

BLIND FLOUR BEETLES: VARIABLE EYE PHENOTYPES
IN THE MICROCEPHALIC (*MC*) MUTANT OF *TRIBOLIUM CASTANEUM*
(COLEOPTERA, TENEBRIONIDAE).

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ABSTRACT

The recessive mutation *microcephalic (mc)* in *Tribolium castaneum* affects primarily the head morphology, but also affects the compound eyes. One or both of the eyes may be missing, and when present, the eyes may vary from a single ommatidium to an almost normal size. This effect is temperature-dependent: blindness and asymmetry increase in frequency at lower temperatures, and the two conditions seem to be related in a complementary way. The critical time for the determination of eye phenotypes seems to be in the embryonic or early larval stage, although the eyes are only visible in the late pupa and adult.

The temperature-dependent expression of the eye phenotypes may be explained using the "morphogenetic substance" theory.

INTRODUCTION

This article is dedicated to Prof. Y. Kugler, in recognition of his contribution to the study of insects in Israel and his role in shaping my scientific career.

Flour beetles (*Tribolium*) are notorious pests of stored products, but they are also excellent organisms for entomological research. Four volumes compiled by Sokoloff (1966, 1972, 1974, 1977) contain reviews of the data on the general biology, ecology, and genetics of *Tribolium*. In particular, these organisms turned out to be very useful for laboratory studies of population ecology, population genetics, and evolutionary processes.

In these fields of research, mutations affecting *Tribolium* morphology are very useful. The *mc* mutation discussed in the present paper is particularly interesting because it has secondary morphological effects with incomplete, temperature-dependent expressivity.

The phenotypic expression of a character depends not only on the genetic information coded in the DNA, but also on complex interactions with other genes and with the environment. The effect of environmental factors on the expression of the genotype are illustrated by, and can best be studied with, mutant genes having incomplete penetrance and/or expressivity.

Penetrance and expressivity of a gene are in fact two aspects of the same

phenomenon (Rendel 1962). When a mutant phenotype appears in only a fraction of the homozygous individuals, it is described as incompletely penetrant (e.g. the *eu* mutant of *Tribolium castaneum*. Wool and Mendlinger, 1973). Individuals which do express the mutant phenotype may vary in the level of its expressivity. Such is the case in the *vestigial* gene of *Drosophila melanogaster*, where the mutant wings may vary in size from small to almost normal (e.g. Nakashima-Tanaka 1967), and in the *lobe* gene of the same organism (see Ayala and Kiger, 1980, for a description). Examples of genes with variable penetrance and/or expressivity are known also in mammals (e.g. Wittman and Hamburgh, 1968), and in plants (e.g. Ashri, 1970; Gottschalk, 1978).

Penetrance and expressivity are known to be affected by environmental factors, in particular by temperature (Nakashima-Tanaka 1967; Wool and Mendlinger 1973).

The present study deals with the effects of temperature on the expressivity of eye phenotypes in the recessive, head-shape mutant *mc* (microcephalic) of the flour beetle *Tribolium castaneum*.

MATERIALS AND METHODS

Strain history. A single mutant female was discovered in 1979 in a *T. castaneum* strain homozygous for the *pearl* (*p*) allele (a recessive eye mutant in linkage group II. Sokoloff, 1966). The shape of the head was deformed and the individual looked completely blind (Fig. 1). A detailed examination revealed that one compound eye was missing entirely and only five ommatidia remained of the other. The phenotypic appearance of these characters corresponded with the description of the mutant "microcephalic" (*mc*), first detected in 1961 by Sokoloff and Lasley (Sokoloff, 1966). The primary effect of this mutation is the deformation of the head ("harpoon-like"). This character was described as recessive with full penetrance. The effects on eye shape, size and number of ommatidia are described as secondary. The *mc* locus is located in linkage group V (Sokoloff, 1966) When tested by F₂ or backcrosses, *mc* caused about 10% reduction in viability, but it behaved as a lethal if introduced as a heterozygote into a population.

