

## EFFECTS OF SPONTANEOUS AND INDUCED MUTATIONS ON SELECTION RESPONSE

Elisabeth Gründl and Leo Dempfle  
Institut für Tierwissenschaften  
Technische Universität München-Weihenstephan  
8050 Freising, FRG

### SUMMARY

Spontaneous and induced mutations may increase selection response. Negative effects of mutations can be overcome by selection. However, it seems to be hardly possible to accumulate induced mutations in unselected populations. In some experiments with *Drosophila* great selection responses could be traced back to effective factors with large effects. This indicates that mutants for bristle number show a leptokurtic distribution which leads to big differences of selection response between replicates.

### INTRODUCTION

The first attempts to induce genetic changes by irradiation or by chemical mutagens were made in the beginning of the 20th century (Wolf (1909), Schiemann (1912), Dewitz (1913) cited by Stubbe 1930)). However, the most famous experiments with *Drosophila* which encouraged radiation genetics and research in chemical mutagenesis have been published by Muller (1927) and by Auerbach and Robson (1944, 1946). After that a tremendous lot of work has been done to classify mutations induced in this manner by various doses and agents. Later on, working with quantitative traits, Scossioli (1953) and Clayton and Robertson (1955) observed additional selection response after irradiation. Clayton and Robertson (1955) published also the first estimates of mutational variance. Buzzati-Traverso (1954) enlarged the number of offspring after treating isogenic strains with x-rays. In most cases selection response caused by induced mutations was small compared to that found in outbred populations. Thus only little work was done in this field. However, since Frankham (1980) stressed the influence of mutations on long term selection response and Hill (1982) looked more closely into the theoretical background interest in this area grew up again.

This paper is mainly restricted to effects of spontaneous mutations and mutations induced by irradiation and by the chemical mutagen ethylmethane sulfonate (EMS). Also transformation is briefly covered. The field of mutations by hybrid dysgenesis is covered by the papers of Mackay and Moran given in an other session.

### EFFECTS OF IRRADIATION AND EMS

#### 1. Effects of irradiation on DNA

Ionizing radiation is able to damage parts of the cell directly or indirectly by building radicals when reacting with water. These radicals may build toxic agents or may react with DNA. Several types of damage may be produced:

- single-strand break
- double-strand break
- alteration or loss of nucleotide-bases
- denaturation.

## 2. Effects of EMS

EMS is an alkylating agent, because it is adding a  $\text{CH}_3\text{-CH}_2$ -group to the DNA base. Alkylation of guanine, the nucleotide base which is alkylated most, leads to mispairing with thymine instead of cytosine. This is called transition, because the pyrimidine cytosine is replaced by the pyrimidine thymine (G:C--A:T). Alkylation of guanine may also cause depurination, the loss of the alkylated base. This gap may be repaired, but if that is not done before replication, adenine will be incorporated into the daughter strand opposite the gap. A pyrimidine is now replaced by a purine what is called transversion (G:C--T:A). The probability of mispairing after alkylation is less than 100%. This results in delayed mutations, i.e. mutations which occur after several replications. This is the reason for the high amount of mosaics created by EMS.

## 3. Types of genetic damage

Table 1 compares the types of genetic damage due to 1 mM EMS fed to adult males, and irradiation with 20 Gy gamma-rays.

**Table 1:** Kinds of mutation (%) induced by EMS fed to adult males and by gamma-rays

	spontaneous	1 mM EMS	20 Gy gamma-rays
sex-linked recessive lethal	0.10	4.0	4.0
dominant lethal	3 - 10	-	30
translocation	0.04	0.2	2.0
minute	0.10	0.3	0.6

Recessive lethal mutations represent a heterogeneous class of genetic damage, consisting of gene mutations, deletions and other structural rearrangements. The amount of sex-linked recessive mutations is estimated as percentage of X-chromosomes which are lethal when hemizygous. Chromosome breakage events are the predominant type of event which results in dominant lethality. The amount of dominant lethals equals one minus the percentage of larvae hatching from eggs or adults emerging from pupae. The meaning of this test is quite doubtful because nongenetic damage, i.e. inactivation or malfunction of sperm, may contribute to what would be scored as "dominant lethal". Translocations lead to production of gametes with duplications and deficiencies for parts of the chromosomes which are usually lethal to the zygote. They are recognized by the absence of two out of four expected phenotypes in the second generation after treatment. When heterozygous, Minute mutants cause short thin bristles and delayed developmental time. They are lethal in homozygous or hemizygous condition.

