Forty Generations of Bidirectional Selection for Mating Frequency in Male Japanese Quail

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ABSTRACT A bidirectional replicated selection experiment for high (H1 and H2) or low (L1 and L2) cumulative number of complete matings (CNCM) in male Japanese quail was conducted for 40 generations. In the S32 generation, a subline was taken from each selected line and selection was relaxed. In the randombred control line (C), CNCM and unselected traits changed significantly over generations. Means of the selected lines were adjusted each generation for deviations from the control means. After 40 generations of selection, there was a 21-fold difference in CNCM (59.4 vs 2.8) between Lines H1 and L1. Whereas means increased and variation decreased in the high lines, means decreased and variation increased in the low lines. Regressions of mean CNCM on generation of Lines H1, H2, L1, and L2 were 1.15 ± 0.08, 0.61 ± 0.08, ±0.26 ± 0.04, and −0.34 ± 0.03, respectively. Although responses to selection were observed throughout the 40 generations in Line H1, the low lines appeared to have reached a limit to selection after the S30 generation. Relaxed lines provided supporting evidence for this conclusion. Mean CNCM decreased in the relaxed high lines to that of the control, whereas the low relaxed lines remained at the same level as their corresponding selected lines. Line H2 went into extinction in the 37th generation as a result of reduced fitness. Realized heritabilities of CNCM were 0.09, 0.07, 0.06, and ±0.15 in Lines H1, H2, L1, and L2, respectively. As correlated responses to the selection, male quail in the high lines were heavier, exhibited greater relative aggressiveness, and had larger cloacal glands than those of the control and low lines.

(Key words: Japanese quail, long-term selection, relaxed selection, mating behavior, genetic plateau)

1998 Poultry Science 77:1469–1477

INTRODUCTION

Reproductive traits are among the least understood traits in quantitative genetics (Frankham, 1990). An important component of reproduction in domestic animals and of fitness in wild populations is sexual behavior (Adkins-Regan, 1996). Quantitative genetic differences have been reported for sexual behavior of several species, including Drosophila (Manning, 1961; Pyle and Gromko, 1981; Gromko and Newport, 1988; Scott, 1994), rodents (Dewsbury, 1975), and chickens (Siegel, 1965, 1972; Dunnington and Siegel, 1983).

Japanese quail (Coturnix coturnix japonica) are popular for use in studies involving long-term genetic selection because of the short generation interval, high fecundity, and small body size. Marks (1996) reported that, after 97 generations of selection for high 4-wk BW in Japanese quail, genetic variation remained and realized heritabilities were consistent with theoretical predictions of loss of additive genetic variation with continuous selection. Nestor et al. (1996) conducted long-term (> 30 generations) divergent selection experiments for BW and yolk precursors in Japanese quail. Responses to selection differed with respect to trait, direction, and generation.

A replicated bidirectional selection experiment was initiated with Japanese quail to study the quantitative inheritance of mating behavior (Sefton and Siegel, 1975). Lines were divergently selected for high or low cumulative number of completed matings (CNCM) of males using a randombred control line as the base population. Differences between high and low selected lines were observed after six generations of selection with realized heritabilities of 0.07 for the high lines and 0.25 for the low lines. Subsequently, Cunningham and Siegel (1978) and Blohowiak and Siegel (1983) reported
results through the S_{12} and S_{29} generations, respectively. Although both the means and CV of CNCM changed, the asymmetrical response observed during the first 12 generations of selection disappeared with additional generations of selection in one pair of replicated lines. This long-term selection experiment has been extended to 40 generations of continuous selection. In addition, selection was relaxed in subpopulations taken from each selected line in the S_{32} generation. The objective of the present paper is to summarize results for the selected and unselected traits in these populations.

