

BILATERAL ASYMMETRY IN CHICKENS OF DIFFERENT GENETIC
BACKGROUNDS

by

Aiming Yang

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Approved:

P. B. Siegel, Chairman
B. Wallace
E. A. Dunnington
K. H. Hinkelmann
C. T. Larsen

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(ABSTRACT)

The dissertation consists of a series of experiments conducted to study developmental stability of various genetic stocks at different stages in the life cycle. The primary measures of stability were type and degree of asymmetry of bilateral traits and heterosis.

Higher relative asymmetry (RA), which was defined as $(|L-R| / [(L+R)/2]) \times 100$, was observed in lines of White Leghorns selected 23 generations for high or low antibody response to sheep red blood cells than in their F_1 crosses. The bilateral traits were 39-day shank length and length and weight of the first primary wing feather. Shank length was again measured on day 49 while body, heart, shank, and lung weights and ceca lengths were obtained on day 56. Heterosis was positive for organ sizes and negative for degree of RA.

Shank length and diameter, weight and length of the first primary wing feather, and distance between the junction of maxilla and mandibles and auditory canal (face length) were used to classify bilateral types and measure RA in six genetic stocks.

The stocks were the S_{23} generation of White Leghorn lines selected for high or low antibody response to SRBC, sublines where selection had been relaxed for eight generations, and reciprocal crosses of the selected lines. Differences were found among all stocks for the traits measured. Rankings among traits for RA in descending order were face length, shank diameter, feather weight, and shank and feather lengths. The RA of shank

and feather lengths did not differ from each other. The mean RA of the five traits was higher for the two selected lines than the crosses between them. The RAs of the two lines where selection had been relaxed was similar to that of selected lines.

In a line of White Rocks selected 39 generations for low eight-week body weight, bilateral traits measured were shank length and diameter, face length, and weight and length of the first primary wing feather of females at 240 days of age. The RAs of individuals that had not commenced egg production by 245 days of age were similar to those that had entered lay. In both cases, these RAs were higher than those of a subline in which selection had been relaxed for four generations.

Broiler sire lines had higher RA than dam lines for lung weight at hatch. Heterosis of RAs suggested superior homeostasis in F_1 crosses than in the sire lines.

Based on populations studied, it may be concluded that RAs were trait specific with the RA of shank length being lower ($0 < RA < 2\%$) than lung weight which was 10% or higher regardless of genetic background. The types of bilateral asymmetry exhibited although less consistent, still had consistency such that feather weight and ceca weight exhibited antisymmetry across different stocks. Length and width of shank and weight of lung, were generally of fluctuating asymmetry.

Heart:lung ratios differed among genetic stocks. In White Leghorns, lungs from late embryonic development to 25 days after hatch were heavier in a line which had heavier juvenile body weight than in one with lower juvenile body weight. In commercial broilers, heart:lung ratios at hatch were lower and thus inferior in parental lines than in their F_1 crosses.

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INTRODUCTION

The poultry industry has reached a level of sophistication so that it uses intensively selected lines and crosses among them that are highly efficient in production of meat or eggs. Responses to selection for production of meat or eggs did not come about without reallocation of resources from other biological functions. Such reallocations can interrupt biological balances and disrupt homeostasis. Disruption of homeostasis may be evidenced by stress responses. Although there has been considerable research on responses to environmental stressors in poultry (Freeman, 1985; Siegel, 1995; Zulkifli and Siegel, 1995), information on responses to genetic stressors is quite limited. Responses to environmental stressors may be measured in a variety of ways, including immunological, behavioral, serological, and endocrine changes (Siegel, 1995; Zulkifli and Siegel, 1995; Freeman, 1985). In contrast, the principal criteria for measuring responses to genetic stressors are bilateral asymmetries (see reviews by Palmer and Strobeck, 1986, 1992; Parsons, 1990).

Physiological and genetic limits to selection in poultry are well documented (Lerner, 1954; Dunnington, 1990). Intensive selection for egg or meat production has increased skeletal and metabolic disorders while the incidence of diseases has decreased. Leg disorders (Cahaner and Siegel, 1986), ascites (Julian, 1993), and sudden death syndrome (Bowes *et al.*, 1988; Chung *et al.*, 1993) are examples of skeletal and metabolic disorders which may be developmental in origin. Breeders face the challenge of paying attention to mechanisms involved with development, and because fluctuating asymmetry may be diagnostic of genetic stressors (Parsons, 1990; 1992), it is a promising candidate for study in poultry populations.

LITERATURE REVIEW

Bilateral asymmetry, the deviation of part of an organism

from perfect symmetry, can be categorized as antisymmetry, directional, or fluctuating (van Valen, 1962). Each of these categories is characterized by a different combination of the mean and variance of the distribution of right minus left (R-L) differences. In an excellent recent review Palmer (1996) provided examples of frequency distributions for fluctuating asymmetry, directional asymmetry, and antisymmetry.

Antisymmetry occurs where asymmetry is normally present but it is variable as to which side has greater development. It is distinguished by a platykurtic (broad peaked) or bimodal distribution of R-L differences about a mean of zero. An example is the oversized signalling claw in male fiddler crab which occurs with approximately equal frequency on both the right and left sides (Palmer and Strobeck, 1986). Directional asymmetry refers to greater development of a character on one side of the plane or planes of symmetry than the other or others. Traits exhibit normally distributed R-L differences about a mean that is significantly either greater or less than zero. An example is the avian heart (King and McLelland, 1984) where for the chicken the right atrium is larger than the left, and the left ventricle is three times that of the right ventricle (Sturkie, 1965). In the chicken, testes and adrenal glands show similar patterns with left greater than right (Siegel and Siegel, 1960; Hocking, 1992). Fluctuating asymmetry (FA) reflects small, random deviations from symmetry in bilateral characters with a normal distribution of R-L differences whose mean is zero (van Valen, 1962; Palmer and Strobeck, 1986; Leary and Allendorf, 1989; Parsons, 1990; Moller and Swaddle, 1997). Any two or all three types of asymmetry may occur together for the same character with FA thought to be ubiquitous (van Valen, 1962; Palmer and Strobeck, 1992).

FLUCTUATING ASYMMETRY

Darwin's statement in *The Variation of Animals and Plants*

Under Domestication, "it might have been anticipated that deviations from the law of symmetry would not have been inherited" (Darwin, 1868 as cited by Palmer and Strobeck, 1986) has stood the test of time. Genetic studies have confirmed that FAs have little or no measurable heritability (Summer and Huestis, 1921; Potter and Nance, 1976; Leamy and Atchley, 1985; Leary and Allendorf, 1989; Parsons, 1990), and it may be reasoned that deviations from perfect symmetry should be minor because the left and the right sides of the body are products of the same genome (Leary and Allendorf, 1989; Zakharov, 1989; 1992). Caused by genetic and environmental stressors, FAs have been studied as a measure of developmental stability (Palmer and Strobeck, 1986; Parsons, 1990; Moller and Swaddle, 1997).

Asymmetry has been observed for variety of traits and organisms including teeth in humans, rats, mice, and extinct horses; palm ridge counts, fingertip ridge counts, and ear lobe lengths in humans; limb bones in humans and martens; cranial traits in large cats, rhesus macaques, muskrats, and kangaroo rats; various skeletal elements and mustachial vibrissae in rats and mice; numerous metric and meristic traits in lizards and fish; sternopleural chaetae number and other features in *Drosophila*; wing lengths in honeybees and houseflies; wings and other features in butterflies; antennal length in dipteran flies; labial palps and siphonal papillae of freshwater bivalves (see reviews by Palmer and Strobeck, 1986; Leary and Allendorf, 1989; Parsons, 1990); as well as bones in birds (Moller *et al.*, 1995; Alexander, *et al.*, 1984; Soule and Couzin-Roudy, 1982). Parsons (1990) proposed that with recombination as a model, genetic and environmental perturbations might be expected to influence FA in analogous ways because both stress categories may be imposed during development. Accordingly, the overall level of FAs in morphology provides an integrated measure of phenotypic quality

of the individual in terms of ability to control stable morphological development (van Valen, 1962; Palmer and Strobeck, 1986; Leary and Allendorf, 1989; Parsons, 1990).

ENVIRONMENTAL STRESS

There is accumulating evidence that variability tends to increase when severe stressors are imposed by physical and biological environments (see reviews by Palmer and Strobeck, 1986; Leary and Allendorf, 1989; Parsons, 1990; Moller and Swaddle, 1997). This variability may be especially important in determining survival, and indirectly at the level of genes controlling protein variation. The assumption is that populations have not undergone selection for phenotypic extremes which could modify variability (Palmer and Strobeck, 1986). For a definition of the severity of a stressor, one may consider a physical stressor that substantially increases variability with such severity that continuous exposure results in rapid lethality. These conditions are exemplified by short bursts of extreme stress occurring at climatic and ecological margins where just a small environmental perturbation could be lethal. Examples of such environmental stressors include extreme temperature (as seen with heat in chickens), protein deprivation, starvation, audiogenic stress, exposure to pollutants and toxicants, and crowding (see reviews by Palmer and Strobeck, 1986; Leary and Allendorf, 1989; Parsons, 1990, 1992; Zulkifli and Siegel, 1995).

In humans, dental asymmetry has been used as an indicator of stress in populations assayed for favorable environments as judged from ethnographic and medical data (Palmer and Strobeck, 1986; Parsons, 1990; 1992). In addition, domestication may be considered as an environmental stressor resulting from artificial selection. Domestication may be contrary to natural selection and thus increase FAs (see reviews by Mitton and Grant, 1984; Palmer and Strobeck, 1986; Leary and Allendorf, 1989; Parsons, 1990,

1992).

GENETIC STRESS

Several types of genetic stress have been shown to influence FA. These include loss of genetic variation, hybridization, incorporation of mutants with major effects, and intense artificial selection (Summer and Huestis, 1921; Palmer and Strobeck, 1986; Clarke and McKenzie, 1987; Leary and Allendorf, 1989; Parsons, 1990).

LOSS OF GENETIC VARIATION. Reduced genetic variation in inbred laboratory strains of animals results in more asymmetry than that found in hybrids between strains (Mather, 1953; Leamy, 1984) or individuals from random mating populations (Leary *et al.*, 1985; Bader, 1965; Alados *et al.*, 1996). It has been reported for several species that populations genetically more variable as measured by enzyme-coding loci exhibit fewer FAs than populations less variable at these loci (Bader, 1965; Soule, 1979; Kat, 1982; Vrijenhoek and Lerman, 1982; Hutchison and Cheverud, 1995). Also, individuals from populations with reduced genetic variation because of bottlenecks or founder events exhibited more FAs than individuals from other conspecific populations or species (Vrijenhoek and Lerman, 1982; Leary *et al.*, 1985; Wayne *et al.*, 1986).

High levels of FAs in a population, therefore, may indicate that loss of genetic variation has resulted in perturbed development. The association between heterozygosity and reduced FAs may be due to either homozygosity of deleterious recessive alleles or a heterozygous advantage (Mitton and Grant, 1984; Palmer and Strobeck, 1986; Leary and Allendorf, 1989; Parsons, 1990).

HYBRIDIZATION. Hybridization may also impose a genetic stress associated with increased likelihood of a breakdown in genomic coadaptation, i.e., homeostasis (Tebb and Thoday, 1958;

Leary *et al.*, 1985; Zakharov, 1989). Crosses between species may reflect disruption of genomic adaptations which have developed since the populations became reproductively isolated and hence reduced fitness. Whether this occurs and to what degree depends on the specific populations involved (Leary and Allendorf, 1989; Parsons, 1990). Although observed both between and within species, such disruption is less likely when populations are genetically quite similar or in hybrid populations that have existed long enough to enable homeostasis to re-evolve (Clarke and McKenzie, 1987; Leary and Allendorf, 1989).

INCORPORATION OF NOVEL MUTANTS INTO THE GENOME. Genomic disruption can occur when a major gene is being incorporated into a population, even when the gene is an advantageous allele (Fisher, 1958; Clarke and McKenzie, 1987). Homeostasis may initially deteriorate and then be restored because of subsequent selection favoring modifiers at other loci. Developmental disruption caused by a new mutant which had been reduced by breeding or natural selection may be restored when the mutant is introgressed into another genetic background. Therefore, genomic recombination may be an appropriate explanation for the modifiers' selection (Parsons, 1990). If so, there may be application to population genetics, conservation biology, and animal breeding (Jones, 1987; Clarke and McKenzie, 1987; Leary and Allendorf, 1989; Moller *et al.*, 1995). Transgenic organisms provide an example (Parsons, 1992) because when the extra macromolecules are incorporated or synthesized, genetic stress occurs with a general destabilization of metabolic processes which have a fitness cost expressed as an energetic burden (Lenski and Nguyen, 1988). Specifically, transgenic pigs with high growth rates have simultaneously a high incidence of gastric ulcers, arthritis, cardiomegaly, dermatitis, and renal disorders (Pursel *et al.*, 1989).

INTENSIVE SELECTION. Both intensive natural (Jones, 1987; Clarke and McKenzie, 1987) and artificial (Reeve, 1960; Moller and Pomiankowski, 1993 a,b) selection can cause deterioration of homeostasis, and genomic disruption may also occur when a population is transferred to a new environment (Leary and Allendorf, 1989). Moreover, intensive directional selection has been hypothesized by Parsons (1992) to increase the overall level of developmental instability as measured by FAs, whereas Moller and Pomiankowski (1993a,b) and Moller *et al.* (1995) hypothesized that stabilizing selection could have the opposite effect. The rationale is that selection directly affects alleles that control developmental homeostasis. Therefore, directional selection for a trait should impose selection against genetic modifiers that reduce the development of extreme phenotypes, whereas stabilizing selection should result in incorporation of modifiers that reduce extreme genotypes (Fisher, 1958; Jones, 1987; Clarke and McKenzie, 1987; Parsons, 1992).

IMPLICATIONS

From a genetic point of view, FA may be used as a measure of development stability caused by environmental and genetic stressors. This means that FA may serve as a monitor of evolution (see reviews by Palmer and Strobeck, 1986; Jones, 1987; Leary and Allendorf, 1989; Parsons, 1990, 1992), domestication (Parsons, 1990), conservation biology (Leary and Allendorf, 1989), and animal welfare and breeding (Moller *et al.*, 1995).

EVOLUTION. There are several reasons why FA may be a valuable tool for making evolutionary inferences. First, because FA is in general negatively correlated with heterozygosity, the level of FA may be used to detect genetic variation among contemporary populations or between ancestral and descendent ones (Palmer and Strobeck, 1986; Jones, 1987). Second, if the level of FA reflects degree of canalization, then FA may reveal the

evolution of increased canalizing ability within taxa over the first several million years of metazoan evolution (Soule, 1982). Finally, if FA reflects a degree of canalization, levels of FA may be used to test the strength of selection for canalization of different traits under the assumption that traits of greater functional significance to an organism undergo stronger selection for canalization. This hypothesis is supported by the report of Gummer and Brigham (1995) that traits can be ranked in order of functional importance according to the degree of FA.

DOMESTICATION. If domestication is considered an environmental stressor it may be a model for studying stress and therefore evolutionary and population genetics (Jones, 1987; Kohane and Parsons, 1988). Assessment of FA may be useful in monitoring the stress of introducing wild organisms into a domesticated environment (Belyaev, 1979; Parsons, 1990).

During domestication, which is an artificial selection process, limited resources are reallocated (Parsons, 1990). For example, increased skeletal strength could reduce the probability of fracture, but involve greater mass and increased energy cost. Hence, there would be an optimum strength for each skeletal arrangement and the symmetry of the mechanical properties of paired long bones would indicate the precision of formation of animal skeletons. Alexander *et al.* (1984) tested homologous limb bones in three species of birds for failure in bending, load at fracture, and work to fracture and found that FA was much lower in gulls than in domesticated pigeons and hens. It would be clearly adaptive to maximize symmetry in the wild especially in relation to minimizing variability of load for fracture. Domestication, however, could reduce the selective advantage of symmetry.

CONSERVATION BIOLOGY. As an indicator of perturbed development due to stressors, FA may have application to

conservation biology programs (Leary and Allendorf, 1989). First, maintaining genetic diversity is often essential for captive populations which serve as a source to establish and augment natural populations. Assuming that environments of captive populations are usually less variable than those of natural populations, an increase in FA within a captive population would be more likely to indicate genetic than environmental stressors. If this assumption is correct, FA could be used to monitor the efficacy of genetic variation maintained in captive populations. Natural populations and environments may be monitored by the choice of organisms and characteristics. Populations with recent histories of hybridization, bottlenecks, or exposure to pollutants exhibit greater FAs than other populations (see reviews by Palmer and Strobeck, 1986; Leary and Allendorf, 1989; Parsons, 1990; Moller and Swaddle, 1997). Since temporal changes or unusually high FAs may also indicate environmental stressors, sedentary organisms or ectotherms may be more useful than nonsedentary organs to assay environmental quality (Leary and Allendorf, 1989).

ANIMAL WELFARE AND BREEDING. The overall level of FA reflects an integrated measure of phenotypic quality of the individual (see reviews by Mitton and Grant, 1984; Palmer and Strobeck, 1986; Leary and Allendorf, 1989; Parsons, 1990, 1992). Therefore, it is not surprising that both genetic and environmental stressors cause FAs in a variety of species, and that individuals or populations under genetic stress are also sensitive to environmental stressors.

Chickens have undergone intensive artificial selection for growth or egg production during the last half century, and metabolic and skeletal disorders have become major concerns (Cahaner and Siegel, 1986; Julian, 1993; Chung *et al.*, 1993). Moller *et al.* (1995) proposed that the level of FA may be a

reliable and objective way to assess general health and welfare status of such stocks. Moller and Pomiankowski (1993a,b) reported that intense directional selection increased overall level of developmental instability as measured by FAs whereas stabilizing selection had the opposite effect.

Since a lack of homeostasis may be a problem in highly selected populations, FA has potential as a useful and inexpensive integrated measure of the phenotypic quality of individuals or populations. Levels of FAs may be useful in breeding programs because individuals with low FAs may be particularly adapted to cope with stressful conditions either in terms of the environmental or their genetic background. An analysis of the genetics of developmental stability revealed that FA and other measures of developmental stability have a moderate to high additive genetic variance (Moller *et al.*, 1995). Therefore, if breeding programs are designed to select for increased overall developmental stability, it may be possible to increase general performance under a range of conditions. Also, because relative FA appears to differ among breeds (Moller *et al.*, 1995), there may be variation in sensitivity in response to genetic stressors. Lastly, as an assessment of environmental and genetic stressors, FA and other measures of development stability may have potential in evaluating rearing conditions for animals (Leary and Allendorf, 1989; Parsons, 1990).

