Genetic Characterization of Stress Responsiveness in Japanese Quail. 2. Analyses of Maternal Effects, Additive Sex Linkage Effects, Heterosis, and Heritability by Diallel Crosses

F. M. Odeh, G. G. Cadd, and D. G. Satterlee

Applied Animal Biotechnology Laboratories, Department of Animal and Poultry Science, Louisiana State University Agricultural Center, Baton Rouge, Louisiana 70803

ABSTRACT Diallel crosses were used to investigate the genetic inheritance of stress responsiveness through examination of population effects in progeny of randombred (RB) quail and quail selected for reduced (low stress, LS) or exaggerated (high stress, HS) plasma corticosterone (CS) response to brief immobilization. The three genotypes were crossed in a $3 \times 3$ factorial arrangement of treatments that allowed all possible crosses between RB, LS, and HS males with RB, LS, and HS females. The nine crosses produced 479 progeny that were, at 28 d of age, stressed by immobilization, and a sample of blood was collected. The following quantitative genetic parameters were estimated for plasma CS: heritability, heterosis, maternal effects, sex-linkage effects, and heterosis due to the sex chromosomes. Genotypic-phenotypic correlations (Key words: corticosterone, stress, heritability, heterosis, Japanese quail)

INTRODUCTION A detailed discussion of the deleterious effects (reductions in production performance and animal welfare) associated with hyperadrenal corticalism and increased fearfulness in fowl was presented in the first paper of this series (Odeh et al., 2002). In elucidating the importance of developing ways to reduce the harmful effects of fear and distress in poultry, the earlier paper also gave the literature justification for using genetic selection for decreased adrenocortical responsiveness and less pronounced fear responses as one method of reducing stress-induced losses in animal productivity and welfare.

Odeh et al. (2002) further discussed the usefulness of studying the physiology and genetics of the divergent Japanese quail adrenal stress response lines of Satterlee and Johnson (1988). Specifically, the value of these quail within RB quail and quail of the two selected lines were also obtained. Maternal effects were different ($P < 0.05$) from zero for the plasma CS responses of all three genotypes. Additive sex linkage effects on plasma CS responses were variable and of much less importance than maternal effects. The correlations between plasma CS response and genotype were: 0.22 for RB quail and 0.37 and 0.55 for quail of the LS and HS lines, respectively. Heterosis effects were low and most likely due to either an increase in homozygosity of genes on the sex chromosomes or an increase of maternal effects within reciprocal crosses. Heritability, estimated for the post-immobilization plasma CS response, was 0.05 for RB quail and 0.14 and 0.30 for quail of the LS and HS lines, respectively.

as a tool for the study of line differences in nonspecific stress responsiveness, as well as how such differences relate to reproductive and other important physiological and behavioral traits, was made evident. The central theme that emerged was that quail selected for reduced (low stress, LS) rather than exaggerated (high stress, HS) plasma corticosterone (CS) response to brief immobilization consistently exhibited desirable outcomes in characteristics correlated to the selected trait. For example, LS quail have shown greater body weight gain, less cortical bone porosity, reduced heterophil:lymphocyte ratios, less developmental instability, an accelerated onset of puberty in males and females, greater copulation efficiency in males, greater sociality, and less underlying fearfulness (Satterlee and Johnson, 1985; Gildersleeve et al., 1987; Satterlee and Roberts, 1990; Jones et al., 1992a,b, 1994, 1999; Satterlee and Jones, 1995; Jones and Satterlee, 1996; Satterlee et al., 2000, 2002; Cadd et al., 2002; Marin and Satterlee, 2002; Marin et al., 2002).

©2003 Poultry Science Association, Inc.
Received for publication March 19, 2002.
Accepted for publication August 27, 2002.
1Approved for publication by the Director of the Louisiana Agricultural Experiment Station as manuscript Number 02-11-0172.
2To whom correspondence should be addressed: dsatterlee@agctr.lsu.edu.

Abbreviation Key: CS = corticosterone; RB = randombred; LS = low stress; HS = high stress; G = generation; $r_{AP}$ = phenotypic-genotypic correlations.
Thus, Odeh et al. (2002) not only made evident the issue of why it is important to study genetic characteristics associated with selection for and against adrenocortical responsiveness to stress, but they also gave estimates of average plasma CS response to immobilization, general combining ability, and specific combining ability for each of nine diallel crosses made from all possible matings between randombred (RB), LS, and HS males with RB, LS, and HS females. Herein, measurements of maternal effects, additive sex linkage effects, heterosis, heritability, and heterosis due to genes on the sex chromosomes from the progeny of the same nine diallel crosses are reported.