The individual was crossed to a normal male and our current *mc* strain was derived from the F₂ offspring of this cross. The preliminary genetic work was carried out in 1979, and remained unpublished. In 1984 we continued this research with emphasis on the regulation of eye phenotypes. The combined results are reported here.

In addition to *mc*, we used two other laboratory strains of *T. castaneum*: *pearl*, homozygous for the *p* allele (linkage group II) and *black*, homozygous for the "black body" (*b*) allele (linkage group III). Sokoloff, 1966).

Environments. All strains are routinely maintained on wheat flour supplemented with 5% brewers' yeast, and in a standard environment of 30°C and about 60% RH.

Experimental design. Two types of experiments were carried out: — a "genetic" type and an "environmental" type. The "genetic" type was set up with single-pair crosses. Pairs of virgin adults of known phenotypes were allowed to oviposit in vials with 3 g medium for 1 week. Then they were removed, and their offspring were classified and counted by phenotype as they became adult. The "environmental" type of experiments was set up with egg samples. Large numbers of *mc* adults were allowed to oviposit for 3 days in jars with standard medium. The eggs were then sifted out, and



Fig. 1. (A) Ventral views of a normal *Tribolium castaneum* adult (left) and a blind (R-L-) *mc* mutant (B) Mutants from left: R+L-, R-L+, Sn. The last individual is normal.

samples of 100 eggs were counted into vials as before. In this type of design, input egg density was constant, and the environmental (temperature) regime during ontogenesis was modified in different treatments.

Phenotypic classes. The eye phenotypes were originally grouped into four classes: both eyes missing (R-L-), right eye missing (R-L+), left eye missing (R+L-), and "almost normal" (Sn). For analysis, the two asymmetrical classes (R+L-, R-L+) were pooled.

Epistatic effects on the eye phenotypes were investigated (with the "genetic" design) by crossing *mc* individuals with strains with a similar (*pp*) and different (*bb*) genetic background and observing the F₂ offspring.

RESULTS

I. *Inheritance of the mc (head shape) mutation.*

Frequencies of wild type and mutant ("harpoon-like") phenotypes in F_2 offspring of crosses of *mc* with *bb* and *pp* were significantly different from the expected 3:1 ratio (Table 1), confirming the results reported by Sokoloff (1966). No significant differences were found between reciprocal crosses.

The observed frequencies of *mc/mc* homozygotes were always significantly lower than expected (Table 1). Simple calculations based on Hardy-Weinberg expectation with selection against a recessive homozygote yield rather low fitness estimates for *mc/mc* relative to the other genotype (W_i in Table 1). The estimates in 1979 and 1984 differ considerably, but are rather similar when *mc* is crossed with different strains (i.e., fitness is not much affected by the genetic background).

In 1984 another experiment was carried out using the "environmental" design. Samples of eggs of *mc* and *pp* were reared to adulthood at 3 temperatures: 23°C, 27°C and 30°C. In all three environments, *mc* survived considerably less than *pp* (Table 2), supporting the former conclusion.

II. *The secondary effects of mc on the eye phenotype.*

In 1979 and again in 1984, "genetic"-type experiments were carried out in which pairs of virgin *mc* adults of known eye phenotypes were crossed. All crosses were held at 30°C. The results are summarized in Table 3. The number of single pairs in each cross were not the same, depending on the availability of adults of each phenotype.

In 1979, the frequencies of offspring were independent of the parental phenotypes, and no heterogeneity was detected among crosses (Sokal and Rohlf 1981) (Table 3A). In 1984, very highly significant heterogeneity in the proportions of phenotypes was detected among parental crosses ($P < 0.001$). The proportions of blind and asymmetrical individuals were high in crosses having at least one blind parent, and low when both parents were of the "semi-normal" phenotype (Table 3B). The possible reasons for the discrepancy between years will be discussed later.

