Reports and Letters

Mass Screening for Disease-Resistant Mutants in Oats

The development of methods for screening large populations of microorganisms for a few spontaneous or induced mutants has led to attempts to obtain useful mutations from higher plants by similar means. Success in such attempts can be expected only if very large numbers, millions or billions, of individuals can be handled in a reasonable length of time. This requires a screening agent that will eliminate from the population all but a few desired mutant types. In screening for disease resistance, it is also desirable that the selection pressure exerted by the screening agent be adjustable to allow selection of moderately resistant, as well as highly resistant or immune, individuals.

In the work reported here (*1*), a toxin produced by cultures of the fungus *Helminthosporium victoriae* Meehan and Murphy was used to screen oats for mutants resistant to the disease caused by this fungus. The disease, known as *Helminthosporium* blight, has caused severe damage to oat varieties possessing the "Victoria type" of resistance to crown rust and has been responsible for the replacement of these varieties with varieties resistant to *H. victoriae* (*2*). This has not provided an entirely satisfactory solution to the problem, because the latter are more susceptible to certain races of crown rust than are the Victoria derivatives that they replaced. Attempts to combine the Victoria type of resistance to crown rust with resistance to *H. victoriae* by conventional breeding methods have indicated that the two characters may be completely linked (*2*). It therefore appeared worth while to attempt to combine the two characters by screening oat varieties susceptible to *H. victoriae* for resistant mutants.

Fig. 1. An oat seedling resistant to *Helminthosporium* blight obtained by screening a susceptible population with a toxic agent produced by the pathogen, *H. victoriae*.

The toxin produced by *H. victoriae* and used as a screening agent in this study has been shown to cause the same disease symptoms and to possess the same host specificity as the pathogen itself (*2, 3*). This toxin is more than 100,000 times more toxic to oat varieties that are susceptible to *Helminthosporium* blight than it is to varieties resistant to this disease (*3*). Details of methods for the production and bioassay of this toxin are given in another report (*3*).

Certified seed oats of two varieties, Victorgrain 48-93 and Fulgrain, both of which are susceptible to *Helminthosporium* blight, were screened in 12-bu lots. In the screening process, the oats were soaked for 30 minutes in tap water and then spread out in ordinary wooden flats to a depth of about ½ inch. The flats (approximately 200 were required for each 12-bu lot of oats) were then placed in a large stack and the latter covered with burlap, which was kept moist by spraying several times each day with water. After 2 days at a temperature of 27°C the stack was opened, and the oats in each flat were drenched with a solution containing 10 units/ml of *H. victoriae* toxin (*3*). By this time the oats had germinated and developed roots 5 to 10 mm in length. After treatment with the toxin, the flats were restacked, maintained at 27°C for 2 more days, and then removed and examined for the presence of seedlings that had survived the treatment.

The survivors, which numbered approximately 50 per bushel, were separated into two categories; those completely unaffected by the toxin (see Fig. 1) were classified "resistant," whereas those showing some degree of injury or stunting were classified as "doubtful." All the surviving seedlings were heavily inoculated with spores and mycelium of *H. victoriae* and were planted in pots of soil. Counts made 30 days later showed that 92 percent of the plants in the resistant category were alive, whereas only 8 percent of those classified as doubtful survived inoculation with the pathogen.

A total of 100 bu (approximately 45 million grains) of oats was screened; from these, 973 seedlings were obtained that survived both treatment with the toxin and inoculation with *H. victoriae*. These seedlings were transferred to a rust nursery, inoculated with race 45 of the crown rust organism, and exposed to natural infection by other races of rust prevalent in the Baton Rouge area. Nearly half (471) of the plants were highly susceptible to rust and these were discarded. Seed from the remaining plants, which gave rust reactions ranging from moderately susceptible to highly resistant, were retained for further testing.

The entire experiment, including production of the toxin, screening, transplanting, and testing for rust reaction, required a total of slightly more than 800 man-hours. Since no expensive facilities or equipment are needed, it is apparent that tremendous numbers of individuals can be processed quickly and inexpensively by this method. The process, therefore, provides not only a promising means for obtaining disease-resistant plants but also a tool that should be useful in quantitative studies of spontaneous or induced mutation at specific loci in oats.

H. E. Wheeler
H. H. Luke*
Department of Botany, Louisiana State University, Baton Rouge

References and Notes

1. This work was performed under contract No. AT-(40-1)-1731 for the U.S. Atomic Energy Commission. The technical assistance of C. H. Driver and K. A. West is gratefully acknowledged.
2. S. C. Litzenberger, *Phytopathology* **39**, 300 (1949).
3. H. H. Luke and H. E. Wheeler, *Phytopathology*, 45, 453 (1955).
* Present address: Agricultural Research Service, U.S. Department of Agriculture, University of Florida, Gainesville.

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Salt Effect on I^{131} Metabolism in the Salamander

Other than metamorphosis, the role of the thyroid gland in the physiology of the adult amphibian is relatively obscure. Little evidence exists suggesting that osmotic changes in the swimming media may influence the thyroidal uptake of radioiodine by the fresh water amphibian. This report summarizes a preliminary study that was designed to explore the influence of sodium chloride changes in the swimming media on the amphibian metabolism of iodine (*1*).

Adult salamanders (*Triturus viridescens*) of a mean weight of approximately 3.0 g were used. All animals were obtained from a breeding pond in north-

Table 1. Twenty-four-hour distribution of I^{131} in *Triturus* exposed to different salinities of swimming media for 5 days. All groups composed of 20 animals; all figures are percentages.