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PAPER I
Late Embryonic And Early Posthatch Growth of Heart and Lung
in White Leghorn Lines of Chickens

ABSTRACT: Growth and development of heart and lungs were measured from day 15 of incubation to 25 days after hatch in lines of chickens that had undergone long-term selection for high (HAS) or low (LAS) antibody titers to sheep erythrocytes. A correlated response to this selection was heavier 28-day body weights for LAS than HAS chickens. In this experiment body weights were heavier in line LAS than HAS from 15 days of incubation to 5 days after hatch and again at 25 days after hatch. Absorption of residual yolk was greater in HAS than LAS chicks. Although there were no differences between lines for absolute heart weights, lungs were heavier in line LAS than HAS at all ages except 20 days after hatch. Relative to body weight, both heart and lung weights declined with age, however, the pattern of decline differed. For this criterion, hearts were larger in line HAS than LAS to 5 days after hatch with no difference thereafter; for lungs lines were similar until 5 days after hatch after which they were larger in LAS than HAS chicks. In all but one case (HAS at hatch) the left:right relationship for lung weights exhibited fluctuating asymmetry with the left minus right character difference having a normal distribution and mean zero. The degree of fluctuating asymmetry, however, varied with age.

INTRODUCTION

Growth is a complex process whereby various systems interact and size of organs change at different rates. Developmental constraints contribute to biological balances which allow for synchrony among systems. Directional selection for specific traits such as body weight and meat yield can disrupt developmental synchrony and result in conditions such as pulmonary hypertension syndrome (ascites) which has become common in young meat-type but not egg-type chickens (Julian, 1989; 1993). Ascites is an example of a biological imbalance which is a

consequence of altering the dynamics of coupling the cardiovascular and the respiratory systems in which lung is derived from entoderm while heart and skeletal muscles are derived from mesoderm. Asymmetries of the lungs and hearts of chickens are different. There are two separate lungs each located to the left or right of the vertebral column, and a single heart which lies partly between the two lobes of the liver, partly cranial to that organ. The left ventricle of the heart is about three-times larger than the right ventricle and the right atrium larger than the left atrium (King and McLelland, 1984). In contrast, size of left and right lungs is symmetrical across a wide range of avian species (Maina *et al.*, 1982). Both sides of an individual are products of the same genome and the degree of relative asymmetry of bilateral traits has been used to measure perturbed development due to genetic and environmental stressors (Bader, 1965; Palmer & Strobeck, 1986; Parsons, 1990; Palmer, 1996).

The genetics of growth and development of the fowl and Japanese quail was recently reviewed by Marks (1995). The late embryonic period and early posthatch period of precocial birds is a time of major changes in the cardiovascular-respiratory interface. The experiment reported in this paper was designed to measure growth and development of the heart and lungs from day 15 of incubation to 25 days after hatch in lines of chickens that were not directionally selected for growth.

METHODS AND MATERIALS

The lines of chickens used in this experiment were White Leghorns selected 23 generations for high (HAS) or low (LAS) antibody response to a single intravenous injection of .1 mL of a .25% suspension of sheep red blood cells administered between 41 and 51 days of age (Siegel and Gross, 1980; Martin *et al.*, 1990).

A correlated response to this selection was that by 28 days after hatch body weights are greater for LAS than HAS chickens.

Eggs from age contemporary parents of both lines were incubated in the same machine. The parents were random samples from each parental line and all progeny data were from a single setting (incubation) of eggs obtained when parents were between 231 and 236 days of age. At 15 and 18 days of incubation, 10 embryos from each line were freed from their yolk sac and other membranes, blotted on a paper towel, weighed (.01g), and their heart and left (L) and right (R) lungs dissected and weighed (.01g). At hatch, random samples of 34 HAS chicks (12 females and 22 males) and 35 LAS chicks (15 females and 20 males) were sacrificed by cervical dislocation to obtain L and R lung, heart, yolk sac, and body weights. Body weight was defined as live body weight minus yolk sac weight. At 5, 10, 15, 20, 25 days after hatch, L and R lung, heart, yolk sac, and body weights were obtained from random samples of 10 individuals from each line. After hatch, chicks were reared in battery brooders with wire floors in a windowless room where lighting was continuous and room temperature was maintained at 21 ± 1 C. Brooder temperatures were 34 ± 1 C to day 7 and 26 ± 1 from days 7 to 14. Brooder heaters were then turned off. A mash diet consisting of 20% crude protein and 2685 kcal of ME/kg in mash form was fed *ad libitum*. Water was always available.

Body, lung $((L+R)/2)$, and heart weights were analyzed by ANOVA with age, line, sex, and the interactions among them as main effects using the GLM procedure (SAS Institute, 1985). The same analysis was used to analyze relative fluctuating asymmetry (RFA) of lungs which was defined as $(|L-R|/(L+R)/2)$. Product-moment correlations were calculated among body, lung $((L+R)/2)$, and heart weights. Changes in weights over ages were determined by polynomial regression analysis using the GLM procedure. Signed

(+ or -) bilateral asymmetry (L-R) was tested for normality with mean zero by the Shapiro-Wilk statistic W and one sample t-test (SAS Institute, 1985). Yolk sacs were scored as completely absorbed (<.07g) or not absorbed. Prior to analyses, body weights and absolute organ weights were transformed to common logarithms and ratios to arc sine square roots.

RESULT

Interactions of lines and of sex with age were significant for body, lung, and heart weights. Accordingly, subsequent analyses of these traits were conducted by line and sex at each age.

Growth patterns according to genetic line

Body weights were heavier for line LAS than HAS from 15 days of embryonic development to 5 days after hatch and again at 25 days after hatch (Figure 1). Polynomial regressions of body weights on age followed a quadratic form for both line HAS and LAS with R^2 of .96 and .98, respectively. Equations are shown in the legend for Figure 1.

There were no differences between lines for absolute heart weight at any age (Figure 2). In contrast, absolute lung weights were heavier for LAS than HAS chicks at all ages except day 20. Growth patterns of hearts and lungs had quadratic significance as seen by the equations shown in the legend for Figure 2. R^2 for heart was .96 for line HAS and .95 for line LAS. For lungs, R^2 was .91 for line HAS and .95 for line LAS.

There were no relationships between body and absolute lung or heart weights at any age. This lack of part-whole relationship was probably because lungs ranged from only .29% to .50% of body weight and hearts ranged from .66% to 1.11% of body weight. When, however, lung and heart weights relative to body weights were examined, differences between lines became evident (Figure 3).

Although both heart and lung weights relative to body weight declined with age, relative heart weights were heavier in line HAS than LAS at 15 days of embryonic development as well as at hatch and 5 days after hatch, while relative lung weights were heavier at 5, 10, 15, and 25 days after hatch in line LAS than HAS. When ratios of absolute lung to absolute heart weight were compared (Figure 4), the ratio in line LAS was higher than that for line HAS at all ages except day 20.

Absorption of residual yolk was greater by HAS than LAS chicks. Although residual yolk had disappeared (*i.e.*, was less than .07g) from all 10 HAS chicks sacrificed 5 days after hatch, 8 of 10 LAS chicks still had residual yolk (mean weight of .206±.032g). Overall, residual yolk had disappeared in 48 of 50 HAS chicks and 29 of 50 LAS chicks. There was no difference between males and females for absorption of residual yolk.

Growth of males and females

There was sexual dimorphism for body weight with males numerically heavier than females by 10 days after hatch and significantly heavier by 20 days (Table 1). Sexual dimorphism was also evident for absolute lung and heart weights which followed a pattern consistent with that for body weight. There were no differences, however, between males and females for lung and heart weights relative to body weight and for the ratio of lung to heart weight.

Developmental stability

Fluctuating asymmetry (FA) may be used as a measure of overall individual ability to overcome developmental stressors. FA, which is one form of bilateral asymmetry, can be confounded by directional asymmetry and antisymmetry which are fundamentally different from FA. In all cases, except for HAS chicks at hatch, Shapiro-Wilk statistic W and one sample t-test of signed L-R character values showed normal distributions with mean zero

(i.e., FA) for lung weights. There was directional asymmetry for the exception because although the distribution was normal, the right lung was heavier than the left.

Although FA of lungs was similar for both lines with means of $.019 \pm .002$ and $.020 \pm .002$ for lines HAS and LAS, respectively, there were significant age effects and an age by sex interaction.

FAs were similar for both sexes to 20 days of age after which, it was greater for males than females (Table 2). For males, FA increased with age. The same pattern, but less definitive, was observed in females.

There were no age, line, sex, or interactions among main variables for RFA which had an overall mean of $.105 \pm .006$. Divergence in RFA among sexes followed that for FA with the difference of $.039$ between sexes (Table 2) approaching significance ($P = .055$).

DISCUSSION

Differences between lines for body weights observed in this experiment agree with previous reports where LAS chicks were heavier than HAS chicks 28 days after hatch (Siegel *et al.*, 1980; Martin *et al.*, 1990). Our results were also consistent with those of Kreukniet *et al.* (1994) who observed lower growth rates in their high than in their low sheep red blood cell antibody responder lines of chickens. Although there were no differences between lines for absolute heart weight, relative to body weight hearts were heavier in HAS than LAS chicks. The heart to body weight ratios observed in this experiment were similar to those reported over seven decades ago by Latimer (1924).

The heavier body weights of LAS than HAS chicks was paralleled by heavier lungs suggesting that lung capacity may be a key factor in growth. Heart weight and lung weight had different time-dependent emphasis. Differences between lines for

heart weight relative to body weight was evident to 5 days after hatch while for lungs differences between lines occurred after hatch. One possible reason is that heart has growth priority during early embryonic development whereas lung development occurs later (Patten, 1951).

It appears that absorption of residual yolk was a correlated response to selection for antibody titers to SRBC in that absorption was greater in HAS than LAS chicks. The rapid absorption of residual yolk by HAS chicks was consistent with reports by Nitsan *et al.* (1991), Murakami *et al.* (1992) and Nir *et al.* (1993) where essentially all residual yolk was absorbed by 4 days after hatch. Greater absorption of yolk in HAS chicks may have the advantage of maternal antibody being available to the young chicks. On the other hand, relative higher reliance on yolk residue as a food source by HAS chicks during the first few days after hatch may account for lower body weights due to slower development of the digestive system and feeding behavior (Turro *et al.*, 1994). Our results and those of Kreukniet *et al.* (1994) on the "trade-offs" of growth and the immune system provide further insights on the allocation of resources of an organism (Siegel and Dunnington, 1997). Moreover, the dynamics of embryonic and early posthatch growth patterns of supply organs observed here and by others (e.g., Lilja, 1983; Katanbaf *et al.*, 1988; Nitsan *et al.*, 1991; Nir *et al.*, 1993) demonstrate a need for further in depth studies in avian species.

Similarities between lines for FA and RFA in lung weight may reflect comparable selection pressure for and against antibody titers to SRBC. This result was consistent with that reported for mean relative fluctuating asymmetry for shank length and diameter, weight and length of the first primary wing feather, and distance between the junction of upper and lower mandibles and auditory canal (face length) in these lines (Yang *et al.*,

1997). Although detection of differences in variation is less powerful than for means, males grow faster than females contributing to their greater FA and RFA. This difference between sexes is consistent with the higher relative fluctuating asymmetry attributed to genetic stress of chickens from intensively selected for growth (Moller *et al.*, 1995) and in parental lines than their F₁ crosses (Yang *et al.*, 1997). Thus, it appears that the degree of fluctuating asymmetry of lung may be a candidate trait to measure developmental stability.

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Table I 1. Mean body, lung, and heart weights (g) for males and females from hatch to 25 days after hatch by sex

Age (days)	Body wt		Lung wt		Heart wt	
	male	female	male	female	male	female
0	27	27	0.12	0.12	0.24	0.24
5	45	45	0.17	0.17	0.40	0.39
10	73	70	0.28	0.25	0.58	0.56
15	104	97	0.35	0.36	0.73	0.71
20	139*	125	0.48*	0.41	1.00*	0.84
25	183*	160	0.60*	0.49	1.20	1.06 ¹

* $P < 0.05$.

¹ $P=0.09$.

In all cases weights at any one age differed significantly ($P \leq 0.05$) from those at any other age.

Table I 2. Fluctuating (FA)¹ and relative fluctuating (RFA)² asymmetry of lung weight (g) from hatch to 25 days after hatch by sex

(days)	Male	Female	Male	Female
0	0.014c	0.017b	0.123	0.145
5	0.016bc	0.016b	0.100	0.100
10	0.022bc	0.026ab	0.081	0.098
15	0.019bc	0.035a	0.056	0.094
20	0.030b	0.026ab	0.064	0.063
25	0.056a*	0.026ab	0.094	0.055 ³

¹ FA = |Left-Right|.

² RFA = (|Left-Right| / [(Left+Right)/2]).

³ P = 0.055.

* P < 0.050.

Treatment means in the same column with the same letter are not significantly ($P \leq 0.050$) different.

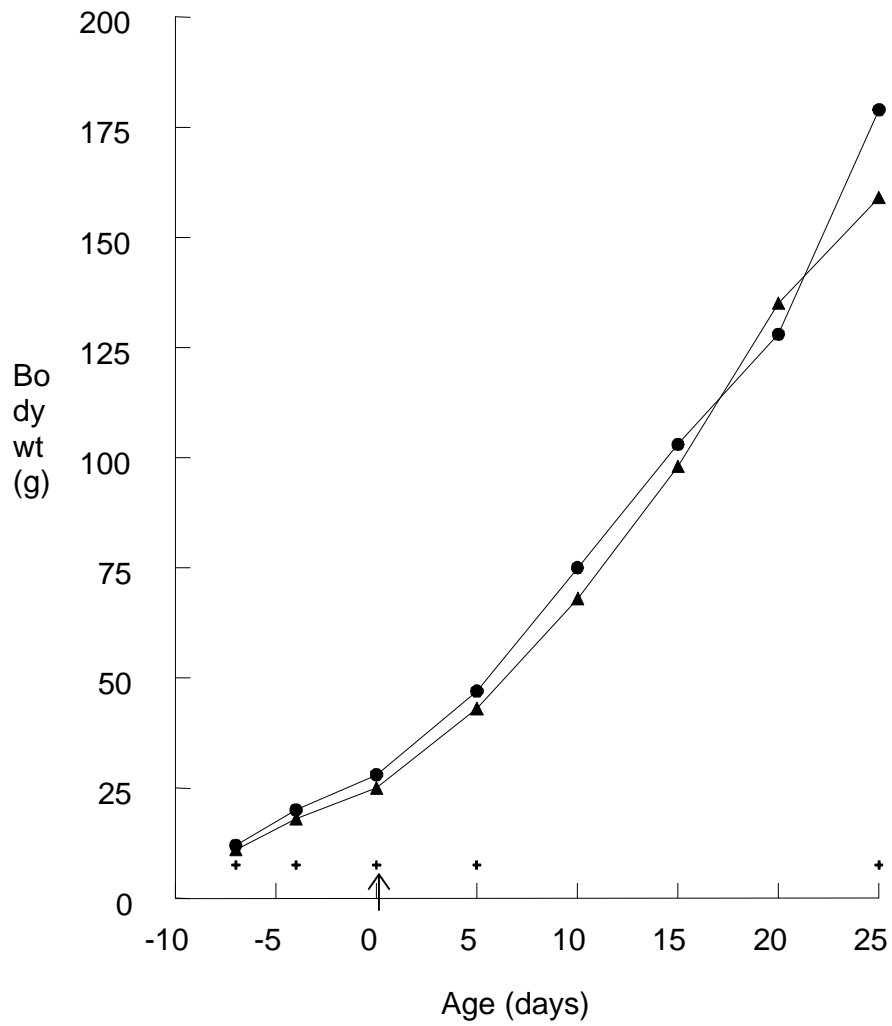


Figure 1. Body weights (sexes pooled) from day 15 of incubation to 25 days after hatch by line (▲-HAS; ●-LAS). + Difference between lines $P \leq .05$.

$$Y_{HAS} = 27.05 + 3.22x + .69x^2.$$

$$Y_{LAS} = 28.82 + 3.14x + .11x^2.$$

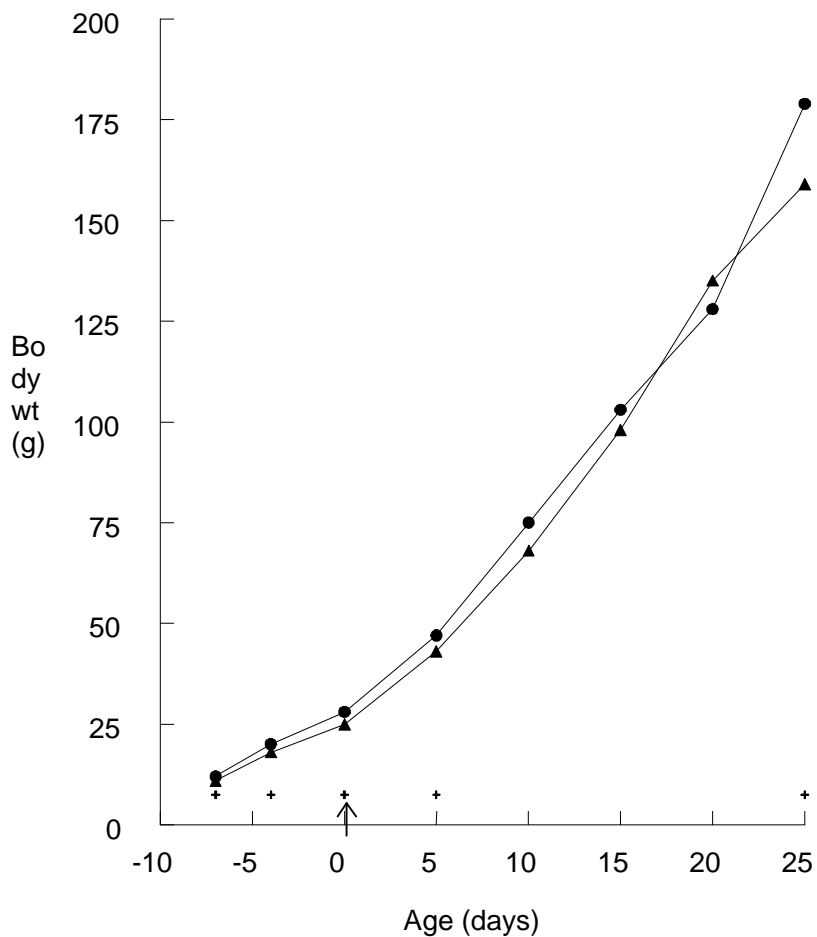


Figure 2. Heart and lung weights from day 15 of incubation to 25 days after hatch by line (\blacktriangle -HAS; \bullet -LAS). There was no difference between lines for heart weight at any age. At all ages, except day 20, lung weight was heavier for LAS than HAS chicks.

