AGONISTIC BEHAVIOR, THE DEVELOPMENT OF THE SOCIAL HIERARCHY AND STRESS IN GENETICALLY DIVERSE FLOCKS OF CHICKENS

by

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INTRODUCTION

The study of agonistic behavior, which consists of both aggressive and submissive components, has established the foundation of much of the current understanding of the social organization of vertebrates. Accordingly, much attention has been centered upon this aspect of social behavior, with research involving both the theoretical and the practical implications of agonistic behavior. Recently, several questions have been raised regarding the actual importance of agonistic behavior in functionally important aspects of social organization (e.g. Rowell, 1974; Syme, 1973).

Two aspects of the agonistic behavior of chickens, a classic experimental animal for such studies, were considered in this research: 1) the genetic influences upon the development of agonistic behavior and the social hierarchy associated with selection for high and low juvenile body weight and with the sex-linked dwarf gene (*dw*), and 2) the relationship of agonistic behavior and social rank with physiological stress during the initial formation of the social hierarchy.

Both aspects of this research represent considerations pertinent to the basic understanding of social organization. In addition, because of the economic importance of the animals which were used in this study, the results are relevant to practical concerns in the broiler industry. In particular, both selection for juvenile body weight and the dwarf gene are factors of importance in commercial breeding programs. Furthermore, animal welfare interests have frequently suggested that agonistic behavior under intensive husbandry conditions is stressful to chickens.

This research was accordingly designed to provide certain basic information in these areas.

REVIEW OF THE LITERATURE

Characteristics of the social hierarchy. The social organization of the domestic fowl (Schjelderuppe-Ebbe, 1913; 1922) is characterized by a social hierarchy based upon peck rights. This system is essentially the function of a stable dominant-subordinant relationship that develops between each pair of individuals within a flock. A high frequency and intensity of agonistic encounters typifies the period of the formation of the social hierarchy. This is followed by a period of decline of such encounters to a lower, relatively stable level, as the relationships between individuals become established and the hierarchy stabilizes (e.g. Siegel and Hurst, 1962).

The residual level of agonistic activity within a flock subsequent to the formation of a stable hierarchy is an important aspect of the social organization of chickens for several reasons. First, a certain amount of reinforcement is essential for the maintainance of the social inertia of the relationship between each pair of individuals, and thus, for the maintainance of a stable social hierarchy (Guhl, 1964). Second, it is through these agonistic encounters that the effects of social rank and the stability of the social order are mediated. Finally, the agonistic behavior within flocks provides a direct means of studying the social order which is superior to other methods, such as paired encounters and competetive trials, since the former tends to yield biased orders (Chase, 1974) and the latter may yield an order which is unrelated to those based upon agonistic behavior (Syme, 1974).

Beyond measuring the reduction in agonistic behavior concommitant with the formation of the social hierarchy, two other measures have been used to determine the stability of the social order. The first is the Landau index (Landau, 1951), $h=12/(n^3-n)\sum_{a=1}^{n}[(Va-(n-1)/2]^2$, in which Va is the number of individuals dominated by the ath individual, and n is the number of individuals in the social order. The portion of the equation within the summation is the variance in social rank, while the factor $12/(n^3-n)$ standardizes that variance to a range of from 0 to 1. This measure estimates the linearity of the hierarchy; a value of 1 representing a completely linear order, while a value of 0 occurs in the case where all individuals have equal rank in the order. Despite its apparent utility as a comparative measure, the Landau index has received little use in studies involving social organization (Chase, 1974; Beckoff, 1977).

A second measure was used by McBride _et al_. (1970) to determine another, partially unrelated aspect of the stability of the social order (Beckoff, 1977), namely, the stability of an individual's rank over time. They utilized the product moment correlation between an individual's rank during a given week and its rank during each subsequent week. The use of the McBride _et al_. (1970) procedure, in combination with the Landau index, facilitates the study of both the linear stability of the social order and the stability of individual relationships within that hierarchy.

The ontogeny of agonistic behavior. The seminal work in this area is that of Guhl (1958), who stated that the centripetal forces of social behavior, imprinting and flocking behavior, develop long before the centrifugal forces of agonistic behavior. He observed that while the earliest aggressive peck occurred at the end of the second week posthatching, avoidance behavior in response to an aggresive peck did not appear until the fifth week of age. Fighting appeared during the sixth week, but did not appear to result in decisions in most cases. Ratner (1965) observed that aggressive pecking and submitting to pecks commenced at approximately 17 days posthatching, and that fighting appeared early in the sixth week. These results are similar to those of Guhl (1958) and of Dawson and Siegel (1967), although it is difficult to evaluate the ontogeny of submissive behavior in the case of Ratner's study. This is because he provided no clear definition of submission, while avoidance was a defined behavior in the studies of Guhl and of Dawson and Siegel. Kruijt (1964) studied the ontogeny of social behavior in the junglefowl, and showed that the development of agonistic behavior was quite similar to that reported for the domestic fowl.

Guhl (1958) observed that males formed stable peck orders between 50 an 55 days of age, while females did not form stable orders until approximately 10 days later. He also observed that during the first six weeks posthatching, that males delivered about ten times more pecks than did females. McBride _et_ _al_. (1970) found that although completely stable peck orders were not established by 19 weeks posthatching, the peck orders estimated from the second through the eighteenth weeks were all highly correlated with that observed during the nineteenth week.

Effects of agonistic behavior and the social hierarchy. Social strife may be characterized by abnormally high levels of agonistic activity. In chickens , social strife may be produced either by disrupting an existing social order or by preventing its initial formation. Several studies have used these methods as means of determining the biological advantages of the social hierarchy and the physiological effects of strife. Guhl and Allee (1944) observed that chickens maintained in disrupted flocks showed reduced body weight, feed consumption, egg production and comb size in comparison to those maintained in organized flocks. Siegel and Siegel (1961) observed that adrenal weights of chickens increased during the period of peck-order formation. Such increases may be considered as an indication of physiological stress (Garren and Schaffner, 1956).

It has been shown in a series of experiments (Gross and Colmano, 1967; 1969; Gross, 1972; Gross and Siegel, 1973) that high levels of social strife result in reduced resistance to viral infection and increased resistance to bacterial infections, and that abnormally low levels of agonistic activity result in an opposite effect. These effects were attributed to respectively high and low levels of physiological stress in response to the degree of social strife. McBride *et al.* (1970) noted a significant negative correlation between the number of pecks given and adrenal weight, but not between adrenal weight and social rank. Williams *et al.* (1977) observed significant increases in plasma corticosterone levels concommitant with the formation of the social order in flocks of cockerels, whereas there was no correlation between social rank and corticosterone titers.

The rank of an individual in the social hierarchy influences its ability to attain important physical resources such as feed and water (Guhl, 1953; Tindell and Craig, 1959). This may provide a partial explanation for the observation that low ranking birds are less productive (Sanctuary, 1932; Guhl, 1953; Tindell and Craig, 1959; Biswas and Craig, 1971), mature later (Guhl, 1953; Tindell and Craig, 1959) and weigh less during development (McBride *et al*., 1970) than higher ranking flockmates. Social rank may also influence reproductive success. Guhl *et al*. (1945) noted that high ranking females mated less frequently than those of lower ranks, a factor which may be related to the aggressive component of courtship behavior eliciting a non-submissive (i.e. non-receptive) response in the high ranking pullets. Guhl and Warren (1946) reported that high ranking males mated with a higher frequency than did lower ranking males, whereas Craig *et al*. (1977) did not observe this relationship.

The genetics of agonistic behavior. Most of the current knowledge of the genetics of agonistic behavior results from experiments in which there was direct selection for either aggressiveness or dominance ability, or when these traits were measured as correlated responses to selection for other traits. From a selection experiment, Guhl *et al.* (1960) calculated heritabilities of 0.22 and 0.18 for the percentage of contests won in initial paired encounters and the number of chickens dominated, respectively. Craig *et al.* (1965) obtained realized heritabilities of 0.16 and 0.28 for White Leghorns and Rhode Island Reds, respectively, for dominance ability based on performance in initial paired encounters. Correlated responses for body weight , survivorship and various production characteristics to artificial selection for social dominance were observed by Craig (1968) and Craig and Toth (1969). Also, selection for early egg production (Lowry and Abplanalp, 1972) and late egg production (Bhagwat and Craig, 1978) appear to result in a correlated response of increased dominance ability.

The effect if the dwarf gene (*dw*) upon agonistic behavior was studied by Faure and Ricard (1973). They found that dwarfs, when compared to normal cockerels, had significantly greater dominance ability and willingness to fight, but were not different in the frequency of pecking.

Agonistic behavior and the housing environment. Interest in the influence of different forms of housing and the density of chickens within the houses on both behavioral and production traits in chickens has increased in recent years. This is because of a possible conflict between the optimization of flocking densities to maximize profits per unit of facilities and a concern for the well-being of the chickens maintained under conditions of high population densities.

Several studies have shown that the frequency of agonistic encounters is lower in battery cages than in deep litter pens (Bareham, 1972; Hughes and Black, 1974; Craig and Bhagwat, 1974; Hughes and Black, 1978). Within cage environments, agonistic behavior diminishes with increased densities (Polley *et al*., 1974). Furthermore, the frequency of agonistic encounters increases with flock size when density is held constant (Hughes and Wood-Gush, 1977), indicating a relationship between the social and physical environment.