NaCl added	Activity* retained in whole body 24 hr	Activity† per 100 mg of tail	Activity in total liver	Non-thyroid‡ activity in jaw	Thyroid§ activity in jaw	Thyroid‖ activity in jaw per gram of body wt.
0	80.9	0.94	0.09	1.09	5.40	1.81
0.2	77.9	1.52	0.06	2.44	4.82	1.63
0.4	72.2	1.59	0.25	2.63	4.66	1.62
0.6	66.8	2.06	0.33	3.25	4.36	1.34
0.8	55.1	2.29	0.53	5.19	1.99	0.59

* Whole body I^{131} activity 24 hours following I^{131} administration (less decay) times 100, divided by whole body I^{131} activity (zero time) immediately after I^{131} administration.
† Assayed I^{131} activity in tail portion expressed per 100 mg of tail tissue.
‡ Calculated I^{131} activity of total weight of jaw expressed as I^{131} activity of an equal weight of tail tissue.
§ Assayed I^{131} activity of total jaw less nonthyroid activity in jaw.
‖ Thyroid activity in jaw divided by grams of total body weight.

Table 2. Influence of prior conditioning on the response of *Triturus* to different salinities of swimming media. All groups composed of 20 animals; all figures are percentages.

NaCl added	Activity* retained in whole body 24 hr	Activity† per 100 mg of tail	Activity in total liver	Non-thyroid‡ activity in jaw	Thyroid§ activity in jaw	Thyroid‖ activity in jaw per gram of body wt.
0	82.0	2.00	0.13	2.96	5.21	1.48
0.8	66.2	4.20	0.61	6.70	1.08	0.57

* Whole body I^{131} activity 24 hours following I^{131} administration (less decay) times 100, divided by whole body I^{131} activity (zero time) immediately after I^{131} administration.
† Assayed I^{131} activity in tail portion expressed per 100 mg of tail tissue.
‡ Calculated I^{131} activity of total weight of jaw expressed as I^{131} activity of an equal weight of tail tissue.
§ Assayed I^{131} activity of total jaw less nonthyroid activity in jaw.
‖ Thyroid activity in jaw divided by grams of total body weight.

western Pennsylvania and were maintained prior to use under identical conditions in fresh lake water. All animals were collected within a 2-week period and were used no later than 2 weeks after collection.

Procedures were similar for all groups. Before adjustment of the salinity of the swimming media, all animals were conditioned to filtered, demineralized lake water for 24 hours (less than 5 ppm as NaCl). Individual animals were then placed in adjusted solutions for periods of time as described in a subsequent paragraph. Twenty-four hours prior to sacrifice all animals were injected intraperitoneally with approximately 10 μc of I^{131} in 0.1 ml of 0.7-percent saline. Immediately after injection, each animal was rinsed in distilled water and assayed for total I^{131} (whole body content) by scintillation counting; this procedure was repeated 24 hours later just prior to sacrifice. After correction for radioactive decay, differences between whole body assays of I^{131} were attributed to excretory loss.

Table 3. Response of *Triturus* to 0.8-percent sodium chloride swimming media for different lengths of time. All groups composed of 20 animals.

Time in 0.8% saline (day)	Thyroid* activity in jaw per gram of body wt. (%)	Activity† retained in whole body (%)
1	0.41	71.1
3	0.52	66.2
5	0.59	55.1

* Assayed I^{131} activity of total jaw less nonthyroid activity in jaw.
† Thyroid activity in jaw divided by grams of total body weight.

At sacrifice, each animal was anesthetized with ether and weighed; the lower jaw, total liver, and a portion of the tail were weighed on a torsion balance and assayed for I^{131} by scintillation counting. The excess of the I^{131} jaw activity over the tail aliquot activity per unit weight was attributed to I^{131} accumulation in the thyroid. This activity was expressed as a percentage of the total activity administered.

1) Groups of 20 salamanders were maintained individually in 100 ml of demineralized lake water containing added NaCl (0, 0.2, 0.4, 0.6, or 0.8 percent) for 5 days prior to sacrifice (Table 1).

2) To determine the prior conditioning effect of added salt, two additional groups of 20 salamanders were maintained for a 3-day interval in 0-percent saline and 0.8-percent saline, after which the swimming media were reversed: those in 0 percent saline were placed in 0.8-percent saline, and those previously in 0.8-percent saline were transferred to 0-percent saline solutions. These animals were sacrificed 3 days after the change in their swimming media by the procedure we have outlined (Table 2).

3) Three other groups of 20 animals were sacrificed after 1-, 3-, and 5-day exposures to 0.8-percent NaCl added to the lake-water swimming media (Table 3).

On the basis of the data presented here, it appeared that whole body retention of radioiodine decreased with increased salinity of the swimming media. In addition, I^{131} activity measured in the thyroid in lower jaw by the methods described also decreased with increased salinity of the swimming media and paralleled the decreased retention of I^{131} in the whole animal. Iodine-131 levels of liver and tail tissue increased with increased salinity.

Prior exposure to different saline media did not prevent this response (Table 2). However, animals exposed to 0.8-percent saline swimming media for 1 to 5 days exhibited progressively decreasing amounts of I^{131} retained in the whole body, while the I^{131} thyroid activity tended to recover somewhat during this time (Table 3).

It was concluded that osmotic changes in the swimming media are capable of altering the metabolism of I^{131} in the adult amphibian *Triturus*. Such changes, ecologically, may play a role in seasonal variations of amphibian species.

JOSEPH B. BOATMAN
JOSEPH H. SUNDER
Addison H. Gibson Laboratory, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

Note

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Source and Origin of Magnetite at Scott Mine, Sterling Lake, New York

In many metamorphic regions of the eastern United States the rocks contain local concentrations of magnetite. These magnetite bodies are today commonly considered to have been formed by solutions from magmatic sources. The associated host rocks are interpreted to be intrusive igneous bodies, and the ores are believed to be genetically related to these