

GENETICS OF INITIAL IMPRINTING RESPONSES:  
SELECTION, AND HETEROSIS

by

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Thesis submitted to the Graduate Faculty of the  
Virginia Polytechnic Institute  
in partial fulfillment for the degree of

DOCTOR OF PHILOSOPHY

in

Genetics

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May, 1968

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## INTRODUCTION

Newly hatched chicks of many species will respond toward, approach, and stay near any outstanding feature of their environment. If the object moves, they attempt to follow, and it has been shown that chicks exposed to such stimuli during a certain early period will subsequently favor that object over their own kind. It is not surprising that this phenomenon has attracted a great deal of attention from naturalists and experimentalists alike, but it is surprising that the basic biological nature of imprinting remains obscure.

Experiments I and II of this dissertation were designed to study the underlying genetical mechanisms of imprinting and to establish foundation stocks for the investigation of related aspects of the phenomenon. Experiment III was designed to investigate effects of stimulation in different sensory modalities just prior to testing and to determine effects of age and prior socialization. The fourth and final section contains brief discussions of peripheral experiments.

## REVIEW OF LITERATURE

### Imprinting

Previous Work and Present Outlook. Imprinting is a form of social behavior that occurs shortly after birth or hatching. Although imprinting is characterized by the formation of parent-offspring and/or offspring-offspring bonds, the innate tendencies to establish these social relationships can be released by a variety of experimental devices. Verplanck (1957) states that imprinting is said to have occurred if an individual exhibits toward an object to which it had been exposed during the first few hours of life, behavior normally exhibited only toward members of its own species.

The biological function of the trait may be primarily to insure that precocial young will approach and stay near their parents when parental care and/or instruction may be advantageous. Mating preferences established by imprinting may also have positive selective value in some species.

Much of the voluminous literature concerning imprinting was reviewed by Hess (1959), Grey (1963), Sluckin (1965), and Bateson (1966). The latter noted that the phenomenon has become increasingly ill-defined in recent years.

The word imprinting was first used by Lorenz (1937), but Grey (1962a, 1963) noted that Spalding (1872, 1873) was the first to study the phenomenon. Charles Darwin (1873) pointed out that Spalding's work with young chickens and turkeys proved that "many special fears, tastes, etc., which must have been acquired at a remote period, are now strictly inherited." Darwin was unaware of the principles of Mendelian genetics, but came close to grasping the idea of particulate inheritance as evidenced by a letter to Huxley in which he stated "Approaching the subject from the side which attracts me most, viz., inheritance, I have lately been inclined to speculate, very crudely and indistinctly, that propagation by true fertilization will turn out to be a sort of mixture, and not true fusion" (in Fisher, 1930). The approach to imprinting taken in this dissertation is from the same viewpoint. Namely, that the determination of the underlying inheritance of a behavior character should be a first step of its study. Surprisingly, there is a dearth of knowledge concerning the genetic aspects of imprinting.

The innateness of imprinting responses was noted by Spalding (1872, 1873), and Lorenz (1937) was perhaps overly impressed by the instinctive nature of the phenomenon. Smith and Templeton (1966) reported that genetic factors do not significantly influence the response of

domestic chicks to a visual imprinting device, and Grey, et al., (1966) reported that "responsiveness to and learning of different models at different ages is differently inherited." To the writer's knowledge, this dissertation is the first comprehensive study of the inheritance of response, approach, and stay-near tendencies of chicks in an imprinting situation.

Measurements of Imprinting. Since imprinting is a complex trait which consists of both tangible and intangible events, investigators either go to great lengths to show that the trait measured is really related to imprinting or, more often, speak of traits such as "the approach response" or "the following response" and assume some relationship with imprinting. It is doubtful that imprinting per se can be directly quantified. Many previous attempts to measure the phenomenon have been criticized on the basis of mathematical and/or biological soundness. For example, Salzen and Sluckin (1959) and Hess (1959) used mobile imprinting devices designed to measure the distance followed, whereas Guiton (1959), Pitz and Ross (1961), and Thompson and Dubanoski (1964) used similar units and measured the time spent in following. These methods have been criticized by Connolly and Moray (1964) on the basis of failure to account for chance following scores.

The approach response has also been used as indicative of imprinting tendencies. James (1960a, 1960b) and Abercrombie and James (1961) suggest that the approach response is determined by the same mechanisms that cause conventional imprinting. Schneirla (1959) feels that imprinting may be a unitary process characterized by a tendency to approach a suitable object.

Smith (1962) investigated relative effectiveness of various visual as well as auditory stimulation. Ramsey (1951), Gottlieb (1963), Gottlieb and Klopfer (1962), and numerous other investigators have attested to the relative importance of auditory stimulation in the imprinting situation. The effectiveness of stimulation in specific sensory modalities in releasing imprinting responses may vary according to the evolutionary history of the species.

### Selection and Heterosis

#### Selection

Selection may be defined as the non-random differential reproduction of genotypes. Individuals with superior reproductive fitness may contribute proportionately more offspring to subsequent generations and thereby cause changes in gene frequencies. This process may occur naturally or artificially, and the expected goals of artificial selection may differ from the real-

ized goals if natural selection is operative. Artificial selection is purposeful and directed. It is usually much stronger than natural selection because emphasis is generally concentrated on changing one or a few traits, whereas natural selection operates on the totality of the fitness components.

The Heritability Concept. Johannsen (1903) was the first to show that selection within isogenic lines did not change population means. This is because genetic variation is essential for selection to be effective, and the amount of genetic change is limited by the amount of heritable variation. Lush (1945) partitioned the phenotypic variance ( $\sigma_P^2$ ) into three main parts according to its causes: (1) that due to the environment, (2) that due to heredity, and (3) that due to joint effects of environment and heredity. These causes have been elaborated on in several texts (eg. Lerner, 1950, 1958; Falconer, 1960) and will be briefly discussed here.

That portion of the total phenotypic variance that is due to genetic effects is the heritability ( $h^2$ ). Since

$$\sigma_P^2 = \sigma_G^2 + \sigma_E^2 + 2r_{GE} \sigma_G \sigma_E + f(GE)$$

where,

$$\sigma_G^2 = \text{genotypic variance,}$$

$$\sigma_E^2 = \text{environmental variance,}$$

$r_{GE}$  = correlation between genotype and environment,

$\sigma_G$  and  $\sigma_E$  = genotype and environmental standard deviations, respectively,

$f(GE)$  = interaction between genotype and environment,

and 
$$\sigma_G^2 = \sigma_A^2 + \sigma_D^2 + \sigma_I^2$$

where  $\sigma_A^2$  = additive genetic variance,

$\sigma_D^2$  = dominance variance, and

$\sigma_I^2$  = epistatic variance,

a broad estimate of  $h^2$  is  $\sigma_G^2 / \sigma_P^2$  and a narrow estimate is  $h^2$  is  $\sigma_A^2 / \sigma_P^2$ . If  $2r_{GE}\sigma_G\sigma_E$  and  $f(GE)$  are considered zero, then  $\sigma_G^2 + \sigma_E^2 / \sigma_P^2 = 1$ . In the purely additive scheme  $\sigma_A^2 + \sigma_E^2 / \sigma_P^2 = 1$ . There are procedures for the estimation of  $\sigma_P^2$ ,  $\sigma_G^2$ , and  $\sigma_A^2$  (Lush, 1945; Lerner, 1950; Falconer, 1960; Kempthorne, 1957) which thus enables calculations of  $h^2$ . Although these estimates for a trait are for a particular population at a certain point in time, they are still valuable as aids to breeders and as keys to understanding the biological nature of the traits.

Correlated Traits. Since a trait cannot be divorced from an organism, it is clear that characters interact. Lerner (1958) noted that the very notions of coadaptation and



homeostatic regulation imply correlations among responses. It can easily be shown (Hazel, 1943; Lerner, 1950, 1958; Falconer, 1960) that a phenotypic correlation ( $r_{P_{xy}}$ ) between two traits is:

$$r_{P_{xy}} = h_x r_{G_{xy}} h_y + e_x r_{E_{xy}} e_y.$$

Algebraic manipulation allows computation of  $r_{G_{xy}}$  and  $r_{E_{xy}}$  if data for calculation of the heritabilities of traits X and Y are available.

Genetic correlations are caused by linkage, which is a transient effect, and pleiotropy, which is a permanent effect. Genetic and environmental correlations are confounded in phenotypic correlations, and therefore it is important that they be calculated. Genetic relationships are of particular importance because they provide information on concomitant changes in other traits. This is particularly valuable in studying complex traits such as imprinting, because all components of imprinting will necessarily be affected if selection is for one component. Genetic correlations may range from +1 to -1 and may be asymmetrical (Siegel, 1962; Nordskog and Festings, 1962; Bohren, et al., 1966).

#### Heterosis

Dobzhansky (1952) noted that heterosis encompasses

several distinct phenomena. These are: (1) mutational euheterosis, which results from the sheltering of deleterious recessive mutants by their selectively superior dominant alleles, (2) balanced euheterosis (overdominance), which is the superiority of heterozygotes over either homozygote class, and (3) luxuriance (pseudoheterosis), which is hybrid superiority resulting from a chance combination of genes which happen to be complementary despite, not because of, the evolutionary history of the parental stocks. Mutational and balanced heterosis result from natural selection modifying gene frequencies and/or expressions of the parental types. Luxuriance is not indicative of evolutionary adaptation.

Although Fisher's (1930, 1941) fundamental theorem of natural selection ("the rate of increase in the average fitness of a population at any time is equal to the genetic variance in fitness at that time.") may not be precisely correct under certain conditions, (Kimura, 1965; Li, 1967), it provides insight into the genetic nature of a trait. Since fitness is made up of many components, natural selection operates on an index basis. Selection pressure is greatest on those traits which contribute most to fitness. Since the heritability ( $\sigma_A^2 / \sigma_P^2$ ) of a trait with no history of artificial selection is low if the trait is a component of fitness,  $\sigma_A^2$  would have been

lowered through natural selection increasing gene frequencies and expressions. Crossing experiments are therefore valuable in determining whether a given trait is a component of fitness.

EXPERIMENT I: BIDIRECTIONAL SELECTION FOR CHICK'S RESPONSE  
TOWARD AN IMPRINTING APPARATUS

Methods and Materials

Stocks and Management. The stocks consisted of lines selected for high and low body weight (Siegel, 1962) and high and low mating behavior (Siegel, 1965). The weight lines originated from crosses of seven inbred lines of White Rocks and the mating behavior lines from the Athens-Canadian Randombreds (Hess, 1962). Initially an attempt was made to determine if related lines of chicks differed in their imprinting responses. I looked for differences between the weight lines and between the mating behavior lines.

Selection was relaxed in a sample of the  $F_6$  generation of the low weight line, and the foundation stock for the bidirectional selection experiment was from this relaxed line. Two lines were then selected on the basis of individual responses toward the imprinting apparatus. Chicks that responded fast formed the parental stock of the fast response line (FR), whereas chicks that responded slowly or not at all during the 300 sec test period formed the parental stock of the slow response line (SR). Thereafter mating assignments were made within lines at random except that full or half sib matings were not

permitted. Generations were discrete, and the effective number of sires and dams are given in Table 1.

Inbreeding was estimated as  $\Delta F = 1/8 N_m + 1/8 N_f$ , where  $\Delta F$  is the rate of inbreeding per generation and  $N_m$  and  $N_f$  the effective numbers of sires and dams, respectively (Wright, 1931). The mean  $F$  in the  $F_4$  generation was .08 in the FR line and .10 in the SR line.

Management procedures were comparable in all generations. Briefly, pedigreed chicks were wing-banded after being tested and were raised in batteries until 15 weeks of age. They were then transferred to individual cages under an increasing light regime to stimulate egg production at an early age.

