

samples of the  $\beta$ -glucosides of coumarinic acid and *o*-coumaric acid, respectively. The action of a preparation of  $\beta$ -glucosidase from almonds (Mann Research Laboratories) on the bound compounds furnished further evidence that the compounds were  $\beta$ -glucosides. Both bound compounds were hydrolyzed by the almond preparation; and, in agreement with reports of other workers (3, 5), the *trans* compound was hydrolyzed more rapidly than the *cis* isomer.

An active  $\beta$ -glucosidase was detected in extracts of tonka bean leaves and cotyledons. Unlike the almond  $\beta$ -glucosidase, however, the tonka bean enzyme rapidly hydrolyzed coumarinyl glucoside and was essentially inert toward *o*-coumarinyl glucoside. In its specificity for the *cis* glucoside, the tonka bean enzyme resembled sweetclover  $\beta$ -glucosidase (3).

The occurrence of the  $\beta$ -glucosides of coumarinic acid and *o*-coumaric acid and the presence of a glucosidase specific for the *cis* glucoside indicate that in the tonka bean, as in sweetclover (3), coumarin is synthesized through the conversion of *o*-coumarinyl glucoside to coumarinyl glucoside which under suitable conditions may be hydrolyzed to yield coumarinic acid. Spontaneous lactonization of coumarinic acid then produces coumarin (11).

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#### "Imprinting" in Nature

**Abstract.** *Much laboratory research is based on the assumption that the learning of parental characteristics by young nidifugous birds is rooted on visual factors. Naturalistic observations of ground- and hole-nesting duck species, augmented by sound amplification equipment, indicate that hatchlings are exposed to the call of their mother for a relatively long period before they are exposed to the sight of her.*

Considering the fact that ethologists have (almost?) returned the naturalistic observation of behavior to its former place of respectability, it was surprising for me to learn that Lorenz's formulation of imprinting was not based on comprehensive natural observation (1). Rather, he appears to have relied almost solely on his own and O. Heinroth's previous observations concerning the following-response of ducklings and goslings hatched in incubators. (Such birds tend to follow humans or the first relatively large moving objects which they see shortly after hatching.) Relying on these and other non-naturalistic observations, Lorenz delineated what he believed to be the major characteristics of imprinting, and it is indeed a tribute to his genius that much of what he described or inferred has been subsequently supported by laboratory research.

With an interest in determining the sensory environment which young precocial (nidifugous) birds experience upon hatching, the length of stay in the nest, and the hen's behavior in enticing the young to leave the nest, I embarked several years ago on naturalistic studies of hole-nesting and ground-nesting duck species. At the time I viewed this venture from the standpoint of only furthering my personal knowledge, but some of the results of these studies seem important enough to warrant public mention.

Since the time of Audobon, naturalists have recurrently observed the dramatic exodus of hole-nesting wood ducklings (*Aix sponsa*) from dark cavities located in trees some 20 to 60 feet above the ground (or water). In response to the hen's call, the ducklings ascend the vertical surface of the cavity, pause momentarily in the exit leading to the outer world, and then leap into space with nonfunctional wings aflutter, thereby joining their parent on the ground or water below.

Naturalists are accustomed to viewing avian behavior in terms of innate or instinctive predispositions, and it has been tacitly assumed that wood ducklings are innately responsive to the exodus call of the wood duck hen (2)—that is, the ducklings' responsiveness to the hen's call has been traditionally viewed as occurring relatively independent of previous experience, being determined by a largely preformed, inherited sensorimotor linkage. Though I personally do not understand this point of view any better than I understand the view that behavior is almost solely a function of experience or learning, I was still surprised by what we found concerning the sensory environment of wood ducklings during and after hatching. In the three instances where we were able to institute sound recording while the eggs were in the early pipping stage, we found the hen uttering a low intensity call ("kuk, kuk, kuk . . .") which she continued with rising amplitude and rate up to and including the exodus some 20 to 36 hours later. Thus there was ample opportunity for the ducklings to associate the hen's call with her presence for a prolonged period prior to the exodus. These observations allow the wood ducklings' responsiveness to the hen's call to be placed, however vaguely, in the category of associative learning. The proximity of the familiar stimulative source (the hen) presumably acts as the rewarding aspect for the ducklings (3).

The fact that auditory stimulation seems to play such an important role in at least the initial stages of "imprinting" in hole-nesting ducklings suggested that we ought to undertake comparative observation of a ground-nesting species. By virtue of differences in nest-site ecology, it has been suggested that visual factors may be more primary than auditory factors in the imprinting of ground-nesting ducklings. Though my wife and I have been able to monitor only one nest of a ground-nesting species (*Anas platyrhynchos*) thus far, I am inclined to mention our findings because they parallel those made by Robert I. Smith on one nest of another ground-nesting species (*Anas acuta*) (4). In both instances, while perched on the nest, the hens vocalized during and after the hatching of the young. In the case of *Anas platyrhynchos* the rate and intensity of the call gradually increased up to and including the exodus. Smith's recording

terminated before the exodus, so we do not know whether the pintail hen used the call in enticing her young to leave the nest. The fact that the Colliases heard ground-nesting hens (*Aythya valisineria* and *Anas discors*) vocalize as they led their young from the nest (5), leads me to believe that in nature auditory stimulation is a typical component of the stimulative complex which at the very least *initiates* imprinting in ground-nesting as well as in hole-nesting species of ducklings (6).