$$\begin{array}{l}
 \text{Lung:} \quad Y_{\text{HAS}} = .1087 + .0100x \quad + \quad .0002x^2. \\
 \quad \quad \quad Y_{\text{LAS}} = .1306 \quad + .0128x \quad + \quad .0002x^2. \\
 \text{Heart:} \quad Y_{\text{HAS}} = .2556 + .0249x \quad + \quad .0003x^2. \\
 \quad \quad \quad Y_{\text{LAS}} = .2515 \quad + .0254x \quad + \quad .0005x^2.
 \end{array}$$

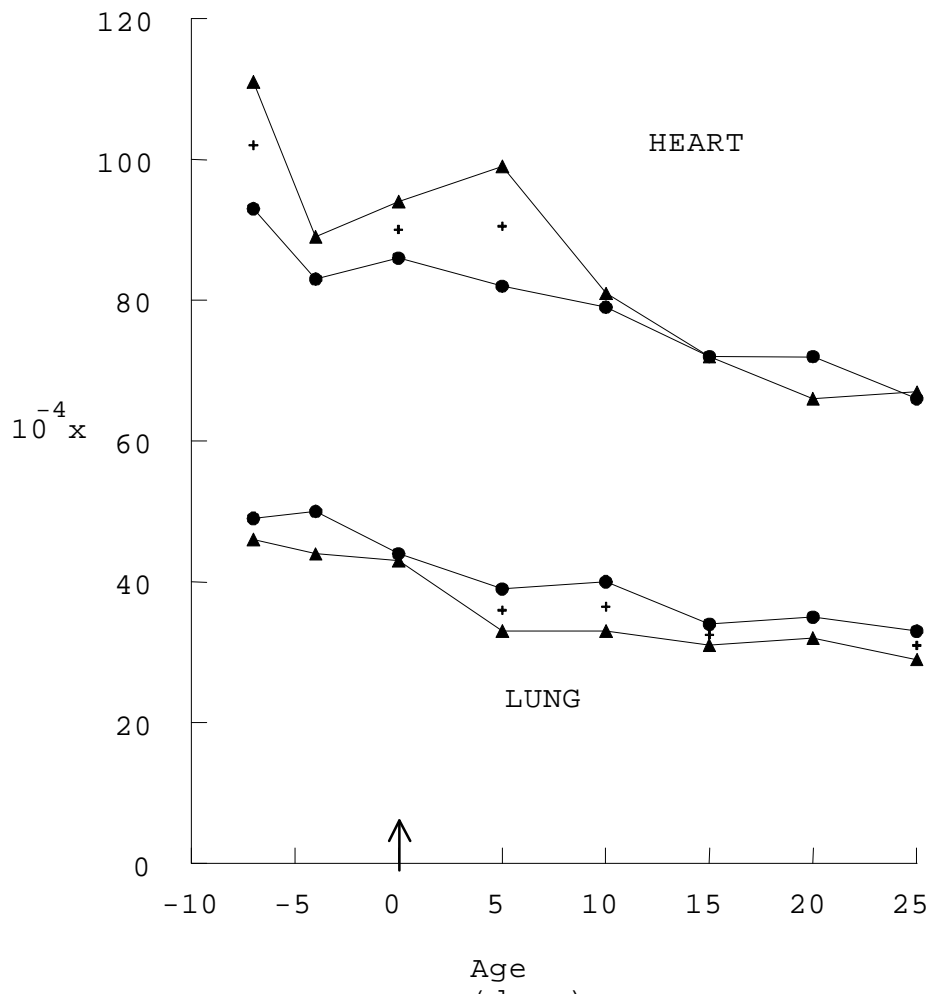


Figure 3. Heart and lung weights relative to body weight from day 15 of incubation to 25 days after hatch by line (▲-HAS;●-LAS).
 + Difference between lines $P \leq .05$.

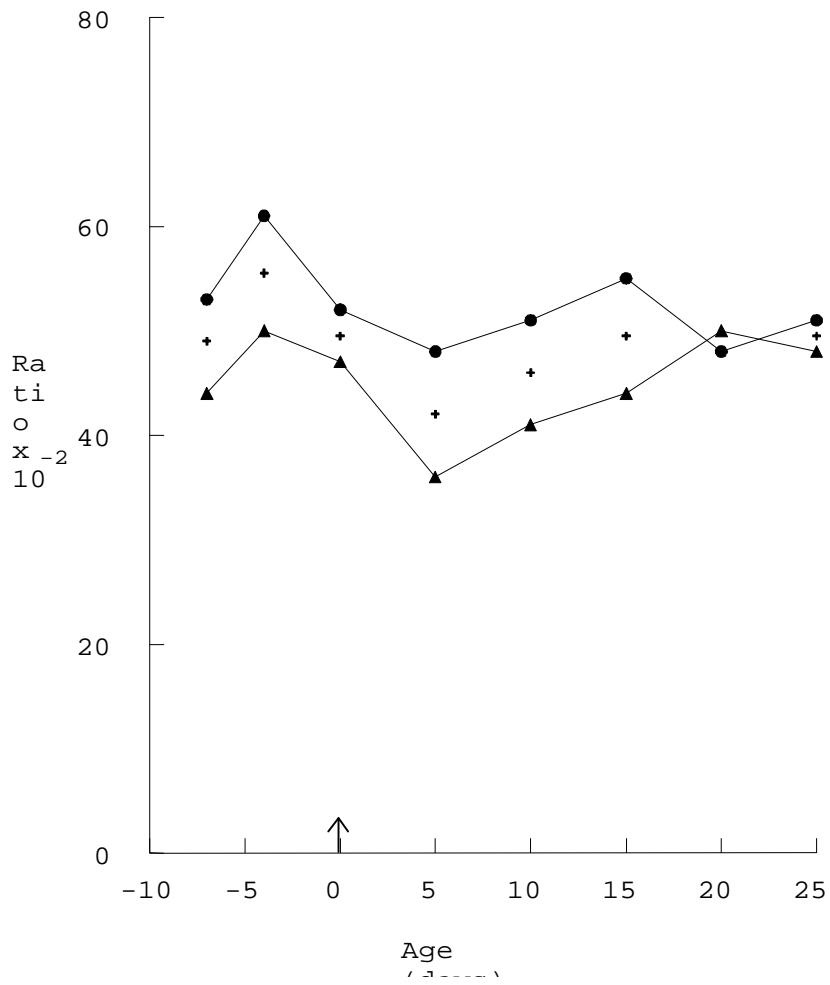


Figure 4. Ratio of lung and heart weights (lung/heart) from day 15 of incubation to 25 days after hatch by line(▲-HAS;●-LAS). + Difference between lines $P \leq .05$.

PAPER II
Asymmetries and Heterosis of Bilateral Traits in Parental Lines
of Chickens and Their F₁ Crosses

Introduction

The developmental axes of embryos are defined in terms antero-posterior and dorso-ventral (PALMER 1996). Fluctuating asymmetry (FA) is deviation from bilateral symmetry (left minus right) in morphological traits in which symmetry is the normal state. FA reflects small random deviations from symmetry in bilateral traits with a zero mean and a normal distribution. It occurs when an individual is unable to undergo identical development on both sides of a bilaterally symmetrical trait. Two other types of bilateral asymmetry are directional asymmetry (DA) defined as having normal distribution with left minus right mean not zero, and antisymmetry (AS) defined as having a left minus right mean zero with a distribution that is not normal (VAN VALEN 1962).

Identical development of bilateral traits can be disrupted by environment stressors, genetic stressors, and/or their interaction. Genetic stressors include loss of genetic variation from inbreeding or bottlenecks and founder events (LEARY and ALLENDORF 1989), hybridization between species or populations that are normally reproductive isolated (GRAHAM and FELLELY 1985; LEARY et al. 1985), and incorporation of novel mutants into the genome (CLARKE and MCKENZIE 1987). It has been proposed that intensive directional selection increases developmental instability (PARSONS 1992), and that stabilizing selection could have the opposite effect (MOLLER and POMIANKOWSKI 1993a,b; MOLLER

et al. 1995). The rationale being that selection directly affects alleles that control developmental homeostasis. Therefore, directional selection for a trait should impose selection against genetic modifiers that reduce the development of extreme phenotypes, whereas stabilizing selection should result in incorporation of modifiers that reduce extreme genotypes (FISHER 1958; JONES 1987; CLARKE and MCKENZIE 1987; PARSONS 1992).

In poultry, selection for specific traits can disrupt homeostasis and cause metabolic disorders (LERNER 1954; SIEGEL and DUNNINGTON 1997; YANG et al. 1997), while heterosis is common for fitness traits (FAIRFULL 1990). Commercial poultry breeding is based mainly on within line selection and line crossing. The purpose of the experiment reported here was to measure asymmetry of bilateral traits and heterosis in selected lines and their F_1 crosses. Such information may have potential in measuring development stability in livestock and poultry breeding.

Materials and Methods

Foundation stocks for this experiment were White Leghorn lines selected 23 generations for high or low antibody response to a single intravenous injection of 0.1 mL of a 0.25% suspension of sheep red blood cells (SIEGEL and GROSS 1980; MARTIN et al. 1990). Matings were made between and within lines, using age contemporary parents, to produce parental line and reciprocal F_1 cross chicks in a single hatch. Hereafter, progeny type will be

denoted showing the sire line first and the dam line second (e.g., HL is a high line sire mated to a low line dam). At hatch chicks were wingbanded, vaccinated against Mareks disease and placed in floor pens with woodshavings as litter. A mash diet containing 20% crude protein and 2685 kcal ME/kg was provided *ad libitum*. Lighting and water were available continuously.

At 38 days of age, 30 males from each stock were randomly selected for measurements of 2 bilateral traits. The traits were left and right metatarsus (shank) length (0.1mm) and left and right first primary wing feather length (0.1cm) and weight (0.01g). To obtain the weight of a primary wing feather required that it be removed from the chicken. Measurements of the length of left and right shanks were again obtained on these males at 49 days of age by the same holder and measurer. Because the chickens had entered feather molt, no data were obtained for any feathers at 49 days. When 56 days of age, random samples of 10 males and 10 females from each progeny type were weighed (1g) and killed by cervical dislocation. Data were obtained for lengths (0.1cm) of the left and right ceca and weights (0.01g) of the left and right shanks (with toes), left and right lungs, and hearts.

Organ data, on an absolute weight or length basis and weight relative to body weight were analyzed by analysis of variance. For data obtained at 38 and 49 days of age the statistical model was $Y_{ij} = u + S_i + e_{ij}$ where, $i=1,2,3,4$ stocks (HH, HL, LH, and LL)

and $j=1,2, \dots, n$ progeny. For 56-day data, sex (male and female) and the interaction of stock by sex were included in the model. Weights were transformed to common logarithms, and ratios and percentages to arc sine square roots prior to analyses. Heterosis was calculated as the deviation of each reciprocal F_1 from the parental line mean and expressed as a percentage.

Each signed (+ or -) bilateral asymmetry (left minus right) was tested for normality with mean zero by the Shapiro-Wilk statistic and one sample t-test (SAS 1985). Contrasts of reciprocal F_1 crosses with parental lines (HH + LL - HL - LH) were conducted for relative asymmetry ($|L-R|/[(L+R)/2]$) $\times 100$. Product-moment correlations between various traits were calculated within stock and sex.

Results

Means and % heterosis

Shanks of the F_1 males were longer than those of males from either parental line at both 38 and 49 days of age with heterosis being 6 and 4% for the HL and LH crosses, respectively (Table 1). Between the parental lines, although LL males had longer shanks than HH males at 38 days of age, there was no difference between lines at 49 days of age. Length of the first primary wing feather was longer for the HL than the LH males and parental-line males which were similar. Heterosis was 4 and 5% for length of the left and right first primary feather respectively for the HL cross,

and essentially zero for the LH cross. Feather weights were similar for all stocks with heterosis ranging from -3 to 9%.

Stock by sex interactions were not significant for any of the traits measured at 56 days of age. Sexual dimorphism with larger values for males than females was present for all traits except lung weight relative to body weight (Table 2). LL chicks were heavier than HH chicks with both F_1 crosses heavier than either parental line. Heterosis for body weight was 11% for the HL cross and 9% for the LH cross. Both left and right shanks were heavier for line LL than HH chicks with the F_1 crosses having heavier shanks than chicks from their HH but not LL parental line. Expressed relative to body weight, shank weight was heavier for the LH cross than for the other 3 stocks that did not differ from each other. The difference in expression of shank weight influenced in the degree of heterosis which ranged from 9 to 16% for absolute weight and 0 to 5% relative to body weight.

At 56 days of age, lung weights (both absolute and relative to body weight) were lower for HH chicks than for HL, LH, and LL chicks which did not differ (Table 2). Heterosis for lung weight ranged from 22 to 28% on an absolute and 10 to 17% on a relative to body weight basis. The relationship among stocks in heart weight differed depending on how heart weight was expressed. On an absolute basis there were no differences in heart weight among stocks HL, LH, and LL, although all were heavier than for stock

HH. In contrast, heart weight relative to body weight was heavier for LL chicks than for the other 3 stocks that were similar. Heterosis was 7% for absolute heart weight and -3% for heart weight relative to body weight. The ratio of absolute heart to absolute lung weight (heart/lung) varied according to stock being .93, .70, .72, and .76 for stocks HH, HL, LH, and LL, respectively.

At 56 days of age, ceca were longer for LL than HH chicken with the crosses intermediate to the parental lines. Heterosis ranged from 1 to 9% for this trait.

Types of asymmetry

Means of left minus right bilateral differences and types of asymmetry are summarized in Table 3. In all cases the left shank was longer than the right shank, however, the type of asymmetry for shank length differed according to age and stock. Line LL males exhibited DA at 38 and FA at 49 days of age while the pattern was reversed for line HH males (FA at 38 and DA at 49 days). DA was evident for the HL cross at both ages and for the LH cross at 49 days. In contrast to shank length, bilateral differences for shank weight at 56 days of age was positive in 4 cases and negative in 4 cases which was consistent with their being FA in 6 of 8 comparisons.

Length and weight of the first primary wing feather were AS in all cases except for weight in the LH cross which was FA

(Table 3). Females of all stocks exhibited FA for lung weight. While there was FA for HH and LH males, HL and LL males were AS. Although the right cecum was consistently longer than the left cecum, males and females of both F_1 crosses exhibited FA for this trait. In both parental lines males were DA, while HH females were AS and LL females FA. Across traits, sexes, and ages of 40 bilateral comparisons, 20 were FA, 12 were AS, and 8 were DA.

For the 40 within stock and sex correlations among signed left minus right (L-R) differences of the 7 bilateral traits, 5 were significant. None, however, was consistent across the 4 stocks. Of the correlations for 40 non-signed ($|L-R|$) associations, 11 were significant with no consistent pattern. Data for these correlations are not shown.

Relative asymmetry

There were differences among stocks in relative asymmetry (RA) for shank length at 38 days of age and ceca length at 56 days of age (Table 4). RA for shank length in LH males was less than that of its parental lines and its reciprocal cross, all of which had similar RAs. This difference between reciprocal crosses was evident in the heterosis of RA which was -9% for HL and -45% for LH. For ceca length, the RA for LL males was lower than for the other 3 stocks. For females, the RAs for both crosses were similar to each other and their HH parental line, but larger than their LL parental line. Heterosis for RA for ceca length ranged

from -34 to -55%. Although differences in RAs among stocks were not significant for the other traits, there was evidence of considerable heterosis with lower RAs in all cases except for shank weight at 56 days of age.

The sum of the RAs of all traits, as an index, showed that the RA of 54.5 for the HL cross was lower than those of the parental lines (Table 4). The RA of 61.4 for the LH cross while lower than the RAs of the parental lines did not differ from them or from the HL cross. Heterosis of -26 and -17% for crosses HL and LH demonstrated considerable hybrid vigor for RA as an index of developmental stability.

Discussion

Heavier body weights for LL than HH chicks agreed with previous reports for these selected lines (SIEGEL and GROSS 1980; MARTIN et al. 1990) as well as for lines selected for high and low antibody response to SRBC in The Netherlands (PARMENTIER et al. 1996). Body weights of crosses were heavier than those of both parental lines, showing overdominance. Positive relationships of shank length and weight with body weight were consistent with results reported for chickens selected mainly for growth rate (LERNER et al. 1947) and for turkeys selected for increased shank width (YE et al. 1997). Overdominance for shank length in turkeys (YE et al. 1997) was also observed in our data. The higher ratio of heart to lung weight in line HH than line LL was consistent

with previous observations for these lines (YANG and SIEGEL 1997).

There was a strong tendency for antisymmetry at both 38 and 49 days for weight and length of the first primary wing feather in the parental lines and the crosses. This pattern of mean zero with a distribution that was not normal was previously noted in lines HH and LL at 150 days of age (YANG et al. 1997). The consistency of fluctuating asymmetry for lung weights observed here was in agreement with that reported for the HH and LL lines from late embryonic to 25 days after hatch (YANG and SIEGEL 1997). Thus, it appears that types of asymmetry of bilateral traits are consistent for stocks across experiments.

Heterosis was positive and of a modest magnitude for most traits demonstrating improved fitness. Heterosis for relative asymmetry of the bilateral traits was considerable and negative demonstrating enhanced fitness and developmental stability of line crosses. These findings suggest potential for use of fluctuating asymmetry as a method of measuring genetic stressors in poultry and livestock.