**MATERIALS AND METHODS**

**Stocks**

The quail used in this study were from replicated lines selected for high or low cumulative number of completed matings (CNCM), sublines in which selection was relaxed, and from an unselected randombred control line. Details of the origin and maintenance of the selected and control lines were reported previously by Sefton and Siegel (1975), Cunningham and Siegel (1978), and Blohowiak and Siegel (1983). Briefly, the foundation population of the four selected lines and the unselected randombred control population was a random mating line with no known history of artificial selection. Twenty-five males and 100 females were randomly selected from the base population to form the control line (C). This line was subsequently reproduced by randomly selecting one male offspring from each sire family and one female from each dam family. Simultaneously, two groups of 50 males each were randomly selected from the same foundation population. Group 1 was the foundation population for High line 1 (H1), and Low line 1 (L1), whereas Group 2 formed the foundation population for High line 2 (H2) and Low line 2 (L2). Within each group, 12 males with the highest and 12 males with the lowest CNCM were chosen and resulted in the pairs of the bidirectionally selected lines.

Approximately 50 males from each selected line and 100 males from the control population were randomly selected and their CNCM measured in each of the first four generations of selection. The number of males tested for Line C after the S_{4} generation was 50. The 12 males with the highest and the 12 males with lowest CNCM were chosen to reproduce their respective lines with the restriction that no more than one male from a half-sib family would be used in any generation. Lines were reproduced during the first four generations of selection by assigning four females from within a line to each male of the same line. In subsequent generations, three dams were assigned to each sire. Full- and half-sib matings were generally avoided.

**Selection Criterion**

The selection criterion was CNCM made by a male tested in eight observation periods between 10 and 14 wk of age (Sefton and Siegel, 1973, 1975). An observation period consisted of placing a male individually and at random within a flock of nonvirgin tester females for an 8-min period. Each tester flock consisted of a randomly chosen group of three age-contemporary females from the four selected lines and the control line. A minimum of 24 h was allotted between observation periods. Except for observation periods, males were housed in single-bird cages and, upon completion of each test period, the male was returned to an individual holding cage.

**Relaxation of Selection**

In the S_{32} generation of selection, a subpopulation was established from each of the four selected lines. Before individuals were chosen as parents of the S_{33} generation, a random sample of 20 males and 20 females was selected from each line to begin a subline in which selection was discontinued. Relaxed lines originating from H1, H2, L1, and L2 were designated HR1, HR2, LR1, and LR2, respectively. These closed populations were then randombred and maintained contemporaneously with the selected and control lines.

**Unselected Traits**

Unselected traits measured in this experiment were BW, cloacal gland area, and relative aggressiveness. Body weights to the nearest 1.0 g were obtained at 4 and 8 wk of age starting in the S_{3} and S_{5} generations, respectively. Cloacal gland area, measured the day after the last mating trial, consisted of dorsal by ventral height × length expressed in square millimeters (Sefton and Siegel, 1975). Beginning in the S_{4} generation, relative aggressiveness was determined a few days after the completion of mating trials by observing each male in eight initial paired encounters in a neutral cage. Pairings among males were made at random and each male received a value based on the percentage of encounters won.

**Statistical Analysis**

Responses of selected and unselected traits were calculated each generation. Traits were control-adjusted to remove environmental effects. For each trait, comparisons among lines were made each generation with the General Linear Models (GLM) procedure (SAS Institute, 1985). Data transformations were made prior to analysis because of heterogeneous variances among lines, as determined by Bartlett’s test (Blohowiak and Siegel, 1983), or because means and variances were correlated. The CNCM were transformed to square roots, and BW to common logarithms. For relative aggressiveness, percentage wins from paired encounters were transformed to arcsine square roots. When significant differences were found among lines, comparisons were made by Duncan’s multiple range test. Regressions of CNCM, BW, cloacal gland area, and relative aggressiveness on generation were calculated for each line.
Realized heritabilities of CNCM for each selected line were estimated as the regression of cumulative response on cumulative selection differentials. Data for CNCM were expressed in standard deviation units to reduce scaling effects.

RESULTS

Changes in the Control Line

Over a period of 40 generations, there was a significant positive regression of CNCM on generations for the randombred control line (Table 1). The regression was not significant from Generations $P_0$ to $S_{29}$, whereas from Generations $S_{30}$ to $S_{40}$ it was significant and negative. The inconsistency in the direction of change for the different periods might be due to irregular changes of CNCM over generations in the C line, which was characterized by a sharp decrease in the $S_4$ and an increase from the $S_{28}$ to $S_{31}$ generation. No time trends in CV of CNCM were observed in the C line (Table 1). Although over all generations, BW at 4 and 8 wk decreased significantly and cloacal gland area and relative aggressiveness increased significantly, there was no change in these traits for the last 11 generations (Table 4). Because the C line was the foundation population for the selected lines and was maintained as a randombred population, means of the selected lines were adjusted each generation for devia-
TABLE 2. Means ± SEM of CNCM, body weight, cloacal gland area, and relative aggressiveness of males in the S_{40} generation of selection\(^1\)

