throline by centrifugation and resuspension of the cells in veronal buffer was followed by an increase in total hemolysis to a level similar to that described by curve 1.

These results indicate that lysis of sensitized sheep erythrocytes by phenanthroline resembles lysis produced by C'9 in requirement for C'8 sites, dependence on number of C'8 sites, dependence on temperature, and in inhibition by 0.09*M* EDTA. Like C'9, phenanthroline can exert its effect on the cell in the presence of 0.09*M* EDTA which inhibits hemolysis. It may be concluded that the two chelating compounds act at different steps of the terminal phase of the hemolytic reaction. Phenanthroline acts, as C'9, following C'8; EDTA acts following C'9 or phenanthroline. Even at a molar concentration which was six times greater than the highest phenanthroline concentration employed, EDTA showed no lytic effect, as evidenced by lack of lysis following removal of EDTA from cells bearing C'8 sites (not shown).

The possible relationship between the hemolytic and the metal-binding capacity of phenanthroline was investigated next. Before addition to C'8 cells, phenanthroline was mixed with solutions of various metal ions; the extent of inhibition of lysis is shown in Table 2. A definite reduction of the hemolytic activity of phenanthroline is apparent, indicating that the ability to bind metals may be essential for its hemolytic function.

As phenanthroline-induced lysis of C'8 cells appeared to be dependent upon the chelating activity of the compound, it was considered possible that C'9 functions similarly, and, hence, the effect of metal ions on C'9 activity was tested. As shown in Table 2, incubation of C'9 for 15 minutes at 37°C with 1×10^{-4} or $5 \times 10^{-5}M$ Fe^{++} ($FeCl_2$ or $FeSO_4$) led to complete inhibition of activity. None of the other ions tested exhibited a comparable effect. In particular, Fe^{+++} was virtually ineffective. Dialysis of C'9 treated with Fe^{++} did not restore its activity.

Two other ion-binding substances, desferrioxamine (*11*) (0.01*M*) and iron-free transferrin (*12*) (1mg/ml), were found to lack the ability to induce lysis of cells containing C'8 sites. These two substances are known to bind iron only in its trivalent form (*13*), whereas phenanthroline is capable of binding iron only in its bivalent form (*14*). Since C'9 could be inhibited by Fe^{++}, but not by Fe^{+++}, it appears that C'9 shares with phenanthroline an affinity for Fe^{++} and a lack of reactivity with Fe^{+++}. It may be postulated that the capacity of C'9 and of phenanthroline to lyse C'8 cells is due to their reactivity with Fe^{++}. Work is needed to determine whether activation of C'8 sites by C'9 is brought about by withdrawal of Fe^{++}.

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Tribolium castaneum: Morphology of "Aureate" Revealed by the Scanning Electron Microscope

Abstract. *Counts of setae in "aureate"* (au), *spontaneous autosomal recessive mutation of good penetrance and viability, show that the* au *gene causes a threefold increase in setation over the normal in the visible abdominal sternites but not in the membranous wings of the tenebrionid flour beetle* Tribolium castaneum. *Micrographs taken with the scanning electron microscope demonstrate that the* au *gene increases setation throughout the body.*

All living stages (eggs, larvae, pupae, and adults) of *Tribolium confusum* can survive being placed in the chamber of the scanning electron microscope (*1*) and exposed to a pressure of about 10^{-4} torr and to an electron beam (whose current and electron energy are, respectively, 2×10^{-11} to 2×10^{-10} amp and 25 kev) for as long as half an hour (*2*). In the scanning electron microscope, the radiation leaving the specimen is not focused in the optical sense, and therefore a great increase of usable radiation is possible.

Secondary electrons, visible light, characteristic x-rays, or specimen current could theoretically be used to build up the image, and the information carried by these various radiations can be resolved at dimensions comparable to those used in electron optical design. The scanning electron microscope separates localization from information content, and we report further uses of this instrument.

We exposed the flour beetles to low pressure (10^{-4} torr) in the chamber of the scanning electron microscope for a few minutes to anesthetize them. One beetle was then selected and mounted in an aluminum "boat" attached to a specimen holder and reintroduced in the scanning electron microscope. Beetles from two strains were used: (i) an unselected wild-type strain derived from Texas, kept in the laboratory by mass matings since 1958, and (ii) a mutant of spontaneous occurrence designated "aureate" (*au*).