1 mM EMS fed to adult males and irradiation with 20 Gy gamma-rays cause the same amount of recessive lethal mutations and an approximate fivefold increase of visible mutations. However, gamma-radiation increases dominant lethal mutations and translocations drastically whereas EMS showed no or much smaller effects. That means that gamma-rays induce much more drastic effects on chromosome structure than EMS or spontaneous mutations.

## MUTATION IN PLANT BREEDING

In the early 1940's the first attempts to utilize induced mutations in modern plant breeding have been made. Annual diploid and allopolyploid self-fertilizing crops and ornamentals have mainly been used in the experiments. Many mutants favourable for the character of interest turned out to have negative effects on other traits. Sometimes these negative effects could be overcome by transferring the mutants into a different genetic background. However, in spite of the amount of work which had been done in this field only a few mutants are practically used. Gottschalk and Wolff (1983) listed 24 released varieties with improved seed storage substances.

Plants seem to be more resistant to chromosome and genome mutations than animals. Many polyploid plants are known. Gottschalk (1978) showed that only one out of 25 *Pisum* strains homozygous for an induced translocation was inferior to the mother variety with regard to vitality and fertility. On the other hand in animals chromosome and genome mutations lead often to sterility or lethality.

## SELECTION EXPERIMENTS WITH ANIMALS

### Chicken

Abplanalp et al. (1964) irradiated sperms of a selected single comb white leghorn strain over a period of seven generations with 10 to 15 Gy x-rays for a total of 80 Gy. Following the seven generations of irradiation the chicken were selected for increasing egg number over six generations. During the irradiation period 60 males were mated to 60 females each generation and during the selection period each of 10 males were mated to 5 females. Within each treatment two replicates were selected. During the irradiation period traits associated with reproduction like hatchability and egg production decreased, indicating that the effects of mutations concerning these traits are not distributed symmetrically around zero. However, hatchability and egg production were restored two generations after the end of irradiation. In subsequent generations there was no difference in selection response between both treatments.

### Rats

Gianola et al. (1979) tried to increase genetic variance in an inbred strain of rats by irradiating males with 4.5 Gy in each of nine consecutive generations. Following irradiation the rats were kept in closed lines without selection for 6 generations. After that each of the two replicates in the irradiated and the control group was split into three sublines for within-litter selection for increased, decreased and random six-week body weight. In irradiated lines small selection responses were realized only for decreasing body weight. Untreated lines remained unchanged. The authors concluded that irradiation does not increase selection response substantially.

### Mice

Womack and Bogart (1968) selected ten random bred mice lines, maintained with four males and twelve females each, over a period of five generations by an index giving equal emphasis to large litter size and large 28-day weights. Within two lines males and females were irradiated with 0, 0.25, 0.5 and 1 Gy x-rays respectively each generation whereas two lines were randomly selected. After four generations the lines receiving 0.5 and 1 Gy x-rays were lost. In five of the six

irradiated lines selection response for litter size was negative. Total selection responses for 28-day weight were smaller in the irradiated lines than in the control lines and average realized heritability was more or less halved. However, variance of selection response was much higher in irradiated than in control lines.

Roberts (1967) tried to overcome a selection plateau which was reached after 35 generations of selection for high 6-week weight in mice by applying 6 Gy x-rays to selected mice once. He did not get any additional gain, but on the other hand litter size, a reproductive trait, was not affected, too.

#### *Tribolium castaneum*

Enfield (1980) crossed two inbred lines of tribolium and selected two sublines of this cross for 130 generations for high pupa weight. A more or less linear selection response was realized during the whole experiment. In a later experiment Enfield (1986,1989) expanded an inbred line of *Tribolium* and maintained it, divided into two subpopulations, for 90 generations in population cages with an effective population size of 100 - 200 animals. Mutational variance, estimated from these experiments could explain the long continuing selection response and the amount of variance left in unselected control lines of the former experiment after 130 generations. It should be pointed out, that spontaneous mutation were accumulated in expanded inbred lines without selection such that after 90 generations, when selection began, heritabilities of approximately 0.20 and 0.10 were realized.

