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## The response of larvae of Trogoderma granarium Everts to -10°C

Some preliminary observations of the response of fully grown larvae of Trogoderma granarium Everts to low temperatures have been made by placing samples of approximately 50 larvae in a deep freeze cabinet at -10°C for various periods of time. The larvae were taken directly to the cabinet from cultures maintained at 30°C and after exposure were placed on food and returned to 30°C. The larvae were then examined, initially at weekly intervals and then fortnightly or three weekly, for six months.

Periods of exposure to -10°C of from two hours to 14 days were tested. Exposures of up to 3.5 hours appeared to have no lasting effect on the larvae. From 7.5 to 72 hours larval mortality increased to 96%. No larvae survived exposures of five days or longer.

All the exposures tested immobilized the larvae. On their return to 30°C some larvae remained immobile for a long time. Such larvae displayed no sign of damage and it was impossible to tell from their appearance whether they would eventually die or resume development. Dead larvae became desiccated and dark in color.

After short exposures some larvae were still immobile at the end of the observation period. Increase in exposure not only increased mortality but also appeared to decrease the period of immobility before the larvae could be seen to be dead.

These findings are demonstrated in Table 1 below. In this summary which combines the results of three separate tests some slight approximations in dates have been made in order to divide the observation period into the five intervals shown. Results for exposures giving less than 100% kill have been combined into groups.

Table 1. Numbers of survivors and dead larvae found during the observation period at 30°C following exposure to -10°C.

| Length of exposure to -10°C | Stages found | Observation period, days from start of exposure | | | | | Total insects found | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0-30 | 31-60 | 61-90 | 91-125 | 126-166 | Adults | Larvae live at end | Larvae dead |
| 0, 1.5, 3.5 h | adults | 129 | 3 | 2 | 0 | 0 | | | |
| | dead larvae | 2 | 1 | 0 | 1 | 0 | 134 | 1 | 4 |
| 7.5-41 h | adults | 33 | 5 | 0 | 1 | 8 | | | |
| | dead larvae | 91 | 65 | 13 | 5 | 4 | 47 | 7 | 178 |
| 48-72 h | adults | 8 | 3 | 1 | 4 | 1 | | | |
| | dead larvae | 170 | 30 | 20 | 2 | 0 | 17 | 4 | 222 |
| 5 days | dead larvae | 47 | 2 | 0 | 1 | 0 | 0 | 0 | 50 |
| 7 days | " | 36 | 8 | 2 | 1 | 0 | 0 | 0 | 47 |
| 10 days | " | 37 | 17 | 1 | 0 | 0 | 0 | 0 | 55 |
| 12 days | " | 38 | 10 | 1 | 0 | 0 | 0 | 0 | 49 |
| 14 days | " | 43 | 7 | 0 | 0 | 0 | 0 | 0 | 50 |

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## Allelism of "mottled" (mt) and "melanotic stink glands" (msg) in Tribolium castaneum

Two mutations of similar phenotype have been reported, "melanotic stink glands," msg (Sokoloff and Hoy, TIB 8:55-56), and "mottled," mt (Englert, TIB 9:59-60). Both of these mutations are affected by apparent deposition of polymerized darkly pigmented secretions composed of ethylquinones in the region of the stink glands.

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Both mutations are inherited as autosomal recessives exhibiting incomplete penetrance and variable expressivity. Under ordinary laboratory conditions "mottled" exhibits an average penetrance of 63 per cent. In the stock received from the Berkeley Stock Center, msg was found to exhibit a penetrance of approximately 60-70 per cent under our laboratory conditions, however, the viability of the stock was less than good. Linkage tests of the two had indicated a relationship with linkage group III (includes "black," b). Therefore, a test for allelism utilizing single pair matings between the two stocks was conducted. From 29 single pair matings, only 16 of 178 beetles (8.9 per cent) exhibited what could be called the mutant phenotype.

A second test was conducted, this time utilizing mass matings in an attempt to increase productivity. A total of 464 progeny were examined, 17 of which exhibited the mutant phenotype (3.7 per cent). Neither of these tests could be considered conclusive, so to further establish allelism, the msg stock was crossed to the Purdue wild foundation stock to give a similar genetic background to that of mt. Mutant progeny from $F_2$ segregants were used to start the "new" msg stock.

Beetles from the reconstituted msg stock were crossed with mt beetles to again check for allelism. The results revealed only 3 of 132 beetles which were of the "mutant" phenotype (0.8 per cent), indicating that similar genetic background did not increase the penetrance of the "mutant" phenotype. Thus, in the classical sense of allelism the two mutants cannot be considered to be allelic.

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The chromosome numbers of some stored product Coleoptera

| Species | Karyotype | |
|---|---|---|
| DERMESTIDAE | | |
| Dermestes maculatus | $8_{II}$ + Xyp | JOHN & SHAW 1966 |
| " " | $8_{II}$ + $Xy_1y_2$ | |
| " " | $8_{II}$ + $Xy_1y_2y_3$ | |
| D. frischii | $8_{II}$ + Xyp | |
| D. frischii | $8_{II}$ + $Xy_1y_2$ | |
| D. ater | $8_{II}$ + Xyp | |
| D. haemorrhoidalis | $8_{II}$ + XY | |
| D. lardarius | $8_{II}$ + Xyp | |
| D. peruvianus | $8_{II}$ + Xyp | |

| Species | Karyotype | |
|---|---|---|
| *Trogoderma parabile* | $9_{II}$ + Xyp | SHAW (Unpublished) |
| *T. glabrum* | $9_{II}$ + Xyp | |
| *Anthrenus verbasci* | $8_{II}$ + Xyp | |
| *A. flavipes* | $8_{II}$ + Xyp | |
| OSTOMATIDAE | | |
| *Tenebroides mauritanicus* | $11_{II}$ + Xyp | |
| TENEBRIONIDAE | | |
| *Alphitobius diaperinus* | $9_{II}$ + XO | |
| *Tenebrio molitor* | $9_{II}$ + Xyp | |
| *T. obscurus* | $9_{II}$ + Xyp | |
| *Gnathocerus cornutus* | $9_{II}$ + Xyp | |

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## *Sex chromosome variation in D. maculatus and D. frischii*

Five cultures of *Dermestes maculatus* were available for cytological examination. The individuals from stocks originating in Australia, South Africa, Nigeria and Sudan all showed a standard Xy parachute sex bivalent but all the individuals examined from an Indian culture were characterized by multiple y's. In 32 out of 33 individuals examined, two y chromosomes were present leading to the formation of a sex multiple of three at meiosis. This multiple shows regular meiotic behavior giving a consistent X-2y segregation with no observed deviation.

In a single individual of the Indian strain, three y chromosomes were found and here the meiotic behavior was less regular. At first metaphase, two principal patterns of orientation were observed.

First, and most frequently (35 out of 40 cells scored), the three y's co-orientated with the single X giving a regular X-3y segregation. In five other cells one of the y's was orientated to the same pole as the X giving an Xy-2y pattern of segregation. The consequences of this irregular behavior were seen at second division for in addition to (8 + X) and (8 + 3y) cell types, (8 + X + y) and (8 + 2y) cells were also seen.

Five of the 25 males of *D. frischii* examined also turned out to be the $Xy_1y_2$ type.

Two possible explanations can be offered to account for these multiple y variants. Either they represent a polymorphism which exists in nature or, alternatively, we are dealing with a system of supernumerary y-chromosomes which have arisen in culture presumably as a consequence of inbreeding coupled with reduced competition.

A 3y strain of D. maculatus has now been selected and it is hoped to isolate females which may possess a y-chromosome due to the irregular behavior of the 3 y's at meiosis. (In conjunction with Dr. B. John, Department of Genetics, Birmingham University, England.)

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*The effects of synchrony of egg batches on fitness characters and competition in Tribolium castaneum.

In a previous study (Sokal and Karten, 1964) the effects of density on three characters affecting fitness (survival to adulthood, dry weight of adults, and length of developmental period) were described for ++, +b and bb individuals in pure culture and in mixed cultures at varying proportions. It was believed that these findings would help explain the results of a selection experiment (Sokal and Sonleitner, 1965; a more extensive manuscript is in preparation) in which by 10 generations the frequency of b increased from 0.25 to 0.53, other cultures started at gene frequency 0.50 had increased to 0.58, while yet others started at 0.75 had maintained themselves approximately at this value. Analysis of the data revealed, however, that the results of the earlier study differed in two important details from those of the selection experiment. In the study of Sokal and Karten (1964) black had consistently higher or at least equal survival to adulthood when compared to ++, while in the selection experiment the wild type strain invariably had appreciably higher survival than bb. Also, developmental period of bb was less than that of the wild type in the experiment of Sokal and Karten (at least at the lower densities), while in the selection experiment, the black strain developed considerably slower than the wild type strain (by as much as 19 days).

Study of these results and another experiment reported later in this issue (Sokal, 1967) ruled out genetic differences between the original stocks and the selected strains as responsible for the differences in survival and developmental period. Another possible cause of the divergent results of the two sets of experiments is that Sokal and Karten used egg batches obtained from 4-hr egg collections while the selection study was based on 3-day egg yields. It is assumed that the latter egg batches are less synchronous and may therefore be responsible for differences in survival and developmental period. The present paper tests this hypothesis.

The materials and techniques employed are identical to those reported in Sokal and Karten (1964). The design differed in that only two densities, 20/g and 100/g were set up with pure cultures of ++, +b and bb, and with mixed cultures representing Hardy-Weinberg proportions for gene frequencies 0.1, 0.5 and 0.9 of black. For each combination of conditions 4-hr as well as 3-day eggs were tested. Replication for each experimental condition was four vials for the pure cultures and $0.5q_b$, and eight vials for the other gene frequencies at density 20/g, while at density 100/g the respective replications were three and six vials. The entire experiment was repeated four times.

Survival to adulthood: The overall survival not broken down by genotype is shown in Table 1. The 4-hr data compare well with previous results by Sokal and Karten (1964) and Sokal and Huber (1963). Percentages of survival at densities 20/g are higher than at 100/g, even for the ++ cultures which previously did not show this trend. At density 20/g the 3-day egg batches did not differ from the 4-hr vials in per cent survival, but at 100/g they are significantly lower than the same eggs at density 20/g and also the 4-hr cultures at density 100/g. The lowest single per cent survival is in the 3-day cultures of ++ which dip to 49.61%.

Table 1. Overall adult survival at different gene frequencies, and two densities for 4-hr and 3-day egg batches.

| Egg batch | Density | Gene frequency 0.00 | 0.10 | 0.50 | +b | 0.90 | 1.00 |
|---|---|---|---|---|---|---|---|
| 4 hrs | 20/g | 81.98 | 79.84 | 85.78 | 87.97 | 83.20 | 85.31 |
| | 100/g | 71.01 | 64.39 | 76.65 | 60.24 | 77.98 | 71.21 |
| 3 days | 20/g | 83.90 | 83.33 | 80.26 | 86.72 | 78.25 | 86.25 |
| | 100/g | 49.61 | 55.43 | 60.75 | 62.85 | 63.76 | 66.79 |

Figure 1 illustrates the survivorships as percentages of input (averaged over the four experiments) for pure and mixed cultures at the three gene frequencies, two densities and for the two types of egg batches. Although the details of this figure differ somewhat from corresponding Figures 1 and 4 in Sokal and Karten (1964), the general trends are identical, showing heterozygous superiority under most conditions and a trend toward higher survival of wild type with increasing gene frequency of black at the highest density. Some instances of genetic facilitation are again demonstrated. However, with respect to the main feature of the experiment, the effect of synchrony of the egg batches on survival, no apparent difference can be illustrated. This is borne out by a factorial analysis of variance which shows only density and replication as significant main

effects. Most significant interactions involve replication as one of the factors, the outcomes of the several experiments having fluctuated considerably.

Dry weight of adults: These results (not illustrated here) are quite comparable to the findings of Sokal and Karten (1964). Again, weights are in the relation +b > ++ > bb. This relation is maintained at both densities and for both types of egg batches. Weight is not affected by synchrony of egg batches. Beetles at density 20/g are considerably heavier than those at 100/g.

Length of developmental period: This variable is affected by density and differs considerably among genotypes. Table 2 shows the relations among developmental periods of the three genotypes in this experiment and in that of Sokal and Karten. Note the reversal in relationship between ++ and bb for the two densities of the 4-hr cultures. However, in the 3-day egg batches, patterned after the selection experiment, this reversal does not occur. Thus, the 4-hr cultures actually come closer to the bb > ++ pattern of the selection experiment (based on 3-day egg yields at high densities) than do the 3-day cultures. Mean developmental period was apparently affected markedly by the synchrony of the egg hatch, yet when mean hatching period, the time from the start of the experiment until the hatching of each individual egg, was calculated it was seen that the increase in developmental period in the 3-day eggs could be accounted for by their greater mean hatch time.

Table 2. Relation among developmental periods of the three genotypes.

| Density | | Egg batch: 4 hours | Egg batch: 3 days |
|---|---|---|---|
| 20/g | | | |
| | This study | ++ > bb = +b | ++ = bb = +b |
| | Sokal & Karten '64 | ++ > bb > +b | |
| 100/g | | | |
| | This study | +b > bb > ++ | ++ > +b > bb |
| | Sokal & Karten '64 | bb > +b > ++ | |

More informative than means are cumulative frequency distributions of emerging adults of the three genotypes shown in Figure 2. Figures 2a and 2b

are representative of the general findings. They illustrate density 100/g of the 3-day batches in pure culture and at gene frequency 0.9, respectively. It is clear from these graphs that the ++ strain lags consistently behind the other two, which for most of the experimental conditions produced coincident curves, or if they did separate, showed the bb to be slightly ahead of the +b. This relationship (of ++ lagging behind bb) was also found in separate, unpublished experiments carried out by F. J. Sonleitner in our laboratory. Notice how in the pure strains (Figure 2a) the emergence patterns are much more diffuse and development takes longer than in the mixed strains at gene frequency 0.9. This culture exhibits genetic facilitation, with all three strains developing faster and closer together as shown by the steeper slope of the curves. Under only one condition (100/g of the 4-hr egg batches in pure culture; see Figure 2c) was ++ not the slowest strain.

Conclusion: While the relations described above have considerable interest, they do not demonstrate any major effects due to greater or lesser synchronization in egg batches and are thus not able to explain the differences between the early results of Sokal and Karten (and now also the present results) and those observed in the selection experiment of Sokal and Sonleitner.

Contribution No. 1351 from the Department of Entomology, The University of Kansas. This is paper No. 5 in a series on the ecological genetics of Tribolium. Numbers 1 through 4 are listed at the end of paper No. 6 (Sokal, 1967, in this issue of TIB). This research was supported by the National Science Foundation under grant GB-2170 and by a Public Health Research Career Program Award (No. 3-K3-GM-22, 021-01S1) from the National Institute of General Medical Sciences. The technical assistance of Miss Yu-Jen Chen, Mrs. Maxine L. Howe and Mrs. Cornella B. Tollefson are very much appreciated.

## Literature Cited

Sokal, R. R. 1967. A comparison of fitness characters and their response to density in stock and selected cultures of wild type and black Tribolium castaneum. TIB 10:142-147

Sokal, R. R. and I. Huber. 1963. Competition among genotypes in Tribolium castaneum at varying densities and gene frequencies (the sooty locus). American Naturalist 97:169-184.

Sokal, R. R. and I. Karten. 1964. Competition among genotypes in Tribolium castaneum at varying densities and gene frequencies (the black locus). Genetics 49:195-211.

Sokal, R. R. and F. J. Sonleitner. 1965. Components of selection in Tribolium (Coleoptera) and houseflies. Proceedings of the XII International Congress of Entomology. 274-275.

