GENETIC STOCK AND HOUSING ENVIRONMENT EFFECTS ON TONIC IMMOBILITY, AVOIDANCE BEHAVIOR AND QUANTITATIVE TRAITS IN WHITE LEGHORN HENS

by

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THIS IS AS RECEIVED FROM THE CUSTOMER.

Dedicated with gratitudes to God to:

my mother, Mrs. Amila Kujiyat,

my wife, Hope Hassana and

my children, Bobai and Nenadi.

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[1] Philippians 4:13; [2] Romans 8:37; [3] Philippians 4:19; [4] Matthew 25:40.

INTRODUCTION

Fearful behavior has been defined as a "psycho-physiological response to a perceived danger" by Jones and Faure (1981a). Natural selection has presumably shaped such behavior so as to enhance survival of the individual and its "fitness" via increased survival of progeny or relatives. Major categories of behavior for dealing with perceived danger include both escape responses and reduced activity, including total immobility (Salzen, 1963; Gallup, 1974a, 1977). Escape behavior should be appropriate for avoiding threatening situations posed either by other animals or by inanimate environmental events. On the other hand, immobility may serve primarily as a defense against other animals by a reduction of attention-attracting properties or, in the extreme case, by causing immobile individuals to appear lifeless, thereby not stimulating further pursuit or attack (Gallup, 1977; Sargeant and Eberhardt, 1975).

Occasionally, avoidance and escape behavior may lead to injuries, feather damage and reduced reproductive functioning, especially for hens kept in high-density, multiple-hen cages in which escape is impossible (Elmslie et al., 1966; Hansen, 1976; Craig et al., 1983). Tonic immobility (TI), induced by restraint, has been generally regarded in recent years as fear-potentiated (Ratner and Thompson, 1960; Gallup, 1977; Jones and Faure, 1981b). On the basis of this assumption Jones and Faure (1981b) suggested that hens kept in crowded cages are more fearful than hens from uncrowded floor pens because they exhibit prolonged TI.

Evidence is available that ease of induction of TI varies between genetic stocks (Gallup et al., 1974; Gallup, 1974b; Jones et al., 1981), and that duration of TI may be readily altered by selection (Gallup, 1974b). Whether relative susceptibility to and duration of TI is associated with well-being in artificial environments is unclear, as is its possible association with escape behavior.

The primary objectives of the presently reported studies were:

a) to investigate further TI and avoidance behavior in adult, laying White Leghorn hens.

b) to evaluate certain genetic influences on these two fear-response measures, and

c) to determine what associations exist among these measures and between them and body weight, feather cover and egg production traits.

LITERATURE REVIEW

Characterization and Measures of Fearfulness

Although Murphy (1978) found no universally accepted definition of fear, Jones and Faure (1981a) defined it as the "psycho-physiological response to a perceived danger." This perceived danger is the fear-inducing stimulus whose effectiveness varies within species, being influenced by genetic strain, sex, age, previous experience and prevailing conditions (Melzack, 1952; Hebb, 1953; Salzen, 1963).

Attempts to define and rank fear responses based on behavior intuitively regarded as fearful such as freezing, fleeing and vocalizing have met with criticism. Murphy (1978) argued that these approaches do not take into account the biological function of fear, the responses shown in the natural habitat and the effect of the artificial environments on fear characteristics. However, Jones *et al.*, (1981) employing remote observation technique and radio telemetry of heart rate to assess the behavioral and physiological responses of hens exposed to a slowly approaching human being, found little evidence to support Murphy's (1978) contention that the absence of overt fear responses was not always indicative of a low level of fear. They found that in most cases where a dramatic increase in heart rate occurred, an associated behavioral response occurred. Nash et al., (1976) reported that following TI onset, heart rate decreased, reaching its lowest level just prior to termination, while respiration rate increased initially but then gradually decreased during the response. They found that body temperature remained lowered throughout the response period. Ookawa (1972) immobilized (hypnotized) adult White Leghorn hens by both ventral and dorsal restraint procedures and reported of a transitional stage (beginning) characterized by both eyes kept open and an electro-encephalogram (EEG) of slow waves with high amplitude, while the hypnotic or immobilized stage was characterized by an EEG of continuous train of slow waves similar to those of slow EEG sleep. He also found that the mean heart rate during the immobilized period was lower than the means for controls. These observations support Jones et al.'s (1981) suggestion that under carefully controlled conditions, Murphy's (1978) reservations can be discounted as the hen's behavioral response is a useful index of its underlying state of fear.

Methods used to measure fearfulness include the nervousness score (Hansen, 1976) or pencil test (Hughes and Duncan, 1972; Hughes and Black, 1974; Sefton, 1976; Sefton and Crober, 1976). For the pencil test, the experimenter faces the bird and slowly moves the pencil from left to right across the front of the cage. A score of 1-5 (variable due to modification) is assigned depending on whether the bird pecks at the pencil (a score of 1) or shows varying levels of avoidance with a maximum score of 5 if it flees to the rear of the cage. Avoidance or withdrawal from humans, novel objects and foods (Murphy, 1977; Murphy and Duncan, 1977) and the hen's behavioral responses to sound (Phillips and Siegel, 1966; Hatton and Thompson, 1975) have been evaluated. Recently, Craig _et al._, (1983) studied latency until recovery from avoidance or escape behavior of hens resulting from having their cage struck and from exposure to a metronome. The tonic immobility response is reported to be a useful measure of fearful behavior (Gallup, 1973, 1974a, 1977; Jones and Faure, 1981a, 1981b). Other reported fear-associated responses include freezing (Andrew, 1956) crouching (Arnold, 1945), vocalizing and defecation (Gallup _et al._, 1976).

Tonic Immobility Test:

Considering the available methods of measuring fearful behavior in the chicken, Gallup (1979) concluded that TI was the best. Jones and Faure (1981b) defined tonic immobility as a "fear-potentiated response induced by physical restraint and characterized by reduced responsiveness to external stimulation." Other responses noticed during TI included intermittent periods of eye closure, changes in heart and respiratory rates, altered electro-encephalographic patterns and Parkinsonian-like tremors in the extremities (Braud and Ginsburg, 1973; Gallup, 1977). TI has been known to last from a few seconds to several hours, for example, Gallup (1977) reported a maximum duration of 5 hrs. and 45 minutes.

Although interest in TI or "animal hypnosis" dates back to the Old Testament days (Ratner, 1967), published literature only spans more than three centuries. Gilman and Marcuse (1949) and Gilman _et al._ (1950) reported that most authors date its inception as an investigated phenomenon at 1646 in which Kircher is reported to have described how by holding a hen in an uncomfortable position and fixing its head, a state of entrancement could be induced when a chalkline was drawn from its beak outward. Labels that have been used to describe TI such as cataplexy, catalepsy, thanatomimesis, death feigning,, fascination, thanatosis, entrancement, rho, akinesis, paroxysmal inhibition, Tostell reflex, mesmerism, fright paralysis, monoideism, bewitchment, and sham death are evidence of the conceptual confusion of what TI really is (Gallup, 1974a). TI has been observed in insects, crustaceans, fish, amphibians, reptiles, birds, rats, rabbits and possibly primates.

Different methods of induction of TI have been used including stroking the subject's ventral surface, forcing it to fixate its gaze o n a chalk line and placing a hood over its head. For an effective induction, some form of physical restraint is necessary (Gilman and Marcuse, 1949; Ratner, 1967; Gallup, 1977). The critical dimension of the restraint technique hinges on certain kinds of tactile and proprioceptive input (Gallup, 1974a). Under manual restraint, the animal struggles and tries to escape, but having been held for a few seconds, struggling subsides and a "frozen" immobilized posture is assumed which persists in the absense of further restraint. This relative lack of responsiveness to external stimuli is associated with a relative state of profound but reversible physical immobility and muscle hypertonicity (Gallup, 1974a).

Termination of TI is usually abrupt and discrete. Naive subjects commonly make an immediate attempt to escape from the experimenter (Ratner and Thompson, 1960). Eye closure during TI is regarded as predictive of a prolonged duration while vocalization usually indicates an impending termination of response (Gallup et al., 1971; Rovee et al., 1973; Rovee and Kleinman, 1974).

Rovee and Luciano (1973) found that social rearing was a prerequisite for ventral TI induction in 3-day-old chicks from which they proposed three levels of immobility that appear to characterize the behaviors of the young chicks: Stage 1, appears at the outset of immobility and immediately prior to spontaneous termination and is characterized by shrill distress calls with eyes continuously opened. Stage 2, a slightly deeper trance is characterized by suppressed vocal behavior and eye flutters. Stage 3, predictive of prolonged reaction is characterized by closed eyes and absence of vocalization, and occasional twitches. Jones and Faure (1981c) using the dorsal restraint induction of TI on adult hens proposed a two-stage TI reaction: inhibition and alert stages. Inhibition stage is characterized by complete immobility from induction till first alert head movement, while alert stage is when the bird might make several head movements before righting itself. They proposed that the term tonic immobility should strictly apply to the inhibition stage, and that latency till first head movement might be a more sensitive measure of disinhibition, with a concomitant decrease in fearfulness, than righting time. Rovee and Luciano's (1973) proposed 3-stage and Jones and Faure's (1981c) proposed 2-stage analyses of the TI reaction are difficult to compare because of the differences in methods of restraint and ages of test subjects.

Different kinds of substrates have been used either singly or in combination to induce TI. These include induction boxes 35 x 20 x 7.5 cm (Braud and Ginsburg, 1973), 0.61 x 0.61 x 0.5 m (Nash and Gallup, 1976), a table (Gallup et al., 1976; Jones and Faure, 1980), cloth (Braud and Ginsburg, 1973; Jones and Faure, 1980), a level ground (Montevechi, 1978 cited by Jones and Faure, 1981c) and a U-shaped wooden cradle 32 x 21 x 27 cm narrowing to 8 cm at a height of 16 cm (Jones and Faure, 1980).

Induction procedures that have been used include placing the bird on its back (dorsal restraint), on its side (lateral restraint) and in an upright position (ventral restraint). The subject is then manually restrained for 15 s before the experimenter removes his hands (Braud and Ginsburg, 1973; Gallup et al., 1970, 1971, 1976; Jones and Faure, 1980, 1981a, 1981b; Gallup, 1974b, 1977). Induction is said to occur if the bird remains immobilized for at least 10 s after restraint is terminated, otherwise the procedure is repeated until induction is achieved (Jones and Faure, 1980, 1981a).

The parameters that most workers have measured in TI testing include number of 15-s restraint periods required to induce TI, number of head movements, latencies until first gross leg and head movements, and until righting (duration of TI), eye closure, vocalization and defecation.

Theories of Tonic Immobility

Gallup (1974a) reported that at least six discernible, though not necessarily mutually exclusive theories have been advanced to account for the phenomenon of TI. These are: (i) it is viewed as the counterpart of human hypnosis. This view is discounted on the basis of anthropomorphism and differences in the induction procedures. (ii) it is viewed to result from inhibition of cerebral activity originally suggested by Pavlov in 1921 cited by Gallup (1974a). (iii) Sleep, analogized to the immobile state. Both (ii) and (iii) fall short of providing an adequate accounting of available data. Klemm (1971) showed differences between TI and paradoxical sleep. (iv) Death feigning (Darwin, 1900, cited by Gallup, 1974a), which though criticized because of supposed teleological overtones has begun to receive increased attention and empirical support because of his basic notion that it reflects an adaptive reaction to predation. (v) TI occurs because of spatial disorientation caused by inversion and restraint, implying vestibular involvement. This theory has been negated on the basis that subjects in an upright posture are readily immobilized. (vi) The fear hypothesis. This was originated by Preyer in 1878 (cited by Gallup, 1974a). The modern version of this theory is that TI represents an innate fear response which is prompted by aversive environmental events. Data exist to support this, and the response may participate in predator-prey relationships (Gallup, 1974a).

The validity of TI as a fearfulness measure stems from the observation that it is potentiated (prolonged duration) and attenuated (shortened duration) by procedures known to potentiate or attenuate the fearful behavioral response in chickens. Potentiating procedures observed included preinduction shock, loud noise (Gallup et al., 1970; Gallup, Creekmore and Hill, 1970; Gallup, 1973), adrenalin injection (Braud and Ginsburg, 1973), a visual cliff (Gallup and Williamson, 1972) and simulated natural predators (Gallup, 1971; Sargeant and Eberhardt, 1975). Conversely, attenuating conditions included absence of human observer (Gallup et al., 1972; Nash, 1977), taming and familiarization (Gilman et al., 1950), conditioned safety signals (Maser et al., 1973), habituation (Gallup, 1974a; Nash and Gallup, 1976; Nash, 1978) and tranquilizers (Gallup, 1973). The aversive properties of TI induction procedures (Nash and Gallup, 1975) and inverse proportionality of TI to the peck-order position in the chicken (Crawford, 1977) also support the use of TI as a measure of fear.

Substantial genetic effect on TI has been demonstrated following strain comparison (Gallup et al., 1974b; Jones and Faure, 1981c) and selective breeding (Gallup, 1974b). Gallup (1974b) found after only one generation of selection a realized heritability of 0.75-0.90 for TI. Genetic differences were also detected using Hansen's (1976) nervousness score and avoidance responses (Craig et al., 1983) and in fearfulness and head shaking (Mauldin and Siegel, 1979).

Type of environment in which subjects are kept has been shown to affect the nature of the fearful behavior. Presence of an experimenter (Gallup, et al.,1972; Nash, 1977), hooded and unhooded simulated hawks (Gallup et al., 1971) and a pair of mounted artificial eyes (Gallup, Nash and Ellison, 1971) all prolonged the duration of TI while their absence attenuated it. Jones and Faure (1981b) reported that hens in multiple-hen cages (4-5 females/cage) exhibited longer latencies of TI as compared to hens kept in floor pens. From this observation they suggested that caged hens were more fearful than penned hens. Elmslie et al. (1966) and Hansen (1976) showed that population size was a primary cause of the build up of nervousness and hysteria in adult hens. Elmslie et al. (1966) encountered hysteria among hens housed at a density of 14 hens per cage (122 x 38 cm) which they eliminated by dividing the cages into 3 units (of 41 x 38 cm each) and reducing group size to 3 hens per cage. Hansen (1976) did not encounter hysteria among 6-hen groups housed with 387 cm^2 per hen in cages, but 5 of 8 30-hen lots in cages (with a space allowance of 464 cm^2 per hen) became hysterical. Hansen eliminated and prevented hysteria by adding nests and perches to the community cages and by moving flocks to less crowded cages. Jones and Faure (1981b) also observed that pen-reared birds appeared more disturbed as compared to caged ones, which indicated that environmental differences affected fearful behavior.

