speculative. The fact that triamterene is actively secreted by the renal tubule (*4*) opens the possibility of active transport by other tissues as well. In this connection, it should be noted that the mechanism of renal secretion of triamterene has not been established. Since the placenta does not actively transport certain typical organic acids or bases (*3*), an alternative transport mechanism could operate both in the kidney and placenta. Purines are among endogenous substances for which a transfer system might exist. Requirements for transfer from the fetal compartment are in some ways analogous to those for transfer from the central nervous system. The recent demonstration of energy-dependent accumulation of purines by the choroid plexus establishes the existence of a transport mechanism (*9*) which could perform this function.

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10 December 1969

Environmental and Genetical Contributions to Class Difference: A Model Experiment

Abstract. *Flies were divided into two groups on the basis of the number of their bristles, and raised under different environmental (temperature) conditions. In each generation, offspring of the two groups were retained in their group or transferred to the other group, depending on the number of their bristles. After nine generations it was found that the genetic component of the intergroup difference was 42 percent; the portion of the intragroup variance that was genetic was 13 percent.*

It is known that human social classes may differ in average values for variables with significant heritabilities. It is not known, however, to what extent the differences between class means have genetic components. In most cases it is not easy to discover whether an observed class difference has a genetic component, still less to measure the proportion of that difference that is genetic, because environmental (especially cultural) effects and genetic differences are confounded and both can give rise to parent-offspring correlations, so that components of social heredity may spuriously inflate "heritability" estimates.

In such a situation the heritability of the variable may be shown to be high within families, or even within classes, but the question of the heritability of between-class or between-group differences remains completely open, because there is no warrant for extrapolating from within- to between-group heritabilities without satisfactory "transplant" experiments (see *1*). Thus with a continuous variable such as I.Q., though we know heritabilities are significant, we are still in a position where we cannot explain the observed class differences in man.

The problem is to decide whether and to what quantitative extent environmental factors directly affecting contemporary individuals cause the class differences, and to what extent the difference is caused by genetic differences that have arisen because differing genotypes have in the past moved into the different classes. Measurement of the relative contributions of these two causes can be made only if measurement of the between-class heritability can be obtained.

Table 1. Mean numbers of bristles of two groups of flies, and intergroup mobility.

Generation	Group		Difference	Mobility (%)
	20°C	25°C		
0	19.8	18.9	0.9	
6	19.5	17.4	2.1	20
7	19.9	17.7	2.2	20
8	19.9	17.4	2.5	30
9	19.8	17.4	2.4	20

Discontinuous variables, such as blood groups, or sickle cell anemia, present a less intractable problem, for with these we may more or less completely classify individuals into genotypes, estimate gene frequencies, and compare the gene frequencies of classes with some precision. Indeed, it is known (*2*) that Africans of different social status in Kampala differ in frequency of the sickle cell gene. This seems likely to be a consequence of greater selection of the ancestors of the contemporary "lower" classes for resistance to malaria, for it is well known that sickle cell heterozygotes are in some measure protected from malaria, a protection that in high-malarial areas counters the serious disadvantages that sickle cell homozygotes suffer.

Though continuous variables present much greater problems than this, it is to be expected on theoretical grounds that if any of them is correlated with social mobility [as I.Q. is known to be to some degree (*3*)] and has significant heritability [as I.Q. has (see, for example, *4*)], then the social mobility will lead to nonrandom transfer of genes from class to class. These classes will therefore be expected to come to differ genetically to some extent. At the same time, however, if nutrition, home background, educational opportunities, and so forth, differ between the classes, then the classes will also differ for immediate environmental (including cultural) reasons. We thus have a theoretical expectation that social classes will come to differ genetically in I.Q. but no adequate basis for estimating whether this might explain a significant part of their phenotypic differences of mean I.Q. or any other relevant variable.

Such theoretical expectations are useless unless they can be tested and quantified. Since the problem they pose is of considerable political significance and also involves emotive ideological questions, it seems important that they be tested, preferably in a context of minimum emotive significance. To this

end we have devised some simple experiments using the fly *Drosophila melanogaster* that are aimed at exploring the relative contributions of genetic and environmental factors to group differences in a variable made to determine mobility between groups whose environments differ. We use the word "group" to avoid the social implications that may be attached to the word "class" and thus to stress that we are dealing with an analogy.

Our experiment, so that the results may be open to simple interpretation, is designed to meet the following general requirements:

1) A continuous variable of significant but incomplete heritability. We have chosen sternopleural bristle number.

2) Between-group environmental differences that affect that variable. We have chosen temperature, for *Drosophila* tend to have more bristles when they develop at lower temperatures (*5*).