Eggs were collected daily and stored at 13° C until incubation. After 18 days of incubation they were transferred to individual hatching tray compartments. Trays were lined with crinolin which assured physical and visual, but not auditory, isolation. Light intensity in the hatcher was maintained below 4.3 luxes. Compartments were inspected at 2-hr intervals, and when a chick had hatched (completely emerged from the egg) it was transferred to a labeled compartment until tested.

Testing Apparatus. The testing apparatus was designed from one described by Smith and Bird (1963). It was

Table 1. Number of selected parents producing progeny in each generation.

Line	Sex	Parents of			
		F <sub>1</sub>	F <sub>2</sub>	F <sub>3</sub>	F <sub>4</sub>
FR	♂♂	8	9	11	10
	♀♀	12	12	15	12
SR	♂♂	6	7	13	9
	♀♀	11	11	16	15

FR - fast response line

SR - slow response line

roughly hexagonal, 90 cm between parallel sides, and the floor and walls were painted black. The visual stimulus consisted of a translucent plastic dome containing moving colored lights which flickered on and off. A continuous tape recording of the clucking of a broody hen furnished an auditory stimulus. The temperature in the testing room was maintained at about 30° C. Each chick was tested individually and was given one 300-sec test period.

Testing Procedure and Traits Studied. Each chick was transported from its isolation box to the testing apparatus at 24±2 hr posthatching and was placed in a circle, 5 cm in diameter, that was drawn on the floor of the compartment opposite the visual stimulus (Figure 1). The selected trait was time in seconds to respond toward the stimulus (move from area A to area B). Time in seconds to approach to within 15 cm of the stimulus (move from area B to area C) and time spent within 15 cm of the stimulus (within area C) were considered correlated behavior traits.

Thus, measurements of response, approach, and stay-near tendencies were obtained for each chick. Numbers of chicks tested per line per generation are shown in Table 2.

Control Line. Each generation eggs from the relaxed line were treated in the same manner as those from the selected lines; these chicks served as controls. Since the selected lines were formed from the relaxed line, and

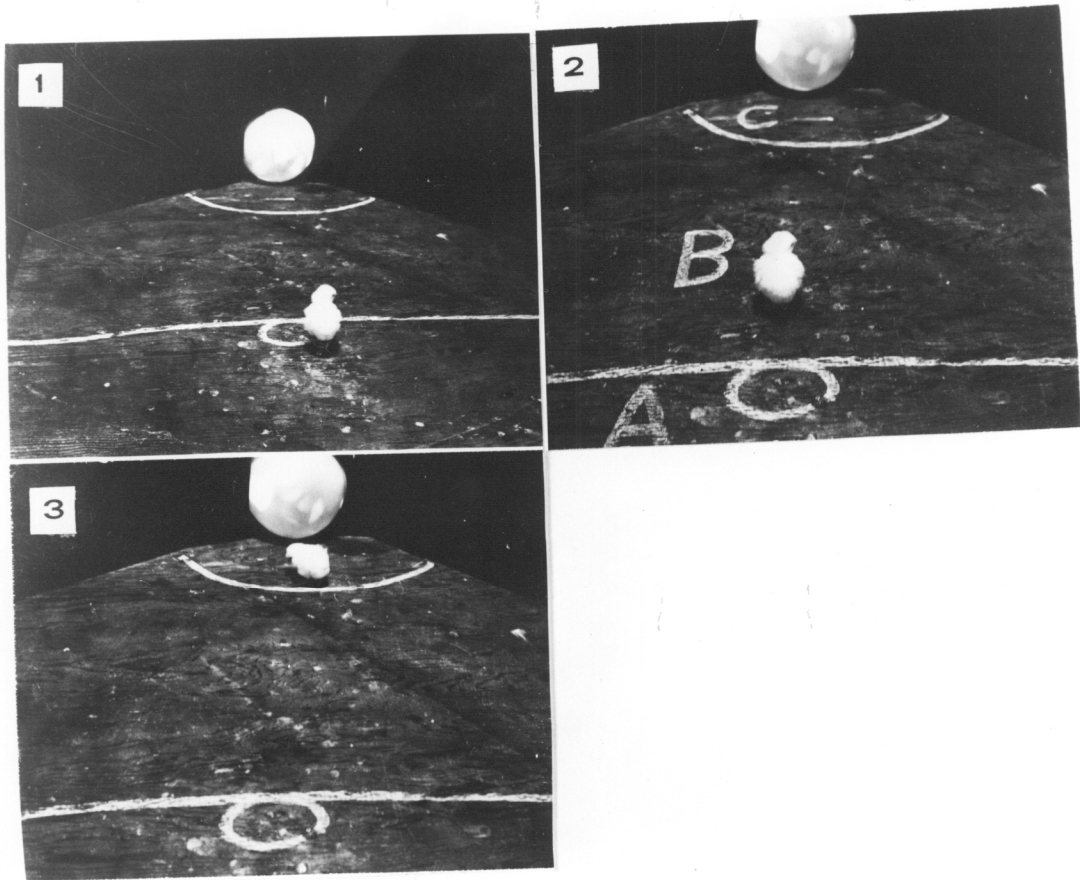


Figure 1. Testing compartment showing criteria for response (A→B), approach (B→C), and time spent near (within C) the apparatus.



Table 2. Number of individuals tested for behavior responses in each generation.

Line	Generation			
	F <sub>1</sub>	F <sub>2</sub>	F <sub>3</sub>	F <sub>4</sub>
FR	51	106	45	79
SR	35	102	102	87
C	72	82	42	31

FR - fast response line  
SR - slow response line  
C - control line

since there was no evidence of regression of the selected trait (body weight) on generations, it may be assumed that gene frequencies in the control line are not changing rapidly. This line should, therefore, serve as an adequate measure of environmental fluctuations during the selection program.

Analyses. Parametric and nonparametric statistics were used because variances were large and often heterogenous. Differences between the lines within generations were analyzed by 2 x 2 chi-squares corrected for continuity (Siegel, 1956).

For the selected trait, response to selection was measured each generation as the deviation from the random-mating controls. The cumulative response was then regressed on the cumulative selection differential (Falconer's 1954a realized heritability). Student's (1934) method of dividing the cumulative response by the cumulative selection differential was also used to determine heritabilities of the selected trait.

Realized correlated heritabilities of the unselected traits were calculated by two methods, (1) the cumulative response of the unselected trait was divided by the cumulative expected secondary selection differential ( $iY_x$ ) for each generation and the means calculated, and (2) the cumulative response of the unselected trait was

regressed on  $iY_x$ . The second method is the more reliable (Carte, 1968). The secondary selection differential as discussed by McGee (1965) and calculated by Ideta and Siegel (1966) is  $iY_x = iXb_{P_{yx}}$  where  $iX$  is the selection differential of the selected trait, and  $b_{P_{yx}}$  is the phenotypic regression coefficient of Y on X.

Genetic correlations ( $r_{G_{xy}}$ ) between the selected and unselected traits were estimated from Falconer (1954b) where

$$r_{G_{xy}} = \Delta G_y / \Delta G_x \cdot h_x / h_y \cdot \sigma_{P_x} / \sigma_{P_y}$$

and  $\Delta G_x$  and  $\Delta G_y$  are the genetic changes in the selected and unselected traits, respectively,  $h_x$  and  $h_y$  are the square roots of the heritabilities of the selected and unselected traits, respectively, and  $\sigma_{P_x}$  and  $\sigma_{P_y}$  are the phenotypic standard deviations of the selected and unselected traits, respectively.

Phenotypic correlations were obtained each generation, tested for homogeneity, and their average weighted values obtained by z transformations (Snedecor, 1946).

### Results

Comparisons Between Related Lines. Response and approach tendencies for four lines of chicks are shown in Figure 2.

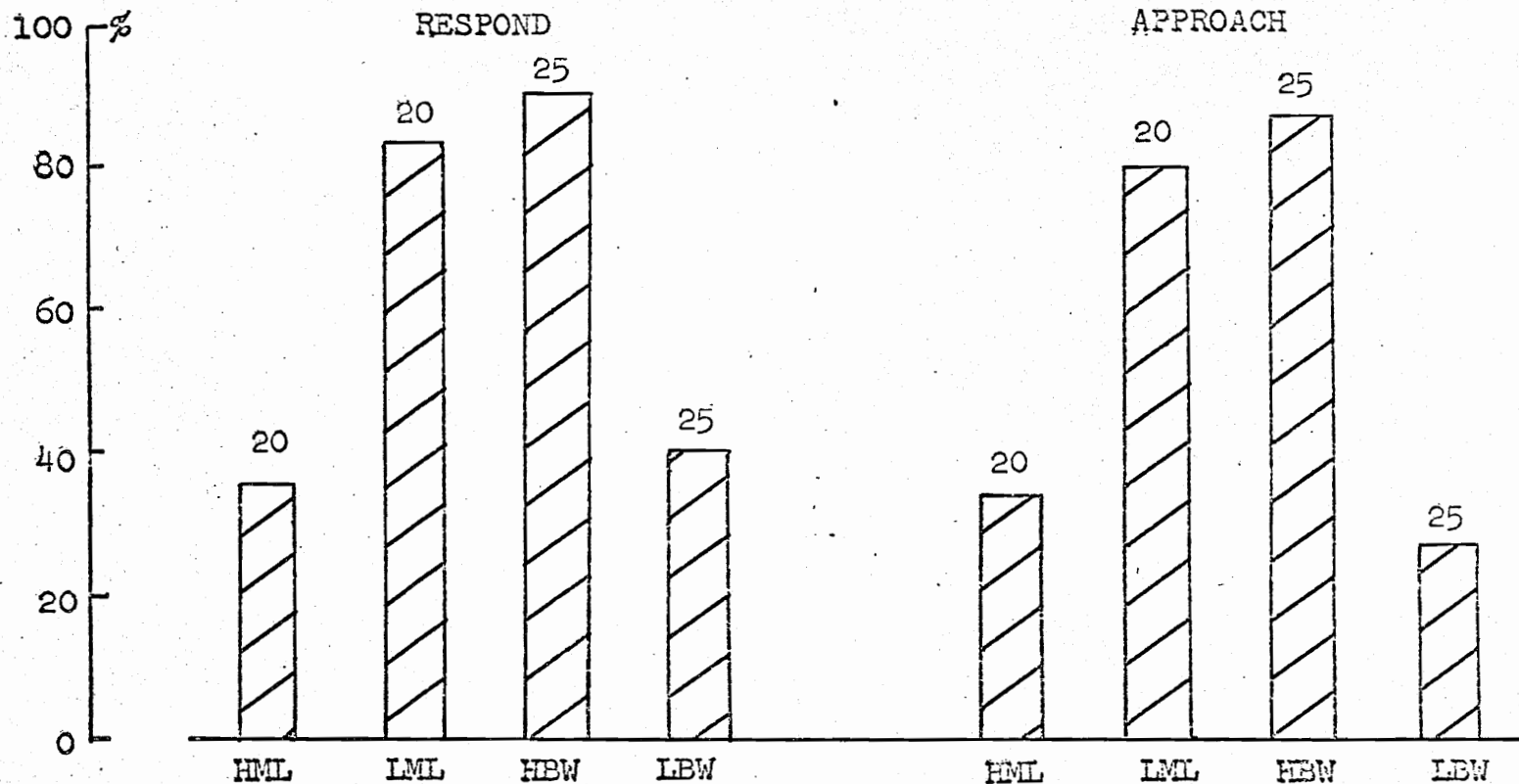


Figure 2. Response and approach tendencies of high and low mating lines (HML and LML) and high and low body weight lines (HBW and LBW) with numbers of chicks tested per line above each column.

Differences for both response and approach were significant between the HBW and LBW lines. This pilot study showed the existence of genetic differences for the behavior traits, and a selection experiment was initiated to study their inheritance in greater detail.