McBride (1970) and Craig *et al*. (1969) hypothesized that the reduction in the frequency of encounters with increased density of housing was due to two factors. One was the inhibitory effect of the presence of dominant individuals on the encounters of their subordinants. The other resulted from the desensitization to normal releasers of aggressive behavior, i.e. the entry of one individual into the personal space of another, since under high density conditions, other individuals are nearly continuously within the personal space of an individual. The former theory has found support in the recent investigations of Bhagwat and Craig (1979) and Ylander and Craig (in press) who studied the effect of dominant individuals upon the agonistic encounters between their subordinants. The latter theory found support in the study by Craig *et al*. (1969).

METHODS AND MATERIALS

Stocks and husbandry. The chickens used in this research were from two lines which had been divergently selected for juvenile body weight and into which the sex-linked dwarf gene (*dw*) had been introduced (Reddy and Siegel, 1977; Siegel, 1978). The matings used to produce these chickens were as follows: homozygous normal males (*DwDw*) were mated to normal females (*Dw-*) to produce homozygous normal males and normal females. Heterozygous males (*Dwdw*) were mated to dwarf (*dw-*) females to produce 1/2 heterozygous and 1/2 dwarf (*dwdw*) males and 1/2 normal and 1/2 dwarf females. Additional homozygous dwarf males and dwarf females were produced through the mating of homozygous dwarf males with dwarf females. The parental generation was the sixth backcross generation following the introduction of the dwarf gene into the selected lines.

The chicks were hatched and wing-banded on May 21,1978. On the following day, they were vent sexed, vaccinated for Marek's disease, segregated into flocks by sex, line and genotype, and housed in brooder cages under constant illumination. Feed, which consisted of the diet used during the course of the selection of these populations (Siegel, 1962), and water were provided ad libitum.

On June 21, 1978, three flocks of eight birds from each of the four line-genotype combinations were randomly selected from their respective populations and housed in 78 by 78 cm wire floor cages which provided 714 cm² of floor space per bird. The dorsal plumage of each chicken was marked with a combination of colored dyes to facilitate the recognition of each individual during behavioral observations. Feed and water were provided ad libitum and the lighting was continuous. During the first week after housing, any chicken which died was replaced from the available stock. Individuals which died or became ill subsequent to this first week were removed from the flock and were not included in the data for purposes of analysis subsequent to the onset of illness. The first replicate of the high weight dwarf pullets was not included in the analyses due to the loss of four individuals to illness or death.

All care and handling of the chickens, as well as all observations, were performed by the author, except where noted.

Behavioral observations. Each flock was observed for twelve 20-minute periods per week from the fifth through the tenth week post-hatching, and with diminishing frequencies through the termination of the experiment which was at the end of the fourteenth week. Each agonistic encounter was recorded in terms of the winner, the loser and the intensity of the encounter (Table 1). The behavioral categories outined in Table 1 were defined as follows:

> Fights were characterized by the initial upright postures of both individuals with aggressive physical contact being made by both pullets.
>
> Pecks included only those blows which were delivered to the top of the head, the comb or the wattles; pecks delivered to other areas were not considered aggressive behaviors for the purposes of this study.
>
> Threats involved directed aggressive movement toward another pullet, and were usually characterized by an upright posture. The front threat display of Kruijt (1964) was not observed during this study.
>
> Avoidances consisted of a lowering and/or averting of the head and occasionally the body with little locomotion away from the aggressor.

Table 1. Scores used in measuring agonistic encounters.

Behavior		
Aggressive	Submissive	Score
Fight	None	8
Peck-chase	Flee	7
Threat-chase	Flee	6
Peck	Flee	5
Threat	Flee	4
Peck	Avoid	3
Threat	Avoid	2
None	Avoid	1

Fleeing consisted of excited locomotion away from an aggressor.

Chases were counted when the aggressor pursued a fleeing individual.

The number of encounters won and lost by each individual was summed weekly. Average intensities were the sum of the intensity scores for the encounters won divided by the number of encounters won, and as the sum of the intensity scores for the encounters lost divided by the number of encounters lost. The number of encounters won and the number lost were converted to frequencies by dividing the cummulative number of encounters by the number of hours of observation. Thus, each individual received four behavioral scores for each week:

the frequency of encounters won,
the frequency of encounters lost,
the average intensity of the enccunters won, and
the average intensity of encounters lost.

The average number of encounters per individual per hour within a flock and the average intensity of those encounters were considered as the measure of the social activity within the flock.

The social order of each flock was determined weekly from the sixth through the thirteenth week post-hatching on the basis of the observed relationships between each pair of individuals in the flock. When no agonistic encounters were observed between a pair of individuals, the dominance relationship was determined on the basis of their relative postures when in close proximity. It was frequently necessary to tap upon the cage to cause the pullets to move about, and thereby bring different pairs into proximity. The use of this procedure was facilitated by the assistance of a second observer. The rank of an individual was taken as the number of other pullets over whom she was dominant. Landau indices (Chase, 1974) were computed weekly for the social orders of each flock. Product moment correlations between individual social ranks for each week from five to ten weeks of age, were computed after determining that the within flock covariance matrices for each line-genotype combination were homogeneous.

Physiological measurements. Data were obtained for characteristics known to be sensitive to stessors. These were growth rate of the body, plasma corticosterone levels, antibody production in response to red blood cell antigens, and resistance to a bacterial challenge.

The body weight of each individual was measured in grams at the beginning of each week, and the weekly changes computed. The percentage weight change was computed as the gain for that week divided by the weight at the beginning of the week. During the sixth, tenth and fourteenth weeks post-hatching, each pullet was given a 0.1 ml intravenous injection of a 0.5% suspension of red blood cells (RBC) in physiological saline. The RBC antigens used were swine, sheep and horse cells in the sixth, tenth and fourteenth weeks, respectively. Five days after injection of the RBC antigen, 0.5 ml of blood of blood was drawn from the brachial vein for determination of antibody titers and plasma corticosterone levels. Antibody titers were measured by the microdilution technique and plasma corticosterone levels were determined using a modification of Murphy's (1967) competetive protein binding radioassay (Mauldin, 1978), with the following exceptions:

> 1) Incubate the samples at 45°C for 10 rather than 5 minutes. 2) Add floracil to complete set of samples (50) and shake as a unit for 60 seconds.

At the end of the sixteenth week, each pullet was innoculated in the posterior-thoracic air sac with 0.1 ml of a 24 hr incubated culture of serotype O1 K1 <u>Escherichia</u> <u>coli</u> in tryptose broth at a dilution of $10^{-3.5}$. Mortality was recorded during the next five days, after which the survivors were sacrificed and post-mortem examinations were performed to determine the extent of heart and air sac lesions. Four levels of infection were considered; mortality, heart lesions, air sac lesions without heart lesions and an absence of lesions (uninfected).

Analyses. Analyses of covariance were used to test for differences between the lines, dwarf genotypes and their interactions in the regression of the average frequency and intensity of agonistic encounters within flocks and of the Landau indices on age. The model used was:

$$Y_{ijkl} = \mu + L_i + G_j + (LG)_{ij} + \beta\,(w_k - \overline{w}) + e_{ijkl}$$

where, i = 1,2 lines, j = 1,2 genotypes, k = 1,2,...,n ages, l = 1,2,...,n individuals, and β the regression coefficient of the dependent variable on age.

Analyses of variance were used to detect differences between the lines, dwarf genotypes and their interactions in antibody titers, plasma corticosterone levels, weight gain and percentage weight gain using the model:

$$Y_{ijkl} = \mu + L_i + G_j + R_k + (LG)_{ij} + (LR)_{ik} + (GR)_{jk} + (LGR)_{ijk} + e_{ijkl}$$

where i = 1,2 lines, j = 1,2 genotypes, k = 1,2,3+ replicates and l = 1,2,...,n individuals. Differences in disease resistance were tested using Bonferroni's Chi square with tau = 3.

The relationship of social rank with the frequency and intensity of agonistic encounters was analyzed using a maximum R^2 improvement stepwise multiple regression procedure (Barr et al., 1976), where rank was the dependent variable and the frequency and the average intensity of encounters were each taken as the independent variables. A preliminary analysis of covariance indicated that the effects of age, line, dwarf genotype and their interactions were not significant. Accordingly, the data were pooled across weeks and flocks.

The stepwise multiple regression procedure cited above was also used to determine the association of the physiological measurements with social rank and agonistic behaviors. Since a preliminary analysis indicated highly significant heterogeneity of the within flock covariance matrices, regression models were computed within flocks for each week's data.

Product moment correlations were computed within flocks for each of the combinations of behavioral and physiological variables. The correlation coefficients were then tested for heterogeneity and pooled when homogeneous.

\+ Only two replicates analyzed for the high-weight dwarf combination (see Stocks and husbandry).

The relationship between the behavior measurements, the stability of the social hierarchies and the physiological variables was examined using product moment correlations computed from the error sums of squares and cross proucts from analyses of variance and covariance where the model was:

$$Y_{ijk} = \mu + L_i + G_j + (LG)_{ij} + e_{ijk}$$

where i = 1,2 lines, j = 1,2 genotypes, and k = 1,2,3+ flocks.

+Only two flocks in the high weight dwarf group.

RESULTS AND DISCUSSION

Agonistic behavior and the development of the social hierarchy.

The mean freqencies and intensities of agonistic encounters within each flock between 5 and 10 weeks of age are shown in Figures 1 and 2, respectively. In general, both the frequency and the intensity of encounters declined with age. During the period between 5 and 7 weeks of age, however, there was considerable variation among flocks and between genotypes, with the general pattern being for a slight increase between 5 and 6 weeks of age in the dwarf flocks and a more erratic pattern in the normal flocks. The difference between normal and dwarf flocks may have been caused by behavior-developmental differences due to the dwarf gene--a factor which should be considered in future behavioral studies of genetically diverse populations.