Salzen (1963) suggested that the occurence of the fearful response in chicks is a function of the difference between the nature of the test situation and the nature and extent of the chick's experience of past situations. He then asserted that fear develops with experience rather than with age. Most fear responses of the chick are not manifested until after the critical period of imprinting. Although Ratner and Thompson (1960) and Salzen (1963) reported that lateral and dorsal TI induced reactions were virtually absent before the 7th day and develop rapidly between the 7th and 10th day, ventral TI induced reactions have been achieved within 12-hr posthatch through 10-days of age (Rovee and Kleinman, 1973; Rovee and Luciano, 1973; Rovee et al., 1973). Possible physiological maturation of lateral and dorsal TI induction and fearfulness is attributed to the delay. Maturation of fearfulness is considered in terms of effects of social experience on development of adrenal secretion and establishment of familiar social environments. Therefore, the effect of previous experience on the observed fear-associated behavioral responses tend to vary. Gallup et al. (1974) reported that repeated elicitation of TI and not just handling was responsible for the reduced duration of the response after multiple exposures to manual restraint. Therefore, habituation becomes a function of the number of stimulus presentations. They detected genetic involvement in the number of trials required to reach the criteria for habituation. They also found that massed trials produced robust sensitization rather than reduced responsiveness. Hughes and Black (1976) found that regular handling from 2 days to 16 weeks of age reduced the avoidance behavior in growers and pullets, but the effects declined with age. Jones and Faure (1981a) reported a decreased TI response (duration) but an increased approach to human beings following regular handling. However, Jones and Faure (1981a) suggested that regular handling does not depress general fearfulness, but specifically reduces the fear of human beings by chickens.

Fearful Behavior, Body weight, Feather Cover and Egg Productivity

Possible associations of measures of fearfulness with feather cover, general body condition and egg production have been reported. Elmslie et al. (1966) and Hansen (1976) found that reduced egg production, feather damage and loss and also injuries were associated with high levels of nervousness and hysteria. Adams et al. (1978) found that birds with the most feather damage were the most fearful. The observed association of feather damage with fearful behavior has been attributed to feather pecking (Hughes and Duncan, 1972). Craig et al. (1983) recently reported that greater nervous and fearful behavior by hens in colony cages (10-14 hen per cage) as measured by Hansen's nervousness score and by latencies to return to feeding or to "normal" activity following avoidance, tended to be significantly associated with greater feather loss and nonsignificantly correlated with earlier sexual maturity and lower egg mass production (on a part-year basis).

Sefton (1976) reported that within matings, fearfulness and egg production as well as fearfulness and livability tended to be negatively related. Because he found no relationship between these factors over matings within cage size or cage tier, a genetic component to relationship between fearfulness and either egg production or livability is indicated. Sefton (1976) also found that hens housed in the top tier of cages layed less and were more fearful than those in a bottom tier. Sefton and Crober (1976) reported that birds housed in larger cages of 516 cm^2 per bird were less fearful than those in the smaller cages of 412 cm^2 per bird. They found that lower fearfulness was associated with higher egg production. Mauldin and Siegel (1979) reported consistently low and non-significant correlations between "fear and production traits". This was consistent with earlier findings in which 1 out of 20 correlations between fear and production traits was significant (Siegel et al., 1978).

MATERIALS AND METHODS

Genetic Stocks:

For Experiment I, the randombred unselected (control) C_1 and C_2 strains were used. For Experiments II-IV, the C_1 and C_2 strains with their reciprocal crosses $C_1 \times C_2$ and $C_2 \times C_1$ as well as the Y_1 and Y_2 strains (most recently described by Craig et al., 1983) with their reciprocal crosses $Y_1 \times Y_2$ and $Y_2 \times Y_1$ were used. The Y_1 and Y_2 strains had been selected for increased part-year egg mass from 30-40 weeks old over 11 generations. All strains were derived from the Kentville White Leghorn Randombred Control population. The Y strains have become differentiated from the unselected (control) C strains, becoming socially dominant to them (Bhagwat and Craig, 1978) and producing greater egg mass (Craig et al., 1982). Behavioral differences have been detected between the replicated strains within the C and Y stocks, presumably due to random genetic drift acting within these relatively small, closed populations.

General Management Procedures

Subjects for Experiment I were hatched on March 28, 1981, while those for Experiments II-IV were hatched on May 9 and 10, 1981. All chicks were wing banded, dubbed (combs removed) and vaccinated at hatching time against infectious bronchitis, Marek's and Newcastle diseases. Chicks within genetic groupings were then placed into randomly assigned brooding-rearing pens. At about 4 weeks old, each chick had 1/3 of its upper beak removed. All birds received about 15 hrs light daily during the entire rearing period. At 19 weeks old, all pullets used for Experiments II-IV were transferred into four contrasting environments within the same large room of a house designed for hens in the egg-laying phase (described in detail later). Pullets for Experiment I were transferred into single-hen cages in the same house at 27 weeks old. All pullets were fed the KSU 18% crude protein layers ration ad libitum. All hens in the egg-laying house (test environment) received natural lighting which was supplemented with artificial lights when pullets were 20 weeks old, so as to provide not less than 14 hrs daily light.

Behavioral Tests

Tonic Immobility:

Two types of wooden cradles (test substrates), one with a base and the other without, were used for Experiment I, but only the one with a base was used for the remaining experiments. The cradles were constructed in a modified U-shape with dimensions as described by Jones and Faure (1980). To prepare for the TI test the cradle was placed on an egg carton placed on its side, so that hens being tested were at a height of about 40 cm from the floor. A piece of black velvet cloth was folded to form the contour of the cradle. The test subject was placed on its back with the head hanging over the edge. Each bird was restrained for a 15 s period after which the experimenter removed his hands. If the bird remained immobile for 10 s TI had been induced, otherwise the procedure was repeated until the immobility response was attained. The two types of cradle and an immobilized hen in place are shown in Figure 1. As soon as TI was attained, a set of 4 stop watches were started to record latencies until first gross leg and head movements and until righting (the hen stood up) and also the cumulative duration of eye closure. Other TI parameters recorded were the number of restraint periods (15 s induction trials) required to induce TI and whether hens vocalized and/or jumped up at termination of TI or whether a quiet righting occurred. The experimenter sat about 1 m away from the test subject and fixed his eyes on the bird throughout the test.

Metronome Avoidance Test

A metronome was used in Experiment IV to assess the recovery of hens from avoidance/escape responses in multiple-hen cages (5 hens per cage) only. The technique was modified from that described by Craig et al. (1983). Feed was covered from 0800 hrs until hens in each cage were tested, beginning at 1400 hrs. The metronome was mounted on a platform temporarily attached to the front of the cage and behind the feeder (Figure 2). As soon as the metronome was started at 120 beats per minute, the feeder was uncovered and 5 stop watches (one for each bird) were started. The tested birds were observed from a distance of 1.2 m by the observer sitting on a

Figure 1. Left, Cradles without and with a base to support the back of a bird during tonic immobility.

Right, Hen in tonic immobility on a cradle with a base covered with a black velvet cloth.

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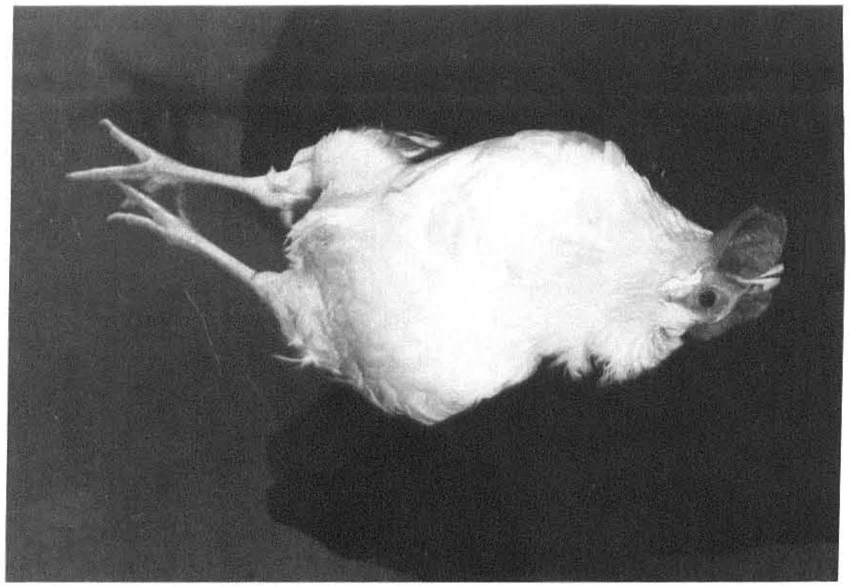


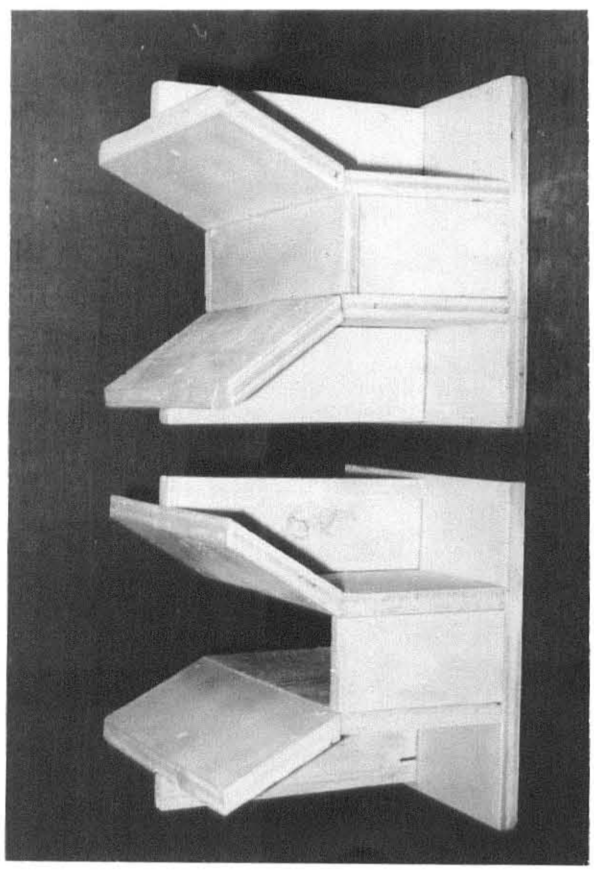
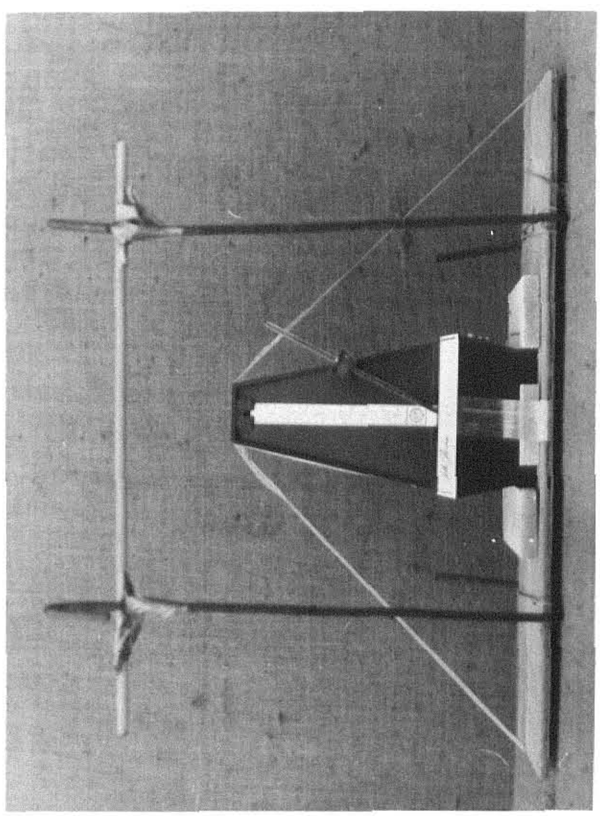


Figure 2. Left, Metronome on a board with hooks suitable for attachment to a cage.
Right, Metronome apparatus in place on a cage.



stool along the aisle between cage rows. Latency until each bird returned to feeding was recorded. The hens beak was required to touch feed in the trough as the criterion of feeding. Each bird was identified by wearing colored (red, white, blue, green or yellow) badges on both wings. Only 4 of the 5 birds could feed simultaneously.

Statistical Analysis

Fixed Effects and Random Variables

Selection (unselected controls vs. selected) for part-year egg mass, and crossing (strains vs. crosses) in Experiments II-IV, and treatments were all assumed to be fixed effects. Replicated strains and recriprocal crosses within selection and crossing were treated as random effects because of the probability of random genetic drift. A completely randomized factorial design was used in all experiments.

Non-parametric Measures

The number of restraint periods required for induction of TI were grouped into the categories of 1 and >1. Eye closure and vocalization and/or jumping were treated as "All-or-None" traits. Therefore these measures were analyzed by the Chi-square frequency distribution procedure in all experiments.

Transformation of Latency Measures

Raw data for all latency measures (until first gross leg and head movements and until righting for the TI test, and latency to return to feeding for the metronome test) were tested for normality of distribution. All were found to deviate significantly; they were highly skewed with many small values and long tails to the right. Because of that, both square root and logarithmic transformations were made and the transformed data were tested for normality. Logarithmic transformation was found to yield a better correction for non-normality and was, therefore, used in further analysis.

Correlation Analysis

Correlation coefficients (r) between all possible pairs of latency measures, based on experimental unit (cage or pen) means, were calculated and tested for heterogeneity among subclasses. The correlation coefficients (r) were transformed to z values using the equation:

$$z = .5\ (\text{Log}_e\ (1 + r) - \text{Log}_e\ (1 - r)$$

(Snedecor and Cochran, 1981; p. 186). The Chi-square distribution procedure was used to test the null hypothesis of a common population correlation. Lack of heterogeneity allowed estimation of the population correlation.

Linear mathematical models used for analysis of variance, treatments and statistical procedures specific to each experiment are described more fully under each experiment.

EXPERIMENT I: GENETIC STOCK AND CRADLE CONTRUCTION EFFECTS ON TONIC IMMOBILITY

INTRODUCTION

Jones and Faure (1980) used a U-shaped wooden cradle in their studies of TI in chickens. From their description it is not clear whether the cradle had a base, giving direct support to the dorsal surface of the bird or not. Therefore, questions arise concerning possible effects of body size and presence or absence of a dorsal support. Would a smaller-bodied hen placed in the trough of the cradle without dorsal support be under greater continuing passive restraint from the sides of the cradle than a larger-bodied hen? Would a greater passive restraint in a cradle without a base delay recovery of birds from TI as compared with birds having dorsal support? This experiment was undertaken primarily to compare the effects on TI of a cradle with a base that directly gave dorsal support to the bird with one without such a base. Because C_1 and C_2 strains hens were available for this comparison of cradles, it was also possible to test for genetic differences between them.

MATERIALS AND METHODS

Singly-caged, 57-week-old hens of the C_1 and C_2 strains were used. For each kind of cradle, 27 C_1 and 23 C_2 hens were randomly selected and tested in random order for the TI response. The number of restraint periods required to induce TI and latencies until first gross leg and head movements and until righting were recorded along with presence or absence of eye closure and vocalization and/or jumping at termination of the response. The test was carried out over a 3-day period.

Statistical Analysis

The fixed treatment was type of cradle used. The following linear mathematical model was used in the analysis:

$$Y_{ijk} = u + S_i + T_j + (ST)_{ij} + E_{ijk}$$

Where: Y_{ijk} = response variables ($\log_{10}$s latencies).

u = overall mean;

S_i = effect of ith strain, i = 1-2;

T_j = effect of jth test treatment, j=1-2;

$(ST)_{ij}$=interaction effect of ith strain with jth test treatment;

E_{ijk}=residual effect.