Elsewhere I have put forth the notion that in ground-nesting species auditory stimulation may serve an attention-directing or activating function, while in hole-nesting species such stimulation not only serves an activating role but also plays a major role in the ducklings' recognition of their parent (7). The main purpose of my present report is to draw attention to the fact that in nature "imprinting" does not appear to be rooted on visual factors, due to the primacy (in time) of auditory factors. Much laboratory research has been based on the premise that the learning of parental characteristics by nidifugous birds is rooted solely or largely on visual factors, and little attention has been given to the role of auditory stimulation in the imprinting process (8). It is a matter of some interest that, at the mammalian level, the departure of young opossums from the den is also initiated by the mother's call (9, 10).

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3. A detailed report of these observations is in press (*J. Comp. Physiol. Psychol.*).
4. These recordings were made during the late evening and night by Smith at the Delta Waterfowl Research Station, Manitoba, Canada, in 1958. I was present for only an insignificant period of the vigil and I appreciate Dr. Smith's kindness in allowing me to copy and analyze portions of the original tapes.
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6. The hens' vocalizations are typically inaudible to the unaided human ear. In addition, as originally pointed out by the Colliases, movement of the bill or throat is not usually discernible as the hens utter their call. Sometimes a slight upward movement of the tail and swelling of the breast accompany the call.
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10. The naturalistic studies were supported by the Chapman Fund of the American Museum of Natural History, the North Carolina Hospitals Board of Control, and research grant M-6039 from the National Institutes of Health. Objective naturalistic observations are difficult and tedious to arrange, record, and analyze. I am grateful to the following persons for help at one stage or another: Dr. J. R. Hester, Dr. Eugene Hester, Jack Dermid, Nora Gottlieb, Frank Lane, Carter Doran, Linda Green, John Whalen.

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### Heritability of Variations in Oil Content of Individual Corn Kernels

**Abstract.** Nuclear magnetic resonance spectroscopy was used to determine oil content of individual corn kernels in order to evaluate this technique as an aid in the development of strains having greater oil content. This method is rapid and does not impair viability. Individual kernels from a selfed single-cross ear ranged from 2.7 to 5.4 percent oil and were significantly correlated ( $r = +0.75$ ) with the oil content of their progeny ears. This indicates that the single-kernel differences in oil content were heritable, and this method may greatly increase selection efficiency in breeding for higher oil content in corn.

Corn oil, an important food fat relatively rich in linoleic acid, has been in strong demand in recent years, but the supply of corn oil has been limited to the demand for products of the corn milling industries (1). The most effective method of increasing the supply of corn oil is through breeding for corn of higher oil content. Sprague *et al.* (2) demonstrated the effectiveness of the recurrent selection procedure for oil improvement in corn by increasing mean oil content of a synthetic variety from 4.7 to 7.0 percent in two selection cycles. However, the selection was perforce based on extraction analytical values obtained on a ground aliquot of composite samples of ears. Such time-consuming procedures limit the scope of breeding work and force the breeder to base his selection decisions on population averages rather than on individual differences. Alexander (3) recently proposed that development of a nondestructive method of measuring oil content of individual corn kernels would provide optimum selection pressure in the recurrent selection procedure.

Conway and Smith (4) recently demonstrated that wide-line nuclear

magnetic resonance (NMR) spectroscopy provides a rapid, accurate, and nondestructive method for determining fat in multiple or single-kernel samples of corn dried to below 5 percent moisture. The basic instrument is a Schlumberger model 104 NMR analyzer and model 104-3 integrator. To facilitate single-kernel analysis, instrument sensitivity was improved by installing a 2-ml "high-gain" radio-frequency coil in place of the standard 40-ml coil and by using a specially fabricated sample cell so that each kernel was positioned at the optimum location in the magnetic field. All kernels analyzed were dried uniformly to 5 percent moisture to suppress the water signal. Samples of corn of known oil content ranging from 3.9 to 8.8 percent oil were used for calibration purposes. Twenty-five individual kernels from each sample were examined with instruments and then were ground collectively and analyzed for fat by a standard solvent extraction procedure (4). The oil content was linearly related to the NMR signal amplitude. Maximum deviations from the calibration line were 0.1 percent fat, absolute. Time required for measurement was 4 minutes for each kernel.

To determine the heritability of single-kernel variations in oil content, analyses were made on 289 kernels from one selfed ear of the single-cross Hy  $\times$  Oh 45, and 189 kernels from one ear of a nearly homozygous inbred designated HO-18. The range in single-kernel values for the single cross was 2.70 to 5.41 percent oil (Fig. 1). The two parents, Hy and Oh 45, of the single

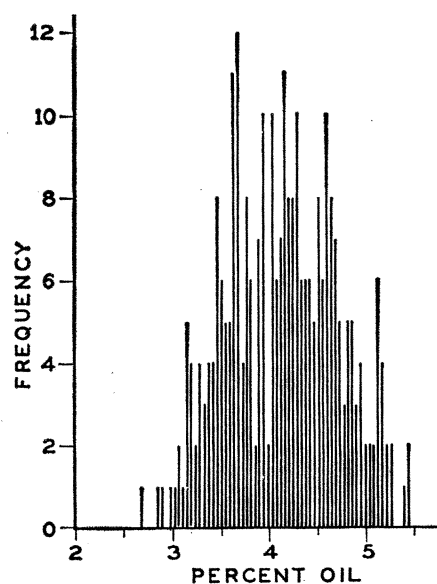


Fig. 1. Frequency distribution of percent oil in single kernels from one selfed ear of the corn single cross, Hy  $\times$  Oh 45.