The regressions of the mean frequency of encounters on age are shown in Figure 3. The analyses of covariance indicated that the slopes of the regressions of the mean frequency of encounters on age differed significantly

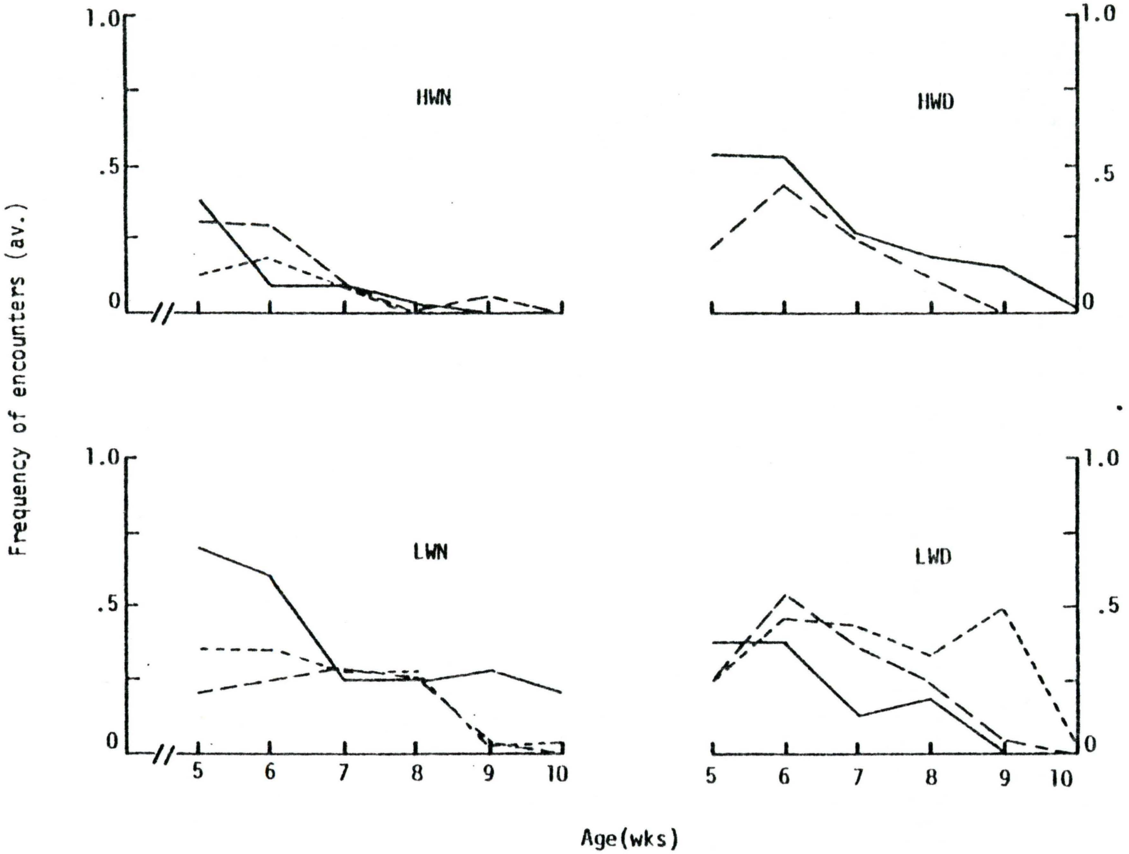

Figure 1. The average number of agonistic encounters per hour for each week, by replicates within genetic groups.

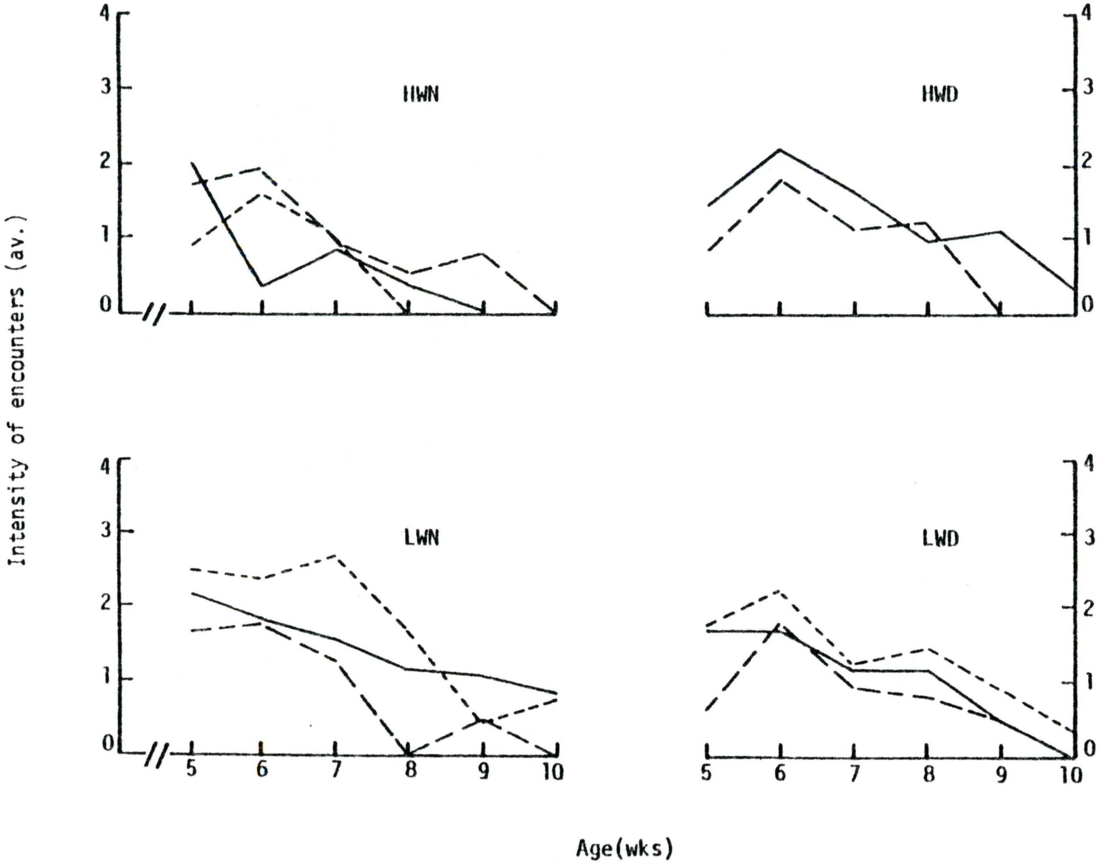

Figure 2. The average intensity of agonistic encounters for each week shown by replicates within genetic groups.

between the dwarf and normal genotypes, but not between the high weight and low weight lines, as tested by the age by genotype and age by line interactions (Appendix 1a). After the effect of the regression on age was removed, both the differences in frequency of encounters between lines and genotypes were significant (Appendices 2b and 2c), with larger means for the low than for the high weight flocks and for the dwarf than for the normal flocks (Figure 3).

The regressions of the mean intensity of encounters on age are presented in Figure 4. While the slopes of the regressions were not significantly different (Appendix 1a), the intercepts were different (Appendix 1c). These differences are principally due to lower mean intensity of encounters in the high weight normal flocks than in the other groups.

These results indicate that divergent selection for juvenile body weight resulted in chickens in the high line which had both a lower frequency and intensity of agonistic encounters than those in the low weight line. Dwarf chickens had a higher frequency of encounters, regardless of line, than normal chickens, while the effect of dwarfism on the the intensity of encounters was expressed only in the genetic background of the high weight line.

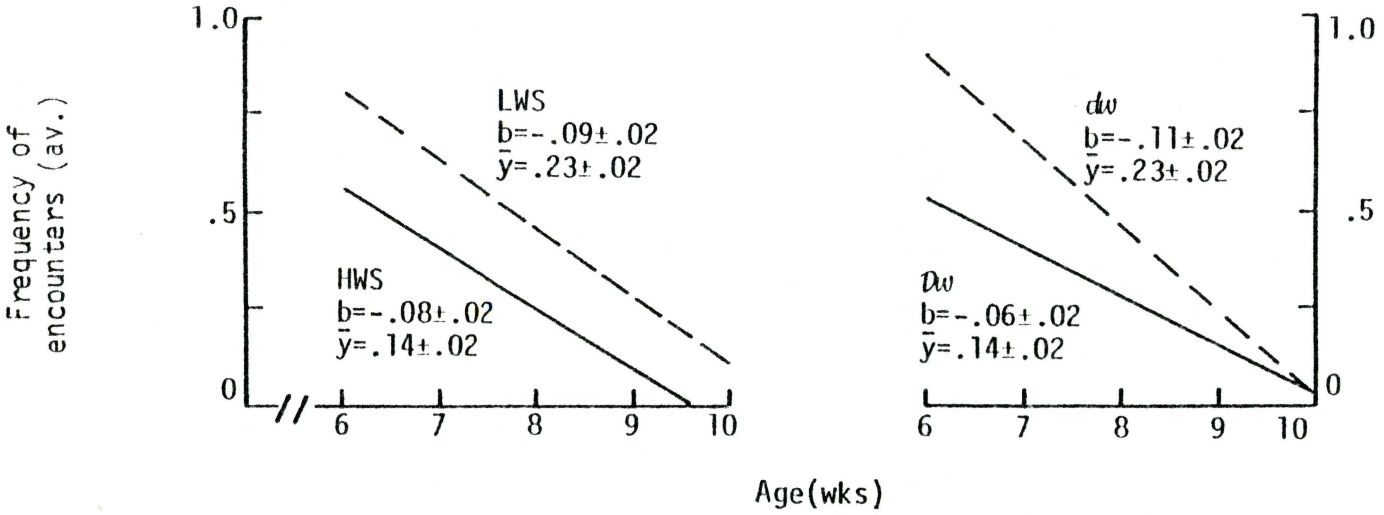

Figure 3. Regressions of the average number of agonistic encounters per hour on age, as computed within lines and genotypes.