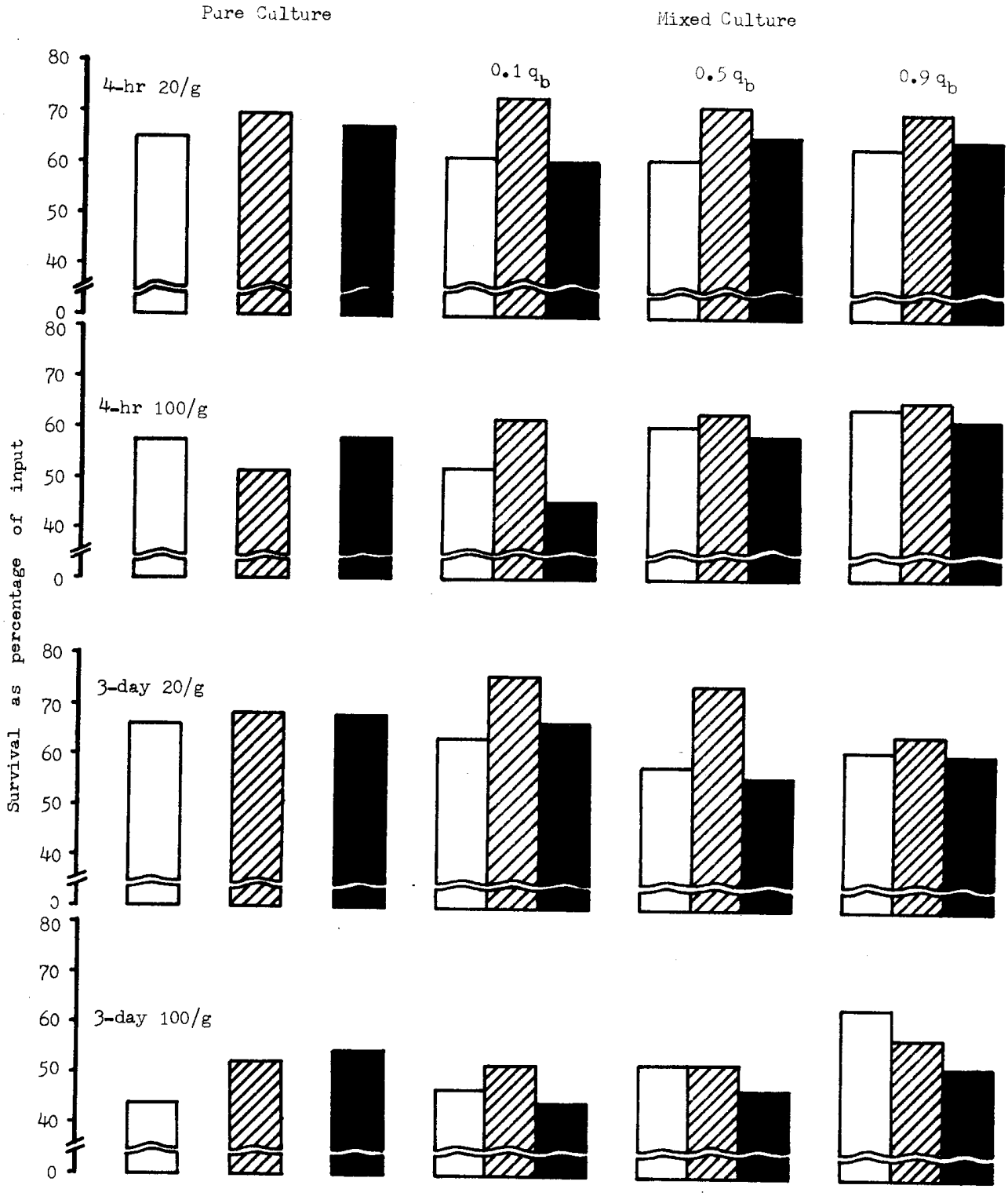
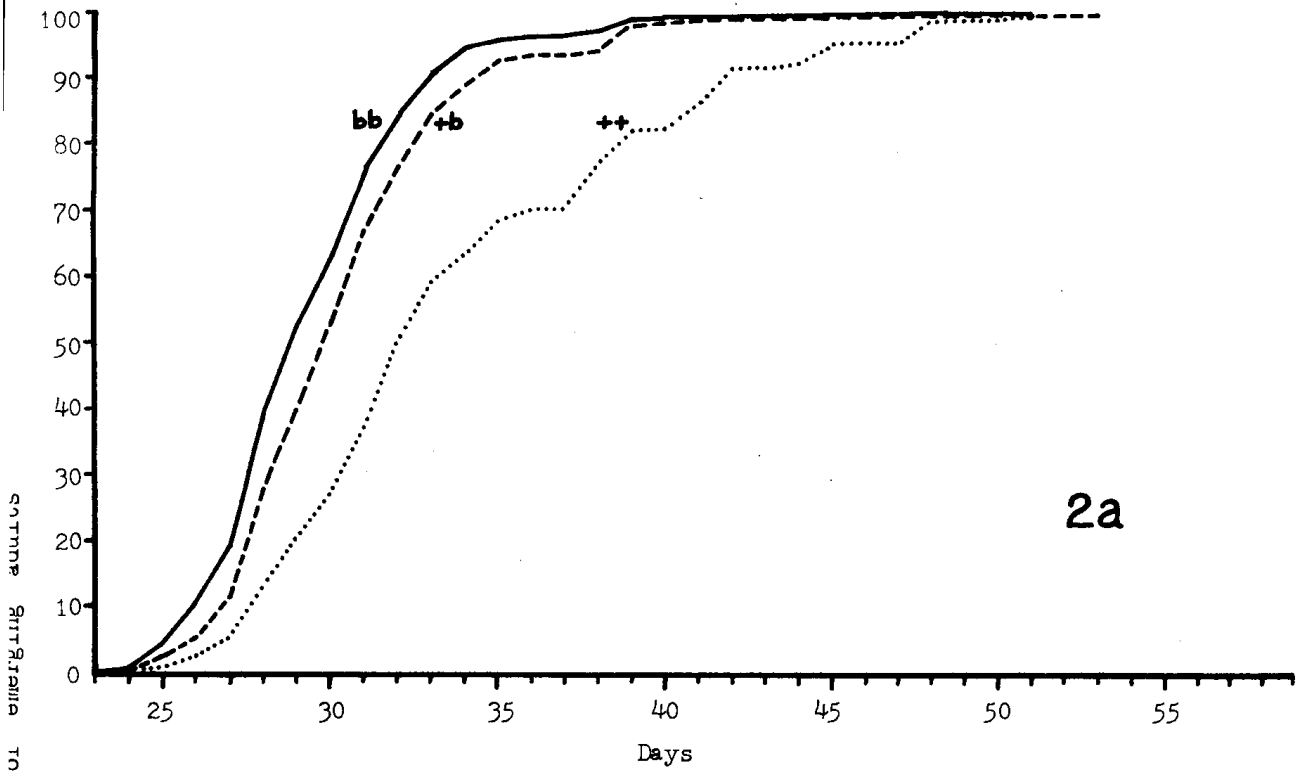
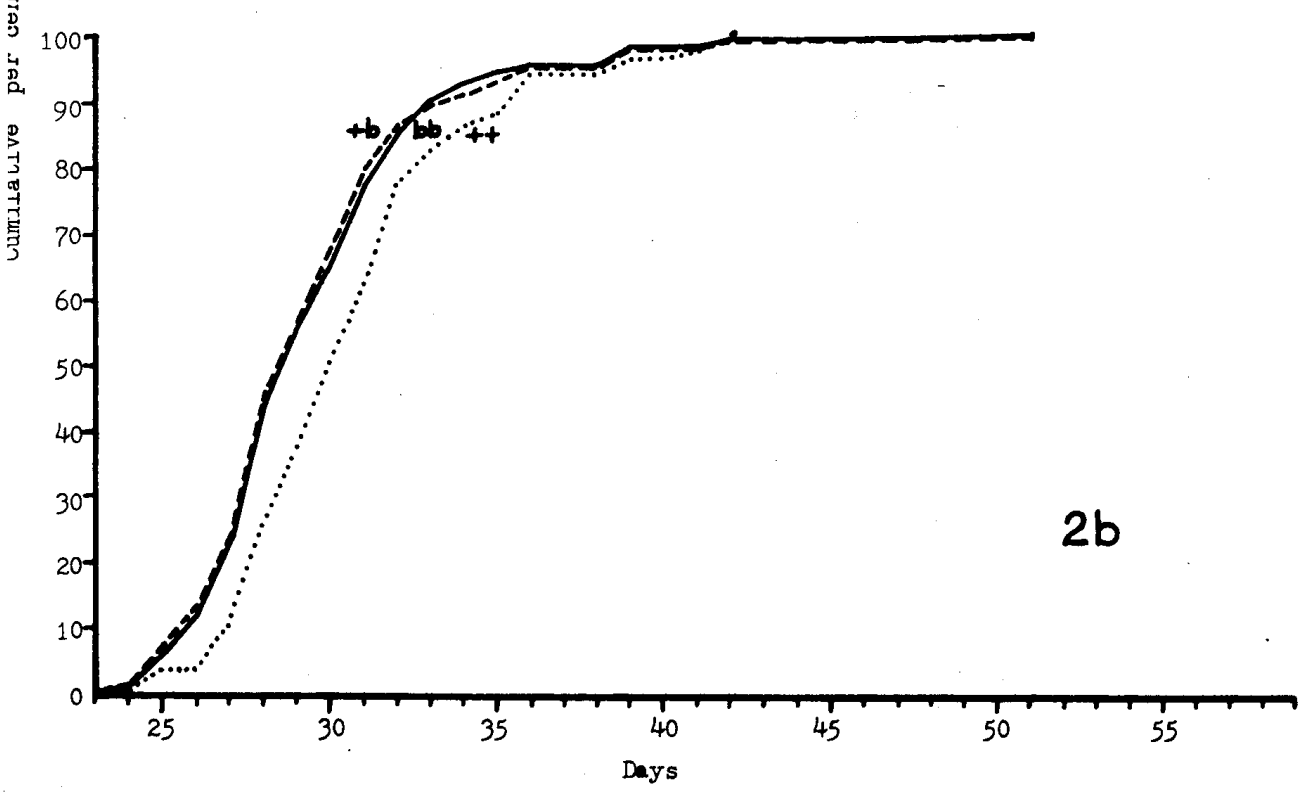



Figure 1. Adult survival expressed as percentages of egg input averaged over the replicates and experiments of the study and shown for the two types of egg batches, two densities and three gene frequencies employed. Leftmost column with noncontiguous bars represents the results of rearing the beetles in pure culture. Hollow bars represent the ++ genotype, hatched bars +b and black bb.


100
90
80
70
60
50
40
30
20
10
0
bb
+b
++
25
30
35
40
45
50
55
Days
2a


Cumulative per cell of emerging adults
100
90
80
70
60
50
40
30
20
10
0
+b
bb
++
25
30
35
40
45
50
55
Days
2b

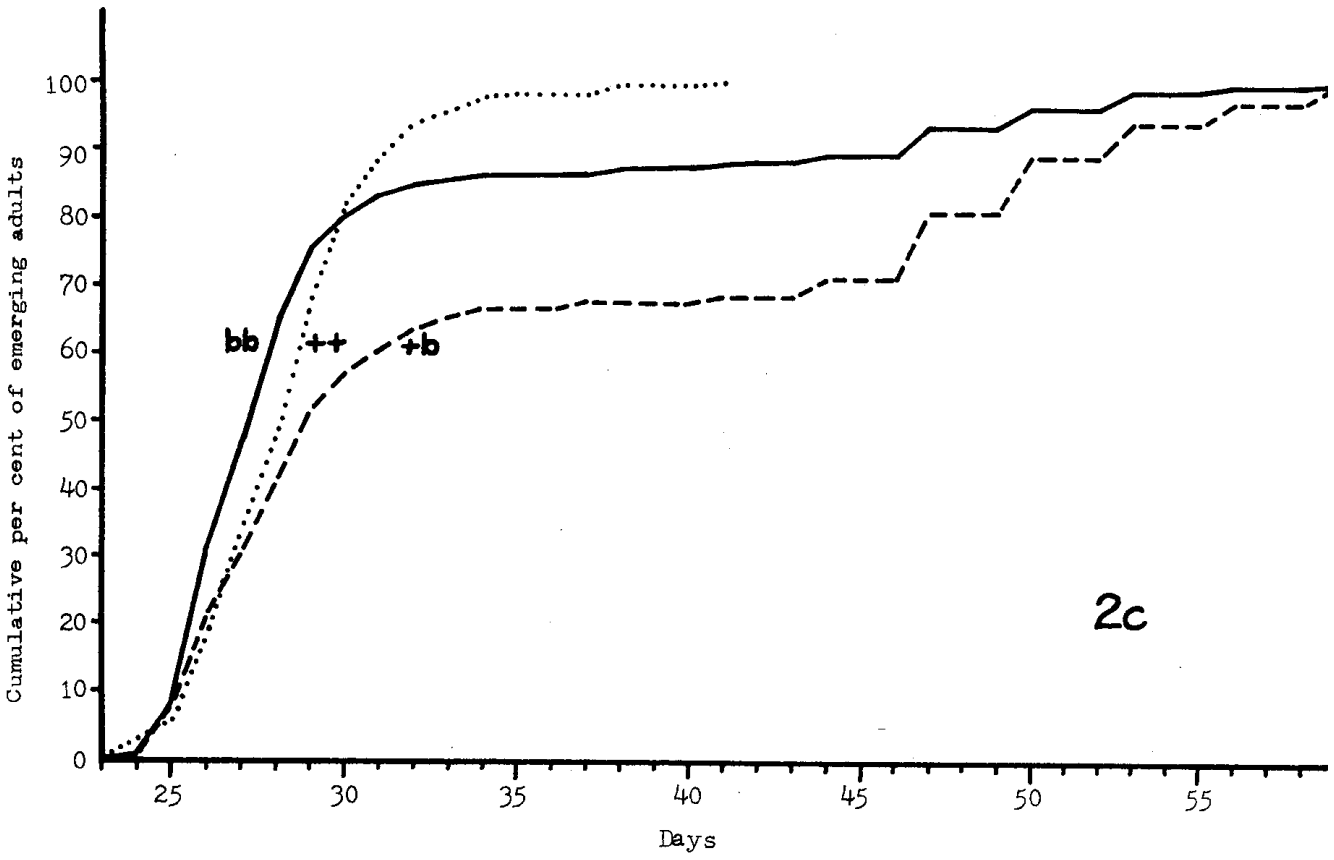



Figure 2. Cumulative frequency distributions showing percent of adult emergence over time (in days).
2a. 3-day, 100/g, pure culture.
2b. 3-day, 100/g, mixed culture $0.9q_b$.
2c. 4-hr, 100/g, pure culture.

++ .....
+b - - - - - - - -
bb ————————

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*A comparison of fitness characters and their responses to density in stock and selected cultures of wild type and black Tribolium castaneum.

The wild type and black control strains of a selection experiment (Sokal and Sonleitner, 1965; a more extensive manuscript is in preparation) differed in two important respects from the stock cultures from which they were derived. Invariably the wild type strain had appreciably higher adult survival than the bb cultures, this difference increasing at the higher densities. By contrast, experiments with the stock cultures carried out by Sokal and Karten (1964) and Sokal (1967) had shown that black had consistently higher, or at least equal, survival to adulthood when compared with ++. However, these experiments were carried out in 8 g of flour in 6-dram shell vials, while the observations in the selection experiment had been made in 40g of flour contained in half-pint Mason jars. A second discrepancy between the two types of experiments is that the developmental period of bb was less than that of the ++ strains under all but one set of conditions in the stock cultures, while in the selection experiment bb took consistently longer to develop than ++.

The purpose of the present experiments is to delineate more sharply the nature of the differences in survival to adulthood, dry weight of adults, and length of developmental period between the controls of the selection experiment and the stock cultures from which they were derived. These comparisons were carried out under conditions of the earlier experiments by Sokal and Karten (1964) and Sokal (1967), i.e., in 8 g of flour in shell vials. While an analysis of these differences is of primary importance for an understanding of the results of the selection experiment, the findings are here interpreted in terms of the treatment to which the controls in the selection experiment had been subjected and are of some general interest from this point of view.

The materials and techniques are identical to those reported in Sokal and Karten (1964). However, only pure strains ++ and bb were tested at four densities, 5/g, 20/g, 50/g, and 100/g. The two strains were the standard UPF wild type and black stock cultures employed in work in our laboratory (see stock list) and the ++ and bb controls from the RSE selection experiment (Sokal and Sonleitner, 1965). The controls were taken from generations 25 and 26, respectively, of the second replicate for these two strains. The eggs for these experiments were obtained during a 4-hour period. Replication for each experimental condition was 10 vials at density 5/g, 4 vials at density 20/g, and 3 vials each at densities 50/g and 100/g. The entire experiment was repeated two times.

Survival to adulthood: Survival as percentage of egg input is graphed in Figure 1, which shows that survival decreases with an increase in density for all tested cultures. In both the stock and selection controls, the

black strain had a consistently higher survival, quite in contrast with our findings under the conditions of the selection experiment. Thus, the genetically determined differences in survival between the black and wild type stocks have not changed in the selection controls, or if they have, these differences are not expressed under the conditions of these experiments (8 g of flour in 6-dram shell vials). It may therefore be that the reverse relationship observed in the selection experiment is due to the environment peculiar thereto (40 g of flour in half-pint jars) or is only expressed in that environment. An overall decrease in survival of the selection controls is noticeable in Figure 1. The survival is expressed in degrees (because of the angular transformation); on the average the selection controls have five degrees lower survival than the corresponding stock cultures. Investigation of per cent hatchability of eggs revealed that these differences in survival are due to differences in larval or pupal survival.