Summary

Asymmetries and heterosis of bilateral traits were measured in two lines of White Leghorns selected for high (HH) or low (LL) antibody response 5 days after an injection with 0.1mL of 0.25% suspension of sheep red blood cell (SRBC) and their reciprocal

crosses. The bilateral traits were 39-day shank length and length and weight of the first primary wing feather. Shank length was again measured on day 49 while body, heart, shank, and heart weights and ceca lengths were obtained on day 56. Heterosis was positive for organ sizes and negative for degree of bilateral asymmetry. Sums of bilateral asymmetries were lower for crosses than their parental lines which, like heterosis, reflected their greater biological developmental stability and fitness.

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Table II 1. Means \pm SEM and % heterosis of bilateral traits at 38 and 49 days of age for males by stock

Trait	Stock ¹				Pooled SEM	Heterosis ²	
	HH	HL	LH	LL		HL	LH
Shank length (mm) at 38 and 49 days of age							
38 days							
Left	62.4 c	67.3 a	66.1 a	64.6 b	0.3	6	4
Right	62.1 c	66.9 a	66.0 a	64.3 b	0.3	6	4
49 days							
Left	75.2 b	79.8 a	78.1 a	75.1 b	0.4	6	4
Right	74.9 b	79.4 a	77.7 a	74.9 b	0.4	6	4
First primary wing feather length (cm) and weight (g) at 38 days of age							
Length							
Left	11.9 b	12.4 a	11.9 b	11.9 b	0.1	4	0
Right	11.7 b	12.3 a	11.9 b	11.8 b	0.1	5	1
Weight ($\times 10^{-3}$)							
Left	57 a	65 a	58 a	62 a	2	9	-3
Right	54 a	62 a	62 a	62 a	2	7	7

a-c Means in a row for stocks with no common letter differ at $P \leq .05$.

¹ The first letter designates the sire line and the second letter the dam line for each stock. H and L lines were selected 23 generations for high and low response to SRBC, respectively.

² $((HL \text{ or } LH - (HH + LL)/2)) / ((HH + LL)/2) \times 100$.

Table II 2. Means ± SEM and % heterosis of traits at 56 days of age by sex and stock

Trait	Sex ¹		Stock ²				Pooled SEM	Heterosis ³		
	male	female	HH	HL	LH	LL		HL	LH	
Absolute weight (g)										
Body	618	520	544 c	624 a	616 a	583 b	6	11	9	
Shank										
Left	13.2	10.1	10.3 c	11.9 ab	12.6 a	11.5 ab	0.3	9	16	
Right	13.3	10.2	10.3 c	12.0 ab	12.7 a	11.6 b	0.2	10	16	
Lung										
Left	2.2	1.7	1.4 b	2.1 a	2.1 a	2.0 a	0.1	24	24	
Right	2.3	1.8	1.4 b	2.3 a	2.2 a	2.2 a	0.1	28	22	
Heart	3.4	2.7	2.6 b	3.1 a	3.1 a	3.2 a	0.1	7	7	
Weight relative to body weight (organ weight/body weight) x 100										
Shank	4.2	3.9	4.0 b	4.0 b	4.2 a	4.0 b	<0.1	0	5	
Lung	0.69	0.67	0.55b	0.74a	0.69a	0.71a	0.01	17	10	
Heart	0.54	0.51	0.52b	0.52b	0.52b	0.55a	0.01	-3	-3	
Ceca length (cm)										
Left	13.7	13.1	11.2 c	13.4 b	14.0 ab	14.4 a	0.2	5	9	
Right	14.2	13.4	11.7 c	13.6 b	14.3 b	15.3 a	0.2	1	6	

a-c Means in a row for stocks with no common letter differ at $P \leq .05$.

¹ Differences between sexes were significant ($P \leq .05$) for all traits except lung weight relative to body weight.

² The first letter designates the sire line and the second letter the dam line for each stock. H and L lines were selected 23 generations for high and low response to SRBC, respectively.

³ $((HL \text{ or } LH - (HH + LL)/2)) / ((HH + LL)/2) \times 100$.

Table II 3. Means \pm SEM of bilateral differences¹ and types of asymmetry² by sex and stock

Trait	Sex	Stock ³			
		HH	HL	LH	LL
Shank length (mm) at 38 and 49 days of age					
38 days	M	29.7 \pm 16.1 FA	37.0 \pm 13.1 DA	15.5 \pm 10.1 AS	29.3 \pm 12.8 DA
49 days	F	37.9 \pm 17.7 DA	45.7 \pm 11.0 DA	37.3 \pm 15.4 DA	19.3 \pm 21.7 FA
Shank weight (g) at 56 days of age					
	M	5.3 \pm 5.7 FA	-2.8 \pm 7.8 FA	-18.5 \pm 15.5 AS	-14.6 \pm 5.9 DA
	F	2.3 \pm 4.4 FA	-8.0 \pm 4.6 FA	4.2 \pm 7.6 FA	1.8 \pm 8.5 FA
First primary wing feather length (cm) and weight(g) at 38 days of age					
Length	M	12.7 \pm 10.7 AS	7.0 \pm 8.1 AS	-3.1 \pm 8.9 AS	4.3 \pm 1.1 AS
Weight	F	0.3 \pm 0.5 AS	0.3 \pm 0.2 AS	1.1 \pm 0.2 FA	0.0 \pm 0.2 AS
Lung weight (g) and ceca length (cm) at 56 days of age					
Lung	M	5.3 \pm 9.0 FA	13.0 \pm 13.5 AS	-15.8 \pm 16.1 FA	-30.9 \pm 17.1 AS
	F	0.9 \pm 7.9 FA	-26.9 \pm 14.7 FA	-0.9 \pm 11.9 FA	-12.9 \pm 13.6 FA
Ceca	M	-51.2 \pm 10.6 DA	-26.0 \pm 20.7 FA	-22.7 \pm 13.9 FA	-13.5 \pm 13.6 DA
	F	-35.6 \pm 9.3 AS	-14.5 \pm 16.7 FA	-32.2 \pm 18.1 FA	-56.7 \pm 26.5 FA

¹ (Left-Right) x 100.

² FA - fluctuating asymmetry; DA - directional asymmetry; AS - antisymmetry.

³ The first letter designates the sire line and the second letter the dam line for each stock. H and L lines were selected 23 generations for high and low response to SRBC, respectively.

Table II 4. Means \pm SEM and % heterosis for relative asymmetry¹ of bilateral traits by sex and stock

Trait	Sex	Stock ²				Heterosis ³	
		HH	HL	LH	LL	HL	LH
Shank length at 38 and 49 days of age							
38 days	M	1.2 \pm 0.2a	1.0 \pm 0.1a	0.6 \pm 0.1b	1.0 \pm 0.1a	-9	-45
49 days	F	1.1 \pm 0.2a	0.7 \pm 0.1a	0.9 \pm 0.1a	1.1 \pm 0.2a	-36	-18
Shank weight at 56 days of age							
	M	1.2 \pm 0.1a	1.5 \pm 0.3a	1.7 \pm 1.1a	1.5 \pm 0.4a	11	26
	F	1.5 \pm 0.2a	1.3 \pm 0.3a	1.7 \pm 0.3a	2.1 \pm 0.5a	-28	-6
First primary wing feather length and weight at 38 days of age							
Length	M	3.2 \pm 0.7a	1.7 \pm 0.6a	2.1 \pm 0.7a	3.0 \pm 0.8a	-45	-32
Weight	F	23.5 \pm 4.8a	12.6 \pm 2.8a	18.6 \pm 2.9a	15.8 \pm 2.3a	-36	-5
Lung weight and ceca length at 56 days of age							
Lung	M	13.7 \pm 2.6a	10.1 \pm 3.8a	13.5 \pm 3.5a	15.6 \pm 5.6a	-31	-8
	F	18.4 \pm 2.6a	18.1 \pm 5.1a	16.0 \pm 4.0a	20.4 \pm 4.1a	-7	-18
Ceca	M	4.4 \pm 0.9b	4.2 \pm 0.8b	3.0 \pm 0.5b	9.0 \pm 0.9a	-37	-55
	F	3.8 \pm 0.4ab	3.2 \pm 0.8b	3.3 \pm 1.0b	6.2 \pm 1.0a	-36	-34
Sum of relative asymmetry of traits							
		72.0 a	54.4b	61.4ab	75.7a	-26	-17

a-b Means in a row for stocks with no common letter differ at $P \leq .05$.

¹ $L-R / [(L+R)/2] \times 100$.

² The first letter designates the sire line and the second letter the dam line for each stock. H and L lines were selected 23 generations for high and low response to SRBC, respectively.

³ $((HL \text{ or } LH - (HH + LL)/2)) / ((HH + LL)/2) \times 100$.

M-Male F-Female

PAPER III

Developmental Stability in Stocks of White Leghorn Chickens

ABSTRACT The degree of asymmetry in bilateral morphological characters may reflect genetic and environmental stressors. Shank length and diameter, weight and length of the first primary wing feather, and distance between the junction of upper and lower mandibles and auditory canal (face length) were used to classify bilateral types and measure relative asymmetry (RA) in six genetic stocks. The stocks were the S₂₃ generation of White Leghorn lines selected for high or low antibody response to SRBC, sublines where selection had been relaxed for eight generations, and reciprocal crosses of the selected lines. Differences were found among all stocks for the traits measured. Rankings among traits for RA in descending order were face length, shank diameter, feather weight, and shank and feather lengths. The RA of shank and feather lengths did not differ from each other. An overall RA composed of mean RA of the five traits showed that the two selected lines exhibited greater RAs than the crosses between them. The RAs of the two lines where selection had been relaxed was similar to that of selected lines. This research suggests that an overall RA created as a combination of RAs of several bilateral traits can be a valid measure of genetic stress in chickens and provides a method of comparing developmental stability among populations.

(Key words: chickens, bilateral asymmetry, genetic stress, homeostasis)

INTRODUCTION

Both sides of a bilateral symmetrical trait of an animal are considered to be under genetic control (Leary and Allendorf, 1989; Parsons, 1990). Although sides may be expected to be identical because they are products of the same genome, this is not always the case as seen in the functional ovary and heart atriums and ventricles of chickens. The developmental axes of the embryo are defined in terms antero-posterior and dorso-ventral (Palmer, 1996), not left-right. Bilateral asymmetry, the deviation of part of an organism from perfect symmetry, can be categorized as antisymmetry, directional asymmetry, or fluctuating asymmetry (van Valen, 1962). Each of these categories is characterized by a different combination of the mean and the distribution of left minus right. Palmer (1996) provided examples of frequency distributions for these three types of asymmetry.

Four genes have been reported to direct development of asymmetry of the internal organs during gastrulation and neurula stages in chick embryos (Yost, 1995). Therefore, asymmetry of bilateral traits may indicate perturbed development due to intra- (genetic) and extra- (environmental) stressors. Studied in a variety of organisms, asymmetries of bilateral traits appear to have promise as a tool in the study of evolution, conservation biology, and animal breeding (van Valen, 1962; Palmer and Strobeck, 1986; Jones, 1987; Parsons, 1990. Moller *et al*, 1995;

Palmer, 1996).

Considerable research has been conducted on environmental stressors in poultry (e.g., Freeman, 1985; Siegel, 1995; Zulkifli and Siegel, 1995). In contrast, information on genetic stressors such as major mutations, selection, inbreeding, and chromosomal imbalance remains quite limited. For poultry breeding, selection for specific traits can disrupt homeostasis (Lerner, 1954), and heterosis is common for fitness traits (Fairfull, 1990). Of growing concern in commercial poultry is an increased incidence of leg disorders (Cahaner and Siegel, 1986), ascites (Julian, 1993), sudden death syndrome (Olkowski and Classen, 1995) in meat stocks, and osteomalacia and fatty liver syndrome (Schwartz, 1994) in layers. Although these conditions are associated with bilateral characteristics, the relationship of current health concerns in poultry and bilateral asymmetry are unclear in poultry breeding programs. Reported in this paper are comparisons of bilateral asymmetry in chickens from selected lines, sublines in which selection was relaxed, and F₁ crosses of the selected lines.

MATERIALS And METHODS

Genetic Stocks And Husbandry

The chickens used in this experiment were White Leghorn females from matings of lines selected for high (HAS) or low (LAS) antibody response to a single intravenous injection of 0.1

mL of a 0.25% suspension of SRBC (Siegel and Gross, 1980; Martin *et al.*, 1990). The respective stocks were the S₂₃ generation of Lines HAS and LAS, sublines of HAS and LAS where selection had been relaxed (HAR and LAR, respectively) for 8 generations, and reciprocal F₁ crosses of the selected lines. All individuals were produced from age contemporary parents. At hatch they were vaccinated against Mareks disease and reared as contemporaries in floor pens to 18 wks of age. They were then transferred to individual cages in an environmentally controlled room.

Traits Measured

At 28, 168, and 240 d of age, BWs were obtained. Antibody titers to SRBC were measured during the 6th wk after hatch. On Day 150, data were obtained for the following bilateral characters: length (0.1mm) of the metatarsus (shank), diameter (0.1mm) of the shank perpendicular to the spur, and distance (0.1mm) between the auditory canal and the posterior junction of upper and lower mandible (face length). The measurer and holder of the chickens were the same for all measurements. Also at this age, the left and right first primary wing feathers were removed and their length (mm) and weight (0.01g) obtained. All first primary wing feathers were mature. Data were obtained for 29 HAR, 30 LAR, 51 LAS, 59 HAS x LAS, 60 HAS, and 60 LAS x HAS females.

There were three categories for left minus right (L-R) bilateral differences. Definitions were mean zero and normal

distribution for fluctuating asymmetry (FA), mean not zero and normal distribution for directional asymmetry (DA), and mean zero with a distribution that was not normal for antisymmetry (AS). Relative asymmetry (RA) was defined as the ratio of the absolute value of asymmetry (L-R) divided by the value for the size of the bilateral trait; $RA = (|L-R| / [(L+R) / 2]) \times 100$.

Statistical Analyses

Analyses of variance (SAS, 1985) were conducted for all measurements using the completely randomized model: $Y_{ij} = u + g_i + e_{ij}$, where $i = 1, 2, \dots, 6$ stocks (HAS, LAS, HAR, LAR, HAR x LAR, and LAR x HAR) or when $i = 1, 2, \dots, 5$ RAs (shank length, shank diameter, face length, feather weight, and feather length) and $j = 1, 2, \dots, n$ individuals. When significant at $P \leq 0.05$, multiple means were separated by Duncan's multiple range test.

Prior to analysis RAs were transformed to arc sine square roots. Each signed (+ or -) bilateral asymmetry (L-R) was tested for normality by Kolmogorov-Smirnov (sample size > 59) or the Shapiro-Wilk statistic W (sample size < 60). One-sample t-tests were used to test if means were zero (SAS, 1985). The mean RA of each trait across stocks was calculated on an individual basis. Also, within each stock the RAs of different characters were calculated on an individual basis for an overall measure of RA. Product-moment correlations between bilateral traits $((L+R)/2)$ and RA with BW and SRBC antibody titers were calculated within

stocks. Correlations were also calculated between shank length and diameter and between feather weight and length.

RESULTS

Sizes of Bilateral Traits

Stocks differed for both length and diameter as well as the length x diameter of the left and right shanks (Table 1). There was a general consistency for both shanks in that they were longest for the F₁ crosses, shortest for Line LAS, and intermediate for Lines HAS, HAR, and LAR. The single exception was for left shank of HAS females which differed only from that of LAS females. For diameter of the shanks a different general pattern was noted among stocks with diameters being greater for Lines HAS and HAR, smaller for Lines LAR and LAS, with the crosses being intermediate but not different from lines HAR (left), LAR and LAS. When shank was expressed as the length x diameter, there was considerable overlapping of values across stocks. Face lengths were different among stocks with the order being LAS x HAS and HAR (left) > HAS x LAS > HAS, LAR, and LAS. Lines HAS and HAR had heavier first primary feathers than the other 4 stocks which did not differ from each other. The ranking of stocks for feather length was HAR > HAS > F₁ crosses > (LAS, LAR) with the exception that LAR (left) did not differ from the F₁ crosses.

Bilateral Asymmetry

Except for cross LAS x HAS which exhibited FA, stocks exhibited directional asymmetry for shank length with the left shank being consistently longer than the right one (Table 2). For shank diameter where left was generally less than right, there was antisymmetry in Line HAS and both crosses, directional asymmetry for Lines HAR and LAS, and FA for Line LAR. For length x diameter of shank, the direction again was left generally less than right with FA for the crosses and Line LAR, directional asymmetry for Lines HAR and LAS, and antisymmetry for Line HAS. For face length, there was FA for Stocks HAS, HAR, LAR and HAS x LAS while LAS and LAS x HAS exhibited antisymmetry. Feather weight and feather length exhibited antisymmetry in all stocks. Neither logarithmic, square root, reciprocal, nor arc sine square root transformations resulted in normality for data that were antisymmetry.

Relative Asymmetry

The average RAs for the 5 bilateral traits (Table 3) was highest for face length and least for shank and feather lengths (which did not differ). The average RA of 3.66 for shank diameter and 2.82 for feather weight differed from each other and the other traits.

Overall RAs for the 5 bilateral traits are presented in Figure 1 by stock. There were no differences among the selected

and relaxed lines. The RA of those 4 lines, however, were larger than those of the F₁ crosses which did not differ from each other. Although heterosis for each of the traits measured in this experiment was low (the highest was 10% for face length), the reduction in RA of the F₁ crosses compared to their parental lines was considerable. Percentage reductions in RA were 38 for shank length, 64 for shank diameter, 31 for face length, 22 for feather weight, and 32 for feather length. For the overall RA, the reduction of the F₁ crosses compared to the parental lines was 39%.

Correlations

Neither bilateral traits nor their RAs were correlated with BW at 28, 168, and 240 days of age or SRBC at 6 wk of age in any of the 6 stocks. Shank length and diameter were significantly correlated and ranged from 0.36 to 0.53 across stocks. The correlations of feather weights with lengths were significant and ranged from 0.74 to 0.96 across stocks.

DISCUSSION

The selected lines used in this experiment had undergone long-term single trait selection for high or low antibody response to SRBC antigen. Correlated responses to this selection were heavier body weights for LAS than HAS chickens (Martin *et al.*, 1990). Thus, it was not surprising that not only were there

differences among the selected lines, relaxed lines, and crosses for growth allomorphic traits, but that there was evidence of heterosis.