III. *Temperature effects on the eye phenotypes.*

To test for possible effects of temperature on the expression of the eyes in *mc*, an "environmental"-type experiment was carried out. Egg samples of *mc*, oviposited at 30°C, were held for development in vials with medium at 3 temperatures: 23°C, room temperature (daily average at that time: 27°C) and 30°C. Offspring of all replicates are pooled in Table 4.

The frequencies of the eye phenotypes significantly depended on temperature ($p < 0.001$). The frequency of blind individuals was higher at 23°C than at 27 or 30°C (Fig. 2). Adding the asymmetrical individuals does not change this pattern.

IV. *Critical time for the temperature effect on eye expression.*

In this "environmental" type experiment, 15 samples of 100 eggs were collected from *mc* stock adults at each of 2 temperatures: 25°C and 30°C. Five of the 15 were allowed to complete development at the oviposition temperature, five were transferred to the alternative temperature at the early larval stage (day 7 after set-up), and five

TABLE 1. Frequencies of normal and mutant (harpoon-like head) offspring in F₂ of the reciprocal crosses *mc/mc* x *p/p* and *mc/mc* x *b/b* compared with the expected 3:1 ratio. All replicates pooled. No significant differences was detected between reciprocal crosses. *W_i* = estimated relative fitness of *mc/mc* homozygotes (for the normal homozygote, *W_i* = 1.0).

| | | Normal | Mutant | Total | <i>W_i</i> |
|---|------|--|--------|-------|----------------------|
| Cross <i>mc/mc</i> x <i>p/p</i> 1979 | obs. | 326 | 57 | 383 | 0.595 |
| | exp. | 287.25 | 95.75 | | |
| | | $\chi^2 = 22.22$ (1df) $p \lll 0.001$ | | | |
| 1984 | obs. | 711 | 40 | 751 | 0.213 |
| | exp. | 563.25 | 187.75 | | |
| | | $\chi^2 = 155.03$ (1df) $p \lll 0.001$ | | | |
| Cross <i>mc/mc</i> x <i>b/b</i> 1979 | obs. | 315 | 59 | 374 | 0.631 |
| | exp. | 280.5 | 93.5 | | |
| | | $\chi^2 = 16.97$ (1df) $p < 0.001$ | | | |
| 1984 | obs. | 637 | 65 | 702 | 0.370 |
| | exp. | 526.5 | 175.5 | | |
| | | $\chi^2 = 92.8$ (1df) $p \lll 0.001$ | | | |

TABLE 2. Egg-to-adult survival (%) of *mc* compared with *pearl* (this strain carries the + allele at the *mc* locus, but is probably similar in genetic background to *mc*). *n* = number of replicates.

| Temp. | <i>mc</i> | | | <i>pearl</i> | | |
|-------|-----------|---------|----------|--------------|---------|----------|
| | Mean | Range | <i>n</i> | Mean | Range | <i>n</i> |
| 23°C | 26.7 | (22-31) | (3) | 64.0 | (56-75) | (3) |
| 27°C | 60.3 | (53-74) | (7) | 83.3 | (80-88) | (3) |
| 30°C | 40.3 | (36-48) | (4) | 56.7 | (50-69) | (3) |

were transferred on day 24, at the late larval stage before pupation.

The frequencies of blind and asymmetrical individuals among the offspring in different treatments were significantly different when oviposition was at 30°C ($p < 0.001$), but not when the eggs were laid at 25°C. In the former group, the frequency of blind individuals was highest when eggs were transferred to 25°C at an early stage in development (Table 5). The differences in frequency of blind and asymmetrical individuals are illustrated in Fig. 3.

