GENETIC ANALYSES OF REPRODUCTIVE BEHAVIOR IN THE DOMESTIC FOWL AND THE JAPANESE QUAIL

by

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INTRODUCTION

Genetic and physiological mechanisms are intricately involved in the reproductive behavior of poultry. Turkeys are commercially produced via artificial insemination because mechanical barriers impede the mating act and result in low fertility. Although most commercial broilers are produced from natural matings, there is increasing evidence of reproductive problems in breeder flocks. Whereas genetic and neural-hormonal factors are of importance with respect to mating behaviors, there is a dearth of information concerning their association in the fowl and how they may be altered by selection. Such information is necessary for understanding reproductive behavior in ground nesting birds such as turkeys, chickens and quail.

The Japanese quail (*Coturnix coturnix japonica*) has become a popular pilot organism for poultry research. This is because of its similarities with the domestic fowl. Through bidirectional selection, lines of chickens and Japanese quail differing in mating frequency, have been established at Virginia Polytechnic Institute and State University. This dissertation involves a series of experiments that were undertaken to examine the interfacing of heredity, behavior and physiology in the reproduction of the fowl and quail. Experiment I was designed to investigate the fertility of lines of chickens known to differ in mating frequency. In Experiment II, the mode of inheritance of mating behavior and blood testosterone levels were examined in chickens using purelines and crosses between them.

Electroencephalographic aspects of the mating behavior of chickens were studied in Experiment III. Lastly, Experiment IV was designed to investigate further the genetics of behavior in Japanese quail utilizing comparisons among lines selected for mating frequency and their respective F_1, F_2 and backcross generations.

LITERATURE REVIEW

The literature involving the genetics, behavior and physiology of reproduction is voluminous. Accordingly, this review will focus primarily on studies involving the chicken and the Japanese quail.

Qualitative genetic factors

A number of loci are known to be involved with the reproductive behavior and fertility of chickens and Japanese quail. Crawford and Smyth (1964a) observed that females with white feathers were socially dominant and courted more frequently than those with barred feathers. In contrast, the frequency of crouching was greater for females with barred than with white plumage. No differences were found between plumage types for courting frequencies of males. Earlier, however, Crawford and Smyth (1961) reported that males with columbian and barred plumage courted columbian-feathered females more frequently than they did barred females. Also, the males with barred feathering courted females more frequently than did cocks having the columbian plumage. Furthermore, although barred females in the lower social positions crouched more frequently than did columbian hens, there was no indication of a preference to the plumage pattern of the cock. Barred birds of both sexes had the highest copulation frequency.

A body of evidence has been accumulated to show a relationship of comb type with mating behavior and fertility. Males with

single combs (*rrpp*) copulated at a higher frequency than those with either homozygous (*RRpp*) or heterozygous (*Rrpp*) rose comb males (Crawford and Smyth, 1965). This effect was attributed to an unknown effect of the *R* allele and not to the appearance or social environment of the birds. Frequencies of sexual displays were not consistently correlated to genotype, although homozygous rose comb (*RRpp*) pullets were courted more frequently than either *Rrpp* or *rrpp* females. The association of rose comb with fertility was first reported over a half century ago when Dunn (1927) observed a higher number of single comb offspring when single and rose comb males were intermingled in flocks with single comb females. Dunn suggested that selective fertilization favored the union of germ cells of nearly related individuals. Subsequent studies (Crawford, 1965; Crawford and Merritt, 1963; Crawford and Smyth, 1964b; 1964c; 1964d; 1964e; Petitjean and Cochez, 1966; Ponsignon, 1951) showed that this was due to an effect when there was homozygosity for the *R* alleles. The comb phenotype may also affect the social order of a flock, which in turn may affect sexual behaviors. Williams *et al.* (1977) presented evidence of social stratification based on comb phenotype, with single comb males having higher ranks than those with walnut combs.

The *epi* gene (Crawford, 1970) which results in *grand mal* epileptic seizures in chickens also affects the mating behavior. This is because epileptic males often are unable to complete matings. Apparently this is the result of visual and/or behavioral excitation

causing a seizure (Crawford, 1980).

Quantitative genetic factors

Polygenic systems have been shown to influence the mating behavior of chickens and Japanese quail. Siegel *et al.* (1958) interpreted results of crosses designed to test the sexual behavior of inbred and incross White Rock cocks as an indication of a genetic basis for courting, mounting, treading and the completing of matings. There was no evidence of heterosis for any of these behaviors. They found a highly significant positive correlation between fertility and number of courts, whereas the correlations between fertility and other types of mating behavior were not significant. These observations are consistent with a positive association between courtship and aggressiveness (Benoff and Siegel, 1977). Siegel (1959a) noted a positive phenotypic association between relative aggressiveness and mating ability in White Rock cocks when males were tested singly with pullets. These observations indicated that males which exhibited the largest number of courts, mounts, and treads also completed the largest number of matings. When several males were intermingled in the flock, differences among males or groups of males for interferences during mounting and treading were not unidirectional with those highest in the social hierarchy not necessarily having the greatest sex drive (Siegel, 1959b).

Earlier, Wood-Gush and Osborne (1956) suggested a genetic basis for mating frequency in chickens, with the top ranking males

for mating frequency having, in general, the highest fertility. In subsequent studies, however, Wood-Gush (1957, 1958) did not observe a positive correlation between sexual activity and aggression. Previously Wood-Gush (1956) suggested that courtship displays were due to a conflict between sex, attack and escape, which differed basically from an agonistic display.

Bhagwat and Craig (1978) reported increased social dominance ability occurred in strains selected for egg production traits when intermingled with females from the unselected control population. They hypothesized that this observation was in some way associated with an earlier sexual maturity in the selected strain. Similarly, Dawson and Siegel (1967) reported that aggressive behavior was more frequent in all-male than in all-female or sex-intermingled flocks. A correlation of 0.30 was found between number of males dominated and the number of completed matings per hour (Kratzer and Craig, 1980). Also, males confined in larger areas had a high frequency of courtings, completed matings and aggressive acts than those maintained in smaller areas.

The results of a bidirectional selection experiment for mating frequency in male chickens was described by Tindell and Arze (1965). Males from the high mating line matured approximately two weeks later than those from the low mating one. The short and long term results of bidirectional selection for cumulative number of completed matings (CNCM) in chickens were reported by Siegel (1965; 1972). As expected, changes in genetic variation and correlated responses

of unselected traits occurred over the course of selection. Correlated responses were found for other mating characteristics and the concentration of spermatozoa, whereas such responses were either absent or of minor importance for relative aggressiveness and body weight. In contrast, Blohowiak *et al.* (1980) noted lower mating frequencies for males selected for high body weight than those selected for low body weight. They attributed this to decreased libido in the extremely heavy males. This would explain the difference between reports as the cocks in the high and low mating lines (Siegel, 1965; 1972) did not approximate the body weights of those studied by Blohowiak *et al.* (1980).

Analyses, based on reciprocal crosses between the high and low mating lines and the randombred control line of chickens (Siegel, 1965; 1972), were described by Cook *et al.* (1972). Dominance and/or epistatic factors and sex-linked loci appeared to influence low mating frequency, whereas additive genetic effects without sex-linked factors appeared to be the main sources of genetic variation for high mating frequency. Also presented was evidence to support the hypothesis of the two genetic systems interacting to influence male mating frequency in chickens. This hypothesis, initially proposed by McCollom *et al.* (1971) and expanded by Cook and Siegel (1974) suggests that the neural system may be of primary importance to behavior, and once neural thresholds were reached, endocrine responses became effective. Selection for CNCM would thus affect neural threshold(s), and hence the sensitivity of the target tissues

to hormones. Subsequently, Van Krey *et al.* (1977) suggested that androgens may have a permissive role which allows the mating potential to be expressed. Thus, selection for high and low CNCM would also influence an inhibitory center, which operates independently of the steroid hormones.

The effects of age on mating activity was measured in Japanese quail by Sefton and Siegel (1973). Sexual activity reaches a peak at about 70 days of age and stays at this high level until about 210 days, when it begins to decline. This follows the pattern of fecundity in this species. Body size may affect fertility in Japanese quail. Marks (1979) observed, in lines of quail selected for four-week body weight, that fertility and hatchability decreased in comparison to the unselected control line. Results of bidirectional selection for CNCM in Japanese quail were reported by Sefton and Siegel (1975) and Cunningham and Siegel (1978). Mating activity and relative aggressiveness are positively related in Japanese quail, which is in contrast to that observed in chickens. This difference in aggressiveness between the species may be due to differences in sexual dimorphism of body weight; male Japanese quail being smaller than females. This may result in physical aggressiveness playing a vital role in completion of the mating act in this species. Additional evidence of the importance of aggressiveness in the mating of Japanese quail may be found in the report of Hughes *et al.* (1980), who found that increasing the sex ratio to one male to three females per cage had no detrimental

effect on fertility. Mating frequency and cloacal gland size are positively correlated in the Japanese quail, with both being positively influenced by androgens (Sacks, 1969). A single physiological factor, possibly androgen, was hypothesized by Ottinger and Brinkley (1979) as affecting sexual characteristics in quail. This conclusion was based on the order of onset of the behaviors and the rapid increase in their frequencies following their initial appearance. This is consistent with the observations of Wilson and Bermant (1972) who found both mating behavior and cloacal gland size were androgen sensitive. The aggressive part of mating in quail was noted with the probability of completing the mating sequence greater in heterosexual than homosexual contacts, due to the behavior of the mounted, not the mountee.

Aggressiveness may also be important in interactions with environment. Seastedt and MacLean (1980) found, in ground nesting longspurs, that the "probability of polygyny is determined, in part, by the interaction of male and female characteristics. An "attractive" male with a high-quality territory might be denied polygynous matings by the successful resistance of the first female, while another, equally "attractive" male mated to a less resistant female (who, perhaps, failed to centre her nest and/or is less aggressive toward other females) might succeed in mating more than once." Similarly, with European wrens, there is evidence that females use characteristics of the male, their nests and their territories in the selection of breeding sites (Garson, 1980). Female choice has also been

reported to be important in redwinged blackbirds (Lenington, 1980).

Physiological studies

Although it is well documented that both the neural and the endocrine systems are involved in the mating behavior of birds, the role of genetic variation in these systems is unclear. Siegel and Siegel (1964) described procedures for detecting family differences in response to FSH and LH, and Siegel *et al.* (1968) used bioassay methods to determine pituitary secretory capabilities for gonadotropins in lines of chickens selected for high and low body weight. The threshold of response to pituitary homogenates, FSH alone and in combination with either LH or stilbestrol was significantly lower in an early than in a late maturing line of chickens (Siegel and Van Krey, 1969).

The effect of an ovulatory inducing hormone (OIH) was found to be inhibited, preventing multiple ovulation, by PMSG or FSH in hypophysectomized hens, supporting the theory that withdrawal of FSH stimulation sensitized the follicles to OIH in the normal hen (Ogawa *et al.*, 1976). Kamiyoshi *et al.* (1977a) noted that diurnal peaks in the gonadotropic activities of caudal pituitary lobes in young chickens and in adult males. Twin peaks observed in the cephalic lobe of the hens were slightly different than those observed in the caudal lobe. Observations with capons indicate that the appearance of diurnal changes in pituitary gonadotropic activities are related to photoperiod (Kamiyoshi *et al.*, 1977b),

and those with hens and cocks suggest that the common peak may be due to a factor(s) related to a photoperiod common to both sexes (Kamiyoshi *et al.*, 1977c). Similar results with young chickens and quail were obtained by Hashiguchi *et al.* (1977a, 1977b) who earlier (Hashiguchi *et al.*, 1976) described the influence of diurnal changes as caused by variation in the amount of testicular RNA, indicating a diurnal pituitary gonadotropic effect. FSH acting in synergism with LH causes estrogen production in the female, and androgen production in the male. Factors affecting the production or release of these hormones may thus affect sexual activity through changes in sexual steroid synthesis, release and/or sensitivity. Thus, as the hypothalamus is related to FSH and LH release by the pituitary, its function may be related to sexual behavior.

Assenmacher *et al.* (1975) and Jallageas and Assenmacher (1979) reported that the seasonal variation of testosterone levels in Pekin drakes was related to the LH and thyroxine levels. Seasonal effects on plasma testosterone titers were investigated in sterile chicken-pheasant hybrids by Purohit *et al.* (1978). They proposed that the low levels of testosterone in the hybrids during winter and spring seasons could be attributed to the impairment of steroid biosynthetic activity of the Leydig cells, and may be causally related to the absence of secondary sexual characteristics and the interruption of spermatogenesis in the hybrids. Seasonal changes in blood testosterone levels, and changes in behavior and body conformation related to the changes in testosterone level have also been observed

in turkeys (Lisano and Kennamer, 1977), while in the male ring dove, stimulatory effects of mating on gonadotropic secretion and gonadal activity were observed in the late fall (Haase *et al.*, 1976).