Antisymmetry occurs when asymmetry is normally present, but variable as to which side has greater development. It is distinguished by a platykurtic or bimodal distribution of L-R differences about a mean of zero. Directional asymmetry refers to greater development of a character on one side of the plane or planes of symmetry than the other side. Fluctuating asymmetry reflects small random deviations from symmetry in bilateral traits with a normal distribution of L-R differences whose mean is zero. Any two or all three types of asymmetry may occur together for the same trait with fluctuating asymmetry thought to be ubiquitous (van Valen, 1962; Palmer and Strobeck, 1992).

Results obtained in this experiment were consistent with those for data from several species showing that not all bilateral asymmetries are FA, *i.e.*, normal distribution with mean zero (Summer and Huestis, 1921; Thoday, 1958; Lacy and Horner, 1996; Palmer, 1996). Also, our results showed that even for the same trait there were different distributions among genetic stocks.

It has been proposed that RA of morphological traits could provide a reliable indicator of genetic stress (Palmer and Strobeck, 1986; Leary and Allendof, 1989). Our results are

consistent with this thesis. The degree of RA varied among bilateral traits being greatest for face length, intermediate for shank diameter and weight of the first primary wing feather, and least for shank length and feather length. This variation in RA among traits suggests the value of an overall RA as a measure of developmental stability. Overall RAs of the 5 bilateral traits showed that the F_1 crosses had better developmental stability than their parental lines and the lines where selection was relaxed. That is, there was less developmental error which may reflect superior buffering against genetic and environmental stressors. Genetic changes made by individual phenotypic selection for a specific trait are primarily due to additive gene effects. That the relaxed lines exhibited RAs similar to those of the selected lines is consistent with their not regressing to the original level. In conclusion, it appears the RAs of 5 bilateral traits reported here could be valid candidates for measuring developmental stability in genetic stocks.

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TABLE III 1. Mean \pm SEM of traits at 150 days of age by genetic stock

Trait	Stock ¹							
	HAS	HAR	LAR	LAS	HAS x LAS	LAS x HAS		
n ²	60	29	30	51	59	60		
Shank (mm)								
length: left	99.47 \pm 0.38 ab	98.04 \pm 0.58 b	98.36 \pm 0.48 b	96.25 \pm 0.43 c	100.45 \pm 0.34 a	100.26 \pm 0.34 a		
right	98.62 \pm 0.37 b	97.61 \pm 0.57 b	98.86 \pm 0.48 b	95.53 \pm 0.44 c	100.13 \pm 0.34 a	100.23 \pm 0.34 a		
diameter:left	10.25 \pm 0.09 a	10.10 \pm 0.09 ab	9.66 \pm 0.14 c	9.72 \pm 0.07 c	9.78 \pm 0.07 bc	9.90 \pm 0.06 bc		
right	10.44 \pm 0.11 a	10.46 \pm 0.11 a	9.70 \pm 0.14 c	10.03 \pm 0.09 b	9.82 \pm 0.06 bc	9.84 \pm 0.06 bc		
length left	1021 \pm 12 a	991 \pm 13 ab	961 \pm 16 bc	936 \pm 10 c	983 \pm 9 b	993 \pm 8 ab		
xdiameter:right	1030 \pm 12 a	1021 \pm 14 a	956 \pm 17 b	956 \pm 11 b	983 \pm 8 b	987 \pm 8 b		
Distance (mm) between junction of upper and lower mandibles and auditory canal (face length)								
left	21.54 \pm 0.26 c	23.57 \pm 0.19 a	20.87 \pm 0.30 c	21.15 \pm 0.23 c	22.81 \pm 0.20 b	23.74 \pm 0.17 a		
right	21.87 \pm 0.23 c	23.65 \pm 0.20 ab	20.26 \pm 0.25 d	20.66 \pm 0.22 d	23.11 \pm 0.15 b	24.06 \pm 0.14 a		
First primary wing feather weight (g) and length (cm)								
weight: left	0.326 \pm 0.006a	0.325 \pm 0.006a	0.269 \pm 0.003b	0.266 \pm 0.004b	0.278 \pm 0.005b	0.278 \pm 0.005b		
right	0.323 \pm 0.006a	0.325 \pm 0.007a	0.267 \pm 0.003b	0.267 \pm 0.005b	0.275 \pm 0.005b	0.276 \pm 0.005b		
length: left	19.37 \pm 0.11 b	19.77 \pm 0.09 a	18.08 \pm 0.08 c	17.85 \pm 0.10 d	18.37 \pm 0.09 c	18.36 \pm 0.10 c		
right	19.30 \pm 0.11 b	19.70 \pm 0.12 a	18.08 \pm 0.08 d	17.87 \pm 0.09 d	18.41 \pm 0.09 c	18.40 \pm 0.10 c		

a-d means in a row with no common letter differ significantly ($p \leq 0.05$).

¹HAS - selected 23 generations for high antibody response to sheep red blood cells (SRBC);

LAS - selected 23 generations for low antibody response to SRBC;

HAR and LAR - sublines of HAS and LAS, respectively, where selection was relaxed for 8 generations;

HAS x LAS and LAS x HAS - F₁ generation with sire line given first and dam line second.

²number of individuals.

TABLE III 2. Mean \pm SEM of bilateral differences (left-right) between traits and types of asymmetry¹ at 150 days of age by genetic stock

Trait	Stock ²							
	HAS	HAR	LAR	LAS	HAS x LAS	LAS x HAS		
Shank (mm)								
length:	0.85 \pm 0.14 DA	0.43 \pm 0.16 DA	1.00 \pm 0.20 DA	0.72 \pm 0.20 DA	0.32 \pm 0.13 DA	0.04 \pm 0.10 FA		
diameter:	-0.19 \pm 0.07 AS	-0.36 \pm 0.10 DA	-0.04 \pm 0.06 FA	-0.30 \pm 0.07 DA	-0.03 \pm 0.05 AS	0.06 \pm 0.05 AS		
length x diameter:	-9.8 \pm 6.6 AS	-30.0 \pm 9.7 DA	-5.3 \pm 7.2 FA	-23.5 \pm 7.4 DA	-0.1 \pm 5.2 FA	6.20 \pm 5.3 FA		
Distance (mm) between junction of upper and lower mandibles and auditory canal (face length)								
	-0.33 \pm 0.25 FA	-0.08 \pm 0.25 FA	0.62 \pm 0.42 FA	0.49 \pm 0.25 AS	-0.29 \pm 0.19 FA	-0.33 \pm 0.17 AS		
First primary wing feather weight (g) and length (cm)								
weight:	0.003 \pm 0.003 AS	0.001 \pm 0.004 AS	0.002 \pm 0.002 AS	-0.001 \pm 0.002 AS	0.004 \pm 0.002 AS	0.003 \pm 0.002 AS		
length:	0.75 \pm 0.55 AS	0.67 \pm 0.73 AS	-0.07 \pm 0.24 AS	0.38 \pm 0.54 AS	0.83 \pm 0.28 AS	0.92 \pm 0.24 AS		

¹ FA-fluctuating asymmetry; DA-directional asymmetry; AS-antisymmetry.

² HAS - selected 23 generations for high antibody response to sheep red blood cells (SRBC); LAS - selected 23 generations for low antibody response to SRBC; HAR and LAR - sublines of HAS and LAS, respectively, where selection was relaxed for 8 generations; HAS x LAS and LAS x HAS - F₁ generation with sire line given first and dam line second.

TABLE III 3. Mean relative asymmetry¹ of each bilateral trait at 150 days of age by genetic stock and when averaged across all stocks

Trait	Stock ²						Average
	HAS	HAR	LAR	LAS	HAS x LAS	LAS x HAS	
Shank (mm)							
length:	1.13	0.76	1.16	1.17	0.80	0.63	0.93 d
diameter:	3.71	4.93	3.04	4.94	2.79	0.30	3.66 b
Distance (mm) between junction of upper and lower mandibles and auditory canal (face length)							
	7.31	4.75	9.06	7.47	5.41	4.81	6.35 a
First primary wing feather weight (g) and length (cm)							
weight:	3.51	4.61	1.80	2.56	2.54	2.22	2.82 c
length:	1.35	1.27	0.55	1.23	0.65	0.70	0.96 d

a-d means in the averaged column with no common letter differ significantly ($P \leq 0.05$).

1 $(L-R / [(L+R) / 2]) \times 100$.

2 HAS - selected 23 generations for high antibody response to sheep red blood cells (SRBC);

LAS - selected 23 generations for low antibody response to SRBC;

HAR and LAR - sublimes of HAS and LAS, respectively, where selection was relaxed for 8 generations;

HAS x LAS and LAS x HAS - F₁ generation with sire line given first and dam line second

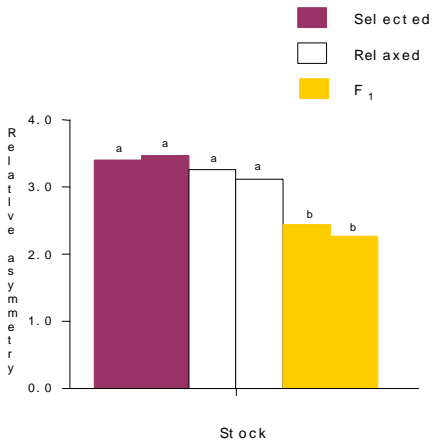


FIGURE 1. Mean of relative bilateral asymmetries for 5 traits measured at 150 days of age [length and diameter of shank, distance between junction of upper and lower mandibles and auditory canal (face length), and weight and length of first primary wing feather] by genetic stock. HAS - selected 23 generations for high antibody response to sheep red blood cells (SRBC); LAS - selected 23 generations for low antibody response to SRBC; HAR and LAR - sublimes of HAS and LAS, respectively, where selection was relaxed for 8 generations; HAS x LAS and LAS x HAS - F₁ generation with sire line given first and dam line second. (a-b) - columns with the same letter are not different ($P \leq 0.05$).

PAPER IV

Mode of Inheritance of Unselected Traits in Lines of Chickens
Selected for High or Low Antibody Response to sheep Red Blood
Cells: 2. Heterophils, Lymphocytes, and Hematocrits

ABSTRACT The nuclear lines for this experiment were White Leghorns that had undergone long-term selection for high (HH) or low (LL) antibody response to sheep red blood cell antigen(s). Sixteen progeny types consisting of parental lines, reciprocal F_1 and F_2 crosses, and backcrosses were produced in a single hatch from age contemporary parents. At 30 days of age blood was obtained from a random sample of 10 males per progeny type ($n=160$) and slides prepared for subsequent determination of number of heterophils and lymphocytes. Twelve days later, blood was collected from random samples of 10 males and 10 females per progeny type ($n=320$) for measuring hematocrits. There were no differences between parental lines for heterophils, lymphocytes, or the heterophil/lymphocyte ratio. Reciprocal effects were evident in the F_1 crosses and directional heterosis was present in one cross but not the other. Neither maternal heterosis nor recombination effects were significant for either heterophils or lymphocytes. Although hematocrits were similar for males and females and parental lines, sex-linked and recombination effects appeared to be important.

(*Key words:* chickens, SRBC, hematocrits, heterophils, lymphocytes).

INTRODUCTION

An animal's protection from disease is based, in part, on phagocytic, cell-mediated, and humoral immunity. In birds the heterophils are phagocytic cells whose main role is protection against invading microorganisms (Powell, 1987), while primary functions of lymphocytes involve cell-mediated and humoral immunity. Heterophils increase and lymphocytes decrease when chickens are stressed resulting in the ratio between them being a good index of response to a stressor (Gross and Siegel, 1983; Siegel, 1995). There is a genetic component to heterophil and lymphocyte responses to stressors (Gross and Siegel, 1985) and their ratio has been used as a heritable selection criterion for heat resistance in chickens (Al-Murrani *et al.*, 1997). Gene action models for heterophils and lymphocytes in nonstressed chickens, however, have not been investigated.

Relative hematocrit values (packed cell volume) are quantitatively inherited with heritabilities of 0.39 and 0.27 from paternal and maternal half-sib correlations, respectively (Washburn, 1967). Although Shlosberg *et al.* (1996) demonstrated in chickens that hematocrit values could be changed through selection, information on gene action models for this trait is lacking. This paper reports on the modes of inheritance of heterophils, lymphocytes, and hematocrits as measured in nuclear lines selected for high and low antibody response to SRBC, their

F₁, F₂, and backcross populations.

MATERIALS AND METHODS

This experiment involved two lines of White Leghorn chicken derived from the same base population but selected divergently for high (HH) and low (LL) antibody response 5 days after a single intravenous injection of 0.1mL of 0.25% suspension of SRBC antigen(s) (Siegel and Gross, 1980; Martin *et al.*, 1990).

The methods of mating and husbandry for the chicks used in this experiment were described by Boa-Amponsem *et al.* (1998). Briefly, matings were made between age-contemporary chickens from the S₂₂ generation of these lines to produce the parental lines and reciprocal F₁ crosses. These 4 populations were then mated to produce 16 progeny types consisting of parental, reciprocal F₁, F₂, and backcrosses. At hatch, 100 straight-run chicks of each progeny type were wingbanded, vaccinated for Marek's disease and placed in floor pens with woodshavings as litter. Chicks were provided *ad libitum* a mash diet containing 20% crude protein and 2685 kcal ME/kg. Lighting and water were available continuously.

At 30 days of age, blood was obtained from the brachial vein of 10 males from each mating combination and mixed with EDTA as the anticoagulant. Slides were prepared for determining the number of heterophils and lymphocytes as described by Gross and Siegel (1983). All slides were coded and a total of 60 cells were classified as heterophils or lymphocytes by the same individual

(AY). At 42 days of age, blood was collected from the brachial vein in heparinized microhematocrit tubes from 10 females and 10 males from each mating combination. Duplicate samples were spun and the percentage packed cell volume was taken as the hematocrit.

Hematocrits and heterophil/lymphocyte ratios were transformed to arc sine square roots prior to analysis. Genetic analysis for heterophils, lymphocytes, and heterophil/lymphocyte ratios were conducted for males only. Genetic analyses for hematocrits were conducted within sexes and with sexes pooled in order to obtain information on sex-linked and maternal effects. The models used for comparing paternal lines, reciprocal effects, heterosis, maternal heterosis, and recombination effects were the same as those described by Boa-Amponsem *et al.* (1998) with specific comparisons shown in Tables 2 and 4. Calculations for scaling tests A, B, and C for an additive-dominance model (Mather and Jinks, 1982) were consistent with those of Boa-Amponsem *et al.* (1998). The formulae were: $A = 2BC_{HH} - HH - [(HL+LH)/2]$, $B = 2BC_{LL} - LL - [(HL+LH)/2]$, and $C = 4F_2 - 2F_1 - HH - LL$ where, $BC_{LL} = (HHHL + HHLH + HLHH + LHHH)/4$ and $BC_{LL} = (LLHL + LLLH + HLLL + LHLL)/4$.

RESULTS

Heterophils, Lymphocytes and the Ratio

Mean heterophils, lymphocytes, and heterophil/lymphocyte ratios for males at 30 d of age are presented in Table 1 with

genetic contrasts in Table 2. There were no differences between parental lines for these traits. Reciprocal effects were significant and of opposite sign, being negative for heterophils and positive for lymphocytes with the negative ratio between them approaching significance ($P < 0.06$). For heterophils and the ratio, values were greater for the LH than the HL cross. In contrast for lymphocytes the mean was greater for the HL than for the LH cross. Directional heterosis was significant for the HL cross but not the LH cross for heterophils, lymphocytes and the ratio between them. The heterosis for the HL cross was negative for heterophils and positive for lymphocytes. Neither maternal heterosis nor recombination effects were significant. None of scaling tests was significant with $A = -0.28 \pm 2.62$, 0.28 ± 2.62 , and -0.02 ± 0.10 , $B = 1.91 \pm 2.69$, -1.91 ± 2.69 , $0.05 \pm .10$, and $C = -3.97 \pm 4.76$, 3.97 ± 4.76 , -0.15 ± 0.18 for heterophils, lymphocytes, and the heterophil/lymphocyte ratio, respectively.

Hematocrits

Mean hematocrits at 42 days of age are presented for progeny types in Table 3 with genetic contrast in Table 4. There were no differences in hematocrits between males ($35.2 \pm 0.2\%$) and females ($35.5 \pm 0.2\%$) nor between parental lines. Reciprocal effects were highly significant for males and when sexes were pooled with values higher for the HL than LH cross. Although heterosis was significant for males but not females of the HL

cross, there was no evidence for heterosis for the LH cross. Maternal heterosis was not significant for either sex or when sexes were pooled. Both measures of recombination effects were strongly positive for males and when sexes were pooled, whereas for females there were significant recombination effects in the comparison of the F_2 populations with backcrosses but not for the F_2 with the F_1 generation comparison. Scaling tests were not significant for A and B. Values were: A = 0.016 ± 0.014 , 0.002 ± 0.012 , -0.007 ± 0.009 , B = -0.013 ± 0.014 , -0.020 ± 0.012 , -0.016 ± 0.009 for females, males, and sexes pooled, respectively. Scaling test C was significant with corresponding values being -0.005 ± 0.025 , -0.064 ± 0.022 , -0.080 ± 0.017 .

DISCUSSION

This study was designed to examine modes of inheritance of heterophils, lymphocytes, and hematocrits under routine husbandry where there were no known stressors. The parental lines differed in response to the SRBC antigen and resistance to several diseases (Gross *et al.*, 1980). The traits measured in this experiment are modified by stressors and disease agents, but information is lacking on genetic variation for them prior to imposition of these environmental insults.

In this experiment, numbers of heterophils and lymphocytes showed no difference between lines. Other reports, however, have described differences among lines for lymphocyte number. In

turkeys, Bayyari *et al.* (1997) found lymphocyte numbers were lower in a line selected for heavier BW than in a line selected for increased egg production. Biozzi *et al.* (1971) reported higher lymphocyte numbers in spleens of mice selected for high than for low antibody response to SRBC. The lack of differences for heterophils, lymphocytes, and the ratio between them may be because under our husbandry there was an optimum level of stress where the ratio was about 0.5, in contrast to ratios of <0.2 and >0.8 which indicate low and high levels of stress, respectively (Gross and Siegel, 1993). Heterophils have been reported to phagocytose and digest *Escherichia coli*, *Bacillus megaterium*, and *Staphylococcus aureus* (Gross, 1962). Even though Line LL resisted these pathogens better than Line HH (Gross *et al.*, 1980), there was no line difference in number of heterophils in this experiment where the chickens were not infected with these organisms. This may be because alterations in numbers of heterophils that may be line specific, occur by infection or stressors (Gross and Siegel, 1983) and suggests that genetic variation may exist in the immunocompetence of heterophils. The modes of inheritance of spleen weight, before and after infection, in these selection lines were also different (Boa-Amponsem *et al.*, 1998).

