonium.

Substance	Rat jejunum			Frog heart		
	α	pD_2	pA_2	α	pD_2	pA_2
Ach	1	7.1		1	7.2	
H fur	1	5.9		1	5.9	
DL-mu	1	6.8		1	6.4	
DL-e/al-mu	1	5.0		1	4.5	
DL-allo-mu	1	4.4		0.4		3.7
DL-epi-mu	1	3.9		0.1		3.8
DL-pr-mu	0		4.7			5.1

partly parasympathomimetic and partly parasympatholytic (see Fig. 2, right). High doses of acetylcholine are antagonized by allomuscarine only to a certain degree, whereas allomuscarine, as such, depresses the heart beat to the same degree. Hence DL-allomuscarine is a parasympathetic drug with a dual action, or a partial agonist. Both DL-muscarine and DL-epiallomuscarine have purely parasympathomimetic action on the frog heart. The affinities, expressed as pD_2 or pA_2 values (*11*), and the intrinsic activities of the whole series are shown in Table 1.

The results establish that the affinity and intrinsic activity depend not only on the molecular structure of the drug but also on that of the receptor (*12*).

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2. J. M. van Rossum and E. J. Ariëns, *Experientia* **13**, 161 (1957).
3. DL-Propyl-demethylmuscarine was generously supplied by Dr. C. H. Eugster, Organic Institute of the University of Zürich.
4. Furtrethonium was generously supplied by Smith, Kline and French, Philadelphia.
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11. H. O. Schild, *Brit. J. Pharmacol.* **4**, 277 (1949); E. J. Ariëns and J. M. van Rossum, *Arch. intern. pharmacodynamie* **110**, 275 (1957).
12. The technical assistance of Miss C. Hurkmans, Miss R. de Groot, and Miss M. Cornelissen is gratefully acknowledged. Part of this report was prepared during a stay in the department of pharmacology, Tulane University School of Medicine, New Orleans, La., on a U.S. Public Health Service grant (2G-363).

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Increased Incidence of Tumor Metastases in Female Mice

Abstract. Most of the tumor cells injected into the tail vein of mice fail to survive at the site of arrest in the lungs, but the percentage of surviving cells is higher in females than in males. The surviving cells, however, grow at a similar and constant rate in both sexes.

The take and the growth of some transplantable tumors are known to be influenced by the sex of the recipient animal, females being usually more susceptible than males (*1*). It has been shown that the incidence of metastases is higher in female than in male mice bearing chemically induced skin tumors (*2*), and higher in estrogen-treated than in control rats bearing spontaneous mammary tumors (*3*). This higher susceptibility of female animals to tumor transplantation and metastasis may be explained through one of two mechanisms: either an increased growth rate of the tumor or an increased survival of the tumor cells originally injected or disseminated through the metastatic pathways.

In previous studies, using tritiated thymidine to label the dividing cells, we investigated the fate and the growth rate of Ehrlich ascites tumor cells injected intravenously into strain CAF_1 female mice (*4*). It was found that 99 percent of the tumor cells lodging in the lungs died in the first 48 hours, and that, from the 3rd day on, the surviving tumor cells grew at a constant rate. About 40 percent of the cells were labeled by a single injection of tritiated thymidine.

In connection with this experiment, a number of male CAF_1 mice, together with the females, were given 6×10^6 Ehrlich ascites tumor cells, from male donors, by injection into the tail vein. The animals were then randomized into four groups, each group receiving a single intraperitoneal injection of tritiated thymidine (30.5 μc per animal) at 1, 48, or 240 hours after the injection of tumor cells. The animals were sacrificed 24 hours after the injection of tritiated thymidine. The lungs were weighed, and the percentage of labeled tumor cells and the uptake of tritiated thymidine were investigated by autoradiography and liquid scintillation counting (*4*).

The results are shown in Table 1. It may be seen that the weight of the lungs increases with time after the intravenous injection of tumor cells, but more so in females than in males. As the weight of the lungs in animals thus treated is correlated with the number of secondary growths (*5*), this weight discrepancy indicates a lower incidence of metastases in the males. The uptake of tritiated thymidine by the lungs, which is essentially a function of the amount of tumor tissue present in these organs (*4*), follows a similar pattern, and on the 10th day it is five times higher in females than in males. It may also be seen that, if the tritiated thymidine label is taken as a criterion of viability of a tumor cell, the great majority of the injected tumor cells fail to survive at the site of arrest in the lungs; the number of surviving cells, however, is 10 times larger in the females than in the males. After 48 hours the percentage of labeled tumor cells, which is then a function of the growth rate of the tumor, is the same in both males and females. On the 10th day after tumor injection, the percentage of labeled tumor cells is again the same in both sexes. It should be added that, in the females, the growth rate of this tumor is known to be constant from the 3rd to the 12th day after the intravenous injection (*4*).

These data indicate that, under the conditions of the experiment reported here, the increased number of metastases in female mice, as evidenced by the heavier weight of the lungs and the higher uptake of tritiated thymidine, is due to an increased survival of injected tumor cells lodging in the lungs. Once established in the lungs, the tumor cells grow at a similar and constant rate in both sexes. It is sug-

Table 1. Uptake of tritiated thymidine in the lungs of mice injected intravenously with tumor cells. All animals were injected intravenously with 6×10^6 Ehrlich ascites tumor cells and, at the indicated intervals thereafter, with 30.5 μc of tritiated thymidine intraperitoneally.

Animals		Interval between injection of tumor cells and tritiated thymidine	Mean wt. of lungs (mg)	Uptake ($\mu c \times 10^2$)	Labeled tumor cells (%)
No.	Sex				
3	M	1 hr	226	1.55	>0.1
3	F	1 hr	232	1.38	0.8
3	M	24 hr	234	1.95	>0.1
3	F	24 hr	219	2.97	10.0
3	M	48 hr	216	5.40	35.0
3	F	48 hr	275	5.73	35.0
3	M	10 day	311	6.98	40.0
3	F	10 day	591	38.15	38.0