The influence of androgens on sexual behavior was studied in lines of chickens selected bidirectionally for mating activity (Van Krey *et al.*, 1977). Although androgen stimulated sexual behavior in all lines, the response was found to be only threshold in this respect, suggesting that despite wide differences between lines in overt sexual behavior, a degree of receptor sight equality and/or effector response existed. They related this to studies of the role of hypothalamic centers and mating activity (Barfield *et al.*, 1975; Crawford and Glick, 1975; Haynes and Glick, 1974) and hypothesized an inhibitory mating center (IMC) dominant to a stimulatory mating center. The IMC was considered not to be dependent upon nor affected by the gonadal steroids, and testosterone implants restricted in activity to the immediate area of the SMC were capable of stimulating copulatory behavior. This implies that the role of androgens with respect to adult male copulatory behavior may be threshold in nature. These inferences are supported by the studies of Snapir *et al.* (1974), which indicated that lesions to the mammillary nuclei of the hypothalamus and the posterior part of the ventromedial nuclei caused impairment of hypothalamic FSH-releasing factor (FRF) and LH releasing factor (LRF), while impairment of FRF activity, and less impairment of LRF, results from lesions of the posterior part of the mammillary nuclei. Balthazart (1976) reported that

the social displays and sexual behavior of drakes corresponded with the highest testosterone levels. It was hypothesized that variation in testosterone levels and in behaviors could be due to a direct effect of the variation of hormone levels on the behavior. Differences in androgen levels between the sexes may start very early since Balthazart and deRycker (1979) observed differences between sexes of chicks at hatch. These inferences are further supported by the results of Benoff *et al.* (1978a) who reported no significant differences in blood and hypothalamic disintegration per minute of radioactive testosterone between hormone level treatments among lines divergently selected for CNCM. The findings suggest that the behavioral differences among the lines differing in mating frequency are not due to a difference in the ability of the hypothalamic tissue to concentrate testosterone.

Also involved may be the aromatization of testosterone to estrogenic compounds. Preliminary results with respect to testosterone propionate, estradiol benzoate and dihydrotestosterone support the observations that the aromatization of testosterone to estrogen is a requirement for mating behavior in quail (Adkins and Nock, 1976; Balander, 1978). The relative rate at which this conversion occurs could be a factor responsible for the regulation of sexual behavior in the high and low mating lines of quail (Cunningham and Siegel, 1978).

The site in the brain upon which the androgen acts may also influence behavior. Barfield (1969) concluded that androgen normally

acts on the preoptic area in the male fowl to activate copulatory behavior, but not courtship or aggressive behavior. Results with radioactively labelled testosterone (Barfield *et al.*, 1978) indicated that the pattern of accumulation of testosterone in the male fowl was comparable to that for sex hormone uptake in vertebrates in general, with accumulation generally found in areas known to be concerned with sex hormone dependent functions. These results were consistent with those of Gardner and Fisher (1968) and provide evidence for an androgen-sensitive mechanism in the anterior hypothalamus, and suggest that neural cells mediating mating behavior in chickens can be primed or triggered by androgen very early in development.

Cunningham *et al.* (1977) described the effect of exogenous testosterone cypionate on the mating behavior of castrated male Japanese quail from lines selected for CNCM. In no case was the mating frequency of androgen treated capons from the low mating line equal to that of males from the high mating line. Furthermore, exogenous testosterone, at the levels used, failed to stimulate capons from the selected lines to mate as frequently as the respective intact males from the particular line. Also, exogenous testosterone had a depressing effect upon the mating frequency of intact males from the high and control lines, and no effect upon males from the low mating line. Further support of the androgen influence hypothesis comes from the observations of Benoff *et al.* (1978b) where there were greater plasma testosterone titers, packed cell volumes and

mating behaviors in males from the high mating line than in those from the low mating line birds.

Mating behavior and fertility

Fertility may be indirectly influenced by genetic variation in mating behavior. Additionally, there are genetic influences on fertility *per se*. Williams and McGibbon (1956) found differences in duration of fertility between two inbred lines of chickens, with considerable variation between males within lines. Duration of fertility improved when males were mated to females of the same line.

Physical ability to mate may be related to fertility in natural mating flocks. Parker (1961) found that the semen from Cornish cockerels yielded good fertility when used for artificial insemination, and concluded that the poor fertility with natural matings was attributable to their physical conformation which reduced their effectiveness during copulation.

In chickens, Siegel and Beane (1963) confirmed the results of Parker and McClusky (1959) and Crawford (1962) who found no difference in the fertility of males kept on the floor and in cages. However, Parker *et al.* (1942) found that males maintained in breeding pens were more sexually active than those confined to batteries, and produced more sperm. Spermatozoa production was influenced by season, and there were marked differences in sexual activity,

spermatozoa production and semen characteristics among individual males. Differences among breeds were also found.

Social factors have also been found to affect mating behavior. McDaniel and Craig (1959) found significant correlations between social aggressiveness, sexual effectiveness, and crouches elicited from hens. Correlations between social aggressiveness and fertility, however, were not significant. Siegel and Siegel (1964b) reared cockerels in sex-intermingled flocks until various ages (58, 70, 84 days) and then placed them into either all-male flocks or individual cages where physical contact was prevented. Mating behaviors measured from 217 to 231 days of age, showed that the age when cockerels were placed in the cages influenced their subsequent mating ability. This suggests the existence of a sensitive period when physical contact at an earlier age may influence future behavior.

Heterosexual or unisexual contact of juvenile males may influence mating behavior in chickens (Cook and Siegel, 1974). It was hypothesized that selection for low CNCM was primarily for higher neural thresholds, whereas selection for high CNCM affected the loci operative after the neural thresholds were reached. The magnitude of the sexual component of a court was found to be related to the genetic background of the population, being lower in the low mating lines than in the high mating lines.

EXPERIMENT I

Fertility of chickens from lines divergently selected for mating frequency.

INTRODUCTION

Fertility of chickens is influenced by genetic and nongenetic factors (Wilson *et al.*, 1979). These include prematal and postmatal barriers such as mechanical factors (Parker, 1961; Blohowiak *et al.*, 1980), behavioral factors (McDaniel and Craig, 1959; Cook and Siegel, 1974), and variation for mating frequency (Wood-Gush, 1960; Tindell and Arze, 1965; Siegel, 1972). Since semen from a single ejaculation can fertilize several eggs, variation in mating frequency may or may not influence fertility.

The experiment presented here was designed to evaluate fertility in natural matings and from artificial insemination in lines of chickens known to differ in mating frequency.

MATERIALS AND METHODS

The chickens used in this experiment were obtained from the S_{20} generation of lines selected for high (HML) and low (LML) cumulative number of completed matings, plus the randombred control population (AC), which served as the base population for the selected lines (Siegel, 1965; 1972). Behaviors considered were the cumulative number of courts, mounts, treads and completed matings measured

between 31 and 33 weeks of age as outlined by Siegel (1965). Briefly, the procedure consisted of releasing a male singly into a flock of females the same age as the male for ten minutes and recording the frequency of these behaviors. This procedure was repeated on eight separate days, and the observational unit was the cumulative number for the 80 minutes of observation. The cumulative number of courts, mounts, treads and completed matings were transformed to square roots prior to analysis by the model:

$$Y_{ij} = \mu + L_i + e_{ij}$$

where i = 1, 2, 3 lines, j = 1, 2 . . . n individuals within a line.

Commencing at 34 weeks of age, comparisons were made among males of these lines for fertility via natural and artificial mating. Males for natural matings were selected at random from each line and placed singly in flocks containing five pullets each. Assignment of males (16 males per line) to the 48 female flocks was made at random. Each male remained in a flock for 10 consecutive days after which he was removed. Eggs were broken and fertility was determined daily by macroscopic examination (Kosin, 1944). Peak fertility was defined as the first day of maximum fertility for the flock. Duration of fertility was defined as the last day after the males were removed from the flock that fertility was more than 20 percent.

For artificial matings, 16 males from each line were preconditioned for semen collection, after which a single ejaculate from each male was divided into three equal parts and inseminated into three virgin females that were randomly assigned to him. Eggs were gathered

daily and broken to determine fertility. Peak and duration of fertility were considered as the first day of maximum fertility and the last day a fertile egg was laid preceeding two infertile eggs for each pullet, respectively.

The males used in natural and artificial matings were switched for the initiation of a second run. That is, the males used in natural matings were used for artificial matings and those used for artificial mating were used in natural mating. Experimental procedures were the same in both runs.

Preliminary analyses revealed no significant differences between runs. Thus, duration of fertility, days to peak fertility, and fertility at peak and on the 2nd, 5th, 10th and 18th day after males were placed in flocks with females or after pullets were inseminated, were analyzed by the model:

$$Y_{ijk} = \mu + L_i + M_j + (LM)_{ij} + e_{ijk}$$

where i = 1, 2, 3 lines, j = 1, 2 methods of mating and k = 1, 2 . . . n individuals per line-method subclass. The proportions for fertility were adjusted using the Freeman-Tukey arc sine transformation for binomial proportions with less than 50 observations (Mosteller and Youtz, 1961).

Correlations between the various behavior and fertility measurements were calculated within lines, converted to z values and tested for homogeneity (Snedecor, 1946) and pooled across lines when homogeneous.

RESULTS AND DISCUSSION

Mating behavior

There were significant differences among lines for the frequency of courts, mounts, treads and completed matings (Table 1). Males from the HML courted more than those from the LML and AC lines, which did not differ from each other. The males from the HML had the most mounts, treads and completed matings, those from the LML the least with the AC males intermediate and significantly different from the selected lines. These results were consistent with previous observations (e.g. Siegel, 1972) and demonstrate that the samples used here were representative of the respective lines.

Phenotypic correlations between behaviors were homogeneous across lines. The highly significant correlations for numbers of mounts and treads with completed matings were .93 and .94, respectively; values were consistent with those from previous generations (Siegel, 1965; 1972). The correlations between numbers of courts with mounts, treads and completed matings were .68, .65 and .43, respectively. Although these correlations were highly significant, they were of a considerably lower magnitude than would be expected if courting were primarily a sexual behavior. This is consistent with the hypothesis that courting in chickens changes from agonistic to sexual behavior (Kruijt, 1964; 1966; Siegel, 1972).

Percentage fertility

There were no significant line-method of mating interactions

Table 1. Means and standard errors for cumulative number of courts, mounts, treads and completed matings

Line	N	Courts	Mounts	Treads	Matings
HML	32	130 ± 7^{a}	37 ± 2^{a}	33 ± 2^{a}	27 ± 2^{a}
LML	32	64 ± 6^{b}	2 ± 1^{c}	2 ± 1^{c}	2 ± 1^{c}
AC	31	70 ± 7^{b}	7 ± 1^{b}	6 ± 1^{b}	6 ± 1^{b}

Means in the same column having the same superscript are not significantly different ($P \leq .05$)

for any fertility measurements. Also, differences among lines were not significant for percentage fertility on days 2, 5, 10, 18 or on the day of peak fertility (Table 2). The absence of differences among lines indicates that when left with a flock of females for an extended undistrubed period, males from these lines, which differed in mating frequency, had similar fertilizing capabilities. Additional support for this was indicated by the nonsignificant correlations between the behaviors and fertility.

Previous work (Siegel, 1965; 1972) indicated that the lower mating frequency of LML males may be compensated for by superior semen quality. Although the HML males may mate more frequently than those from the LML and AC lines, the sperm host glands of the females may have been filled to capacity in all lines (Compton *et al.*, 1978) independently of the number of matings or semen dose (Compton and Van Krey, 1979). The sperm in the glands may be released over time at an equal rate depending only upon the capacity of the female glands.

Although differences between natural mating and artificial insemination for percentage fertility were not significant on days 2, 5, 10 or on the day of peak fertility, there were significant differences between methods at day 18 and for number of days to peak fertility. The reasons for this will become obvious in the next section on duration of fertility.