The full data set of the frequencies of blind individuals (%), including 6 treatments with 5 replicates each, was subjected to a 2-way analysis of variance (with angular transformation; Sokal and Rohlf, 1981) to test for interaction between the

TABLE 3. Frequencies of eye phenotypes in offspring of crosses between blind, asymmetrical and other parents.

| A. 1979 data. | | | | | |
|--|----------------------|--------|--------|---------|--------|
| Cross | Total # Offspring | Number | | Percent | |
| | | Blind | Asymm. | Blind | Asymm. |
| Blind x Blind | 51 | 15 | 21 | 29.4 | 41.2 |
| Asymm. x Asymm. | 207 | 61 | 79 | 29.5 | 38.2 |
| Blind x Asymm. | 42 | 14 | 16 | 33.3 | 38.1 |
| Sn x Asymm. | 44 | 11 | 18 | 25.0 | 40.1 |
| Heterogeneity G = 0.991, 6 df (ns) | | | | | |
| B. 1984 data. | | | | | |
| Cross | Total # Offspring | Number | | Percent | |
| | | Blind | Asymm. | Blind | Asymm. |
| Blind x Blind | 76 | 35 | 24 | 46.1 | 31.6 |
| Asymm. x Asymm. | 253 | 70 | 59 | 27.7 | 23.3 |
| Blind x Asymm. | 97 | 43 | 20 | 44.3 | 20.6 |
| Sn x Asymm. | 101 | 12 | 6 | 11.9 | 5.9 |
| Heterogeneity G = 82.49, 6 df, p < 0.001 | | | | | |

TABLE 4. Frequencies of offspring of different eye phenotypes at 3 temperatures (1984 data).

| Temperature | Total # Offspring | Number | | Percent | |
|--|----------------------|--------|--------|---------|--------|
| | | Blind | Asymm. | Blind | Asymm. |
| 23°C | 75 | 53 | 7 | 70.7 | 9.3 |
| 27°C | 422 | 153 | 120 | 36.3 | 28.4 |
| 30°C | 159 | 6 | 21 | 3.8 | 13.2 |
| Heterogeneity G = 176.5, 6 df, p < 0.001 | | | | | |

main effects (oviposition temperature, and stage of transfer to the alternative temperature). None was found. Oviposition temperature (25° vs. 30°) was the only significant factor (F = 5.17, 1,24 df; p < 0.05). A similar analysis on percentage of asymmetrical individuals yielded no significant differences.

DISCUSSION

Characters with incomplete penetrance or expressivity serve as a reminder that the determination of the phenotype involves complex interactions of the genotype with the environment during ontogenesis (the "epigenetic landscape" of Waddington 1957). Using such characters, the effect and the timing of particular environmental

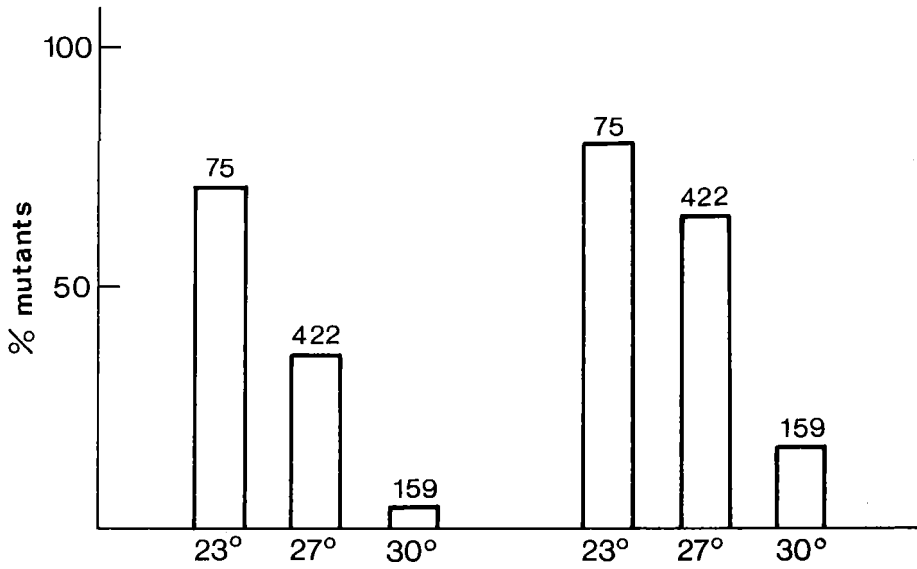


Fig. 2. Frequency of blind (left) and asymmetrical (right) individuals at 3 temperatures. All replicates pooled. Total number of individuals – above each column.