Response to Selection. The response to individual phenotypic selection for time to respond toward the imprinting apparatus is shown in Figure 3. No progress for time to respond was evident in the SR line, whereas steady progress was made in the FR line (upper graph). The lower graph of Figure 3 shows that the regressions of cumulative responses on cumulative selection differentials (realized heritabilities) were  $.32 \pm .08$  for the FR line and  $-.06 \pm .06$  for the SR line. These regressions were significantly different from each other. The regression for the FR line was significantly different from zero while that for the SR line was not.

Means and Standard Errors. Means and standard errors for the selected and correlated behavior traits are presented in Table 3. Standard errors for all traits were large relative to their means and demonstrate the high inherent variability of these characters. It is also seen from Table 3 that selection in the FR line was effective in decreasing time to respond and time to approach while steadily increasing the time spent near

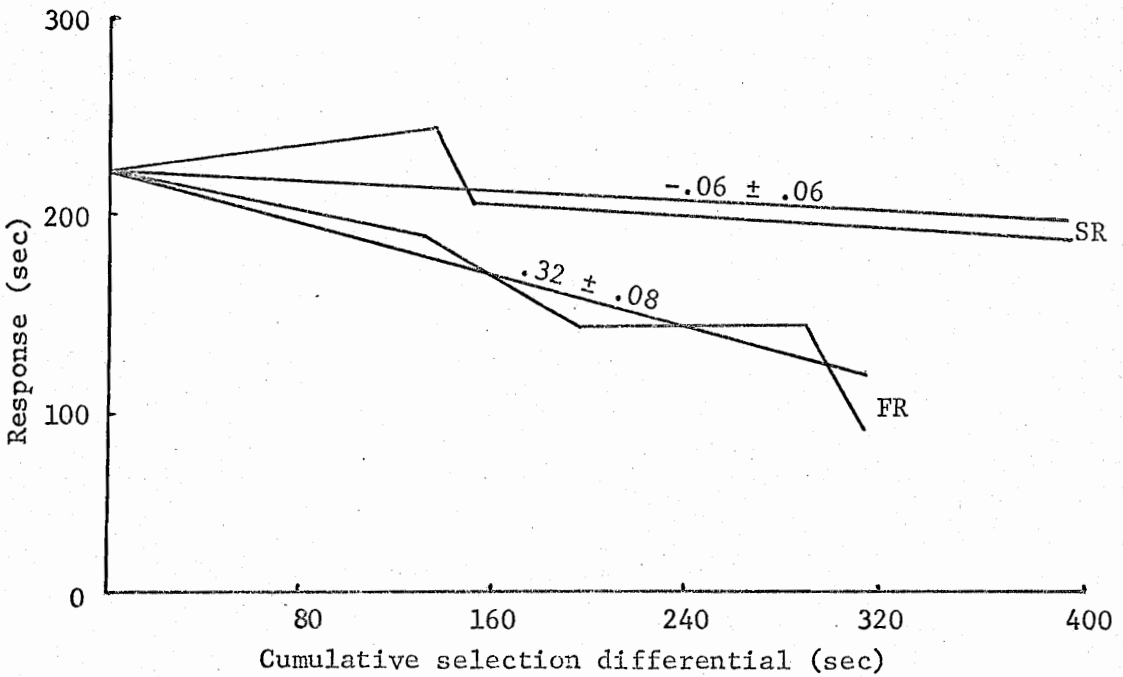
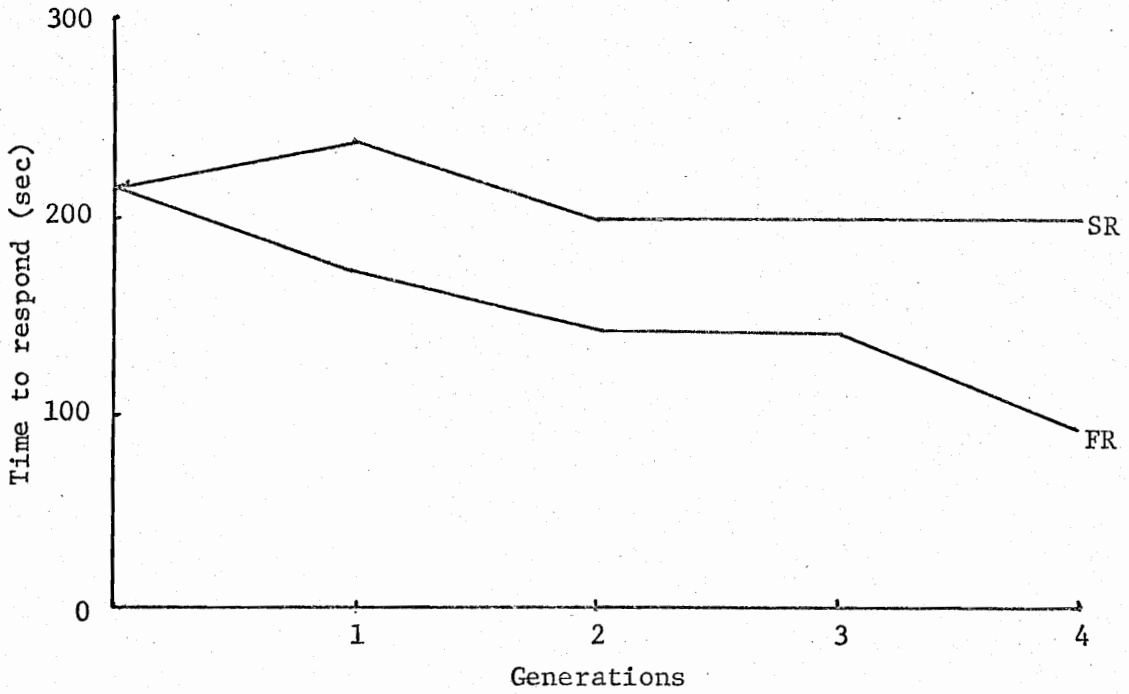


Figure 3. Bidirectional selection for time to respond. Control adjusted response by generations (upper), and response by selection differential with linear regression lines fitted to units of response (lower).

Table 3. Means and standard errors (se) for the selected and correlated traits.

Gen.	Line	Time to respond	Time to approach	Time near apparatus
		$\bar{x} \pm se$	$\bar{x} \pm se$	$\bar{x} \pm se$
F <sub>1</sub>	FR	177 ± 16	148 ± 19	101 ± 15
	SR	239 ± 16	215 ± 22	46 ± 14
	C	210 ± 17	184 ± 21	47 ± 8
F <sub>2</sub>	FR	160 ± 10	138 ± 12	96 ± 9
	SR	216 ± 10	201 ± 13	50 ± 8
	C	230 ± 11	212 ± 14	44 ± 9
F <sub>3</sub>	FR	121 ± 16	101 ± 17	146 ± 16
	SR	173 ± 10	133 ± 12	88 ± 9
	C	184 ± 20	185 ± 21	81 ± 16
F <sub>4</sub>	FR	96 ± 10	72 ± 11	171 ± 11
	SR	196 ± 11	163 ± 15	79 ± 10
	C	213 ± 15	172 ± 23	52 ± 10

FR - fast response line

SR - slow response line

C - control line

the imprinting apparatus. Surprisingly, progress in the SR line seems to be opposite the direction of selection, but examination of the data shows that the means fluctuated among generations with no consistent trend.

Comparisons of Selected Lines. Intrageneration comparisons among lines for response and approach tendencies were made by Chi-square analyses. Although stay-near tendencies could be classified in an all or none scheme, chicks that approach automatically spend some time near the apparatus, resulting in identical nonparametric values for approach and stay-near tendencies. The FR line was significantly different from controls for both response and approach in the  $F_2$  and all subsequent generations (Table 4). The SR line differed from the control line for chicks approaching only in the  $F_3$  generation, and this was ascribed to chance. Differences between FR and SR lines for response and approach tendencies were significant in all filial generations except the  $F_3$ .

Regression Coefficients, Correlations, and Heritabilities.

Phenotypic regression coefficients for approach and stay-near tendencies on time to respond are shown in Table 5. All were highly significant with regressions of time to approach on time to respond being positive and those for time spent near the apparatus on time to respond being



Table 4. Chi-square comparisons among lines for response and approach tendencies within each generation.

Gen.	Line	Proportion of chicks	
		responding	approaching
F <sub>1</sub>	FR	67 <sup>a</sup>	57 <sup>a</sup>
	SR	37 <sup>b</sup>	31 <sup>b</sup>
	C	49 <sup>ab</sup>	42 <sup>ab</sup>
F <sub>2</sub>	FR	74 <sup>a</sup>	67 <sup>a</sup>
	SR	47 <sup>b</sup>	38 <sup>b</sup>
	C	43 <sup>b</sup>	35 <sup>b</sup>
F <sub>3</sub>	FR	84 <sup>a</sup>	76 <sup>a</sup>
	SR	71 <sup>ab</sup>	66 <sup>a</sup>
	C	57 <sup>b</sup>	45 <sup>b</sup>
F <sub>4</sub>	FR	90 <sup>a</sup>	87 <sup>a</sup>
	SR	59 <sup>b</sup>	51 <sup>b</sup>
	C	61 <sup>b</sup>	52 <sup>b</sup>

FR - fast response line

SR - slow response line

C - control line

Table 5. Phenotypic regression coefficients (b) and their standard errors (se) of unselected behavior traits on time to respond.

Line	Gen.	<u>Time to approach</u>	<u>Time near apparatus</u>
		b ± se <sup>1</sup>	b ± se <sup>1</sup>
FR	F <sub>1</sub>	109 ± 6	-91 ± 4
	F <sub>2</sub>	92 ± 6	-79 ± 5
	F <sub>3</sub>	93 ± 7	-92 ± 4
	F <sub>4</sub>	83 ± 6	-98 ± 6
SR	F <sub>1</sub>	118 ± 9	-84 ± 16
	F <sub>2</sub>	105 ± 6	-57 ± 7
	F <sub>3</sub>	93 ± 6	-82 ± 4
	F <sub>4</sub>	111 ± 6	-84 ± 4

FR - fast response line

SR - slow response line

<sup>1</sup> 10<sup>-2</sup>; all regressions were highly significant (P ≤ .01)

negative.

Figure 4 shows realized correlated heritabilities for time to approach (upper) and time spent near the apparatus (lower), and Table 6 shows heritabilities for the selected and correlated behavior traits. Traits were heritable in the FR line and nonheritable in the SR line.

Table 7 shows correlations between the selected and correlated traits. Phenotypic correlations were very high and in the direction expected. Genetic correlations were very high in the FR line but could not be calculated in the SR line because of the zero heritabilities in that line. That is, in the SR line the denominator of the term  $h_x/h_y$  becomes zero and is undefined.

Natural Selection. The role of natural selection in this experiment was not evident. Table 8 shows ratios of effective to expected selection differentials. All the values are greater than one. In addition, mortality to 9 weeks of age, fertility, and hatchability were similar for both lines.

