Table 2. Curve-fitting and test of goodness of fit : X = time and Y = percent of breakage of chromosomes

Concentration level (%)	Regression equation	χ^2	Remarks
0.1	$Y = -1.78 + 1.61\ X$	2.19 (2 df)	not significant
0.2	$Y = -5.83 + 10.59\ X$	0.84 (3 df)	not significant
0.3	$Y = -5.60 + 18.57\ X$	6.15 (4 df)	not significant
0.5 (a)	*$Y = -4.82 + 29.34\ X$	22.71 (4 df)	highly significant
0.5 (b)	*$Y = -36.41 + 71.76\ X - 9.51\ X^2$	3.045 (3 df)	not significant

*The 2 equations are the line and parabola respectively, fitted to data by the 'method of least squares'.

duced breakage at frequencies which became increasingly important as a function of the dose and of the duration of treatment. At 0.1%, breakage was first observed after 3 h of treatment; at 0.5% after 1 h already.

From statistical analysis (table 2) it was found that a more or less linear relationship exists between time and the percentage of breakage induced by colchicine at 0.1, 0.2 and 0.3% concentrations. However, at 0.5% concentration level the relationship is no longer linear but it is parabolic. It should also be mentioned here that no other types of chromosomal aberrations except chromosome breaks were observed in the colchicine-treated material. Earlier workers[5-7] found that the breaks caused by colchicine are not at random but are confined to the centromeric region. For instance, Levan and Rhoades[5] observed that certain chromosomes in maize broke at the centromere and gave rise to isochromosomes. Darlington[6] made similar observation in *Fritillaria camschatcensis* and Karpechenko[7] in barley. The present results show that the chromosome breaks may occur at random and are not confined to the centromeric region alone. One of the reasons why random breaks were detectable in *V. fischeri* may be that this material has much larger chromosomes than those which earlier workers dealt with.

The present results may explain why colchicine-induced 'raw' polyploids are invariably sterile. These results led us to believe that colchicine, besides duplicating the chromosome number, also damages certain chromosomes in the complement, the damage in most cases being undetectable. Further indirect evidence that colchicine may cause damage to chromosomes has been obtained in the colchicine-treated seedlings of the Asiatic cotton, *Gossypium arboreum*, where 1 out of 100 treated seedlings did not show chromosome duplication upon maturity and yet it was completely sterile with no bolls[11]. Pollen mother cells of this unresponsive diploid plant showed regular meiosis of 13 bivalents at metaphase I, and wellstained pollen grains. Such an observation indicates that colchicine may cause damage to chromosomes with or without chromosome duplication; thus undetectable chromosome damage could perhaps be the main cause for seed sterility. It is possibly for this reason that the spontaneously-arizing polyploids are more fertile than the colchicine-induced ones.

In the light of the above results it is suggested that a) when used to induce duplication of plant chromosomes, colchicine should be used only in low concentrations (if a less toxic polyploidising agent is not available for the purpose) and b) it should not be used in the treatment of gout during pregnancy as there is likelihood of congenitally defective births from the prolonged administration of this drug.

11 Unpublished work of the first author in collaboration with his students in Sind University, Pakistan.

Interaction of genotype and learning in the food preference of the flour beetle, *Tribolium castaneum*

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Summary. Selection was combined with training to study learning in the flour beetle, *Tribolium castaneum*. The response was rapid when selecting for both normal (N) and garlic-aversive (G) food preference – which increased for N and decreased for G. N adults can be conditioned to go toward the medium on which they were raised. Preference of G females is explained by habituation and that of N males by conditioning. This suggested that conditioning or habituation depends on the genetic background and sex.