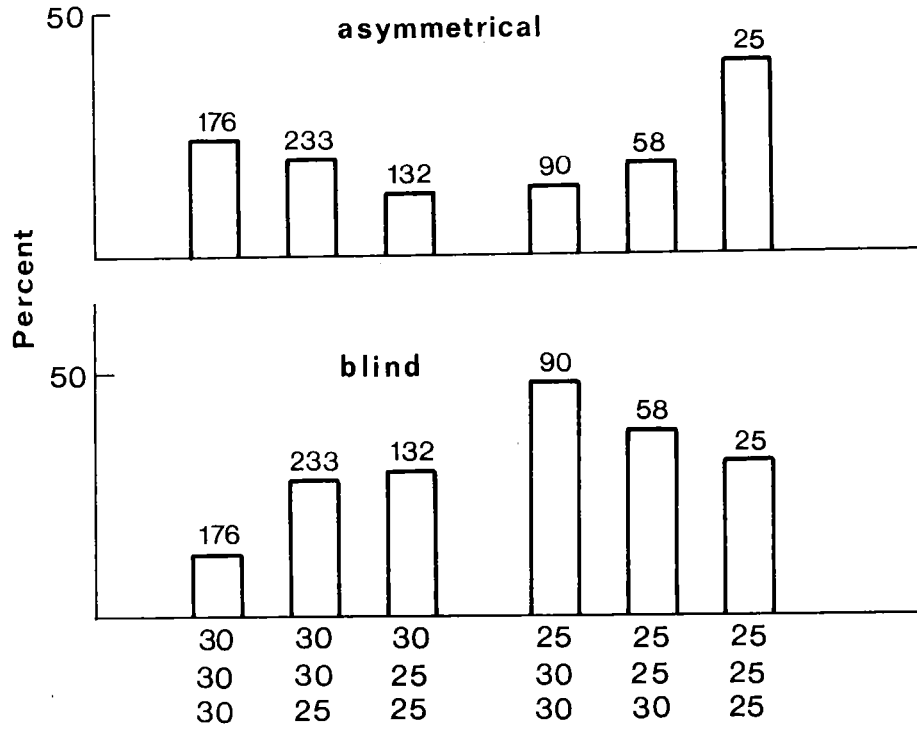


Fig. 3. Frequencies of blind (lower histogram) and asymmetrical (upper histogram) individuals in the six treatments of experiment IV. (All replicates pooled. Total number of individuals – above each column.) Treatment codes – beneath each column – as in table 5 (and see text).

TABLE 5. Distribution of eye phenotypes in the six temperature treatments of exp. IV. Data pooled from 5 replicates per treatment. Treatment codes are temperatures during early larval, late larval and pupal periods.

| Oviposition Temperature | Treatment Code | Total // Offspring | Blind | Phenotypes | |
|--|----------------|--------------------|-------|--------------|---------|
| | | | | Asymmetrical | % Blind |
| 30°C | 30,30,30 | 176 | 23 | 43 | 13.1 |
| | 30,30,25 | 233 | 65 | 47 | 27.9 |
| | 30,25,25 | 132 | 40 | 17 | 30.3 |
| Heterogeneity G = 21.3, 4 df (p < 0.001) | | | | | |
| Oviposition Temperature | Treatment Code | Total // Offspring | Blind | Phenotypes | |
| | | | | Asymmetrical | % Blind |
| 25°C | 25,30,30 | 90 | 43 | 13 | 47.8 |
| | 25,25,30 | 58 | 22 | 11 | 37.9 |
| | 25,25,25 | 25 | 8 | 10 | 32.0 |
| Heterogeneity G = 8.1, 4 df (ns) | | | | | |

factors on the phenotype may be studied in detail.