#### Discussion

Line Comparisons. Although genetic variation is necessary for progress in a selection experiment, the absence of

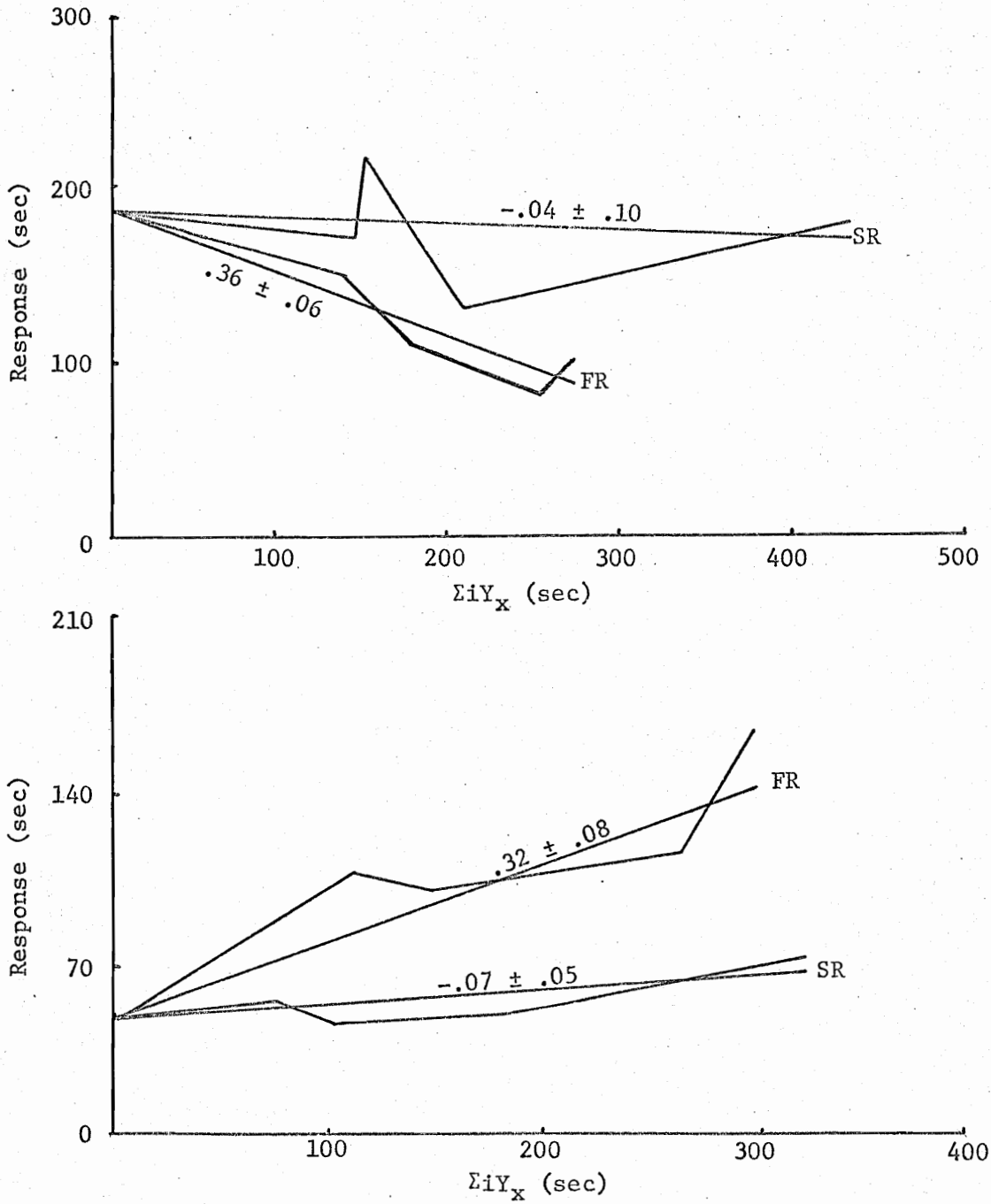


Figure 4. Regression of control adjusted responses on expected secondary selection differentials ( $\Sigma iY_x$ ) for time to approach (upper) and time near apparatus (lower).

Table 6. Heritabilities of selected and correlated behavior traits within selected lines.

Line	Heritability	Time (sec)		
		to respond	to approach	near apparatus
FR	Realized <sup>1</sup>	.30	.35	.38
	Realized <sup>2</sup>	.32 ± .08	.36 ± .06	.32 ± .08
SR	Realized <sup>1</sup>	.00	-.04	-.04
	Realized <sup>2</sup>	-.06 ± .06	-.04 ± .10	-.07 ± .05

<sup>1</sup> Response ÷ cumulative selection differential

<sup>2</sup> Regression of response on cumulative selection differential

Table 7. Phenotypic ( $r_{p_{xy}}$ ) and realized genetic ( $r_{G_{xy}}$ ) correlations between time to respond and unselected behavioral traits within selected lines.

Line	Unselected trait	$r_{p_{xy}}^1$	$r_{G_{xy}}$
FR	Time to approach	.84**	.77
	Time near apparatus	-.93**	-.96
SR	Time to approach	.82**	
	Time near apparatus	-.87**	

FR - fast response line

SR - slow response line

1 - average phenotypic correlation for generations  $F_1 - F_4$

genetic variation does not mean that a character is not inherited. Since there were interline differences for response and approach tendencies, genetic changes through selection would be predicted. The demonstration of interline differences show the need for careful comparisons of experimental and control groups and suggest that much of the variation in results from one laboratory to another may be due to differences in stocks. For example, if we were attempting to determine the effectiveness of our imprinting apparatus our conclusions would vary if we used only HML or only LML chicks (Figure 2).

Selection Progress. In the FR line response, approach, and stay-near tendencies were steadily increased over generations whereas in the SR line selection had no noticeable effect on these traits (Figures 3 and 4; Tables 3, 4, and 6). At first these results were not surprising since asymmetry in bidirectional selection programs is common. However, it soon became evident that no progress was being made in the SR line and that the usual causes of differences in rates of response did not explain the asymmetry. Moreover, while asymmetry is often expected, a complete lack of response to selection is not.

These results could explain those found by Smith and Templeton (1966). They attempted to increase within-family variation by mating cocks to hens with high and

low scores and then partitioning variances by component analyses. If progress was made in only one direction  $\sigma_A^2$  would be underestimated by about one-half, and their estimate of .18 would be comparable to the estimate of .32 for  $\sigma_A^2$  in the FR line.

Phenotypic Variation. The phenotypic variability of behavior traits is usually quite large, making reliable statistical evaluation difficult. Sluckin (1965) reviewed imprinting and early learning and pointed out that individuals within any given species probably differ greatly among themselves in approach and stay-near behavior. Jaynes (1956), Gottlieb (1961), Fabricius and Boyd (1954) and others have noted great variability in imprinting responses. When the phenotypic variation of a trait is high, it may be difficult to differentiate among genotypes on the basis of phenotypic measurements. Table 3 shows that standard errors of traits measured in this experiment were large relative to the means, but in the FR line the steady genetic progress indicates that in spite of the high variability genotypes were distinguished on the basis of phenotypic measurements.

Relationships Among the Selected and Correlated Traits.

In Experiment II of this dissertation it is shown that phenotypic correlations among response, approach, and stay-near tendencies are very high and are involved in



the initial phases of imprinting. This experiment shows that genetic correlations among the traits are also very high (Table 7), strengthening the argument that they are sequential and should be discussed in terms of imprinting behavior.

Phenotypic and genetic correlations, phenotypic regression coefficients, and heritabilities may all contribute information about the underlying inheritance of traits, but the calculation of these parameters may be influenced by a number of factors. The interrelationships of the expressions may, therefore, provide information about the nature of the traits measured.

The Role of Natural Selection. Ratios of effective to expected selection differentials provides an estimate of the effect of natural selection. When the ratio is unity, natural selection has had no obvious effect on artificial selection; a value smaller than one indicates that natural selection has opposed artificial selection. The ratios in both the selected lines were essentially unity (Table 8). This indication that natural selection was unimportant in helping or hindering selection progress was substantiated by the observation that fertility, hatchability, and mortality to 9-weeks of age were similar within lines.

Genetic and Environmental Relationships. The lack of

Table 8. Ratios (effective÷expected) of selection differentials.

Line	Generation			
	F <sub>1</sub>	F <sub>2</sub>	F <sub>3</sub>	F <sub>4</sub>
FR	1.07	1.04	1.10	1.09
SR	1.04	1.07	1.04	1.27

FR - fast response line

SR - slow response line

response to selection in the SR line meant a zero heritability. It is generally accepted that any trait which can be measured can be selected for, but the situation seen in this selection experiment may be a special case. The zero heritability made it impossible to calculate genetic correlations in the SR line, and the phenotypic correlations were seemingly due to environmental causes, i.e.

$$r_{P_{xy}} = r_{E_{xy}}$$

and

$$b_{P_{yx}} = r_{E_{xy}} \div b_{P_{xy}}$$

This is true because

$$r_{P_{xy}}^2 = (b_{P_{yx}}) (b_{P_{xy}})$$

and

$$b_{P_{yx}} = (h_x r_{G_{xy}} h_y + e_x r_{E_{xy}} e_y)^2 \div b_{P_{xy}}$$

These formulas demonstrate the relationships among the phenotypic regression coefficients ( $b_p$ ), the phenotypic correlation coefficients ( $r_p$ ), and the environmental correlation coefficients ( $r_E$ ). Since response may lead to spending time near the apparatus and the sequence can not be reversed,  $b_{P_{xy}}$  (the regression of the unselected trait on the selected trait) is meaningless. Moreover, the lack of response to selection in the SR line makes calculation of various genetic parameters in that line

meaningless.

Asymmetry in Response to Selection. The response of the FR and SR lines to selection were asymmetrical. Several reasons for different rates of response in bi-directional selection experiments were presented by Falconer (1954). They were (1) differences in selection differentials, (2) scale effects, (3) maternal effects, (4) inbreeding depression, (5) directional dominance, and (6) unequal initial gene frequencies.

Regressing the cumulative response on the cumulative selection differential eliminates differences in selection differentials as a source of asymmetry (Figure 3, lower, and Figure 4). Such differences may arise from natural selection, selection of unequal proportions of the divergent lines, and the phenotypic standard deviations of the traits.

Scale effects causing differences in selection differentials may be eliminated when progress is plotted as a realized heritability; but the choice of an appropriate scale of measurement may still be in error, and asymmetry may be due to these "scale effects." Skewed distributions of data, variances related to means, or high coefficients of variation may indicate scale effects. Distributions for the behavior data presented here cannot be determined because testing time per chick was

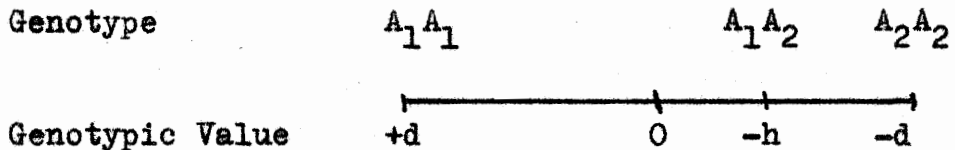
limited to 300 sec, thereby eliminating a portion of the distribution curve. Usually, chicks that did not respond in 300 sec would not respond in 480 sec, and it would be experimentally impractical to obtain actual values for these traits. In the FR line distributions could be estimated since most chicks responded and approached, and the curves approximated the normal in all filial generations. Coefficients of variation were high, but this is not unusual for behavior traits. Therefore, while it is impossible to exclude the role of scale effects, it is doubtful that they were the major causes of asymmetry.

Maternal effects may be dismissed as a cause of the asymmetry since such effects would appear to be unimportant for these traits because birds are oviparous.

Inbreeding depression will be present when directional dominance has evolved and has modified the frequencies and/or the expressions of genes. Therefore, these sources of asymmetry will be discussed together in general terms.

From the discussions of Mather (1949) and Falconer (1954a, 1960), two alleles,  $A_1$  and  $A_2$ , can be assigned initial frequencies of  $p$  and  $q$ , respectively. The three genotypes  $A_1A_1$ ,  $A_1A_2$ , and  $A_2A_2$  can be assigned values of  $+d$ ,  $h$ , and  $-d$ , respectively. The midpoint between the homozygote classes is zero, and the value of the heterozygote depends on the direction and degree of dominance.