All terms of the model are assumed uncorrelated. The terms S_i, $(ST)_{ij}$ and E_{ijk} are considered random terms and assumed independently distributed with mean zero and variance as σ^2_S, σ^2_{ST} and σ^2_E respectively. Table 1 identifies sources of variance about the mean (μ), indicates the degrees of freedom and gives the expected composition of mean squares for analysis from which appropriate error terms were deduced.

Table 1. Source of variance, degrees of freedom and expected composition of mean squares for comparisons of genetic stocks and type of cradle

Source of variance	df	Expected composition of mean squares
Strain (S)	1	$\sigma^2 + k\sigma^2_{ST} + 50\sigma^2_{S}$
Type of Cradle (T)	1	$\sigma^2 + k\sigma^2_{ST} + \Sigma T^2_j$
S x T	1	$\sigma^2 + k\sigma^2_{ST}$
Error	96	σ^2

RESULTS

Genetic Differences

Genetic strains differed (P=.053) for latency until righting with the C_1 strain pullets taking more than twice as long to recover as did the C_2 strain pullets, Table 2. C_1 strain birds also had about twice as long latencies until first gross leg and head movements, but those differences were not significant. Roughly 70% of both C_1 and C_2 strain pullets required only a single period of restraint to induce TI, Table 3. Strains differed in eye closure, where 15% of C_1 strain pullets closed their eyes, but none of the C_2 strain pullets did. However, 87% of C_2 strain pullets compared to 70% of C_1 strain pullets vocalized and/or jumped at righting.

Type of cradle effect

There were no cradle differences on TI latencies, Table 2. However, 78% of hens tested on the cradle without a base required only a single period of restraint to induce TI as compared to the 60% tested on the cradle with a base, Table 3.

Table 2. Mean squares and least squares means indicating strain and type of cradle effects on latencies until recovery from TI of singly-caged hens

Source of variance	MS for F-test	df	Leg movement	Head movement	Righting
			Latencies, $\log_{10}$ seconds		
			Mean Squares		
Strain (S)	S x T	1	1.49	3.36	1.50*
Type of Cradle (T)	S x T	1	.05	.39	.01
S x T	Error	1	.09	.40	.01
Error		96	.14	.19	.15
			Least squares means, seconds		
Strain: C_1			223	128	254*
C_2			127	55	145
Type of cradle:	with base		177	97	188
	without base		159	73	196

*P=.053

Table 3. Genetic strain and type of cradle effects on percentages of singly-caged hens requiring a single restraint period for TI induction, closing eyes and vocalizing and/or jumping on recovery

	Required single restraint period	Closed eyes	Vocalized and/or jumped
Strain:			
C_1	70	15**	70
C_2	67	0	87*
Type of cradle:			
With a base	60	12	78
Without a base	78**	4	78

*$P \leq .05$ from Chi-square test based on actual numbers.

**$P \leq .01$ from Chi-square test based on actual numbers.

Interactions

There were no significant interactions between strains and type of cradle for TI latencies.

Correlations Among Latencies

Correlation coefficients were calculated among the 3 latency measurements within the four strain-cradle type subclasses and tested for heterogeneity. All correlation coefficients for the two strains tested in the cradle with a base were positive, high and differed between the strains, while only those between leg movement and righting time for the strains tested in the cradle without a base differed, Table 4. C_1 strain hens tested on the cradle with a base had a coefficient of r=.99 as compared with r=.51 for the same strain tested in the cradle without a base for leg movement with righting time. Absence of heterogeneity allowed means to be estimated for the two strains tested on the cradle without a bse. Coefficients for leg movement with head movement and for head movement with righting time were smaller for hens tested on the cradle without dorsal support as compared to hens tested on the cradle with a base.

Table 4. Correlation coefficient estimates of latency measures of TI for strains tested in cradles with and without a base

Type of cradle	Strain		Head movement	Righting
With base:				
	C_1	Leg movement	.71**	.99**
	C_2	Leg movement	.62**	.95**
	C_1	Head movement	-	.71**
	C_2	Head movement	-	.67**
Without base:[1]				
		Leg movement	.41**	(see below)
		Head movement	-	.25**
	C_1	Leg movement		.51**
	C_2	Leg movement		.92**

**$P \leq .01$.

[1]Mean correlation coefficients were calculated for C_1 and C_2 strains when they did not differ between strains.

EXPERIMENT II: GENETIC STOCK, CHASING AND IMMEDIATE VS DELAYED TESTING EFFECTS ON TONIC IMMOBILITY

INTRODUCTION

Chickens kept in individual-bird cages are easily caught and tested for the TI response as compared to hens kept in floor pens that must ordinarily be chased before catching. Jones and Faure (1981c) placed a wire cage over each bird 15 min before testing to facilitate ease of catching. Objectives of the presently reported study were to investigate TI measures on hens from floor pens as affected by:

(a) genetic strain differences,

(b) amount of chasing prior to testing, and

(c) immediate testing after catching vs briefly confining a pullet in a single-bird cage away from its pen, before testing.

MATERIALS AND METHODS

Hens of the 8 genetic stocks previously described which had been kept in floor pens with a space allowance of 2323 cm^2 per hen, were used for TI testing at 38 weeks old. A total of 96 layers were tested; 2 were randomly selected from each of 24 pens for immediate TI testing and another 48 were similarly chosen for delayed testing. All TI measures were made over a 4-day period. A stop watch was used to record the time it took from the moment the experimenter opened the door of the pen until the hen was caught (catching time). After catching, the bird was immediately tested for the TI response as described earlier. For the delayed test, each bird was caught and confined in a single-hen cage away from its pen for as long as it took to test and return the preceding the subject and to catch the hen to be tested subsequently. Time that elapsed from placing the hen in the cage until removed for TI testing (holding time) was recorded. Therefore, data collected included catching time and holding time together with the TI measures previously described, except for vocalization and/or jumping.

Statistical Analysis

The following linear mathematical model was chosen to compare immediate vs delayed testing effects and the other variables.

$$Y_{ijklm} = u + Se_i + C_j + SeC_{ij} + S_{k(ij)} + T_l + SeT_{il} + CT_{jl} + SeCT_{ijl} + (ST)_{k(ij)l} + E_{ijklm}$$

where:

Y_{ijklm} = response variables ($\log_{10}$ seconds of latencies)

u= overall mean;

Se_i = effect of the ith form of selection, i = 1-2;

C_j = effect of the jth form of crossing, j= 1-2;

SeC_{ij} = interaction effect of the ith form of selection with the jth form of crossing;

$S_{k(ij)}$ = effect of the kth strain within the ith form of selection and jth form of crossing, k= 1-8;

T_l = effect of the lth type of test treatment, l = 1-2;

SeT_{il} = interaction effect of the ith form of selection with the lth type of test treatment;

CT_{jl} = interaction effect of the jth form of crossing with the lth type of test treatment;

$SeCT_{ijl}$ = interaction effect of the ith form of selection with the jth form of crossing, with the lth type of test treatment;

$(ST)_{k(ij)l}$ = interaction effect of the kth strain with the ith form of selection and jth form of crossing with the lth type of test treament;

E_{ijklm} = residual effect.

All the terms of the model are assumed to be uncorrelated. The terms $Sk_{(ij)}$, $(ST)_{k(ij)l}$ and E_{ijklm} are considered random terms which are assumed to be independently distributed with mean zero and variances as σ^2,σ^2_{ST} andσ^2E respectively. Table 5 identifies sources of variance about the mean (u), indicates degrees of freedom, and gives expected composition of mean squares from which appropriate error terms were deduced.

Regression of transformed latencies on catching time and holding time was carried out using the linear regression model $Y_i = \beta_0 + \beta_1 X_i$.

Where:

Y_i = response variable (transformed latency);

βo = intercept ($\log_{10}$ seconds)

β_1 = Slope

X_i = independent variable (catching time, holding time)

Table 5. Sources of variance, degrees of freedom and expected composition of mean squares for comparison of latency measures of genetic stocks and methods of TI testing

Source of variance	df	Expected composition of mean squares
Selection (Se)	1	$\sigma^2 + k\sigma^2_{ST} + 6\sigma^2_S + \Sigma Se^2_i$
Crossing (C)	1	$\sigma^2 + k\sigma^2_{ST} + 6\sigma^2_S + \Sigma C^2_j$
Se x C	1	$\sigma^2 + k\sigma^2_{ST} + 6\sigma^2_S + \Sigma SeC^2_{ij}$
Strain w/n Se and C (S)	4	$\sigma^2 + k\sigma^2_{ST} + 6\sigma^2_S$
Type of Test (T)	1	$\sigma^2 + k\sigma^2_{ST} + \Sigma T^2_l$
Se x T	1	$\sigma^2 + k\sigma^2_{ST} + \Sigma SeT^2_{il}$
C x T	1	$\sigma^2 + k\sigma^2_{ST} + \Sigma CT^2_{il}$
Se x C x T	1	$\sigma^2 + k\sigma^2_{ST} + \Sigma SeCT^2_{ijl}$
S x T	4	$\sigma^2 + k\sigma^2_{ST}$
Error	47	σ^2

1/ Strains within unselected controls (C_1, C_2), within selected (Y_1, Y_2) and reciprocal crosses between control strains (C_1 x C_2, C_2 x C_1) and selected strains (Y_1 x Y_2, Y_2 x Y_1).

Correlation Analysis

Correlation coefficients among latency measures were calculated based on pen means. Heterogeneity among the correlation coefficients for strains were tested as described earlier. Where heterogeneity was indicated, the correlation coefficients are presented by test and individual strain subclass.

RESULTS

Genetic Differences

Strains previously selected for part-year egg mass had shorter latency till first gross head movement as compared with unselected control stocks (47s vs 71s), Table 6. However, differences for latencies till first gross leg movement and until righting did not approach significance. Similarly, differences were not found between selected and control stocks for those requiring a single restraint period for TI induction, nor for incidence of hens closing their eyes, Table 7. There were no significant crossing effects on any of the measures.

Although C_1 pullets did show longer latency until righting (288s) as compared with C_2 pullets (235s) the difference was not significant in this experiment. A large difference was observed between the Y strains; Y_1 pullets remained immobile for more than twice as long as Y_2 pullets in terms of righting time (456 and 180s, respectively), Table 6. No other significant differences were found between replicated strains or their crosses, Tables 6 and 7.

Table 6. Mean squares and least squares means indicating effects of genetic stock and type of test (immediate vs. delayed) on latency measures of TI

			Latencies, $\log_{10}$ seconds		
Source of Variance	MS for F test	df	Leg movement	Head movement	Righting
			Mean squares		
Selection (Se)	S	1	.07	.38*	.00
Cross (C)	S	1	.00	.09	.10
Se x C	S	1	.18	.16	.02
Strain w/n Se x C (S)	S x T	4	.04	.05	.13**
Type of Test (T)	S x T	1	.02	.07	.04
Se x T	S x T	1	.59*	.49	.04†
C x T	S x T	1	.02	.00	.09*
Se x C x T	S x T	1	.02	.24	.00
S x T	Error	4	.05	.12	.01
Error		32	.14	.15	.05
			Least squares means, seconds		
Selection: Unselected controls			167	71*	304
Selected			141	47	303
Cross: Strains			152	65	273
Crosses			155	53	337
Strains: C_1			136	62	288
C_2			152	77	235
$C_1 \times C_2$			228	90	397
$C_2 \times C_1$			166	60	317
Y_1			197	51	456**
Y_2			132	70	180
$Y_1 \times Y_2$			135	36	340
$Y_2 \times Y_1$			113	40	303
Type of Test: Immediate			161	63	323
Delayed			147	53	285

† $P < .10$
* $P \leq .05$
** $P \leq .01$

Table 7. Genetic strain and type of test (immediate vs delayed) effects on percentages of penned hens requiring a single restraint period to induce TI and closing their eyes during the response

	Required single period of restraint	Closed eyes
Selection:		
Unselected control	52	15
Selected	63	10
Cross:		
Strain	50	15
Crosses	65	10
Type of Test:		
Immediate	69*	13
Delayed	46	13
Strain:		
C_1	42	8
C_2	50	25
$C_1 \times C_2$	58	0
$C_2 \times C_1$	58	25
Y_1	50	0
Y_2	58	25
$Y_1 \times Y_2$	67	8
$Y_2 \times Y_1$	75	8

*$P<.05$ from Chi-square test based on actual numbers.

Types of Test (Immediate vs Delayed)

Hens were chased during catching for periods ranging from 5 to 100 seconds (mean 26s) for the immediate test. Hens were similarly caught but temporarily confined for periods that ranged from 300 to 3360 seconds (mean 761s) for the delayed TI test. Although the hens confined before testing had a tendency for shorter latencies, differences were not significant, Table 6. Sixty-nine percent of hens tested immediately after catching required a single period of restraint for induction as compared to the 46% for the previously confined ones, Table 7.

Interactions

Only 2 out of 15 interaction terms tested reached the .05 level of probability. Subclass means and differences associated with the Selection x Type of test interaction for first gross leg movement ($P < .05$) and for the other latencies (lack significance) are shown in Table 8A. There was an absence of consistency for the different latency measures. For example, while control hens tested immediately after catching showed a more prompt leg movement as compared with selected hens, the opposite occurred with delayed tested hens. However, relative recovery rates for righting time were reversed for the two kinds of genetic stocks under the same types of testing conditions, Table 8A.

Subclass least squares means associated with Crossing x Types of test (C x T) interactions (Table 8B) also reveal inconsistencies among latency results. The significant interaction for righting time was associated with a large difference between crosses and strains when tested immediately after

Table 8. Least squares means and differences of latency measures for subclasses associated with Genetic stock x Type of test interactions

Genetic stock-test subclasses	Latency, seconds					
	Leg movement		Head movement		Righting	
	Mean	Difference	Mean	Difference	Mean	Difference
A: Selection x type of test						
Control-immediate	136	- 55	62	- 3	346	+ 44
Selected-immediate	191		65		302	
		*				†
Control-delayed	206	+122	83	+48	267	- 37
Selected-delayed	104		35		304	
B: Crossing x type of test						
Cross-immediate	171	+ 19	57	-14	396	+133
Strain-immediate	152		71		263	
						*
Cross-delayed	141	- 12	48	-11	287	+ 5
Strain-delayed	153		59		282	

† $P \leq .10$ for interaction term.

* $P \leq .05$ for interaction term.

catching and essentially no difference when confined temporarily in cages before testing.

Regression Analysis

There were significant and negative regression coefficients for latency till first gross leg and head movements on catching time (immediate test) but not for righting time, nor for any latencies on holding time (delayed test) prior to testing, Table 9. Predicted latencies until leg and head movements associated with arbitrarily chosen catching times demonstrating the negative regression coefficients are shown in Table 10.

Correlation Analysis

Correlation coefficients involving latencies between first gross leg and head movements and between first gross leg movement and righting time were positive and significant with values of .66 and .60, respectively (Table 11).

Heterogeneity was found when correlations of different subclasses were compared for latencies between head movement and righting. Further testing revealed that coefficients differed between strains within the delayed testing procedure only (Table 11). Head movement did not appear to be associated with righting time for the immediately tested group of hens. However, there was great variability among hens of the different strains which were confined prior to TI testing. Those correlation coefficients ranged from -.62 to .96 and 4 out of 8 were significant.