Rendel (1962) and Sang (1963) developed theoretical models explaining the phenomena of incomplete penetrance and expressivity using the concept of threshold, suggested many years earlier by R. Goldschmidt. The models assume that a quantity of a "morphogenetic substance" accumulates during development. A minimal threshold quantity must be present to produce the normal phenotype. If this threshold is not reached, a mutant phenotype results. (The theory assumes a normal distribution of the quantity of the "morphogenetic substance", while the discrete appearance of the phenotypes is a result of the threshold effect.) In these models, an upper threshold is also assumed to account for an "exaggerated" phenotype (and thus include cases of dominance). Whitten (1966) used only a single threshold to account for the phenotypic expression of the eye mutant *Witty* in *Drosophila melanogaster*. The concept of the morphogenetic substance is very useful in thinking about the phenomena: Rendel (1962) offers the following analogy with physics (p. 299).

"Just as force is that which moves, or tends to move, an object, so morphogenetic substance is that which gives rise to, or tends to give rise to, the development of a phenotype. It includes all influences affecting a phenotype".

The mutation we discovered in 1979 is morphologically very similar to the *mc* mutation described by Sokoloff and Lasley in 1961. Genetic tests for allelism were not performed, however, and although it seems to be a case of recurrent mutation, this similarity should be regarded only as indicative, but not proof, of genetic identity.

In the present study, we were mainly concerned with the secondary temperature-dependent, pleiotropic effects of *mc* on the eye: when the eggs develop at 25°C, a larger proportion of adults emerge with one or both eyes missing than at the standard 30°C, and the critical period seems to be the embryonic or early larval stage. Although

the 1984 data seem to suggest that some genetic (epistatic) interactions affect the phenotype of the eye, certainly the temperature effect is far more important.

In normal *Tribolium*, lower temperatures slow down all developmental processes, but it does not change the phenotype of the eyes in the adult (the compound eyes are not present in the larval stages, and only become apparent in the pupa. See review on *Tribolium* embryology in Sokoloff 1972). The *mc* mutation disrupts the regular development and separates the eye from the development of other organs such that it becomes more sensitive to environmental control. If we assume that a "morphogenetic substance" is required in some threshold quantity for normal eye formation, then the *mc* mutation seems to have created a bottleneck – a rate limiting step. When ontogenesis is slowed down at the lowered temperature, the effect of the bottleneck is more pronounced.

Another interesting phenomenon, which is attractive for further research, is the *asymmetrical* expression of the eyes (individuals having either the left or the right eye but not both). Asymmetry is mentioned occasionally in the literature as a measure of environmental stability. Soulé (1967, 1979) measured asymmetry in scale numbers between the sides of the body of lizards and suggested that it should be negatively correlated with the level of heterozygosity if heterozygotes are more homeostatic than homozygotes (as suggested by Lerner, 1954).

The proportions of asymmetrical individuals in *mc* show an interesting trend (Fig. 3). When eggs are laid at 30°C, asymmetry decreases upon transfer to 25°C. When eggs are laid at 25°C, asymmetry decreases upon transfer to 30°C. It is as if the *change* in temperature during development, and not either temperature alone, determines asymmetry. It is also interesting that the proportion of *blind* individuals show the *exact reverse* pattern, as if blindness and asymmetry are complementary in some way (Fig. 3).

A mutation affecting the eyes asymmetrically was discovered in *Drosophila melanogaster* (the *Witty*, *Wi*, gene on chromosome II). The effects of the major gene and its modifiers were studied by Whitten (1966, 1968). Whitten (1966) offers two alternative theoretical models and favors the one which assume that each eye develops independently and produces its own "morphogenetic substance", which may or may not suffice to reach the threshold for producing a normal eye. If the level of the morphogenetic substance are originally not far from the threshold, random environmental variation near the critical period in development may result in asymmetry.