Dry weight of adults: These relations (not illustrated) are generally consistent with previous findings in various experiments (e.g., Sokal and Karten, 1964). The ++ strain is heavier than the bb strain and there are no differences between the stock cultures and the selection controls.

Length of developmental period: Here, relations in the stock cultures are as described in earlier studies, with the ++ strain having a longer developmental period than bb at the low densities. While even at density 100/g the average developmental period of the ++ is half a day longer than that of the bb, the steeper increase of the developmental period of bb in response to density is evident. Studies by Sokal and Karten (1964) and Sokal (1967) have shown that under these conditions of high density bb has a longer developmental period than ++. This relationship is illustrated in the selection controls in Figure 2. Since the actual selection experiment was run at asymptotic densities, between 82/g and 90/g, it may simply be that differences in developmental period observed in that experiment are reactions to the density in the cultures. Notice, however, that the selected strains have on the average a developmental period two days longer than their counterparts from stock cultures. These findings can be corroborated by unpublished data by F. J. Sonleitner who, using the earlier generations 12 and 13 of the selection controls at density 12.5/g, found that the ++ control had a mean of 30.5 days while the bb controls had a mean of 29.3 days. Comparable figures obtained by him for stock cultures were 29.8 days versus 28.5 days.

Conclusions: This experiment is unable to explain the reversion of survival of ++ and bb in the selection experiment. This may be due to the difference in environmental conditions in the jars as contrasted with the vials of this experiment. This point is now being investigated. As for length of developmental period, the genotype-density interaction evident for the selection controls in Figure 2 and matching experiences in stock cultures by Sokal and Karten (1964) and Sokal (1967) may be able to explain the longer developmental period of bb in the selection experiment, although the differences observed there are far greater than the difference of about two days noted in this experiment.

Of general interest are the overall differences in survival and developmental period between the stock cultures and the selection controls. The selection controls had been subjected to a pattern of stock-keeping in which adults from a culture were permitted to oviposit for three days, then removed from the culture and the eggs reared until almost all of the adults had emerged. This has apparently resulted in inadvertent selection for long developmental periods. No effort was made to use only the earliest emerging beetles as progenitors, such as might be done in a Drosophila experiment where the investigator is eager to carry out as many generations as possible. Such selection might have led to short developmental period (see **Hunter,** 1959, for a striking example). In our selection experiment, the most successful beetles were those which remained as relatively small larvae for a considerable period of time, not pupating until most other pupae had already done so. Thus, their chances of being cannibalized were minimized. Selection for such slow-developing larvae would, of course, retard the mean developmental period of the entire strain, which appears to have taken place both in the wild type and bb selection controls (also in hybrid strains not reported on here). The lower overall survival of the selection controls vis-a-vis the stock cultures may simply be due to their longer developmental period, during which they are exposed to more vicissitudes of the environment or may reflect that more of the earlier pupae are cannibalized by the larger numbers of remaining larvae in the selection controls.

Contribution No. 1352 from the Department of Entomology, The University of Kansas. This is paper No. 6 in a series on the ecological genetics of Tribolium. Nos. 1 through 5 are Schlager (1963), Sokal and Huber (1963), Sokal and Karten (1964), Karten (1965), and Sokal (1967), respectively. This research was supported by the National Science Foundation under grant GB-2170 and by a Public Health Research Career Program Award (No. 3-K3-GM-22, 021-01S1) of the National Institute of General Medical Sciences. The technical assistance of Mr. Young-chen Chang, Mrs. Maxine L. Howe, and Mrs. Cornella B. Tollefson are very much appreciated.

## Literature Cited

Hunter, P. E. 1959. Selection of Drosophila melanogaster for length of larval period. Zeitschrift für Vererbungslehre 90:7-28.

Karten, I. 1965. Genetic differences and conditioning in Tribolium castaneum. Physiological Zoology 38:69-79.

Schlager, G. 1963. The ecological genetics of the mutant sooty in populations of Tribolium castaneum. Evolution 17:254-273.

Sokal, R. R. 1967. The effects of synchrony of egg batches on fitness characters and competition in Tribolium castaneum TIB 10:135-141.

Sokal, R. R. and I. Huber. 1963. Competition among genotypes in Tribolium castaneum at varying densities and gene frequencies (the sooty locus). American Naturalist 97:169-184.

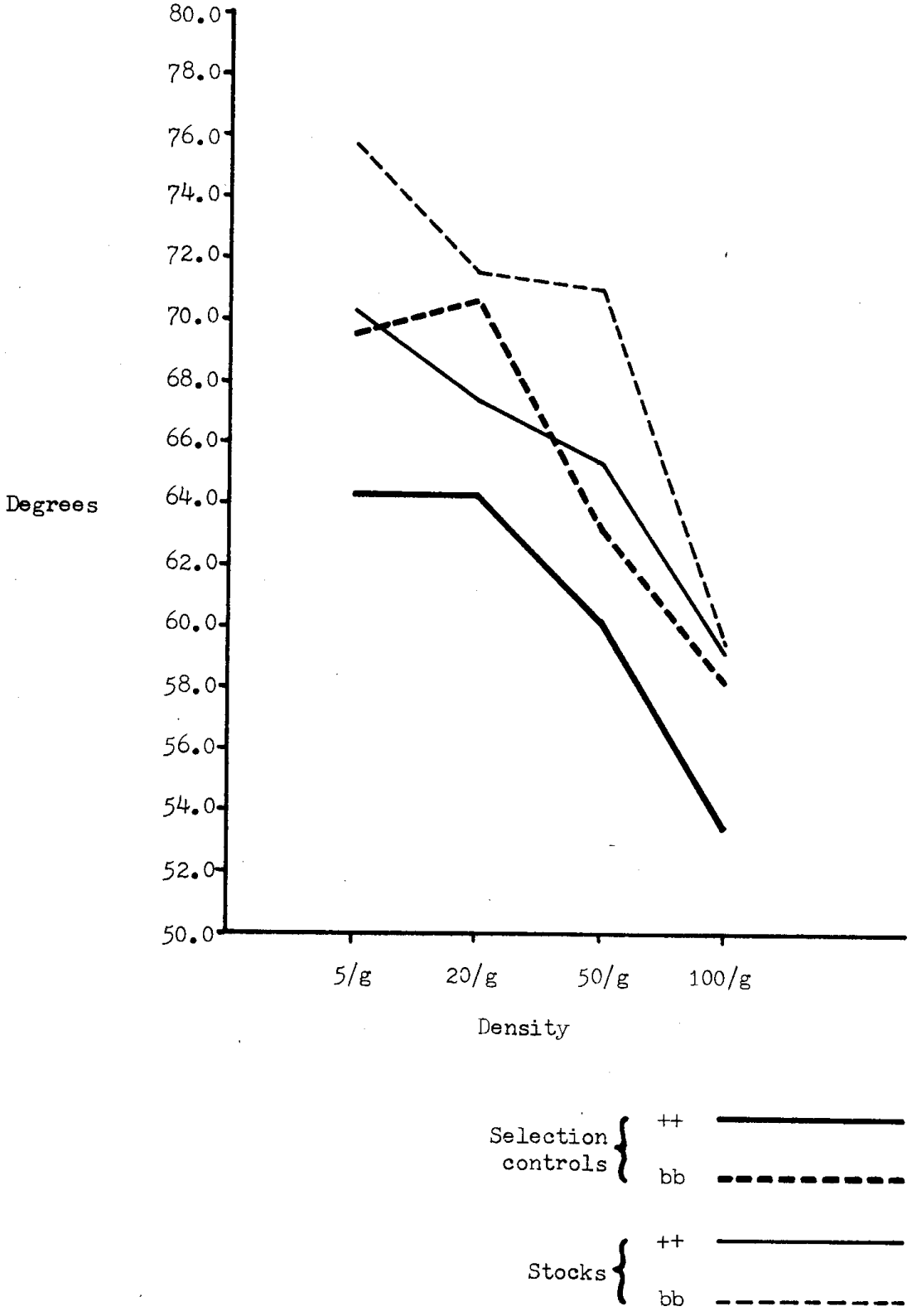


Figure 1. Adult survival expressed as degrees (angular transformatio of percentage of egg input) averaged over the replicates and experiments of the study. Results are shown for the four strains tested at the four densities.

Table 1. Phenotype and gene frequency of paddle. (The initial population consisted of 25 + ♂, 25 + ♀ 25 pd ♂, and 25 pd ♀.)

| Replicates | Months elapsed | Adults found: Live + ♂ | Live + ♀ | Live pd ♂ | Live pd ♀ | Dead + ♂ | Dead + ♀ | Dead pd ♂ | Dead pd ♀ | Gene frequency ♂ | Gene frequency ♀ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 343 | 467 | 285 | 172 | 24 | 43 | 25 | 13 | .4538 | .5272 |
| 2 | 2 | 376 | 471 | 269 | 125 | 13 | 25 | 15 | 7 | .4170 | .4579 |
| 3 | 2 | 313 | 516 | 278 | 119 | (50) | | (26) | (3) | .4704 | .4379 |
| 4 | 2 | 241 | 559 | 362 | 105 | 13 | 17 | 10 | 16 (4) | .6003 | .3976 |
| 5 | 2 | 213 | 624 | 382 | 46 | (4) | | (10) | (9) | .6420 | .2620 |
| 6 | 2 | 272 | 449 | 301 | 129 | (38) | | (21) | (8) | .5253 | .4724 |

The founding population of each of the six replicates involving pd consisted of 25 + ♂♂, 25 + ♀♀, 25 pd ♂♂, 25 pd ♀♀. The data are given in Table 1, where wild type and paddle progeny, whether living or dead, are given according to sex. The gene frequencies, obtained directly from the males, and estimated for the females are also given. It is clear that for the short period of observation there is no consistent trend, male's frequencies sometimes exceed those of females and vice versa. It is clear, however, that the gene frequencies obtained for the two sexes are clearly different.

The pd gene is not very useful if one wants to identify the genotype or phenotype of dead males and females, because the antennae and/or tarsi often break off.

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Preliminary population studies with mutants of Tribolium castaneum Herbst. II. The black gene.

Two allelic mutations were available: the black mutant derived from the Chicago wild type strain, and the black mutant derived from the McGill wild type strain. Eight replicate populations were set up with 25 +/+ ♂♂; 25 +/+ ♀♀ (Chicago wild) and 25 b/b ♂♂; 25 b/b ♀♀ (derived from Chicago +/+). Eight replicate populations were set up with 25 +/+ ♂♂, 25 +/+ ♀♀ (Chicago wild) and 25 b/b ♂♂; 25 b/b ♀♀ derived from the much more productive McGill wild type strain (Sokoloff, Shrode and Bywaters, 1965? Phys. Zool). The data as well as estimated and real frequencies are given in Tables 1 and 2, respectively. Half of the replicates in each had to be discontinued at the end of three months because of incubator failure, and the last two observations 17 and 23 months after the experiments were begun, were made after the populations had been taken out of the incubator and subjected to room and lower temperatures while they were being transported across the country. The gene frequencies (estimated and real) of the two populations are, however, initially different, the McGill black being greater than the Chicago black. At the end of two years, however, the Chicago black populations consist of black at a gene frequency between 0.40 and 0.50 while the McGill black populations ended up with a gene frequency between 0.10 and 0.40.

Aside from these differences it is clear that the two populations differ in:

Table 1. Phenotype and gene frequency of black. (The initial population consisted of 25 ♂ + 25 ♀ Chicago wild type and 25 ♂ + 25 ♀ Chicago black.)

| Replicates | Months Later | Adults found +/+ live | +/+ dead | +/b live | +/b dead | b/b live | b/b dead | Gene frequency estimated | Gene frequency real |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 3 | 109 | 17 | 490 | 35 | 282 | 29 | .5657 | .5982 |
| | 9 | 142 | 47 | 575 | 109 | 350 | 51 | .5727 | .5975 |
| | 17 | 117 | 203 | 334 | 519 | 121 | 377 | .4599 | .5035 |
| | 23 | 194 | 86 | 295 | 232 | 152 | 121 | .4869 | .4672 |
| 2 | 3 | 238 | 69 | 684 | 62 | 389 | 31 | .5447 | .6576 |
| 2 repeat | 3 | 188 | 11 | 513 | 21 | 317 | 20 | .5579 | .5634 |
| | 9 | 187 | 26 | 724 | 141 | 383 | 88 | .5441 | .6757 |
| 3 | 3 | 161 | 16 | 500 | 38 | 251 | 14 | .5246 | .5493 |
| | 9 | 180 | 35 | 568 | 156 | 282 | 66 | .5233 | .5495 |
| | 17 | 132 | 173 | 389 | 530 | 202 | 296 | .5286 | .5484 |
| | 23 | 179 | 104 | 308 | 261 | 118 | 312 | .4416 | .4496 |
| 4 | 3 | 242 | 41 | 639 | 23 | 507 | 20 | .6044 | .5955 |
| 5 | 3 | 146 | 18 | 422 | 28 | 190 | 30 | .5006 | .5290 |
| | 9 | 156 | 33 | 559 | 134 | 232 | 46 | .4950 | .5401 |
| | 17 | 92 | 141 | 376 | 521 | 146 | 252 | .4876 | .5440 |
| | 23 | 120 | 90 | 258 | 338 | 120 | 158 | .4909 | .5000 |
| 6 | 3 | 265 | 13 | 658 | 32 | 401 | 28 | .5503 | .5514 |
| 7 | 3 | 125 | 19 | 543 | 50 | 305 | 56 | .5599 | .5925 |
| | 9 | 158 | 41 | 528 | 108 | 304 | 69 | .5542 | .5737 |
| | 17 | 128 | 128 | 463 | 453 | 209 | 200 | .5111 | .5506 |
| | 23 | 96 | 190 | 208 | 447 | 34 | 305 | .3172 | .4083 |
| 8 | 3 | 154 | 14 | 605 | 25 | 346 | 24 | .5596 | .5869 |

Table 2. Phenotype and gene frequency of black. (The initial population consisted of 25 ♂ + 25 ♀ Chicago wild type and 25 ♂ + 25 ♀ McGill black.)

| Replicates | Months Later | Adults found +/+ live | +/+ dead | +/b live | +/b dead | b/b live | b/b dead | Gene frequency estimated | Gene frequency real |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 3 | 188 | 10 | 743 | 21 | 594 | 28 | .6241 | .6331 |
| | 9 | 272 | 23 | 950 | 149 | 688 | 217 | .6002 | .6089 |
| | 17 | 231 | 162 | 706 | 494 | 304 | 547 | .4950 | .5294 |
| | 23 | 187 | 288 | 285 | 536 | 65 | 312 | .3479 | .3864 |
| 2 | 3 | 345 | 28 | 1220 | 88 | 705 | 39 | .4716 | .5793 |
| 3 | 3 | 104 | 4 | 724 | 17 | 633 | 42 | .6583 | .6810 |
| | 9 | 232 | 32 | 957 | 78 | 777 | 166 | .6286 | .6386 |
| | 17 | 196 | 111 | 737 | 551 | 276 | 634 | .4778 | .5330 |
| | 23 | 170 | 368 | 229 | 541 | 30 | 381 | .2644 | .3968 |
| 4 | 3 | 422 | 8 | 1128 | 40 | 765 | 7 | .5749 | .5741 |
| 5 | 3 | 118 | 5 | 746 | 21 | 692 | 25 | .6669 | .6844 |
| | 9 | 292 | 24 | 918 | 81 | 880 | 181 | .6489 | .6407 |
| | 17 | 253 | 774 | 963 | 510 | 305 | 772 | .4478 | .5171 |
| | 23 | 203 | 275 | 257 | 632 | 15 | 325 | .1778 | .1302 |
| 6 | 3 | 446 | 18 | 1008 | 50 | 803 | 23 | .5965 | .5791 |
| 7 | 3 | 123 | 8 | 791 | 19 | 534 | 26 | .6073 | .6419 |
| | 9 | 281 | 23 | 1041 | 95 | 171 | 115 | .5930 | .6069 |
| | 17 | 223 | 103 | 779 | 285 | 223 | 338 | .4266 | .5000 |
| | 23 | 186 | 307 | 246 | 552 | 35 | 300 | .2737 | .1012 |
| 8 | 3 | 457 | 14 | 1343 | 19 | 732 | 14 | .5377 | .5543 |
| | 10 | 584 | * | 1355 | * | 728 | 322 | .5225 | .5270 |

* (Total of 642 +/+ and +/b)

(1) density (i.e. number of adults observed at census).
(2) mortality.

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Preliminary population studies with mutants of Tribolium castaneum Herbst. III. The jet gene.

The autosomal recessive body color gene jet, and the Chicago wild type (from which it was originally derived) were introduced in equal numbers in regard to sex and genotype (25 + ♂; 25 + ♀; 25 j ♂; 25 j ♀). Half of the cultures were continued for 23 months in the same manner as the other populations. The data and gene frequencies are summarized in Table 1.