This mutant is autosomally recessive, having good penetrance and viability; it is characterized by an increase in the number of setae over the whole body, with the result

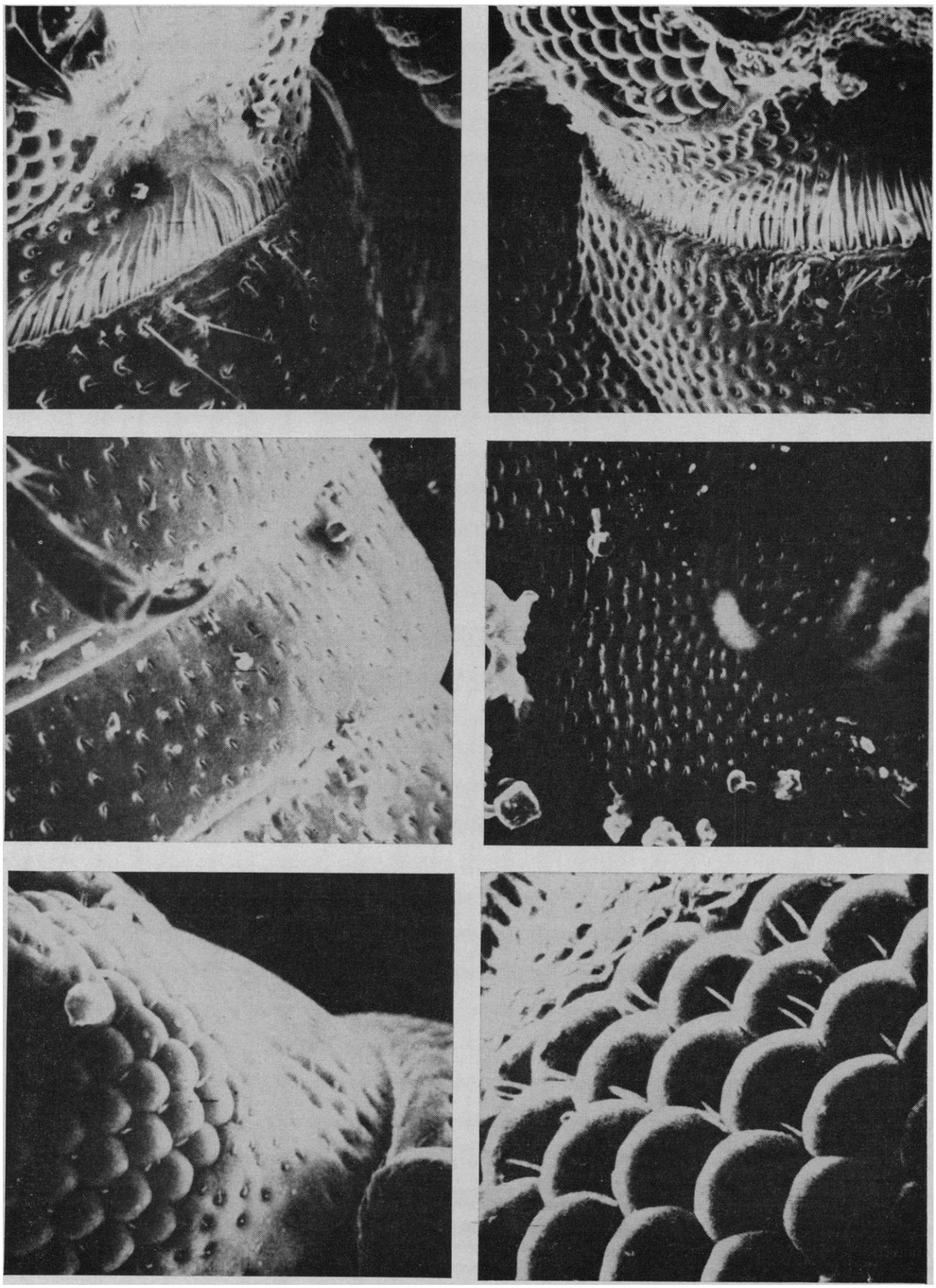

Fig. 1. Micrographs of *Tribolium castaneum*; normal (left) and aureate (right).

that the beetle acquires a golden appearance (which is absent in strains that are not *au*) that is evident even in the presence of body-color genes such as *sooty* (*3*).