**MATERIALS AND METHODS**

**Birds, Husbandry, Treatment Applications, and Corticosterone Assay**

The quail used in the present study were the same progeny that resulted from the nine diallel crosses made by Odeh et al. (2002). Briefly, the test subjects were RB quail and two quail lines (LS and HS) divergently selected for reduced or exaggerated plasma CS response to brief immobilization, respectively (Satterlee and Johnson, 1988; Satterlee et al., 2000, 2002). These three genotypes were crossed in a $3 \times 3$ factorial arrangement of treatments that allowed all possible combinations between RB, LS, and HS males with RB, LS, and HS females. The birds were full sibs of breeders from Generation (G)27 families used to maintain the aforementioned stocks. The genetic history that attests to the maintenance of line differences in phenotypic responses at G27 was also described by Odeh et al. (2002). The nine different genetic combinations studied were: HH, RH, LH, HR, HL, RR, LR, RL, and LL, where HH represents a cross between a HS male and a HS female, RH represents a cross between a RB male and a HS female, and so on.

Egg incubation, chick battery brooding, feeding, lighting, and other general husbandry conditions were similar to those described elsewhere (Jones and Satterlee, 1996; Odeh et al., 2002). In order to maintain the cross identity of each bird, leg bands (placed on chicks at hatching) were replaced with permanent wing bands at 14 d of age.

At 28 d of age, the 479 progeny produced from the nine diallel crosses were randomly and individually captured from the battery brooder and subjected to the genetic selection stressor used by Satterlee and Johnson (1988), immobilization in a crush cage for not less than 4 min, but not more than 10 min. Immediately following restraint, blood samples were collected, plasmas were harvested, stored frozen, and subsequently assayed for their CS content, and sex was determined as previously described (Odeh et al., 2002).

**Genetic Parameter Calculations and Statistical Analyses**

From the post-immobilization plasma CS responses measured in the progeny of the nine diallel crosses, the following genetic effects were estimated: heterosis, maternal effects, sex linkage effects, and heterosis due to the genes on the sex chromosomes. In addition, heritability and additive genetic variance of stress responsiveness were estimated. In general, maternal (Eisen et al., 1983) and sex linkage effects (Carbonell et al., 1983) were estimated according to procedures outlined by Barbato and Vasilotas-Youenken (1991). Specifically, least-squares means of plasma CS responses estimated for the males or females or male-female combinations within each diallel cross (Odeh et al., 2002) were used in a series of contrast and estimate models to compute heterosis, maternal effects, additive sex linkage effects, and heterosis due to genes on the sex chromosomes.

Heterosis effects for each line were estimated using the GLM procedure of SAS (SAS Institute, 1995). The model representing the mean plasma CS response for a given offspring that resulted from a specific diallel cross was as follows:

$$ Y_{ijkl} = g_{ij} + g_{ik} + g_{jk} + h_{ij} + h_{ik} + h_{jk} + \text{error}_{ijkl} $$

where $Y_{ijkl}$ is the plasma CS value for progeny $l$ that resulted from cross $ij$ or cross $ik$ or cross $jk$; $g_{ij}$, $g_{ik}$, and $g_{jk}$ is genetic effect of the sires (assumed to be 1/2 of the additive genetic variance) of lines i, j, or k, respectively; $h_{ij}$, $h_{ik}$, and $h_{jk}$ is genetic effects of the dams (assumed to be 1/2 of the additive genetic variance) of lines i, j, or k, respectively; $\text{error}_{ijkl}$ is a random error.

The maternal effect ($M_i$) is an effect common to all the offspring derived from females of the line $i$ and it includes maternal genetic and nongenetic effects (William, 1972). Herein, $M_i$ was calculated as the mean deviation of the progeny plasma CS response for a particular dam from the mean plasma CS response estimated from the nine diallel crosses.

Additive sex-linked effects ($a_{ij}$) were estimated from the difference between the sexes in their respective maternal effects. That is, $a_{ij} = M_{i}^{m} - M_{i}^{f}$, where $M_{i}^{m}$ is maternal effect of the males in the line $i$, and $M_{i}^{f}$ is maternal effect of the females in the line $i$.