Since in Experiment II it was shown that crossbreeding decreased time to respond toward the imprinting apparatus,  $A_2$  must be dominant to  $A_1$  and  $h$  would lie on the negative side of the scale. Diagrammatically,



Thus, in the FR line, attempts were made to increase the frequency of  $A_2$  alleles (decrease the phenotype). Such attempts in this line would be opposed by inbreeding effects. Selection in the SR line attempts to increase the frequency of  $A_1$ , and inbreeding effects would be complementary. This is because inbreeding depression acts to increase response and approach time and to decrease time spent near the apparatus, and attempts to correct for the possible effects of inbreeding would increase asymmetry. The expressions given above allow for determining the mean of a random breeding population as follows:

Genotype	Frequency	Value	Frequency x Value
$A_1A_1$	$p^2$	+d	$p^2d$
$A_1A_2$	$2pq$	h	$2pqh$
$A_2A_2$	$q^2$	-d	$-q^2d$

Since  $(p + q) = 1$ , the sum of the last column may be written as  $d(p - q) + 2pqh$  and equals the population

mean. Each locus will then contribute to the population mean a value  $d(p - q)$  due to homozygotes and a value  $2pqh$  due to heterozygotes. Mather (1949) has extended these basic expressions to apply to multiple loci, and Falconer (1954a) points out that  $d(p - q) + 2pqh$  specifies the deviation of the initial phenotypic level from the midpoint between the two limits. Its value, relative to the level of the limit, determines the direction and degree of asymmetry in responses to selection. The term  $d(p - q)$  represents the effect of unequal initial gene frequencies and the term  $2pqh$  represents the effect of directional dominance on the initial phenotypic level. In this experiment  $h$  was negative, and  $p$  and  $q$  were either zero or positive, making the term for directional dominance negative. Since directional dominance has evolved favoring the expression of alleles lowering time to respond in an imprinting situation, natural selection would have favored the accumulation of alleles favoring fast response ( $A_2$  alleles in the description above). This means that the frequency of  $A_2$  type alleles ( $q$ ) should be greater than the frequency of  $A_1$  type of alleles ( $p$ ) and the term for the effect of unequal initial gene frequencies,  $d(p - q)$ , would be negative. Again we are confronted by the fact that the direction of the asymmetry observed

here cannot be accounted for by the more usual factors. The graphs constructed by Falconer (1954a) allow one to plot expected results of selection starting at any desired initial gene frequency and with no dominance or with complete dominance. In no case is the expectation zero progress, and in no case would progress be greater in the direction opposite that of inbreeding depression. The usual causes of asymmetry did not explain that observed in this experiment. This would not be surprising if we are dealing with threshold traits (Wright, 1934a, 1934b; Lush, et al., 1948; Robertson and Lerner, 1949). Quantification would then be possible only if chicks responded.

Manning (1961, 1967, 1968) discussed mating speed in Drosophila and reported that the control of female receptivity to male courtship is of a dual nature. The change from the unreceptive to the receptive state was an all-or-none phenomenon; but, when a female was receptive, courtship summation could be modified by changing the rate at which males delivered courtship stimulation. He also noted more variability in the slow mating lines because matings were spread out over a number of hours, whereas in the fast mating lines matings usually occurred within 3 to 4 minutes.



In several ways response, approach, and stay-near behavior may be considered all-or-none traits. Once a chick responds toward the stimulus its behavior is modifiable and highly predictable. It is shown in Experiment II that although incubation period, mating type, etc. affected behavior toward an imprinting apparatus, these factors were of negligible importance as compared with the "switch" from unresponsiveness to responsiveness. A chick that responds will usually approach and stay near an imprinting apparatus, but the factors triggering response itself are yet to be determined.

Through selection, responsiveness per se and the rate of consummatory behavior has been modified in both sexes of the FR line. At the phenotypic level it is not possible from the existing data to determine if genotypic changes have occurred in the SR line. Experimentation is necessary to ascertain if we are working with threshold traits. If so, and this is probable, the testing scheme will require modification if we are to make substantial progress in the SR line, and the explanation of the asymmetry of response to selection may lie in the nature of the traits themselves.

#### Summary

Comparisons of related lines disclosed that significant differences in response and approach tendencies

exist in chickens. A bidirectional selection experiment was initiated with time to respond toward a distant audio-visual imprinting apparatus as the selected trait. Approach and stay-near tendencies were measured as correlated traits. In the FR line selection was effective, and the heritabilities of response, approach, and stay-near tendencies were 0.32, 0.36, and 0.32, respectively. Selection was completely ineffective in the SR line for the selected and correlated traits. Reasons for the asymmetry in rates of response to selection are discussed, and it is hypothesized that responsiveness is a threshold trait. Genetic and phenotypic relationships among the traits provided evidence that response, approach, and stay-near tendencies are initial components of imprinting.

EXPERIMENT II: NONADDITIVE INHERITANCE OF CHICK'S  
RESPONSE TOWARD AN IMPRINTING APPARATUS

Methods and Materials

Subjects. The subjects were 669 domestic chicks from five 2 x 2 mating sets (Table 9). Since the pit games (G in Table 9) were used in two mating sets, there were 9 types of  $F_1$  purebreds (290 chicks) and 10 types of  $F_1$  crossbreds (379 chicks). Chicks from the FR line, the SR line, and their reciprocal crosses were excluded from all analyses except those concerning heterosis because these lines had undergone artificial selection for the traits measured i.e. response tendencies toward the imprinting apparatus. In the parts of this study not directly concerning genetic factors, 535 chicks were used.

The high and low weight lines and the high and low mating lines have been described (Siegel 1962, 1965). The pit games have been maintained for several generations in our laboratory, and the inbred Leghorn lines were samples from lines maintained by Dr. C. E. Holmes at Virginia Polytechnic Institute. The fast and slow response lines are described in Experiment I.

Management, Apparatus, and Procedure. Management of eggs and chicks and the testing apparatus and procedure were the same as described in Experiment I except that

Table 9. Number and types of chicks tested.

Purebred	Number	Crossbred	Number
WL <sub>1</sub> <sup>†</sup>	27	WL <sub>1</sub> x G	18
WL <sub>2</sub> <sup>††</sup>	21	G x WL <sub>1</sub>	13
G	37	WL <sub>2</sub> x G	30
HML*	30	G x WL <sub>2</sub>	46
LML*	47	HML x HWL	43
HWL*	36	HWL x HML	36
LWL*	40	LML x LWL	47
FR*	27	LWL x LML	37
SR*	25	FR x SR	50
		SR x FR	59
	Σ 290		Σ 379

Inbreeding coefficients: <sup>†</sup>0.54; <sup>††</sup>0.27; \* < 0.16

after testing each chick was weighed, sacrificed, and sexed by direct gonadal examination. Three hatches were required. Testing was conducted 24 hours a day, and the length of the incubation period was known for each chick. Testing was  $24 \pm 2$  hours after hatching. Thus, the known variables which might have affected response, approach, and stay-near tendencies were (1) mating type, (2) time of day tested, (3) incubation period, (4) 24-hr body weight, (5) sex, and (6) hatch.

Analyses. An IBM 7040 computer was used to obtain correlations, curvilinear regressions, and multiple regressions; correlations were then tested for homogeneity, and their average weighted values were obtained by z transformations (Snedecor, 1946). Nonparametric statistical tests were used when parametric tests were found inappropriate.

#### Results and Discussion

Heterosis and Evolutionary Considerations. Response, approach, and stay-near tendencies of purebred and crossbred chicks are shown in Figure 5. A trend is obvious because in 4 of 5 comparisons crossbreds were superior to the purebreds in response and approach tendencies. Chi-squares comparing all crossbreds with all purebreds

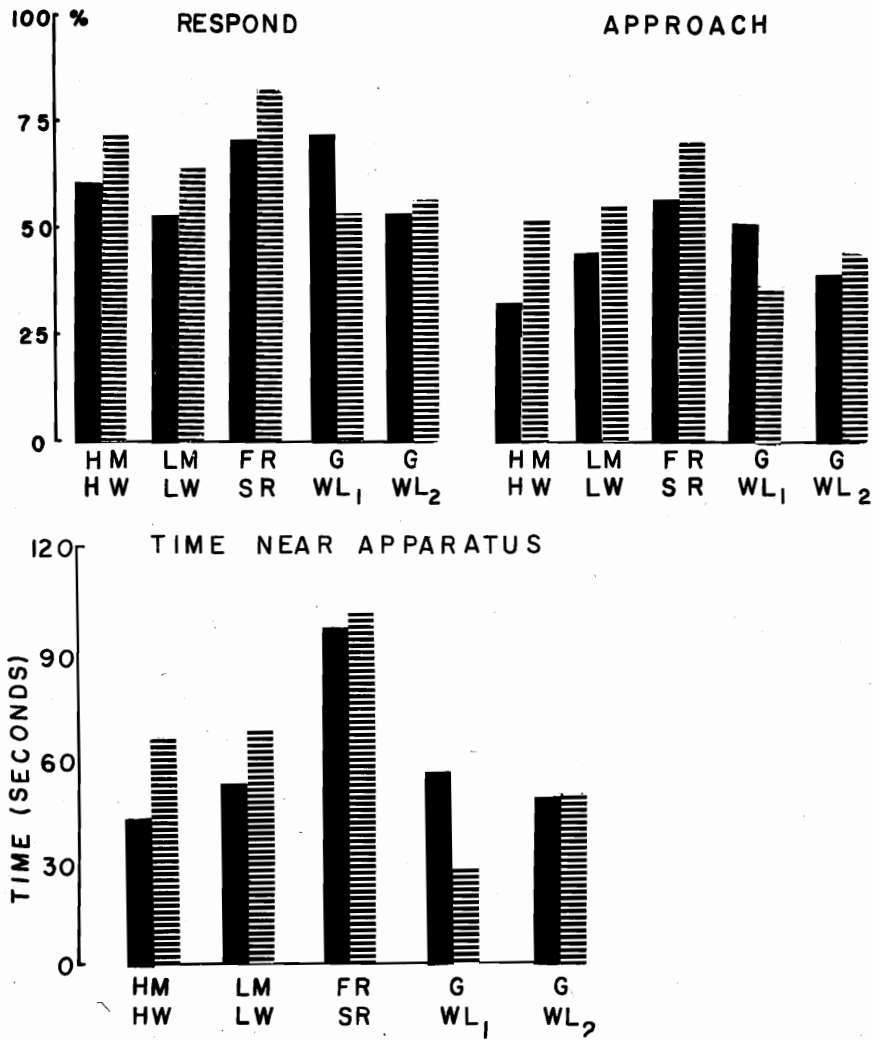


Figure 5. Average response, approach, and time spent near the imprinting apparatus for purebreds (solid bar) and crossbreeds (striped bar) N = 669.

showed that significantly more crossbreds responded and approached than purebreds. Differences within mating sets were not significant, however, this is not surprising since variances for these traits are large. Essentially the same pattern is seen for the tendency of 24-hr-old chicks to stay near the imprinting apparatus. Variances were homogenous for this trait, but were still very large. F tests comparing crossbreds and purebreds within 2 x 2 sets were not significant, but pooling data over sets showed that crossbreds spent significantly more time near the apparatus than purebreds.

Although the degree of inbreeding of the lines involved was low in most cases (Table 9), results showed that tendencies to respond toward, to approach, and to stay near a distant imprinting apparatus at 24 hr post-hatching are traits which are inherited in a heterotic manner. These results do not exclude additive inheritance which were shown in Experiment I to have a low, but significant, effect on response tendencies.

Fisher's (1930) fundamental theorem of natural selection predicts that traits which are major components of fitness will contain little additive genetic variation ( $\sigma_A^2$ ) when the population concerned is in relative equilibrium with its environment. Such traits will have been subjected to extensive prior selection

which reduces  $\sigma_A^2$  but favors the accumulation of (or evolution of) nonadditive genetic variation. According to this theory, the traits measured in our test situation must have been adaptive during the evolution of the domestic fowl.