Duration of fertility

No significant differences were found among lines for duration

Table 2. Means and standard errors for percentage fertility, peak fertility and days to peak fertility under natural and artificial mating, by lines

Method of Mating		Percentage at Day				Peak	
		2	5	10	18	%	Day
Natural	LML	50.7 ± 8.8	74.3 ± 6.8	74.2 ± 5.8	44.5 ± 11.1	92.7 ± 3.6	4.8 ± 0.5
	HML	48.5 ± 8.0	70.1 ± 6.0	61.0 ± 6.6	28.2 ± 8.6	96.0 ± 2.0	4.2 ± 0.3
	AC	49.0 ± 7.9	77.7 ± 5.8	74.4 ± 4.9	30.9 ± 8.7	88.8 ± 3.8	4.6 ± 0.5
	Pooled	49.4	74.1	70.3	34.2**	92.5	4.6**
Artificial	LML	56.9 ± 6.3	90.5 ± 3.2	64.4 ± 7.4	16.6 ± 12.6	92.8 ± 3.6	3.0 ± 0.1
	HML	53.4 ± 7.0	75.0 ± 7.3	62.4 ± 7.2	0 ± 0	97.2 ± 1.6	3.4 ± 0.2
	AC	62.0 ± 7.5	69.5 ± 7.6	60.5 ± 8.1	0 ± 0	93.8 ± 3.9	3.5 ± 0.3
	Pooled	57.3	78.9	62.4	0.6**	94.7	3.3**

**Differences between methods of mating was significant ($P \leq 0.01$)

of fertility (Table 3). There were, however, significant differences between artificial insemination and natural matings with the duration of fertility being longer for the former than for the latter. Since duration of fertility for natural mating was determined from the day after the males were removed from the flock, and considering the lack of differences in percentage fertility at days 2, 5 and 10 between the methods of mating, it appears that not all females were mated on the last day males were with females. Thus, the base point for measuring duration was variable in the natural situation and constant in the artificial one. This is supported by the number of days to peak fertility differing significantly between natural and artificial matings. Peak fertility for the former occurred approximately 1½ days after the peak for artificial insemination, suggesting that all females were not mated the first day.

The later peak in fertility with natural insemination would most likely be due to male-female interactions in the natural mating pens interfering with immediate fertilization. These interactions may include unequal mating with all females (including delayed and complete lack of mating) and, matings with no transfer of semen. The duration of fertility plus the number of days to peak fertility for natural matings was approximately equal to the duration of fertility noted for artificial insemination. This provides further support for the thesis that although fertility was similar for all lines, there were differences between mating methods which may be due to a failure for all females within a flock to be fertilized on the

Table 3. Means and standard errors by line for duration of fertility (days) under artificial and natural mating

Line	Artificial	Natural
HML	11.4 ± 0.5	6.8 ± 0.5
LML	10.9 ± 0.7	6.5 ± 0.5
AC	10.9 ± 0.5	7.0 ± 0.4
Pooled	11.1**	6.8**

**Differences between methods of mating was significant ($P \leq .01$)

same day as occurred with artificial insemination. The reverse of this reasoning would also account for the slower decline in fertility that was observed in natural matings than in artificial insemination (see days 10 and 18 in Table 2).

SUMMARY

Fertility comparisons, of males under natural and artificial mating situations, were made among lines of chickens selected for high and low mating frequency and the randombred population from which the selected lines originated. Although highly significant differences existed among lines for the frequency of sexual behaviors, there were no differences in fertility among lines either when males were with females for extended periods of time or when hens were artificially inseminated. Differences were noted between mating systems for days to peak and duration of fertility.

EXPERIMENT II

Crosses of lines of chickens selected for mating frequency.

INTRODUCTION

Several short-term selection experiments for mating frequency have been conducted in chickens (Wood-Gush, 1958; 1960; Tindell and Arze, 1965; Galpren and Dukhno, 1974). From these experiments, and those of Siegel (1965; 1972), a model has been developed (Cook and Siegel, 1974; Van Krey *et al.*, 1977) to describe the mode of inheritance of mating behavior in male chickens. The model proposes that selection for low mating frequency is primarily for higher neural thresholds, which acts through an inhibitory mating center that is primary to a secondary mating center, which is stimulatory. Under this model, selection for high mating frequency changes the frequency of alleles at loci operative after the neural thresholds are attained, with the inhibitory mating center not being dependent upon nor influenced by androgens. Thus, in the presence of androgens, mating will occur but only to the extent dictated by the inhibitory mating center.

Long-term selection for mating frequency and crosses among populations differing in this behavior can provide additional insights into the genetic architecture of mating behavior in the male fowl. The experiment presented here contains the results

of reciprocal crosses between lines of chickens that have undergone 20 generations of divergent selection for high and low mating frequency of males, and of reciprocal crosses between the selected lines and the random mating population from which they originated.

MATERIALS AND METHODS

Stocks and Husbandry

F_1 and S_{21} generation progeny were produced from reciprocal crosses and pureline matings of S_{20} generation parents from lines selected for high (H) and low (L) cumulative number of completed matings (CNCM) by males and the randombred control (C) which was used as the base population for the selected lines. Descriptions of these lines were presented by Siegel (1972). Mating combinations used in this experiment are designated with the first letter denoting the sire and the second letter denoting the dam. All matings were made via artificial insemination starting when the sires and dams were 47 weeks of age. Progeny were obtained from two hatches, which were on March 6 and 13, 1979. Chicks were removed from the hatcher, wing-banded, vaccinated for Marek's disease and brooded in floor pens with feed and water provided *ad libitum*.

Traits

Body weights were obtained at eight weeks of age and behavioral data were obtained between 28 and 32 weeks of age. The behavioral

data included the frequency of courting, mounting, treading and completed matings as outlined by Siegel (1972). Briefly, the procedure for measuring behaviors consisted of releasing a male, that has been maintained in an all-male flock since eight weeks of age, singly into a flock of six females for 10 minutes and recording the cumulative number of courts, mounts, treads and completed matings. He was then returned to his home flock. This procedure was repeated on eight separate days and the cumulative value was considered as the observation for the male.

At 34 weeks of age five ml of blood was obtained by cardiac puncture from a random sample of males from each line. The blood, which was to be assayed for testosterone, was collected in heparinized syringes, spun in a refrigerated centrifuge, and the plasma stored in a frozen state until thawed for assay. Extraction and assay methods followed those described by Benoff (1977) and Kattesh *et al.* (1979). Ten microliters of 1200 cpm double label testosterone (1, 2-^{3}H(N) testosterone, New England Nuclear) were air dried in the extraction tube, and one ml of plasma added. Incubation, at 45° C for ten minutes to allow for equilibration, was followed by a five ml addition of ether. The samples were then frozen, following which the ether was extracted after which a second five ml volume of ether was added, frozen, and extracted. The two ether extractions were pooled as one and dried down in a water bath and 0.2 ml of 9:1 benzene:methanol solvent was added. The redissolved extract was applied to 50 mm columns packed with

Sephadex LH-20 and washed with four ml of benzene:methanol (9:1 v/v). The third and fourth ml portions known to contain testosterone were saved and 0.3 and 0.6 ml fractions were pipetted into assay tubes and air dried for radioimmunoassay. A 0.2 ml fraction was dried in a scintillation vial for recovery determination.

The testosterone contents in the samples were determined using a radioimmunoassay with rabbit antibody (obtained from Dr. H. D. Hafs, Michigan State University, East Lansing, Michigan) diluted with phosphate buffer saline. To the sample tube 200 µl of antibody was added and the contents incubated at room temperature for 15 minutes, after which 100 ml of 1,2-^{3}H(N) testosterone was added and the mixture held overnight (15 to 16 hrs) in a cooler at 4° C. Just prior to removal from the cooler, samples were placed in an ice bath. Upon removal from the cooler, one ml of cold dextrane coated charcoal was added to the tubes. Within ten minutes, the samples were centrifuged for ten minutes at 4099 G at a temperature of 4° C. Upon removal from the centrifuge, 0.5 ml aliquots were pipetted into scintillation vials and five ml of Omnifluor scintillation cocktail was added. Samples were then counted for five minutes in a liquid scintillation detector. Standards ranging from 0.005 ng to 0.5 ng plus two water and a plasma blank were run with each 40 vials that were assayed. Linear regressions computed on a within assay basis from these standards were used to determine the testosterone concentrations of the unknowns which were then adjusted to a one ml plasma basis according to

the size of the assay aliquot and recovery determination.

Prior to undertaking assays of actual samples, validation techniques using a series of plasma volumes and added testosterone were conducted to evaluate the procedure. It may be seen from the upper portion of Table 4 that the quantities of added testosterone recovered ranged from 76 to 82 percent (e.g., .38 ÷ .50 = 76%). Data for the extraction quantity phase are presented in the lower part of Table 4 for two different plasma samples. In Sample 1, the extraction method appears to have a lower recovery as the volume of plasma extracted was increased. The extraction of 1.5 ml of plasma resulted in significantly less testosterone recovery than did 0.3, 0.5 or 1.0 ml extractions. No significant differences were found among the other three quantities. With Sample 2, no significant differences were found among the extraction quantities used. Pooled plasma from Samples 1 and 2 were used as the standard in the actual assays. When the added testosterone quantities and plasma extraction volumes were observed together, assay volume of one ml was chosen (Gwazdauskas, 1980).

Statistical Analyses

The statistical analyses used to compare genetic combinations followed those described by Cook *et al.* (1972), which was a modified analysis for testing contrasts (Scheffe, 1959; 1970). The nonorthogonal linear contrasts (with unequal group numbers) for each trait are shown in Table 5. Contrasts 1, 2 and 3 evaluate pureline effects (additive genetic effects), 4, 5 and 6 evaluate

Table 4. Means and standard errors of testosterone assay validation procedures[1]

	Quantity of testosterone added (ng)	n	Quantity of added testosterone recovered
	0.5	12	0.38 ± 0.07
	1.0	13	0.82 ± 0.07
	2.0	14	1.67 ± 0.16
	Quantity of plasma extracted (ml)	**n**	**Quantity of testosterone (ng/ml)**
Sample 1	0.3	4	1.22 ± 0.12
	0.5	4	1.21 ± 0.12
	1.0	4	1.00 ± 0.13
	1.5	4	0.81 ± 0.15
Sample 2	0.3	4	2.00 ± 0.18
	0.5	4	2.22 ± 0.15
	1.0	4	1.99 ± 0.26
	1.5	4	2.31 ± 0.50

[1]Different plasma samples used for 1 and 2

maternal influences among males of reciprocal crosses, contrasts 7, 8, 9 and 11 evaluate nonadditive genetic effects while differences in contrast 10 may be due to nonadditive genetic effects and/or unequal initial gene frequencies. Differences among male progeny of reciprocal crosses are attributable to maternal effects (contrasts 4,5 and 6) and not sex linkage (Eisen *et al.*, 1966). This is because the homogametic (XX) males have comparable sex chromosomes in reciprocal crosses. Contrasts were calculated using all males, and using data where those males that did not complete any matings during the eight 10-minute observation periods were excluded. Hereafter, these males will be referred to as nonmaters.

Mating efficiency was considered as the ratio x 100 of courting, mounting, treading, and completed matings to the preceding behaviors in the sequence. Percentages were adjusted using the Freeman-Tukey arc sine transformation for binomial proportions with less than 50 observations (Mosteller and Youtz, 1961), and the frequency of courts, mounts, treads, and completed matings were transformed to square roots prior to analysis. Analyses of variance were conducted on behavioral and endocrine data with the inclusion of all males and with the exclusion of nonmaters. Chi-square analyses were used to compare the frequency of maters and nonmaters. Since data from a particular mating combination was used more than once, tau was set as 11 and 0.05 alpha level was determined from the tables presented by Jensen *et al.* (1968).

Table 5. Nonorthogonal linear contrasts used in analyzing data

Contrast Code	Contrast
1	HH - LL
2	HH - CC
3	LL - CC
4	HC - CH
5	HL - LH
6	CL - LC
7	(HH + CC) - (CH + HC)
8	(HH + LL) - (LH + HL)
9	(CC + LL) - (LC + CL)
10	2CC - (HH + LL)
11	2CC - (HL + LH)

RESULTS AND DISCUSSION

Body weight

As is shown in Tables 6 and 7, the LL males were significantly heavier at eight weeks of age than HH males (contrast 1) with those from the CC line significantly heavier than those from either selected line (contrasts 2 and 3). There were no significant differences between reciprocal crosses (contrasts 4, 5 and 6) indicating that maternal effects were not important, an observation consistent with those obtained for the S_{11} generation (Cook *et al.*, 1972). When reciprocal crosses were pooled and compared to midparent values, the crosses were heavier in all cases, with the difference of that involving the selected lines and their crosses being significant (contrast 8). This suggests that although there may be heterosis for juvenile body weight, the degree varies with the particular populations studied. This inconsistency in response among various crosses agrees with results obtained from the literature (e.g., Cook *et al.*, 1972; Malone *et al.*, 1980).