Divergent selection for antibody response to SRBC did not result in a correlated response in hematocrits which may be an

example of stabilized selection. Chickens may benefit from intermediate hematocrit values, as shown by Shlosberg *et al.* (1996) who reported that selection for high hematocrits was associated with an increased incidence of ascites while mortality from causes other than ascites was greater when selection was for lower hematocrits. In this experiment, the lack of sexual dimorphism for hematocrits was consistent with those of Washburn and Siegel (1963) where, in BW selection lines, values for males were similar to those for females until sexual maturity. Also, Shlosberg *et al.* (1992) did not observe sexual dimorphism for hematocrits at 42 and 49 days of age in a fast-growing strain of broilers.

Although sex-linked and maternal effects may influence reciprocal effects, maternal but not sex-linked effects, should affect both sexes similarly. For hematocrits, analyses for sexes showed that sex-linkage was important. Also, recombination effects appeared to be important for hematocrits, as evidenced by measures unbiased by maternal heterosis as well as the significant scale test C. The effects, however, were probably a reflection of the sex-linkage for this trait.

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TABLE IV 1. Mean number of heterophils, lymphocytes, and heterophil/lymphocyte ratios at 30 days of age for cockerels from all mating combinations of lines HH¹ and LL¹

Progeny ²	Heterophils ³	Lymphocytes ³	H/L ratio ³
Parental lines and F ₁			
HHHH	20.7	39.3	0.56
HHLL	14.4	45.6	0.33
LLHH	19.9	40.1	0.51
LLLL	17.7	42.3	0.45
Backcross to HH parental line			
HHHL	19.8	40.2	0.53
HHLH	18.6	41.4	0.47
HLHH	18.5	41.5	0.47
LHHH	18.3	41.7	0.47
Backcross to LL parental line			
LLHL	20.2	39.8	0.52
LLLH	17.5	42.5	0.45
HLLL	18.4	41.6	0.45
LHLL	17.3	42.7	0.42
F ₂			
HLHL	20.2	41.6	0.47
HLLH	17.5	41.1	0.47
LHHL	18.4	45.6	0.34
LHLH	17.3	43.0	0.41
Pooled SEM	0.4	0.4	0.2

¹ HH and LL were selected for high and low antibody response to SRBC, respectively.

² The first two letters designate the sire and the second two letters the dam population for the mating combination.

³ Genetic contrasts are shown in Table 2.

TABLE IV 2 Genetic effects (in %) on heterophils, lymphocytes, and heterophil/lymphocyte ratios at 30 days of age for males according to contrasts involving various mating combinations of lines HH¹ and LL¹

Contrast	Heterophils	Lymphocytes	H/L ratios
Parental lines (P)			
HH-LL	17	7	25
Reciprocal effects			
HL-LH	-28 *	14 *	-36 †
Heterosis			
HL-(HH+LL)/2	-25 *	12 *	35 *
LH-(HH+LL)/2	4	-2	1
(HL+LH)-(HH+LL)	-11	5	-17
Maternal heterosis ²			
HHF ₁ -F ₁ HH	4	-2	8
LLF ₁ -F ₁ LL	4	2	11
$(4\overline{BC} - 2\overline{F}_2 - \overline{F}_1 - P)/20$	5	2	6
Recombination			
$2(\overline{F}_2 - \overline{F}_1)0$	0	0	1
$4(\overline{F}_2 - \overline{BC})0$	8	3	-1

¹ HH and LL were selected for high and low antibody response to SRBC, respectively.

² HHF₁-F₁HH = (HHHL+HHLH)-(HLHH+LHHH).
LLF₁-F₁LL = (LLHL+LLLH)-(HLLL+LHLL).

† $P < 0.06$; * $P \leq 0.05$.

TABLE IV 3. Mean hematocrits (%) at 42 days of age by sex and with sexes combined for chicks from all mating combinations of lines HH¹ and LL¹

Progeny ²	Male	Female	Sexes pooled ³
Parental lines and F ₁			
HHHH	35.9	36.2	36.0
HHLL	39.2	34.8	37.0
LLHH	34.3	35.3	34.8
LLLL	36.5	36.8	36.6
Backcross to HH parental line			
HHHL	35.2	36.2	35.7
HHLH	35.7	35.7	35.7
HLHH	36.2	36.0	36.1
LHHH	35.0	35.1	35.0
Backcross to LL parental line			
LLHL	35.2	35.0	35.1
LLLH	38.1	34.9	36.5
HLLL	34.0	34.1	34.0
LHLL	36.6	35.8	36.2
F ₂			
HLHL	33.3	34.0	33.6
HLLH	32.9	33.0	33.0
LHHL	34.0	34.4	34.2
LHLH	36.2	35.3	35.8
Pooled SEM	0.2	0.2	0.2

¹ HH and LL were selected for high and low antibody response to SRBC, respectively.

² The first two letters designate the sire and the second two letters the dam population for the mating combination.

³ Genetic contrasts are shown in Table 4.

TABLE IV 4. Genetic effects (in %) for hematocrits at 42 days of age by sex and sexes combined according to contrasts involving various mating combinations of lines HH¹ and LL¹

Contrast	Male	Female	Sexes pooled
Parental lines (P)			
HH-LL	-2	-2	-2
Reciprocal effects			
HL-LH	14 **	-1	6 **
Heterosis			
HL-(HH+LL)/2	8 **	-5	2
LH-(HH+LL)/2	-5	-3	-4
(HL+LH)-(HH+LL)	2	-4	-1
Maternal heterosis ²			
HHF ₁ -F ₁ HH	-1	1	<1
LLF ₁ -F ₁ LL	4	0	2
$(4\overline{BC} - 2\overline{F}_2 - \overline{F}_1 - P)/20$	1	1	1
Recombination			
$2(\overline{F}_2 - \overline{F}_1)0$	-7 **	-2	-5 **
$4(\overline{F}_2 - \overline{BC})0$	-5 **	-3 *	-4 **

¹ HH and LL were selected for high and low antibody response to SRBC, respectively.

² HHF₁-F₁HH = (HHHL+HHLH)-(HLHH+LHHH).
LLF₁-F₁LL = (LLHL+LLLH)-(HLLL+LHLL).

* $P \leq 0.05$; ** $P \leq 0.01$.

Paper V

Developmental Stability in Different Genetic Stocks of White Rock
Chickens

Abstract

Asymmetries were determined for several bilateral traits in females from a line of chickens selected for 39 generations for low 56-day body weight (LWS) and in a subline of LWS where selection had been relaxed for four generations (LWR). Because of reduced food intake under *ad libitum* feeding, some LWS females do not commence egg production, a condition that can be overcome by relaxing selection for a generation or two. Bilateral traits, measured at 240 days of age in LWS nonlayers, LWS layers, and LWR layers, were shank length and diameter, distance between the auditory canal and the posterior junction of the upper and lower mandible, and weight and length of the first primary wing feather. Other traits measured were body weights at 56, 168, and 240 days of age and age at first egg. Fluctuating asymmetry, a good overall measure of developmental stability, was lower in the relaxed than selected line. Means of relative asymmetries were also lower for LWR females than LWS layers and nonlayers which were similar.

INTRODUCTION

Fluctuating asymmetry (FA) occurs when an individual is unable to undergo identical development on both sides of a bilaterally symmetrical trait. FA has been studied in a variety of organisms and appears to have promise as a tool in studies of evolution, conservation biology, and animal breeding (Moller *et al.* 1995; Palmer 1996; Palmer and Strobeck 1986; Parsons 1992; van Valen 1962).

As a developmental stability measure of environmental and genetic stressors, FA reflects small random deviations from symmetry in bilateral characters with a normal distribution and a zero mean. Considerable research has been conducted on environmental stressors in poultry (e.g., Ben-Nathan *et al.*, 1976; Freeman 1985; Garren and Shaffner, 1956; Siegel 1995; Wolford and Ringer, 1962; Zulkifli and Siegel 1995). Although Lerner (1954) in experiments with chickens pointed out that selection for specific traits disrupted homeostasis, information on genetic stressors in poultry remains quite limited. Genetic stressors include loss of genetic variation from inbreeding or from bottlenecks of founders, hybridization, incorporation of a new mutant or major gene, and directional selection (Leary and Allendorf, 1989; Moller *et al.*, 1995; Parsons, 1990).

Intense directional selection has been hypothesized to increase the overall level of developmental instability as measured by FA because it directly affects alleles that control developmental homeostasis and against genetic modifiers that control development of extreme phenotypes (Moller *et al.*, 1995).

Dunnington and Siegel (1996) reported negative correlated responses in reproductive capabilities of chickens selected for 38 generations for low 56-day body weight. As responses in the selected trait progressed, noticeable alterations occurred in the proportion of individuals that survived during the early

posthatch period (Noble *et al.* 1993), and of the survivors, in the proportion that achieved sexual maturity (Siegel and Dunnington 1987). Those chicks that died did so because they simply did not eat. Since generation 25, from 25% to 50% of the females failed to reach sexual maturity by 275 days of age (Dunnington and Siegel 1996). Those which did not mature when provided food *ad libitum* could be brought into egg production by force-feeding (Zelenka *et al.* 1988). Also after only one or two generations of relaxed selection essentially all pullets would enter lay when fed *ad libitum* (Liu *et al.* 1995) suggesting enhanced developmental stability. To evaluate this, we report in this paper on asymmetry among pullets from the selected line that had either commenced or not commenced egg production and for pullets in a subline where selection had been relaxed.

Materials And Methods

Genetic Stocks and Husbandry

The chickens used in this experiment were from generation S₃₉ of a line mass selected for low 56-day body weight (LWS) and a subline of LWS where selection had been relaxed (LWR) for 4 generations. The base population for the selected line consisted of crosses of 7 inbred lines of White Plymouth Rocks (Siegel 1962). The numbers of sires and dams selected to produce the LWS line were 8 and 48 respectively through the S₄ generation, 12 and 48 from the S₅ to S₂₅ generation, and 14 and 56 from the S₂₆ generation onward (Dunnington and Siegel 1996).

In generation 35, random samples from the LWS line were taken before choosing parents for reproducing the line and selection was relaxed to form line LWR. This line has been maintained as a closed population by artificially inseminating 30 to 35 females with pooled semen from 10 to 15 males in each of 4 generations.

For this experiment chicks from both lines were hatched on

the same day (March 5, 1996) from age-contemporary parents. Upon removal from the hatcher chicks were wingbanded, vaccinated for Marek's disease and placed in floor pens. At 56 days of age sexes were separated and the females were transferred to floor pens in another building where they remained until 140 days of age. They were then randomly assigned to individual cages in a light controlled room where they remained until the end of the experiment. Lighting to 56 days of age was artificial and continuous. From 56 to 140 days females were exposed to natural daylength. Thereafter, the photoperiod was from 0600 to 2000 hours. Water and feed were always available. Crude protein (%) and metabolizable energy (kcal/kg) of diets were 20.0 and 2685 to day 56, 14.0 and 2827 from day 56 to 140, and 16.1 and 2572 from day 140 onward.

Traits Measured

Body weights were obtained at 56, 168, and 240 days of age, and age at first egg was recorded for each individual that commenced egg production by 245 days of age. All chickens were considered healthy. At 240 days of age, data were obtained for several bilateral characters. These were length (.1mm) of the metatarsus (shank), diameter (.1mm) of the shank perpendicular to the spur, and distance (.1mm) between the auditory canal and the posterior junction of upper and lower mandible (face length). The same measurer and holder made all measurements. After removal, the length (.1mm) and weight (.01g) of the left and right first primary wing feather were obtained. When blood was present in the quill of a feather, the hen was classified as in molt and feather length and weight were not recorded. FA was defined as mean zero with normal distribution. Directional asymmetry (DA) was defined as mean not zero with normal distribution. Antisymmetry (AS) was defined as mean zero with a distribution that was not normal.

Statistical Analyses

Analyses of variance (SAS Institute 1985) were conducted for all measurements using the completely randomized model: $Y_{ij} = u + g_i + e_{ij}$, where $i = 1, 2, 3$ groups (LWS layer, LWS nonlayer, LWR layer) and $j = 1, 2 \dots n$ individuals. All LWR females had commenced lay by 245 days of age. When means were significantly different at $P < .05$, multiple means were separated by Duncan's test. Asymmetries were tested by Levene's test (Palmer 1996). Body weights were transformed to common logarithms prior to analysis. Relative asymmetries ($|L-R| / [(L+R) / 2] \times 100$) expressed as a ratio were transformed to arc sine square roots prior to analysis. Each signed (+ or -) bilateral asymmetry (L-R) was tested for normality with mean zero by the Shapiro-Wilk statistic W and by one-sample t -tests (SAS Institute 1985). The mean of the relative asymmetries of the different characters was calculated as an overall measure of relative asymmetry. Product-moment correlations between bilateral traits $(\text{left} + \text{right}) / 2$ and relative asymmetry with body weights at various ages and age at first egg were calculated within groups.

Results

Effects of selection

In line LWS 58% (25/43) of the females had commenced lay by 245 days of age. The mean age of 207 days at sexual maturity of this group was not different from the mean of 200 days in line LWR where all 33 females had entered lay (Table 1). Body weights of LWS nonlayers were consistently lower than those of LWS layers at 56, 168, and 240 days of age. Between layers, body weights were lower at 56 and 168 days but not at 240 days for line LWS than LWR. Shank lengths of LWS nonlayers were shorter than those of LWS layers which did not differ from LWR layers. Shank diameters differed among groups with ranking in descending order being greatest for LWR layers > LWS layers > LWS nonlayers. Face length as well as feather weight and feather length was similar

for the three groups. There were, however, 5 LWS nonlayers with molted feathers and 1 LWS layer in molt. No LWR pullets were in molt.

Normality of bilateral traits

Bilateral differences for shank length and shank diameter had a normal distribution, zero mean, and exhibited FA (Table 2).

Face length also showed FA in line LWR and for LWS nonlayers, with DA in LWS layers where the distribution was normal but the mean was not zero. Feather weight and feather length in LWS nonlayers exhibited FA. LWS layers exhibited DA whereas for LWR females there was AS with a mean of zero and a distribution that was not normal.

Effects of selection and relaxed selection on the level of asymmetry

Relative asymmetry was similar among the three groups for shank length, shank diameter, and face length (Table 3). Values for feather weights and lengths were higher for both LWS groups than for LWR. The overall mean of asymmetries was higher for LWS layers and nonlayers than for LWR females.

Effects of selection on proportional development

Correlations of bilateral traits with body weight and age at first egg calculated within groups are presented in Table 4. There were highly significant positive correlations of shank length at 240 days of age with body weight at 56, 168, and 240 for LWR pullets. Although these correlations were positive, they were lower at each age for both LWS layers and nonlayers. Correlations of shank length with age at first egg showed a similar negative pattern for both the selected and relaxed lines, with that for the relaxed line being highly significant. For shank diameter, the pattern among groups was a highly significant positive correlation with 56-day body weight for LWR but not LWS layers and nonlayers. At 168 and 240 days, the correlations were

highly significant and positive for all three groups. The negative correlation for shank diameter and age at first egg was not significant for LWS pullets and was highly significant for LWR pullets. Although correlations for face length with body weights were positive and with age at first egg negative, none was significant.

Of the 27 correlations (data not shown) between relative asymmetry of shank length, shank diameter, and face length with body weight at 56, 168, and 240 days of age, only the correlation of .41 for shank length and 56-day body weight for LWS layers was significant ($P \leq .05$) and it may be due to chance. Of the 6 correlations between relative asymmetry of these bilateral traits with age at first egg, 2 were positive, 4 were negative, and none was significant.

Discussion

Developmental priorities

For any organism there will be a developmental order which will influence prioritization of resources. Juveniles preferentially allocate resources to skeletal and muscular growth and development and adults to reproduction. These early associations among traits may also be noted at older ages when resources allotted to them are primarily for maintenance. Thus, differences among groups in shank length and shank diameter at 240 days of age corresponded to the pattern observed for body weight at all ages. That is, lower body weights were associated with smaller shanks.

Face length and weight and length of the first primary wing feather were similar for all groups. For the former, it is reasonable that the general area of the head which includes the brain should have priority development with little change (Oyan and Anker-Nilssen 1996). As far as similarity among groups for the primary wing feather at 240 days of age, the juvenile molt

and growth occur after the age of 56 days when selection was made for body weight. Perhaps more importantly from a resource allocation point-of-view, resources allocated to feather development would be at a critical physiological margin essential for thermal stability with little room for variation in chickens (Dunnington and Siegel 1984).

Selection and different types of FAs

From the results reported in this paper, overall FA provided a good measure of developmental stability in these chickens. Feather weight, in particular, showed a relative asymmetry of 11.5% in selected line LWS pullets and only 4.5% when selection was relaxed in line LWR. Relative asymmetries for the other traits measured were in the 1% to 6% range. Although shank length and diameter exhibited FA in all groups, feather weight and length exhibited FA in LWS nonlayers, AS in LWR, and DA in LWS layers. Neither logarithmic, square root, arcsine, nor other transformations improved the fit to normality for size or asymmetry measures. The observation that not all traits were normally distributed was consistent with reports by Lacy and Horner (1996) and Thoday (1958), but not that of Moller *et al.* (1995). The former two reports involved the effects of genetics (selection and inbreeding) in *Drosophila* and mice, respectively. The report of Moller *et al.* (1995) involved specimens of Red Jungle fowl and commercial meat-type chickens which were crosses of selected lines.

Homeostasis and FA

In long-term single trait directional selection experiments, reduced and irregular responses may be directly or indirectly due to greater sensitivity to environmental factors which could be reflected by developmental error (Clayton *et al.* 1957; Lerner 1954; Moller *et al.* 1995; Parsons 1990; van Valen 1962). Increased FA implies disturbances in developmental homeostasis at

the molecular, chromosomal, and epigenetic levels (Palmer 1996; Parsons 1990). How much and when selection responses occur are less predictable in later than earlier generations of artificial selection. Hence, overall FA and homeostatic thresholds could aid in evaluating consequences of selection.