In mammals, heterosis has different interpretations in males ($h_{ij}^{m}$) and females ($h_{ij}^{f}$) (Carbonell et al., 1983). They are as follows: for males, $h_{ij}^{m} = h_{ij}^{m} + a_{ij}^{m} + a_{ij}^{hy}$, and for females, $h_{ij}^{f} = h_{ij}^{f} + a_{ij}^{f}$, where $h_{ij}^{m}$ is specific heterosis of autosomal genes, $h_{ij}^{f}$ is specific heterosis of genes on the sex chromosomes, and $a_{ij}^{m}$ and $a_{ij}^{f}$ are additive-by-dominant heterosis. It follows then that the difference between heterotic effects in males ($h_{ij}^{m}$) and heterotic effects in females ($h_{ij}^{f}$) is due to the heterosis of genes on the sex chromosomes ($h_{ij}^{s}$) (i.e., $h_{ij}^{s} = h_{ij}^{m} - h_{ij}^{f}$). To account for the fact that avian females possess the sex-determination chromosome, the above heterotic formulas were modified as follows: for males, $h_{ij}^{m} = h_{ij}^{m} + a_{ij}^{hy}$ for females, $h_{ij}^{f} = h_{ij}^{f} + a_{ij}^{hy}$, and for the heterosis due to genes on the sex chromosomes, $h_{ij}^{s} = h_{ij}^{f} - h_{ij}^{m}$.

Heritability of plasma CS response to immobilization for each of the three genotypes was estimated using the GLM procedure of SAS (SAS Institute, 1995). Plasma CS responses of the progeny ($Y_{ijk}$) were analyzed for varia-
tion between the diallel crosses and within a cross using mixed model analyses to estimate the variance components associated with the following model: \( Y_{ijkl} = \text{sex}_i + \text{sire}_j + \text{dam(sire)}_{kij} + \text{error}_{ij(k)} \), where \( Y_{ijkl} \) is the plasma CS value for progeny that resulted from cross \( ij \) or cross \( ik \) or cross \( jk; \) \( \text{sex}_i \) is a fixed effect (used to adjust the variation due to the gender effect); \( \text{sire}_j \) is the sire effect for the line \( j \) (used to calculate the variation between the sires within the cross \( \sigma^2_{\text{sire}} \)); \( \text{dam(sire)}_{kij} \) is the dam effect from the line \( k \) mated to the sire from the line \( i \) (used to measure the variation between the dams within a sire \( \sigma^2_{\text{dam(sire)}} \)); and, \( \text{error}_{ij(k)} \) is random error (used to calculate the variation between the progeny within each cross \( \sigma^2_{\text{progeny}} \)).

Heritability \( (h^2) \) has been defined as the ratio of genotypic to phenotypic variance (Falconer and Mackay, 1996). Therefore, \( h^2 \) was estimated from the SAS-derived estimates of variance components as follows: \( h^2 = \frac{4\sigma^2_{\text{sire}}}{\sigma^2_{\text{sire}} + \sigma^2_{\text{dam(sire)}} + \sigma^2_{\text{progeny}}} \). An estimate of the correlation \( (r_{\text{AP}}) \) between genotype and phenotype (stress responsiveness) was calculated as the square root of the \( h^2 \).

### RESULTS AND DISCUSSION

Maternal (dam) and sire effects estimated for plasma CS responses among the RB, LS, and HS lines are given in Table 1. The dam and sire effects on post-immobilization plasma CS responses were significantly different from zero for the RB, LS, and HS quail. The relative strengths of both maternal and sire effects on the three genotypes were HS > LS > RB. Within the HS line, the magnitudes of the maternal and sire effects were similar, suggesting that both parents contribute equally to the HS phenotype. However, dam effects were greater \( (P < 0.05) \) than sire effects in RB and LS quail. The presence of augmented dam effects in the RB and LS quail suggests that their progeny have become more sensitive to maternal influences. In avians, maternal effects can include sex linkage effects, incubation environment, egg composition, maternal antibodies, and cytoplasmic or mitochondrial inheritance. It was also found that RB and LS quail followed the same trend of stress response inheritance in other genetic effects, such as general combining ability and line effects (Odeh et al., 2002), as well as sex linkage effects (Table 1). From this point of view, the genotype in the LS line may be more closely related to the genotype of RB quail. Although there was a trend of higher maternal effects in female than male progeny of all genotypes, there were no significant differences in maternal effects between males and females within RB, LS, and HS quail (Table 2). These findings suggest that a major gene or genes controlling adrenocortical stress responsiveness may not be located on the female sex chromosome. The findings also argue that any likelihood of significant cytoplasmic or mitochondrial inheritance within each line is diminished. Sire effects in female progeny were higher \( (P < 0.05) \) than in male progeny only within the LS line. This result suggests that the genetic selection program conducted on the LS line produced an accumulation of alleles that somehow interact differently with the products of the gene(s) that are located specifically on female sex chromosomes.