<table>
<thead>
<tr>
<th>Line</th>
<th>CNCM</th>
<th>BW at 4 wk</th>
<th>BW at 8 wk</th>
<th>Cloacal gland area</th>
<th>Relative aggressiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>59.4 ± 4.4(^a)</td>
<td>83.7 ± 0.9(^b)</td>
<td>106.3 ± 1.3(^a)</td>
<td>219.5 ± 14.4(^a)</td>
<td>74.7 ± 3.6(^a)</td>
</tr>
<tr>
<td>HR1</td>
<td>10.2 ± 2.9(^b)</td>
<td>85.7 ± 1.4(^b)</td>
<td>104.2 ± 1.4(^b)</td>
<td>107.2 ± 15.9(^cd)</td>
<td>58.5 ± 6.3(^b)</td>
</tr>
<tr>
<td>HR2</td>
<td>12.2 ± 4.7(^b)</td>
<td>82.2 ± 2.4(^bc)</td>
<td>92.0 ± 1.9(^de)</td>
<td>90.4 ± 23.9(^cd)</td>
<td>75.0 ± 8.9(^b)</td>
</tr>
<tr>
<td>C</td>
<td>13.6 ± 1.8(^b)</td>
<td>76.8 ± 0.8(^de)</td>
<td>95.4 ± 0.9(^cd)</td>
<td>177.6 ± 13.9(^ab)</td>
<td>67.9 ± 4.0(^d)</td>
</tr>
<tr>
<td>L1</td>
<td>2.8 ± 0.8(^c)</td>
<td>73.7 ± 1.2(^d)</td>
<td>99.3 ± 1.3(^bc)</td>
<td>94.9 ± 10.7(^ab)</td>
<td>34.6 ± 5.0(^d)</td>
</tr>
<tr>
<td>LR1</td>
<td>3.5 ± 1.3(^c)</td>
<td>89.6 ± 1.8(^b)</td>
<td>103.5 ± 2.0(^ab)</td>
<td>139.7 ± 23.3(^bc)</td>
<td>46.7 ± 6.3(^bc)</td>
</tr>
<tr>
<td>L2</td>
<td>1.0 ± 0.5(^c)</td>
<td>78.8 ± 1.0(^cd)</td>
<td>89.2 ± 1.0(^e)</td>
<td>33.9 ± 6.7(^c)</td>
<td>24.6 ± 2.9(^d)</td>
</tr>
<tr>
<td>LR2</td>
<td>2.3 ± 1.2(^c)</td>
<td>77.1 ± 1.2(^de)</td>
<td>90.7 ± 1.1(^de)</td>
<td>76.3 ± 18.8(^de)</td>
<td>22.5 ± 4.0(^d)</td>
</tr>
</tbody>
</table>

\(^a\)\(^e\)Means within trait with no common superscript differed significantly (\(P \leq 0.05\)).

\(^1\)Lines H1 and H2 were selected for high cumulative number of complete matings (CNCM); Lines L1 and L2 were selected for low CNCM; Line C was a randombred control line; Selection was relaxed in Lines HR1, HR2, LR1, and LR2 for generation S_{33} through S_{40}.

Responses to selection for increased or decreased CNCM are presented in Figure 1 for the selected lines. After 40 generations of selection, there was a 21-fold difference in mean CNCM between Lines H1 and L1 (Table 2). The consequence of selection is further demonstrated in Figure 2, which shows the distributions of CNCM in Lines H1, L1, and C in the S_{40} generation. Even though long-term selection had greatly increased CNCM in Line H1, considerable variation remained and occasional nonmaters were still observed in that line.