The attraction of animals towards specific foods or odours may depend on either inherited or learned preferences[1]. Genetic variation in preference has been demonstrated by many selection studies, e.g., those for ethanol preference in rodents[2]. But in insects there is a situation where learning can impede the response to selection. This is the phenomenon of 'pre-imaginal conditioning' whereby insects, reared on a normally aversive medium, lose their aversion[3], making it very difficult to select for increased aversion. In *Drosophila melanogaster*, such 'pre-imaginal conditioning' has been explained as habituation to the presence of the aversive substance, rather than as conditioning through an association of the substance with the food medium[4]. The present experiment involved a

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1 P. Rozin, Adv. Stud. Behav. *6*, 21 (1976).

2 G. Lindzey, J. Loehlin, M. Manosevitz and D. Thiesen, A. Rev. Psychol. *22*, 39 (1971).

3 W. H. Thorpe, Learning and Instinct in Animals, 2nd ed. Methuen, London 1973.

4 A. Manning, Nature *216*, 967 (1967).

Learning to distinguish standard and garlic supplemented media at different generations of selection for either medium

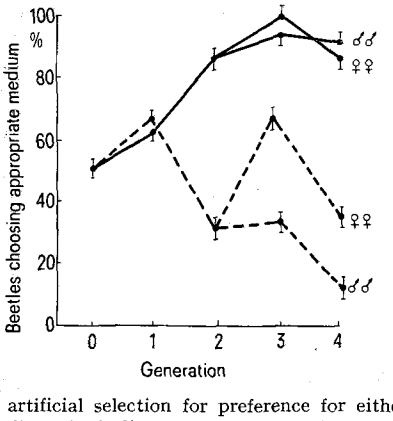
Generation	Sex	Garlic line Trial 1	Trial 2	Trial 3	Normal line Trial 1	Trial 2	Trial 3
0		41% (7.3)	67% (12.1)	36% (17.0)	58% (7.1)	87% (7.0)	94% (5.9)
3	♂♂	71% (17)	100% (0.0)	100% (0.0)	93% (4.7)	100% (0.0)	100% (0.0)
	♀♀	71% (9.9)	64% (12.8)	67% (15.7)	93% (6.6)	100% (0.0)	100% (0.0)
4	♂♂	9% (2.3)	0% (0.0)	–	98% (0.9)	98% (1.1)	99% (0.9)
	♀♀	2% (1.3)	–	–	97% (1.2)	99% (0.9)	96% (2.3)

Percentages are based on number of beetles selecting the given medium out of the number of beetles leaving the source arm. The value in brackets is the SD calculated as in the figure.

selection study with *Tribolium castaneum* where conditioning, and not just habituation, occurs and where this conditioning interacts with the genetic preference.

Methods. Beetles were selected over 4 generations for a preference either for the standard medium (95% whole wheat flour and 5% dried yeast) or for a garlic medium (standard medium supplemented with 40% garlic powder). The latter constitutes an aversive medium in that it is frequently avoided by *T. castaneum* and has deleterious effects on fertility and viability. Similar effects were previously observed in beetles for another odourant, phenyl-thio-carbamide[5].

All beetles were incubated at 30 °C and 70% RH and were less than 1 week old when tested. They were starved for 24 h before being introduced in groups of not more than 50 into the empty arm (source arm) of a Y-shaped maze, described elsewhere in detail[6]. The beetles selecting the arm of the maze that contained the standard medium (normal line) in the initial generation, G0, and in subsequent generations were reared on that medium. Beetles selecting the other arm of the maze with the garlic medium (garlic line) were bred on garlic up to G3, when viability became so low that the standard medium had to be used for G4. In each generation after the maze trial, the selected insects were left on the appropriate medium for 3 days, at which time eggs were collected over the next 24 h. Males and females were tested separately in G3 and G4.



Response to artificial selection for preference for either standard *Tribolium* medium (solid line) or medium supplemented with 40% garlic powder (dashed line). The bars represent 1 SD above and below the actual value and were calculated from the formula SD = $\sqrt{P(100-P)/N}$, where P is the percentage of beetles choosing a given medium out of N, number of beetles that left the source arm. N varies between 29 and 261 depending upon viability with an average of 105.1.