This work was supported in part by USPHS grant GM 08942.

Table 1. Phenotype and changes in gene frequency of jet. (The founding population consisted of 25 + ♂, 25 + ♀; 25 j ♂, 25 j ♀.)

| Replicate | Months later | Adults found +/+ live | +/+ dead | j/j live | j/j dead | Estimated gene frequency |
|---|---|---|---|---|---|---|
| 1 | 3 | 923 | 95 | 164 | 12 | .3883 |
| | 9 | 699 | 383 | 143 | 58 | .4121 |
| | 17 | 565 | 741 | 121 | 153 | .4200 |
| | 23 | 321 | 359 | 34 | 37 | .3952 |
| 2 | 2 | 1411 | 98 | 333 | 28 | .4369 |
| | 10 | 1071 | 1242 | 258 | 238 | .4406 |
| 3 | 3 | 928 | 87 | 187 | 12 | .4095 |
| | 9 | 754 | 505 | 166 | 89 | .4247 |
| | 17 | 576 | 840 | 107 | 165 | .3959 |
| | 23 | 589 | 815 | 72 | 75 | .3300 |
| 4 | 2 | 1341 | 180 | 321 | 83 | .4893 |
| 4 repeat | 2 | 1042 | 43 | 388 | 12 | .4209 |
| | 8 | 1234 | 429 | 384 | 90 | .4871 |
| 5 | 3 | 754 | 80 | 215 | 27 | .4711 |
| | 9 | 547 | 380 | 166 | 85 | .4825 |
| | 17 | 520 | 658 | 136 | 165 | .4553 |
| | 23 | 376 | 448 | 45 | 71 | .3270 |

Table 1. (cont.)

| Replicate | Months later | Adults found +/+ live | +/+ dead | j/j live | j/j dead | Estimated gene frequency |
|---|---|---|---|---|---|---|
| 6 | 3 | 1277 | 120 | 367 | 15 | .4724 |
| 7 | 3 | 871 | 79 | 247 | 15 | .4700 |
| | 9 | 676 | 471 | 215 | 134 | .4912 |
| | 17 | 544 | 696 | 106 | 211 | .4039 |
| | 23 | 380 | 948 | 33 | 85 | .2826 |
| 8 | 2 | 1672 | 72 | 475 | 22 | .4703 |
| | 10 | 1316 | 1393 | 291 | 360 | .4702 |

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Preliminary population studies with mutants of Tribolium castaneum Herbst. IV. The pearl gene.

In these experiments the gene was introduced as homozygotes and as heterozygotes according to the following scheme:

| Set 1 | | | | Set 2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| +/+ | | p/p | | +/p | | +/+ | | p/p | |
| ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| 50 | 50 | -- | -- | -- | 1 | 50 | 49 | -- | -- |
| 50 | 49 | -- | 1 | -- | 10 | 50 | 40 | -- | -- |
| 50 | 45 | -- | 5 | -- | 50 | 50 | -- | -- | -- |
| 50 | 25 | -- | 25 | 50 | 50 | -- | -- | -- | -- |
| 50 | -- | -- | 50 | 25 | -- | -- | 50 | 25 | -- |
| -- | 50 | 50 | -- | -- | 25 | 50 | -- | -- | 25 |
| -- | 25 | 50 | 25 | -- | 10 | 50 | -- | -- | 40 |
| -- | 5 | 50 | 45 | -- | 1 | -- | -- | 50 | 49 |
| -- | 1 | 50 | 49 | | | | | | |
| -- | -- | 50 | 50 | | | | | | |

The results are summarized in Tables 1 and 2. Essentially the results show that in most of the replicates set up the frequency of the pearl gene remains as that introduced almost ad infinitum.

Table 1. Phenotype and gene frequency of pearl in populations with varying initial gene frequencies of pearl introduced as homozygotes.

| Founders | Months elapsed | Adults found +/+ live | +/+ dead | p/p live | p/p dead | Gene frequency |
|---|---|---|---|---|---|---|
| A. 50 + ♀; 50 + ♂ | | | | | | |
| 1 | 3 | 849 | 136 | --- | --- | |
| 2 | 3 | 1367 | 47 | --- | --- | |
| 2 repeat | 3 | 1343 | 78 | --- | --- | |
| 3 | 3 | 844 | 161 | --- | --- | |
| 4 | 3 | 1665 | 71 | --- | --- | |
| 4 repeat | 3 | 1051 | 70 | --- | --- | |
| B. 49 +/+ ♀; 1 p/p ♀; 50 +/+ ♂ | | | | | | |
| 1 | 3 | 807 | 165 | --- | --- | |
| 2 | 3 | 1653 | 56 | 1 | 1 | .02408 |
| | 4 | 1419 | 354 | --- | 1 | .02366 |
| | 6 | 1255 | 643 | --- | 4 | .04584 |
| 3 | 3 | 883 | 142 | 1 | --- | .03122 |
| 4 | 3 | 1425 | 40 | --- | --- | |
| | 6 | 941 | 81 | 1 | --- | .03125 |
| C. 45 +/+ ♀; 5 p/p ♀; 50 +/+ ♂ | | | | | | |
| 1 | 3 | 942 | 101 | 3 | --- | .05355 |
| 2 | 3 | 1508 | 55 | 3 | 1 | .05053 |
| | 4 | 1262 | 124 | 2 | 2 | .05364 |
| 3 | 3 | 847 | 148 | 4 | --- | .06328 |
| 4 | 3 | 1565 | 47 | 5 | --- | .05561 |
| | 4 | 1386 | 205 | 3 | 3 | .06129 |

Table 1. (cont.)

| Founders | Months elapsed | Adults found +/+ live | +/+ dead | p/p live | p/p dead | Gene frequency |
|---|---|---|---|---|---|---|
| D. 25 +/+ ♀; 25 p/p ♀; 50 +/+ ♂ | | | | | | |
| 1 | 3 | 981 | 138 | 24 | --- | .1449 |
| 2 | 2 | 1229 | 50 | 24 | 1 | .1385 |
| | 4 | 1615 | 146 | 49 | 13 | .1844 |
| 3 | 3 | 997 | 124 | 25 | | .1477 |
| 4 | 2 | 1271 | 43 | 25 | | .1366 |
| | 5 | 1512 | 189 | 40 | 13 | .1738 |
| E. 25 + ♀; 25 p ♀; 25 + ♂; 25 p ♂ | | | | | | |
| 1 | 3 | 954 | 79 | 135 | 16 | .3571 |
| 2 | 3 | 1016 | 76 | 298 | 35 | .4834 |
| | 5 | 1194 | 135 | 346 | 43 | .4758 |
| | 6 | 1365 | 345 | 391 | 76 | .4631 |
| | 14 | 1058 | 1832 | 297 | 326 | .4211 |
| 3 | 3 | 893 | 107 | 109 | 12 | .3285 |
| 3 repeat | 3 | 846 | --- | 208 | --- | .4442 |
| | 7 | 1177 | 318 | 214 | 85 | .4095 |
| 4 | 3 | 1122 | 62 | 367 | 16 | .4917 |
| | 4 | 1164 | 97 | 378 | 44 | .5007 |
| 4 repeat | 3 | 946 | 128 | 255 | 53 | .4730 |
| | 9 | 796 | 855 | 173 | 191 | .4250 |
| 5 | 3 | 747 | 102 | 135 | 12 | .3841 |
| 6 | 2 | 1111 | 43 | 304 | 15 | .4653 |
| | 4 | 1151 | 128 | 310 | 40 | .4636 |
| | 6 | 1348 | 507 | 361 | 124 | .4553 |
| | 9 | 1139 | 774 | 340 | 177 | .4613 |
| | 14 | 760 | 872 | 220 | 194 | .4498 |
| 7 | 3 | 766 | 73 | 124 | 11 | .3723 |
| | 9 | 740 | 428 | 121 | 49 | .3565 |
| | 17 | 632 | 814 | 105 | 175 | .3955 |
| | 22 | 240 | --- | 40 | --- | .3793 |

Table 1. (cont.)

| Founders | Months elapsed | Adults found +/+ live | +/+ dead | p/p live | p/p dead | Gene frequency |
|---|---|---|---|---|---|---|
| E. 25 + ♀; 25 p ♀; 25 + ♂; 25 p ♂ (cont.) | | | | | | |
| 7 repeat | 3 | 736 | --- | 206 | --- | .4677 |
| | 7 | 1352 | 294 | 288 | 54 | .4147 |
| 8 | 2 | 961 | 66 | 329 | 21 | .5041 |
| | 4 | 915 | 106 | 279 | 43 | .4897 |
| 8 repeat | 2 | 884 | 57 | 266 | 19 | .4822 |
| | 5 | 1184 | 456 | 277 | 130 | .4459 |
| | 10 | 1032 | 858 | 238 | 178 | .4247 |
| F. 25 +/+ ♀; 25 p/p ♀; 50 p/p ♂ | | | | | | |
| 1 | 3 | 652 | 53 | 244 | 25 | .5255 |
| 2 | 2 | 642 | 35 | 489 | 29 | .6584 |
| | 4 | 736 | 59 | 553 | 59 | .6595 |
| | 6 | 675 | 520 | 495 | 509 | .6767 |
| 3 | 3 | 653 | 54 | 257 | 29 | .5367 |
| 4 | 3 | 819 | 51 | 551 | 32 | .7080 |
| | 4 | 360 | 457 | 114 | 380 | .6139 |
| | 6 | 1252 | 89 | 543 | 35 | .5488 |
| G. 5 +/+ ♀; 45 p/p ♀; 50 +/+ ♂ | | | | | | |
| 1 | 3 | 208 | 26 | 558 | 82 | .8557 |
| 2 | 2 | 129 | 27 | 978 | 53 | .9320 |
| | 4 | 139 | 13 | 1015 | 132 | .9397 |
| 3 | 3 | 124 | 11 | 438 | 84 | .8913 |
| 4 | 2 | 106 | 3 | 709 | 52 | .9353 |
| | 4 | 151 | 30 | 929 | 184 | .9274 |
| H. 1 +/+ ♀; 49 p/p ♀; 50 p/p ♂ | | | | | | |
| 1 | 3 | 11 | 4 | 694 | 96 | .9814 |
| 2 | 2 | 17 | --- | 1049 | 39 | .9821 |
| | 4 | 24 | 8 | 1083 | 148 | .9873 |
| 3 | 3 | 28 | 7 | 674 | 128 | .9789 |

Table 1. (cont.)

| Founders | Months elapsed | Adults found +/+ live | +/+ dead | p/p live | p/p dead | Gene frequency |
|---|---|---|---|---|---|---|
| H. 1 +/+ ♀; 49 p/p ♀; 50 p/p ♂ (cont.) | | | | | | |
| 4 | 2 | 20 | --- | 989 | 25 | .9903 |
| | 4 | 29 | 3 | 1122 | 227 | .9883 |
| | 10 | 41 | 17 | 972 | 916 | .9850 |
| I. 50 p/p ♀; 50 p/p ♂ | | | | | | Pearl frequency |
| 1 | 3 | --- | --- | 610 | 148 | 1.0 |
| 2 | 2 | --- | --- | 1034 | 44 | 1.0 |
| | 4 | --- | --- | 1122 | 219 | 1.0 |
| 3 | 3 | --- | --- | 469 | 105 | 1.0 |
| 4 | 2 | --- | --- | 1072 | 54 | |
| | 4 | --- | --- | 869 | 262 | |

Table 2. Phenotype and gene frequency of pearl in populations with various initial gene frequencies of pearl introduced as heterozygotes.

| Founders | Months later | Adults found +/+ live | +/+ dead | p/p live | p/p dead | Pearl frequency |
|---|---|---|---|---|---|---|
| J. 1 +/p ♀; 49 +/+ ♀; 50 +/+ ♂ | | | | | | |
| 1 | 3 | 926 | 150 | 0 | 0 | |
| 2 | 3 | 1342 | 45 | 0 | 0 | |
| | 4 | 1285 | 228 | 0 | 0 | |
| | 6 | 1321 | 485 | 0 | 0 | |
| 3 | 3 | 989 | 173 | 0 | 0 | |
| 4 | 2 | 1111 | 38 | 0 | 0 | |
| | 4 | 1562 | 248 | 0 | 0 | |
| K. 1 +/p ♀; 49 +/+ ♀; 1 +/p ♂; 49 +/+ ♀ | | | | | | |
| 1 | 3 | 988 | 136 | 0 | 0 | ? |
| 2 | 3 | 1170 | 33 | 2 | | .04074 |
| | 5 | 1568 | 245 | 3 | 1 | .04691 |
| | 7 | 1531 | 93 | 1 | 0 | .02481 |
| 3 | 3 | 921 | 140 | 0 | 0 | ? |
| 4 | 3 | 1148 | 64 | 0 | 0 | ? |
| | 4 | 1553 | 191 | 0 | 0 | ? |
| | 6 | 1296 | 673 | 1 | 0 | .02254 |
| L. 5 +/p ♀; 45 +/+ ♀; 5 +/p ♂; 45 +/+ ♀ | | | | mutant live | mutant dead | Gene frequency |
| 1 | 3 | 1038 | 117 | 0 | 0 | ? |
| 2 | 2 | 1082 | 48 | 2 | 0 | .04204 |
| | 4 | 1331 | 298 | 4 | 5 | .07412 |
| | 6 | 1358 | 425 | 5 | 3 | .06684 |
| 3 | 3 | 903 | 132 | 0 | 0 | ? |
| 4 | 3 | 1398 | 41 | 8 | 0 | .07439 |
| | 3 | 1736 | 218 | 13 | 2 | .08728 |
| | 6 | 1488 | 659 | 12 | 4 | .08801 |

Table 2. (cont.)

| Founders | Months later | Adults found +/+ live | +/+ dead | mutant live | mutant dead | Gene frequency |
|---|---|---|---|---|---|---|
| M. 25 +/p ♀; 25 +/+ ♀; 25 +/p ♂; 25 +/+ ♂ | | | | | | |
| 1 | 3 | 1000 | 85 | 69 | 4 | .2511 |
| 2 | 3 | 1406 | 69 | 90 | 8 | .2516 |
| | 4 | 1543 | 186 | 104 | 10 | .2487 |
| 3 | 3 | 976 | 114 | 81 | 5 | .2708 |
| 4 | 2 | 1060 | 28 | 70 | 2 | .2626 |
| | 5 | 1431 | 215 | 103 | 5 | .2481 |
| | 6 | 986 | 547 | 78 | 29 | .2554 |
| N. 25 +/p ♀; 25 p/p ♀; 25 +/p ♂; 25 p/p ♂ | | | | | | |
| 1 | 3 | 741 | 77 | 360 | 39 | .5719 |
| 2 | 3 | 599 | 37 | 493 | 33 | .6728 |
| | 5 | 590 | 146 | 414 | 131 | .6522 |
| | 7 | 858 | 426 | 517 | 238 | .6085 |
| 3 | 3 | 531 | 82 | 364 | 36 | .6284 |
| 4 | 3 | 708 | 29 | 637 | 29 | .6889 |
| | 5 | 599 | 117 | 532 | 150 | .6984 |
| | 6 | 632 | 266 | 522 | 212 | .6780 |
| O. 5 +/p ♀; 45 p/p ♀; 5 +/p ♂; 45 p/p ♀ | | | | | | |
| 1 | 3 | 112 | 13 | 427 | 85 | .8965 |
| 2 | 2 | 177 | 6 | 1033 | 43 | .9244 |
| | 4 | 153 | 23 | 1025 | 188 | .9345 |
| 3 | 3 | 181 | 22 | 497 | 86 | .8612 |
| 4 | 3 | 181 | 11 | 994 | 72 | .9205 |
| | 5 | 195 | 31 | 1071 | 147 | .9184 |

Table 2. (cont.)

| Founders | Months elapsed | Adults found +/+ live | +/+ dead | mutant live | mutant dead | Gene frequency |
|---|---|---|---|---|---|---|
| P. 1 +/p ♀; 49 p/p ♀; 1 +/p ♂; 49 p/p ♂ | | | | | | |
| 1 | 3 | 16 | 3 | 555 | 146 | .9867 |
| 2 | 2 | 24 | 2 | 979 | 49 | .9876 |
| | 4 | 24 | 8 | 1119 | 343 | .9892 |
| 3 | 3 | 65 | 6 | 562 | 106 | .9507 |
| 4 | 2 | 10 | 2 | 872 | 43 | .9935 |
| | 4 | 18 | 3 | 1028 | 208 | .9916 |
| | 6 | 25 | 6 | 1062 | 508 | .9903 |
| Q. 1 +/p ♀; 49 p/p ♀; 50 p/p ♂ | | | | | | |
| 1 | 3 | 8 | 0 | 631 | 150 | .9949 |
| 2 | 2 | 12 | 0 | 841 | 38 | .9932 |
| | 4 | 22 | 6 | 996 | 378 | .9899 |
| 3 | 3 | 5 | 2 | 656 | 168 | .9958 |
| 4 | 2 | 8 | 1 | 1077 | 47 | .9960 |
| | 4 | 7 | 1 | 1062 | 198 | .9968 |

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Preliminary population studies with mutants of Latheticus oryzae Waterh. I. The pearl gene.