Phenotypic Correlations. Correlations between selected and unselected traits may change during the course of selection, and Rendel (1963) has shown that genetic correlations may actually change sign during a selection experiment. For this reason, the FR, SR, and their reciprocal crosses were excluded from the correlation analysis. These lines are being selected for response tendencies and are discussed in detail in Experiment I.

Table 10 shows weighted phenotypic correlations among the traits studied. Both quantitative and qualitative measurements of response and approach tendencies are shown. Response toward the imprinting apparatus is highly correlated with approach and with time spent near the apparatus, indicating that chicks which respond generally follow a pattern of approach and stay near behavior. This sequence of steps would seem to be meaningless except in terms of the concept of imprinting. The evidence that non-additive gene action has evolved for response, approach, and stay near behavior during the critical period for imprinting in this neonatal



Table 10. Phenotypic correlations among components of the approach response (N = 535).

	Respond or not respond	Time to approach	Approach or not approach	Time near apparatus
Time to respond	.81	.75	.80	-.81
Respond or not respond		.67	.70	-.54
Time to approach			.96	-.83
Approach or not approach				-.78

species, coupled with evidence that such behaviors are sequential, or at least highly related, leaves little doubt that these traits are indicative of tendencies to imprint.

Time of Day, Developmental Age, and Sex. Grey (1962b) and Grey, et al., 1963, 1964) reported cyclic fluctuations in chicks' preferences for a hen or an age-mate. Grey, et al., (1964), stated that in imprinting responses developmental age is either isomorphic with or confounded by time of day effects and then proceeded to differentiate these factors. Since incubation periods within and among lines vary more than 24 hours, the statement that calculation of age from incubation is isomorphic with a time of day effect is incorrect. At any rate, evaluation of a time of day effect was possible here because chicks were tested constantly through day and night.

Developmental age effects were discussed by Klopfer (1961), Gottlieb and Klopfer (1962), and Gottlieb (1961, 1963). Gottlieb (1963) reported that the error associated with the calculation of developmental age could be slightly reduced in ducks and chickens by refrigeration of the eggs after at least 12 hours of incubation. Developmental rates during incubation are influenced by a number of factors (Bohren et al., 1961; Coleman et al., 1963), and the length of incubation period may therefore

reflect developmental age. Since incubation period is known to have a genetic basis (Smyth and Howes, 1949; Coleman et al., 1963; Siegel et al., 1968; Table 13), refrigerating eggs after x hours of incubation may selectively eliminate certain genotypes.

Phenotypic correlations of behavior traits with time of day and with incubation period were obtained within each mating type, and the pooled values are presented in Table 11. None of the correlations with time of day were significant whereas those with incubation period were negative and of a low, but significant, order.

Cyclic fluctuations in chicks' preferences for imprinting objects could result in both positive and negative associations at different points in a cycle and thereby cancel each other. To ascertain if this was the case, polynomial equations were fitted to the data. In no instance were we able to describe the relationship between the behavior traits and the independent variables, incubation period and time of day tested. The proportions of the total variation of the behavior traits accounted for by each independent variable are shown in Table 12. No important association was found, demonstrating that such relationships may be statistically significant but experimentally meaningless. While our results confirm Gottlieb's (1961, 1963) findings of a significant

Table 11. Phenotypic correlations (r) of behavior traits with time of day tested and with incubation period.

	r with	
	Time of day tested	Incubation period
Time to respond	.06	-.11*
Respond or not	.04	-.09*
Time to approach	.05	-.17**
Approach or not	.06	-.16**
Time near apparatus	-.07	-.13**

\*P .05

\*\*P .01

Table 12. Proportions of variation of behavior traits explained ( $R^2$ ) by fitting polynomials.

Behavior trait (Dependent variable)	Polynomial (Degree)	$R^2$	
		Time of day tested	Incubation period
Time to respond	1st	.003	.013
	2nd	.008	.013
	3rd	.016	.014
	4th	.016	
Time to approach	1st	.004	.023
	2nd	.020	.023
	3rd	.026	.023
	4th	.026	
Time near apparatus	1st	.005	.019
	2nd	.019	.020
	3rd	.021	.020
	4th	.022	

relationship between developmental age and imprinting tendencies, I question the importance of it as a bias in experimental situations. This is because the magnitude of the bias is so small as to make it unimportant as a source of error.

Table 13 shows comparisons among mating types and between sexes for incubation period and 24-hr body weight. Differences in incubation time were significant among mating types but not sexes. There were significant differences among mating types for body weights, and males were significantly heavier than females. There were no significant differences between sexes for any of the behavior traits.

All Factors Considered. The role of the 6 known factors was assessed by a series of multiple regressions. Hatch effects contained an upward bias because one of the hatches consisted mainly of crossbred chicks. Also, the validity of attempts to partition the contributing factors according to their relative importance may not be strictly acceptable in a statistical sense because assumptions for parametric tests may not be met by all data. However, it is experimentally valuable to know the relative order of importance of these factors. With this in mind, the most important factors influencing time to respond are hatch and incubation period (Table 14).

Table 13. Comparisons among mating types and between sexes for incubation period and for 24-hr body weight.

Mating type	Incubation period (Hours)	24-hr body wt. (Grams)
WL	491 ± 11 <sup>bc</sup>	36.3 ± 3.1 <sup>bc</sup>
WL	495 ± 11 <sup>bc</sup>	36.6 ± 2.4 <sup>bcd</sup>
G	492 ± 10 <sup>bc</sup>	35.2 ± 2.8 <sup>d</sup>
HML	493 ± 11 <sup>b</sup>	40.4 ± 3.5 <sup>a</sup>
LML	491 ± 10 <sup>bcd</sup>	37.3 ± 4.6 <sup>bc</sup>
HWL	499 ± 9 <sup>ef</sup>	40.0 ± 2.8 <sup>a</sup>
LWL	498 ± 8 <sup>ef</sup>	33.3 ± 2.7 <sup>e</sup>
WL x G	497 ± 9 <sup>bcd</sup>	35.8 ± 2.1 <sup>cd</sup>
G x WL	488 ± 9 <sup>a</sup>	32.6 ± 1.5 <sup>e</sup>
WL x G	491 ± 9 <sup>bc</sup>	35.8 ± 3.2 <sup>cd</sup>
G x WL	486 ± 10 <sup>a</sup>	38.1 ± 3.5 <sup>b</sup>
HML x HWL	501 ± 7 <sup>f</sup>	40.3 ± 3.5 <sup>a</sup>
HWL x HML	492 ± 9 <sup>cd</sup>	40.5 ± 3.4 <sup>a</sup>
LML x LWL	497 ± 7 <sup>de</sup>	33.1 ± 3.6 <sup>e</sup>
LWL x LML	493 ± 10 <sup>cde</sup>	37.6 ± 2.7 <sup>b</sup>
Males	493 ± 10	37.4 ± 3.9
Females	494 ± 10	36.9 ± 4.3

ns - not significant; \*\*P<.01

Table 14. Multiple regression equations for each behavior trait.

Dependent Variable	$R^2$		Equation
	All contributors	Main contributors	
$X_9$	$X_1 + \dots + X_8 = .7874$	$X_8 = .6932$ $X_7 = .0940$	$\hat{X}_9 = 188.76 - 0.3678X_8 - 0.2931X_7$
$X_8$	$X_1 + \dots + X_7 = .5886$	$X_7 = .5599$ $X_4 = .0230$	$\hat{X}_8 = 445.06 + 0.9341X_4 - 9.0180X_7$
$X_7$	$X_1 + \dots + X_6 = .0343$	$X_2 = .0166$ $X_4 = .0108$	$\hat{X}_7 = 653.61 + 1.3667X_2 - 9.5443X_4$

Variables:  $X_1$  = Mating type       $X_4$  = Incubation period       $X_7$  = Time to respond  
 $X_2$  = Hatch                       $X_5$  = 24-hr wt                       $X_8$  = Time to approach  
 $X_3$  = Sex                               $X_6$  = Time of day tested               $X_9$  = Time near apparatus



The variation in time to approach is explained mainly by response time, but incubation period (developmental age) is also of some minor importance. Likewise, the primary influence on time near the apparatus was the previous behavior trait, time to approach. This series of equations supports the hypothesis advanced in Experiment I that response is a threshold trait, and that once a chick responds its subsequent behavior is highly predictable. The independent variables evaluated here, while demonstrating the sequential nature of response, approach, and stay-near behavior, are of little value in predicting what triggers response tendencies.

#### Summary

Six-hundred sixty-nine crossbred and purebred chicks from 15 mating types were tested for response, approach, and stay-near tendencies in an imprinting situation. These traits were found to be inherited in a heterotic manner and were highly correlated. It was concluded that these characters are components of fitness and should be discussed in terms of imprinting.

Time of day tested, sex, hatch, developmental age, 24-hr body weights, and mating type were considered independent variables that could influence the behavior responses. Although the initiation of response was not

predictable from any or a combination of these variables, once a chick responded the subsequent behavior was highly predictable. Evidence presented supports the hypothesis that the traits are sequential and highly dependent on initiation of responsiveness.

### EXPERIMENT III: PRIOR EXPERIENCE AND THE APPROACH RESPONSE

#### Methods and Materials

Apparatus. The approach response was elicited by an apparatus which combined several imprinting devices operating simultaneously. Four Christmas tree light bulbs set in a diamond pattern of holes, 11.5 cm apart, blinked on and off approximately 60 times per minute. The pattern for light flicker was similar to that described by Abercrombie and James (1961). A white styrofoam cube mounted in front of the diamond pattern of lights was attached to a pendulum which oscillated at the rate of 60 cycles per minute. A continuous tape recording of the clucking of a broody hen furnished an auditory stimulus.

The testing compartment was patterned after that utilized by Smith and Bird (1963). The run was hexagonal, 90 cm between parallel sides, and the floor and walls were painted black. The sides of the run were 60 cm high, and the stimulus was attached to one side of the run.

Subjects and Husbandry. Subjects for the experiments were chicks from  $F_6$  matings of a White Rock line selected for low body weight (Siegel, 1962). Eggs were incubated for 18 days in a forced-draft incubator after which they were placed in individual compartments in a dimly-lighted hatcher (maximum light intensity of 4.3 luxes) for

hatching. The compartments assured visual and physical but not auditory isolation. Compartments were inspected at hourly intervals, and when a chick had hatched it was transferred to a labeled compartment until time for stimulation and/or testing. Hence, the maximum error in determining the time of hatching was one hour. Chicks were randomly assigned to treatment categories so that position in hatch and time of day at testing would not influence the results in any systematic manner.

General Procedure. The chicks in each trial were divided into handled and non-handled categories. Each chick in the handled category was removed from its compartment at some specific age, held in the investigator's hands, and stroked gently from head to tail for 3 minutes. Handling was in the light or in complete darkness according to treatment category. The chick was then either returned to its compartment or placed individually in the testing area, depending upon its particular treatment group. Each bird in the non-handled category was removed from its compartment and either placed individually in the testing area with a minimum of handling and visual experience (controls) or exposed to visual stimulation and then placed in the testing area. Visual stimulation was accomplished by placing a chick on a table while the experimenter gyrated his hand all about the chick for

3 minutes without ever contacting the subject. Each subject was allowed 5 minutes to respond toward the stimulus. Response was recorded as time in seconds to move toward the apparatus from a circle, 5 cm in diameter, in which the subject was initially placed. Time to approach within 30 cm of the apparatus was also recorded in trials 1,3,4, and 5. Response was measured as a positive reaction toward the stimulus, while approach involved a movement of about 40 cm toward the apparatus.

Data were analyzed by Mann-Whitney U-Tests or by one-tailed chi-squares calculated by 2 x 2 contingency tables corrected for continuity.