Testosterone

None of the contrasts revealed differences among mating combinations for plasma testosterone levels; an observation consistent whether or not nonmaters were included in the analyses (Tables 6 and 7). The circulating testosterone titers obtained in this experiment were lower than those obtained for the HH, LL and CC lines by Benoff (1977). Coefficients of variation for plasma testosterone levels

Table 6. Means and standard errors for body weight at eight weeks of age, testosterone levels and behaviors by mating combination[1]

Mating	N[2]	Body wt.	Testosterone (ng/ml)	% Maters	Number of Courts	Mounts	Treads	CNCM
HH	44 (11)	834 ± 12	2.5 ± 0.7	100	96 ± 3	31.2 ± 1.9	25.5 ± 1.5	24.4 ± 1.4
LL	77 (11)	900 ± 9	2.5 ± 1.1	16	29 ± 2	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
CC	63 (11)	974 ± 9	3.1 ± 1.3	82	69 ± 4	7.2 ± 0.8	5.8 ± 0.6	5.2 ± 0.6
HC	15 (13)	957 ± 16	2.0 ± 0.7	100	121 ± 9	32.3 ± 3.9	25.5 ± 3.6	23.1 ± 3.2
CH	13 (12)	858 ± 40	2.5 ± 0.8	100	131 ± 8	32.7 ± 5.7	26.6 ± 3.5	24.5 ± 3.3
HL	17 (15)	970 ± 12	1.6 ± 0.3	100	106 ± 5	24.0 ± 2.8	20.4 ± 2.3	18.9 ± 2.2
LH	14 (13)	973 ± 13	2.2 ± 0.7	93	75 ± 8	18.1 ± 3.8	15.4 ± 3.1	15.0 ± 3.0
LC	15 (15)	993 ± 28	2.8 ± 0.1	87	65 ± 7	7.4 ± 1.3	6.5 ± 1.2	5.8 ± 1.2
CL	15 (12)	966 ± 21	2.1 ± 0.6	93	83 ± 10	10.3 ± 2.3	8.7 ± 2.0	8.1 ± 1.8

[1]Values include maters and nonmaters
[2]Number in brackets is number of samples for testosterone assays

Table 7. Summary of Scheffe's analyses for linear contrasts[1] for body weight, testosterone and behaviors

	Contrast	Body wt.	Testos-terone	% Maters[2]	Courts	Mounts	Treads	CNCM
1	**HH** - **LL**	**LL**	--	**HH**	**HH**	**HH**	**HH**	**HH**
			--		*HH*	*HH*	*HH*	*HH*
2	**HH** - **CC**	**CC**	--	**HH**	**HH**	**HH**	**HH**	**HH**
			--		*HH*	*HH*	*HH*	*HH*
3	**LL** - **CC**	**CC**	--	**CC**	**CC**	**CC**	**CC**	**CC**
			--		*CC*	--	*CC*	*CC*
4	**HC** - **CH**	--	--	--	--	--	--	--
			--		--	--	--	--
5	**HL** - **LH**	--	--	--	--	--	--	--
			--		--	--	--	--
6	**CL** - **LC**	--	--	--	--	--	--	--
			--		--	--	--	--
7	(**HH** + **CC**) - (**CH** + **HC**)	--	--	--	**CH** + **HC**	**CH** + **HC**	**CH** + **HC**	**CH** + **HC**
			--		*CH* + *HC*	*CH* + *HC*	*CH* + *HC*	*CH* + *HC*
8	(**HH** + **LL**) - (**LH** + **HL**)	**HL** + **LH**	--	**HL** + **LH**	**HL** + **LH**	**HL** + **LH**	**HL** + **LH**	**HL** + **LH**
			--		--	--	--	--
9	(**CC** + **LL**) - (**LC** + **CL**)	--	--	**LC** + **CL**	**LC** + **CL**	**LC** + **CL**	**LC** + **CL**	**LC** + **CL**
			--		--	--	--	--
10	2**CC** - (**HH** + **LL**)	**CC**	--	**CC**	**CC**	**HH** + **LL**	**HH** + **LL**	**HH** + **LL**
			--		*CC*	*HH* + *LL*	*HH* + *LL*	*HH* + *LL*
11	2**CC** - (**HL** + **LH**)	--	--	--	**HL** + **LH**	**HL** + **LH**	**HL** + **LH**	**HL** + **LH**
			--		*HL* + *LH*	*HL* + *LH*	*HL* + *LH*	*HL* + *LH*

[1]All birds in bold type, while those for maters only in italics
[2]Contrast was made using actual numbers

were quite high, with values ranging from 0.04 to 12.66 ng/ml. These values bracketed the range of 0.84 to 7.83 ng/ml obtained by liquid gas chromatography procedures for a different population of cockerels by Furr and Thomas (1970).

Correlations of testosterone with the number of courts and CNCMs were calculated within each mating combination. None of the correlations were significant except those involving the CH mating combination (Table 8). Correlations were tested and found to be homogeneous (Snedecor, 1946). Since nonmaters were included in the correlations, and the radioimmunoassay showed all birds to have circulating testosterone, it appears that the threshold testosterone level (McCollum *et al.*, 1971) was reached in these populations and that differences in courting and CNCMs are due to other factors.

Behavioral traits

Maters vs nonmaters. The percentage of males that completed matings were 100, 82 and 16 for the HH, CC and LL purelines, respectively (Table 6), with the differences being significant for each pureline comparison as shown by contrasts 1, 2 and 3 in Table 7. There were no significant differences between reciprocal crosses for percentage maters (contrasts 4, 5 and 6), indicating that maternal effects are not important. The proportions of males from the reciprocal crosses of the selected lines that mated were significantly greater than the midparent values in two of three comparisons (contrasts 7, 8 and 9), indicating significant heterotic effects (see Figure 1). The significant difference between

Table 8. Correlations of testosterone levels with courting and CNCMs by mating combinations

Mating	Correlations between testosterone and Courts	CNCM
HH	0.15	0.07
LL	-0.53	0.00
CC	-0.09	-0.06
HC	-0.21	0.05
CH	0.62*	0.66*
HL	-0.21	0.14
LH	-0.24	-0.11
LC	-0.05	0.07
CL	-0.25	0.10

*($P \leq 0.05$)

the CC males and the mean value for the selected lines (contrast 10) lend credence that we are dealing with a threshold trait. Assuming a continuum for mating frequency after the threshold is reached, the lower average for the selected line than for the unselected control line may be attributed to the large number of nonmating LL males. Accordingly, this effect would not be expected (and was not seen) in contrast 11, which involved a comparison of the control line to the reciprocal crosses of the selected lines.

Courts, mounts, treads and CNCMs. HH males completed significantly more courts, mounts, treads and CNCMs than LL and CC males (contrasts 1 and 2) regardless of whether or not nonmaters were included in the analyses (Table 7). Also, CC males courted, mounted, treaded, and mated significantly more than LL males (contrast 3) when nonmaters were included in the analysis. When nonmaters were excluded from the analysis, the difference existed only for courts and not for the other behaviors. The significant differences among the selected and control lines for these behaviors indicate that individual phenotypic selection for CNCMs was effective. Evidence for additive genetic variation for high and low mating behavior may be seen from the high mating line having consistently greater values than the control line with the latter values in turn being consistently larger than those from the low line, and are consistent with the results of Siegel (1972).

No significant differences were noted between reciprocal crosses

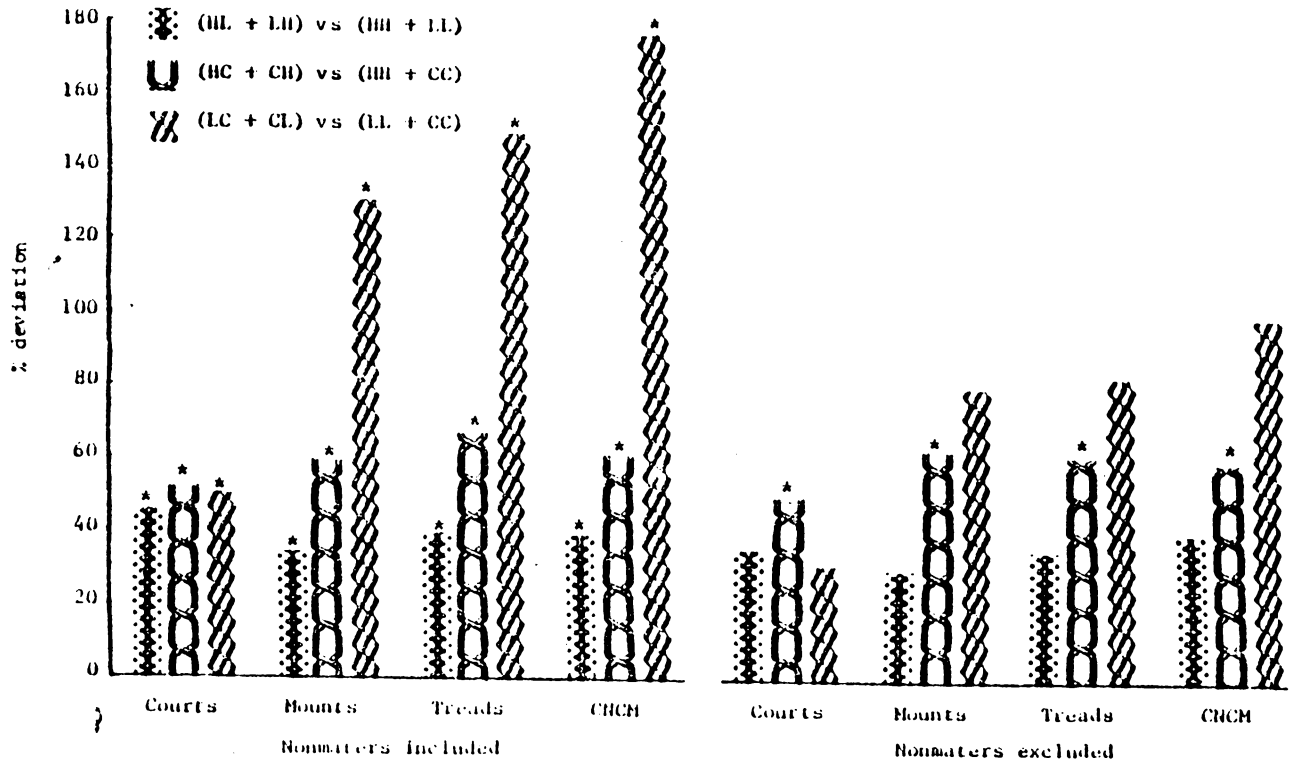


Figure 1. Percentage heterosis expressed as the deviation of crosses from their midparent mean (*$P \leq 0.05$)

(contrasts 4, 5 and 6) regardless of whether or not nonmaters were included in the analysis. Although the crosses had significantly more courts, mounts, treads and CNCMs than their midparent values in all comparisons (contrasts 7, 8 and 9) when nonmaters were included in the analysis, this difference was only significant for contrast 7 when nonmaters were excluded. In contrasts 8 and 9, the elimination of the large number of LL nonmaters served to increase the value of the midparent means to such an extent as to eliminate significant differences in these comparisons. In all comparisons involving possible heterosis, the means for the crosses were at least 30 percent greater than those for their respective midparent values (Figure 1), suggesting nonadditive gene action for these behaviors.

When the midline value of the selected lines was compared to that of the randombred base population (CC) in contrast 10, the CC line was significantly greater for courts and less for mounts, treads and CNCMs. This pattern existed whether or not nonmaters were included and supports the thesis that the response to selection was asymmetrical. Such could result from nonadditive genetic effects and/or unequal initial gene frequencies in the P_1 generation. Further evidence for heterotic effects may be seen in contrast 11, where the midline means were greater for the crosses of the selected lines than for the control population.

The results obtained here differ from those obtained in the S_{11} generation (Cook *et al.*, 1972) where the values for the mating

traits of crosses between the HH and LL lines were significantly lower than their respective midparent values. Now, after an additional ten generations of selection, the progeny of the high and low crosses and low and control crosses exhibited frequencies of mating behavior that were significantly larger than their midparent means. This may be the effect of changing the threshold for mating behavior in the selected lines. The higher values of crosses suggest that high sexual threshold in the low line was overridden in the crosses by some heterotic effect which allowed the expression of increased mating behavior. The evidence of a heterotic effect for increased mating frequency allows for speculation that genes for a low mating threshold are recessive to those for high mating.