Table 9. Regression coefficients of latencies of TI on catching time (immediate tests) and holding time (delayed tests) of floor-pen layers

Response variable, $\log_{10}$seconds	Independent variable, seconds	β_0	β_1	P of β_1
		$\log_{10}$seconds		
Leg movement	Catching time	2.5983	-0.0149	.02*
	Holding time	2.2607	-0.0001	.52
Head movement	Catching time	2.1874	-0.0144	.05*
	Holding time	1.7172	1.4588	.94
Righting	Catching time	2.4886	0.0008	.83
	Holding time	2.4957	-5.3962	.70

*$P \leq .05$

Table 10. Predicted latencies until first gross leg and head movements associated with arbitrarily chosen catching times

Catching time, seconds	Latency, seconds	
	Leg movement[1]	Head movement[1]
10	281	109
20	200	78
30	142	56
40	101	40
50	71	29
60	51	21
70	36	15
80	25	11
90	18	8
100	13	6

[1]Predicted from the regression equation:

$Y_i = \beta_0 + \beta_1 X_i$ where β_0 and β_1 have the values given in Table 9.

Table 11. Correlation coefficient estimates among latency measures of TI for immediate and delayed tested groups of hens

Kind of test	Head movement	Righting
Combined[1]		
Leg movement	.66**	.60**
Immediate[2]		
Head movement		.06
Delayed		
Head movement		
C_1		-.45
C_2		.96**
$C_1 \times C_2$		.83**
$C_2 \times C_1$		-.62*
Y_1		.46
Y_2		.67*
$Y_1 \times Y_2$		.24
$Y_2 \times Y_1$		-.11

*$P \leq .05$

**$P \leq .01$

[1]Mean correlation coefficients were calculated for all strain-kind of test subclasses because heterogeneity was not present.

[2]A mean correlation coefficient was calculated over all genetic stocks because heterogeneity was not present.

EXPERIMENT III: GENETIC STOCK AND HOUSING ENVIRONMENT EFFECTS ON TONIC IMMOBILITY

INTRODUCTION

Jones and Faure (1981b) reported that hens kept 4 per cage, with a floor area of 700 cm^2 (108 in^2) per hen, showed longer duration of TI as compared to hens kept 4 per floor pen, with floor area of 9300cm^2 (1440 in^2) per hen. From this observation, they suggested that caged birds were more fearful than those kept in floor pens.

Gallup (1974b) and Gallup et al. (1976) reported both breed and strain within breed differences on the TI phenomenon. Their test subjects have mostly been sexually immature birds. This study was carried out on adult laying hens to further investigate environmental and genetic effects on TI.

MATERIALS AND METHODS

The genetic stocks described earlier were used. Table 12 indicates for each stock the number of pullets per flock or experimental unit, number of replicated flocks (supplying hens for testing) per stock in each environment, and feeder and floor space allowances. Deep-litter floor pens and single-hen cages provided generous feeder and floor space by current commercial criteria. Floor pens had roosts and nests, while all cages were of welded-wire construction with the sloping floor and barren design common to commercial cages. Feed and water for caged hens were reached by individuals extending their heads through openings. Colony cages (17 hens per

Table 12. Laying-house environments, pullets per flock and number of flocks per housing-genetic stock subclass

Item	Laying-house environments: Floor pen	Single-hen cage[1]	Multiple-hen cage (5/C)	Colony cage (17/C)
Feeder space per hen, cm	16	20	8	5
Floor space per hen, cm^2	2323	930	372	382
Pullets per flock	15	15	5	17
Flocks per genetic stock[2]	3	3	3	3

[1]Fifteen adjacent, single-hen cages were considered as an experimental unit or "flock".

[2]Genetic strains and crosses were: C_1, C_2, Y_1, Y_2, $C_1 \times C_2$, $C_2 \times C_1$, $Y_1 \times Y_2$ and $Y_2 \times Y_1$.

cage) though similar to single-bird cages, were deeper (71 cm compared to 46 cm) as well as wider, and provided less feeder and floor space per pullet. Multiple-hen cages (5 hens per cage) were made by removing the partition between two single-bird cages. Floor space per hen was essentially equal for cages holding 5 and 17 hens.

For Round 1 (mean age 47 weeks), 3 birds were randomly selected from each unit of each housing environment. The multiple-hen environment had 23 cages so that whereas 72 birds were tested from each of the other environments, 69 were tested from it.

For Round 2 (mean age of 55 weeks), 3 naive subjects were again randomly selected from each environment, except for the multiple-hen cages, from which the two remaining naive subjects were tested from each cage. Thus, a total of 144 birds were tested from each environment except the multiple-hen environment that had 112 (instead of 115, because of mortality) tested.

TI testing was carried out over a 3-week period within each round.

Procedures

Hens from the floor pens were caught and tested immediately. All TI testing involved the cradle with a base as described earlier. The same TI parameters were measured as in Experiment I.

Statistical Analysis

The general procedures have been described earlier. However, the treatments specific to this experiment were housing environments and rounds.

The following linear mathematical model was chosen to compare the effects of the variables indicated.

$$Y_{ijklmn} = u + Se_i + C_j + SeC_{ij} + S_{k(ij)} + E_l + SeE_{il} + CE_{jl} + SeCE_{ijl} + (SE)_{k(ij)l} + R_m + SeR_{im} + CR_{jm} + SeCR_{ijm} + SeER_{ilm} + CER_{jlm} + SeCER_{ijlm} + (SR)_{k(ij)m} + (SER)_{k(ij)lm} + \varepsilon_{ijklmn}$$

Where:

Y_{ijklmn} = Response variables (Latencies)

u = overall mean

Se_i = effect of the ith form of selection, i = 1-2;

C_j = effect of the jth form of crossing, j = 1-2;

SeC_{ij} = interaction effect of the ith with the jth forms of selection and crossing respectively,

$S_{k(ij)}$ = effect of the kth strain within the ith and jth forms of selection and crossing, respectively, k = 1-8;

E_l = effect of the lth housing environment (environment) treatment, l = 1-4;

SeE_{il} = interaction effect of the ith form of selection with the lth environment treatment;

CE_{jl} = interaction effect of the jth form of crossing with the lth environment treatment;

$SeCE_{ijl}$ = interaction effect of the ith form of selection with the jth form of crossing with the lth environment treatment;

$(SE)_{k(ij)l}$ = interaction effect of the kth strain within the ith and jth forms of selection and crossing respectively with the lth environment treatment;

R_m = effect of the mth round treatment, m = 1-2;

SeR_{im} = interaction effect of the ith form of selection with the mth round treatment;

CR_{jm} = interaction effect of the jth form of crossing with the mth round treatment;

$SeCR_{ijm}$ = interaction effect of the ith form of selection with the jth form of crossing with the mth round treatment;

$SeER_{ilm}$ = interaction effect of the ith form of selection with the lth environment treatment with the mth round treatment;

CER_{jlm} = interaction effect of the jth form of crossing with the lth environment treatment with the mth round treatment;

$SeCER_{ijlm}$ = interaction effect of the ith form of selection with the jth form of crossing with the lth environment treatment with the mth round treatment;

$(SR)_{k(ij)m}$ = interaction effect of the kth strain within the ith and jth forms of selection and crossing respectively, with the mth round treatment;

$(SER)_{k(ij)lm}$ = interaction effect of the kth strain within the ith and jth forms of selection and crossing respectively with the lth environment treatment, with the mth round treatment;

ε_{ijklmn} = residual effect;

All the terms of the model are assumed to be uncorrelated. The terms $S_{k(ij)}$; $(SE)_{k(ij)l}$; $(SR)_{k(ij)m}$; $(SER)_{k(ij)lm}$ and ε_{ijklmn} are considered random terms which are assumed to be independently distributed with mean zero and variances as $\sigma^2_S, \sigma^2_{SE}, \sigma^2_{SER}$ and σ^2_ε respectively. Table 13 indicates sources of variance about the mean (u), degrees of freedom and expected mean squares from which appropriate error terms were deduced. Synthetic error mean squares and associated degrees of freedom required for testing mean

Table 13. Sources of variance, degrees of freedom, and expected composition of mean squares for comparisons of TI latency measures on hens of genetic stocks kept in different laying-house environments

Source of variance	df	Expected composition of mean squares
Selection (Se)	1	$\sigma^2 + k_3\sigma^2_{SER} + k_2\sigma^2_{SR} + k_1\sigma^2_{SE} + 24\sigma^2_S + \Sigma Se^2_i$
Cross (C)	1	$\sigma^2 + k_3\sigma^2_{SER} + k_2\sigma^2_{SR} + k_1\sigma^2_{SE} + 24\sigma^2_S + \Sigma C^2_j$
Se x C	1	$\sigma^2 + k_3\sigma^2_{SER} + k_2\sigma^2_{SR} + k_1\sigma^2_{SE} + 24\sigma^2_S + \Sigma SeC^2_{ij}$
Strain w/n Se and C (S)	4	$\sigma^2 + k_3\sigma^2_{SER} + k_2\sigma^2_{SR} + k_1\sigma^2_{SE} + 24\sigma^2_S$
Synthetic 1/		$\sigma^2 + k_3\sigma^2_{SER} + k_2\sigma^2_{SR} + k_1\sigma^2_{SE}$
Environment (E)	3	$\sigma^2 + k_3\sigma^2_{SER} + k_1\sigma^2_{SE} + \Sigma E^2_l$
Se x E	3	$\sigma^2 + k_3\sigma^2_{SER} + k_1\sigma^2_{SE} + \Sigma SeE^2_{il}$
C x E	3	$\sigma^2 + k_3\sigma^2_{SER} + k_1\sigma^2_{SE} + \Sigma CE^2_{il}$
Se x C x E	3	$\sigma^2 + k_3\sigma^2_{SER} + k_1\sigma^2_{SE} + \Sigma SeCE^2_{ijl}$
S x E	12	$\sigma^2 + k_3\sigma^2_{SER} + k_1\sigma^2_{SE}$
Round (R)	1	$\sigma^2 + k_3\sigma^2_{SER} + k_2\sigma^2_{SR} + \Sigma R^2_m$
Se x R	1	$\sigma^2 + k_3\sigma^2_{SER} + k_2\sigma^2_{SR} + \Sigma SeR^2_{im}$
C x R	1	$\sigma^2 + k_3\sigma^2_{SER} + k_2\sigma^2_{SR} + \Sigma CR^2_{jm}$
Se x C x R	1	$\sigma^2 + k_3\sigma^2_{SER} + k_2\sigma^2_{SR} + \Sigma SeCR^2_{ijm}$
Se x E x R	1	$\sigma^2 + k_3\sigma^2_{SER} + k_2\sigma^2_{SR} + \Sigma SER^2_{ilm}$
S x R	4	$\sigma^2 + k_3\sigma^2_{SER} + k_2\sigma^2_{SR}$
E x R	3	$\sigma^2 + k_3\sigma^2_{SER} + \Sigma ER^2_{lm}$
C x E x R	3	$\sigma^2 + k_3\sigma^2_{SER} + \Sigma CER^2_{jlm}$
Se x C x E x R	3	$\sigma^2 + k_3\sigma^2_{SER} + \Sigma SeCER^2_{ijlm}$
S x E x R	12	$\sigma^2 + k_3\sigma^2_{SER}$
Error	125	σ^2

1/ Synthetic mean squares calculated by Satterthwaite's method; i.e. linear combination of mean squares as follows:

(S x E) + (S x R) - (S x E x R).

squares for strains within selection and crossing were obtained by Satterthwaite's method from a linear combination of mean squares (Snedecor and Cochran, 1981; pp 320-325).

RESULTS

Genetic Differences

Selection for part-year egg mass was associated with shorter latency until first gross head movement (Table 14) and reduced incidence of eye closure as compared with that of unselected controls (7% vs 12%) as shown in Table 15.

Crossing effects and strain differences within strains and reciprocal crosses were not found for any of the latency measures, Table 14. Differences between strains and crosses were absent for number of birds that required a single period of restraint for TI induction, closed their eyes during the response and vocalized and/or jumped on recovery from the response, Table 15. However, reciprocal crosses between C_1 and C_2 exhibited differences for eye closure (16% vs 6%), while reciprocal crosses for Y_1 and Y_2 exhibited a difference in the incidence of those that required a single period of restraint to induce TI (58% vs 82%).

Table 14. Mean squares and least squares means indicating effects of genetic stock, laying-house environment and round on latency measures of TI

Source of variance	MS for F test	df	Leg movement	Head movement	Righting
			Latencies, $\log_{10}$ seconds		
			Mean squares		
Selection (Se)	S	1	.58	1.47**	.01
Cross (C)	S	1	.00	.01	.01
Se x C	S	1	.02	.07	.02
Strain w/n Se and C (S)	Syn	4	.13	.04	.27
Synthetic[1] (Syn)			.03	.28	.13
Environment (E)	S x E	3	.61**	1.93**	.53*
Se x E	S x E	3	.02	.08	.10
C x E	S x E	3	.03	.02	.03
Se x C x E	S x E	3	.21**	.12	.27†
S x E	S x E x R	12	.02	.06	.10*
Round (R)	S x R	1	.72*	1.91†	.26
Se x R	S x R	1	.44*	.26	.01
C x R	S x R	1	.01	.02	.00
Se x C x R	S x R	1	.00	.09	.19
S x R	S x E x R	4	.05	.28*	.06
E x R	S x E x R	3	.12*	.05	.01
Se x E x R	S x E x R	3	.18*	.21†	.04
C x E x R	S x E x R	3	.10	.02	.05
Se x C x E x R	S x E x R	3	.01	.06	.01
S x E x R	Error	12	.05	.06	.03
Error		125	.07	.11	.06
			Least squares means, seconds		
Selection: Unselected controls			148	80**	209
Selected			115	53	214
Cross: Strains			131	64	215
Crosses			130	66	208

(continued next page)

Table 14. con't

Strains:	C_1	165	86	257
	C_2	141	78	168
	$C_1 \times C_2$	145	85	239
	$C_2 \times C_1$	143	71	186
	Y_1	141	50	284
	Y_2	89	50	176
	$Y_1 \times Y_2$	114	51	194
	$Y_2 \times Y_1$	121	62	217
Laying-house Environment:				
Floor pen		141[a]	83[a]	185[a]
Single-hen cage		127[a]	54[c]	170[a]
Multiple-hen cage(5/C)		92[c]	37[d]	217[a]
Colony cage (17/C)		175[b]	108[b]	294[b]
Round: 1		113	52	231
2		150*	82	194

[1] Synthetic mean squares calculated as indicated in Table 12, i.e. linear combination of mean squares: (S x E) + (S x R) - (S x E x R).

† $P \leq .10$
* $P \leq .05$
** $P \leq .01$

[a,b,c,d] means within columns with different superscripts differ within treatment classification ($P \leq .05$).

Housing Environment Effects

Housing environments affected all latency measures, Table 14. Hens kept in colony cages took longer to recover from TI for all measures as compared to hens in any of the other 3 environments. Latencies until righting did not differ among the other 3 environments. Birds in multiple-hen cages showed shorter latencies to first gross leg and head movements as compared to hens from floor pens and single-bird cages. Hens in the latter 2 environments differed in latency until first gross head movement only.

Although the percentages of hens from floor pens and colony cages did not differ for those that required a single period of restraint to induce TI (72% vs 73%, respectively) and those that vocalize and/or jumped on recovery (93% vs 90%, respectively), both differed from those kept in single-bird and multiple-hen cages for the same traits (56% 55% and 73%, 79%, respectively), Table 15.