Over all of the generations of selection, regressions of mean CNCM on generation in the selected lines differed significantly from zero (Table 1). Selection for increased CNCM resulted in more abrupt changes in CNCM than selection for low CNCM, especially during the later generations. Line H1 showed persistent response to selection with the regression for generations S_{30} to S_{40} almost the same as that for the first 29 generations (Figure 1). Means for Line H2 were consistently smaller than those for Line H1 after the S_{9} generation. Mean CNCM for Line H2 dropped significantly after the S_{35} generation and the line eventually went into extinction.

Responses of both Lines L1 and L2 to selection for low CNCM were similar throughout the 40 generations (Figure 1). Means of both lines decreased linearly during the first 30 generations, after which the response turned to the opposite direction and CNCM increased. This reversal, which was significant (Table 1), occurred even though selection pressures were applied to lower CNCM.

**FIGURE 2.** Frequency distributions of cumulative number of complete matings (CNCM) for male Japanese quail at the S_{40} generation of selection. Lines H1 and L1 were selected for high and low CNCM, respectively and the control line was maintained by random mating.
Variation of CNCM also changed significantly with selection. Selection for increased CNCM resulted in less variation of the trait, whereas selection for low CNCM increased the variation. The CV of CNCM was 55% in the base population. In Generation S40, CV were 48, 182, and 290% in Lines H1, L1, and L2, respectively. Just prior to extinction of Line H2, the CV of CNCM increased from 34% in the S35 to 128% in the S37 generation. Significant regressions of CV of CNCM on generation were positive in Lines L1 and L2, and negative in Line H1 (Table 1).

**Realized Heritability**

Realized heritabilities (h²) of CNCM through the S29 generation, although low, were different from zero for Lines H1, H2, and L1, but not for Line L2 (Table 3). As selection progressed, h² of CNCM in Lines H1 and H2 decreased and was not different from zero for the period after the S30 generation. During these latter generations, h² for low CNCM were negative and different from zero. For the 40 generations of selection, h² of CNCM for Lines H1, H2, and L1 were low, significant, and consistent in magnitude with each other, whereas h² of CNCM for Line L2 was negative (−0.15) and significant. When comparisons were made within replicates, h² of CNCM were similar in the H1 and L1 lines, whereas those in the H2 and L2 lines differed from each other.

**Nonmaters**

Selection for increased or decreased mating frequency may change the proportion of nonmaters, which was defined as males that did not complete at least one mating during testing. The frequency of nonmaters in the P0 was 2.5% (Figure 3). There was no change in the control line with the regression of percentage nonmaters on generations being −0.11 ± 0.08. With divergent selection, the frequency of nonmaters increased in the low lines and decreased in the high lines. Regressions of percentage nonmaters on generations were 0.79 ± 0.20, 1.52 ± 0.19, and −0.12 ± 0.04 for Lines L1, L2, and H1, respectively. For Line H2, the regression of 0.02 ± 0.16 was likely caused by the sharp increase in percentage nonmaters after the S35 generation. There was an immediate increase in the frequency of nonmaters in the low CNCM lines with the percentages in Lines L1 and L2 reaching 51 and 22, respectively, by the S4 generation. Between 40 and 70% of the males in these lines were nonmaters, with the highest percentage being 86 in the S38 generation for Line L2. On the other hand, the low frequencies of nonmaters in Line H1 throughout the experiment and in Line H2 to generation S35 meant that changes in CNCM in the high lines was not a result of...
Correlated Responses

Correlated responses for unselected traits will be presented both in the context of differences among lines and as changes within lines as measured by regressions of means on generations.

Body Weights at 4 and 8 wk of Age. Regressions of 4- and 8-wk BW on generation were significant and negative for the C line (Table 4). Overall, quail in the high lines were heavier than those in the control and low lines (Table 2) with L1 quail lightest at 4 wk of age and L2 quail lightest at 8 wk of age in most generations. Regressions (P0 through S40) of 4-wk BW on generation were positive and significant in Line H1, and not different from zero in Lines L1, L2, and H2 (Table 4). Regressions of 8-wk BW on generation were positive and significant in Lines H1, H2, and L1, and not significant in Line L2.

Cloacal Gland Area. Since the first measurements in the S3 generation, cloacal glands were consistently larger in H1 than L1 males. This relationship was consistent with that observed between H2 and L2 males. By the S40 generation, the size of cloacal glands of H1 males were more than double those of L1 and L2 males (Table 2). Regressions of mean cloacal gland size on generation, although not significant in the H1 line, were significant and negative in the low lines (Table 4). The negative regression found in the H2 line was associated with the abrupt change of the trait that occurred before the extinction of the line in the S37 generation.