These experiments were designed to test, in a comparative way, the performance of pearl in populations. For this reason the founders in the various sets were introduced in a manner similar to the experiment reported above for Tribolium castaneum. The first notable difference between the two species is that the number of adults produced by Latheticus is far lower than that obtained in Tribolium cultures. Furthermore, the developmental period of Latheticus in these cultures is astonishingly slow. Cultures set up six to eight months before may contain only the original adults, the progeny being in the late larva or in the pupa stage. The cultures appear as if the larvae are all of the same age, and they remain thus for a long period of time. Eggs and small larvae apparently are eaten by the older larvae as they are produced. At the other end, the first few pupae forming are destroyed by the younger larvae. The small size of the populations as well as the long developmental period makes it impractical to pursue population studies with this organism.

The data, summarized in Tables 1 and 2, insofar as they go, appear to indicate that when pearl is frequent and wild type infrequent the results in the two species are comparable.

Table 1. Frequency changes of pearl in Latheticus oryzae introduced initially at frequencies from 0-1.0 into each of six replicates as homozygotes.

| Founders | Months later | Adults found +/+ live | +/+ dead | p/p live | p/p dead | Gene frequency |
|---|---|---|---|---|---|---|
| A. 50 + ♀; 50 + ♂ | | | | | | |
| 1 | 8 | 114 | 29 | --- | --- | 0 |
| 2 | 6 | 124 | 44 | --- | --- | 0 |
| | 11 | 218 | 176 | --- | --- | 0 |
| 3 | 8 | 93 | 7 | --- | --- | 0 |
| 4 | 7 | 188 | 92 | --- | --- | 0 |
| | 11 | 241 | 179 | --- | --- | 0 |

Table 1. (cont.)

| Founders | Months later | Adults found +/+ live | +/+ dead | p/p live | p/p dead | Gene frequency |
|---|---|---|---|---|---|---|
| A. 50 + p; 50 + ♂ (cont.) | | | | | | |
| 5 | 8 | 93 | 7 | --- | --- | 0 |
| 6 | 7 | 136 | 91 | --- | --- | 0 |
| | 11 | 190 | 178 | --- | --- | 0 |
| B. 49 + ♀; 1 p/p ♀; 50 + ♂ | | | | | | |
| 1 | 8 | 111 | 40 | 1 | --- | |
| 2 | 5 | 74 | 31 | 1 | --- | |
| | 7 | 231 | 39 | --- | --- | |
| 3 | 8 | 102 | 18 | 1 | --- | |
| 4 | 7 | 186 | 66 | 1 | --- | |
| | 11 | 166 | 162 | 0 | 2 | |
| 5 | 8 | 96 | 4 | 2 | --- | |
| 6 | 7 | 124 | 55 | 1 | --- | |
| | 11 | 256 | 105 | --- | 1 | |
| C. 45 +/+ ♀; 5 p/p ♀; 50 + ♂ | | | | | | |
| 1 | 8 | 99 | 11 | 6 | --- | |
| 2 | 5 | 72 | 23 | 5 | --- | |
| | 7 | 204 | 68 | 9 | 2 | .1972 |
| 3 | 8 | 101 | 18 | 4 | 1 | |
| 4 | 7 | 112 | 79 | 4 | --- | |
| | 11 | 209 | 152 | 2 | 3 | .1189 |
| 5 | 8 | 89 | 7 | 5 | --- | |
| 6 | 7 | 142 | 92 | 4 | 1 | |
| | 11 | 174 | 177 | 3 | 2 | .1185 |

Table 1. (cont.)

| Founders | Months later | Adults found +/+ live | +/+ dead | p/p live | p/p dead | Gene frequency |
|---|---|---|---|---|---|---|
| D. 25 +/+ ♀; 25 p/p ♀; 50 +/+ ♂ | | | | | | |
| 1 | 8 | 85 | 32 | 25 | --- | |
| 2 | 5 | 67 | 6 | 25 | --- | |
| | 7 | 131 | 131 | 21 | 12 | .3345 |
| 3 | 8 | 70 | 20 | 26 | --- | |
| 4 | 7 | 66 | 43 | 21 | 4 | |
| | 11 | 175 | 95 | 22 | 8 | .3162 |
| 5 | 8 | 73 | 9 | 26 | --- | |
| 6 | 7 | 200 | 96 | 21 | 2 | |
| | 11 | 178 | 97 | 21 | 13 | .3317 |
| E. 25 +/+ ♀; 25 p/p ♀; 25 +/+ ♂; 25 p/p ♀ | | | | | | |
| 1 | 8 | 55 | 17 | 49 | --- | .6364 |
| | 20 | 47 | 19 | 34 | 5 | .6094 |
| | 26 | 85 | 56 | 33 | 36 | .5732 |
| 2 | 5 | 32 | 18 | 41 | 9 | |
| | 7 | 194 | 61 | 37 | 5 | .3761 |
| 3 | 8 | 55 | 7 | 50 | --- | .6681 |
| | 20 | 19 | 33 | 23 | 7 | .6984 |
| | 26 | 0 | 15 | 13 | 27 | .8528 |
| 4 | 7 | 34 | 16 | 47 | 2 | |
| | 11 | 74 | 76 | 37 | 25 | .5408 |
| 5 | 8 | 59 | 11 | 48 | 2 | .6455 |
| | 20 | 23 | 18 | 20 | 5 | .6155 |
| | 26 | 54 | 37 | 32 | 19 | .5993 |
| 6 | 7 | 113 | 98 | 41 | 5 | .4231 |
| | 11 | 163 | 74 | 29 | 20 | .4139 |

Table 1. (cont.)

| Founders | Months later | Adults found +/+ live | +/+ dead | p/p live | p/p dead | Gene frequency |
|---|---|---|---|---|---|---|
| F. 50 +/+ ♀; 50 p/p ♂ | | | | | | |
| 1 | 8 | 61 | 20 | 47 | 1 | |
| 2 | 5 | 46 | 4 | 44 | 6 | |
| | 7 | 145 | 65 | 35 | 9 | .4162 |
| 3 | 8 | 68 | 21 | 48 | 2 | |
| 4 | 7 | 87 | 43 | 35 | 11 | |
| | 11 | 222 | 105 | 11 | 33 | .3444 |
| 5 | 8 | 56 | 10 | 45 | 4 | |
| 6 | 7 | 94 | 79 | 41 | 10 | |
| | 11 | 221 | 111 | 31 | 25 | .3799 |
| G. 25 +/+ ♀; 25 p/p ♀; 50 p/p ♂ | | | | | | |
| 1 | 8 | 30 | 5 | 82 | 10 | |
| 2 | 6 | 21 | 4 | 63 | 12 | |
| | 8 | 102 | 14 | 164 | 32 | .7926 |
| 3 | 8 | 29 | 4 | 71 | 7 | |
| 4 | 7 | 49 | 42 | 71 | 51 | |
| | 11 | 102 | 65 | 144 | 62 | .7432 |
| 5 | 8 | 25 | 4 | 75 | 7 | |
| 6 | 7 | 54 | 27 | 102 | 37 | |
| | 11 | 46 | 56 | 75 | 80 | .7766 |
| H. 5 +/+ ♀; 45 p/p ♀; 50 p/p ♂ | | | | | | |
| 1 | 8 | 4 | 4 | 97 | 7 | |
| 2 | 5 | 3 | 2 | 75 | 20 | |
| | 7 | 10 | 2 | 194 | 60 | .9772 |
| 3 | 8 | 10 | 4 | 92 | 8 | |

Table 1. (cont.)

| Founders | Months later | Adults found +/+ live | +/+ dead | p/p live | p/p dead | Gene frequency |
|---|---|---|---|---|---|---|
| H. 5 +/+ ♀; 45 p/p ♀; 50 p/p ♂ (cont.) | | | | | | |
| 4 | 7 | 13 | 6 | 121 | 52 | |
| | 11 | 15 | 10 | 204 | 85 | .9594 |
| 5 | 8 | 5 | --- | 90 | 6 | |
| 6 | 7 | 6 | 2 | 84 | 27 | |
| | 11 | 6 | 5 | 145 | 86 | .9770 |
| I. 1 +/+ ♀; 49 p/p ♀; 50 p/p ♂ | | | | | | |
| 1 | 8 | 1 | --- | 96 | 15 | |
| 2 | 5 | 1 | --- | 86 | 13 | |
| | 7 | 3 | 2 | 150 | 76 | .9891 |
| 3 | 8 | 1 | --- | 95 | 11 | |
| 4 | 7 | --- | 1 | 88 | 15 | |
| | 11 | --- | --- | 282 | 133 | |
| 5 | 8 | 2 | --- | 98 | 10 | |
| 6 | 7 | 1 | --- | 79 | 20 | |
| | 11 | 2 | 1 | 108 | 85 | .9923 |
| J. 50 p/p ♀; 50 p/p ♂ | | | | | | |
| 1 | 8 | --- | --- | 101 | 18 | |
| 2 | 7 | --- | --- | 131 | 40 | |
| | 11 | --- | --- | 229 | 116 | |
| 3 | contaminated with wild type | | | | | |
| 4 | 7 | --- | --- | 85 | 23 | |
| | 11 | --- | --- | 202 | 82 | |
| 5 | 8 | --- | --- | 96 | 5 | |
| 6 | 7 | --- | --- | 92 | 48 | |
| | 11 | --- | --- | 216 | 128 | |

Table 2. Phenotype and gene frequency of pearl in populations with various gene frequencies of pearl introduced as heterozygotes.

| Founders | Months later | Adults found +/+ live | +/+ dead | p/p live | p/p dead | Gene frequency |
|---|---|---|---|---|---|---|
| K. 1 +/p ♀; 49 +/+ ♀; 50 +/+ ♂ | | | | | | |
| 1 | 8 | 101 | 10 | --- | --- | |
| 2 | 7 | 173 | 92 | 2 | --- | |
| | 11 | 257 | 130 | --- | 1 | .05075 |
| 3 | 8 | 100 | 9 | --- | --- | |
| 4 | 5 | 104 | 19 | --- | --- | |
| | 9 | 278 | 101 | --- | --- | |
| 5 | 8 | 101 | 5 | --- | --- | |
| 6 | 5 | 116 | 24 | --- | --- | |
| | 9 | 207 | 152 | --- | --- | |
| L. 10 +/p ♀; 40 +/+ ♀; 50 +/+ ♂ | | | | | | |
| 1 | 8 | 93 | 7 | 1 | --- | |
| 2 | 7 | 94 | 11 | --- | --- | |
| | 11 | 206 | 164 | 2 | --- | .07332 |
| 3 | 8 | 102 | 15 | --- | --- | |
| 4 | 5 | 87 | 13 | --- | --- | |
| | 9 | 181 | 97 | --- | --- | |
| 5 | 8 | 97 | 8 | --- | --- | |
| 6 | 5 | 90 | 9 | 1 | --- | |
| | 9 | 207 | 130 | 1 | --- | .05439 |
| M. 50 +/p ♀; 50 +/+ ♂ | | | | | | |
| 1 | 4 | 97 | 4 | 1 | --- | |
| 2 | 4 | 94 | 5 | 1 | --- | |
| | 8 | 152 | 90 | 6 | 1 | .1676 |
| 3 | 5 | 97 | 2 | 1 | --- | |

Table 2. (cont.)

| Founders | Months later | Adults found +/+ live | +/+ dead | p/p live | p/p dead | Gene frequency |
|---|---|---|---|---|---|---|
| M. 50 +/p ♀; 50 +/+ ♂ (cont.) | | | | | | |
| 4 | 5 | 97 | 3 | --- | --- | |
| | 9 | 59 | 39 | --- | --- | |
| 5 | 5 | 98 | 3 | --- | --- | |
| 6 | 4 | 95 | 3 | 2 | --- | |
| | 8 | 172 | 106 | 9 | --- | .1771 |
| N. 50 +/p ♀; 50 +/p ♂ | | | | | | |
| 1 | 5 | 98 | 2 | --- | --- | |
| 2 | 4 | 95 | 5 | --- | --- | |
| | 8 | 181 | 90 | 47 | 9 | .4139 |
| 3 | 5 | 98 | 2 | --- | --- | |
| 4 | 4 | 95 | 3 | 1 | --- | |
| | 8 | 114 | 69 | 16 | 6 | .3491 |
| 5 | 5 | 99 | 3 | --- | 1 | |
| 6 | 4 | 99 | --- | 1 | --- | |
| | 8 | 71 | 1 | 28 | --- | |
| P. 50 +/+ ♀; 25 +/p ♂; 25 p/p ♂ | | | | | | |
| 1 | 6 | 74 | 8 | 24 | 1 | |
| 2 | 5 | 72 | 4 | 23 | 1 | |
| | 9 | 184 | 123 | 10 | 15 | .2744 |
| 3 | 6 | 76 | 4 | 25 | 1 | |
| 4 | 5 | 59 | 16 | 19 | 6 | |
| | 9 | 150 | 113 | 5 | 14 | .2858 |
| 5 | 6 | 77 | 5 | 24 | 1 | |
| 6 | 5 | 70 | 5 | 22 | 3 | |
| | 9 | 163 | 137 | 6 | 23 | .2967 |

Table 2. (cont.)

| Founders | Months later | Adults found +/+ live | +/+ dead | p/p live | p/p dead | Gene frequency |
|---|---|---|---|---|---|---|
| Q. 25 +/p ♀; 25 p/p ♀; 50 +/+ ♂ | | | | | | |
| 1 | 6 | 75 | 6 | 26 | | |
| 2 | 5 | 64 | 9 | 25 | | |
| | 9 | 91 | 55 | 19 | 6 | .3824 |
| 3 | 6 | 80 | 8 | 25 | | |
| 4 | 5 | 69 | 4 | 23 | 1 | |
| | 9 | 129 | 108 | 14 | 8 | .2914 |
| 5 | 6 | 66 | 6 | 25 | | |
| 6 | 5 | 73 | 2 | 20 | 5 | |
| | 9 | 178 | 91 | 19 | 9 | .3071 |
| S. 10 +/p ♀; 40 p/p ♀; 50 p/p ♂ | | | | | | |
| 1 | 6 | 10 | --- | 85 | 7 | |
| 2 | 5 | 9 | 1 | 82 | 7 | |
| | 9 | 12 | 8 | 96 | 62 | .9421 |
| 3 | 6 | 10 | --- | 90 | 5 | |
| 4 | 5 | 9 | --- | 83 | 7 | |
| | 9 | 17 | 10 | 83 | 130 | .9421 |
| 5 | 6 | 10 | 2 | 86 | 6 | |
| 6 | 5 | 10 | --- | 86 | 4 | |
| | 9 | 18 | 6 | 118 | 89 | .9466 |
| T. 1 +/p ♀; 49 p/p ♀; 50 p/p ♂ | | | | | | |
| 1 | 6 | 1 | 3 | 97 | --- | |
| 2 | 5 | 1 | --- | 97 | 22 | |
| | 9 | 2 | 1 | 151 | 109 | .9943 |
| 3 | 6 | 2 | --- | 97 | 3 | |
| 4 | 5 | 1 | --- | 90 | 8 | |
| | 9 | 2 | --- | 176 | 75 | .9960 |
| 5 | 6 | 2 | --- | 96 | 3 | |
| 6 | 5 | 1 | --- | 87 | 66 | .9967 |

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*Additions to established linkage groups

I. T. castaneum

A. X-chromosome

1. lethal-5 (l-5), located about 25 units to the left of py (away from r). Allelic with $l_2$ and $l_4$.

2. lethal-6 (l-6), located about 12 units to the left of py (away from r).

3. lethal-7 (l-7), located about 20 units to the left of py (away from r).

B. Autosomes

aureate is located about 42 units away from black. Three-point crosses to locate it in respect to other genes are now in progress.

II. T. confusum

A. X-chromosome

1. alate prothorax, apt, is between $es^{lt}$ and lp, about four units to the left of lp.

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*Additional sex-linked lethals in Tribolium castaneum Herbst.

In an experiment designed to determine the frequency of lethals as a regression on the age of the maternal grandfather (see Lerner and Inouye in the present issue of TIB) several females gave aberrant sex-ratios and two of these, on retesting, proved to be heterozygous for lethals. The material was originally derived from the Berkeley synthetic strain marked with sooty (for details on its construction and maintenance see Lerner and Ho, 1961, Am. Nat. 95:329).