Round Effects

Hens tested in Rounds 1 and 2 differed significantly in latency until first gross leg movement only, Table 14. Seventy-five percent of birds tested in Round 2 required a single period of restraint to induce TI as compared with 55% for those of Round 1, Table 15. Eighty-eight percent of Round 2 birds vocalized and/or jumped on recovery from TI as compared to 80% of those of Round 1. For eye closure, a reversal of rank was exhibited where 13% of Round 1 birds compared to 6% of Round 2 birds closed their eyes during the test, Table 15.

Table 15. Genetic stock, laying-house environment and round effects on percentages of hens requiring a single period of restraint, closing their eyes, and vocalizing and/or jumping on recovery in TI tests

	N	Required single restraint period	Closed eyes	Vocalized/ jumped
Selection:				
Unselected	276	61	12*	85
Selected	268	62	7	83
Crossing:				
Strains	273	66	8	84
Crosses	271	63	11	84
Environment:				
Floor pen	144	72[a]	5[a]	93[a]
Single hen cages	144	56[b]	7[a]	73[b]
Multiple-hen	112	55[b]	11[a]	79[b]
Colony cages	144	73[a]	15[b]	90[a]
Round:				
1	285	55	13*	80
2	259	75**	6	88*
Strains:				
C_1	69	65	17	77
C_2	69	54	9	93
$C_1 \times C_2$	69	67	16*	90
$C_2 \times C_1$	69	58	6	80
Y_1	64	66	5	84
Y_2	69	68	12	83
$Y_1 \times Y_2$	69	58	6	77
$Y_2 \times Y_1$	66	82*	5	89

*$P \leq .05$ from X^2-test based on actual numbers.

**$P \leq .01$ from X^2-test based on actual numbers.

a,b,c% within columns with different superscripts differ significantly ($P \leq .05$).

Interactions

Leg movement: Selection x Round and Environment x Round interactions were found for latency until first gross leg movement, Table 14. Subclass least squares means presented in Table 16A show that unselected control hens exhibited nearly a minute longer latency (54 sec) than selected hens in Round 1, but the difference was minimal in Round 2 (4 sec). Round 1 latencies tended to be shorter than those for Round 2 in floor pens, multiple-hen and colony cages (by 32, 68 and 33 seconds, respectively), but no difference between singly-caged hens tested in the 2 rounds, Table 16B.

Absence of consistent patterns of differences among subclasses for latency to first leg movement was detected by the three-way interactions of selection x cross x environment (Table 16C) and selection x environment x round (Table 16D).

Head Movement: Replicated strains within strain-cross subclasses performed consistently over rounds as indicated by the S x R interactions, Table 14. Paired comparisons of the replicated stocks in Table 17 reveals reversals in ranks for latency until first gross head movement between Round 1 and Round 2 in all cases. Differences between replicated strains (or crosses) in the 2 rounds ranged from values of a few seconds only (e.g. Y_1 and Y_2) to over a minute (e.g. crosses of C strains).

Righting Time: Inconsistency of latencies until righting of hens of replicated strains (or crosses) from one environment to another are evident in Table 18 and are associated with the significant S x E interaction (Table 14).

Table 16. Least squares means for subclasses involved in interactions for latency until first gross leg movement

	Round 1	Round 2
A. Selection-round subclasses		
Unselected control	143[1]	152
Selected	89	148
B. Environment-round subclasses		
Floor pen	125	157
Single-hen cages	127	127
Multiple-hen (5F/C)	64	132
Colony cages (17F/C)	159	192

C. Selection-cross-environment subclasses

	Floor pen	Cages: Single-hen	Cages: Multiple-hen	Cages: Colony
Control - strains	187	168	95	180
Selected- strains	109	86	105	160
Control - crosses	133	135	104	228
Selected- crosses	144	132	70	142
D. Selection-environment-round subclasses				
Control - round 1	164	165	91	172
Selected- round 1	96	97	45	147
Control - round 2	151	138	108	239
Selected- round 2	164	117	161	154

[1]All mean latencies are in seconds.

Table 17. Least squares means for replicated strains within strain-cross-round subclass for latency until first gross head movement

Strains[1]	Round 1	Round 2
	Seconds	
C_1	67	110
C_2	79	77
$C_1 \times C_2$	56	130
$C_2 \times C_1$	76	66
Y_1	36	70
Y_2	32	78
$Y_1 \times Y_2$	50	53
$Y_2 \times Y_1$	39	98

[1]Replicated strains or reciprocal crosses are presented as paired comparisons within rounds.

Table 18. Least squares means for replicated strains within strain-cross-environment subclasses for latency until righting

	Environments			
		Cages		
Strains[1]	Floor pen	Single-hen	Multiple-hen (5/C)	Colony (17/C)
	Seconds			
C_1	198	251	198	444
C_2	216	135	129	206
$C_1 \times C_2$	145	145	342	453
$C_2 \times C_1$	152	174	150	303
Y_1	209	149	573	366
Y_2	161	129	164	283
$Y_1 \times Y_2$	194	176	240	172
$Y_2 \times Y_1$	227	239	163	252

[1]Replicated strains or reciprocal crosses are presented as paired comparisons within environments.

Correlation Analysis

Correlation coefficients of latencies between first gross leg and head movements and between first gross head movement and righting time were positive and significant (r = .68 and r = .50, respectively), Table 19.

Heterogeneity was detected when correlation of different subclasses were compared for latencies till first gross leg movement and righting time. Further testing revealed the difference was among strains tested in Round 2. First gross leg movement was positively and significantly associated with righting time in Round 1 (r = .50), Table 19. However, correlation coefficients differed among the strains in Round 2 and ranged from .59 to .99 (all were significant), Table 19.

Table 19. Correlation coefficients among latency measures of TI

			Head Movement	Righting
	Leg movement[1]		.68**	
	Head movement[1]			.51**
Round 1:	Leg movement[2]			.50**
Round 2:				
	Stocks			
	C_1	Leg movement		.59*
	C_2	"		.99**
	$C_1 \times C_2$	"		.65*
	$C_2 \times C_1$	"		.90**
	Y_1	"		.92**
	Y_2	"		.89**
	$Y_1 \times Y_2$	"		.76**
	$Y_2 \times Y_1$	"		.87**

*P $\leq$ .05

**P $\leq$ .01

[1]Mean correlation coefficients were calculated from genetic stock-round subclass correlations when heterogeneity was not detected among them.

[2]A mean correlation coefficient was calculated for Round 1 because heterogeneity was not detected among genetic stocks in this round.

EXPERIMENT IV: GENETIC STOCK, GROUP AND ROUND EFFECTS ON MEASURES OF FEARFUL BEHAVIOR AND ASSOCIATIONS AMONG MEASURES AND QUANTITATIVE TRAITS

INTRODUCTION

Craig et al. (1983) compared Y_1 and Y_2 strain pullets kept in colony cages by Hansen's nervousness score (Hansen, 1976) and by latency to recover from avoidance response brought about by striking of the cages and also by exposure to a metronome. The presently reported study compared hens of 8 genetic stocks for fear-associated measures by the TI and metronome techniques. The possibility of habituation was assessed and associations among behavioral responses (latencies and other traits) were also estimated.

MATERIALS AND METHODS

Genetic Stocks

The 8 genetic stocks described earlier were used.

Procedures

Each genetic stock was assigned to 3 randomly located sets of 3 adjacent cages. Cages held 5 females each and had 8 cm feeder space and 372 cm^2 floor space per hen. The 3 sets of cages for each stock were randomly assigned to treatment "groups" with 1 set per group and with each of the 3 cages per set serving as a strain-group subclass.

Both TI and metronome tests were used to estimate level of fearfulness. Both kinds of tests were conducted within a 6-week period in each of 3 rounds (see below). Groups differed in order of testing and/or kinds of tests as follows:

Group 1: Metronome test first, followed 3 weeks later by TI test;

Group 2: Metronome test first, followed 3 weeks later by a second metronome test;

Group 3: TI test first, followed 3 weeks later by metronome test.

Within each round, the first 3 weeks involved the metronome test for Groups 1 and 2 and TI test for Group 3. During the second 3-week period, Group 1 was given the TI test and Group 2 and 3 the metronome test.

Rounds of testing were conducted at mean hen ages of 35, 48 and 61 weeks. With 13-week intervals between rounds 1, 2 and 3, rounds coincided with winter, spring and summer months, respectively.

Body Weight, feather score and Egg-production Traits

Body weight and feather cover scores were obtained twice (Rounds 1 and 2), body weights at 32 and 63 weeks, and feather cover scores at 44 and 63 weeks of age. Feather cover was scored 1-9 by matching the feather condition of each bird with a series of photographs showing differing feather damage and loss (Adams et al., 1978). The highest score of 9 indicated complete coverage and undamage feathers, while a score of 1 indicated total feather loss to the back and dorsal side of the wings. Body weight change was the difference between body weight at 63 and 32 weeks. To avoid negative values, 1000 was added to the difference so that a weight change value below 1000 indicated a loss in body weight while values above 1000 indicated a gain.

Past egg productivity of individual hens was estimated by a pigmentation score based on amount of yellow color of the vent, eye ring, beak and shank. Scores of 0-9 were assigned at 32 and 63 weeks (Rounds 1 and 2, respectively) based on criteria shown in Table 20. Actual egg-production records were available as cage totals 3 days per week from 20-69 weeks. Mean hen-day rate of egg production was calculated from 30-69 weeks while the other traits, hen-housed rate of egg production, egg weight and egg mass were based on the 20-69 week period. Egg mass was calculated for each cage by multiplying the total number of eggs laid during egg collection days by mean egg weight and dividing by the number of hen-housed egg collecting days. Mean egg weight was based on eggs collected when layers were 25, 35, 45, 55, and 65 weeks old. Mean age of sexual maturity was estimated for pullets in each cage by means of age when 50% hen-day rate of production was first obtained.

Table 20. Pigmentation score criteria[1]

Score	Description
0	Fully pigmented beak; vent dry and puckered yellow
1	Vent bleached; eye ring, earlobe and beak yellow
2	Eye ring and ear lobe bleached; beak bleached at base only
3	Beak bleached 2/3rd from base toward tip
4	Beak completely bleached
5	Shank yellow (#4 of Cargill Yolk Pigmentation Meter)[2]
6	Shank medium yellow (#3 of Cargill Yolk Pigmentation Meter)
7	Shank lemon yellow (#2 of Cargill Yolk Pigmentation Meter)
8	Shank light yellow (#1 of Cargill Yolk Pigmentation Meter)
9	Shank completely bleached

[1]When hen's ration is based on milo.
[2]From: Cargill-Nutrena Research Farm
Cargill Incorporated
200 Grain Exchange
Minneapolis 15, Minnesota 55121

Statistical Analysis

Treatment fixed effects were Group (order of tests) and Round (age). Because of repeated measures on the same subjects (in different rounds) for TI and metronome tests and for body weights, feather cover and pigmentation scores, a split-plot factorial design was used. The following linear mathematical model was chosen to compare the effects of the variables.

$$Y_{ijklmn} = u + Se_i + C_j + SeC_{ij} + S_{k(ij)} + G_l + SeG_{il} + CG_{jl} + SeCG_{ijl} + (SG)_{k(ij)l} + R_m + SeR_{im} + CR_{jm} + SeCR_{ijm} + SeGR_{ilm} + CGR_{jlm} + SeCGR_{ijlm} + (SR)_{k(ij)m} + GR_{lm} + (SGR)_{k(ij)lm} + E_{ijklmn}$$

Where:

Y_{ijklmn} = *Response variables (latencies for TI and metronome measures),* body weight, feather and prigmentation scores).

u = over all mean

Se_i = effect of the ith form of selection, i = i-2;

C_j = effect of the jth form of crossing, j = 1-2;

SeC_{ij} = interaction effect of the ith form of selection with thejth form of crossing;

$S_{k(ij)}$ = effect of the kth strain within the ith form selection and jth form of crossing, k=1-8;

G_l = effect of the lth group treatment, l = 1-3, except for TI l = 1-2;

SeG_{il} = interaction effect of the ith form of selection with the lth group treatment;

CG_{jl} = interaction effect of the jth form of crossing with the lth group treatment;

$SeCG_{ijl}$ = interaction effect of the ith form of selection with jth form of crossing and with lth group treament;

$(SG)_{k(ij)l}$ = interaction effect of the kth strain within the ith form of selection and jth form of crossing with the lth group treatment;

R_m = effect of the mth round treatment, m= 1-2, except for TI and metronome, m = 1-3;

SeR_{im} = interaction effect of the ith form of selection with the mth round treatment;

CR_{jm} = interaction effect of the jth form of crossing with the mth round treatment;

$SeCR_{ijm}$ = interaction effect of the ith form of selection with the jth form of crossing with the mth round treatment;

$SeGR_{ilm}$ = interaction effect of the ith form of selection with the lth group treatment, with the mth round treatment;

CGR_{jlm} = interaction effect of the jth form of crossing with the lth group treatment with the mth round treatment;

$SeCGR_{ijlm}$ = interaction effect of the ith form of selection with the jth form of crossing with the lth group treatment with mth round treatment;

$(SR)_{k(ij)m}$ = interaction effect of the kth strain within the ith form of selection and jth form of crossing with the mth round treatment;

GR_{lm} = interaction effect of the lth group treatment with the mth round treatment;

$(SGR)_{k(ij)lm}$ = interaction effect of the kth strain within the ith form of selection and jth form of crossing with the lth group treatment with the mth round treatment;

E_{ijklmn} = residual effect.

The model chosen to compare the effects of variables on traits measured only once lacked the round treatment effects and all interactions with round as follows:

$$Y_{ijklm} = u + Se_i + C_j + SeC_{ij} + S_{k(ij)} + G_l + SeG_{il} + CG_{jl} + SeCG_{ijl} + (SG)_{k(ij)l} + E_{ijklm}$$

Where:

Y_{ijklm} = respnse variables (change in body weight hen-day and hen-housed rates of egg production, egg weight, egg mass and age of 50% production).

In both models all terms are assumed uncorrelated. The terms $S_{k(ij)}$, $(SG)_{k(ij)l}$, $(SR)_{k(ij)m}$, $(SGR)_{k(ij)lm}$ and $E_{ijklm(n}$ are considered random and assumed to be independently distributed with mean zero and variances as σ^2_S, σ^2_{SG}, σ^2_{SR}, σ^2_{SGR} and σ^2_E, respectively. Table 21 identifies the sources of variance about the mean (u), indicates degrees of freedom, and gives expected composition of mean squares for analysis from which error terms were deduced. Synthetic mean squares and asociated degress of freedom required for testing mean squares for strains within selection and crossing (except strains k for egg producting traits measured only once) were obtained by Satterthwaite's method, i.e. by a linear combination of mean squares (Snedecor and Cochran, 1981; pp 320-325).