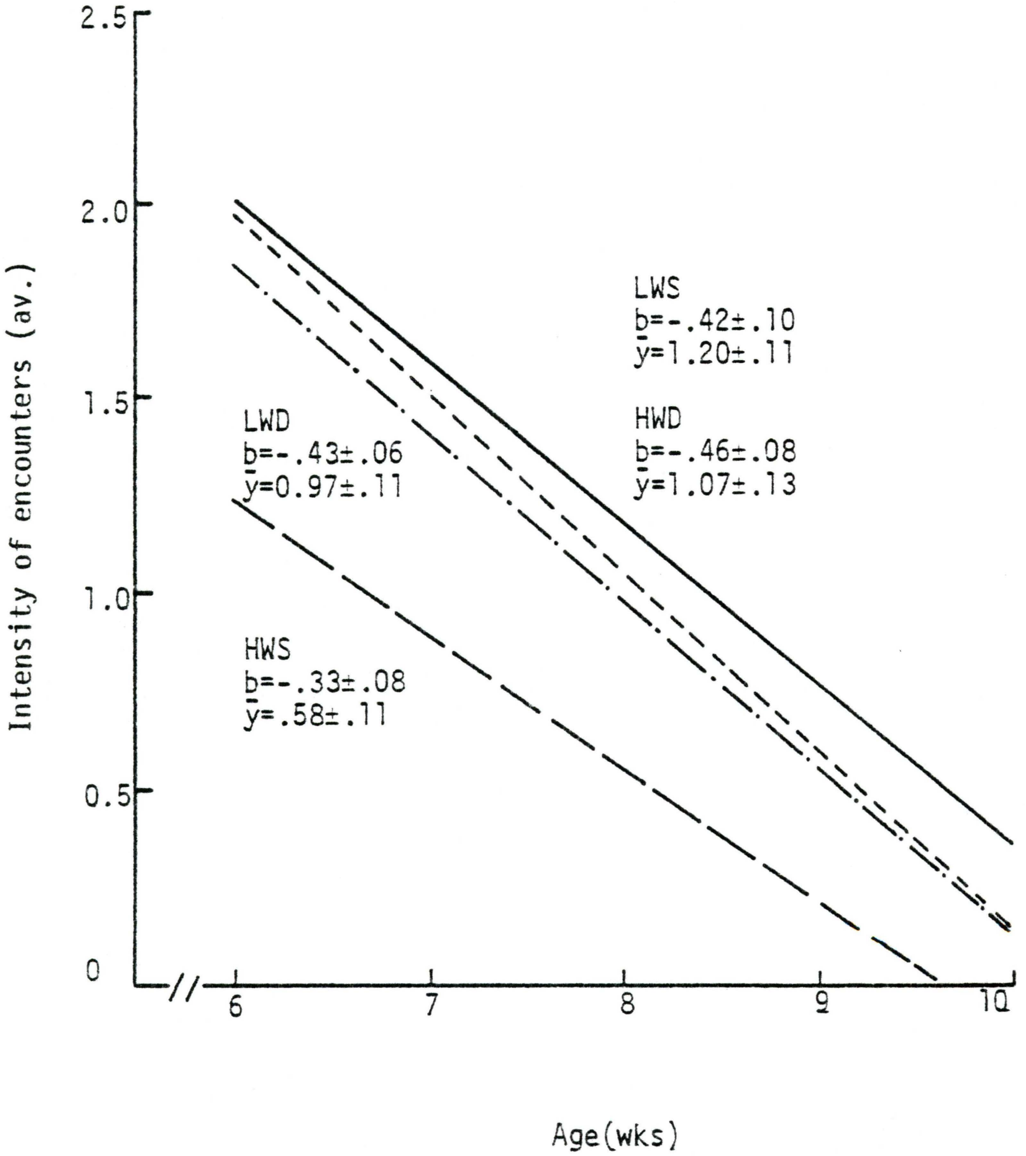

Figure 4. Regressions of the average intensity of encounters on age, as computed within genetic groups.

These results differ from those of Dawson and Siegel (1967), who found no differences between these lines for agonistic encounters in the second generation of selection in these lines. This is not surprising, however, since correlated responses not obvious in the early generations may appear subsequently during long term selection. These results appear to be inconsistent with those of Faure and Ricard (1973), who found no overall increase in the frequency of encounters due to the introduction of the dwarf gene into an unspecified genetic background. It is interesting that selection for increased growth rate decreased agonistic activity, while selection for egg production characteristics, which generally have negative genetic correlations with body weight (Kinney, 1969), tends to increase such activity (Craig *et al.*, 1975; Bhagwat and Craig, 1977; Lowry and Abplanalp, 1972; Bhagwat and Craig, 1978).

The development of the social hierarchy. There were no significant differences among the lines, dwarf genotypes or their various combinations for either the slope or the intercept of the regression of the Landau index on age (Appendices 2a, 2c). The regression was significant ($b = .03 \pm .01$, $p < .05$). Landau indices were high at six weeks and subsequent ages in most flocks (Figure 5). Furthermore, the proportion of index values which exceeded 0.9, a value suggested by Chase (1974) as the minimum delimiting a stable hierarchy, increased from 10 of 44 values during the first four weeks of observation to 23 of 44 during the last four weeks of this study.

The correlations between the social ranks at different ages (Table 2) were moderately high, and of magnitudes similar to those obtained by McBride *et al.* (1970) for pen-reared flocks. Correlations between the ranks of individuals at 6 and 7 weeks of age with those at later ages were considerably higher in dwarf than in non-dwarf flocks. This pattern suggests that social ranks estimated at 6 and 7 weeks of age were less stable in normal than in dwarf flocks. Although this difference may be due to several reasons, two appear most likely. First, the higher frequency and intensity of encounters in dwarf flocks might result in more firmly established dominance-subordinance relationships between individuals. Second, the higher frequency and intensity of encounters in dwarf flocks may have resulted in a more accurate assessment of the social relationships within each flock, i.e. reduced the observational error.

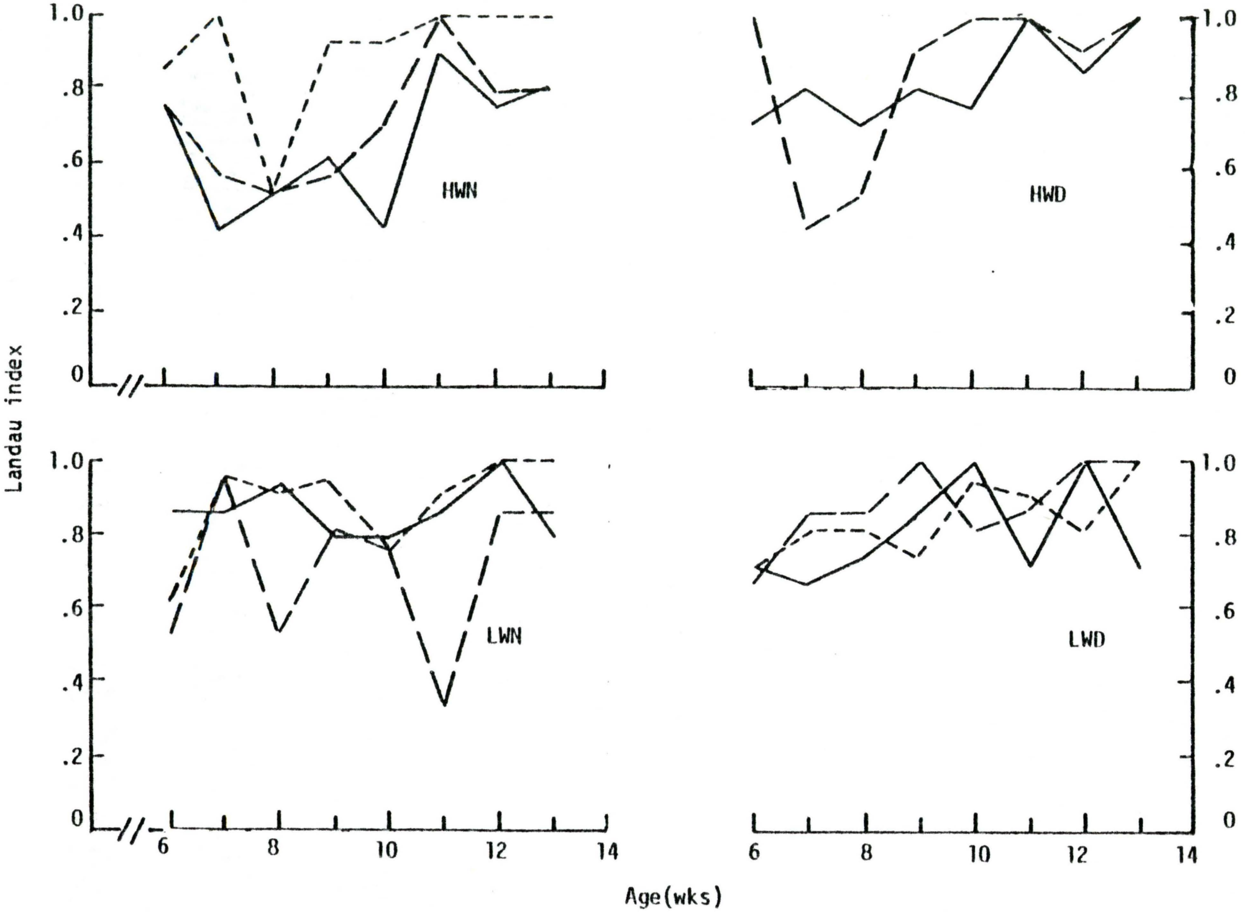

Figure 5. Weekly Landau indices shown by replicate within genetic groups.