In order to locate these lethals and determine possible allelism, four virgin females from stock 29c and eight from stock 68a were mated with py r males. Because of lack of time it was not possible to set up the sequential matings immediately with a consequent overlap in generations. Female virgins were then mated with py r males, four from 29c and eight from 68a. In the former, one female proved to be heterozygous for the lethal producing 1+ and 4 py r males, and 6+ and 4 py r females. The "+" virgin females (py r +/+ + $l_x$) were placed in individual creamers and remated with py r males, allowed to lay eggs for a week and transferred to fresh medium four times at intervals of a week to increase the number of progeny. From 68a two females designated as 68a-1 and 68a-6 and producing 9 py r ♂♂ : 14+, 1 py, 6 py r ♀♀, and 3+, 1 py, 13 py r ♂♂ : 18 +, 1 r, 1 py, 10 py r ♀♀, respectively, were the source of the carriers of the other lethals. In 68a-1 eight "+" females and in 68a-6 twelve "+" females were heterozygous for the lethal. These females were separated in individual creamers and allowed to remain with their progeny until the latter emerged as adults.

## Results and Conclusion

### Experiment 29c

The various broods in experiment 29c have been tested and found homogeneous. Therefore, the data have been pooled and they are shown in Table 1. They make it possible to determine that the crossover frequency for the three genes involved is:

| | Males | Females |
|---|---|---|
| r - py | 44/425 = 10.35 | 78/917 = 8.51 |
| r - $l_{29c}$ | 136/425 = 32.00 | |
| py - $l_{29c}$ | 104/425 = 24.47 | |

Therefore, the order is $l_{29c}$ - py - r. Previous studies have located $l_2$ at 22.78 units from py (from 90/395 crossovers detected between py and $l_2$) and $l_4$ at 25.45 units from the same gene (from 70/275 crossovers between py and $l_4$). $l_2$ and $l_4$ have been found allelic (Sokoloff and Dawson, 1963, Can. J. Genet. Cytol. 5:138). Chi square tests of homogeneity between $l_2$, $l_4$ and $l_{29c}$ indicate the data are homogeneous. Therefore, $l_{29c}$ must be considered as a recurrence of $l_2$ and it is designated as l-5. (cf. section on New Mutants.)

Tables 2 and 3 summarize the data for experiment 68.

The data for 68a-1 indicate the crossover frequencies are:

| | Males | Females |
|---|---|---|
| py - r | 47/398 = 11.81 | 92/613 = 15.01 |
| r - lethal | 87/398 = 21.86 | |
| py - lethal | 46/398 = 11.56 | |

so the order is: lethal - py - r.

The data for 68a-6 give the following crossover values:

| | Males | Females |
|---|---|---|
| py - r | 30/416 = 7.21 | 84/822 = 10.22 |
| r - lethal | 99/416 = 23.80 | |
| py - lethal | 81/416 = 19.47 | |

and the order of the three genes is lethal - py - r.

68a-1 and 68a-6 have been tested for allelism by the Chi square test for homogeneity with $l_2$, $l_4$ and $l_5$. The statistical tests suggest these lethals are not allelic with them nor with each other. Neither are they allelic with $l_3$. Therefore, 68a-1 is redesignated $l_6$ and 68a-6 $l_7$.

We do not know whether the original female whose progeny indicated the presence of a sex-linked lethal carried both lethals or whether a second lethal occurred during the course of these experiments.

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Table 1. Progeny of py r +/++ 29c × py r +/.

| Phenotype | Male | Female |
|---|---|---|
| py r | 283 | 266 |
| + + | 98 | 573 |
| py | 38 | 38 |
| r | 6 | 40 |
| Total | 425 | 917 |

Table 2. Progeny of py r +/++ 68a-1 × py r + (nine successful creamers)

| Phenotype | Male | Female |
|---|---|---|
| py r | 308 | 216 |
| + + | 43 | 305 |
| py | 44 | 56 |
| r | 3 | 36 |
| Total | 398 | 613 |

Table 3. Progeny of py r +/++ 68a-6 × py r +/. (12 successful creamers)

| Phenotype | Male | Female |
|---|---|---|
| py r | 311 | 268 |
| + + | 75 | 470 |
| py | 24 | 36 |
| r | 6 | 48 |
| Total | 416 | 822 |

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## Further studies of productivity of Tribolium castaneum and Tribolium confusum in homo- and heterospecific matings

In a previous paper (Sokoloff, Shrode and Bywaters, 1965, Phys. Zool. 38:165), it was shown that productivity (defined as the number of adult progeny per female within a specific interval or the number of fertile eggs per female surviving to the adult stage) is a reliable genetic character. It was used in a previous study by Sokoloff and Inouye (TIB 6:61) to determine whether females of T. castaneum (CS) or T. confusum (CF) are affected in their reproductive capacities by the presence of males of the other species. It was found that when CF females are in association with CS males their productivity drops somewhat, but not as much as when CS females are in creamers together with CF males. This previous experiment left unanswered the question whether this drop in productivity, observed particularly for CS, was not the result of depriving the females from their mating partners. Tagarro and Rico (TIB 9:120) showed that when males are removed from cultures where females are already fecundated, they continue to lay fertile eggs at a slightly lower rate than when males were present, but that this difference is not significant. The period they tested was between days 7 and 11 after the females had eclosed. Since in the Sokoloff and Inouye experiments the females were left without males for a much longer period, the present experiments were performed.

For each of the species or species combination the experimental set-up was as follows: into each of 10 creamers were introduced four pairs of beetles 10 days old (females and males had been isolated as pupae) on a Thursday. At the same time on Monday the imagoes were transferred into new containers, the old medium with eggs being returned to the original creamers after counting the eggs. The adults were moved to new quarters every twenty-four hours for three days, and the eggs were counted before returning them to their respective creamers. Every week the beetles were manipulated in the following way: the first week the females in all 10 creamers had males of the same species as partners. The second week the set was broken into two subsets. From subset A the males were removed altogether, and these females are referred as "widows." From subset B half of the males were removed and replaced by males of the other species. We refer to these females as "bigamists." On the third week the females of subset A are supplied with males of the other species, and refer to them as "miscegynists, type I." From subset B the remaining two males of the same species are removed and replaced by males of the other species, and the females referred to as "miscegynists, type II." Finally, on the fourth week, the foreign males are removed from both subsets and males of the same species re-introduced. (The two subsets will be referred to as "repurified I" and "repurified II," respectively.)

Table 1. Mean number of eggs and larvae produced by four females of T. confusum (CF) and T. castaneum (CS) in a three-day period following homo-, heterospecific, or mixed-species mating in succeeding weeks (N = number of observations).

| Week | Type of mating | CF | | | | CS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | eggs | | larvae | | eggs | | larvae | | |
| | | $\bar{m}$ | ± S.E. | $\bar{m}$ | ± S.E. | $\bar{m}$ | ± S.E. | $\bar{m}$ | ± S.E. | N |
| 1 | "Pure species" | 48.73 | 2.55 | 44.73 | 2.35 | 57.53 | 2.16 | 48.93 | 2.36 | 30 |
| 2 | "Widows" | 48.73 | 2.38 | 39.60 | 1.80 | 46.47 | 2.50 | 40.73 | 2.53 | 15 |
| 3 | "Bigamists" | 44.07 | 2.35 | 38.60 | 2.27 | 40.13 | 3.43 | 31.33 | 3.13 | 15 |
| 3 | "Miscegynists I" | 45.53 | 1.76 | 35.60 | 2.71 | 28.27 | 2.54 | 15.80 | 2.97 | 15 |
| 3 | "Miscegynists II" | 45.73 | 1.84 | 41.33 | 1.72 | 28.33 | 2.67 | 15.67 | 2.18 | 15 |
| 4 | "Repurified I" | 44.73 | 1.74 | 38.47 | 2.07 | 51.73 | 1.47 | 43.13 | 2.55 | 15 |
| 4 | "Repurified II" | 43.80 | 2.17 | 38.00 | 2.14 | 43.07 | 3.21 | 36.20 | 3.07 | 15 |

If any dead beetles were found, they were sexed and replaced by beetles of the same age, sex and species.

Fertility of the eggs was determined by counting larvae when they were three weeks old.

The data have been analyzed to determine any significant difference between the three successive 24-hour periods. Since no significant difference has been found, the values of the three successive days have been pooled providing 30 observations for the pure species (10 replicates × 3 days) and 15 for the subsets (5 replicates × 3 days). The results are summarized in Table 1.

Comparisons of the means by t-test indicates that none of the differences observed for CF are significant. For CS significant differences can be shown at the .02 level for the means of eggs produced by "pure species" and "widows" and at the .01 level for "pure species" vs. "miscegynists-I", "miscegynists-II," and "repurified." Significant differences are obtained at the .01 level for fertility of eggs of "pure species" vs. "bigamists," "miscegynists-I" and "miscegynists-II." The values obtained for "pure species" and "re-purified II" are significantly different at the .02 level.

This experiment appears to indicate that when inseminated CF females are introduced with CS males either they reject them, or if copulation takes place between them, the foreign sperm play no role in fertilization of CF eggs. On the other hand, if CS females are confined with CF males (and no CS males are present) they inseminate them, and the foreign sperm may fertilize a fairly large number or all of CS eggs. The result is that females exhibit partial or complete sterility. These sterile or semisterile CS females recover almost immediately from any "damage" the foreign males may have produced if CS males are reintroduced.

Therefore, it is concluded that in mixed species cultures heterospecific matings should not influence greatly the outcome of competition if the numbers of the two species are large. Where the numbers of CS are small (and CF large), the absence of sufficient CS males to service the CS females present might result in the loss of CS from those cultures.

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Influence of collecting frequency on egg-laying rate of fecundated and virgin females in Tribolium castaneum

Egg-laying rate is the quantitative character we are using more in our genetic research with Tribolium castaneum. We have defined a period

comprised between the 7th and the 11th days after adult emergence to measure that character.

Egg collecting and counting is a time-consuming task, so we were interested to reduce as much as possible the number of collections in such testing period. At 33°C and 70% RH larvae hatch 66 ± 6 hours after the eggs are laid. So with fecundated females it is necessary to collect no later than every other day (two collections in the four-day period). With virgin females this problem does not exist, so it is possible to collect once at the end of the testing period.

However, we wondered whether collecting every day, every other day, or at the end of the four-day period could have some influence in the total number of eggs laid or counted. Favorable effect of fresh medium after a collection, disturbing effect of the suction sifting, cannibalism, etc., could contribute to find different figures in the total lay of the four days.

Four trials were run comparing 24 vs. 48 hours collecting frequencies both with fecundated and with virgin females. Two more trials were run to compare 48 vs. 96 hours collecting frequencies only with virgin females. We used our "Consejo" strain of Tribolium castaneum. Fecundated females were mated at adult emergence and the males (one per female) were maintained during the testing period. At day 7th after emergence the medium in each vial was changed. Egg collecting was made by suction sifting every day, every other day or at day 11, according to the corresponding treatment, and afterwards fresh medium was added. The figure analyzed was the total number of eggs laid per female during the four-day testing period.

Table 1 includes the analysis of each of the four experiments run with fecundated females comparing 24 vs. 48 hours collecting frequencies, together with a pooled analysis of the four as a whole. We can see that in no case there exist differences between treatments.

Table 2 includes similar information as in Table 1 but with the data obtained with virgin females. The results in this case are not as clear as with fecundated females because in the A trial we find significant differences to the .05 level in favor of 48 h. and in the B one slightly significant (only to .10 level) but in favor of the 24 h. frequency. However, when considering the pooled analysis we do not find significant differences to any level between treatments and the significance for the interaction reflects the difference of results obtained in A and B. The greater influence of uncontrolled effects in egg laying of virgin females as compared with the lay of fecundated ones, widely observed by us in many experiments, could explain the anomalous results of A. In a practical sense we can consider the non-significant differences found for treatments in the pooled analysis as favorable.

Table 3 contains the analysis of the two experiments comparing 48 vs. 96 hours collecting frequencies with virgin females and the pooled analysis

Table 1. Means, standard errors, df, MS and F values of the analysis of four experiments studying the influence of 24 vs. 48 hours collecting frequencies on the laying rate of fecundated females.

| Experiment | Means and S.E. | | Sources | df | MS | F |
|---|---|---|---|---|---|---|
| A | 24 h. | 63.5 ± 2.7 | Treatments | 1 | 46.04 | 0.27 |
| | 48 h. | 65.5 ± 2.7 | Error | 45 | 173.50 | |
| B | 24 h. | 76.9 ± 3.6 | Treatments | 1 | 218.11 | 0.69 |
| | 48 h. | 72.5 ± 4.0 | Error | 43 | 317.11 | |
| C | 24 h. | 34.4 ± 2.9 | Treatments | 1 | 154.47 | 0.96 |
| | 48 h. | 38.3 ± 2.8 | Error | 37 | 161.00 | |
| D | 24 h. | 61.0 ± 3.3 | Treatments | 1 | 108.15 | 0.40 |
| | 48 h. | 58.0 ± 3.5 | Error | 45 | 273.09 | |
| Pool | 24 h. | 60.4 ± 1.6 | Experiments | 3 | 10,881.46 | 46.61* |
| | 48 h. | 58.9 ± 1.6 | Treatments | 1 | 110.06 | 0.47 |
| | | | Exp. × Treat. | 3 | 138.90 | 0.59 |
| | | | Error | 170 | 233.47 | |

* Significant to .005 level.

Table 2. Means, standard errors, df, MS and F values of the analysis of four experiments studying the influence of 24 vs. 48 hours collecting frequencies on the laying rate of virgin females.

| Experiment | Means and S.E. | | Sources | df | MS | F |
|---|---|---|---|---|---|---|
| A' | 24 h. | 9.8 ± 1.1 | Treatments | 1 | 252.01 | 6.62† |
| | 48 h. | 13.7 ± 1.1 | Error | 62 | 38.04 | |
| B' | 24 h. | 19.4 ± 1.7 | Treatments | 1 | 243.95 | 3.77* |
| | 48 h. | 14.9 ± 1.6 | Error | 46 | 64.65 | |
| C' | 24 h. | 9.3 ± 1.5 | Treatments | 1 | 21.03 | 0.48 |
| | 48 h. | 7.9 ± 1.5 | Error | 38 | 44.17 | |
| D' | 24 h. | 14.1 ± 1.9 | Treatments | 1 | 89.78 | 1.01 |
| | 48 h. | 16.8 ± 1.9 | Error | 48 | 89.05 | |
| Pool | 24 h. | 12.9 ± 0.8 | Experiments | 3 | 635.57 | 10.93‡ |
| | 48 h. | 13.6 ± 0.8 | Treatments | 1 | 25.59 | 0.44 |
| | | | Exp. × Treat. | 3 | 193.73 | 3.33† |
| | | | Error | 194 | 58.17 | |

* Significant to .10 level; † to .04 level; ‡ to .005 level.

Table 3. Means, standard errors, df, MS and F values of the analysis of two experiments studying the influence of 48 vs. 96 hours collecting frequencies on the laying rate of virgin females.

| Experiments | Means and S.E. | | Sources | df | MS | F |
|---|---|---|---|---|---|---|
| E | 48 h. | 12.8 ± 1.2 | Treatments | 1 | 0.66 | 0.01 |
| | 96 h. | 13.0 ± 1.2 | Error | 96 | 69.09 | |
| F | 48 h. | 28.4 ± 2.3 | Treatments | 1 | 0.66 | 0.00 |
| | 96 h. | 28.3 ± 2.3 | Error | 96 | 250.33 | |
| Pool | 48 h. | 20.6 ± 1.3 | Experiments | 1 | 11,694.88 | 73.23* |
| | 96 h. | 20.6 ± 1.3 | Treatments | 1 | 0.00 | 0.00 |
| | | | Exp. × Treat. | 1 | 1.30 | 0.01 |
| | | | Error | 192 | 159.71 | |

* Significant to .005 level.

of both. No differences at all are found between treatments in any experiment nor in the pooled analysis. This result also helps to interpret the figures in Table 2 in the sense that the significant differences observed in A and B must be due to chance effects.

In all sets of trials differences between experiments are always significant. This is not surprising because many uncontrolled effects influence the number of eggs laid by females of Tribolium castaneum. Pooling the analysis of different trials is quite fair because we have proportional or nearly proportional subclass numbers; analysis with means of treatments × experiments cells give the same results reported here.

Therefore, we can conclude that under our experimental conditions, when we are interested to measure the egg-laying rate from day 7 to day 11 after adult emergence, it does not make any difference whether we collect eggs every day or every other day in both fecundated and virgin females and even in doing only one collection at the end of the four days with virgin females. The longest "between collections" period with fecundated females is 48 hours because larvae may hatch before three days.