Correlation Analysis:

Correlation coefficients (r) were calculated for strain-group-round subclasses on the basis of all pairs of measures on individuals (latencies from TI and metronome tests, body weights, feather and pigmentation scores) and between all pairs of measures based on cage means. Those r values were then transformed to z values as described earlier and used to test for

Table 21. Sources of variance, degrees of freedom and expected composition of mean squares for all TI latency measures on pullets kept in multiple-hen (5/C) cages

Source of variance	df	Expected composition of mean squares
Selection (Se)	1	$\sigma^2 + k_3\sigma^2_{SGR} + k_2\sigma^2_{SR} + k_1\sigma^2_{SG} + 17.5\sigma^2_S + \Sigma Se^2_i$
Cross (C)	1	$\sigma^2 + k_3\sigma^2_{SGR} + k_2\sigma^2_{SR} + k_1\sigma^2_{SG} + 17.5\sigma^2_S + \Sigma C^2_j$
Se x C	1	$\sigma^2 + k_3\sigma^2_{SGR} + k_2\sigma^2_{SR} + k_1\sigma^2_{SG} + 17.5\sigma^2_S + \Sigma SeC^2_{ij}$
Strain w/n Se and C (S)	4	$\sigma^2 + k_3\sigma^2_{SGR} + k_2\sigma^2 SR + k_1\sigma^2_{SG} + 17.5\sigma^2_S$
Synthetic 1/		$\sigma^2 + k_3\sigma^2_{SGR} + k_2\sigma^2_{SR} + k_1\sigma^2_{SG}$
Group (G)	1	$\sigma^2 + k_3\sigma^2_{SGR} + k_1\sigma^2_{SG} + \Sigma G^2_l$
Se x G	1	$\sigma^2 + k_3\sigma^2_{SGR} + k_1\sigma^2_{SG} + \Sigma SeG^2_{ij}$
C x G	1	$\sigma^2 + k_3\sigma^2_{SGR} + k_1\sigma^2_{SG} + \Sigma CG^2_{jl}$
Se x C x G	1	$\sigma^2 + k_3\sigma^2_{SGR} + k_1\sigma^2_{SG} + \Sigma SeCG^2_{ijl}$
S x G	4	$\sigma^2 + k_3\sigma^2_{SGR} + k_1\sigma^2_{SG}$
Round (R)	2	$\sigma^2 + k_3\sigma^2_{SGR} + k_2\sigma^2_{SR} + \Sigma R^2_m$
Se x R	2	$\sigma^2 + k_3\sigma^2_{SGR} + k_2\sigma^2_{SR} + \Sigma SeR^2_{im}$
C x R	2	$\sigma^2 + k_3\sigma^2_{SGR} + k_2\sigma^2_{SR} + \Sigma CR^2_{jm}$
Se x C x R	2	$\sigma^2 + k_3\sigma^2_{SGR} + k_2\sigma^2_{SR} + \Sigma SeCR^2_{ijm}$
S x R	8	$\sigma^2 + k_3\sigma^2_{SGR} + k_2\sigma^2_{SR}$
Se x G x R	2	$\sigma^2 + k_3\sigma^2_{SGR} + \Sigma SeGR^2_{ijlm}$
G x R	2	$\sigma^2 + k_3\sigma^2_{SGR} + \Sigma GR^2_{lm}$
C x G x R	2	$\sigma^2 + k_3\sigma^2_{SGR} + \Sigma CGR^2_{jlm}$
Se x C x G x R	2	$\sigma^2 + k_3\sigma^2_{SGR} + \Sigma SeCGR^2_{ijlm}$
S x G x R	8	$\sigma^2 + k_3\sigma^2 SGR$
Error	96	σ^2

1/ Synthetic mean square calculated by Satterthwaite's method; i.e. a linear combination of mean squares as follows: (S x G) + (S x R) - (S x G x R).

heterogeneity. The analysis of variance procedure was used to test selection, crossing, group and round differences on the individual-hen data while the Chi-square procedure described by Snedecor and Cochran (1981; pp 186-188) was used in testing for differences among the strains within rounds using the cage means data.

RESULTS

Genetic Differences

Measures of Fearful Behavior: No significant differences were detected between hens of selected and unselected stocks, between strains and their crosses, or among strains within selection and cross subclasses for the latency measures in the TI and metronome tests,Table 22.

Of the non-parametric traits, hens of unselected stocks vocalized and/or jumped more (67%) as compared with hens of selected stocks (57%), Table 23. For strains within selection and cross subclasses, fewer C_1 strain hens (58%) vocalized and/or jumped as compared with C_2 strain hens (79%). Y_1 strain hens were more readily induced to show TI than Y_2 strain hens as indicated by percentages that required only a single period of restraint (66 and 39%, respectively). Differences in incidence of eye closure were found between Y_1 and Y_2 strains hens (12 and 26% respectively) and differences were also detected between Y_1x Y_2 and Y_2 x Y_1 21 and 11%, respectively).

Feather Scores and Body weights: Feather coverage (estimated by the feather score), body weight and weight change were not affected significantly by selection, crossing, or strains within selection and cross subclasses, Table 24.

Table 22. Mean squares and least squares means indicating effects of genetic stocks, groups and round on latency measures for the TI and metronome tests

			Tonic immobility			Metronome	
			Latencies, $\log_{10}$ seconds				
Source of variance	MS for F test	df	Leg movement	Head movement	Righting	Return to feed	df
			Mean squares				
Selection (Se)	S	1	.20	.19	.00	.60	1
Cross (C)	S	1	.04	.00	.12	1.80	1
Se x C	S	1	.02	.10	.10	.50	1
Strain w/n Se and C(S)	Syn	4	.20	.24	.16	.50	4
Synthetic[1] (Syn)			.06	.03	.07	.66	
Group (G)	S x G	1	.14	.37*	.03	1.25†	2
Se x G	S x G	1	.41*	.04	.02	.01	2
C x G	S x G	1	.00	.01	.01	.08	2
Se x C x G	S x G	1	.21†	.03	.00	.06	2
S x G	S x G x R	4	.04	.03	.06	.38†	8
Round (R)	S x R	2	.41*	.11	1.39**	1.04	2
Se x R	S x R	2	.10	.18*	.00	.09	2
C x R	S x R	2	.01	.01	.03	.07	2
Se x C x R	S x R	2	.03	.04	.01	.23	2
S x R	S x G x R	8	.05	.04	.03	.43*	8
G x R	S x G x R	2	.06	.02	.02	.78**	4
Se x G x R	S x G x R	2	.05	.03	.01	.02	4
C x G x R	S x G x R	2	.05	.07	.01	.21	4
Se x C x G x R	S x G x R	2	.00	.06	.06	.04	4
S x G x R	Error	8	.03	.04	.02	.15	16
Error		93	.08	.08	.04	.19	138
			Least squares means, seconds				
Selection: Unselected controls			128	46	218	11	
Selected			108	39	225	9	
Crossing: Strains			122	42	237	12	
Crosses			113	43	207	8	

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Table 22 continued

Strains: C_1	114	42	261	19
C_2	164	56	236	12
$C_1 \times C_2$	135	51	239	7
$C_2 \times C_1$	106	33	154	9
Y_1	133	30	279	7
Y_2	88	44	183	12
$Y_1 \times Y_2$	88	38	211	10
$Y_2 \times Y_1$	129	47	238	6
Group (order of tests)				
1 (Met-TI)	109	38	214	13^{a}
2 (Met-Met)	-	-	-	7^{b}
3 (TI-Met)	126	48*	230	10^{b}
Round (age)				
1 (35 wks)	145^{a}	43	336^{a}	12
2 (48 wks)	119^{ab}	47	214^{b}	7
3 (61 wks)	94^{b}	38	152^{c}	11

[1] Synthetic mean squares calculated by Satterthwaite's method, i.e. linear combination of mean squares as follows: (S x G) + (S x R) - (S x G x R)

† $P \leq .10$
* $P \leq .05$
** $P \leq .01$

[a,b,c] Least squares means in the same column with different superscripts differ significantly, ($P \leq .05$).

Table 23. Genetic stock, group (order of tests) and round (age) effects on percentages of hens requiring a single period of restraint to induce TI, closing eyes and vocalizing and/or jumping on recovery in TI tests

	N	Required single restraint period	Closed eyes	Vocalized and/or jumped
Selection: Unselected	356	55	17	67**
Selected	331	50	18	57
Crossing: Strain	333	52	14	65
Crosses	354	53	20	60
Strain w/n Selection-cross subclasses: C_1	90	58	21	58
C_2	89	49	20	79**
$C_1 \times C_2$	90	54	16	70
$C_2 \times C_1$	87	59	9	63
Y_1	67	66*	12	54
Y_2	87	39	26*	47
$Y_1 \times Y_2$	90	56	11	61
$Y_2 \times Y_1$	87	44	21*	66
Group (order of tests)				
1 (Met-TI)	351	53	18	71**
3 (TI-Met)	336	52	16	54
Round (age): 1 (35 wk)	231	31^a	20	42^a
2 (48 wk)	229	60^b	18	69^b
3 (61 wk)	227	67^b	13	76^b

* $P \leq .05$ (from Chi-square test based on actual numbers)
** $P \leq .01$ (from Chi-square test based on actual numbers)
a, b, % with different superscripts within columns differ significantly ($P \leq .05$)

Table 24. Mean squares and least squares means indicating effects of genetic stock, group and round on feather cover and pigmentation scores, body weight and egg productivity traits

Source of variance	MS for F test	df	Feather score	Weight	Wt change	Pigmentation score	Hen-day rate	Hen-house rate	Age of 50% production	Egg weight	Egg mass
				$\log_{10}$ gm							
			Mean Squares								
				$x10^{-3}$							
Selection (Se)	S	1	.00	7.33	4.62	1.43	497.81†	25.07	.33	368.83*	189.50†
Cross (C)	S	1	.19	5.78	1.83	2.31	795.86*	648.12	16.33	48.58	330.77*
Se x C	S	1	23.65†	3.72	1.92	.03	16.86	42.06	.33	1.29	14.62
Strain w/n Se and C (S)	Syn	4	3.65	1.51	-	4.68*	-	-	-	-	-
Strain w/n Se and C (S)	(S x G)[1]	4			1.59		84.77	190.07*	33.12*	42.11**	30.84
Synthetic[2]			4.28	.84		.87					
Group	S x G	2	.69	3.86*	5.37†	.51	24.40	80.50	11.38	4.09	16.90
Se x G	S x G	2	.31	.15	3.20	.48	157.74†	132.82*	.75	7.05	25.56
C x G	S x G	2	1.04	1.27	2.16	.00	172.50*	37.09	.53	2.79	7.06
Se x C x G	S x G	2	1.16	.18	1.06	.18	.24	55.06	7.12	2.61	12.92
S x G	S x G x R	8	2.26†	.58	-	.64	-	-	-	-	-
(S x G)[1]	Error	8			.62		38.56	30.47	6.48	3.05	13.94
Error[1]		46			.75		55.03	42.26	5.46	2.09	12.75
Round	S x R	1	93.37**	5.80*		748.32**					
Se x R	S x R	1	.43	.81		.89					
C x R	S x R	1	.05	.35		.34					
Se x C x R	S x R	1	2.09	.17		.05					
S x R	S x G x R	4	2.83†	.41		.58					
G x R	S x G x R	2	.45	.55†		.00					

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Table 24 continued

Se x G x R	S x G x R	2	.51	.37		.29					
C x G x R	S x G x R	2	.13	.22		.12					
Se x C x G x R	S x G x R	2	.94	.19		.73					
S x G x R	Error	8	.81	.15		.32					
Error		92	.85	.69		.29					
			Least squares means								
			score	gm	gm	score	%	%	wks	gm	gm
Selection: Unselected			6.70	1649	24	5.03	60.45	56.51	27.36	53.51	30.20
Selected			6.70	1705†	63	5.23	65.82†	57.71	27.22	58.13*	33.51†
Crossing: Strain			6.74	1652	31	5.00	59.74	54.05	27.78	54.98	29.67
crosses			6.66	1702	56	5.26	66.53*	60.17	26.81	56.66	34.05*
Strains: C_1			5.86	1670	-1	5.46*	59.50	57.39*	25.22	50.94*	29.23
C_2			6.79	1629	50	4.37	53.63	47.93	30.33	54.14	25.88
$C_1 \times C_2$			7.12	1654	0	5.89*	67.31	63.64*	26.44	54.32	34.58
$C_2 \times C_1$			7.03	1654	47	4.70	61.37	57.05	27.44	54.65	31.12
Y_1			7.48	1637	26	4.91	62.58	53.49	28.44	60.12*	32.17
Y_2			6.82	1684	51	5.27	63.26	57.38	27.11	54.74	31.41
$Y_1 \times Y_2$			6.55	1708	62	5.36	67.57	62.39	26.22	58.08	36.21
$Y_2 \times Y_1$			5.95	1796	117	5.40	69.86	57.59	27.11	59.59	34.27

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Table 24 continued

Group (Test Order)									
1 (Met-TI)	6.67	1643[a]	15	5.14	63.86	58.59	26.63	55.37	32.38
2 (Met-Met)	6.60	1713[b]	62	5.23	61.95	55.01	28.02	56.19	30.87
3 (TI-Met)	6.84	1675[ab]	55	5.02	63.59	57.73	27.23	55.91	32.32
Round (age): 1 (32/44 wks)[3]	7.52*	1652*		2.80**					
2 (63 wks)	5.88	1702		7.46					

1 Pertains to variables measured only once.

2 Synthetic mean squares calculated by Satterthwaite's method, i.e. a linear combinatin of mean squares as follows: (S x E) + (S x R) - (S x E x R)

3 Weight and pigmentation scores at 32 wks; feather score at 44 wks.

† $P \leq .10$
* $P \leq .05$
** $P \leq .01$

a, b Least squares means in the same column with different superscripts differ significantly.

Egg productivity measures: Pigmentation score (past egg production estimator) was *not affected by selection*. However, selected stock hens showed a moderately higher hen-day rate of production as compared to the unselected stock hens (65.82 and 60.45%, respectively; $P<.10$), Table 24. Selection did not show any significant effect on hen-housed rate of egg production nor on age of sexual maturity as estimated by age of 50% production. Selected stock hens laid heavier eggs (58.13 gm) and moderately greater daily egg mass (33.51 gm, $P<.10$) as compared with unselected stock hens (53.51 gm and 30.20 gm, respectively).

Although hens of strains and crosses did not differ in pigmentation scores, hen-housed rate of egg production, age of sexual maturity and egg weight, hens of the crosses had 6.79% higher hen-day rate of production and 4.38 gm greater daily egg mass as compared to hens of the pure strains, Table 24.

Comparisons of strains within selection and cross subclasses revealed that C_1 strain hens had higher pigmentation score and hen-housed rate of production, but laid lighter weight eggs as compared with C_2 strain hens. The $C_1 \times C_2$ cross had a higher pigmentation score and hen-housed rate of egg production as compared to the $C_2 \times C_1$ cross. The only significant difference found between the Y_1 and Y_2 strains was that Y_1 strain hens laid heavier eggs than Y_2 strains (60.12 gm vs 54.74 gm, respectively). Reciprocal crosses between the Y strains did not show differences in any of the traits.

Group (order of testing) Effects

Measures of Fearful Behavior: Order of testing affected latency to first gross head movement in TI tests ($P<.05$), and latency to return to feed in the metronome tests ($P<.10$), Table 22. Thus Group 3 (TI test first) exhibited longer latency until first gross head movement during TI as compared to Group 1 (metronome test first) (48 s and 38 s, respectively). Longer latency to return to feed in the metronome test was shown by Group 1 hens (13 s) as compared to hens of the other 2 groups (7 and 10 s). Group 2 hens which were tested twice with the metronome during each round had the shortest latency based on the second test.