It is evident from the two measures of the stability of the social orders of these flocks of chickens were relatively stable, even at their initial appearance. In comparing the stability of the social orders between dwarf and normal flocks, however, the stability of ranks appeared to remain constant over time in the dwarf flocks, and after the first two weeks of observation, in the non-dwarf flocks as well.

The multiple regression of social rank on the agonistic behaviors was highly significant (F = 25.0, d.f. = 4, 409, $p < .01$) The partial regression coefficients for the frequency of encounters won (.43 ± .10) and lost (-.34 ± .08) were highly significant, while the coefficients for the average intensity of encounters won (.13 ± .08) and lost (-.16 ± .08) were not significant. Thus, while it appears that the frequency of agonistic encounters is more important than their intensity in determining social rank, the multiple regression model accounted for only 20 percent of the variation in social rank. This suggests that, under the conditions of this experiment, agonistic behavior accounted for only a small part of the differences among individuals in social rank.

Table 2. Product moment correlations[†] of individual social ranks at 6, 7, 8, 9 and 10 weeks of age by lines and genotypes.

	HWN				HWD				LWN				LWD			
Age (wks)	7	8	9	10	7	8	9	10	7	8	9	10	7	8	9	10
6	.26	.26	-.04	-.36	.80	.68	.72	.79	.22	.35	.32	.07	.54	.46	.56	.49
7		.52	.67	.17		.79	.75	.81		.58	.73	.36		.77	.77	.55
8			.54	.08			.74	.69			.80	.55			.64	.56
9				.49				.77				.61				.66

[†] df_{22}, $r = .404$ for $p < .05$ and $r = .515$ for $p < .01$.

Physiological measurements. The analyses of variance of the weekly data for weight gain, percentage weight gain, antibody titers and plasma corticosterone titers are presented in Appendix 2. Characteristic differences for weight gain, existed between the lines and genotypes and for the line by genotype interaction in all weeks (Appendix 2a). These were that the pullets from the high weight line gain more than those from the low weight line, non-dwarfs gained more than dwarfs and the effect of the *dw* gene was greater in the low than in the high weight line (Blohowiak *et al.*, in press). The results for percentage weight gain were less consistent. Although the genotype effect was significant at all ages, the line, replicate, line by replicate interaction and genotype by replicate interaction effects were significant in some weeks and not in others (Appendix 2b).

The highly significant line and significant line by genotype interaction effects for antibody titers (Appendix 2c) are consistent with the results of Mauldin *et al.*, (1978). The line by genotype interaction was of the crossover type at six weeks of age with dwarf pullets having higher mean antibody titers (6.75 ± .34) than the normal ones in the high weight line (5.88 ± .27), while in the low weight line, dwarf pullets had lower mean antibody titers (7.33 ± .32) than the normal pullets (8.55 ± .41).

No differences in plasma corticosterone titers were noted until 14 weeks of age, when the line and line by replicate and genotype by replicate effects were highly significant (Appendix 2c). These results are in contrast to those of Mauldin _et_ _al._, (1978) who found a highly significant line by dwarf genotype interaction for plasma corticosterone at 455 days of age in the fifth backcross generation of these populations.

The two lines differed in survivorship following the bacterial challenge, with higher survivorship in the high weight line (Table 3). It is apparent that this is almost exclusively due to the very high mortality among the low weight dwarf pullets. These results are similar to those of Reddy _et_ _al._ (1975), who also found a difference between these weight lines resulting from a differential effect of dwarfism upon survivorship in the genetic backgrounds of the two lines. These results are also comparable to those of Mauldin _et_ _al._ (1977) who found similar results in the fifth backcross generation of these lines, but who also found contrary results in the fourth backcross generation where the experiment was confounded by a prior disease outbreak. These results indicate two important practical considerations for the study of single gene effects. First, prior history of the population studied may exert a profound

Table 3. Contingency table analyses of survivorship to *E. coli* challenge.

	HWS	LWS	Total	*Dw*	*dw*	Total
Dead	7	20	27	8	19	27
Live	33	26	59	33	26	59
Total	40	46	86	41	45	86

	HWS			LWS		
	Dw	*dw*	Total	*Dw*	*dw*	Total
Dead	3	4	7	5	15	20
Live	16	17	33	17	9	26
Total	19	21	40	22	24	46

influence upon the observed results, and second, the pleiotropic effects of a gene may exhibit considerable variation among the the genetic backgrounds in which it is expressed.

Agonistic behavior, social rank and physiological stress. In order to determine whether the pullets in these flocks were stressed by the agonistic activity typical of the development of the social hierarchy, both simple and multiple correlations were computed to determine the association of social rank and the behavioral measures with the physiological variables. The pooled correlations for each pair of measurements considered, and the proportion of the weekly within flock correlations which were significant are summarized in Table 4. The correlations between the frequency of encounters won and the average intensity of encounters won, and between the frequency of encounters lost and the average intensity of encounters lost were heterogeneous. These correlations were positive in all cases (ranging from .10 to 1.0 and from .04 to 1.0, respectively) and were significant nearly two thirds of the time (Table 4). The correlations between weekly weight gain and percentage weight gain were generally large and positive (Table 4). This was expected, since in all three cases, the variables are clearly not independent.

Table 4. Pooled correlations between various measurements, computed weekly within flocks (above diagonal) and the percentage of values included in the pooled correlation which were significant (below diagonal)+

Trait	F_w	F_1	I_w	I_1	Rank	WG	PG	AB	CS
F_w	--	-.08	*	-.00	.44	.20	.07	-.05	.06
F_1	2	--	-.00	*	-.35	*	-.07	-.03	.05
I_w	64	2	--	-.03	.34	.04	.01	-.16	.10
I_1	2	64	0	--	-.32	-.04	-.11	-.14	.13
Rank	12	14	5	7	--	.39	.10	-.04	-.11
WG	2	13	8	6	13	--	.85	-.05	-.23
PG	4	11	4	6	5	70	--	-.12	.05
AB	0	0	5	9	9	5	0	--	.05
CS	5	9	0	5	9	5	5	9	--

+Only correlations from those flocks in which agonistic behaviors were observed. for a given week are included in the pooled correlations.

*Correlation coefficients were significantly heterogeneous.

Correlations between the frequency of encounters lost and weekly weight gain were heterogeneous and ranged from -.88 to .86. The distribution of these correlations appeared to be centered near zero, suggesting that there may be a unique relationship within each flock.

The values of the remaining pooled correlations were low, and the percentage of the within flock correlations which were significant generally failed to exceed the level which would be expected by chance (i.e. 5 percent). The correlations with the highest values and most frequent significance were those between the behavioral measurements and social rank.

Evaluation of the hypothesis that agonistic behavior and low social rank are stressful during the ontogeny of the developmental social hierarchy requires the examination of not only the relative magnitudes of the correlations, but also of their signs. The pooled correlations were generally in the directions which would be predicted under the hypothesis. The pooled correlations between frequency and intensity of encounters lost with weight gain, percentage weight gain and antibody titers were all negative, whereas their correlations with the corticosterone titers were positive. Furthermore, while the correlations of the physiological variables with the measurements of the encounters lost were of consistently greater magnitude for intensity than for frequency, indicating that the intensity of encounters lost may have acted as a stronger stressor than did the frequency. The relationship was more erratic, however, for the correlations with the measurements involving encounters won. Although the frequency of encounters won does not appear to behave as a stressor in relation to the growth variables, the lower correlations of the average intensity of encounters won with growth may suggest that there was an increased physiological cost in increasing the intensity of the encounters, even if those encounters are won.

The correlations of social rank with the physiological measures were in the predicted direction with the exception of the small negative correlation of rank with antibody titers.

Thus, it appeared that, in general, agonistic activity had a slightly stressful effect, whether the encounters were won or lost. This effect, however, was not as clear when the associated trait was growth as it was with other measurements. This observation is consistent with that of Freeman and Manning (1975), who found that growth rate was less sensitive than adrenal measures to stress.

Although the product moment correlations between the behavioral and physiological measures were generally low and seldom significant, multiple regression analyses revealed significant effects of the four behavioral measures on each of the growth measures and on antibody and plasma corticosterone titers. The multiple correlation coefficients, although variable, were generally quite high (Tables 5). When the partial regression coefficients in Tables 5 were examined to determine whether the behavioral variables had the directional influences which would be predicted of stressors, it was immediately apparent that these coefficients were extremely heterogeneous. Not only was there a lack of general directional trends for these coefficients, but the coefficient of a given behavior frequently had opposite signs for the same physiological measurement between different flocks in the same week, and between different weeks for the same flock. These results may be atributed, in part, to statistical instability in the regression model due to the high correlations that inherently exist between the intensity of agonistic encounters and their frequency.

Table 5 a. Partial regression coefficients and R^2 values of the best fitting multiple regression models of weight gain and percentage weight gain (AB & CS) on behavioral variables, as computed within flocks at 5 weeks of age.