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*Sex pheromones and defensive secretions from Tenebrionid beetles

Studies on the sex pheromones and defensive secretions of Tenebrionid beetles are under way in this laboratory. Sex pheromones have been demonstrated in Tenebrio molitor and Zophobas rugipes (a large Central American species) and a biological assay for the pheromone of Tenebrio molitor has been devised (Tschinkel, Willson and Bern, 1967). Attempts to isolate the sex pheromone of Tenebrio molitor are in progress.

The secretion of the prothoracic defensive glands of Zophobas rugipes is being studied by various chromatographic and spectral techniques. These glands secrete phenols, but are otherwise homologous to the quinone-secreting prothoracic stink glands of Tribolium (Roth, 1943).

Disturbed larvae of Zophobas rugipes frequently squirt hemolymph along with an acrid odor. This phenomenon and an effect of larval crowding on pupation are under study.

Attempts are being made to establish a number of Tenebrionid beetles (especially subfamily Tenebrioninae) in laboratory cultures for comparative studies on their sex pheromones and defensive secretions. Success has been limited. The results are summarized in Table 1.

## Literature Cited

Roth, L. M. 1943. Studies on the gaseous secretion of Tribolium confusum Duval. II. The odoriferous glands of Tribolium confusum. Ann. Ent. Soc. Am. 36:397-424.

Tschinkel, W. R., C. D. Willson and H. A. Bern. 1967. Sex pheromone of the mealworm beetle (Tenebrio molitor). J. Exp. Zool. 164:81-85.

Table 1. Success of laboratory culture of Tenebrionid beetles

| Classification | | | Degree of Success | | | |
|---|---|---|---|---|---|---|
| Subfamily | Genus and Species | Source | Adult Survival in Lab. | Egg Production | Larval Survival | Successful Metamorphosis |
| Tenebrioninae | Tenebrio molitor | Museum Vert. Zool., Berkeley | + | + | + | + |
| | Tenebrio obscurus | Entomol. Dept., Berkeley | + | + | + | + |
| | Argoporis ? | S. Arizona, near Yuma | + | + | + | + |
| | Amphidora ? | Concord, Calif., salt marshes | + | + | + | + |
| | 1-? ? | Concord, Calif. | + | + | + | + |
| | Zophobas rugipes | Near San José, Costa Rica | + | + | + | + |
| | Eleodes ? | Near Fairview Pk., Nevada | + | + | + | - |
| | Eleodes ? | Near Yuma, Arizona | + | + | + | ± (poor survival) |
| | Eleodes ? | California, near Berkeley Marina | + | + | + | + |
| | Eleodes ? | California, near Berkeley Marina | + | - | - | - |
| | Coelocnemis ? | California, Del Puerto Canyon | + | - | - | - |

Table 1. (cont.)

| Classification | | | Degree of Success | | | |
|---|---|---|---|---|---|---|
| Subfamily | Genus and Species | Source | Adult Survival in Lab. | Egg Production | Larval Survival | Successful Metamorphosis |
| Tenebrioninae | Cibdelis blaschkeii | California, Berkeley Hills | + | + | + | + |
| | Alobates ? | California, Sunol Park | + | + | + | - |
| | Tribolium brevicornis | (Sokoloff) | + | + | + | + |
| | Tribolium castaneum | (Sokoloff) | + | + | + | + |
| | Tribolium confusum | (Sokoloff) | + | + | + | + |
| | Tribolium destructor | (Sokoloff) | + | + | + | + |
| Asidinae | 2-? ? | California, San Diego | Several months (Annual species) | + | + | - |
| | Cryptoglossa verrucosa | near Yuma, Arizona | + | + | - | - |
| | Zopherus sp. | near Yuma, Arizona | + | - | - | - |
| | Coniontus sp. | California, near Antioch | + | - | - | - |
| | 3-? ? | ? | + | - | - | - |
| Tentyrinae | Edrotes rotundata | California, Mojave River | Few months | - | - | - |
| | Triophorus sp. | near Yuma, Arizona | + | - | - | - |

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*Some observations on mating frequencies in Tribolium castaneum strains

Introduction. In a series of papers, Ehrman and associates (Ehrman, 1965, 1966; Ehrman et al., 1965) described a phenomenon observed earlier by Petit (1958) with important evolutionary implications. When two strains of Drosophila are mated in unequal proportions, in some cases the rarer strain, regardless of genotype, will mate more often than expected on the basis of its frequency in the observation chamber. The series of experiments reported here was designed to look for a similar phenomenon in two strains of Tribolium. In the course of the experiment, some interesting observations on the mating habits of these strains were made, which are also reported here.

Materials and Methods. The standard UPF wild type and black strains of T. castaneum employed in work in this laboratory (see stock list) served as experimental material. Large numbers of pupae were recovered from multiple cultures of the two stocks, sexed and then put, in unisexual groups of 10, in 6-dram holding vials containing about 4g of flour. The bb beetles emerged two days later than the ++ adults and therefore were two days younger throughout the experiment. The first experiment was set up four days after the emergence of bb adults, and the last one two weeks later.

Mating was observed in two-inch Syracuse dishes with 20 ♂♂ and 20 ♀♀ in varying genotypic proportions. All the males were marked by paint at least three days before the experiment to distinguish true mating pairs from the frequent ♂ × ♂ mounts (see results). The genotype of each sex and time of mating was recorded. Each observation dish was watched constantly for two consecutive 30-minute periods, during which a considerable number of copulations was observed. Two series of observations were made. In the first, after the end of the fixed observation period, all the males were removed, and the females were transferred individually to vials with about 4g of flour, where they were left to oviposit for three days, after which they were discarded. The genotype of the ♀ was recorded on the vial, and the resulting brood was classified by genotype to infer the type or types of males that had sired it.

In the second series, the ♀♀ and ♂♂ were separated after the first 30 minutes' observation period. The "old" ♂♂ were given a new group of virgin ♀♀ and the "old"♀♀, a fresh group of ♂♂ (the previous genotypic proportions being maintained). The two dishes were observed simultaneously for another 30 minutes, after which all the beetles were discarded. No brood analysis was carried out in this series.

The first series was set up at proportions 10:90 and 25:75, with both wild type and black being rare in turn. The second series was run at

10:90 only. Two 50:50 observations were carried out as controls.

Results. Previous to the main experiment, couples (in copula) were recovered from large wild type and black populations and the sex of the participants determined by microscopic examination. It appeared that wild type or black ♂♂ will mate with any Tribolium available, regardless of sex. Mounting of ♂♂ on other ♂♂ occurred in 30.5% of the cases among the wild type and 34.5% among the blacks (based on 127 pairs); a test of independence yielded $X^2 = 0.277$ and fails to show any difference in behavior between the strains in this respect. In all subsequent experiments the males were marked, and only true (♂ × ♀) matings are considered in the following sections.

The observations in the Syracuse dishes may be summarized in the following form; the asterisks refer to total $X^2$ for all replicates.

| Proportion | Combination | Number of replicates | Rare genotype ++ | | Rare genotype bb | |
|---|---|---|---|---|---|---|
| | | | ♂♂ | ♀♀ | ♂♂ | ♀♀ |
| 25:75 | new ♂♂ × new ♀♀ | 8 | *(++) | -- | *(++) | **(bb) |
| | old ♂♂ × old ♀♀ | 8 | -- | -- | -- | *(bb) |
| 10:90 | new ♂♂ × new ♀♀ | 10 | -- | -- | **(++)$^h$ | ***(bb) |
| | old ♂♂ × old ♀♀ | 4 | **(++)$^h$ | -- | -- | -- |
| | new ♂♂ × old ♀♀ | 6 | **(++) | -- | -- | ***(bb)$^h$ |
| | old ♂♂ × new ♀♀ | 6 | -- | -- | -- | ***(bb) |

* .05 > P > .01; ** .01 > P > .001; *** P < .001.

The symbols (++) or (bb) following asterisks indicate the genotype that mated more often. Significant heterogeneity among the replicates is shown by the superscript h.

All new × new cases were observed in the first 30-minute periods. All other combinations were in the second 30-minute periods.

The mean number of copulations in 30 minutes for 10:90 proportions differed greatly among the four combinations: new ♀♀ × new ♂♂ (21.7), new ♀♀ × old ♂♂ (12.5), old ♀♀ × new ♂♂ (15.0), and old ♀♀ × old ♂♂ (6.75). Analyzing the variance of the 26 replicates, the following table shows a highly significant mean square among combinations.

| Source of variation | df | MS |
|---|---|---|
| Genotype of rare strains | 1 | 1.78 |
| Combinations | 3 | 248.26*** |
| Interaction | 3 | 28.74 |
| Error | 18 | 17.35 |

Single degree of freedom comparisons showed a highly significant difference between the mean number of copulations between new ♀♀ × new ♂♂ and the mean of the other three taken as a whole ($P \ll .001$), and a significant difference between old ♀♀ × old ♂♂ and the two (old ♀♀ × new ♂♂ and new ♀♀ × old ♂♂) combinations taken together ($P < .01$). No significant difference was found between the mean of new ♀♀ × old ♂♂ versus old ♀♀ × new ♂♂.

Similar results were obtained in the eight 25:75 replicates, which contained only old ♀♀ × old ♂♂ and new ♀♀ × new ♂♂ combinations.

Surprisingly, the results of the brood analysis of the 10:90 proportions showed that very few of the ♀♀ in the observation dish were fertilized although, based on the average mating frequency, every one of the 20 ♀♀ could have been fertilized. (Similar results were obtained in the 25:75 series.)

| Rare strain | Number of matings observed | Number of fertilized females |
|---|---|---|
| ++ | 25 | 6 |
| | 29 | 7 |
| bb | 24 | 12 |
| | 27 | 13 |
| control (50:50) | 23 | 5 |

In a few cases, a ♀ was mated in sequence to a heterotypic ♂ and then a homotypic ♂. Only 5 of 10 ♀♀ were fertilized by any of the males: 3 bb ♀♀ and 2 ++ ♀♀. The brood of the bb ♀♀ contained only the offspring of the first (++)♂♂. The brood of the ++ ♀♀ contained mainly the first (bb) male's offspring, although 3.5 - 8.5% of the offspring were from the second mating.

Discussion. As can be seen from the results presented above, the Petit-Ehrman phenomenon does not occur in these two strains of Tribolium under our test conditions. However, it was found that black ♀♀, when rare,

consistently mated much more frequently than expected, mostly with wild type males. In all cases where a significant deviation from expected mating frequencies for ♂♂ or ♀♀ was present, the frequency of ++ ♂♂ × bb ♀♀ matings was very much higher than expected ($P \ll .001$ by the G-test). This trend in the bb ♀♀ was consistent for all replicates. The only case in which heterogeneity was significant for the ♀♀ was caused by an exceptionally large deviation from expectation in one of the six replicates. No clear trend could be shown for the ♂♂.

Such a behavior, if reflected in the offspring, could result in an increasing gene frequency of black.

Virgin pairs showed the highest mating frequency per half hour. When both ♂♂ and ♀♀ had mated in the half hour preceding the experiment, they showed the lowest number of mating. In cases where one sex was virgin, while the other had mated in the half hour preceding the experiment, the mating frequency was in-between, with no significant difference between the reciprocal crosses. Possibly mated ♀♀ may resist a subsequent mating shortly after the first one, and the ♂♂ may need a period of rest between matings.

It is interesting to note that even in the old ♀♀ × new ♂♂ and new ♀♀ × old ♂♂ combinations, the black females, when rare, mated significantly more often than expected. Presumably they are unable to resist the males as effectively as the wild-type females.

Why the number of fertilized females should be so much lower than the number of observed copulations is obscure. When b was rare (wild-type common) more ♀♀ were fertilized than when wild type was rare. Some of the matings could have been infertile because of the unusual conditions of light and substrate in the observation dish. By contrast, when in another experiment 20 ♂♂ and 20 ♀♀ at proportions 10:90 and 50:50 were left in flour for 12 hours, 18, 18 and 20 females were fertilized in the cases where + was rare, the two alleles equally frequent, and b rare, respectively.

The brood analysis data are not helpful in testing the Petit-Ehrman phenomenon because the evidence shows a considerable number of double matings (mating of one female with more than one male). Regrettably, this can be demonstrated only if the female mated with both types of males. Ignoring all evident double matings, no significant higher mating frequency of the rare form can be shown.

Contribution No. 1350 from the Department of Entomology, The University of Kansas. This is paper No. 7 in a series on the ecological genetics of Tribolium. Numbers 1 through 5 are listed at the end of paper No. 6 (Sokal, 1967) in this issue of TIB. This research was supported by a National Science Foundation Grant GB-2170 (Robert R. Sokal, principal investigator).

## Literature Cited

Ehrman, L. 1965. Direct observation of sexual isolation between allopatric and sympatric strains of the different Drosophila paulistorum races. Evolution 19:459-464.

Ehrman, L. 1966. Mating success and genotype frequency in Drosophila. Animal Behavior 14:332-339.

Ehrman, L., B. Spassky, O. Pavlovsky, T. Dobzhansky. 1965. Sexual selection, geotaxis, and chromosomal polymorphism in experimental populations of Drosophila pseudoobscura. Evolution 19:337-346.

Petit, C. 1958. Le dèterminisme gènètique et psycho-physiologique de la compètition sexuelle chez Drosophila melanogaster. Bull. Biol. France Belgique 92:248-329.

Sokal, R. R. 1967. A comparison of fitness characters and their response to density in stock and selected cultures of wild-type and black Tribolium castaneum. Tribolium Information Bulletin 10:142-147.

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### *Selection for 13-day larval size in Tribolium under two nutritional levels

Selection experiments for larval growth under two nutritional levels were conducted for sixteen generations so as to evaluate the effectiveness of various selection methods in the presence of genetic-environmental interaction.

A strain of Tribolium castaneum, Purdue "+" Foundation stock was used as the experimental organism. The two levels of nutrition, so-called Good and Poor rations, which were originally formulated by R. H. Hardin, were used as the environments. The primary difference between the two rations depends on the content of brewer's dry yeast and corn oil. The Good contains 10% of dry yeast and 5% corn oil but none at all in the Poor.

The character for selection is the 13-day larval weight in two directions. Genetic parameters of the initial population for the character were: 0.40 in heritability under both environments and the genetic correlation between Good and Poor performance was 0.80.

The experimental populations which were originated from the base population by random sampling are listed in Table 1.

Table 1. Symbols of experimental populations

| | |
|---|---|
| GL | = Selected for large under the Good level every generation. |
| PL | = Selected for large under the Poor level every generation. |
| $\overline{GPL}$ | = Selected for large on average performance under both levels every generation. |
| GPL | = Selected for large under Good and Poor in alternating generations. |
| GS | = Selected for small under Good every generation. |
| PS | = Selected for small under Poor every generation. |
| $\overline{GPS}$ | = Selected for small on average performance under both levels every generation. |
| GPS | = Selected for small under Good and Poor in alternating generations. |
| C | = Unselected controls consisted of 20 pair matings, each contributes one male and one female to the next generation. |

The experiment was repeated twice but one week apart. Each set of replicated experimental populations was sampled from two different sublines derived from the base population a few generations prior to the initiation of selection.

The mating and selection were made in such a way that each pair produces eggs in the creamer which contains standard wheat medium for 48 hours, and then the parents are transferred to Good medium for 24 hours, Poor ration for 24 hours and additional Good or Poor ration for 24 hours, so as to have two creamers of the same ration for selection purpose depending on the population, until the 7th generation. For instance, GL had two Goods and one Poor, while PL had two Poors and one Good. The $\overline{GPL}$ and $\overline{GPS}$ had only one Good and one Poor. Each mating randomly sampled five larvae for each creamer weighed and the sum of two creamers was used as the selection criterion. The measurement taken under the opposite environment rather than for selection was used as the measure of correlated trait. Since the 8th generation inclusively, two Goods and two Poors were measured for all populations in the same manner. Once the families for selection were decided, the full sibs raised under the standard medium were picked up randomly and sexed for mating to produce the next generation. Therefore, the parental individuals in all lines were never exposed to either Good or Poor but standard medium. The individuals measured were discarded after weighing. This technique eliminates any carry-over environmental effect from parental generation.