A greater percentage of birds in Group 1 as compared to Group 3 vocalized and/or jumped at termination of TI (71 and 54%, respectively), Table 23. No other group differences were found for eye closure or for incidence of those requiring a single period of restraint.

Feather Score, Body Weights and Egg Production Traits: Group 1 hens had lower body weights than those of Group 2, but no other differences were detected among groups for feather cover scores or egg production traits, Table 24.

Round (age) Effects

Measures of Fearful Behavior: The same birds tested for TI response showed decreasing latencies until first gross leg movement (145, 119, 94 s) and until righting (336, 214, 152 s) over Rounds 1, 2 & 3, respectively, Table 22. Round did not affect latency to return to feeding in the metronome test.

More restraint periods were required to induce TI in Round 1 testing as compared to Rounds 2 and 3. Percentages of hens that required only a single period of restraint were 31, 60, and 67% for Rounds 1, 2 and 3 respectively, Table 23. Similarly, fewer birds vocalized and/or jumped on recovery from TI in Round 1 as compared to Rounds 2 and 3 (42, 69 and 76%, respectively).

Feather Score, Body Weights and Egg-production Traits: Birds had more feather coverage (based on feather score) in Round 1 (at 44 weeks) than in Round 2 (at 63 weeks), Table 24. Body weights increased by 50 gm from Round 1 to Round 2. Pigmentation decreased with continuing egg production as indicated by pigmentation scores of 2.80 and 7.46 in Rounds 1 (32 weeks) and 2 (63 weeks), respectively.

Interactions

Only 3 out of 56 interaction terms considered for measures of fearful behavior (5%) and 2 out of 78 for body weights, feather scores and egg production traits (3%) reached $P<.05$, Tables 22 and 24, respectively. Because these incidences are expected by chance alone, it appears doubtful whether those interactions should be considered further.

The interaction of Group x Round for latency to return to feed in the metronome test reached $P<.01$. Subclass means associated with this interaction are presented in Table 25. Of primary interest is the observation that pullets tested in Group 1 of Round 1, which were tested by the metronome procedure before any of the others, had a latency to return to feed which ranged from twice to four times greater than that of any other group-round subclass. Hens in all other subclasses, having shorter latencies to return to feed had either been exposed directly or indirectly to the metronome previously. Those in Group 2 were previously exposed within each round and those in Group 3 were indirectly exposed to the metronome by its being used on adjacent cages prior to being tested themselves.

Table 25. Least squares means for Group-Round subclasses for latency to return to feed (metronome test)

Round:	Group 1 (Met-TI)	Group 2 (Met-Met)	Group 3 (TI-Met)
1 (35 wk)	27[1]	6	10
2 (48 wk)	7	6	9
3 (61 wk)	13	10	10

[1]Seconds.

Correlation Analysis

Among Measures of Fearful Behavior: No heterogeneity was found for correlation coefficients of fear-associated measurements among strains within groups and rounds. Positive correlation coefficients were obtained for leg movement with head movement ($r = .55$) and for leg movement with righting ($r = .62$), as well as for head movement with righting ($r = .39$), Table 26A. A positive and significant correlation coefficient was obtained for latency until righting of the TI test with latency to return to feed of the metronome test ($r = .23$), although of small magnitude. A positive correlation coefficient between cumulative eye closure and righting time ($r = .60$; $P<.01$) was detected.

Correlation Between Measures of Fearful Behavior and other Quantitative Traits: Where heterogeneity between strains was absent, population estimates were calculated. A small but significant correlation ($r = .22$) was found for body weight and latency until righting, Table 26B. The great variability among strains and crosses for correlations of body weight-change with latencies until first gross leg and head movements is shown in Table 26C. There was of a lack of heterogeneity among strains for correlation of egg mass with righting time in Rounds 1 and 3; the population estimate, though essentially zero in Round 1, was found to be negative and significant in Round 3, Table 26D. Extreme variability among the strains and crosses in Round 2 for the egg mass and righting time correlation is shown in Table 26E. All the correlation coefficients for selected strains and their crosses were positive, while 3 of 4 for the unselected strains and their crosses were negative.

Table 26. Correlation coefficient estimates among latency measures of fearful behavior and between latencies and measures and between latencies and measures of quantitive traits.

	Leg movement	Head movement	Righting	Return to feed (Metronome)
A[1]				
Leg movement		.55**	.62**	.16
Head movement			.39**	.02
Righting				.23**
B[1]				
Body weight	.07	-.12	.22**	.04
Weight-change	see below	see below	-.18	-.18
Feather score	.06	-.05	-.10	-.01
Pigmentation score	.15	-.04	-.09	.03
Hen-day rate	.03	-.10	-.04	-.03
Hen-housed rate	.09	-.07	-.05	.05
Egg weight	.07	-.09	.08	-.04
Egg mass	.09	-.07	see below	.05
Age 50% hen-day	.02	.06	.01	-.07
C[2] Strains and crosses				
Weight change				
C_1	-.81	.56		
C_2	.61	-.90*		
$C_1 \times C_2$	.07	.77†		
$C_2 \times C_1$	.88*	.52		
Y_1	.09	-.52		
Y_2	.24	.46		
$Y_1 \times y_2$	.02	-.58		
$Y_2 \times Y_1$	.88*	.24		

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Table 26 continued

D[3]

Egg mass	
Round 1	.01
Round 2	see below
Round 3	-.46**

E[4]

Egg mass	
C_1	-.87*
C_2	-.30
$C_1 \times C_2$	-.18
$C_2 \times C_1$	.53
Y_1	.99**
Y_2	.18
$Y_1 \times Y_2$	.81*
$Y_2 \times Y_1$	.55

† $P \leq .10$
* $P \leq .05$
** $P \leq .01$

1 Mean correlation coefficients were calculated when heterogeneity was not found among strain-round subclasses.

2 Mean correlation coefficients were calculated for strains over rounds; heterogeneity was present among strains.

3 Mean correlation coefficients were calculated within rounds when heterogeneity was not found among strains.

4 Correlation coefficients are presented by strains for Round 2 because of heterogeneity.

Correlations Among Body Weight, Feather Score and Egg-production Traits: Heterogeneity tests were conducted for correlation coefficients based on strain-round subclasses and where that was not detected, estimates of population correlations were calculated with results as presented in Table 27A. Thirty-three correlations coefficients were tested for significance and 5 were indicated as occuring only by chance at the .05 level. Because those were all of small absolute size (<.25) and because of the large number tested, they should be regarded with some skepticism.

Negative associations were found between body weight and feather score (r= -.20), between pigmentation and feather scores (r= -.22). Age of sexual maturity, estimated by age of 50% production was negatively associated with pigmentation score (r= - .24), hen-housed rate of egg production (r= - .24) and egg mass (r = - .25).

Because heterogeneity among the strains and crosses for correlations of hen-day rate with hen-housed rate of egg production, and for correlations of hen-housed rate of production with egg mass was detected, the individual stock values are presented in Tables 27B and 27C, respectively. All were positive.

DISCUSSION

Techniques Used in Tonic Immobility Tests

Cradle Construction. Cradles were constructed with dimensions as given by Jones and Faure (1980) and covered with black velvet clothes during TI tests. Jones and Faure indicated that chickens restrained in the dorsal position on such a support were reliably induced into the immobile state. However, as it was not clear from their description whether a support was directly under the bird's back or not, cradles were built both with and without such a support for testing (Figure 1). TI was induced in 78% of hens with a single 15-s restraint

Table 27. Correlation coefficients among body weight, weight change, feather score and egg-production traits

A[1]	Body Weight	Weight change	Pigment score	Feather score	Henday rate	Henhouse rate	Egg wt	Egg mass	Age 50%
Body weight	-	.02	.13	-.20*	-.02	.03	.08	.05	-.13
Weight change		-	.02	-.21	-.02	-.08	.21	-.03	.05
Pigment score			-	-.22*	.10	.15†	-.03	.14	-.24*
Feather score				-	-.10	-.15†	.07	-.13	.09
Hen-day rate					-	see below	-.11	see below	-.14
Hen-housed rate						-	-.13	see below	-.24*
Egg weight							-	-.002	-.01
Egg mass								-	-.25*
B. Hen-day rate with hen-housed rate and egg mass									
Strain:									
C_1	Hen-day					.87**		.68*	
C_2	Hen-day					.69*		.69*	
$C_1 \times C_2$	Hen-day					.60†		.49	
$C_2 \times C_1$	Hen-day					.56		.58†	
Y_1	Hen-day					.32		.35	
Y_2	Hen-day					.47		.38	
$Y_1 \times Y_2$	Hen-day					.60†		.52	
$Y_2 \times Y_1$	Hen-day					.14		.10	
C. Hen-housed rate with egg mass									
Strain:									
C_1	Hen-housed							.74*	
C_2	Hen-housed							.83**	
$C_1 \times C_2$	Hen-housed							.74*	
$C_2 \times C_1$	Hen-housed							.85**	
Y_1	Hen-housed							.96**	
Y_2	Hen-housed							.74*	
$Y_1 \times Y_2$	Hen-housed							.75*	
$Y_2 \times Y_1$	Hen-housed							.83**	

† $P < .10$
* $P \leq .05$
** $P \leq .01$

1 Mean correlation coefficients were calculated when heterogeneity was not found among subclasses.

period when the back was not directly supported and in 60% of hens when the back was supported. Latencies, eye closure and vocalization and/or jumping up at righting did not differ for the two cradle types.

Although TI was more readily induced in the cradle lacking direct support for the bird's back, it was decided that a back support would be used in further testing because hens differing considerably in body size would be tested in the year-long study period. Lack of a back support could have a differential effect depending on body size. Although this hypothesis was not tested directly, observations from a previous pilot study revealed that small bodied hens sank into the trough of the cradle and usually made several struggling attempts at righting time. It is of interest to note that mean TI induction with a single restraint period ranged from 53 to 63% over the four experiments. When TI was not induced in the first restraint period it usually occurred in the second, third, or fourth. Thus, of 50 hens tested in a cradle with a base in Experiment 1, percentages of TI inductions were 60, 32, and 8 with 1, 2 and 3 restraint periods, respectively. Similar results were obtained in the other experiments and Gallup et al. (1971) reported that they used a standard criterion of 1-5 restraint periods with 3-4 week old chicks. If TI was not induced in the 5th trial the subject was considered to have zero duration of TI.

Immediate and Delayed Testing. Whether a hen was chased, caught and tested immediately or temporarily confined before testing appeared only to affect the ease of induction but not latencies nor eye closure once induced. TI was more readily induced in hens tested immediately after capture. Perhaps the chasing immediately before testing provoked a more fearful reaction resulting in increased TI susceptibility, while temporarily confining them allowed some recovery from the more fearful response elicited by chasing.

When latencies to recover from TI were regressed on catching and holding times, only catching time was found to have a significant effect, and that was limited to first gross leg and head movements. Hens which were chased for longer periods showed leg and head movements more promptly after TI induction, but there was no measureable effect on righting. Because righting time appeared to be insensitive to amount of chasing prior to capture (within the range of catching times in Experiment II), it may be a more reliable measure of genetic and housing effects on fearful behavior. Different catching times may be associated with different genetic stocks and housing systems and those should not be confounded with underlying differences in the genetic or housing effects.

It was decided that TI tests could be carried out immediately after capture of hens from floor pens with relatively reliable results if righting time was used as the primary criterion of recovery from TI. Jones and Faure (1981c) confined their hens for about 15 min when caught in floor pens before TI testing, but they presented no justification or evidence bearing on the desirability of that practice.

Associations Among Latency Measures

Within Subclass Correlations. Latencies of individuals to recover from TI were available in all experiments and to return to feeding after avoiding the metronome in Experiment IV. First gross leg and head movements after TI induction yielded consistently positive and significant coefficients ranging from .55 to .71. Correlations between latency till leg movement and righting time were more variable, but coefficients were again consistently positive and significant with values ranging from .50 to .99. First gross head movement and righting time were less consistently correlated among subclasses; some negative values were obtained in Experiment II, but most were positive. Excluding the heterogeneous values of Experiment II, values ranged from .25 to .71.

Jones and Faure (1981c) proposed that TI should apply strictly to the state of inhibition, i.e. from induction till first alert head movement. However, determination of both first gross leg and head movements required much subjective judgement to differentiate between those subtle changes in position associated with breathing and slow movements which could result in marked changes in positions of the legs or head over a period of a minute or more. In constrast, righting response was typically a clear-cut change, occurring relatively rapidly, which could be determined with ease. Of the correlations between latency measures of TI and latency to return to feed in the metronome test, only latency till righting showed a significant but small (.23) association. This suggests that to a limited extent righting time and recovery from an avoidance response have some elements in common.

<u>Consistency of Measures Associated With Genetic Differences</u>. C_1 hens required 75, 23, 53 and 11% more time to right themselves than did C_2 hens in Experiment I, II, III, and IV, respectively. Only the difference in Experiment I was significant, perhaps because more hens of those two strains were compared in that experiment and because only one kind of housing environment (single-hen cages) was involved. Comparison of C_1 and C_2 hens involving latency to leg and head movements failed to detect differences and strain responses were inconsistent in sign from experiment to experiment.

Y_1 strain hens had longer righting time than Y_2 hens in the three experiments where they were compared; they remained immobile 153% ($P<.01$), 61% and 52% longer in Experiments II, III and IV respectively. Leg and head movement latencies gave in consistent results for the Y strain comparisons, as they did also for those between the C strains.

Further comparisons of TI latencies among genetic stocks revealed that hens of selected stocks consistently showed shorter periods until first gross head movement than did hens of unselected stocks. A similar, but non-significant trend was apparent for leg movements but not for righting time.

Because most TI studies reported have been by lateral restraint inductions (Gallup, 1973, 1977, 1979; Gallup et al., 1971, 1972, 1976) and by ventral restraint induction (Rovee and Luciano, 1973; Rovee et al., 1973), only duration of TI (comparable to our latency until righting) has been the main criterion of the phenomenon. However, because of the use of dorsal restraint technique Jones and Faure (1981b) were able to report comparison of three latency measures (leg and head movement and righting time). In other TI studies, head movement and righting time were the only latency measures compared (Jones and Faure, 1980, 1981a,c).

When Jones and Faure (1981a) compared the effect of regular handling vs non-handling on TI in three strains (Broiler, Warrens and Nick chicks), they found consistently shorter latencies till first alert head movement and righting time with regularly handled chicks than with non-handled chicks in all three strains. Only righting time differences in Warren strains were non-significant. However, Jones and Faure (1981c) found no difference in latency till first head movement and till righting time between adolescent "T" (Rhode Island Red x Light Sussex) and "S" (White Leghorn) lines but both head movement and righting time were considerably shorter in "J" line birds (Brown Leghorn). Gallup et al. (1976) reported substantial strain differences breeds breeds of chickens, with hybrids exhibiting intermediate durations.

