

two kinds of variation may well be subjected to a critical test. If there are taken at least two independent observations on each individual, the method of analysis of variance¹ furnishes a convenient mode of testing the relation. Two examples are presented below.

Stark² investigated the relation between hardness of apple varieties and the percentage of unfrozen water in shoots of one season's growth. The data were secured from fifteen varieties during each of eleven months. In each month the shoots from one variety were mixed and subjected to a temperature of -20°C . From the composite sample two determinations of unfrozen water were made by the heat-of-fusion method. How much of the observed variation may be attributed to the heat-of-fusion technique of measurement, and how much to the biological differences in the samples? The analysis of variance is as given in Table 1.

TABLE 1

Source of variation	Degrees of freedom	Mean square
Within composite samples	165	70
Between means of months	10	40,463
Between means of varieties	14	1,429
Month-variety interactions	140	203

It is assumed that the mean square for interactions is a valid estimate of experimental error.³ If so, it may be further assumed that this mean square is the result of the addition of two estimates of variance: (i) that due to errors of measurement by the heat-of-fusion method, designated by V_M , and (ii) that associated with the biological variation in percentage of unfrozen water, V_B . The value of V_M is given directly by the mean square "within composite samples"; that is, $V_M = 70$. Hence,

$$2 V_B + 70 = 203,$$

and therefore, $V_B = 66$. The conclusion is that the errors of measurement and the biological sources of variation in this experiment are almost equally represented in experimental error. So long as the former are so great, any increase of precision may well be

¹ R. A. Fisher, "Statistical Methods for Research Workers," Oliver and Boyd, Edinburgh, 1932; George W. Snedecor, "Calculation and Interpretation of Analysis of Variance and Covariance," Collegiate Press, Inc., Ames, 1934.

² Arvil L. Stark, "Unfrozen Water in Apple Shoots as Related to their Winter Hardiness," a thesis submitted for the degree of doctor of philosophy, Iowa State College, 1934.

³ George W. Snedecor, *loc. cit.*, pages 45-47.

sought in the improvement of the technique of measurement as well as in replication of the composite samples.

Smith and Brown⁴ measured the percentage of carbon dioxide in the soil air at three positions in each of six 14 by 56 foot experimental plots on Carlington loam. At each position determinations were made in triplicate. The analysis of variance is shown in Table 2.

TABLE 2

Source of variation	Degrees of freedom	Mean square
Within positions	36	3.68
Between plot means	5	760.15
Between positions within plots.....	12	63.11

It may be assumed that the mean square "between positions within plots" is the sum of variances attributable to (i) errors in measuring the percentage of carbon dioxide in the soil air at each determination, $V_M = 3.68$; and (ii) variation in the concentration of carbon dioxide from one position to another in the plot, V_B . Then,

$$3 V_B + 3.68 = 63.11,$$

from which, $V_B = 19.81$. This indicates a fairly satisfactory method of measurement. Increased precision in experiments of this kind lies in replication of the plots, accompanied by such experimental designs as will enable the investigator to separate from experimental error the natural variation among the plots.

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POLYEMBRYONY IN THE DOMESTIC FOWL

COMPLETE monovular twins and even triplets are occasionally found among chick embryos. Incomplete posterior or anterior duplications are more frequent. Quantitative data on the incidence of duplication appear to be lacking.

During the years 1929-1933, inclusive, a large number of incubated eggs of known history have been broken incident to hatchability studies at the U. S. Animal Husbandry Experiment Farm, Beltsville, Md. Eggs containing embryos which had died during the first week of development were broken on the seventh day of incubation. Those dying later and the hatched chicks were examined on the fourteenth day or at the close of the incubation period. All embryos which

⁴ F. B. Smith and P. E. Brown, "The Concentration of Carbon Dioxide in the Soil Air under Various Crops and in Fallow Soils," *Iowa State College Journal of Science*, 8: 1-16, 1933.

had lived to the fourth day of incubation or longer were examined for possible duplication.