#### A. Prior Tactile and/or Visual Stimulation

The purpose of the first 3 trials was to determine (1) if handling in the light (tactile-plus-visual stimulation) affected a chick's approach response, (2) if tactile stimulation alone was equivalent to tactile-plus-visual stimulation, and (3) if effects of tactile stimulation, tactile-plus-visual stimulation, and visual stimulation could be dissociated. All handling and/or visual stimulation was done just prior to testing at 24±1 hours posthatching. Controls in each experiment were non-handled chicks which had received no experimental

stimulation prior to testing. All chicks were maintained in physical and visual isolation compartments until time for stimulation and/or testing. All testing occurred at  $24 \pm 1$  hours posthatching.

Trial 1. Twenty-two chicks were randomized at hatching into the two treatment categories, handled and non-handled. The handled category consisted of 7 chicks, and the non-handled category consisted of 15 chicks. Handling was in the light.

Trial 2. Thirty-three subjects were randomized at hatching into 3 treatment groups: handled in the light, handled in darkness, and non-handled controls. Each group contained 11 chicks.

Trial 3. Eighty-eight chicks were randomized at hatching into 4 treatment groups of 22 chicks each. Groups were handled in light, handled in darkness, non-handled controls, and visual stimulation (without handling).

#### Results and Discussion

Trial 1. A Mann-Whitney U-Test of the results of this pilot study disclosed no significant difference between handled and non-handled birds for time to respond (handled median 300 sec.; non-handled median 115 sec.;

$P > .05$ ). The difference between handled and non-handled birds for time to approach was significant (handled median 300 sec.; non-handled median 67 sec.). Although the number of subjects used in this experiment was small it appeared that tactile-plus-visual stimulation (handling in light) decreased a chicks tendency to approach the apparatus. Trial 2 was designed to study this hypothesis further and to determine if tactile stimulation per se was the salient feature of handling chicks in the light.

Trial 2. A chi-square analysis indicated that handling chicks in light just prior to testing decreased the number of responses observed ( $P < .04$ ). Handling in darkness had no observable effect on the number of chicks responding toward the apparatus ( $P > .34$ ).

These results indicate that tactile-plus-visual stimulation decreases approach responses, whereas tactile stimulation per se, i.e. handling in darkness, does not appear to have any inhibition on these responses. A third trial was therefore designed to differentiate the modalities involved and to investigate the effects of such prior experience on a larger scale.

Trial 3. Figure 6 shows the relative proportions of chicks responding toward and approaching the apparatus

by treatment category. When compared with controls, it is evident that prior handling in the light decreases the number of chicks responding ( $P < .02$ ). Prior visual stimulation increases the number of responses ( $P < .02$ ), while handling in the dark has no effect on the number of responses observed ( $P > .38$ ).

The same trend is obvious when the number of approaches per treatment category are compared. Chicks which received tactile-plus-visual stimulation prior to testing approached fewer times ( $P < .01$ ); those receiving prior visual stimulation approached more times ( $P < .06$ ); and those chicks which had received only tactile stimulation approached equally well ( $P > .28$ ), when each treatment group was compared with non-handled controls.

The results of this experiment were consistent with those of Trials 1 and 2 in that prior handling in the light lowered the approach response. These experiments indicated a definite reluctance of chicks which had prior experience of simultaneous visual and tactile stimuli, i.e. handling in the light, to respond to or to approach the stimulus.

Although several hypotheses may be proposed from these results, a drive satiation hypothesis is suggested. The genetic investigations in Experiments I and II of



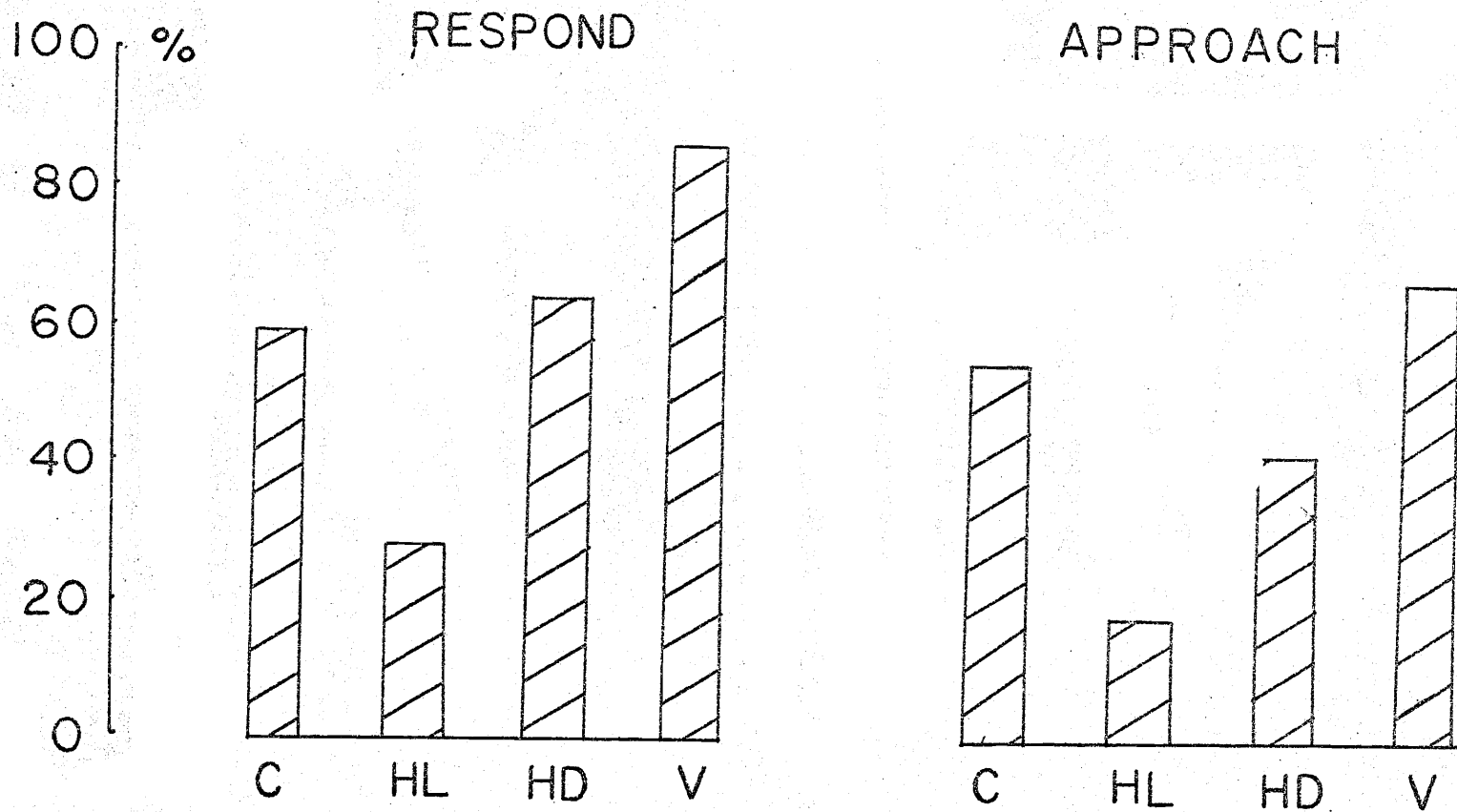


Figure 6. Responses toward and approaches to an imprinting stimulus by treatment categories. Handled in light (HL), handled in darkness (HD), and visual stimulation (V) are each compared with non-handled controls (C) N = 88.

the approach response of chicks indicates that time to respond toward a distinct audio-visual imprinting stimulus is a quantitative trait containing a significant amount of additive genetic variation. This means that a chick's tendency to respond toward the stimulus may be greatly affected by heredity, and the stimuli which most effectively satiate the drive to approach and remain near a parent surrogate, i.e. an imprinting device, must furnish stimulation in modalities which have undergone prior selection for such sensitivity during the evolution of the species.

The chicks generally emitted contentment chirps while being handled in the light, and the propensity to imprint and/or to approach an imprinting apparatus was, at least temporarily, satisfied only by the apparently pleasant combination of stimulation in both modalities. Recent evidence by Ettliger and Blakemore (1966) and Moffett and Ettliger (1966) suggests that monkey neuronal systems for tactile learning are different from those for visual learning. Similar systems in avian neonates could explain why simultaneous stimulation in both modalities decreases the approach response while stimulation in either modality alone does not decrease such responses; that is, a neonate whose drive to respond is satiated by the perception of information

through one of two or more independent learning paths is at a biological disadvantage in the wild since a transfer of information between the modalities may not occur.

Visual stimulation alone may not furnish sufficient stimulation in the proper modality to satiate the approach drive. It does, however, increase such tendencies, possibly due to a "training period effect"; that is, the sort of visual stimulation to which the chicks in these experiments were exposed resembled the apparatus in as much as both involved motion and a flickering light (hand movements between the bird and the overhead light source constituted a flicker effect). Thus, the results may offer support to James' (1960a) report that chicks which have been exposed to a flickering light will subsequently follow objects associated with such a stimulus.

That such generalization may occur is evidenced by the results of Hess (1964) and Polt and Hess (1964, 1966). They showed that chicks with prior social experience follow well but later show no evidence of imprinting to the testing apparatus.

Tactile stimulation alone, i.e. handling in darkness, may have no effect on the approach response at

24  $\pm$  1 hours because of no obvious similarity between such stimulation and the device used to elicit a response. Hence, generalization from the physical stimulation of handling to the visual and auditory stimulation from the apparatus did not occur, and the chicks' tendencies to respond and to approach were not increased. Handling in the dark may not decrease such responses because the biological drive to approach and imprint to a parent surrogate is not met by stimulation with which parental associations cannot be formed.

#### B. Effects of Age and Prior Socialization

The purpose of Trials 4 and 5 was to determine (1) if social experience would confound comparisons of the approach response of chicks handled in the light and non-handled chicks, and (2) to determine the effect on the approach response of handling chicks in the light at times other than just prior to testing at 24  $\pm$  1 hours.

Trial 4. Twenty-eight subjects were randomized at hatching into handled or non-handled categories of 14 birds each. Each category consisted of 7 isolate birds and 7 group birds. Handling and testing of the subjects in this experiment were conducted at 24  $\pm$  1 hours post-hatch age.

Trial 5. This experiment was conducted to measure the effect of handling chicks in the light at times other than just prior to testing at  $24 \pm 1$  hours on the approach response.

Seventy-two chicks were raised in physical and visual isolation. The handled category was subdivided into 5 age groups of 12 birds each. The non-handled, or control, category consisted of one group of 12 birds. Ages at handling were 5, 9, 13, 17, and 23 hours of age.

All birds were returned to their individual compartments after handling to await testing at  $24 \pm 1$  hours of age. Assignment of chicks to their respective treatment groups was at random.

#### Results and Discussion

Trial 4. The chicks grouped prior to testing usually did not respond (median 300 sec.) during the 5-minute test period regardless of whether they were handled or not. Likewise, the chick handled prior to testing generally did not respond (median 300 sec.) whether they were grouped or not. Thus, effects of tactile-plus-visual stimulation by handling in the light and by grouping the chicks prior to testing were equivalent in our test situation. Table 15 shows that a significant

difference in time to respond and time of approach was found between handled and non-handled treatments when chicks were maintained in isolation. Within the non-handled treatment the median for the isolated chicks was significantly less than the median for the group chicks in both time to respond and time of approach.

These data indicated that chicks maintained in isolation had a significantly lower threshold for the approach response than chicks maintained in groups. It was therefore decided to conduct the fifth experiment using only subjects maintained in physical and visual isolation to determine further the effect on the approach response of handling chicks in the light.

Trial 5. The results of Mann-Whitney U-Tests presented in Table 16 show that significant differences existed for time to respond to the apparatus between chicks handled at 13, 17, or 23 hours of age in comparison to non-handled chicks. At these ages the medians for the handled birds were larger than those for the non-handled controls suggesting that prior handling in the light inhibited the response. Handling at 5 hours and 9 hours of age produced no observable effect.