Additive sex linkage effects detect differences in maternal effects between male and female progeny within a line. Additive sex effects for plasma CS responses among RB, LS, and HS quail are given in Table 1. Not surprisingly, when one considers the above definition of additive sex linkage effects and the fact that no significant differences in maternal effects were found between male and female progeny within RB, LS, and HS quail (Table 2), all three quail genotypes showed additive sex linkage effects that were not significantly different from zero.

Heritability estimates for post-immobilization plasma CS responses among the RB, LS, and HS quail are given in Table 1. Heritability was moderate for the LS line, high for the HS line, and quite low for the RB line. Interestingly, \( h^2 \) estimates for the selected lines obtained herein are markedly similar to earlier \( h^2 \) estimates reported by Satterlee and Johnson (1988). They found realized \( h^2 \), calculated as the ratio of cumulative genetic responses to cumulative selection differentials, to be between 0.15 to 0.19 for LS quail and between 0.25 to 0.33 for birds of the HS line. Similarly, realized \( h^2 \) estimates for blood CS response to cold stress were 0.14 for the LS and 0.25 for the HS turkey lines of Brown and Nestor (1973).

Although selection pressure was relaxed periodically for several generations in the present study (Satterlee et al., 2000, 2002), the additive genetic variance for stress response did not change, indicating that the allelic frequencies at quantitative trait loci for the LS and HS lines have most likely become fixed. The variation of \( h^2 \) estimates of the selected lines compared to RB quail suggests that the selection programs resulted in a rapid accumula-

### TABLE 1. Maternal, sire, and additive sex linkage effects, heritability \( (h^2) \) estimates, and phenotypic-genotypic correlations \( (r_{\text{AP}}) \) for plasma corticosterone responses to immobilization for the randombred (RB) and selected (LS = low stress; HS = high stress) lines

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>RB</th>
<th>LS</th>
<th>HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal effect</td>
<td>-2.48*</td>
<td>-4.60*</td>
<td>6.35*</td>
</tr>
<tr>
<td>Sire effect</td>
<td>-0.91</td>
<td>-3.14*</td>
<td>6.55*</td>
</tr>
<tr>
<td>Additive sex linkage</td>
<td>1.71</td>
<td>2.16</td>
<td>-1.27</td>
</tr>
<tr>
<td>( h^2 )</td>
<td>0.05</td>
<td>0.14</td>
<td>0.30</td>
</tr>
<tr>
<td>( r_{\text{AP}} )</td>
<td>0.22</td>
<td>0.37</td>
<td>0.55</td>
</tr>
</tbody>
</table>

*Values with asterisks are different from zero \( (P < 0.05) \).
The correlations between genotype and phenotype (adrenocortical stress responsiveness) for RB quail and the two selected lines are given in Table 1. The correlation coefficients \( r_{AP} \) suggest that the strongest relationship between plasma CS response and genotype resides in the HS line. The order of \( r_{AP} \) strength was HS > LS > RB. These findings support the hypothesis that different alleles exist for the control of low vs. high adrenocortical responsiveness within each of the selected lines. Furthermore, the low \( h^2 \) and \( r_{AP} \) in RB quail suggests that heterozygous alleles most likely exist in the RB base population that provide intermediate adaptive mechanisms for stress responsiveness.

The current study and others (e.g., Satterlee and Johnson, 1988; Jones et al., 1994; Jones, 1996; Odeh et al., 2002) have consistently demonstrated significant line differences (HS > LS) in plasma CS response to many different stressors. Physiologically, this result suggests that differences in nonspecific systemic stress responsiveness exist in the lines. Genetically, the ever-evident line differences in plasma CS responsiveness implies that there are genes that affect variability of this phenotypic response and these genes have some degree of directional dominance for increased homeostasis. The consistent detection of line differences in plasma CS response also suggests that there are different allelic forms of proteins produced in the lines that have a wide range of activity under different environmental conditions. For example, Sgro and Hoffman (1998) suggested that the expression of additive variance in *Drosophila melanogaster* was increased by stressful conditions, and therefore, the genetic response to selection was augmented. The major factor underlying these observations may be that different genotypes do not respond similarly to different environments because of genetic-environment interactions (Siegel, 1979). Arguably then, the gene-environment interaction may have a significant effect on stress response, and this interaction most likely triggers the expression of certain genes. Other researchers have argued that exposure to stressful environmental conditions can result in phenotypic changes due to the expression of new genetic variation, whose expression eventually remains “switched on” in the absence of any stressor (Waddington, 1961).