The completion of the mating act is based on a sequence of behaviors, and mating efficiency may be considered as the ratio of a behavior to those preceding it. When comparisons were made for these ratios (Table 9), the only ones which were significant were those where courts were the denominator. This is not surprising in view of the very high correlations between mounts and treads with CNCMs (Siegel, 1972). HH males had a significantly greater mating efficiency than LL and CC males (contrasts 1 and 2) as measured by mounts:courts, treads:courts and CNCMs:courts. This occurred whether or not nonmaters were included in the analyses. The mating efficiency of CC males was significantly greater than that of LL males (contrast 3) only when nonmaters were included in the comparison.

Table 9. Summary of Scheffe's analyses for linear contrasts for mating efficiency

		Ratio x 100					
	Contrast	Mounts / Courts	Treads / Courts	CNCM / Courts	Treads / Mounts	CNCM / Mounts	CNCM / Treads
1	HH - LL	**HH**	**HH**	**HH**	--	--	--
		HH	*HH*	*HH*	--	--	--
2	HH - CC	**HH**	**HH**	**HH**	--	--	--
		HH	*HH*	*HH*	--	--	--
3	LL - CC	**CC**	**CC**	**CC**	--	--	--
		--	--	--	--	--	--
4	HC - CH	--	--	--	--	--	--
		--	--	--	--	--	--
5	HL - LH	--	--	--	--	--	--
		--	--	--	--	--	--
6	CL - LC	--	--	--	--	--	--
		--	--	--	--	--	--
7	(HH + CC) - (CH + HC)	--	--	--	--	--	--
		--	--	--	--	--	--
8	(HH + LL) - (LH + HL)	**HL + LH**	**HL + LH**	**HL + LH**	--	--	--
		--	--	--	--	--	--
9	(CC + LL) - (LC + CL)	**LC + CL**	**LC + CL**	**LC + CL**	--	--	--
		--	--	--	--	--	--
10	2CC - (HH + LL)	--	--	--	--	--	--
		--	**HH + LL**	--	--	--	--
11	2CC - (LH + LH)	**HL + LH**	**HL + LH**	**HL + LH**	--	--	--
		HL + LH	*HL + LH*	*HL + LH*	--	--	--

[1]All birds in bold type, while those for maters only in italics

There were no differences between reciprocal crosses (contrasts 4, 5 and 6). Contrasts 8 and 9 were significant for mounts:courts, treads:courts and CNCMs:courts when nonmaters were included but not when they were omitted from the analysis, while contrast 11 was significant regardless of whether or not nonmaters were included. In only one comparison (treads:courts, when nonmaters were excluded) was contrast 10 significant. No other significant differences were observed.

The significant positive differences in contrast 1 (HH vs LL) and contrast 2 (HH vs CC) and the significant negative difference in contrast 3 (LL - CC) indicate that the HH males carried courtship through to a completed mating more often than either LL or CC males. Furthermore, courting behavior resulted in a completed mating more often in CC than in LL males. When nonmaters were excluded from the calculations, however, no significant differences existed between CC and LL males indicating that some LL males did carry through the complete mating behavior. Since the only ratios which were significant were those in which courts were the denominator, suggests that once a mount occurs there is a high probability that the mating will be completed, regardless of the population. Since courting initially occurs as an aggressive act and then switches to a sexual act (Siegel, 1959b;Kruijt, 1966), one could expect genetic differences among populations for the incidence of this switching.

The results presented here suggest that the threshold for

low mating frequency has been lowered or overridden by high mating frequency genes in the high mating lines and that the primacy of the inhibitory mating center to the stimulatory mating center that was proposed by Van Krey *et al.* (1977) may not be exerting its full effect. An increase in heterotic expression of genes affecting high mating frequency after the neural threshold has been attained is a possible mechanism for this. If these genes result in an increase of the proportion of courts that turn from aggressive to sexual behavior, the increase in CNCMs which occurred in the hybrids would be expected.

Whereas behavioral differences were found between the selected lines and crosses between them, there were no significant differences in plasma testosterone levels. Also, correlations between circulating testosterone and the behaviors were low. This suggests the behavioral differences noted are due to differences in brain activity control that occur at normal physiological levels of testosterone.

SUMMARY

The mode of inheritance of mating behavior and blood testosterone levels were examined in lines of chickens selected for high and low mating frequency and the randombred population from which the selected lines originated. Comparisons involved purelines and crosses between them. Circulating plasma testosterone levels

appeared to be of a sufficient magnitude to preclude its effect on the behaviors measured. Males from the line selected for high mating frequency exhibited a larger number of courts, mounts, treads and completed matings than either control or low line males. The mean for the control line was intermediate and significantly different from those of the selected lines. Means for crosses were significantly larger than their pureline midparent means, indicating a heterotic effect. It appears that the threshold for mating has been lowered or maybe overridden by genetic variation for increased mating frequency in the HH line and the line crosses. If courting turns from an aggressive to a sexual behavior, then the observed increase in mating would be expected in these mating combinations.

EXPERIMENT III

Electroencephalographic aspects of mating behavior in chickens.

INTRODUCTION

Genetic and phenotypic variation for mating behavior of chickens were described in Experiment II. Such variation is not independent of influences by the central nervous system on physical activities. A hypothesis of brain involvement with mating behavior through an inhibitory (IMC) and a stimulatory mating center (SMC), with the former dominant to the latter, was proposed for chickens by Van Krey *et al.* (1977). Subsequently, Benoff *et al.* (1978a) observed an equality of receptor site and/or effector response in the hypothalamus from lines of chickens that had undergone divergent selection for mating frequency (Siegel, 1965; 1972).

Brain activities associated with the allele for epilepsy was described for chickens by Crichlow and Crawford (1974). The resting electroencephalographic (EEG) pattern of epileptic chickens is markedly different from that of either normal or carrier birds, and epileptic males are frequently unable to naturally complete the mating act because of seizures caused by excitation (Crawford, 1980). Although it is well documented that visual stimuli are important in the courtship behavior of birds, direct visual stimulation may not be necessary in chickens since surgically blinded cocks and hens exhibit sexual

behavior (Ookawa, 1970; 1979).

The experiment reported here was designed to measure EEG patterns when males from lines of chickens, known to be widely diverse in mating behavior, were exposed to females.

MATERIALS AND METHODS

Males from the S_{20} generation of lines selected for high (HML) and low (LML) cumulative number of completed matings and the randombred control (AC) population from which the selected lines were developed were used in this experiment. Descriptions of these lines and the selection procedures used to produce them were provided by Siegel (1965; 1972). The number of males used was 3, 2 and 3 for the HML, LML and AC lines, respectively.

Each male was implanted with five electrodes. Two were located in the cortical area (one over the left and the other over the right hemisphere), two were placed over the optic lobes (one over the left and the other one over the right optic tectum), the fifth electrode which was the reference electrode was implanted as far posteriorly as possible. For implantation of electrodes, each cock was anesthetized with sodium pentobarbital and placed with his head in a stereotaxic instrument. The scalp was incised and two holes for the cortical implants were drilled equidistant from the midline in the foreregion of the skull; two other holes for implants in the optic tectum were drilled equidistant from the midline at the rear of the optic lobes. The site for the reference electrode was a fifth hole located

as far posteriorly as possible. Insulated brass electrodes were implanted with acrylic dental cement in the skull such that the exposed tip was in contact with the surface of the brain menages. Following implantation, the scalp was stitched closed and the birds returned to their cages.

After a recovery period of approximately two weeks, each cock was placed in a wooden retainer measuring 28 cm wide, 59 cm long and 38 cm high within a copper shielded crate measuring 2.8 x 2.8 x 2.8 m (See Appendix Figures 1 and 2). This procedure was repeated twice on different days to allow for adjustment of males to the test situation. The adjustment trials included exposing the male to the entire testing apparatus including being attached to recording wires that were connected to a Narco Desk Model DMP-4B physiograph (see Appendix Figure 3).

Monopolar recordings were obtained for each male in each of the following three test situations: (1) just prior to exposing the male to a female, (2) while the female was present and, (3) after the female was removed. Brain activities from each electrode were recorded on three separate days during the preexposure, exposure and postexposure periods. The hens were in a restrained sexually receptive posture when presented to the cocks (see Appendix Figure 4).

The EEG recordings were measured as the number of positive and negative peaks, and the voltage movement for each peak. Data for each male were adjusted to a 60-second time period for statistical analysis. The statistical model used was:

$$Y_{ijkl} = \mu + A_i + E_j + (AE)_{ij} + L_k + (AL)_{ik} + (EL)_{jk} + (AEL)_{ijk} + e_{ijkl}$$

where Y_{ijkl} is the mean of three observations for the lth individual from the kth line (L) during the jth exposure (E) period from the ith electrode location (A), μ is the population mean, and e_{ijkl} is random error. Main effects were considered as fixed. When significant differences were found means were compared using Duncan's multiple range test.

RESULTS

Location

There were highly significant differences among electrode implantation locations for the number of positive peaks and the combined number of positive and negative peaks, but not for the number of negative peaks (Table 10). The greatest number of positive peaks were from the right optic tectum, the least from the left optic tectum, with the left and right cortical areas being intermediate to these extremes and not significantly different from each other (Table 11). No significant differences were found among locations for voltage measurements (Tables 10 and 11).

Exposure periods

No significant differences were found among exposure situations for the frequency of positive and negative peaks (Table 10) with both following the same general pattern. When the number of positive and negative peaks were combined there was a significant difference (Table 10) with the mean being greater for the preexposure period than for

the other two periods (Table 12). Comparisons among exposure periods for voltages revealed no significant differences among them (Tables 10 and 12).

Lines

Although there were no significant differences among lines for the number of positive peaks, males from the control line had significantly fewer negative peaks than those from either of the selected lines which did not differ from each other (Tables 10 and 13). Furthermore, highly significant differences were found among lines for voltages (Table 10). Each line was significantly different from each other with the lowest voltage for the low line, the intermediate voltage for the high line and the greatest for the control line (Table 13).

Interactions

First and second order interactions were not significant with a single exception; implant location x line when the measurement was the combined number of positive and negative peaks (Table 10). Accordingly, an analysis was made to measure differences among lines within each implant location. As shown in Table 14, there was a lack of parallelism, with differences among lines being significant for the right cortical area but not for the other electrode locations.

DISCUSSION

The pattern whereby a lower number of peaks is associated with

Table 10. Analyses of variance for EEG measurements

Source of Variation	df	Mean Square: Peak Number: Positive	Peak Number: Negative	Peak Number: Combined	Voltage: Positive	Voltage: Negative	Voltage: Combined
Exposure (E)[1]	2	379	878	2411*	0.8	0.2	0.1
Location (A)[2]	3	2436**	205	3660**	4.0	2.8	3.4
E x A	6	433	74	814	1.6	0.6	0.7
Line (L)	2	693	2197**	2739*	40.9**	26.4**	35.1**
E x L	4	63	173	166	1.0	0.7	0.9
A x L	6	396	493	1666*	4.2	1.9	2.8
E x A x L	12	243	52	228	0.7	0.9	0.5
Error	56	348	325	658	2.5	1.6	1.8

[1]Exposure is the period of exposure to the female (before, during, after)
[2]Left cortical area, right cortical area, left optic tectum, right optic tectum
*($P \leq 0.05$)
**($P \leq 0.01$)

Table 11. Means[1] and standard errors for EEG measurements by electrode location

Measurement	Cortical area		Optic tectum	
	Left	Right	Left	Right
Numbers of peaks				
Positive	43.3 ± 5.4[b]	42.1 ± 3.2[b]	31.3 ± 2.0[a]	55.9 ± 3.3[c]
Negative	40.3 ± 3.3[a]	44.4 ± 4.2[a]	37.9 ± 4.2[a]	43.3 ± 3.3[a]
Voltage (mv)				
Positive	2.2 ± 0.5[a]	2.1 ± 0.3[a]	2.4 ± 0.4[a]	1.4 ± 0.2[a]
Negative	1.8 ± 0.3[a]	1.7 ± 0.2[a]	2.4 ± 0.4[a]	1.7 ± 0.3[a]
Combined[2]	2.0 ± 0.4[a]	1.9 ± 0.3[a]	2.4 ± 0.4[a]	1.5 ± 0.3[a]

[1]Means in the same row with the same superscript are not significantly different ($P \leq 0.05$)