Differences in time of approach were not significant until chicks were handled at 23 hours of age and compared

Table 15. Median time to respond and time to approach.

		<u>Time in seconds to</u>	
		<u>respond</u>	<u>approach</u>
Isolated	Handled	300	300
	Non-handled	82	88
		* / \	* / \
Non-handled	Grouped	300	300
	Isolated	82	88
		* / \	* / \

\*p < .05

Table 16. Mean time to respond and time to approach.

Treatment	Time in seconds to	
	respond	approach
24-hr control	176	171
Age at handling		
5-hr	251	259
9-hr	201	252
13-hr	280*	279
17-hr	279*	258
23-hr	292*	279*

\*Significantly different ( $P < 0.05$ ) from controls. N=72



to controls.

This age effect suggests a maturation process within the bird which makes age an important factor in the response obtained. An alternate hypothesis could be that the differences found between ages might reflect the length of time between handling and testing. That is, brief visual and tactile stimulation may temporarily (at least 5 minutes) satiate the drive to respond, but prolonged isolation after such stimulation may cause the chick to forget its prior experience. Hence, the approach response would not be affected.

The explanation of our results is not at odds with Guiton's (1959) interpretation of his study of drive-satiation vs. generalization in the imprinting of chicks. Chicks in our experiment which received short periods of stimulation probably did not imprint (and/or learn) sufficiently to generalize to a different stimulus after several intervening hours of isolation; Guiton's chicks, on the other hand, received prolonged socialization before subsequent isolation and testing.

The results of Trials 4 and 5 were consistent with those of Trials 1, 2, and 3 in that prior handling in the light lowered the approach response. The chicks that received such prior experience at 13, 17, or 23 hours

generally froze when placed in the testing area. The results of the experiments reported here therefore offer some support to Salzen's (1962) statement that fear responses appeared to be independent of age, but dependent upon the difference between the test situation and the previous environment. Sluckin and Salzen (1961) suggested that fear responses, at least in the form of avoidance, are the response that imprinted chicks make to foreign objects. Our experiments show that such responses can occur as a result of handling and/or socialization as early as 13 hours and that these responses lower the approach response.

Guiron (1959), Hess (1964), and others found that prior socialization lowers imprinting strength in chicks. James (1960b) found that extended periods of socialization inhibited responses toward an imprinting device. It may therefore be postulated that relatively short periods of tactile-plus-visual experience, even at early ages, may temporarily satiate a chick's tendency to respond toward a parent surrogate and that any decrement of response or approach tendencies resulting from prior handling or socialization is a result of satiation that occurred at the time of prior stimulation. Thus, early imprinting could make the birds fearful of foreign objects, or could simply increase their selectivity to

a particular stimulus. In the context of such a hypothesis, it is assumed that a response and subsequent approach toward a distant imprinting device constitutes an initial phase of imprinting. It seems biologically unrealistic to assume otherwise.

### Summary

Since banding chicks prior to testing inhibited the approach response it was hypothesized that the tactile and visual stimulation of such treatment satiated, at least temporarily, the drive to respond to an imprinting apparatus. Trial 1 was designed to determine if handling in the light inhibited a chick's response toward and approach to the apparatus. Chicks handled took significantly longer to approach than non-handled controls. Trial 2 was designed to test this difference further and to dissociate effects of handling in darkness and in light. It was found that handling in darkness had no effect on the number of responses while handling in light decreased the number of responses. A third trial was designed to dissociate the sensory modalities involved. It was concluded (1) that tactile stimulation per se, i.e. handling in darkness, had no effect on the approach response, (2) that visual stimulation alone increases numbers of responses and appro-

aches, and (3) that tactile-plus-visual stimulation inhibited a chick's tendency to respond toward and approach an imprinting apparatus.

A trial designed to test effects of prior socialization and handling indicated that isolate chicks show better approach responses than socials, and that prior handling could mask this effect.

When chicks were handled in the light at times other than just prior to testing, an age effect and/or a memory factor was introduced. Handling at 5 and 9 hours had no effect on the approach responses, while handling at 13, 17, or 23 hours post-hatching decreased such responses.

Results were explained on the basis of a drive satiation hypothesis.

#### IV Peripheral Experiments

This section contains brief phases that constituted a part of the total research effort but were not incorporated into the discussions of Experiments I, II, and III.

Additional Correlated Responses. Experiment I was concerned with bidirectional selection for time to respond toward a distant audio-visual imprinting apparatus. Preliminary trials of this experiment had shown a difference in response time between Siegel's (1962) high and low weight lines (HBW and LBW). Since his lines were selected in opposite directions for juvenile body weight and also differed in hatching weight, these traits were measured in the FR and SR lines. Genetic relationships could not be calculated in the SR line for reasons discussed in Experiment I. Correlations were found in the FR line in the  $F_1$  generation but not in subsequent generations. This is suggestive of linkage. Age to first egg and rate of egg production were also measured in the selected lines. Since the  $F_4$  generation has not reached sexual maturity no discussion concerning egg records is presented here.

Developmental Age and the Critical Period. Intergroup differences for incubation periods were presented

(Table 13) with a discussion of the relationship between developmental age and incubation period. If imprinting occurs during a certain critical period of development, lines differing in imprinting responses at the same chronological age may be at different maturational stages. Therefore, selection for imprinting responses could cause concomitant changes in developmental rates. To evaluate this hypothesis somite counts were made at 42-hrs incubation, and thermoregulatory abilities were measured during the first few days posthatching. Mean 42-hr somite numbers in the  $F_4$  generation were  $9.8 \pm .42$  and  $11.7 \pm .54$  in the FR and SR lines, respectively. The difference of 1.9 somites was highly significant. Further, the FR chicks hatched significantly slower than SR chicks ( $506 \pm 1.05$  hr vs.  $502 \pm .95$  hr). This relationship between somite number and incubation period was consistent with that found in other lines of chickens (Siegel et al., 1968).

The changes in developmental rate that occurred with selection for imprinting suggests that the critical period for the latter may have been modified by selection. Preliminary data indicate no critical period in the SR line up to 40 hours posthatching whereas in the FR line strongest imprinting tendencies were observed at about 24 hours. These data indicate that the concept of

a critical period for imprinting requires further detailed study.

Genetic differences have been demonstrated in the maturation of temperature regulating ability in chicks (Hutt and Crawford, 1960). Thermoregulatory data were obtained initially for the HBW and LBW lines and subsequently for the FR and SR lines. Twenty-three HWL and twelve LWL chicks were placed in individual wire compartments and left in a 13° C room for 30 minutes. Cloacal temperatures were recorded with an electric thermometer on each of the first six days posthatching. Lines, days, and the line-day interaction were highly significant. HBW chicks (which exhibit stronger imprinting responses at 24 ± 2 hr posthatching) were significantly more efficient at maintaining their body temperatures on days 2, 4, 5, and 6. Also, the thermoregulatory abilities developed more rapidly in the HBW than in the LBW line. These results suggest that chicks' response to the imprinting stimulus may be correlated with their developmental rates.

To investigate this possibility F<sub>4</sub> generation chicks from the FR and SR lines were tested for thermoregulatory abilities. To increase the sophistication of the recording technique a 24 channel Honeywell Multipoint Recorder was used to automatically record subcutaneous

temperatures of FR and SR chicks maintained in small hatchers. Ambient temperatures were varied four times daily and recordings obtained from thermocouple implants. Unfortunately, with this procedure intragroup variation was too large to allow meaningful comparisons. The more pedestrian method of obtaining temperatures via cloacal measurements was more efficient than the more elaborate method of obtaining subcutaneous temperatures. Further study is necessary in this area.

Memory and Prior Experience. Handling chicks at 5 and 9 hours of age produced no observable effect whereas handling at 13, 17, or 23 hours of age decreased response tendencies (Experiment II). Testing was at 24 hours, and it was not clear whether the effect was due to age itself or memory, i.e. handling could have temporarily satiated response tendencies but prolonged isolation after handling could result in forgetting. Experiments were designed to differentiate the age and/or memory factor.

LBW chicks were randomized into treatment groups of 8 chicks each as follows:

<u>Age handled (hr)</u>	<u>Age tested (hr)</u>
8	12 16 20 24
16	20 24 28 32



Nonhandled control chicks were tested at all ages. The assumption was that comparisons within columns would disclose whether chicks handled at 8 hr and 16 hr of age differed from controls or from each other while comparisons within rows with control chicks would disclose the importance of the interval between handling and testing (i.e. memory).

Analyses were attempted using parametric and non-parametric tests including an attempt to fit curvilinear regression lines to group means with an IBM 7040 computer. All attempts to analyze the experiment failed because of the magnitude of intragroup variation.

This entire experiment was repeated using HBW chicks which were known to exhibit better imprinting responses. Again there was no solution to the statistical problems associated with analyses. The conclusion is that this question can not be resolved until a line is developed with a low phenotypic variability for imprinting responses. Continued selection in the FR line could provide such a population.

## ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks and appreciation to Dr. Paul B. Siegel for his role as major advisor during the course of this study.

Dr. Siegel's unique abilities for guiding and encouraging students is exceeded only by his understanding and tolerance for their errors and shortcomings.

Thanks are also expressed to Dr. Perry C. Holt, Dr. C. E. Howes, Dr. H. P. VanKrey, and Dr. D. A. West for their help in reviewing this dissertation and to Dr. C. E. Holmes for furnishing some of the birds used in the study.

Special thanks are extended to my wife, Jane, for her encouragement and support during the years of my graduate education and for her patience in typing this manuscript.

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## VITA

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GENETICS OF INITIAL IMPRINTING RESPONSES:  
SELECTION AND HETEROSIS

by

Hannon B. Graves

ABSTRACT

Domestic chicks were tested for initial response, approach, and stay-near tendencies during one 5-minute test exposure to a distant audio-visual imprinting apparatus. Significant interline differences demonstrated genetic variation.

A bidirectional selection program was initiated with time to respond as the selected trait. Approach and stay-near tendencies were measured as associated traits. Results through the  $F_4$  generation disclosed that in the fast response line the realized heritability of the selected trait was .32 and the correlated realized heritabilities for time to approach and for time spent near the apparatus were .36 and .32, respectively. In the slow response line selection was ineffective, and heritabilities for the three traits were zero. Genetic and phenotypic relationships among the traits were high in the fast response line, but genetic relationships in the slow response line could not be determined because

of the zero heritabilities. The asymmetrical response to selection could not be explained by the usual causes, and it is hypothesized that responsiveness is a threshold trait.

Comparisons of 290 purebred chicks with 379 crossbred chicks for response, approach, and stay-near tendencies provided evidence of heterosis. This demonstration of non-additive effects implies fitness roles for these traits and supports the idea that they indicate imprinting tendencies.

Time of day tested, sex, hatch, mating type, developmental age, and 24-hr body weight were considered independent variables influencing the behavior traits. Multiple regressions showed that the initiation of responsiveness is not predictable. However, once a chick responded, its behavior was highly predictable; this supports the hypothesis from the selection experiment that responsiveness is a threshold trait.

Sensory modalities involved in satiating the behavior traits were differentiated by comparisons of non-handled chicks with those exposed to visual, tactile, and visual-plus-tactile stimuli just prior to testing. Visual stimulation alone increased responses and approaches, whereas tactile stimulation alone had no

effect on these behaviors. Tactile-plus-visual stimulation just prior to testing inhibited response and approach tendencies. Evaluation of the effects of prior socialization and handling indicated that isolation enhanced approach responses, and that prior handling had the opposite effect. An age and/or memory factor was introduced when chicks were handled in the light at times other than just prior to testing. Handling at 5 and 9 hours had no effect on the approach response, whereas handling at 13, 17, or 23 hours post-hatching decreased such responses. Results were explained on a drive satiation hypothesis.