Heterotic effects are the most important non-additive genetic effects having an impact on strain performance (Fairfull et al., 1983). They reflect the existence of full or partial dominance within a single locus (dominance) or among loci (epistasis). Therefore, for loci with overdominance, the heterozygous state is considered to be superior. The average heterotic effects of plasma CS response for male and female progeny from the reciprocal crosses made in the present study (HS × LS, LS × RB, and HS × RB) are given in Table 3. No significant heterosis was noted for plasma CS response for females or males in any reciprocal cross. The total heterosis values (sexes combined) were also not different from zero for any reciprocal cross. The failure of any of the three reciprocal crosses to show heterosis may be attributed to epistatic interactions. Falconer and Mackay (1996) indicated that when two populations adapted to different conditions are crossed, the hybrids are adapted to neither. They noted that, if some genes are dominant in one direction and some in the other, their effects would tend to cancel out, and no heterosis may be observed. The present study showed that heterosis due to genes on the sex chromosomes was insignificant for all reciprocal crosses. This implies that there is an increase of homozygosity of genes on the sex chromosomes within the lines. Therefore, the other factors that may cancel out the heterosis effects are due to either the increase homozygosity of genes on the sex chromosomes or the increase of maternal effects within reciprocal crosses.

To the authors’ knowledge, the present report and its companion study (Odeh et al., 2002) constitute the first published, comprehensive quantitative genetic characterization of stress responsiveness in avians. These studies suggest that the inheritance of stress response is highly additive and, further, can be broken down into additive variation at the level of sex chromosomes, especially in RB and LS quail. Therefore, the development of a specialized female line would likely prove essential for improving (decreasing) nonspecific stress responsiveness in birds. It is also apparent that heterozygotes are less sensitive to environment variables (maternal effects) than are homozygotes. Finally, the findings indicate that the primary advantages of decreasing stress responsiveness, and thus enhancing the bird’s ability to cope with stressors, come from maternal effects. Results of the phenotypic-genotypic correlations, calculated for plasma CS responses, suggest that specific selection programs could effectively alter stress responsiveness.

### Table 2. Maternal and sire effects and heterosis separated by sex for plasma corticosterone responses to immobilization for the randombred (RB) and selected (LS = low stress; HS = high stress) lines

<table>
<thead>
<tr>
<th>Genetic parameter</th>
<th>RB Male</th>
<th>RB Female</th>
<th>LS Male</th>
<th>LS Female</th>
<th>HS Male</th>
<th>HS Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal effect</td>
<td>−1.66</td>
<td>−3.37*</td>
<td>−3.59*</td>
<td>−5.75*</td>
<td>5.85*</td>
<td>7.12*</td>
</tr>
<tr>
<td>Sire effect</td>
<td>−0.82</td>
<td>−1.00</td>
<td>−1.18*</td>
<td>−5.61*</td>
<td>6.11*</td>
<td>7.11*</td>
</tr>
</tbody>
</table>

*Means of a genetic parameter within a line with no common superscript letters are different \( P < 0.05 \).
*Values with asterisks are different from zero \( P < 0.05 \).
ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of Shane Castille and Lisa Geagan in the collection of blood samples. This work was partially supported by Louisiana Board of Regents’ Grant No. LEQSF (1998-01)-RD-A-01.

REFERENCES


<table>
<thead>
<tr>
<th>Reciprocal cross</th>
<th>Heterosis</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>h₀</td>
<td>h₁</td>
<td>h₂</td>
</tr>
<tr>
<td>HS × LS</td>
<td>−1.57</td>
<td>−0.67</td>
<td>−0.92</td>
</tr>
<tr>
<td>LS × RB</td>
<td>−0.53</td>
<td>1.94</td>
<td>0.51</td>
</tr>
<tr>
<td>HS × RB</td>
<td>−2.43</td>
<td>−1.15</td>
<td>−1.86</td>
</tr>
</tbody>
</table>