Bartlett (1966) investigated the influence of radiation on selection response in *Tribolium*. He selected an outbred strain and a strain which had been previously selected for high pupa weight and had reached a second selection plateau after 44 generations. During the experiment 50 pairmatings were made each generation and the best ten families were selected. Three levels of x-irradiation were chosen: 0, 1, and 10 Gy each generation. After eleven generations of selection, untreated lines responded most, lines treated with 10 Gy responded less. In some of the irradiated lines heritability and phenotypic variance increased, but decreasing reproductive fitness lead to smaller selection differentials and selection responses.

#### *Drosophila melanogaster*

A lot of experiments with *drosophila* have been carried out to investigate the influence of spontaneous and induced mutations on selection response. Table 2 summarizes the results. In order to make experiments using abdominal and sternopleural bristles comparable cumulated selection responses ( $C_t$ ) are standardized with  $\sigma^2_p$  of the base population.

Selecting outbred populations over a period of 20 generations for increased sternopleural bristles one would expect to reach a selection plateau with a standardized cumulated selection response of approximate 5 units.

On average, selection responses based on spontaneous mutations are much smaller. However, in some replicates of the experiments of Mather and Wigan (1942), Frankham (1980) and Gründl and Dempfle (1987) after 20 generations of selection a standardized cumulated selection response of 2 to 4 units was observed. These results were even surpassed by selection responses due to induced mutations. On the other hand it should be pointed out, that there are big differences between replicates. In many experiments lines can be observed which show no selection response. Hill (1982) emphasized that a leptokurtic distribution of mutational

effects leads to big variances of selection response. His results from Monte Carlo simulation are supported by experimental results where great selection response could be traced back to effective factors with large effects (Frankham 1980, Gründl and Dempfle 1989).

#### TRANSFORMATION

Mutations, spontaneous or induced, increase genetic variability in a random manner. A similar effect might be achieved with transformation. The basic phenomenon there seems to be that the sperms can be so heavily irradiated that the DNA is "pulverised" but motility is still present. The eggs are penetrated by the irradiated sperms but no fertilisation occurs. The eggs can then still be fertilized by untreated sperms. In some cases genetic material from the irradiated sperms is taken up and expressed. Pandey & Patchell (1982) report such an experiment with chicken, where they used White Leghorn, (white feathers and pure white eggs) and Sykes (a strain with brown/black feathers and brown eggs). The White Leghorn females were inseminated with irradiated sperms from the Sykes and some of them were inseminated next day with semen from Leghorn. There were no offspring from hens only inseminated with irradiated sperms. Among the offspring of the hens inseminated twice, some produced tinted eggs. The transformation was further corroborated by the results from backcrosses.

A very similar experiment with chicken is reported by Tomita (1987) where the results of Pandey & Patchell is confirmed. Also the technique was used in mice, with some indication of an effect.

Bumstead et al. (1987) reported an experiment which critically checked the results of Pandey & Patchell (1982) using among others the haplotypes of the major histocompatibility complex as markers. They clearly corroborated the findings of Pandey & Patchell (1982) and showed that the aquired genetic information is transmitted to the progeny.

#### DISCUSSION

As shown by the results with *Drosophila* mutations may increase selection response. If mutations with large effects occur, responses are comparable to that found in experiments with outbred populations. However, experience in plant breeding and experiments with mammals have not given very optimistic results. Why do results of irradiation experiments with chicken, rats and mice not agree with those using *Drosophila* as test animal? There might be several reasons.

1. *Drosophila* is more resistant to the harmful effects of irradiation than vertebrates are.
2. Gianola and Abplanalp first irradiated the lines and selected in subsequent generations. So natural selection was able to eliminate created variance before selection begun.