Results and discussion. The results of the selection are shown in the figure. Despite its effects on viability, the garlic medium did not decrease locomotor activity, the average percentage of beetles leaving the source arm being 82.4% compared with 73.1% for the normal line, averaged over all generations. The figure shows that the preference of the normal line for the standard medium increased from an initial 50% in G0 to 90–100% in G3 and G4. The garlic line has a more complex response with a decreased preference for garlic in G2, and a subsequent significant increase in the females in G3. The fact that this preference decreased again in G4 when a standard medium had to be used, suggested that 'pre-imaginal conditioning' or some learning in early adulthood may have made the G3 females less aversive to garlic. Although not previously described in the flour beetle, *Tribolium*, retention of learning from larva to adult is known in the grain beetle, *Tenebrio molitor*[7].

An explanation, based on conditioning, of the differences between generations and between sexes in the garlic line requires some additional evidence of a sex difference in learning in the G3 beetles. This is provided by tests carried out on additional beetles in G0, G3 and G4, there being insufficient numbers in G1 and G2. These beetles were run through the maze 3 times, each trial using only the beetles that had chosen the specified medium on the previous trial; 24 h rest was given between each trial. These data are given in the table. Because of the rapid response to selection in the normal line, by G3, almost all beetles preferred this medium in the initial trial; so we find an increased preference over trials only in G0. Because the standard medium was always presented in the same arm of the maze, the results in G0 could either be explained in terms of learning to turn in a particular direction (the beetles associating the turning and/or the smell with the accustomed food medium) or due to some beetles having an innate bias to turn in a specific direction[8]. The latter is unlikely because the garlic line in G0 showed no increased preference over the 3 trials. If a turning bias were important, an equal increase in both lines would have been expected. The most interesting results are those in G3 and G4 of the garlic line (table). In G3 few males were available for testing and their initial trial gave a preference for garlic which is almost ($p = 0.1$) significantly higher than that of the larger sample in the selection experiment. However, on the 2nd and 3rd trials all males went to the garlic medium, a result of which probability of occurrence by chance

5 M. H. Soliman, Ent. exp. appl. *17*, 454 (1974).
6 M. H. Soliman, J. stor. Prod. Res. *11*, 203 (1975).
7 T. M. Alloway, Am. Zool. *12*, 471 (1972).
8 D. A. Hay, Nature *257*, 44 (1975).

alone is less than 0.001. In contrast, the preference of the females remained unchanged over the 3 trials, so we can conclude that adult males of this selection line, but not females, can be conditioned to go towards the medium on which they were reared. Such sex differences in beetles on a learning task have been reported in another selection experiment involving running speed[9].

This result can be confirmed by altering the medium on which they were reared, so for G4, where the standard medium was used and where larger samples (150 of each sex) were available, the table shows that few males now preferred the garlic medium on the 1st trial and none on the 2nd. So few females went to the garlic medium on the 1st trial that there was an insufficient number for a 2nd trial.

These results can be contrasted with those obtained for *D. melanogaster*, where flies had to choose between a blank and a geraniol-baited tube. There, no evidence was obtained for conditioning (defined by an increased proportion going toward the normally aversive geraniol) at the larval or at the adult stage, but only for habituation (defined by little or no increase in preference predicted on successive trials). An explanation based on habituation is sufficient here to account for the G0 preference and the G3 and the G4 preferences of the females in the garlic line, however, conditioning may explain the performance of the G0 and the G3 and G4 males of the normal line. Thus, whether or not a preference indicates conditioning or habituation depends on genetic background and sex. Considering the biological restraints on learning in this situation[10], we might expect conditioning to occur more slowly in the Garlic line because of the aversiveness of the medium, and this tallies with the very rapid changes in preference between G3 and G4 when the medium was changed. It is not clear why the sex difference should occur, but it may involve the fact that the female uses the medium as an ovipositing site and not just as food. In view of the current interest in the genetics of learning in insects[11], and since a wide variety of antennal mutants of *Tribolium* are available, food-seeking and selection being mediated through these receptors[6], the present technique which combines selection with training may offer a convenient model for the study of learning.