In the experiment reported here, attempts were made to minimize environmental changes by reproducing lines at the same time each year (the first Tuesday in March) and feeding the same dietary formulations throughout all generations. No environment trends were noted for 56-day body weight in a control population during this experiment (Liu *et al.* 1994). Therefore, the sharp escalation of FA in line LWS may be due to genetic stress *per se* or genetic stress resulting from more sensitivity to environmental factors because selection may have been against genetic modifiers that influenced developmental homeostasis (Parsons 1990; Moller *et al.* 1995). Thirty-nine generations of selection have reduced mean 56-day body weight from 710 to 160 g.

Relaxing selection quickly reduced relative asymmetry and increased body weight. Because a proportion of the selected line (those having lowest body weights) did not enter lay, there is a reversal of selection due to reaching a selection limit (Siegel and Dunnington 1987). This suggests that selecting individuals with lower FA may provide a way to enhance recovery of homeostasis.

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Table V 1. Mean \pm SEM of traits for females that had either commenced (layer) or not commenced (nonlayer) egg production by 245 days of age

Trait	LWS ¹		LWR ²
	Nonlayer	Layer	Layer
Body weight (g) at age in days			
56	135 \pm 8 c	188 \pm 9 b	225 \pm 11 a
168	532 \pm 33 c	849 \pm 39 b	976 \pm 38 a
240	878 \pm 46 b	1241 \pm 26 a	1317 \pm 31 a
Age at first egg (days)	-	207 \pm 4 a	200 \pm 4 a
Shank ³ (mm)			
length:left	86.55 \pm .81 b	91.56 \pm .58 a	90.98 \pm .51 a
right	86.70 \pm .87 b	91.82 \pm .64 a	90.96 \pm .50 a
diameter:left	8.83 \pm .19 c	9.74 \pm .10 b	10.16 \pm .09 a
right	8.82 \pm .17 c	9.70 \pm .10 b	10.04 \pm .09 a
Distance (mm) between junction of upper and lower mandibles and auditory canal (face length) ³			
left	20.59 \pm .40 a	20.74 \pm .26 a	20.80 \pm .22 a
right	20.82 \pm .39 a	21.32 \pm .30 a	21.18 \pm .26 a
First primary wing feather weight (g) and length (cm) ³			
weight:left	.262 \pm .010a	.248 \pm .008a	.269 \pm .008a
right	.261 \pm .012a	.272 \pm .008a	.272 \pm .007a
length:left	17.50 \pm .20 a	17.46 \pm .16 a	17.87 \pm .14 a
right	17.51 \pm .22 a	17.76 \pm .15 a	17.89 \pm .14 a

a-c means in a row with no common letter differ significantly ($P \leq .05$).

¹ LWS - selected 39 generations for low 56-day body weight.

² LWR - subline of LWS where selection was relaxed for 4 generations; all had entered egg production.

³ Measured at 240 days of age.

Table V 2. Mean \pm SEM of bilateral difference (left minus right) and type of asymmetry¹ for females that had either commenced (layer) or not commenced (nonlayer) egg production by 245 days of age

Trait ⁴	Nonlayer	LWS ²	Layer	LWR ³	Layer
Shank (mm)					
length	-.150 \pm .375 FA		-.264 \pm .250 FA		.021 \pm .261 FA
diameter	.011 \pm .082 FA		.048 \pm .094 FA		.127 \pm .066 FA
Distance (mm) between junction of upper and lower mandibles and auditory canal (face length)					
	-.2278 \pm .3235 FA		-.5800 \pm .2672 DA		-.3818 \pm .2481 FA
First primary wing feather weight (g) and length (cm)					
weight	.0015 \pm .0107 FA		-.0242 \pm .0065 DA		-.0029 \pm .0037 AS
length	-.0077 \pm .1689 FA		-.3042 \pm .1388 DA		-.0129 \pm .0771 AS

¹ FA - fluctuating asymmetry; DA - directional asymmetry; AS - antisymmetry.

² LWS - selected 39 generations for low 56-day body weight.

³ LWR - subline of LWS where selection was relaxed for 4 generations; all had entered egg production.

⁴ Measured at 240 days of age.

Table V 3. Relative asymmetry¹ for females that had either commenced (layer) or not commenced (nonlayer) egg production by 245 days of age

Trait ⁴	LWS ²		LWR ³	
	Nonlayer	Layer	Layer	Layer
Shank (mm)				
length	1.39± .27a	1.15± .15a	1.32± .17a	
diameter	2.90± .61a	3.88± .57a	3.14± .42a	
Distance (mm) between junction of upper and lower mandibles and auditory canal (face length)				
	5.42± .88a	5.38± .82a	5.21± .78a	
First primary wing feather weight (g) and length (cm)				
weight	11.78±2.21a	11.22±2.10a	4.46±1.21b	
length	2.40± .64a	3.03± .61a	1.39± .34b	
Mean ⁵	4.78± .63a	4.93± .52a	3.10± .35b	

a,b means in a row with no common letter differ significantly ($P \leq 0.05$).

¹ $(L-R / [(L+R) / 2]) \times 100$, where L is left and R is right.

² LWS - selected 39 generations for low 56-day body weight.

³ LWR - subline of LWS where selection was relaxed for 4 generations; all had entered egg production.

⁴ Measured at 240 days of age.

⁵ Average of the 5 relative asymmetry values.

Table V 4. Correlations of bilateral traits with body weight and age at first egg for females that had either commenced (layer) or not commenced (nonlayer) egg production by 245 days of age

Traits	LWS ¹		LWR ²
	Nonlayer	Layer	Layer
Shank length ³ (mm) with Body weight (g) at age in days			
56	.33	.19	.47 **
168	.46	.30	.64 **
240	.24	.52 **	.62 **
Age at first egg (days)	--	-.39	-.47 **
Shank diameter ³ (mm) with Body weight (g) at age in days			
56	.05	.18	.43 **
168	.88 **	.41 *	.68 **
240	.83 **	.56 **	.73 **
Age at first egg (days)	--	-.23	-.50 **
Distance (mm) between junction of upper and lower mandibles and auditory canal (face length) ³ with Body weight (g) at age in days			
56	.20	.30	.22
168	.45	.20	.32
240	.10	.13	.26
Age at first egg (days)	--	-.07	-.27

*, ** significant at P<.05 and P<.01, respectively.

¹ LWS - selected 39 generations for low 56-day body weight.

² LWR - subline of LWS where selection was relaxed for 4 generations; all had entered egg production.

³ Measured at 240 days of age. (left + right)/2.

Paper VI

COMPARISONS OF PARENTAL LINE BROILER BREEDERS AND CROSSES
AMONG THEM FOR WEIGHT OF BODY AND ORGANS AT HATCH

Abstract 1. Body, yolk sac, left and right shanks with toes, empty left and right ceca, left and right lungs, heart, and bursa weights were obtained for 50 chicks at hatch from each of five commercial broiler parental lines and three F₁ crosses involving.

2. Effects of stock and sex were inconsistent among mating combinations.

3. Correlations of yolk-free chick weight with shank weight was high, with heart and lung weights, intermediate, and with ceca and bursa weights low.

4. Heart/lung ratios of all F₁ crosses were greater than those for their respective parental lines.

5. Heterosis for most traits was different among populations.

6. Development as measured by % relative asymmetry was less stable in two sire parental lines than in their respective dam lines and F₁ crosses.

INTRODUCTION

In the poultry industry marketing changes are common and require continuous changes in the design and implementation of breeding programs. A delay of years between development of initial selected populations and production of saleable products, however, requires that poultry breeders predict future markets. Strategies for developing specific sire lines, dam lines, and crosses among them which differ in production traits (Moav, 1966 a,b,c; Moav and Hill, 1966; Moav and Moav, 1966) continue today (Ewart, 1993). Implementation of this breeding strategy has facilitated rapid genetic changes in broiler traits (Havenstein *et al.*, 1994).

Intense within-line selection with emphasis on specific traits can result in lines that may be very different, with complementary effects occurring in crosses among them. Intensive selection for rapid growth not only compromises reproductive ability and immunocompetence, but also contributes to skeletal and metabolic disorders as a consequence of a disruption of homeostasis (Siegel and Dunnington, 1997). Also, asymmetries of bilateral traits and developmental instabilities can be influenced by both genetic and nongenetic factors (Moller and Swaddle, 1997). Lacking are comparisons of the homeostasis of commercial sire and dam lines and their crosses which may be estimated from heterosis of various traits and by measuring

asymmetries of bilateral traits (Palmer and Strobeck, 1986; Parsons, 1992; Moller *et al.*, 1995; Palmer, 1996).

This paper reports on comparisons made at hatch for various traits from five commercial pure lines and three crosses involving them. Criteria for measuring developmental stability and overall quality were heterosis and asymmetries of bilateral traits. Traits included yolk-free body weight and representative components from the skeletal, circulatory, digestive, respiratory, and immune systems.

MATERIALS AND METHODS

Stocks and traits

The stocks used in this experiment included three sire lines (AA, C1C1, C2C2), two dam lines (BB, DD), and three F₁ crosses between the sire and dam lines (AB, C1D, C2D). In matings the sire line is shown first and the dam line second. Eggs from the eight stocks were shipped to Virginia Tech and incubated in one machine for a single hatch. Data were obtained from 50 chicks from each stock with a minimum number of 20 for each sex. At hatch, each chick was weighed and then killed by cervical dislocation. Weights (to nearest 0.01 g) were obtained for yolk sac, left and right shanks with toes, empty left and right ceca, left and right lungs, heart, and bursa. Sex was determined by macroscopic inspection of gonads.

Statistical analysis

Data were analyzed by analysis of variance with stock, sex, and the interaction between them as main effects. When differences among stocks were significant, means were separated by Duncan's multiple range test. When interactions of sex by stock were significant, comparisons between sexes were made within each stock and among stocks within each sex. Significance of heterosis was determined by the contrast of the F_1 with the average of its parental lines.

Three categories were defined for left minus right (L-R) bilateral differences. Definitions were mean zero and normal distribution for fluctuating asymmetry (FA), mean not zero and normal distribution for directional asymmetry (DA), and mean zero with a distribution that was not normal for antisymmetry (AS). Relative asymmetry (RA) was defined as the ratio of the absolute value of asymmetry (L-R) over bilateral trait weight: $RA = (|L-R| / [(L+R)/2]) \times 100$.

Prior to analysis RAs were transformed to arc sine square roots. Each signed (+ or-) bilateral asymmetry (L-R) was tested for normality with mean zero by Shapiro-Wilk statistic W (sample size <60) and by one-sample t-tests (SAS, 1985).

RESULTS

Preliminary analyses showed that results for most traits were specific for each mating combination. Therefore data are presented by mating combination *i.e.*, parental lines and the F_1

cross between them.

AA, AB, and BB matings

Means and percentage heterosis for traits from matings AA, AB, and BB are shown at Table 1. Sex by stock interactions were not significant for any of the traits measured. The only traits where sexual dimorphism was present were absolute left lung weight and lung weight relative to body weight. In both cases weights were heavier for males than females.

Body and absolute shank weights were lower for AA and AB than BB chicks for these matings. Yolk sacs of AB and BB chicks were heavier than those of AA chicks. Absolute weights of other organs were similar among matings.

Correlations of yolk-free body weight with other traits varied. Those between body weight and shank weight were highly significant correlations which ranged from 0.80 to 0.86. Correlations of body weight with bursa and with ceca weights, while positive, were not different from zero for AA and BB matings. For the AB cross, however, there were significant correlations of body weight with bursa (0.36) and body weight with ceca (0.31). Correlations of body weight with lung weight were highly significant for AA chicks (0.45) and significant for BB chicks (0.37). Heart with body weight correlations were highly significant being 0.45, 0.44, and 0.61 for AA, AB, and BB chicks, respectively.

Relative to body weight, yolk sacs of AA chicks were lighter than those of AB and BB chicks, while ceca and bursa weights were heavier for AA than AB and BB chicks. Shanks of BB chicks were heavier than those of AA and AB chicks.

Heterosis, both on an absolute and a relative-to-body-weight basis was low with left and right shank weights being the only traits showing significant heterosis.

C1C1, C1D, DD matings

Means and percentage heterosis of traits where there was no interaction of sex by stock for matings C1C1, C1D, and DD are presented in Table 2. Absolute weights of ceca and of ceca, shank, and bursa weights relative to body weight did not differ among matings, and heterosis for these traits was low. Left and right lungs of males were heavier than those of females. In both sexes, lungs of C1C1 chicks were heavier than those of DD chicks which were heavier than those for C1D chicks. This same pattern was observed for absolute weights of the yolk sac and left and right shanks, as well as yolk sac weight relative to body weight. Heterosis for all of these traits was negative and significant with the exception of right lung weight for males. Absolute weight of the bursa was also greater for C1C1 than DD and C1D chicks which did not differ from each other. The overall correlation of body weight with shank weight was 0.80 with a range from 0.77 for C1C1 chicks to 0.82 for C1D and DD chicks.

Means and heterosis of traits where sex by stock interactions for these matings were significant are presented at Table 3. C1C1 males were heavier than C1D and DD males, while C1C1 females were heavier than DD females which, in turn, were heavier than the F₁ cross. Although there was no sexual dimorphism for body weight of C1D and DD chicks, C1C1 males were heavier than C1C1 females. Heterosis for body weight was negative and highly significant for both sexes.

On an absolute basis, hearts of C1C1 chicks were heavier than those of C1D and DD chicks which did not differ. The interaction resulted from sexual dimorphism (male>female) for DD chicks and no difference between sexes for C1C1 and C1D chicks. In contrast, relative to body weight, hearts of C1C1 and C1D chicks were heavier than those of DD chicks. The pattern for sexual dimorphism, however, was the same as that observed for absolute heart weights, with the only significant heterosis being that for absolute heart weight of females.

Although weights of lungs relative to body weight were similar for males of the three stocks, heterosis was negative and significant. Relative to body weight, lungs were heavier for C1C1 females than for the other two matings with heterosis being significant and negative. Within each mating, lung weights relative to body weight were heavier for C1D and DD males than females while there was no sexual dimorphism for C1C1 chicks,

causing the significant sex by stock interaction. Ratios of heart to lung weights for males were greatest for C1D chicks and least for DD chicks with C1C1 chicks intermediate. For females, ratios were lower for C1C1 chicks than for C1D and DD chicks. Sexual dimorphism was noted only in DD chicks with values higher for females than males resulting in the sex by stock interaction. Although heterosis was positive for both sexes, it was significant only for males.

Correlations of body weight with ceca and with bursa weight for C1C1, C1D, and DD matings were low and not different from zero. For body and shank weights, correlations were highly significant for all matings ranging from 0.77 to 0.82. Although of a lower magnitude than those with shank, correlations between body and lung weights were significant for C1C1 (0.32) and DD (0.35) chicks and highly significant for C1D (0.51) chicks. Of a similar magnitude were correlations between body and heart weights which were significant for C1C1 (0.30) and highly significant for C1D (0.40) and DD (0.38) chicks.

C2C2, C2D, and DD matings

Means, SEM, and % heterosis for matings C2C2, C2D, and DD are presented in Table 4 for traits when the sex by stock interactions were not significant, and in Table 5 when the interaction was significant. There were no differences among stocks for absolute weights of left shank, left and right ceca,

and right lung weight as well as shank ,ceca, and bursa weight relative to body weight. Body weights of males, and yolk sac and heart weights for both males and females were lower for DD than for C2C2 and C2D chicks. Body weights of females and bursa weights were lower for DD than C2C2 chicks, with C2D chicks intermediate and not different from either parental line. Right shank weight was heavier for C2C2 than C2D and DD chicks.

Left lung weights of C2D males and females were lighter than those of their parental lines. Relative to body weight, lungs were heavier for DD chicks of both sexes than those of chicks from the other two stocks. In contrast, heart weights relative to body weight were heaviest in C2D and lightest in DD chicks, with those for C2C2 chicks intermediate and different from the other two. Yolk sac weights relative to body weight were lighter in DD than in C2D chicks with weights for C2C2 chicks intermediate and not different from chicks of the other two matings. Heterosis was positive and significant for absolute yolk sac and heart weights of males, as well as their weights relative to body weight for yolk sac, lungs of males, and hearts in both sexes.

Correlations between body and organ weights were stock specific. For the C2C2 mating the correlation of body weight with shank weight was highly significant (0.77) while correlations of body weight with ceca (0.06), lung (0.03), heart (0.23) and bursa (0.19) were not significant. The association between body weight

and organ weights was similar for C2D and DD matings. Correlations of body weight with shank, ceca, lung, heart, and bursa were 0.76, 0.17, 0.48, 0.41, and 0.10, respectively, for C2D chicks. Respective correlations for DD chicks were 0.82, 0.12, 0.35, 0.38, and 0.25. All were significant or highly significant except those involving ceca and bursa.

The sex by stock interaction for ratio of heart-to-lung weight was significant (Table 5) because in males ratios were highest for C2D and lowest for DD matings with the value for C2C2 intermediate and different from the other two stocks. In contrast, the ratio was lower for DD than C2C2 and C2D females. Comparisons of sexes within matings revealed higher ratios for C2C2 and DD males than females with no difference between sexes for C2D chicks. Heterosis for the lung to heart ratio was positive for males and females with the former being highly significant.

Bilateral asymmetry

Asymmetry types and heterosis for % RA were generally specific for each mating combination (Table 6). For AA, AB and BB matings, shank weight exhibited AS in the parental lines and FA in the F₁ cross. Cecal weights were AS for both parental lines and the F₁. There was AS for lungs in the BB parental line and the F₁ with FA for the AA parental line. Percentage RA, while low for shank weight, exhibited significant heterosis. Although %

RAs were higher for ceca and lung than for shank, heterosis was not significant.

Asymmetries of shank weight for matings C1C1, C1D, and DD were FA for C1C1 and C1D chicks and DA for DD chicks. Cecal weight exhibited AS for all matings. For lung, there was FA for C1D and DD chicks and DA for C1C1. Heterosis of % RA for shank weight was negative and significant. Percentage RA did not differ among matings for ceca, while for lungs parental line C1C1 had a higher % RA than matings C1D and DD. Heterosis of % RA was positive for ceca and negative for lung weight with neither being significant.