Two sets of complete, monovular twins, 27 anterior duplications and 63 posterior duplications were found among 64,716 Single Comb Rhode Island Red embryos and chicks examined. Among 57,646 embryos and chicks from various other breeds and crosses, there were one set of complete monovular twins, 12 anterior and 31 posterior duplications. Thus there was a total of 92, or 0.142 per cent. of duplication among Single Comb Rhode Island Reds but only 44, or 0.076 per cent. among the others. The probability that the difference between these two groups is due to chance is less than one in a hundred as judged by the X^2 test. Further, the incidence of duplication among the Rhode Island Reds was greater than that for the mixed group in each of the five years during which data were collected.

Stockard¹ attributed polyembryony in birds to interruption of development before the completion of gastrulation. Riddle² sought to test Stockard's hypothesis by retarding the development of prematurely laid (4 to 24 hours) eggs of pigeons and doves but was unable to produce duplications. The difference in incidence of duplication between the two groups of data presented in the present communication may indicate the presence of inherited factors influencing duplication among chick embryos. Obviously, the presence of such factors has not been demonstrated.

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SOME NEW RECORDS OF OCCURRENCE OF NORTH AMERICAN FRESH- WATER SPONGES

So little is known of the distribution of fresh-water sponges on our western coast that it seems wise to make available all findings as a matter of record.

Through the kind cooperation of Dr. L. E. Griffin, of Reed College, Portland, Oregon, we have been enabled to examine two specimens from his collections. The first is a specimen of *Spongilla fragilis*, taken from a flume leading from a pond on the college campus. It was collected in October, 1929. The sponge is full of very abnormal spicules. The skeletal ones are smooth amphioxi, many of them bearing ball-like enlargements in the centers; other types of irregularities are also numerous. The gemmule spicules are extremely variable in size and are also often quite abnormal in structure, enlarged in the center or provided with angular projections. There are large numbers of small balls of silica, several

times the diameter of the skeleton spicules, scattered through the sponge; some of them are regular smooth spheres, while others are distorted in shape and bear spines projecting at right angles to their surfaces—the spines vary a great deal in size and in number from one or two to many. The second specimen is *Spongilla lacustris*, collected by M. R. Clare in August, 1928, from Mud Lake, west of Bend, Oregon, in the Cascade Mountains, at an elevation of about 4,500 feet. The specimen bore no gemmules.

Dr. Trevor Kincaid has kindly given me bits of three specimens from his collection. Two specimens were collected from Lake Ozette in the extreme north-west corner of the state of Washington on May 29, 1932. One of these is *Spongilla lacustris*, with thin skeleton spicules, and the other is *Spongilla fragilis*, with very variable gemmule spicules. The third specimen from Dr. Kincaid was taken from the interior of a wooden pipe on the shore of Lake Washington, not far from the University of Washington, where Dr. Kincaid is head of the department of zoology. This specimen unfortunately does not bear any gemmules and can not be identified.

Dr. Jacques Rousseau and Dr. F. M. Victorin, of Quebec, Canada, have kindly sent me three more specimens of Canadian fresh-water sponges from new localities. One is a specimen of *Spongilla lacustris* from "Lac Jaune, near Quebec City, Province of Quebec, August 5th, 1931: collected by Br. Anselme." A second is also a *Spongilla lacustris*, collected by Dr. Victorin in "St. Theodore Co., Joliette, in Laurentides, North of Quebec, September 5th, 1931"; this sponge has somewhat heavier skeleton spicules than those of the first specimen. The third specimen is *Spongilla fragilis*, collected by Dr. F. M. Victorin and Jules Brunel in "Canal de Chambly, Conté Chambly, Province de Québec."

The writer has also collected some very small, young sponges from a lake in Seattle, Washington, in the fall of 1933, but since the skeleton spicules are regular smooth amphioxi and no gemmules are present, the sponge can not be finally identified. In the fall of 1932, the writer also collected *Spongilla fragilis* in very great abundance from a pond near Spring Valley, N. Y., where it was covering the stems of plants and the wall of a stone-lined runway with a thin coat over large areas. The pond had been drained and the sponge was very full of gemmules.

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SWARMING BEETLES

On July 15, 1934, on the summit of Mount Pisgah, in North Carolina, I observed a swarming of Coc-

¹ C. R. Stockard, *Am. Jour. Anat.*, 28: 115-277, 1921.

² O. Riddle, *Am. Jour. Anat.*, 32: 199-252, 1923.