9 I. M. Lerner and N. Inouye, in: Haldane and Modern Biology, p. 51. Ed. K. R. Dronamraju, John Hopkins, Baltimore 1968.
10 R. A. Hinde and J. Stevenson-Hinde, Eds., Constraints on Learning. Academic Press, London 1973.
11 W. G. Quinn and Y. Dudai, Nature *262*, 576 (1976).

Genome size in the green toad (*Bufo viridis*) group

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Summary. Specimens of the green toad, *Bufo viridis*, from Morocco to Kirgizistan, have identical DNA amounts per erythrocyte nucleus. 1 specimen from Kirgizistan is a tetraploid. Of 3 closely related species, *B. calamita* and *B. brongersmai* have about the same DNA amount as *B. viridis*, while *B. latastii* from Afghanistan has 36% more DNA per nucleus.

The DNA content of the genome appears to be a constant for every plant and animal species, while it varies considerably even among closely related species wherever it has been studied. This variation is not random, but appears to be related to adaptive characters of morphology and physiology. From all indications, the mere amount of DNA in a genome is a decisive genetic determinant[1,2]. Since the variation within any species seems to be so much less than interspecific variation, changes in DNA amounts during speciation could be large and rapid ('saltatory changes'), and genome size within any species might be subject to regulatory influences. There is very little information on the evolutionary dynamics of the change in DNA content, and very few species have been investigated throughout their geographical range for DNA amounts. Therefore we wish to present a set of measurements on the nuclear DNA content of green toads covering nearly the whole distributional area of this widespread species. These measurements show that, within the accuracy of determination (10%), *Bufo viridis* has the same nuclear DNA content throughout. 1 of 2 specimens from Kirgizistan has twice this amount in erythrocyte nuclei. 2 related species, *B. calamita* and *B. brongersmai* from Morocco[3], where it is sympatric with *B. viridis*, have essentially the same DNA amount, while *B. latastii* from Afghanistan[4] has 36% more DNA in its erythrocytes.

Material and methods. Blood was obtained from living specimens[5] by puncturing the angular vein of the mouth with a micropipette. Blood smears were fixed in 10% formalin, then air-dried and stored dry for up to a few days before staining. Feulgen staining was preceded by various methods of DNA hydrolysis in order to exclude systematic errors stemming from the preparation[6]. Dye content of Feulgen-stained nuclei was determined at 558 nm using a Barr and Stroud Integrating Microdensito-

1 K. Bachmann, O. B. Goin and C. J. Goin, Brookhaven Symp. Biol. *23*, 419 (1972).
2 H. J. Price, Bot. Rev. *42*, 27 (1976).
3 M. S. Hoogmoed, Zoöl. Meded., Leiden *47*, 49 (1972).
4 H. Hemmer, J. F. Schmidtler and W. Böhme, Zool. Abh. Dresden *34*, in press (1978).
5 Sources of the animals are: *B. viridis*, Frunze, Kirgizistan, coll. Hemmer; Haifa, Israel, commercial dealer; Izmir, Turkey, coll. Prof. Dr Özeti, Izmir; Corsica, coll. Dr Back, Bonn; Germany, coll. Hemmer; Tafraoute, Morocco, coll. Schulte, Neuhaus; *B. brongersmai*, Tafraoute, Morocco, coll. Schulte, Neuhaus; *B. latastii*, Afghanistan, coll. Scheffler, Mühlheim; *B. calamita*, Germany, coll. Hemmer; Holland, coll. Dr Hoogmoed, Leiden; *B. bufo*, Northern Spain, coll. Gutsche, Aachen.
6 K. Bachmann and J. P. Bogart, Cytogenet. Cell Genet. *15*, 186 (1975).