3) The population as a whole is not exposed to artificial selection for the variable. It is admitted that social classes may differ in fertility, and that fertility differences correlated with I.Q. are known; but the introduction of artificial selection in the experimental design would merely complicate interpretation without helping us to answer our primary questions. The particular design we have chosen (see below) permits natural selection between families of different fertility, and of course we "select" the individuals for allocation to their group, and the group division involves assortative mating because mating occurs after mobility between groups. But, since all individuals are given the opportunity to breed, there is no overall artificial selection pressure.

4) Mobility between groups is determined solely by the chosen variable, and the environmental effect acts in the same direction as the intergroup mobility, so that between-group parent-offspring environmental and genetic correlations have the same sign.

The experimental design based on these principles may be varied in many ways, but for our first experiment we have chosen to have two groups only, of equal sizes, each comprising the flies grown in one culture vessel. One, which receives the flies with higher bristle numbers, is grown at 20°C; the other, which receives the flies with lower bristle numbers, is grown at 25°C. The population is maintained as follows. In each generation we count the bristles on each of ten virgin females and ten males from each culture vessel. The ten flies with the highest bristle numbers from each sex from the 20 counted are then placed in a 20°C environment to produce the next generation of one group. The ten with the lowest bristle numbers are placed in 25°C to produce the next generation of the other group. (Where a choice has to be made to determine which of a number of similar flies shall go into which group, it is made at random.) We have run such a population for nine generations, starting from our "Barton" wild stock.

Table 2. Mean numbers of bristles of flies of generation 8 raised in two environmental conditions.

Environment	Progeny of flies with high number of bristles	Progeny of flies with low number of bristles
20°C	19.9	18.5
25°C	18.3	17.4

The means for the two cultures and the intergroup mobilities, that is, the percentage of flies that, because of their bristle numbers, changed group in each generation, are given in Table 1 for the base population and the more recent generations.

The results clearly show that the group grown at 20°C has the higher mean, that the difference in means is greater than that produced by temperature alone on the base population, and that mobility is less than the 50 percent which would be expected if the groups were not different. An analysis of variance carried out in triplicate assays of generation 7 gave no evidence of difference between samples, but a highly significant ($P < .001$) difference between the 20°C and 25°C groups, and, as is usual for this variable, between sexes. There was a small interaction involving sexes, but no other significant term.

It remained to determine the degree to which the groups differed for genetic or environmental reasons. With this organism we can readily make transplant experiments. To do this, the extra samples that had been scored in generation 7 were used. A culture of progenies of flies with high bristle number (the 20°C group) was set up at 25°C, and a culture of progenies of flies with low bristle number (the 25°C group) was set up at 20°C, so that with the population itself there were four kinds of progeny in the next generation. We could thus discover what part of the difference of group means would remain when the difference of environmental conditions in which the groups were grown was removed. The results are set out in Table 2.

Table 3. Analysis of variance of the test data.

Source	*N*	Mean square
Group	1	84.02*
Temperature	1	114.82*
Sex	1	64.07*
Group × temperature	1	3.3†
Other interactions	3	3.2†
Within sex, group and temperature	232	2.06

* $P < .001$. † Not significant.

The difference between the means of the two groups when grown in their "own" environmental conditions is $19.9 - 17.4$, or 2.5 bristles per individual. When the environmental conditions are made the same, the difference is reduced to an average of 1.15 bristles per fly, giving us a between-group heritability of $1.15/2.5 = 0.42$.

Table 3 gives a full analysis of variance of these data and shows that the group difference, environmental difference, and sex difference are all highly significant.

This analysis of variance clearly demonstrates that in the conditions of this experiment the groups have come to differ for *both* environmental *and* genetic reasons. We have, however, used these data to attempt a partition of the variance of bristle number in the population with a view to estimating the relative importance of the two causes of variation in bristle number. To do this we needed in addition an estimate of the heritable proportion of the within-group, within-temperature variance. This we have obtained from assortative mating tests to give the regression of offspring mean on mid-parent value within groups in their "own" environment. The heritability estimates average 0.13.

Table 4 gives the resulting components of variance. A high proportion of the genetic variance is between groups, and the environmental variance arising from temperature (the group environmental difference) is about the same as that arising from other environmental causes. The proportion of the group difference that is genetic (0.42) is larger than the proportion of the within-group variance that is genetic (0.13), which underlines the dangers referred to above of equating within- and between-group heritabilities. The overall heritability (non-sex) is

Table 4. Components of variance (exclusive of the between-sexes variance) as percentage variance.

Source of variance	Phenotypic variance	Heritability	Genetic component	Environmental component
Between groups	60.4	0.42	25.4	35.0
Within groups	39.6	0.13	5.1	34.5
Total	100	0.30	30.5	69.5
(Between sexes)	(7.9)	(1)	(7.9)	

0.30; this is of similar order to that at the beginning of the experiment, when it was tested and found to be 0.25.