Shank weights of C2C2 and C2D chicks exhibited AS while DD chicks showed DA. Ceca and lung weights were AS and FA, respectively, in all matings. Percentage RA was much lower for shank than for ceca and lung weights. Heterosis for % RAs for shank and ceca were negative and positive, respectively, with neither being significant. For lungs, % RA was greater for C2C2 than C2D and DD chicks with heterosis being negative and significant.

DISCUSSION

Growth of specific organs may occur simultaneously or sequentially. Although developmental control is specific for traits rather than for the whole organism, allomorphic growth of each trait is an orderly process. Temporal development of organs

should be recognized when measuring asymmetries of several traits at a specific chronological age (Kimball *et al.*, 1997). In our experiment, asymmetries and heterosis of traits involved comparisons among genetic stocks at the same chronological age and physiological stage (i.e., at hatch) for weights of components representing the skeletal (shanks), circulatory (heart), digestive (ceca), respiratory (lungs), and immune systems (bursa). The sire and dam parental lines in this experiment had undergone intense selection for growth and associated traits with F₁ crosses among them based on economic considerations of combining ability. That results were mating combination specific was not surprising. Yolk-free body weight at hatch was inconsistent across matings. There were cases of no sexual dimorphism, males being heavier than females, and females being heavier than males. This lack of generality of sex dimorphism for body weight at hatch was consistent with that reported by Burke (1992). Lower percentage yolk sac weight relative to body weight corresponded to lower body weight stocks which agreed with the report of Yang and Siegel (1997) in lines of White Leghorns divergently selected for sheep red blood cells.

Heterosis for yolk-free body and shank weights was variable reflecting the trait and population specific characteristics of heterosis. Neither bursa nor ceca weights exhibited heterosis. Heterosis for lung and heart weights both on an absolute and

relative to body weight basis was considerable and significant for some matings with the direction of heterosis being negative for lung and positive for heart. The relevance of this may have practical implications to metabolic disorders in broilers.

Lungs and hearts, critical organs for the incidence of ascites (Julian, 1993), exhibited sexual dimorphism with an interaction of sex by stock. Specifically, lung size may be especially important because, due to skeletal limitations, it cannot be enlarged proportionally with increasing rapid growth that requires higher oxygen exchange. In all cases heart/lung ratios were higher in F_1 crosses than in their respective parental lines suggesting a poorer anatomical structure for resistance to ascites. For the bilateral traits measured, regardless of mating combinations, % RA was considerably less for shank than ceca and lung weights which were similar. Heterosis for % RA of shank varied in significance among mating combination, while for ceca it was consistently positive and for lung consistently negative. Lines C1C1 and C2C2 had significantly higher RAs for lung weights than dam line DD and F_1 progeny suggesting poor homeostasis in response to genetic stressor(s) which may reflect higher selection intensities in sire lines.

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Table VI 1. Mean, SEM, and % heterosis for traits at hatch for mating combinations AA, AB, and BB

Trait ¹	Mating combinations			Pooled	
	AA	AB	BB	SEM	Heterosis
Absolute wt (g)					
Body ²	37.8 b	38.4 b	40.7 a	0.3	-2
Yolk sac	3.9 b	4.9 a	5.2 a	0.1	7
Shank, L ³	1.11 b	1.14 b	1.25 a	0.01	-3 *
R ³	1.11 b	1.13 b	1.25 a	0.01	-4 *
Ceca, L	0.067	0.060	0.065	0.002	-9
R	0.067	0.060	0.065	0.002	-6
Lung, L M	0.186	0.183	0.195	0.004	-4
F	0.169	0.167	0.185	0.004	-6
R	0.173	0.179	0.181	0.003	-3
Heart	0.33	0.33	0.34	0.04	-2
Bursa	0.050	0.049	0.043	0.002	5
(Heart/lung) x 100	0.89	1.02	0.91	0.01	2
Relative wt = (organ/body) x 100					
Yolk sac	10.38 b	12.78 a	12.69 a	0.37	7
Shank	5.88 b	5.91 b	6.15 a	0.03	-2
Ceca	0.35 a	0.31 b	0.32 b	0.01	-7
Lung, M	0.98	0.94	0.94	0.02	-2
F	0.88	0.90	0.84	0.02	2
Heart	0.87	0.85	0.83	0.01	0
Bursa	0.127a	0.110 b	0.112 b	0.005	8

¹ Sexes pooled except when significant (P < 0.05) difference between _ and _.

² Live wt minus yolk sac wt.

³ L = left; R = right.

Within a row means with different letters differ (P < 0.05).

P < 0.05.

M=male F=Female

Table VI 2. Mean, SEM, and % heterosis for traits at hatch for mating combinations C1C1, C1D, and DD

Trait ^{1,2}	Mating combinations			Pooled SEM	Heterosis
	C1C1	C1D	DD		
Absolute wt (g)					
Yolk sac	4.8 a	2.7 c	3.9 b	0.1	-38**
Shank, L ³	1.20 a	1.04 c	1.11 b	0.01	-10**
R ³	1.21 a	1.03 c	1.10 b	0.01	-11**
Ceca, L	0.066	0.058	0.059	0.01	-7
R	0.063	0.060	0.058	0.01	-1
Lung, L M	0.210a	0.163 c	0.183 b	0.004	-17**
F	0.206a	0.147 c	0.170 b	0.005	-22**
R M	0.195a	0.165 c	0.182 b	0.003	-12
F	0.189a	0.150 c	0.163 b	0.004	-15*
Bursa	0.047a	0.041 b	0.039 b	0.001	-5
Relative wt = (organ/body) x 100					
Yolk sac	11.72 a	7.49 c	10.30 b	0.3	-32**
Shank	5.84	5.78	5.76	0.02	0
Ceca	0.31	0.33	0.30	0.01	8
Bursa	0.115	0.114	0.102	0.003	5

¹ Sexes pooled except when significant (P < 0.05) difference between Male and Female.

² Mating combination by sex interactions were significant (P < 0.05) for body and heart wt, heart/lung ratio, and lung and heart wt. relative to body wt (see Table 3).

³ L = left; R = right.

Within a row means with different letters differ (P < 0.05).

P < 0.05; ** P < 0.01.

M=Male F=Female

Table VI 3. Mean, SEM, and % heterosis for traits at hatch for mating combinations C1C1, C1D, and DD where the mating combinations by sex interactions were significant

Trait	Mating combinations			Pooled	
	C1C1	C1D	DD	SEM	Heterosis
Absolute wt (g)					
Body ¹ , M	42.0 a	36.1 b	37.6 b	0.4	-9 **
	*	NS	NS		
F	40.4 a	35.4 c	38.7 b	0.4	-10 **
Heart, M	0.35 a	0.29 b	0.27 b	0.01	-6
	NS	NS	**		
F	0.33 a	0.29 b	0.31 ab	0.01	-9 *
(Heart/lung) x 100					
M	0.87 b	0.90 a	0.74 c	0.02	12 **
	NS	NS	**		
F	0.85 b	0.98 a	0.97 a	0.01	7
Relative wt = (organ/body) x 100					
Lung, M	0.97	0.91	0.98	0.01	-7 *
	NS	*	**		
F	0.98 a	0.84 b	0.85 b	0.02	-8 *
Heart, M	0.83 a	0.81 a	0.71 b	0.01	5
	NS	NS	**		
F	0.82 a	0.81 a	0.79 b	0.01	1

¹ Live wt minus yolk sac wt.

Within a row means with different letters differ (P < 0.05).

P < 0.05; ** P < 0.01; NS P > 0.05.

M=Male F=Female

Table VI 4. Mean, SEM, and % heterosis for traits at hatch for mating combinations C2C2, C2D, and DD

Trait ^{1,2}	Mating combinations						Pooled	
	C2C2		C2D		DD		SEM	Heterosis
Absolute wt (g)								
Body ³	M	39.2 a	39.2 a	37.6 b	0.3	2		
	F	40.9 a	39.1 ab	38.7 b	0.3	-2		
Yolk sac		4.8 a	5.0 a	3.9 b	0.1	15 *		
Shank, L ⁴		1.14	1.11	1.11	0.01	-1		
	R ⁴	1.14 a	1.10 b	1.10 b	0.01	-2		
Ceca, L		0.058	0.055	0.059	0.001	-6		
	R	0.057	0.059	0.058	0.002	3		
Lung, L M		0.183a	0.170 b	0.183 a	0.003	-7		
	F	0.164a	0.154 b	0.170 a	0.004	-8		
	R M	0.071	0.175	0.182	0.004	-1		
	F	0.062	0.156	0.163	0.004	-2		
Heart, M		0.32 a	0.35 a	0.27 b	0.01	19 **		
	F	0.35 a	0.34 a	0.31 b	0.01	8		
Bursa		0.048a	0.045 ab	0.039 b	0.001	3		
Relative wt = (organ/body) x 100								
Yolk sac		11.83 ab	12.85 a	10.30 b	0.30	6 *		
Shank		5.68	5.65	5.76	0.03	-1		
Ceca		0.29	0.29	0.30	0.01	-2		
Lung, M		0.90 b	0.88 b	0.98 a	0.02	-6 *		
	F	0.80 b	0.79 b	0.85 a	0.02	-4		
Heart, M		0.83 b	0.88 a	0.71 c	0.01	12 **		
	F	0.85 b	0.89 a	0.79 c	0.01	8 *		
Bursa		0.119	0.116	0.102	0.003	5		

¹ Sexes pooled except when significant (P < 0.05) difference between _ and _.

² Mating combinations by sex interaction was significant (P < 0.05) for heart/lung ratio (see Table 5).

³ Live wt minus yolk sac wt.

⁴ L = left; R = right.

Within a row means with different letters differ (P < 0.05).

P < 0.05; ** P < 0.01.

M=Male F=Female

Table VI 5. Mean, SEM, and % heterosis for heart/lung ratio at hatch for mating combinations C2C2, C2D, and DD where the mating combinations by sex interaction was significant

Sex	Mating combinations			Pooled SEM	Heterosis
	C2C2	C2D	DD		
M	0.94 b	1.03 a	0.74 c	0.02	23 **
F	1.09 a	1.11 a	0.97 b	0.01	14

Within a row means with different letters differ (P < 0.05).
 ** P < 0.01; NS P > 0.05.
 M=Male F=Female

Table VI 6. Asymmetry types¹, % relative asymmetry (RA)², and % heterosis of RA for shank, ceca, and lung weights at hatch by mating combinations

Mating combinations	Asymmetry type	Shank		Asymmetry type	Ceca		Asymmetry type	Lung	
		% RA	% heterosis		% RA	% heterosis		% RA	% heterosis
AA	AS	1.5		AS	15.7		FA	15.3	
AB	FA	2.0	29 *	AS	20.5	18 NS	AS	11.1	-16 NS
BB	AS	1.6		AS	18.8		AS	11.1	
C1C1	FA	1.8		AS	15.9		DA	15.8 a	
C1D	FA	1.0	-35 *	AS	19.6	15 NS	FA	11.4 b	-17 NS
DD	DA	1.3		AS	18.3		FA	11.7 b	
C2C2	AS	1.6		AS	18.9		FA	20.3 a	
C2D	AS	1.2	-17 NS	AS	20.8	12 NS	FA	10.9 b	-32 *
DD	DA	1.3		AS	18.3		FA	11.7 b	

1 AS = antisymmetry (mean zero, not normal distribution);

FA = fluctuating asymmetry (mean zero, normal distribution);

DA = directional asymmetry (mean not zero, normal distribution).

2 $RA = (|L-R| / [(L + R) / 2]) \times 100$.

Mean % RA within a mating combination with different letters differ ($P < 0.05$).

* $P < 0.05$; NS $P > 0.05$.

GENERAL SYNTHESIS

During the last half of this century, the poultry industry has developed into very sophisticated complexes for producing meat and eggs. Breeding programs consist of crossing specialized sire and dam lines in various combinations to produce stocks that differ in their complement of production traits. Intense within line selection is an essential component of the strategies used in breeding poultry for either meat or eggs. Extremely intensive selection for rapid growth to market weight or high intensity and persistency of egg production has increased skeletal and metabolic disorders such as leg disorders, cage fatigue, ascites, and sudden death syndrome. Thus, breeders face the continuous challenge of paying attention to mechanisms involved with homeostasis.

Homeostasis, indicating the tendency of the internal environment of an organism to be maintained as a constant, can be broken down into two elements: canalization and developmental stability. Canalization is regarded as a property of the genome that tends to ensure that a developmental pathway remains on its intended trajectory, whereas developmental stability is the phenotypic outcome of that genetic property.

Ways in which developmental stability has been assessed include monitoring the frequency of deviant phenotypes and measuring variation in trait size. Indices constructed from comparisons of bilateral in the same individual provide estimates of developmental stability that control for genetic and environmental differences. Fluctuating asymmetry, a minor deviation from otherwise perfect symmetry, is most likely to reflect for genetic and environmental differences as the two sides of a bilateral trait grow simultaneously. This concurrency minimizes the probability that environmental conditions are different during the development of each element.

In this dissertation (Papers I, II, III, and IV), fluctuating asymmetry of bilateral traits was investigated during different stages of the life cycle in White Leghorn chickens selected for high or low antibody response to SRBC antigen(s). Other measurement criteria used in the research included heterosis of several traits, the ratio of heterophils to lymphocytes, and hematocrits. In Paper V of this dissertation, asymmetries of bilateral traits were measured at 240 days of age in females from a line of White Plymouth Rock chickens selected for low 56-day body weight (LWS) and in a subline of LWS where selection had been relaxed for four generations. In Paper VI, relative asymmetry (an index of relative differences among several bilateral traits) was measured at hatch in commercial meat-type chickens. The experiment involved three sire lines, two dam lines, and three crosses between them.

Results obtained in Paper I showed that with one exception (at hatch)lungs exhibited fluctuating asymmetry. The degree of fluctuating, but not relative, asymmetry varied with age. Growth patterns of the lungs and heart differed with lung weights heavier in line with heavier body weight while there were no differences between lines for heart weights. Relative to body weight, both heart and lung weights declined with age, with hearts larger in the lower versus heavier body weight line to five days after hatch with no differences thereafter. Lungs, however, were similar in both lines until five days after hatch, after which they were larger in the line with heavier body weights. Bilateral traits measured in Paper II were 39-day length and weight of the first primary wing feather, 39-and 49-day shank length, ceca length and 56-day weights of shank, and lung. Heterosis was positive for organ sizes and negative for degree of bilateral asymmetry. Sum of bilateral asymmetries was lower for crosses than their parental lines which, like heterosis,

reflected greater biological stability and homeostasis.

In Paper III, shank length and diameter, weight and length of the first primary wing feather, and face length were measured at 150 days in the two lines selected for response to SRBC, their respective relaxed lines, and reciprocal crosses (HL and LH) between them. Relative asymmetry of the five traits showed that the two selected lines and their respective relaxed lines exhibited greater relative asymmetry than the F_1 crosses. The experiment described in Paper IV compared results obtained from relative asymmetry measures to those obtained for heterophils, lymphocytes, heterophil/lymphocyte ratios and hematocrits which are routine criteria for measuring stressors. Sixteen progeny types consisting of parental lines, reciprocal F_1 and F_2 crosses, and backcrosses were produced in a single hatch from age contemporary parents. Chicks from the parental lines and reciprocal F_1 crosses were randomly chosen from the same stocks described in Paper II. Similar to results reported in Paper II, parental lines were similar for these traits. Reciprocal effects in the F_1 crosses and directional heterosis were present in cross HL but not in its reciprocal. Cross HL had negative heterosis for relative asymmetry (Paper II) and both negative heterosis of heterophil/lymphocyte ratios and positive heterosis of hematocrits (Paper IV).

There are several points to be drawn from these four papers: (1) The types of bilateral asymmetry have strong trait associated tendencies which vary with stages of life cycle. Lung weight and length, width, and weight of shank generally exhibited fluctuating asymmetry regardless of age, while first feather length and weight showed antisymmetry at both 39 and 150 days of age. (2) Relative asymmetries were less age related than trait related. Shank length and weight exhibited less than 2% relative asymmetry in all experiments while relative asymmetries for lungs

were generally 10% or higher. (3) F_1 crosses exhibited lower relative asymmetries than their parental lines (4) While heterosis for traits was positive, it was negative for relative asymmetry demonstrating greater biological developmental stability for crosses as well as hybrid vigor. (5) Relative asymmetry is an effective measurement of genetic stressors.

In Paper V, females within a line were separated as to whether or not they had commenced egg production by 245 days of age. Previous studies had shown that the nonlaying condition at this age could be overcome by force-feeding or by relaxing selection. Although relative asymmetry was similar for layers and nonlayers, in both groups it was higher than in a subline where selection for low body weight was relaxed.

Results presented in Paper VI showed that at hatch relative asymmetry in commercial broiler parent lines and crosses between them was different from that in White Leghorn chickens selected for high or low response to SRBC. The relative asymmetries of F_1 crosses which would be the parents of commercial broilers were not significantly lower than their parental dam lines. Fluctuating asymmetry for lung weight was higher in sire lines than in the F_1 crosses and dam lines. The results demonstrate that the extremely high selection intensity in sire lines reduced their developmental stability.

In conclusion, morphological traits such as shanks and primary wing feathers exhibited lower relative asymmetries than traits such as lungs; results of detecting genetic stresses by relative asymmetries were supported by those from heterosis and heterophils/lymphocytes ratios; the relative asymmetry of bilateral traits is a reliable indicator of developmental stability.

VITA

Aiming Yang, a daughter of Tinggui Yang and Qiumei Yu, was born on April 17th, 1967 in Emin, Province of Xinjiang, China. She received her Bachelor's degree in Animal Science from Xinjiang Shihezi University in July, 1987. After working for a year as research assistant in Nongjushi Sheep Breeding Farm, she enrolled in Sichuan Agriculture University for her master's degree in Animal breeding and Genetics with specialization in Sheep Science. After receiving her master's in July, 1991, she worked as a research assistant in Chengdu Agriculture Company for one year and then enrolled at Sichuan Agriculture University as a candidate for the Doctorate of Philosophy in Genetics. In August, 1995, she transferred her graduate studies to Virginia Polytechnic Institute & State University in Department of Animal and Poultry Sciences under the guidance of Prof. P. B. Siegel.