[2]Combined voltage is the mean voltage per peak of positive and negative peaks

Table 12. Means[1] and standard errors for EEG measurements by exposure period

Measurement	Exposure		
	Pre	During	Post
Number of peaks			
Positive	47.4 ± 4.2[a]	41.5 ± 2.8[a]	40.2 ± 3.7[a]
Negative	47.6 ± 2.9[a]	39.0 ± 3.0[a]	37.6 ± 3.7[a]
Combined[2]	95.0 ± 5.4[b]	80.5 ± 5.0[a]	77.8 ± 4.8[a]
Voltage (mv)			
Positive	1.8 ± 0.2[a]	2.1 ± 0.3[a]	2.1 ± 0.4[a]
Negative	2.0 ± 0.3[a]	1.9 ± 0.3[a]	1.9 ± 0.3[a]
Combined[3]	1.9 ± 0.2[a]	2.0 ± 0.3[a]	2.0 ± 0.3[a]

[1]Means in the same row with the same superscript are not significantly different ($P \leq 0.05$)

[2]Combined peak numbers is the addition of positive and negative number of peaks

[3]Combined voltage is the mean voltage per peak of positive and negative peaks

Table 13. Means[1] and standard errors and number of tracings for EEG measurements by line

Measurement	Line		
	HM	AC	LM
Number of tracings	24	36	32
Number of peaks			
Positive	37.4 ± 2.5[a]	43.4 ± 3.9[a]	47.1 ± 3.7[a]
Negative	47.3 ± 2.2[b]	32.8 ± 2.9[a]	47.0 ± 3.6[b]
Voltage (mv)			
Positive	2.1 ± 0.3[b]	3.0 ± 0.1[c]	0.8 ± 0.3[a]
Negative	1.9 ± 0.2[b]	2.7 ± 0.1[c]	1.0 ± 0.3[a]
Combined[2]	2.0 ± 0.2[b]	2.9 ± 0.3[c]	0.9 ± 0.1[a]

[1]Means in the same row with the same superscript are not significantly different ($P \leq 0.05$)

[2]Combined voltage is the mean voltage per peak of positive and negative peaks

Table 14. Means[1] and standard errors of total number of peaks (positive plus negative) where the implant location x line interaction was significant

Location	Line		
	HML	AC	LML
Left cortical area	75.7 ± 4.0[a]	79.9 ± 8.7[a]	99.6 ± 14.7[a]
Right cortical area	74.9 ± 6.4[a]	67.5 ± 7.9[a]	113.2 ± 14.8[b]
Left optic tectum	84.8 ± 7.9[a]	57.0 ± 7.2[a]	70.8 ± 7.1[a]
Right optic tectum	103.7 ± 7.4[a]	100.0 ± 8.9[a]	95.1 ± 5.2[a]

[1]Means in the same row with the same superscript are not significantly different ($P \leq 0.05$)

a higher output of voltage is consistent with the negative relationship of low-voltage fast-activity EEGs in normal chickens (Crichlow and Crawford, 1974). Such a negative relationship may be observed in Figure 2, which shows EEG patterns for the HML, AC and LML lines during different exposure periods.

Models have been proposed to describe the mechanisms involved with the mating behavior of various organisms (Toates, 1980). Van Krey *et al.* (1977) presented a model for the domestic fowl that included genetic components. They proposed that there are two centers, one inhibitory (IMC) and the other stimulatory (SMC) with the former being dominant and having a tonic inhibitory influence on the latter. Thus, selection for low mating frequency would decrease the neural thresholds acting through the IMC which would neither be dependent upon nor affected by gonadal hormones. Under this model, selection for high mating frequency would affect the frequencies of alleles at loci operative after the neural thresholds were attained. This reasoning is in agreement with observations that administration of exogenous testosterone does not stimulate mating activity above that of gonadally intact birds and mammals. The Van Krey *et al.* (1977) model is consistent with the controls suggested by Lisk (1966) whereby the mammillary region of the brain contains an inhibitory center and that overt sexual behavior depends upon a balance of activity between a facilitory system and a regulatory inhibitory system.

Experiment II of this dissertation showed that although the selected lines did not differ significantly in blood testosterone levels, males

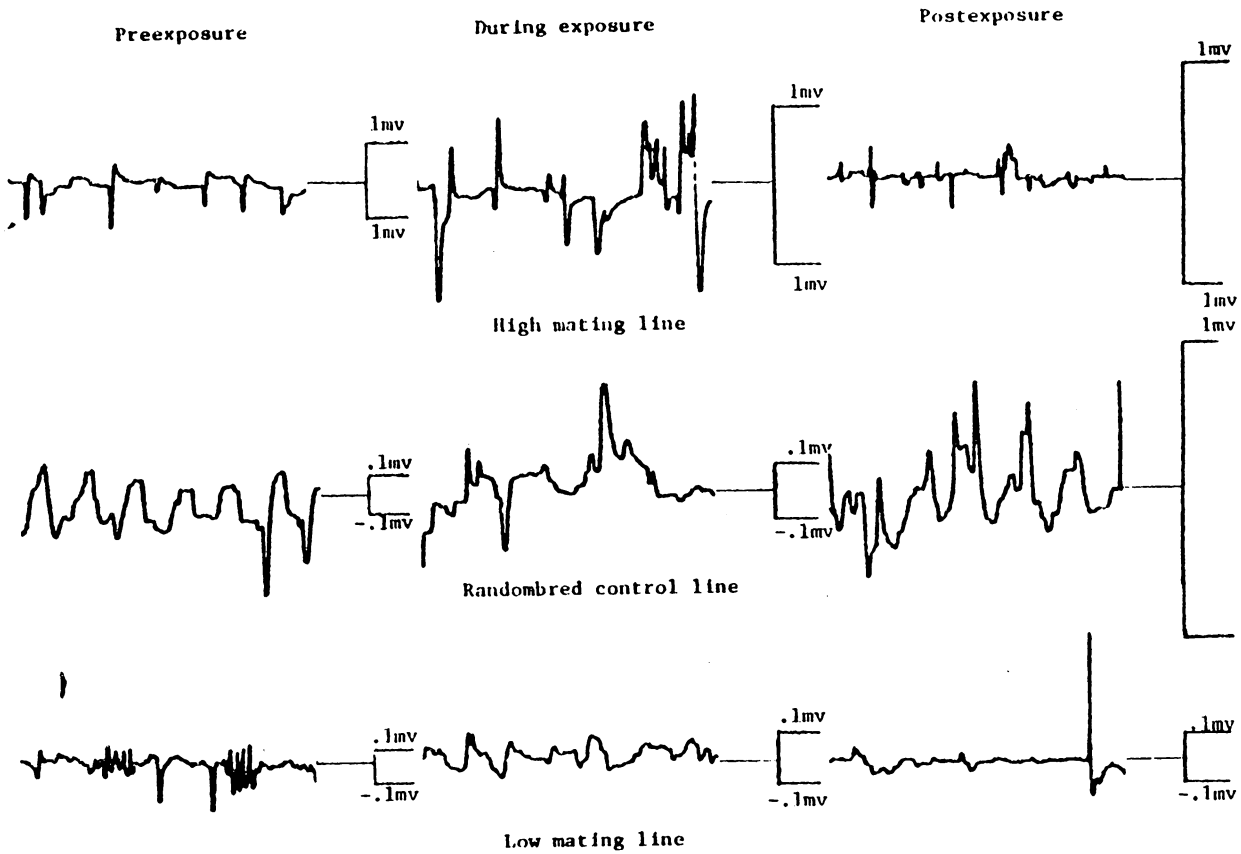
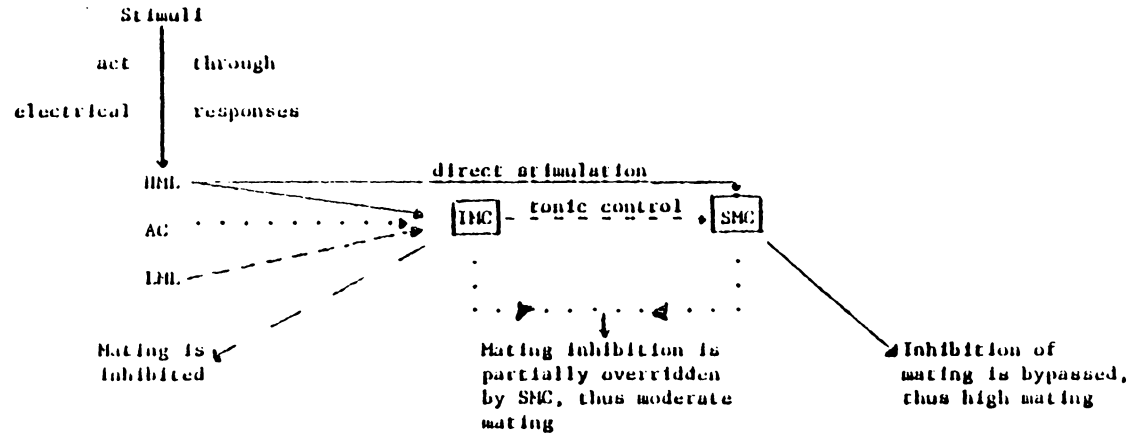


Figure 2. Fifteen sec EEG tracings of HML, AC and LML males before, during and after exposure to a receptive positioned female. The scale for the Y axis are graduated as ± 1 mv or .1 mv.



HML, AC and LML refer to the high mating, unselected, and low mating lines, respectively.

IHC and SMC refer to the inhibitory and stimulatory mating centers, respectively.

Figure 3. A model for brain electrical activity in lines of chickens that are widely diverse for mating behavior.

from the HML had significantly more courts, mounts, treads and completed matings than those from the LML and the AC populations, with the values for the latter being significantly greater than those for the LML males. Thus, factors other than testosterone must be exerting an effect on mating behavior in these lines. The differences in EEGs among lines and the inverse relationship between voltage and number of peaks support, in general, the IMC-SMC hypothesis. This support is outlined in Figure 3. Assuming that the IMC dominates the SMC and that selection has lowered the IMC threshold in the LML, then the lower voltage observed in the LML males would stimulate the IMC and suppress mating behavior. The higher voltage for the HML males in a situation where selection has increased the IMC threshold and lowered the SMC threshold would result in increased mating frequency. In a population such as the unselected control line a higher voltage may be necessary to override the active IMC via an increased stimulation of the SMC.

SUMMARY

Electroencephalographic patterns of males from lines of chickens known to be widely diverse in mating behavior when exposed to females were measured. The right optic tectum area of the brain had significantly more peaks than the left optic tectum (least peaks) or the cortical areas, indicating that visual input may play a role in the bird. The number of peaks were significantly higher before exposure to females

than during or after exposure, indicating a lower voltage relaxed state before the exposure turned to an excited state during and after exposure. The patterns observed were interpreted that if an inhibitory mating center (IMC) dominates a stimulatory mating center (SMC), the low voltage in the low mating line males may act upon an IMC with a low threshold, thus suppressing mating behavior. The higher voltage, lower number of peaks for males from the high mating line would act upon an IMC with a high threshold and may also influence the SMC directly. The high voltages in the unselected control males may be required to override an active IMC.

EXPERIMENT IV

Genetics of mating behavior in Japanese quail.

INTRODUCTION

There is voluminous literature on the genetics, behavior and physiology of reproduction of insects and vertebrates with a modest body of data having been reported for Japanese quail (*Coturnix coturnix japonica*). Kovach (1974) summarized the literature on the behavior of Japanese quail, and Truax (1979) recently reviewed the literature on sexual behavior in this species. Japanese quail are polygamous under domestic conditions (Wetherbee, 1961) and although novelty is not important in the sexual performance of male Japanese quail, there is a shorter latency period to "neck grab" the replacement females suggesting that males distinguish between females (Schein and Carter, 1972). Schein *et al.* (1972) measured the role of the Coolidge effect on the sexual performance of male Japanese quail and found that experience had a positive influence on sexual performance.

Sexual activity of male quail is present at an early age. It reaches a peak at about 70 days of age and remains at this high level until about 210 days, after which it declines (Sefton and Siegel, 1973). The onset of androgen dependent behaviors in quail occurs sequentially, with crowing beginning at about 33 days, mating attempts at about 34 days and actual mating

at about 37 days of age (Ottinger and Brinkley, 1979). This temporal sequence is consistent with that noted in the fowl (Noble and Zitrin, 1942). In contrast, the correlated response of relative aggressiveness to selection for mating activity which is positive in the Japanese quail (Cunningham and Siegel, 1978) is essentially zero in the chicken (Siegel, 1972). This difference between chickens and quail may be due to differences in sexual dimorphism of body weight; male Japanese quail being smaller than females and male chickens being larger than females.

Genetic variation for mating behavior in Japanese quail was reported by Mahn (1968), Sefton (1972), Sefton and Siegel (1975) and Cunningham and Siegel (1978). The experiment reported here was designed to investigate further the genetic architecture of mating behavior in Japanese quail that had been divergently selected for mating frequency.