In order to assess the effect of the *au* gene, counts of setae were carried out on two parts of the body of ten beetles derived from the *au* and ten from the normal Texas stock, namely, the central portion of each visible abdominal sternite and the distal portion of amputated and mounted membranous wings which is free of veins. In the sternites, the setae were counted within a single area 0.01 mm^2 in the middle of the sternite, with the aid of a reticle in a 15× ocular in combination with a 10× objective. In the membranous wings the setae within five areas of each wing selected at random, each 0.02 mm^2, were recorded with a 15× ocular and 44× objective.

In the sternites of the normal beetles the mean setal number in a sample of ten specimens varied from 9.4 ± 0.2 to 10.7 ± 0.4 setae. In *au* these values ranged from 23.7 ± 2.1 to 30.6 ± 1.4 setae, or approximately two to three times as many as in the normal beetles. In the membranous wings the mean number of setae in the sample of 50 measurements in each strain was 10.84 ± 2.36 for the normal and 11.10 ± 1.66 for the mutant. The difference in the two means is not statistically significant ($P > .4$).

The micrographs obtained with the scanning electron microscope are shown in Fig. 1. At the top, on the left, is shown part of the head and the prothorax of the normal beetle, and on the right that of the *au* mutant. Clearly, the number of pits and associated bristles are greatly increased in these two parts of the body. The cervical bristles on the anterior margin of the prothorax are also greatly increased in number.

The micrographs of the abdominal sternites (in the middle of Fig. 1) show to what extent the number of pits and bristles is increased in the mutant. Finally, on the bottom of the figure are two micrographs which contrast the compound eye of the normal and the mutant. In the normal beetle (left) there are only single bristles between the ommatidia, while in the mutant (right) the interommatidial bristles are often doubled.

The cytogenetic basis of most mutants other than those from *Drosophila melanogaster* is not known. In *Drosophila* the sex-linked dominant Hairy-wing (*Hw*) increases the number of hairs present in the normal wing. For example, in *Hw*/+ females the number is increased by about 17 hairs, in *Hw*/*Hw* by about 21 and, combined with a duplication (*Dp*), in *Hw*/*Hw*/*Dp* by about 33 extra hairs. The increase in hair number was less marked in males.

Cytological examination and genetic data revealed that the increase in hair number resulted from a duplication. The duplication essentially doubled the number of extra hairs on the wing (*4*). The autosomal recessive "hairy," which increases the numbers of hairs on the wings and other parts of the body, interacted with *Hw* to increase the number of hairs on the wings even further (*5*).

In *Tribolium* the cytogenetic basis of the aureate mutation has not been investigated because, even with the most powerful compound microscope, the chromosomes are too small to detect chromosomal aberrations such as duplications or deletions. As techniques are developed in conjunction with the scanning electron microscope, it should be possible to examine cytological material and determine whether chromosomal aberrations (such as duplications in *Drosophila*) are responsible for the modification of the phenotype of *Tribolium*.

Be that as it may, our data indicate that the aureate mutation in its effect appears to be unique so far, not only for the genus *Tribolium* but for the order Coleoptera (*6*). Furthermore, because the scanning electron microscope gives micrographs of high resolving power even at high magnifications, it has been possible to obtain a detailed record of the phenotype of normal and mutant to a degree not previously attainable.

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Chromosome Studies on Marshall Islanders Exposed to Fallout Radiation

Abstract. *Cytogenetic studies of blood lymphocytes of Marshall Islanders, 10 years after their exposure to radiation from fallout in 1954, show chromosome-type aberrations in 23 of 43 exposed persons. Half the aberrations are of the exchange type. An unexpectedly large number of acentric fragments, but no exchange-type aberrations, appear in a few unexposed people on the same island.*

Chromosome aberrations in blood lymphocytes have been demonstrated in several population groups exposed to ionizing radiation, including patients during and after radiotherapy for ankylosing spondylitis (*1*) or malignant tumors (*2*), persons exposed during diagnostic procedures (*3*), and others exposed in the course of their work (*4*). Similar findings have been reported from individuals involved in radiation accidents (*5*) and in survivors of the atomic bombings of Hiroshima and Nagasaki in 1945 (*6–8*). One of the more interesting and possibly more significant points in all these studies was the observation that chromosome aberrations can persist in circulating lymphocytes for many years after the exposure. It seemed of interest to determine whether residual damage of this type also occurs in Marshall Islanders