The results of Enfield (1988) and Gründl and Dempfle (1989) indicate, that spontaneous mutations can be accumulated in unselected populations. On the other hand the experiments of Gründl and Dempfle (1989) indicate, that this is not true for induced mutations. However, the negative effects of mutations can be overcome by selection. Appraising this effect, one should keep in mind, that selection responses in outbred populations are often correlated with decrease in fitness, too.

The occurrence of mutants with large effects violates the assumption of Hill's (1982) formula for estimation of mutational variance. If mutational effects are not additive, estimates of  $\sigma^2_M$  are biased. So, to get realistic estimates, distribution of mutant effects must be known and the model should be adapted to it.

Hill assumes mutant effects to be distributed symmetrically around zero. That means that there is no limitation for positive mutants to occur, however, perhaps the low estimates for  $\sigma^2_M$  for fitness traits found by Lynch (1988) indicate that this is not true for traits which have been selected for over a long period.

**Table 2:** Selection responses in *Drosophila* based on spontaneous and induced mutations. Selection was done on several base populations; in refers to an inbred, is to an isogenic and p to a plateaued base population. Generation gives the number of generations with artificial selection, Gy the amount of radiation given each generation to males and females

a: sternopleural bristles

reference	base population	generation	$C_t/\sigma_p$	range	pairs scored/selected	number of lines	Gy
Mather & Wigan (1942)	in	21	0.4	0.3-0.5	20/3	2	0
Mackay (1988)	in	19	1.4	1.2-1.5	40/10	3	0
Gründl & Dempfle (1987)	in	41	1.3	0.0-2.7	50/10	8	0
	in	3 <sup>2</sup>	0.8	0.3-1.4	50/10	4	10 <sup>1</sup>
Scossiroli & Scossiroli (1959)	is	5 <sup>2</sup>	0.0	-	15%	1	0
	is	11	0.7	-	15%	1	0
	is	5 <sup>2</sup>	1.6	-	15%	1	30
	is	11	4.5	-	15%	1	30
Scossiroli (1953)	p	17 <sup>2</sup>	0.7	0.0-2.4	30/5	4	0
	p	17 <sup>2</sup>	3.2	0.0-8.9	30/5	4	30
Clayton & Robertson (1964)	p	8 <sup>2</sup>	0.6	0.2-0.7	25/10	4	0
	p	8 <sup>2</sup>	0.7	0.5-0.9	25/10	4	18
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Gründl & Dempfle (1987)	in	32	2.8	0.6-7.3	50/10	8	EMS <sup>3</sup>

<sup>1</sup> only males irradiated

<sup>2</sup> selection was carried out in cycles; one generation random selection followed by one generation artificial selection.

<sup>3</sup> 0.75 mM resp. 1 mM EMS fed to males during the first 20 generations

Table 2:

b: abdominal bristles

reference	base population	generation	$C_t/\sigma_p$	range	pairs scored/selected	number of lines	Gy
Mather & Wigan (1942)	in	17	1.4	0.5-2.2	20/3	3	0
Clayton & Robertson (1955)	in	17	0.03	-	25/10	2	0
	in	17	0.8	-	25/10	2	18
Clayton & Robertson (1964)	in	5	0.1	0.0-0.2	25/10	3	0
	in	5	0.1	0.0-0.3	50/10	4	0
	in	5	0.3	0.1-0.7	50/10	6	0
Kitagawa (1967)	in	20	0.1	-	30/6	2	0
	in	20	1.4	-	30/6	2	15 <sup>1</sup>
	in	20	0.7	-	30/6	2	15 <sup>2</sup>
	in	20	1.3	-	30/6	2	15
Hollingdale & Barker (1971)	in	20	0.2	0.0-0.3	200/100	3	0
	in	20	0.6	0.3-0.7	200/100	5	10
Frankham (1980)	is	50	3.7	1.0-6.0	10/3	6	0
Clayton & Robertson (1964)	p	7 <sup>3</sup>	0.3	0.0-0.6	25/10	3	0
	p	7 <sup>3</sup>	0.6	0.1-1.0	25/10	3	18

<sup>1</sup> only males irradiated<sup>2</sup> only females irradiated<sup>3</sup> selection was carried out in cycles; one generation random selection followed by one generation artificial selection.

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