Weight Gain

	HWN			HWD		LWN			LWD		
Ind. Var.	1	2	3	1	2	1	2	3	1	2	3
Freq. wins	---	12.4^{ns}	-4.3^{ns}	-15.6*	---	1.6^{ns}	-9.5^{ns}	---	---	24.0*	---
Freq. losses	-19.6**	---	---	13.5**	-22.4*	-6.7**	---	---	---	---	---
Int. wins	---	---	---	24.4*	---	---	6.7^{ns}	---	---	-42.5*	-1.7^{ns}
Int. losses	11.62*	---	---	-21.7*	---	17.0**	---	-3.0^{ns}	-5.4^{ns}	---	---
Rank	---	---	---	---	---	---	---	---	---	---	---
R^2	.775*	.213^{ns}	.009^{ns}	.953*	.590*	.942*	.548^{ns}	.301^{ns}	.244^{ns}	.628^{ns}	.419^{ns}

% Weight Gain

	HWN			HWD		LWN			LWD		
Ind. Var.	1	2	3	1	2	1	2	3	1	2	3
Freq. wins	-0.8^{ns}	---	2.0^{ns}	---	---	---	---	10.6^{ns}	---	---	---
Freq. losses	---	---	---	---	-2.5^{ns}	-4.4**	---	---	---	---	---
Int. wins	---	-0.7^{ns}	---	---	---	12.2**	---	-5.2^{ns}	-2.1^{ns}	-3.4^{ns}	-0.7^{ns}
Int. losses	---	---	---	-8.3**	---	---	-3.0^{ns}	---	---	---	---
Rank	---	---	---	---	---	---	---	---	---	---	---
R^2	.439^{ns}	.114^{ns}	.024^{ns}	.703**	.344^{ns}	.951**	.301^{ns}	.392^{ns}	.365^{ns}	.148^{ns}	.052^{ns}

^{ns} Not significant, * $p < .05$, ** $p < .01$

[1] No agonistic interactions observed for this week.

Table 5 b. Partial regression coefficients and R^2 values of the best fitting multiple regression models of weight gain and percentage weight gain (AB & CS) on behavioral variables, as computed within flocks at 6 weeks of age.

	HWN			HWD		LWN			LWD		
Ind. Var.	1	2	3	1	2	1	2	3	1	2	3
Weight Gain											
Freq. wins	---	44.0*	-18.0*	4.9^{ns}	15.2^{ns}	---	---	---	---	---	---
Freq. losses	---	-30.8^{ns}	---	---	---	---	-11.8^{ns}	---	-6.8*	-3.1^{ns}	---
Int. wins	---	-37.0^{ns}	8.6*	---	-23.6^{ns}	---	---	-3.4^{ns}	4.7*	---	5.2*
Int. losses	---	---	13.2**	---	---	-6.2*	---	---	3.2^{ns}	---	---
Rank	7.7^{ns}	---	---	---	6.7^{ns}	7.0*	---	---	---	---	---
R^2	.233^{ns}	.877*	.884*	.248^{ns}	.613^{ns}	.871*	.501^{ns}	.107^{ns}	.806^{ns}	.247^{ns}	.574*
% Weight gain											
Freq. wins	-2.2^{ns}	5.0*	---	---	3.1^{ns}	---	---	---	---	---	0.8**
Freq. losses	---	-3.8^{ns}	---	---	---	-2.0*	---	---	---	-0.9^{ns}	---
Int. wins	---	-4.7*	---	---	-4.2*	---	-1.0^{ns}	-2.0^{ns}	1.2^{ns}	---	1.1**
Int. losses	---	---	---	---	---	---	-1.7^{ns}	---	---	---	-0.9**
Rank	1.3*	---	-0.6^{ns}	-1.7	---	---	---	-1.6^{ns}	---	---	-0.3**
R^2	.580^{ns}	.897*	.481na	.379^{ns}	.644^{ns}	.755*	.797^{ns}	.444^{ns}	.458^{ns}	.173^{ns}	.999**

^{ns} Not significant, * $p < .05$, ** $p < .01$

[1] No agonistic interactions observed for this week.

Table 5 b, contd.

	HWN			HWD		LWN			LWD		
Ind. Var.	1	2	3	1	2	1	2	3	1	2	3
					Antibody Titers						
Freq. wins	-1.0*	---	---	---	1.2*	---	---	1.0^{ns}	---	---	---
Freq. losses	---	1.2**	-0.6^{ns}	---	---	0.5^{ns}	6.2^{ns}	---	---	0.4^{ns}	-0.9^{ns}
Int. wins	---	---	---	---	-0.7^{ns}	---	---	---	---	---	-1.5^{ns}
Int. losses	---	-1.7**	---	---	0.8*	---	-3.4^{ns}	0.9^{ns}	-0.5*	---	1.6^{ns}
Rank	0.5*	---	---	0.3^{ns}	---	---	-2.1*	0.3^{ns}	---	---	1.0^{ns}
R^2	.782*	.953**	.234^{ns}	.333^{ns}	.776^{ns}	.129^{ns}	.924^{ns}	.775^{ns}	.660*	.390^{ns}	.778^{ns}
					Corticosterone						
Freq. wins	---	---	---	-0.1*	-0.7*	-0.5*	1.6*	0.2^{ns}	0.8	---	---
Freq. losses	-0.4^{ns}	0.6**	-0.4^{ns}	---	---	0.8**	-0.7^{ns}	-0.8**	---	---	---
Int. wins	---	---	---	---	---	1.0**	---	---	---	---	---
Int. losses	---	---	---	---	---	---	0.3^{ns}	0.6**	---	-0.1^{ns}	---
Rank	-0.1^{ns}	---	---	0.2**	0.6*	0.8*	-0.1^{ns}	-0.3*	---	---	-0.1^{ns}
R^2	.601^{ns}	.784**	.472^{ns}	.862**	.777*	.984*	1.000*	.997**	.786**	.121^{ns}	.176^{ns}

^{ns} Not significant, * $p < .05$, ** $p < .01$

^{1} No agonistic interactions observed for this week.

Table 5 c. Partial regression coefficients and R^2 values of the best fitting multiple regression models of weight gain and percentage weight gain (AB & CS) on behavioral variables, as computed within flocks at 7 weeks of age.

	HWN			HWD		LWN			LWD		
Ind. Var.	1	2	3	1	2	1	2	3	1	2	3
					Weight Gain						
Freq. wins	---	-113.1*	-12.3^{ns}	---	-8.7^{ns}	---	---	---	---	---	---
Freq. losses	79.7^{ns}	---	-59.8*	---	---	58.0*	-62.2*	16.3*	-42.8^{ns}	---	---
Int. wins	-34.7^{ns}	53.4**	35.2*	---	---	---	5.6*	-9.2*	-6.4**	---	4.6^{ns}
Int. losses	---	-28.0**	12.4*	10.4^{ns}	---	-46.0*	15.2*	-17.5^{ns}	15.4*	---	---
Rank	56.7*	---	---	5.9^{ns}	5.5^{ns}	---	32.3*	6.5^{ns}	7.2**	4.4^{ns}	---
R^2	$.718^{n}$	.984**	$.984^{ns}$	$.405^{ns}$	$.296^{ns}$	$.730^{ns}$	1.00*	.984*	.973*	$.363^{ns}$	$.375^{ns}$
					% Weight Gain						
Freq. wins	---	---	-5.7*	---	-1.0^{ns}	---	---	---	---	-1.4^{ns}	-4.2*
Freq. losses	---	12.8^{ns}	---	---	1.0^{ns}	25.4**	4.3*	---	---	---	---
Int. wins	---	---	2.9*	---	---	1.2^{ns}	---	-2.3*	---	---	10.2*
Int. losses	1.0^{ns}	-6.2^{ns}	---	---	---	-18.1**	---	---	---	---	2.3*
Rank	---	---	0.6^{ns}	-0.7^{ns}	---	---	---	2.5*	0.9^{ns}	1.4^{ns}	-3.5^{ns}
R^2	$.168^{ns}$	$.691^{ns}$	$.762^{ns}$	$.395^{ns}$	$.294^{ns}$	.957*	.820*	$.724^{ns}$	$.232^{ns}$	$.513^{ns}$	$.920^{ns}$

^{ns} Not significant, * $p < .05$, ** $p < .01$

[1] No agonistic interactions observed for this week.

Table 5 d. Partial regression coefficients and R^2 values of the best fitting multiple regression models of weight gain and percentage weight gain (AB & CS) on behavioral variables, as computed within flocks at 8 weeks of age.

Weight Gain

	HWN			HWD		LWN			LWD		
Ind. Var.	1	2	3	1	2	1	2	3	1	2	3
Freq. wins	38.3^{ns}	-42.1^{ns}	---	10.9^{ns}	---	7.5^{ns}	---	---	---	-4.1*	3.9^{ns}
Freq. los	43.3^{ns}	---	---	11.0^{ns}	15.0^{ns}	---	---	---	---	10.8**	---
Int. wins	---	---	---	---	---	---	---	---	-9.6*	---	---
Int. losses	---	---	---	---	---	---	---	8.5^{ns}	---	---	---
Rank	---	27.3*	7.2^{ns}	---	---	---	3.2^{ns}	---	---	5.1**	---
R^2	.503^{ns}	.837*	.172^{ns}	.589^{ns}	.168^{ns}	.426^{ns}	.038^{ns}	.505^{ns}	.556*	.982**	.366^{ns}

% Weight Gain

	HWN			HWD		LWN			LWD		
Ind. Var.	1	2	3	1	2	1	2	3	1	2	3
Freq. wins	---	---	---	1.6^{ns}	---	---	---	0.4^{ns}	---	-1.0*	1.5^{ns}
Freq. losses	---	---	---	---	---	---	---	---	---	---	---
Int. wins	---	---	---	---	1.4^{ns}	---	---	---	-1.5^{ns}	1.8*	---
Int. losses	---	-1.2^{ns}	---	1.4*	---	---	---	---	1.6^{ns}	0.6*	1.2^{ns}
Rank	-0.7*	1.1^{ns}	-0.1^{ns}	-1.1^{ns}	---	-0.4^{ns}	-0.1^{ns}	---	---	0.6**	---
R^2	.615*	.613^{ns}	.005^{ns}	.802^{ns}	.384^{ns}	.072^{ns}	.001^{ns}	.076^{ns}	.420^{ns}	.971*	.525^{ns}

^{ns} Not significant, * $p < .05$, ** $p < .01$

[1] No agonistic interactions observed for this week.