MATERIALS AND METHODS

The populations involved in this experiment consisted of replicated lines that had been divergently selected for high and low mating frequency and the randombred control which served as the base population for the selected lines (Cunningham and Siegel, 1978). Matings, within replicates, involved the production of the purelines and F_1, F_2 and backcross generations. The initial matings consisted of S_{21} generation parents that were

mated to produce the purelines and the F_1 generation crosses. These chicks were wingbanded and reared as sex-intermingled flocks in brooder batteries until three weeks of age when they were transferred to grower batteries. Sexes were separated at four weeks of age and males remained in unisexual flocks until placed in individual cages at 10 weeks of age. Feed and water were provided *ad libitum* and lighting was continuous. When approximately 16 weeks of age, the quail were mated to produce the F_2 and backcross generations, concomitant with producing a repeat of the previous matings. The mating design is presented in Table 15.

Body weights were obtained at four and eight weeks of age and mating behavior data were obtained between 13 and 16 weeks of age following the procedure of Sefton and Siegel (1975). Briefly, the procedure for measuring mating behavior consisted of releasing a male singly into a cage of three females for an 8-minute period and recording the number of completed matings. The male was then returned to a home cage. The procedure was repeated eight times and the cumulative value was considered as the observation for the male. The size of the cloacal glands were measured as width x height in mm on the day following the last mating trial. Relative aggressiveness, expressed as percentage of encounters won, was measured following the procedure outlined by Sefton and Siegel (1975).. It consisted of eight random

Table 15. Mating combinations within a replicate

Pure line	F_1	F_2	Backcross[1]
H x H	H x L	HL x HL	HL x L
	H x C	HC x HC	HC x C
L x L	L x H	LH x LH	LH x H
	L x C	LC x LC	LC x C
C x C	C x H	CH x CH	CH x H
	C x L	CL x CL	CL x L

[1]In combinations the male line is expressed first and the female line second

initial paired encounters in neutral cages with the subordinate male of each pairing considered as the loser and the dominant male as the winner. These data were obtained the day following the day which the cloacal glands were measured. Data for the proportion of wins were adjusted using the Freeman-Tukey arc sine transformation for binomial proportions with less than 50 observations (Mosteller and Youtz, 1961).

Comparisons among mating combinations were made using nonorthogonal linear contrasts (Scheffe, 1959; 1970). The specific contrasts and genetics effects measured (Cook *et al.*, 1972; Eisen, 1973) are outlined in Tables 16 and 17. Since all matings used to produce the backcrosses involved F_1 males on pureline females, the effect of a hybrid mother was precluded. Contrasts 1, 2 and 3 measured pureline effects (additive genetic effects), contrasts 4-6 and 15-17 maternal effects, contrasts 7-9 and 11 nonadditive effects, contrast 10 nonadditive effects and/or gene frequency differences, and contrasts 12-14 and 18-26 recombination effects. Contrasts were made separately within each replication.

RESULTS AND DISCUSSION

Sample sizes plus means and standard errors are presented by mating combinations and replicates in Table 18 for body weights at 4 and 8 weeks of age and in Table 19 for CNCMs, cloacal gland sizes and relative aggressiveness. Comparisons, as made by nonorthogonal linear contrasts, are presented in Table 20.

Table 16. Nonorthogonal linear contrasts used in analyzing quail data

Contrast Code	Contrast
1	HH - LL
2	HH - CC
3	LL - CC
4	HC - CH
5	HL - LH
6	CL - LC
7	(HH + CC) - (CH + HC)
8	(HH + LL) - (LH + HL)
9	(CC + LL) - (LC + CL)
10	2CC - (HH + LL)
11	2CC - (HL + LH)
12	(HH + HL + LH + LL) - 2(HLHL) + 2(LHLH)
13	(HH + HC + CH + CC) - 2(HCHC) + 2(CHCH)
14	(CC + CL + LC + LL) - 2(LCLC) + 2(CLCL)
15	(HLHL) - (LHLH)
16	(HCHC) - (CHCH)
17	(LCLC) - (CLCL)
18	(HL + LH) - (HLHL) + (LHLH)
19	(CL + LC) - (CLCL) + (LCLC)
20	(CH + HC) - (CHCH) + (HCHC)
21	(LH + HH) - 2(LHHH)
22	(HL + LL) - 2(HLLL)
23	(CH + HH) - 2(HCCC)
24	(HC + CC) - 2(HCCC)
25	(CL + LL) - 2(CLLL)
26	(LC = CC) - 2(LCCC)

Table 17. Expected contribution of genetic effects to the mean of each line[1]

Type	Line	Genetic effects[2]			
		g	m	s	r
Parental	P_i	P_i	P_i	0	0
	P_j	P_j	P_j	0	0
F_1	P_iP_j	$(P_i + P_j)/2$	P_j	P_iP_j	0
	P_jP_i	$(P_i + P_j)/2$	P_i	P_iP_j	0
F_2	$(P_iP_j)_2$	$(P_i + P_j)/2$	$(P_i + P_j)/2$	$(P_iP_j)/2$	P_iP_j
	$(P_jP_i)2$	$(P_i + P_j)/2$	$(P_i + P_j)/2$	$(P_iP_j)/2$	P_iP_j
Backcross	P_iP_j (P_j)	$(3P + P_j)/4$	$(P_i + P_j)/2$	$(P_iP_j)/2$	$(P_iP_j)/2$
	P_jP_i (P_i)	$(P_i + 3P_j)/4$	$(P_i + P_j)/2$	$(P_iP_j)/2$	$(P_iP_j)/2$

[1]Adapted from Eisen (1973)

[2]Letter designation of effects

g - average direct genetic effect of parental line

m - average maternal effect

s - heterosis for direct effects in F_1 cross due to increased average homozygosity and epistasis

r - additional epistasis for direct effects in F_2 individuals due to gametic redistribution of chromosomes from the parental lines

Body weight

Males from the high mating line were significantly heavier at four weeks of age than those from the control and low mating lines in replicate 1, but not replicate 2 (contrasts 1 and 2). Evidence for maternal (contrasts 4-6 and 15-17) and nonadditive genetic effects (contrasts 7-11) were lacking in both replicates, and recombinant effects were indicated in only three cases (contrasts 20, 21 and 23, replicate 1). Means are presented in Table 18.

When comparisons were made for body weight at eight weeks of age, males from the high and control lines were significantly heavier than those for the low line (contrasts 1 and 3) in replicate 2, but not in replicate 1. As with body weight at four weeks of age, maternal, nonadditive and recombination effects were not in evidence.

Large maternal effects for body weight are expected at early ages in the Japanese quail. This is because the correlation between egg weight and the weight of the chick at hatching ranges from .72 (Ghany *et al.*, 1966) to .80 (Sefton and Siegel, 1973). The results from this dissertation support those of Sefton and Siegel (1974) that these maternal effects have largely dissipated by four weeks of age. The general lack of heterosis and recombinant effects infer that variation in body weight of these lines of Japanese quail is primarily due to additive genetic effects. This observation is consistent with those of Marks (1971), Blow and Briggs (1973) and Marks (1979).

Table 18. Number of observations, means and standard errors of four and eight week body weight by mating combination and replicate

Mating Combination	N		Body weight (g)			
			4 weeks		8 weeks	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
L x L	49	42	82.6 ± 0.8	83.9 ± 1.2	106.6 ± 0.9	102.1 ± 1.1
H x H	48	35	92.4 ± 1.2	87.3 ± 1.2	113.3 ± 1.4	110.3 ± 1.0
C x C	97	97	83.4 ± 0.8	83.4 ± 0.8	108.4 ± 0.8	108.4 ± 0.8
H x L	13	14	85.7 ± 1.7	78.2 ± 2.1	109.8 ± 1.6	108.7 ± 1.6
H x C	14	13	89.0 ± 1.4	88.2 ± 1.6	113.4 ± 1.7	111.8 ± 2.8
L x H	13	14	80.0 ± 2.2	82.3 ± 1.1	106.4 ± 2.1	107.5 ± 1.4
L x C	14	14	81.9 ± 1.1	87.7 ± 0.8	107.0 ± 1.5	108.7 ± 1.2
C x H	14	13	97.4 ± 3.3	87.6 ± 1.9	117.8 ± 1.7	110.2 ± 2.1
C x L	15	12	79.6 ± 1.6	82.3 ± 1.4	101.3 ± 2.0	103.5 ± 1.5
HL x HL	14	13	73.6 ± 5.7	81.7 ± 0.9	122.9 ± 8.5	107.9 ± 1.3
HC x HC	14	12	87.5 ± 2.1	86.4 ± 2.5	108.0 ± 1.6	103.2 ± 7.4
LH x LH	13	12	75.7 ± 2.6	80.4 ± 1.8	104.3 ± 1.6	100.9 ± 2.2
LC x LC	13	16	76.2 ± 2.1	85.3 ± 1.0	98.4 ± 1.6	107.4 ± 2.0
CH x CH	14	14	73.8 ± 2.1	76.1 ± 1.4	95.6 ± 2.1	102.4 ± 1.6
CL x CL	14	13	82.0 ± 1.7	77.5 ± 2.2	106.1 ± 7.5	102.1 ± 1.9
HL x LL	14	13	85.1 ± 1.3	81.2 ± 1.2	112.6 ± 1.3	98.0 ± 1.4
HL x CC	4	13	82.5 ± 2.1	84.4 ± 2.1	105.0 ± 3.9	107.2 ± 1.6
LH x HH	14	14	79.4 ± 2.2	84.0 ± 2.4	105.1 ± 7.5	112.7 ± 2.5
LC x CC	14	12	81.1 ± 2.0	82.1 ± 1.5	106.5 ± 2.2	111.4 ± 7.7
CH x HH	10	14	76.6 ± 8.6	88.0 ± 1.6	127.0 ±17.3	108.6 ± 1.7
CL x LL	13	14	75.7 ± 1.2	81.6 ± 1.5	99.5 ± 1.4	98.1 ± 2.1

CNCMs

As shown in contrasts 1 and 2, males from the lines selected for high CNCMs completed significantly more matings than those from lines selected for low CNCMs and the unselected control which served as the base population for the selected lines (Tables 19 and 20). Furthermore,males from the control line completed significantly more matings than those from the low line (contrast 3). These significant differences among lines are consistent with the results of Cunningham and Siegel (1978) who showed, via realized heritabilities, additive genetic variation for CNCMs in these populations of Japanese quail. Contrasts 4-6 and 15-17 were not significant, indicating that maternal effects were not important for mating frequency of male quail. Also, contrasts 7-11 provided general evidence that nonadditive genetic variation had minor effects on the inheritance of this trait. Contrast 8 was significant in replicate 1 with the midparent mean for the selected lines being significantly greater than that for the reciprocal crosses. This may be attributed to the asymmetrical response to selection noted here and by Cunningham and Siegel (1978) for selection for CNCMs. The asymmetry may be seen in Table 19 where the response to selection for low CNCMs was 9.3 in replicate 1 and 9.8 in replicate 2, while the response to selection for high CNCMs was 28.1 and 22.2 in replicates 1 and 2, respectively. In addition, the lack of recombinant effects (contrasts 12-14, 18-26) provides further evidence that most of the genetic variation of this polygenic trait is additive.