## Experimental results

### Average gain per generation

Average selection and correlated responses of the selected character under two environments in terms of change per generation is listed in Table 2. As is seen from the table, direct selection responses exceeded

Table 2. Selection gains per generation over sixteen generations by replication ($10^{-2}$ mg)

| Replication | Population | Good | Poor | Average |
|---|---|---|---|---|
| I | $GL_1$ | 5.6 ± 0.4 | 5.5 ± 5.3 | 5.6 ± 0.5 |
| | $PL_1$ | 3.9 ± 0.5 | 9.3 ± 0.9 | 6.6 ± 0.6 |
| | $\overline{GPL}_1$ | 5.2 ± 0.5 | 8.7 ± 0.7 | 7.0 ± 0.4 |
| | $GPL_1$ | 4.9 ± 0.4 | 8.5 ± 1.2 | 6.7 ± 0.7 |
| | $GS_1$ | -8.7 ± 0.6 | -5.5 ± 0.9 | -7.1 ± 0.6 |
| | $PS_1$ | -8.8 ± 0.7 | -7.9 ± 1.0 | -8.4 ± 0.8 |
| | $\overline{GPS}_1$ | -9.5 ± 0.5 | -6.3 ± 0.8 | -7.9 ± 0.5 |
| | $GPS_1$ | -9.7 ± 0.7 | -6.2 ± 0.8 | -7.9 ± 0.7 |
| II | $GL_2$ | 7.3 ± 0.7 | 6.5 ± 1.0 | 6.9 ± 0.6 |
| | $PL_2$ | 6.9 ± 0.7 | 10.4 ± 0.6 | 8.6 ± 0.4 |
| | $\overline{GPL}_2$ | 5.4 ± 0.8 | 5.7 ± 0.7 | 5.5 ± 0.6 |
| | $GPL_2$ | 6.7 ± 0.6 | 7.6 ± 0.6 | 7.2 ± 0.4 |
| | $GS_2$ | -12.5 ± 0.8 | -4.9 ± 0.8 | -8.7 ± 0.7 |
| | $PS_2$ | -8.6 ± 1.0 | -6.4 ± 0.8 | -7.5 ± 0.9 |
| | $\overline{GPS}_2$ | -7.4 ± 0.9 | -5.5 ± 0.6 | -7.4 ± 0.7 |
| | $GPS_2$ | -9.3 ± 0.7 | -5.5 ± 0.6 | -7.4 ± 0.5 |
| Average of I + II | GL | 6.5 ± 0.5 | 6.0 ± 0.7 | 6.2 ± 0.5 |
| | PL | 5.4 ± 0.4 | 9.9 ± 0.7 | 7.6 ± 0.4 |
| | $\overline{GPL}$ | 5.3 ± 0.3 | 7.3 ± 0.4 | 6.2 ± 0.4 |
| | GPL | 5.8 ± 0.3 | 8.1 ± 0.7 | 7.0 ± 0.5 |
| | GS | -10.6 ± 0.6 | -5.2 ± 0.6 | -7.9 ± 0.5 |
| | PS | -8.7 ± 0.8 | -7.2 ± 0.8 | -8.0 ± 0.7 |
| | $\overline{GPS}$ | -8.4 ± 0.6 | -5.9 ± 0.6 | -7.2 ± 0.5 |
| | GPS | -9.5 ± 0.6 | -5.8 ± 0.5 | -7.7 ± 0.5 |

indirect or correlated gains except that the populations selected for average ($\overline{GPL}$ and $\overline{GPS}$) were inferior to others in most cases. It is also interesting to note that asymmetrical selection responses in two directions after pooling all lines with the same direction of selection are clearly dependent upon the environment tested. That is, asymmetry is favored to small selection under Good ration but the reverse is true under Poor ration.

Realized heritabilities in large and small populations were 0.33 for the large and 0.44 for the small populations and this difference being significant at 5% level. The difference of heritability in two directions suggests that selection for different directions may involve different genetic and physiological mechanisms of the trait. Nevertheless, realized heritabilities of 0.33 ~ 0.44 agreed closely with the estimated heritability in the base population.

Genetic correlation between larval weights under two environments by taking the square root of the product of two genetic regressions obtained from two lines selected in the same direction but under two environments was 0.77, which is close to the estimated correlation in the initial generations.

Generally speaking, genetic changes resulting from our selection experiment agreed fairly well with those expected from the analyses of the initial population.

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*Relative fitness of selected strains under different environments

Sixteen populations selected for large and small larval size and reported in the preceding report were tested for their relative fitness under Good and Poor media. The measure of fitness was the number of adults of a strain expressed as the deviation from that of a tester stock, b. This measure of fitness may be called competitive ability relative to the tester stock. In each set of competition trials, fifty eggs each from the tester and a competent strain were placed together into a vial which contained 20 grams of either Good or Poor medium. After 30 days the number of black and wild type beetles were counted. Hatchability was tested prior to the trial in all populations but no difference among populations was recognized. Repeatability of this measure of fitness was 0.907 and 0.917 under Good and Poor respectively. This suggests that the measure was very consistent with replications.

Statistical analysis showed that there were highly significant differences among strains but not between rations. Strain by environment interaction was also highly significant. Those differences were, however,

non-existent among large populations, while more striking among small populations. The order of relative fitness in the small populations is summarized as follows based on the Duncan test at 1% level:

Under Good environment,

$$GS_2 = \overline{GPS}_2 < PS_2 < \overline{GPS}_1 = PS_1 < GPS_1 < GS_1 = GPS_2$$

Under Poor environment,

$$PS_1 < \overline{GPS}_2 = PS_2 < GS_1 = \overline{GPS}_1 = GPS_2 < GPS_1 = GS_2$$

The striking strain by environment interaction was ascribed primarily to a very characteristic behavior of $GS_2$, whose 13-day weight was much larger under Poor than under Good.

NOTES TECHNICAL

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*A method for isolating C. turcicus to obtain virgin females

Virgin females of C. turcicus can easily be obtained by isolating larvae in gelatin capsules and incubating until the adults emerge. This is more efficient than isolating pupal cases which may be empty or "inhabited" by non-virgin adults.

Gelatin capsules of size No. 2 are separated and the larger "bottom" end is placed in a board drilled with rows of shallow holes (1/4 in. size drill bit). This "bottom" half is then filled with flour and yeast medium with an ordinary "eye dropper" type of bulb pipette. Large larvae close to the prepupal instar are placed singly in individual capsules with jeweler's forceps or a brush, and the smaller "top" half of the capsule firmly replaced. The capsules are then incubated in the rack or in labeled, net covered stock jars until the adults emerge. Then they can be sexed and maintained individually until needed. If large larvae are used there will generally be enough medium left in the capsule for the adult to subsist on for several days.

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*A method for enhancing propagation of poorly viable flour beetles

One often encounters flour beetles whose elytra or membranous wings droop at the sides, are oriented ventrally, or extend beyond the end of the abdomen. These appendages may interfere with copulation or with the tunneling activities of the beetle. One way to enhance the chances of mating is to remove the elytra of the mutant, or the hind legs of the mating partner if it is a female. This should not be done indiscriminately, however, because if the legs or wings are pulled or broken off there may be severe damage and the beetle may bleed to death. A more effective procedure is to cut the appendage off, and for this operation McLure iris scissors are very useful.

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Technique to determine the contents of cocoons of Cryptolestes turcicus

In the course of experiments to determine the effects of low temperature on the various stages in the life history of C. turcicus (Coleoptera: Cucujidae) it was necessary to know whether cocoons contained pupae, or young adults without damaging the cocoon. The adult remains in the cocoon one or two days after it emerges from the pupal skin.

The method adopted was to place cocoons on a plate glass stage of a microscope which had the oculars removed. The stage was covered with black paper and a hole was cut in the paper large enough to accommodate the glass dish containing the cocoons. A light from a 125 W bulb was reflected up through the plate glass and the cocoons from a convex mirror. Best results were obtained in a darkened room using a 2X magnifier. With very little practice it was possible to distinguish two classes of cocoons: (1) those that appeared to be uniformly clear and (2) those that had a dark shadow inside of them. The clear cocoons contained pupae and the others contained young adults. This method was over 98% reliable.

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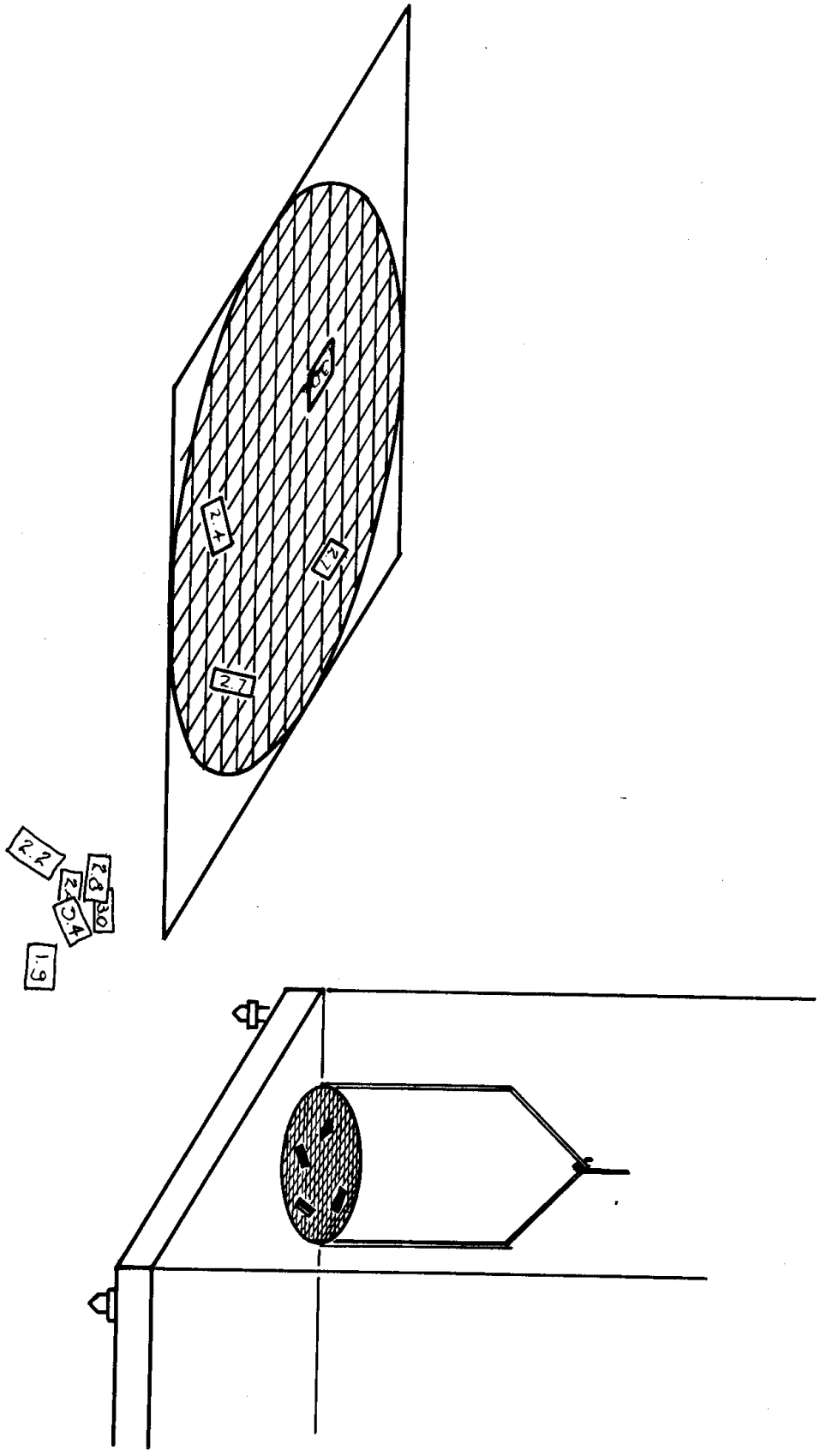
*New method for weighing Tribolium pupae

During a recent selection experiment with Tribolium castaneum, pressure of work created the need for an unusually efficient method of weighing large numbers of pupae singly. The conventional method is to place a pupa on the balance pan, measure its weight, then remove the pupa and replace it with another. This involves considerable time and possibly also wear on the switch mechanism. The alternative system described below, and used successfully at Massey, is adaptable to any weighing balance with a moving scale and a fairly large balance pan.

The balance pan is marked with a grid and a cardboard map of this pan is placed on the laboratory bench alongside the weighing balance. A system of "weighing by successive difference" is used, whereby pupae are added one by one to the pan and their weights noted by successive changes in the scale reading. As each reading is made, a small cardboard chip carrying the weight symbol is placed on the map in the position corresponding to the pupa it represents. When all pupae in a selection replicate are so weighed the author merely has to scan the map, refer from it back to the pan and so


2.4
2.1
2.7
2.2
2.3
2.C.4
3.0
1.9

collect the desired pupae for selection. The chips are then collected, their weights recorded, and returned to the pool.

The pupae show no tendency to roll about on the pan provided the weighing room is kept warm but, if necessary, the pan grid can be constructed of a wire mesh to prevent such movement. The number of pupae which can be weighed together is then limited only by the size of pan or size of scale.

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A method for rearing Eleodes (longicollis?) (Coleoptera: Tenebrionidae) in the laboratory

The stink beetle Eleodes (longicollis?), an inhabitant of the desert areas of the Southwestern United States and Mexico, when irritated does a "headstand" (lowering its head and raising the posterior end of the abdomen) and discharges quinones (see illustrations in Eisner, T. and J. Meinwald, 1966. Defensive secretions of Arthropods. Science 153:1341-1350). Because of repeated encounters with this beetle outdoors as well as indoors (janitors often place specimens on my desk should I want to keep them), attempts have been made to maintain the adults in captivity and to find a medium in which these beetles can be maintained in stock in the laboratory.

The adult specimens were initially kept in baby food jars provided with grapes as sources of food and moisture. This food appeared to be adequate since the beetles lived on it for about a month and the female began to lay eggs in clusters. The latter were transferred to another jar containing standard flour beetle medium (whole wheat flour + brewer's yeast in a proportion of 19:1). The larvae were seen eating this medium, but a few days later they had died, still in the first larvae instar.

In order to stimulate oviposition, the adult female was placed in flour beetle medium, and this proved to be a highly satisfactory food for Eleodes, provided that a source of moisture was available. This was supplied by adding a piece of apple or some other fruit. The increase in moisture induces the growth of mold, so adult beetles have to be transferred rather frequently to fresh medium. Nonetheless, the effort pays off, for at this writing, the beetles have lived in this medium for about five months, and the female continues to lay eggs. The larvae also require transferring to fresh medium every so often because of the tendency of the medium to cake and eventually become moldy when pieces of fruit are added as a source of moisture. The eggs, somewhat larger than those in Tribolium but alike in this genus of a white color, are laid in clutches of about 20-30. The larvae were only about 1-1/2 to 2 mm long in the first instar. At about 24°C, the largest larvae have reached a size of almost three centimeters in three

months, and they resemble the larvae of Tenebrio molitor. Out of 110 small larvae introduced in this jar, only about 10 per cent survive because the immature stages of Eleodes are highly cannibalistic. It was possible to observe the cannibalistic activities of these organisms when the larvae were about 1 cm long. At least two tunnels within which the larvae traveled were evident. They were against the glass wall of the container, and a larva from the upper gallery had made its way to the lower gallery where it had encountered a larva of about the same size; it apparently had dealt the latter a bite on the tergite of the mesothorax which largely immobilized it--at least there was no apparent effort on the part of the larva preyed upon to escape--and the predator larva had half of its head within the body of the larva it was eating. When the larvae are removed from the medium one does not find any pieces of dead larvae, unless the larvae was recently killed.

The medium, insofar as it has been possible to observe, provides larvae the necessary nutritional requirements for good health and development. It is possible, therefore, that the tendency toward cannibalism is a trait characteristic of the family.

It is evident from the above that beetles in this family other than Tribolium and other flour beetles have potentialities of being raised in the laboratory and thus become potentially useful organisms in research population genetics and population ecology.

The writer has not tried it, but it comes to mind that Tenebrio molitor, the mealworm, and Zophobus, another Tenebrionid which normally lives on bat guano, can be reared on bran provided a source of water such as a slice of potato, is provided to the beetles every so often. The survival of Eleodes could also be enhanced by rearing this species in bran medium distributed in a container in layers between paper toweling and to which wood shavings are provided so the smaller larvae can escape the predatory activities of larger larvae. The lack of an incubator at San Bernardino for this particular purpose has prevented the exploration of the question of the optimal conditions of temperature and R.H. for the development of this beetle from egg to adult.

P.S. A visit to the laboratory of Dr. Clyde Willson, Biochemistry Department, University of California, Berkeley, after the above note was written, revealed that Mr. Walter Tschinkel is extremely interested in developing techniques for rearing a wide variety of Tenebrionidae (see Research Note elsewhere in this issue). Mr. Tschinkel informs me that according to Dr. Eisner, Eleodes can be kept on bran for as long as six or seven years in the laboratory (if supplied with water). During this time they produce numerous progeny, but they fail to reach the adult stage. Whether the present diet will prove to be more suitable so Eleodes will undergo metamorphosis remains to be seen.