Table 5 e. Partial regression coefficients and R^2 values of the best fitting multiple regression models of weight gain and percentage weight gain (AB & CS) on behavioral variables, as computed within flocks at 9 weeks of age.

	Weight Gain										
	HWN			HWD		LWN			LWD		
Ind. Var.	1[1]	2	3[1]	1	2[1]	1	2	3	1[1]	2	3
Freq. wins	---	-23.3*	---	73.4*	---	58.5**	23.9^{ns}	---	---	-76.9*	---
Freq. losses	---	---	---	67.1*	---	---	---	-34.2^{ns}	---	---	10.8*
Int. wins	---	---	---	-43.4**	---	-67.0**	---	---	---	---	---
Int. losses	---	---	---	-59.9*	---	---	---	---	---	-36.2^{ns}	---
Rank	-5.9^{ns}	6.3^{ns}	-2.5^{ns}	---	-3.7^{ns}	---	6.6^{ns}	---	-0.6^{ns}	16.0*	---
R^2	.116^{ns}	.788*	.027^{ns}	.959*	.046^{ns}	.903**	.437^{ns}	.540^{ns}	.005^{ns}	.808*	.617*
	% Weight Gain										
Freq. wins	---	---	---	---	---	11.0*	---	---	---	-16.7^{ns}	---
Freq. losses	---	-2.2^{ns}	---	---	---	-11.8*	---	---	---	---	2.6*
Int. wins	---	---	---	-2.5*	---	---	1.5^{ns}	---	---	---	---
Int. losses	---	---	---	---	---	1.5^{ns}	---	---	---	-7.7^{ns}	---
Rank	-1.0*	---	-0.5^{ns}	---	-0.6^{ns}	---	---	-0.4^{ns}	-0.4^{ns}	2.8^{ns}	---
R^2	.517*	.502^{ns}	.138^{ns}	.636*	.094^{ns}	.867^{ns}	.375^{ns}	.013^{ns}	.013^{ns}	.684^{ns}	.644*

^{ns} Not significant, * $p < .05$, ** $p < .01$

[1] No agonistic interactions observed for this week.

Table 5 f. Partial regression coefficients and R^2 values of the best fitting multiple regression models of weight gain and percentage weight gain (AB & CS) on behavioral variables, as computed within flocks at 10 weeks of age.

	Weight Gain										
	HWN			HWD		LWN			LWD		
Ind. Var.	1^1	2^1	3^1	1	2^1	1	2^1	3	1^1	2^1	3
Freq. wins	---	---	---	---	---	---	---	106.0*	---	---	---
Freq. losses	---	---	---	---	---	80.0^{ns}	---	---	---	---	---
Int. wins	---	---	---	---	---	---	---	-35.0*	---	---	---
Int. losses	---	---	---	-46.0**	---	-70.0^{ns}	---	---	---	---	---
Rank	6.1^{ns}	22.5*	-7.3^{ns}	---	6.9^{ns}	---	6.5^{ns}	---	3.9^{ns}	0.4^{ns}	7.0*
R^2	$.110^{ns}$	.669*	$.047^{ns}$	.771**	$.379^{ns}$	$.503^{ns}$	$.473^{ns}$	.834*	$.214^{ns}$	$.000^{ns}$	.575*
	% Weight Gain										
Freq. wins	---	---	---	8.0*	---	---	---	2.8^{ns}	---	---	---
Freq. losses	---	---	---	-14.6**	---	---	---	---	---	---	---
Int. wins	---	---	---	---	---	---	---	---	---	---	---
Int. losses	---	---	---	---	---	-3.1^{ns}	---	---	---	---	---
Rank	0.5^{ns}	1.1^{ns}	-0.5^{ns}	---	0.6^{ns}	---	1.2^{ns}	---	1.2^{ns}	0.3^{ns}	1.2*
R^2	$.145^{ns}$	$.423^{ns}$	$.941^{ns}$	.872**	$.274^{ns}$	$.505^{ns}$	$.184^{ns}$	$.515^{ns}$	$.270^{ns}$	$.004^{ns}$	.529*

^{ns} Not significant, * $p < .05$, ** $p < .01$

[1] No agonistic interactions observed for this week.

Table 5 f, contd.

	Antibody Titers										
	HWN			HWD		LWN			LWD		
Ind. Var.	1[1]	2[1]	3[1]	1	2[1]	1	2[1]	3	1[1]	2[1]	3
Freq. wins	---	---	---	-1.9**	---	---	---	---	---	---	---
Freq. losses	---	---	---	-1.3*	---	-8.3**	---	---	---	---	---
Int. wins	---	---	---	---	---	-0.6*	---	-1.5*	---	---	---
Int. losses	---	---	---	---	---	6.0**	---	---	---	---	1.2^{ns}
Rank	0.2^{ns}	0.3^{ns}	-0.7^{ns}	-0.3	0.5*	---	-0.7^{ns}	---	-0.2^{ns}	0.1^{ns}	---
R^2	.026^{ns}	.160^{ns}	.272^{ns}	.947**	.614*	.960*	.373^{ns}	.646*	.163^{ns}	.047^{ns}	.146^{ns}
	Corticosterone										
Freq. wins	---	---	---	---	---	-8.4*	---	---	---	---	-0.2^{ns}
Freq. losses	---	---	---	---	---	---	---	11.8**	---	---	---
Int. wins	---	---	---	---	---	---	---	---	---	---	---
Int. losses	---	---	---	---	---	-3.3**	---	-3.35*	---	---	---
Rank	0.3^{ns}	0.1^{ns}	-0.0^{ns}	-.756*	0.0^{ns}	---	0.5^{ns}	---	-0.4^{ns}	-0.2^{ns}	---
R^2	.016^{ns}	.124^{ns}	.003^{ns}	.551*	.005^{ns}	.933*	.163^{ns}	.929**	.452^{ns}	.245^{ns}	.051^{ns}

^{ns} Not significant, * $p < 0.05$, ** $p < .01$

[1] No agonistic interactions observed for this week.

Although the R^2 values for the regression models were high, the heterogeneity of the regression coefficients indicated unpredictability of the associations. Thus, conclusions regarding the hypothesis that agonistic behavior and low social rank are stressors during this stage of the behavioral ontogeny of chickens should be based principally upon the product moment correlations. Conclusions from this base are:

1) Under the conditions of this experiment, pullets were not significantly stressed by agonistic activity or low social rank during the formation of the developmental social order.

2) The behavioral measures and social rank generally exhibited the influences characteristic for what would be predicted of stressors.

3) Directional influences were more consistent when the criteria were frequency and intensity of encounters lost than when they were the frequency and intensity of encounters won.

4) The magnitude of behavioral influences appeared to be greater for the intensity of encounters than for their frequency.

To these conclusions it should be added that the uncertainty of results from the multiple regressions might be interpreted to support the hypothesis that low social rank and high levels of agonistic activity are stressful. It is my judgement, however, that such an interpretation is unwarranted without further study.

The question may also be asked whether the differences among flocks in the level of agonistic behavior and the stability of the social order are associated with differences among flocks in average growth rate, antibody titers and corticosterone titers? To investigate this, product moment correlations were computed using the residual sums of squares and cross products from an analyses of variance and covariance which removed the effects of age, line, genotype and all of their interactions (model presented in Methods and Materials). The variables included were the mean frequency of agonistic encounters within each flock, the mean intensity of encounters, the weekly Landau indices, and the weekly means for weight gain, percentage weight gain, antibody titers and plasma corticosterone titers.

It can be seen that the correlations of the stress sensitive criteria with the social and behavioral measures were small, although the correlation between the Landau index and percentage weight gain was significant (Table 6). In addition, the correlation between the mean frequency of encounters and antibody titers was positive, which was contrary to the expected. The conclusion based upon these correlations is that the frequency and intensity of agonistic encounters and the stability of the social order as measured by the Landau index are not associated with physiological stress at the flock level.

Table 6. Product moment correlations computed from error sums of squares and cross products. (df = 14 for all correlations including antibody titers or plasma corticosterone, df = 35 for all other correlations).

	Trait[1]						
Trait[1]	Freq.	Int.	Landau	WG	PG	AB	CS
Frequency	--	.58**	.18	.16	.27	.12	.27
Intensity		--	.19	.09	.21	.01	.14
Landau			--	.23	.38*	.34	-.17
WG				--	.77**	.05	-.51
PG					--	-.18	-.61
AB						--	.14

* $p < .05$, ** $p < .01$

GENERAL COMMENTS

This research provides empirical evidence that selection for high and low juvenile body weight resulted in significant divergence in agonistic behavior between the lines, with pullets from the high weight line having both fewer and less intense agonistic encounters than those from the low weight line. In addition, segregation at the sex-linked dwarf locus caused significant differences within both lines. While the frequency of encounters among dwarf pullets was greater than among normal pullets in both lines, the influence of the dwarf gene on the intensity of encounters varied with the background genome. The differing effects of the dwarf gene in the two genetic backgrounds used in this study demonstrate the necessity of care in drawing inferences regarding single gene effects from restricted genetic populations.