Relative Aggressiveness. Relative aggressiveness, which was first measured in the S4 generation, was generally greater in the H lines than in the low lines. Males in the H2 line exhibited the greatest relative aggressiveness from the S9 through S22 generations, whereas among lines the relative aggressiveness of L2 males was least for most generations. Selection for high or low CNCM resulted in a twofold difference in relative aggressiveness between high and low line males (Table 2). Regressions of mean relative aggressiveness on generations were negative and significant in the low lines and not significant in the high lines (Table 4).

Relaxed Lines

Four relaxed lines were maintained from the 33rd to the 40th generation. Changes in control-adjusted mean CNCM after relaxation of selection differed in the high and low relaxed lines (Figure 4). In the HR1 and HR2 lines, CNCM decreased significantly over generations eventually reaching the same level as that for the control line (Table 2). The CNCM of HR1 males (10.2) was significantly less than that of H1 males (59.4) in the S40 generation of selection. In contrast, no significant differences in the CNCM were observed between the selected and relaxed low CNCM lines in the S40 generation. These four lines showed a similar trend from S33 through S40 (Figure 4). Although regressions of mean CNCM on generations were negative in the HR1 and HR2 lines and positive in the LR1 and LR2 lines, only that for the HR1 line was significant (Table 1). Although variation of CNCM in the relaxed lines exhibited a similar trend to that of their corresponding selected lines, none of the regressions was significant. Values of unselected traits were in most cases similar in the relaxed lines to those in their corresponding selected lines (Table 2).

Discussion

Forty generations of bidirectional selection for CNCM in male Japanese quail were effective in creating extreme diversity between high and low lines. Heritability of CNCM fell in a narrow range of 0.06 to 0.09 in three selected lines, providing evidence of existence of small additive genetic variance for that trait. The low heritability of CNCM makes it phenotypically vulnerable to environmental changes, which could explain to a certain extent why the responses to selection for CNCM changed so abruptly over generations. The apparent lack
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FIGURE 4. Response to relaxed selection of cumulative number of complete matings (CNCM) adjusted to control line in male Japanese quail following 32 generations of selection for CNCM. Line H1 was selected for high CNCM and Lines L1 and L2 were selected for low CNCM. Selection was relaxed in Lines HR1, HR2, LR1, and LR2 for generation S33 through S40.

of response to selection for high CNCM in the early generations of selection suggests the involvement of linkage (Cunningham and Siegel, 1978). As linkage groups broke up, alleles previously unselected for would be increasing in frequency and then would enhance the subsequent responses shown in Figure 1.

Selection limits are important in long-term selection experiments (Robertson, 1960; Eisen, 1980). As most selection experiments have dealt with traits of high or moderate heritability (e.g., BW and bristle number), results from selection for low heritability traits are valuable in experimental evaluation of the theory of selection limits. Forty generations of selection did not result in a plateau of response during the later generations for one of the high lines, whereas both low lines appeared to have reached a selection limit after the S30 generation. Actually, most of the total responses of the high lines occurred after the S20 generation. The response from the S30 to S40 generation in Line H1 did not suggest the approach to a plateau. Comparisons of the relaxed lines with their high line counterparts presented clear evidence that abundant additive genetic variance existed in the high lines after long-term selection. In a similar selection experiment for mating frequency of chickens (Dunnington and Siegel, 1983), additive genetic variance for CNCM was not exhausted in the high line after 23 generations of selection. The low lines of quail, however, showed a different picture with a steady decrease in CNCM through the S31 generation, after which no further selection response was observed, suggesting that a plateau had been reached.

Falconer (1989) suggested that two situations should be distinguished: limits at which no genetic variance remains and limits at which genetic variance is present but the population fails to respond. The relaxed lines in present study addressed this issue. In Lines LR1 and LR2 there was no response to the suspension of selection, suggesting that after long-term selection no genetic variance of mating frequency remained in the low lines. It could then be concluded that it required more generations and total response for the high CNCM lines to reach selection limits than the low CNCM lines.