The *mc* results may be explained using Whitten's model. Disturbance of development at a critical time by transfer to a different temperature may increase asymmetry. The temperature switch does not necessarily affect survival: in the present experiment, eggs oviposited at 25°C survived much less than those oviposited at 30°C (see Total offspring, Table 5). We have encountered this phenomenon in earlier work with other *Tribolium* strains (Wool and Bergerson, 1979): Eggs of *eu*, (a strain with incomplete penetrance of another gene), *bb* and a mixed population were oviposited at 25°C and transferred to 30°C for development. Initially survival was low, but continuation of this practice for 10 generations resulted in a linear increase of survival with time. This suggests some genetic control of the ability of embryos to survive the change in temperature.

Temperature is not the only environmental factor which affects gene expression. Food (Nakashima-Tanaka 1967 a,b; Mampell 1965, 1968) and other ecological factors

which modify developmental rate, may have an effect on the final phenotype of the eyes. An important problem remains unsolved: how does the temperature regime at the embryonic stages affect the eye in the adult, a month later, when the character is not expressed in the larva? To answer this question, a much more detailed knowledge of the biochemical and developmental processes controlling eye formation in *Tribolium* is required than is presently available.

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REFERENCES

- Ashri, A. 1970. A dominant mutation with variable penetrance and expressivity induced by diethyl sulphate in peanuts, *Arachis hypogaea* L. *Mutation Research* 9:473-480.
- Ayala, F.J. and Kiger, J.A. 1980. *Modern Genetics*. Benjamin/Cummings Publ. Co.
- Gottschalk, W. 1978. The dependence of the penetrance of mutant genes on environment and genotypic background. *Genetica* 49:21-29.
- Lerner, I.M. 1954. *Genetic homeostasis*. Wiley.
- Mampell, K. 1965. Gene expression and developmental rate. *Genetica* 36:135-146.
- Mampell, K. 1968. Differentiation and extragenic transmission of modified gene expression. *Genetica* 39:553-566.
- Nakashima-Tanaka, E. 1967a. The effects of chemicals on the phenotypic expression of a vestigial mutant in *Drosophila melanogaster*. *Genetica* 38:459-470.
- Nakashima-Tanaka, E. 1967b. The effect of temperature and genetic background on the phenotypic expression of several vestigial strains of *Drosophila melanogaster*. *Genetica* 38:447-458.
- Rendel, J.M. 1962. The relationship between gene and phenotype. *Journal of Theoretical Biology* 2:296-308.
- Sang, J.H. 1963. Penetrance, expressivity and thresholds. *Journal of Heredity* 54:143-151.
- Sokoloff, A. 1966. *The genetics of Tribolium and related species*. Academic Press.
- Sokoloff, A. 1972. *Biology of Tribolium*, Vol. I. Oxford University Press.
- Sokoloff, A. 1974. *Biology of Tribolium*, Vol. II. Oxford University Press.
- Sokoloff, A. 1977. *Biology of Tribolium*, Vol. III. Oxford University Press.
- Soulé, M. 1967. Phenetics of natural populations II. Asymmetry and evolution in a lizard. *American Naturalist* 101:141-160.
- Soulé, M. 1979. Heterozygosity and developmental stability. *Evolution* 33:396-401.
- Waddington, C.H. 1957. *The strategy of the genes*. Allen & Unwin
- Whitten, M.J. 1966. The quantitative analysis of threshold characters using asymmetry: a study of the Witty character in *Drosophila melanogaster*. *Genetics* 54:465-483.
- Whitten, M.J. 1968. Genetic control of penetrance and the evolution of dominance in *Drosophila*. *Heredity* 23:263-278.
- Whittman, K.S. and Hamburg, M. 1968. The development and effect of genetic background on expressivity and penetrance of the Brachyury mutation in the mouse: a study in developmental genetics. *Journal of Experimental Zoology* 168:137-146.
- Wool, D. and Bergerson, O. 1979. Analysis of selection processes using the incompletely-penetrant mutant *eu* of *Tribolium castaneum*. *Canadian Journal of Genetics and Cytology* 21:405-415.
- Wool, D. and Mendlinger, S. 1973. The *eu* mutant of the flour beetle, *Tribolium castaneum* Herbst: Environmental and genetic effects on penetrance. *Genetica* 44:496-504.