Latency to return to feeding following exposure to the metronome failed to reveal genetic differences in Experiment IV. Craig et al. (1983) found a large difference between the Y strains in their study with Y_2 hens taking longer to return to normal activity and feeding. A similar, though non-significant difference was found in this study; Y_2 hens required about 70% more time to return to feeding than Y_1 hens. Although the differences were non-significant in Experiment IV, it may be noted that the TI test indicated Y_1 hens to be more fearful (as found also in Experiment II and III) but the metronome test gave the opposite result, i.e. it indicated greater avoidance by Y_2 hens (as found by Craig et al., 1983). Thus, it appears that these two types of tests for fearful responses may be measuring different characterisitics of fearful behavior.

Consistency of Measures Associated With Treatment Effects. TI latency measures revealed no differences associated with cradle construction (Experiment I) or immediate vs delayed TI inducttion (Experiment II). However, housing environments (Experiment III) and order and round of testing (Experiment IV) did have measurable effects.

Hens from colony cages (17/C) had consistently longer latencies after TI induction than did hens from any other environment. Hens from multiple-bird cages (5/C) had the shortest leg and head movement latencies but longer righting times than those from single-hen cages or floor pens. Jones and Faure (1981b) found consistencly significant shorter latencies to first leg and head movements and till righting time among hens housed in floor pens with floor area of 9300 cm^2 per hen than among hens housed in multiple-bird cages (5/C) with floor area of 700 cm^2 per hen.

In considering the results of Experiment IV, it is seen that order of applying metronome and TI tests to the same hens did not appear to have any consistent influence within rounds. When comparing rounds (ages) there was a clear-cut stepwise reduction in latency until first gross leg movement and righting time, but latency to first head movement showed relatively little reduction with repeated testing and no significant differences were found for that measure. Most of the habituation studies reported (Nash, 1978; Nash, Gallup and Czech, 1976; Nash et al., 1976) have all been on the duration of TI (same as righting time if dorsal restraint technique is employed) and have consistently shown a stepwise decrease in duration with repeated testing.

The only significant association between latency measures of TI and metronome tests was righting time with latency to return to feeding ($r = .23$).

Relationships between latencies to first gross leg and head movements and righting time need clarification. Righting time or duration appears to be the most desirable measure of TI because it is a clear-cut response, less influenced by period of chasing and catching (as in floor pens and colony cages), gives more consistent evidence of genetic differences and housing effects and yields convincing evidence of habituation effects. In most of the studies of TI reported, duration of the response, which in the original context of the term "tonic immobility," has been the main criterion used (Gallup, 1973, 1977, 1979; Gallup et al., 1971; Rovee et al., 1973, Nash et al., 1976). Therefore, further consideration of TI duration will be restricted to righting time.

Non-parametric Measures of Tonic Immobility

Susceptibility to Induction. Hens tested in a cradle without a base were more susceptible to TI induction than those tested in a cradle with a base. Hens in floor pens chased, caught and tested immediately were also more easily induced than those similarly caught but temporarily confined before testing.

The only genetic effect noticed was that presumed due to random genetic drift; Y_1 strain hens were more susceptible than Y_2 strain hens. However that effect was detected as significant in only one of three experiments. Hens from both floor pens and colony cages (17/C) were more susceptible to TI induction than hens from either single-hen or multiple-bird (5/C) cages. This result may have been associated with the chasing and greater difficulty of capturing hens in these environments in which 15 or 17 hens were present as compared to cages containing only one or 5 hens from which capture was easy.

Order of testing had no apparent influence on susceptibility to TI induction within rounds, but hens tested in Round 2 and 3 were more susceptible than when first tested in Round 1. This result is contrary to expectation if greater ease of induction is associated with a more fearful state; decreasing fearfulness associated with repeated TI testing should have led to habituation and more difficulty in induction rather than less (Nash and Gallup, 1976; Nash *et al.*, 1976; Nash, 1978).

Eye closure. Eye closure was not influenced by type of cradle used, nor by immediate vs delayed testing.

The only genetic effect was apparently associated with random genetic drift in that significantly more C_1 and Y_2 strain hens closed their eyes during the response than did C_2 and Y_1 hens. Strain within selection scheme differences were detected in only one of 4 and one of 3 experiments for the C and Y strains, respectively.

There was no indication of housing environment, order of testing, round or habituation effects on eye closure.

Vocalization and/or Jumping. Vocalization and/or jumping on recovery from TI was not influenced by the type of cradle used. The only genetic differences found were that selected strain hens of Experiment IV were less likely to vocalize and/or jump up on recovery and there were indications of random genetic drift in that C_1 strain hens of Experiments I and IV were less likely to vocalize and/or jump than were C_2 strain hens.

Hens from both floor pens and colony cages were more more likely to vocalize and/or jump up at righting than hens from either single-hen or multiple-bird cages.

In Experiment IV it was found that birds exposed to the metronome test before TI testing were more likely to vocalize and/or jump up than birds tested for TI first. When tested in a second and/or a third round, hens were more likely to vocalize and/or jump than during the first test.

Association Among Non-parametric Measures. In evaluating the relationship among the non-parametric measures of TI, it was apparent that for round treatment effects of Experiments 3 and 4 and housing environment effects of Experiment 3, hens that were more susceptible to TI induction were also more likely to vocalize and/or jump up on recovery from TI. It was observed in only one experiment that higher incidence of eye closure appeared in the group that were less likely to vocalize and/or jump up on recovery. Only in the round treatment effect of Experiment III was it observed that the more susceptible birds were more likely to keep their eyes opened during the TI response. Gallup, Nash and Wagner (1971) found that duration of eye closure was highly associated with latency of vocalization ($r = .84$, $P<.05$).

Associations Among Non-parametric Measures and Righting Time. Non-parametric measures failed to show consistent associations with latency until righting time. However based on cumulative eye closure in Experiment IV, a significant positive association ($r = .60$; $P<.01$) with righting time was found. Rovee and Luciano (1973) suggested that eye closure was predictive of prolonged reaction. Gallup, Nash and Wagner, (1971) also found a positive association between duration of TI and duration of eye closure ($r = .91$; $P<.05$).

Body Weight, Feather Cover and Egg-production Traits

Genetic Stock Effects. Selected strain hens had higher hen-day rate of lay ($P<.10$), heavier eggs and greater egg mass ($P<.10$) than unselected strain hens. Craig et al. (1982) reported that selection for part-year egg mass resulted in increased egg mass with correlated responses of decreased age of sexual maturity, increased egg weight, and increased hen-housed rate of lay. In the current study, selection effects were not detected for age of sexual maturity, hen-housed rate of lay, feather cover, body weight and pigmentation. Crosses were found to have higher hen-day rate of lay and greater egg mass than the strains, which was indicative of hybrid vigor for these traits.

C_1 strain hens had higher egg production as estimated by pigmentation score, and also higher hen-housed rate of lay, but laid lighter eggs than C_2 strain hens. Y_1 strain hens laid heavier eggs than Y_2 strain hens. These differences are presumably due to random genetic drift as also reported by Craig et al. (1982) for most traits in within-generation comparisons.

Associations Among Quantitative Traits. Of 33 mean correlation coefficients calculated, only 5 were significant. Those were all negative and of small magnitude (values ranged from -.20 to -.25). Poorly feathered hen were slightly heavier in body weight and appeared to be better layers as indicated by pigmentation score. Earlier maturing birds had lost more pigmentation as expected and had slightly higher hen-housed rate of lay and egg mass. Craig et al. (1982) also reported a negative association between egg mass and age of sexual maturity.

Associations Among Latency Measures of Fearfulness and Quantitative Traits (Experiment IV). Few significant correlation coefficients were found between latency measures of fearfulness and other quantitative traits in Experiment IV. Heavier body weight was associated with slightly longer righting time ($r = .22$; $P < .01$). Egg mass for the year was associated with shorter righting time in Round 3 TI tests. Egg mass showed no association with righting time in Round 1, but associations between these variables were heterogeneous among genetic stocks in Round 2. Using other measures of fearfulness, Siegel et al. (1978) and Mauldin and Siegel (1979) found essentially no significant correlation between fearfulness and production traits. However, Sefton (1976) and Sefton and Crober (1976) reported negative correlations between fearfulness and production traits; for example lower fearfulness was associated with higher egg production.

In the current study, no significant association was found between feather cover and avoidance response, although such an association was reported by Craig et al. (1983). This discrepancy may be due to differences in the housing environments since their tests were carried out in colony cages containing 14 hens each, while in this study, hens were housed 5 per cage.

Environmental and Testing Effects on Latency Measures of Fearful Behavior

Housing Enviroment (Experiment III)

Hens in colony cages (382 cm^2 per hen) exhibited longer latency till righting time than hens in floor pens (2323 cm^2 per hen), single-hen (930 cm^2 per hen) and multiple-bird (372 cm^2 per hen) cages. This suggests that hens in colony cages were the most fearful. This partially supports Jones and Faure's (1981b) results in which hens in 4-hen cages exhibited longer latency till righting than hens in floor pens. However, their hens had much more space, i.e. 700 cm^2 and 9300 cm^2 per hen in cages and floor pens, respectively.

Order of Testing (Experiment IV)

Hens of Group 1 that were first exposed to a metronome test before TI exhibited longer latency to return to feed but shorter latency till righting (duration of TI) than hens of Group 3 that were first TI tested before metronome exposure. However, Group 2 hens that were exposed to the metronome test twice, and second test data used for analysis, exhibited the shortest latency to return to feed. Previous experience therefore seemed to have determined the strength of response to the stimulus.

Habituation (Experiment IV)

Nash and Gallup (1976) defined habituation as a "relatively permanent, stimulus-specific decrement in response strength that occurs as a consequence of repeated response elicitation." In the current study, repetition of testing was much less frequent and longer intervals were used than in the studies of Nash and Gallup (1976), Nash et al. (1976) and Nash (1978) who did TI testing on 4 consecutive days with 5 inductions per day per subject at 15 sec. intertrial interval. Also no regular handling exercise was employed in the current study which Jones and Faure (1981a) found to be effective in reducing latency until righting. Nevertheless, the results show clear evidence of habituation in TI testing.

Short-term habituation was evident with the group of hens tested twice with the metronome at 3-week intervals within each round. Data from the second test indicated that these hens exhibited a shorter latency to return to feed as compared to those of Group 1 tested once only with the metronome within each round.

Although both TI and metronome tests were repeated over three rounds at 13-week intervals, long-term habituation was evident only in the TI tests. Repeated testing resulted in stepwise decrease of latency until righting over the three rounds.

Interactions

Only 13 out of 198 (7%) interaction terms tested in analyses of variance had $P<.05$. Although some of these 13 interactions may be of importance, this frequency of occurrence is nearly that expected by chance alone. Therefore, those interactions do not warrant further discussion at this time.

CONCLUSION

Cradle construction had no effect on latency measures of TI, but hens tested on a cradle without back support were more susceptible to TI induction. Immediate vs delayed testing for TI response also did not influence any of the latency measures, but hens tested immediately following capture in floor pens were more susceptible to induction. Longer catching periods were associated with shorter latencies until first gross leg and head movements.

Hens housed in colony cages exhibited longer latencies and closed their eyes more than hens from any other housing environment. However, hens in both floor pens and colony cages were more susceptible to TI induction and were more likely to vocalize and/or jump upon recovery than hens from single-hen and multiple-bird cages.

Hens exposed to the metronome test before TI testing, exhibited longer latency to return to feed, but shorter latency to righting time in the TI test and were more likely to vocalize and/or jump upon recovery as compared to those TI tested before the metronome test. Hens tested twice by the metronome with a 3-week interval between tests, showed short-term habituation to the tests. Although long-term habituation at 13-week intervals was absent for metronome tests, it was evident in TI tests where there were stepwise decreases of latency till righting over the three rounds.

Selected strain hens had shorter latency to first gross head movement in one of 3 experiments, higher hen-day rate of lay, heavier eggs and greater egg mass. Hybrid vigor was absent for measures of fearful behavior but evident for hen-housed rate of lay and egg weight. Random genetic drift caused strain differences within selection schemes for righting time of the TI test and also for some quantitative traits.

Associations among TI latency measures were significant and positive. Only righting time of TI was associated with latency to return to feed in the metronome test. Only one measure of fearful behavior (righting time in TI testing) was associated with body weight and egg mass for hens in 5-bird cages.

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GENETIC STOCK AND HOUSING ENVIRONMENT EFFECTS ON TONIC IMMOBILITY, AVOIDANCE BEHAVIOR AND QUANTITATIVE TRAITS IN WHITE LEGHORN HENS

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In a series of 4 experiments carried out on 8 genetic stocks of White Leghorn layers, two measures of fearful behavior, tonic immobility (TI) and recovery from the avoidance response following exposure to a metronome were investigated.

Hens tested on a cradle without support for the back of the hen were more easily induced into TI (more susceptible) than those tested on a cradle with a back support. However, cradle construction had no effect on any of the latency measures of TI. Hens from floor pens chased, caught and immediately subjected to the TI tests were also more susceptible to the TI induction than those chased, caught and temporarily confined before testing. However, there were no significant differences between the immediate and delayed testing for any of the latency measures of TI. Nevertheless, regression analysis indicated that longer catching periods were associated with shorter latencies till first gross leg and head movements.

In housing environment comparisons, hens in colony cages (17/C) exhibited longer latencies and closed their eyes more during TI than hens in floor pens, single-hen and multiple-bird (5/C) cages. Although hens in multiple-bird cages showed the shortest latencies till first gross leg and head movements, they exhibited an intermediate latency till righting between those in colony cages and those in both floor pens and single-hen cages. Birds in both colony cages and floor pens were more easily induced into TI than birds in either single-hen or multiple-bird cages.

Hens that were exposed to the metronome test before being tested for TI showed longer latency to return to feed but exhibited shorter latency till righting with more of them vocalizing and/or jumping on recovery from TI response than those tested for TI before the metronome test. The group of hens tested twice at 3-week intervals within each round had significantly shorter latency to return to feeding during the second test as compared to hens previously unexposed to either TI or metronome testing. Although habituation was evident for the metronome tests conducted twice at 3-week intervals, it was not evident between the same tests conducted at 13-week intervals. Habituation was found for TI when tests were conducted at 13-week intervals; there was a step-wise decrease in latencies until righting time over Rounds 1, 2 and 3.

Although hens of strains selected for part-year egg mass consistently exhibited shorter latency till first gross head movement than unselected strain hens, in three experiments, the difference was significant in only one. Selected strain hens had higher hen-day rate of lay and laid heavier eggs with greater egg mass. Although strain-cross hens did not differ behaviorally from the mean of strains used in crossing, hybrid vigor was evident for hen-housed rate of lay and egg weight.

Random genetic drift influenced both TI and some quantitative traits. C_1 and Y_2 strain hens, consistently exhibited longer latency till righting than C_2 and Y_1 strain hens, respectively. However, only in one of four experiments for the C strains and in one of three experiments for the Y strains were the differences significant. C_1 strain had a higher pigmentation score (estimating past production record) and hen-housed rate of lay and laid heavier eggs than C_2 strain hens in the one experiment where those traits were measured. Y_1 strain hens laid heavier eggs than Y_2 strain hens.

Associations among latency measures of TI were all positive and significant. Only righting time after TI induction showed a significant association (r=.23) with latency to return to feeding after avoidance of a metronome. In examining correlations between fear-associated latency measures and other quantitative traits in the final experiment, only righting time appeared to be associated. The value of righting time and body weight was .22 ($P<.05$). Righting time in Round 3 (61 weeks of age) was negatively associated with egg mass ($r= -.46$; $P<.01$).