Involved in this behavior may be inheritance of a threshold for expression of mating and of mating frequency after the threshold is reached. In the low lines, the threshold for mating may have risen to such a level that there was little opportunity for post threshold responses to be measured.

Blohowiak and Siegel (1983) suggested that the apparent asymmetrical response observed during the first 12 generations of CNCM selection (Cunningham and Siegel, 1978) disappeared in the H1 and L1 lines after 29 generations. Through 40 generations, however, a renewed asymmetry in response to selection was evidenced both phenotypically by different regressions of response on generation and genetically by different realized $h^2$. In contrast to the initial asymmetry, in which the low CNCM lines had greater responses (Cunningham and Siegel, 1978), the high CNCM lines had greater responses in terms of the regression and $h^2$ over the long term. The results of the present experiment were not necessarily consistent with the conclusion of asymmetrical responses to selection for fitness traits with greater responses in the direction of lowered fitness (Frankham, 1990). As his conclusion has been based mainly on short-term selection, long-term selection may reveal other natures of the asymmetry in reproductive fitness traits.

It was suggested by Clayton et al. (1957) that the behavior of selected lines on the relaxation of selection is
of interest because 1) it provides a measure of the natural selection that is opposing artificial selection, and 2) it shows the strength of the evolutionary forces holding the mean in its original position. In the present experiment, high line males could not maintain high levels of mating frequency without continuous selection for CNCM. The rapid regression of CNCM in the relaxed high lines to that of the original level for the control line provided strong evidence that artificial selection for increased mating frequency was contrary to natural selection, although mating frequency may be considered a part of the general fitness. This result can, at least partially, explain why the H2 line went into extinction. On the other hand, the low lines in which selection was relaxed maintained means similar to those of the corresponding selected lines, demonstrating the fixation of genes controlling low mating frequency of male quail.

Extinction of the H2 line provided an example of the risk involved in long-term artificial selection. Although the response of Line H2 to the selection for increased CNCM was less than that for Line H1, responses before the S35 generation did not appear unusual. Dramatic changes happened quickly, with the mean CNCM dropping from 40.5 in the S35 to 20.8 in the S37 generation. CV increasing from 34 to 128%, and the percentage of nonmating increasing from 0 to 50.

There can be several causes for the extinction of a population. As stated above, selection for increased mating frequency at this high level may have been contrary to natural selection and thus reduced fitness. A second reason could be inbreeding. In the H2 line, the rate of inbreeding was estimated as 0.013 with the cumulative inbreeding coefficient through the S35 generation being 0.38 according to Falconer’s formulas (Falconer, 1989). Actual inbreeding, however, must have been higher because the numbers of sires dropped to 9, 4, 5, and 4 in the S36, S35, S36, and S37 generations, respectively. Japanese quail are sensitive to inbreeding depression (MacNeil et al., 1984). Other environmental factors, such as disease, could be ruled out, because all selected and control lines were age-contemporaries reared in the same environment. There is a complicated relationship between total fitness and its components as well as among fitness components per se. Some fitness components may be contrary to other components or even to total fitness. Although we lack complete data to support the hypothesis that mating frequency is negatively associated with other components of total fitness, a trend was clearly shown in this experiment.

The importance of replication in selection experiments was demonstrated by Clayton et al. (1957) in their selection experiment for bristle number in Drosophila melanogaster, and Hill (1980) has provided theoretical considerations of the replication in selection experiments. Although in the present experiment, the responses of the replicated low CNCM lines were consistent with each other, significant differences were noted in the two high CNCM lines. The loss of the H2 line also revealed the risk of long-term selection for specific traits. It is common to have long-term selection experiments with relatively small sample sizes, which increases genetic drift. Therefore, results from this experiment demonstrated that the replication of selected lines may enhance understanding of the underlying genetic basis of traits selected.

The results presented in this paper have practical implications. The feasibility of genetic selection as an efficient tool to modify sexual behavior and improve reproductivity was fully demonstrated. In improving reproduction performance, however, attention should be directed to total reproductive fitness rather than one or a few components of fitness.

ACKNOWLEDGMENTS

Appreciation is expressed to S. I. Jackson for preparation of manuscript and to the many graduate students who assisted in collection of data over these many generations.

REFERENCES