The results indicated that while the frequency of agonistic encounters was more important than their intensity in determining social rank, their combined influences accounted for only a relatively small proportion of the variation in social rank. There was a tendency toward an increasing linearity of the social order and toward an increasing stability of individual social ranks, yet that tendency was slight. Even at its initial appearance, the social hierarchy showed signs of stability.

Although agonistic behaviors caused the directional tendencies characteristic of stressors (as per. the general adaptation syndrome, Selye, 1950), the statistical associations of individual agonistic behaviors and social rank with physiological measures were small. The relative magnitude of the correlations indicated that intensity had a greater influence than the frequency of agonistic encounters upon these physiological measurements. When examined at the flock rather than at the individual level, the results were similar, with the exception of the low but significant correlation of the Landau indices with the percentage weight gain. This suggests that the pullets in this study were not stressed by the levels of agonistic activity which accompanies the formation of the developmental social hierarchy. Even though this study was designed to detect relatively low level stress, considerably below the level of the general adaptation syndrome, it would be surprising to find significant social stress at this stage in behavioral development. This is because relatively high agonistic activity is a normal part of this developmental stage and it would be expected that natural selection would have resulted in an adaptive buffering against social stress during this period.

The design and methodology of this experiment provided a situation which induced relatively low levels of agonistic behavior. In particular, pullets interact less frequently than do cockerels (e.g. Guhl, 1953), and cage housing has a negative effect upon the frequency of encounters in comparison to pen housing (e.g. Bareham, 1972). In view of these factors, and the tendencies observed in this research, it is clear that further study of the physiological effects of agonistic behavior, especially under "natural" (i.e. undisrupted) social conditions would be worthwhile. Furthermore, there is a need to integrate the already broad data base regarding the effects of sex, genetic background and the housing environment with continued interdisciplinary research into the behavioral and physiological characteristics and dynamics of chickens. Such an endeavor will lead to deeper understanding in various areas, whose benefits may extend from the practical aspects of commercial management and humane husbandry to the theoretical study of the complex adaptations of the social organisms.

Finally, and perhaps most important, the heterogeneity among flocks of the covariances between the variables studied points to a major consideration in research involving different social groups. It is not surprising to find a diversity of responses when a variety of traits are considered in different social groups since variation in the social environments among such groups may result in apparently different relationships among the variables.

SUMMARY

Agonistic behavior was studied in two lines of chickens which had been divergently selected for juvenile body weight, into which the sex-linked dwarf gene (dw) had been introduced. The frequency and intensity of agonistic encounters were studied during the intial formation of the social hierarchies of young pullets. In addition, individual social ranks were determined and Landau indices and the correlations between individual ranks at different ages were used as measures of the stability of the social hierarchies. The frequency and intensity of encounters won and lost by individuals, and their social rank were correlated with weekly gain in body weight, plasma corticosterone titers and antibody production in response to red blood cell antigens to determine whether agonistic activity and low social rank were stressors.

The regressions of the mean frequency and intensity of encounters with age were negative and highly significant. The regression of the Landau index on age was positive and significant. There were significant differences between the lines for both the frequency and the intensity of encounters, with the low weight flocks showing a greater

frequency and intensity of encounters than those from the high weight line. The dwarf and normal genotypes differed in both the average frequency of encounters and the slope of the regession of the frequency of encounters on age, with dwarf flocks having both more frequent encounters and a steeper regression. Flocks of the dwarf genotype had higher average intensities of encounters than normal flocks in the high weight line, but the two did not differ in the low weight line.

The frequency of agonistic encounters was more important than their intensity in determining social rank, but the multiple regression of the frequency and intensity of encounters won and lost accounted for only 20 percent of the variation in social rank.

The correlations of the agonistic social order measurements with the physiological variables were generally not significant at either the individual or flock level. Among flock heterogeneity of the covariances among the variables studied may have indicated a differential influence due to differing social environments in the various flocks.

Although the directional tendencies of the correlations between the behavioral and physiological variables indicated that the agonistic behaviors might be stressors, individuals were not stressed by the level of agonistic activity associated with the initial formation of the social hierarchy.

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Appendix 1 a. Analyses of covariance of the regressions of the average number of encounters per hour, the average intensity of encounters and the Landau index on age.

Source of variation	df	MS		
		Encounters	Intensity	Landau
Among ages (A)	1	.80**	17.84**	0.40**
Between lines (L)	1	.01	0.09	0.04
Between genotypes (G)	1	.09**	0.18	0.00
A x L	1	.00	0.02	0.03
A x G	1	.06*	0.12	0.01
L x G	1	.02	0.26	0.00
A x L x G	1	.01	0.08	0.00
Error	47	.01	0.19	0.02

* $p < .05$, ** $p < .01$

Appendix 1 b. Analysis of covariance of the average number of encounters per individual per hour after correcting for differences of regressions on age.

Source of variation	df	MS
Among ages (A)	1	.77**
A (Line)	1	.11**
A (Genotype)	1	.07*
A (Line x Genotype)	1	.02
Error	50	.01

* $p < .05$, ** $p < .01$

Appendix 1 c. Analyses of covariance of the regressions of the average intensity of enounters and the Landau index after correction on age.

Source of variation	df	MS	
		Intensity	Landau
Among ages (A)	1	18.0**	.38**
Between lines (L)	1	0.9*	.02
Between genotypes (G)	1	0.2	.06
L x G	1	1.7**	.01
Error	50†	0.2	.02

† df for Landau are 61.

* $p < .05$, ** $p < .01$

Appendix 2a. Analyses of variance for weekly weight gain during age periods† 4 through 10.

		MS					
Source	df	4-5†	5-6	6-7	7-8	8-9	9-10
Between lines (L)	1	171583**	10588**	131557**	129684**	62414**	63607**
Between genotypes (G)	1	26701**	19013**	21672**	38323**	53439**	85411**
Among replicates (R)	2	1749	1326	1134	50	1216	8718**
L x G	1	14165**	6007*	12136**	16582**	18966*	14215**
L x R	2	124	573	732	885	130	103
G x R	2	64	267	214	531	226	2250
L x G x R	1	317	25	155	156	3813	262
Error	††	747	912	482	635	796	1653

†Age period 4-5 is 4 to 5 weeks of age; 5-6 is 5 to 6 weeks of age, etc.

††Error df 73, 69, 72, 72, 71, 65 for columns, respectively.

* $p < .05$, ** $p < .01$

Appendix 2b. Analyses of variance for percentage weekly weight gain during age periods[†] 4 through 10.

		MS					
Source	df	4-5[†]	5-6	6-7	7-8	8-9	9-10
Between lines (L)	1	86.7	73.0	9.0	282.5**	409.5**	8.8
Between genotypes (G)	1	261.7*	160.8**	49.3*	91.9**	234.9**	693.2**
Among replicates (R)	2	412.6**	12.6	24.2	9.6	22.4	114.4*
L x G	1	40.6	0.0	31.6	14.1	22.6	5.3
L x R	2	6.1	26.9	21.9	76.6**	2.5	4.1
G x R	2	38.7	40.0	46.0*	65.2**	13.7	64.7
L x G x R	1	3.3	42.9	0.3	12.9	46.5	9.0
Error	††	45.0	23.3	14.2	11.6	22.6	28.5

†Age period 4-5 is 4 to 5 weeks of age; 5-6 is 5 to 6 weeks of age, etc.

††Error df 73, 69, 72, 72, 71, 65 for columns, respectively.

*p < .05, ** p < .01

Appendix 2c. Analyses of variance for Antibody titers (AB) and plasma corticosterone titers (CS) at 6, 10 and 14 weeks of age.

		MS					
		Antibody			Corticosterone		
Source	df	6	10	14	6	10	14
Between lines (L)	1	64.79**	28.1**	0.00	0.00	5.2	21.89**
Between genotypes (G)	1	2.37	7.2	1.14	0.00	11.6	9.78
Among replicates (R)	2	0.00	6.0	0.35	0.21	9.9	2.61
L x G	1	11.84*	0.2	154.49**	0.94	4.3	72.59**
L x R	2	0.02	15.2*	9.95	0.08	0.7	51.76**
G x R	2	6.67	13.0*	3.84	0.27	1.1	16.94
L x G x R	1	2.75	7.5*	0.83	0.77	0.3	0.25
Error	†	2.42	2.8	1.17	0.49	3.6	4.35

†Error df 69, 65, 71 by columns, respectively, for each variable.

* p < .05, ** p < .01

AGONISTIC BEHAVIOR, THE DEVELOPMENT OF THE SOCIAL HIERARCHY AND STRESS IN GENETICALLY DIVERSE FLOCKS OF CHICKENS.

by

Frederick Arell Marsteller, Jr.

(ABSTRACT)

The agonistic behavior and development of the social hierarchy of juvenile pullets were studied in two lines of chickens which had been divergently selected for juvenile body weight and in which the dwarf gene (dw) was segregating. Correlations between the behavioral measurements and physiological variables were computed to determine whether the pullets were stressed by the agonistic activity during the development of the social hierarchy.

The lines differed significantly in both the frequency and intensity of agonistic encounters. Dwarf and normal genotypes differed in the frequency of encounters in both lines, whereas for the intensity of encounters, the difference was only in the high weight line. Although the frequency was more important than the intensity of encounters in determining social rank, agonistic behaviors accounted for only 20 percent of the variation in rank. The stability of the social orders increased slightly between 6 and 10 weeks of age.

Although the correlations between behavioral and physiological variables agreed in sign with those predicted of stressors, their values were low. Therefore, it was concluded that the pullets were only minimally stressed by normal agonistic activity during the development of the social hierarchy.