The experiment has therefore confirmed the various points that were under test. Intergroup mobility dependent upon a variable does lead to genetic differences between groups even under conditions where there are strong environmental differences between groups. In fact, in the particular conditions of this experiment the genetic and environmental differences between groups are not of very different importance. On the other hand, despite the environmental difference, much of the genetic variation has sorted out *between* groups at the expense of within-group variance.

The human situation is much more complex than that in our experiment. For example, there is intra- as well as interclass social heredity in the human situation, whereas in our experiment the parent-offspring environmental correlation is entirely between groups; the correlation between human social mobility and any particular variable is incomplete, whereas we have tried to make it complete in our experiment; and the environmental difference between human classes is complex and doubtless very heterogeneous, whereas in our experiment we have made the controlled environmental difference between groups simple and have made it correlate completely with groups. Further, we stress that no importance should be attached to the actual heritabilities or components of variance obtained in our experiment, for they would have differed had we used a smaller or greater temperature difference, a base stock with different initial heritability, or one showing genotype-environment interaction with respect to temperature. It is therefore obvious that extrapolation from our experiment, or indeed from any other animal experiment, must be made only with extreme caution.

Nevertheless, we do feel that our experiment is relevant to the human situation inasmuch as it strengthens the expectation that social mobility related to a heritable variable will give rise to some genetic difference between class means despite strong parent-offspring environmental correlation. We therefore believe that our experimental results support those who hold the view that neither cultural nor genetic approaches alone are likely to lead to adequate explanations of social class phenomena.

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3 December 1969

Bladder Cancer Induction by Aromatic Amines: Role of N-Hydroxy Metabolites

Abstract. *Repeated installation of* N-*hydroxy-2 naphthylamine into dog bladders produced tumors of this organ. There is a correlation of degree of carcinogenicity of 1-naphthylamine, 2-naphthylamine, and 4-aminobiphenyl with both methemoglobin production in the blood and concentration of* N-*oxidation products in the urine. Thus,* N-*hydroxylation appears to be the key to bladder cancer production by aromatic amines.*

After the discovery of the role of *N*-hydroxylation in hepatocarcinogenesis by 2-acetylaminofluorene (*1*), the search for the urinary metabolite active in the induction of bladder cancer by 2-naphthylamine in man and the dog has centered on *N*-hydroxy-2-naphthylamine. The discovery of this substance in the urine of dogs that were given 2-naphthylamine supported this concept (*2*). However, the demonstration of the occurrence of *N*-hydroxy-1-naphthylamine in the urine of dogs that were given large doses of 1-naphthylamine (*3*), coupled with the observation that *N*-hydroxy-1-naphthylamine is more carcinogenic than *N*-hydroxy-2-naphthylamine when tested by intraperitoneal administration to rats (*4*), casts serious doubt on the relevance of *N*-hydroxylation to the induction of bladder cancer.

In an attempt to resolve the dilemma, we found that in the dog the *N*-hydroxynaphthylamines are further oxidized to nitroso compounds (Fig. 1). With thin-layer and gas chromatography, we found both 1- and 2-nitrosonaphthalene in urine. Gas chromatography is a rapid and sensitive way to determine these substances in urine. After the administration of 2-naphthylamine to dogs, the urine, collected by catheter, was adjusted to *p*H 4. Ferricyanide was added to convert the *N*-hydroxy-2-naphthylamine to 2-nitrosonaphthalene. The urine was then extracted with petroleum ether, and the extract was injected on the gas chromatograph. The peak obtained, therefore, represented the sum of *N*-hydroxy and nitroso compounds present which we refer to as total *N*-oxidation products. Ferricyanide could not be used in the measurement of the *N*-oxidation products of 1-naphthylamine. Adjustment of the *p*H to 6.5, however, allowed the extraction with petroleum ether of both *N*-hydroxy and nitroso metabolites. *N*-hydroxy-1-naphthylamine is quantitatively converted to 1-nitrosonaphthalene on the column at 125°C. Studies of mixtures of *N*-hydroxy and nitroso derivatives of both amines added to urine were performed. Recoveries in the range of 80 to 100 percent were obtained. Sensitivity of detection on the electron-capture detector was 100 pg of these substances, and thus we were able to measure 40 parts per billion in 5 ml of urine. This procedure was also applicable to the determination of the *N*-hydroxy metabolites of 4-aminobiphenyl, a bladder carcinogen recently shown in this laboratory to be distinctly more potent than 2-naphthylamine (*5*).

The urine of dogs that had been given the maximum acute dose of these amines that they could tolerate (70 mg/kg) was examined for *N*-oxidation products by gas chromatog-