Table 19. Means and standard errors for CNCM, cloacal gland size (mm^2) and relative aggressiveness (% encounters won) by mating combination and replicate[1]

Mating Combination	CNCM		Cloacal gland		Relative aggressiveness	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
L x L	2.5 ± 0.7	2.0 ± 0.5	167 ± 6.8	183 ± 6	25 ± 3	26 ± 3
H x H	39.9 ± 2.4	34.0 ± 2.3	280 ± 8.8	240 ± 7	60 ± 4	68 ± 4
C x C	11.8 ± 0.8	11.8 ± 0.8	231 ± 5.9	231 ± 6	67 ± 2	67 ± 2
H x L	14.7 ± 5.5	8.4 ± 2.0	131 ± 8.1	123 ± 10	56 ± 11	50 ± 7
H x C	15.9 ± 2.4	9.3 ± 3.2	263 ± 14.6	221 ± 11	63 ± 7	64 ± 5
L x H	6.2 ± 2.3	3.8 ± 1.5	126 ± 7.8	117 ± 14	43 ± 9	47 ± 6
L x C	3.3 ± 1.3	4.6 ± 1.1	188 ± 16.4	223 ± 9	51 ± 8	54 ± 6
C x H	13.2 ± 1.8	14.5 ± 2.3	260 ± 18.8	246 ± 17	67 ± 7	70 ± 5
C x L	5.2 ± 1.3	5.8 ± 1.6	203 ± 7.8	226 ± 14	36 ± 8	53 ± 7
HL x HL	15.4 ± 4.9	5.4 ± 2.2	147 ± 12.5	135 ± 15	63 ± 8	59 ± 5
HC x HC	12.6 ± 4.2	10.2 ± 3.3	122 ± 10.5	117 ± 7	58 ± 8	57 ± 7
LG x LH	9.5 ± 3.7	6.2 ± 2.5	126 ± 7.7	141 ± 7	38 ± 9	40 ± 9
LC x LC	3.2 ± 1.5	1.5 ± 1.0	85 ± 4.5	109 ± 8	36 ± 8	29 ± 4
CH x CH	7.3 ± 3.5	16.7 ± 4.5	106 ± 10.5	156 ± 10	50 ± 8	71 ± 5
CL x CL	11.7 ± 6.2	6.3 ± 1.7	133 ± 12.6	136 ± 14	50 ± 7	38 ± 10
HL x LL	7.1 ± 2.4	1.5 ± 1.4	125 ± 9.8	113 ± 9	70 ± 10	31 ± 8
HC x CC	11.5 ± 2.5	8.9 ± 2.8	117 ± 18.0	125 ± 10	62 ± 8	40 ± 8
LH x HH	26.2 ± 6.4	15.2 ± 3.6	140 ± 17.3	126 ± 6	70 ± 6	61 ± 7
LC x CC	3.7 ± 1.8	3.4 ± 1.8	133 ± 13.8	95 ± 14	59 ± 12	39 ± 11
CH x HH	19.6 ± 3.9	17.1 ± 5.2	134 ± 12.1	121 ± 10	57 ± 4	64 ± 6
CL x LL	3.9 ± 1.9	0.2 ± 0.2	111 ± 8.8	98 ± 7	46 ± 8	17 ± 6

[1]N is shown in Table 18

Table 20. Summary of Scheffe's analyses for linear contrasts for body weight, completed matings, cloacal gland size and relative aggressiveness[1]

Contrast Code	Contrast	Body weight 4 wk.	Body weight 8 wk.	CNCM	Cloacal gland size	Relative aggressiveness
1	HH - LL	HH	--	HH	HH	HH
		--	HH	HH	HH	HH
2	HH - CC	HH	--	HH	HH	--
		--	--	HH	--	--
3	LL - CC	--	--	CC	CC	CC
		--	CC	CC	CC	CC
4	HC - CH	--	--	--	--	--
		--	--	--	--	--
5	HL - LH	--	--	--	--	--
		--	--	--	--	--
6	CL - LC	--	--	--	--	--
		--	--	--	--	--
7	(HH + CC) - (CH + HC)	--	--	--	--	--
		--	--	--	--	--
8	(HH + LL) - (LH + HL)	--	--	(HH + LL)	(HH + LL)	--
		--	--	--	(HH + LL)	--
9	(CC + LL) - (LC + CL)	--	(CC + LL)	--	--	--
		--	--	--	--	--
10	2CC - (HH + LL)	--	--	--	--	CC
		--	--	--	--	CC
11	2CC - (HL + LH)	--	--	CC	CC	--
		--	--	--	CC	--
12	(HH + HL + LH + LL) - 2 ((HLHL) + (LHLH))	--	--	--	(HH + HL + LH + LL)	(HLHL + LHLH)
		--	--	--	--	--
13	(HH + HC + CH + CC) - 2 ((HCHC) + (CHCH))	--	--	--	--	--
		--	--	--	(HH + HC + CH + CL)	--
14	(CC + CL + LC + LL) - 2 ((LCLC) + (CLCL))	--	--	--	--	(CC + CL + LC + LL)
		--	--	--	(CC + CL + CC + LL)	--

Table 20 cont'd. Summary of Scheffe's analyses for linear contrasts for body weight, completed matings, cloacal gland size and relative aggressiveness

Contrast Code	Contrast	Body weight 4 wk.	Body weight 8 wk.	CNCM	Cloacal gland size	Relative aggressiveness
15	(HLHL) - (LHLH)	--	--	--	--	--
		--	--	--	--	--
16	(HCHC) - (CHCH)	--	--	--	--	--
		--	--	--	--	--
17	(LCLC) - (CLCL)	--	--	--	--	--
		--	--	--	--	--
18	(HL + LH) - ((HLHL) + (LHLH))	--	--	--	--	--
		--	--	--	--	--
19	(CL + LC) - ((CLCL) + (LCLC))	--	--	--	(CL + LC)	--
		--	--	--	*(CL + LC)*	--
20	(CH + HC) - ((CHCH) + (HCHC))	(CH + HC)	--	--	(CH + HC)	--
		--	--	--	*(CH + HC)*	--
21	(LH + HH) - 2 (LHHH)	(LH + HH)	--	--	(LH + HH)	--
		--	--	--	*(LH + HH)*	--
22	(HL + LL) - 2 (HLLL)	--	--	--	--	HLLL
		--	--	--	*(HL + LL)*	--
23	(CH + HH) - 2 (CHHH)	(CH + HH)	--	--	(CH + HH)	--
		--	--	--	*(CH + HH)*	--
24	(HC + CC) - 2 (HCCC)	--	--	--	(HC + CC)	--
		--	--	--	*(HC + CC)*	--
25	(CL + LL) - 2 (CLLL)	--	--	--	(CL + LL)	--
		--	--	--	*(CL + LL)*	--
26	(LC + CC) - 2 (LCCC)	--	--	--	(LC + CC)	--
		--	--	--	*(LC + CC)*	--

[1]Replicate 1 in bold type and Replicate 2 in italics

Cloacal gland size

Males from the high mating line had significantly larger cloacal glands than those from the low mating line in both replicates (contrast 1) and males from the control line in replicate 1 (contrast 2). In addition, males from the control line had significantly larger cloacal glands than those from the low mating line (contrast 3). These data are consistent with the correlated responses noted for cloacal gland size to selection for CNCMs (Cunningham and Siegel, 1978).

The size of the cloacal gland was not influenced by maternal effects as shown by contrasts 4-6 and 15-17.

Midparent values of the selected lines were larger than the mean of the F_1 cross of these lines in both replicates (contrast 8). This, plus the observation that cloacal glands for the control line were significantly greater than the F_1 mean of the selected lines (contrast 11), may be a reflection of the asymmetry in the correlated response of this trait concomitant to the asymmetrical response to selection for CNCMs. There was recombination loss in replicate 1 (contrast 12) with the mean for the selected lines and the F_1 reciprocal crosses being significantly larger than that for the F_2 crosses. Further evidence for recombinant effects was also found in contrasts 13 and 14 of replicate 2, with the parental and F_1 means being significantly greater than the respective F_2 values. Additionally, recombination effects were found in both replicates for contrasts 19-21 and 23-26, while contrast 22 was significant in only replicate 2. Here the

F_2 means were significantly lower than the F_1 means (contrasts 19 and 20), and the backcross means were significantly lower than the F_2 means (contrasts 21-26). The similarity between replicates for comparisons (contrasts 1, 2 and 3) which involved additivity may be due to common factor(s) for CNCMs and cloacal gland size. The nonadditive effects (contrasts 8 and 11) as well as those for recombinance (contrasts 19-26) indicates the presence of nonadditive and/or asymmetrical responses.

Relative aggressiveness

Males from the high mating and control line, while not differing from each other (contrast 2), were significantly more aggressive than those from the low mating line in both replicates (contrasts 1 and 3). This pattern was consistent with that noted by Cunningham and Siegel (1978) who observed a correlated response of relative aggressiveness when selection was for low but not for high CNCMs. Such correlated responses were further shown in contrast 10 where the males from the control line were more aggressive in both replicates than the midparent value for selected line values.

Relative aggressiveness was not influenced by maternal effects (contrasts 4-6, 15-17), and F_1 crosses did not differ from their midparent means (contrast 7-9). Significant differences due to recombination effects were found in contrasts 12, 14 and 22 for replicate 1 but not for replicate 2. This inconsistency between replicates was compounded by a lack of a consistent pattern among the three contrasts that were significant in replicate 1. In contrast 12 the F_2 mean was significantly

larger than the F_1-pureline mean, while in contrast 14 the F_2 mean was significantly less than the F_1-pureline mean. Also, the backcross mean was significantly greater than the F_1-pureline mean. This lack of a pattern in the recombination effects, plus the low number of significant contrast causes one to speculate that they may be chance occurrences.

General

The patterns observed from the analysis of contrasts for body weight, CNCMs, size of cloacal gland and relative aggressiveness were consistent with those reported for the effects of divergent selection for CNCMs on the selected trait and on unselected traits (Cunningham and Siegel, 1978). Those data plus those obtained in the crossing experiment reported here provide evidence of considerable heritable variation for mating frequency in the Japanese quail. Although fitness traits are usually associated with a lack of additive genetic variation, such would not be the case if an intermediate was the optimum. This may be the situation for CNCMs because although a male needs to mate frequently enough to inseminate the females, there may be costs for excessive mating such as sperm depletion, vulnerability to predators and energy expenditures.

Selection for CNCMs resulted in correlated responses for relative aggressiveness and the size of the cloacal gland. Whether these correlated responses are due to pleiotrophy and/or linkage for the traits *per se* or to a common physiological effect(s) is unknown.

Although the phenotypic expressions of these traits are influenced by androgens (Ottinger and Brinkley, 1979), and mating and cloacal gland growth are androgen dependent (Cunningham *et al.*, 1977; Sacks, 1969), target response may also be involved. For example, Cunningham *et al.* (1977) observed that while injections of testosterone cypionate stimulated mating activity of caponized males, it decreased mating activity of intact males from the high mating line and had no influence on those from the low mating line. In addition, the data obtained in Experiment II of this dissertation suggest that circulating blood androgen titers have not been influenced by selection for CNCMs in the fowl. Thus, it appears that target sensitivity may be implicated and the model for mating frequency in the Japanese quail may not be unlike that described for the chicken in Experiment III.

The observation that the relationship between relative aggressiveness and CNCMs is greater in the quail than in the chicken is explainable in the context of the differences in sexual dimorphism for body weight that exist in these species. Since courtship behavior of Japanese quail in laboratory situations is not elaborate, and since the male quail does not exhibit the passive dominance of a male greater in size than a female (Blohowiak *et al.*, 1980), increased aggressiveness by males would enhance the probability of females exhibiting submissive posture(s). This would increase the probability of matings. The possibility of physiological factor(s) affecting both mating frequency and cloacal gland size, when considered in the context of the relationship

between relative aggressiveness and mating success, indicate that selection for mating frequency in Japanese quail modifies physiological and/or behavioral activities commonly affecting mating activity, cloacal gland size and relative aggressiveness.

SUMMARY

The genetics of mating behavior in Japanese quail was investigated utilizing comparisons among replicated lines selected for high or low number of completed matings and the randombred control population which served as the base for the selected lines, and their respective F_1, F_2 and backcross generations. Results indicate that the primarily heritable influence on mating frequency is additive genetic variation. The relationship between mating behavior, cloacal gland size and relative aggressiveness suggests that selection for mating frequency influences factors commonly affecting these traits.

GENERAL SYNTHESIS

Males from lines of chickens that had undergone divergent selection for high and low mating frequency and the randombred control line which served as the base population for the selected lines were compared for reproductive behavior and fertility. Although there were large differences among lines for number of matings, their fertility was similar under both natural and artificial mating situations. Significantly more days were required under natural mating for flocks to reach peak fertility than when the pullets were artificially inseminated. The difference in days to peak fertility may be due to male-female interactions, whereby all of the females in the flock would not be fertilized on the same day when reproduction is by natural means. Thus, although the lines may not differ in fertility, under natural mating situations, genetic variation for mating behavior may indirectly affect flock fertility because of male-female interactions.

Blood testosterone levels were compared for cocks from the purelines described in the previous paragraph and F_1 crosses of these populations. No significant differences were found, indicating that circulating testosterone levels were not the cause of the differences in mating behavior among these mating combinations. The data indicate that mating behavior in the fowl is influenced by both additive and nonadditive genetic variation with the heterotic effect associated with the threshold for the mating behavior.

Significant differences in the number of peaks in electroencephalographic tracing were found among lines for implant location and behavioral situations (prior, during and postexposure to a female). The inverse relationship between the number of peaks and the voltage per peak may be considered in the context of a genetic-physiological model involving stimulatory (SMC) and inhibitory (IMC) mating centers. It is proposed that selection for low mating frequency alters the response of the IMC, which controls the SMC, while selection for high mating frequency alters the threshold of the IMC and perhaps the SMC. Via natural selection, the situation in the unselected control line may be proposed to have reached an equilibrium whereby stimulation of the SMC is at a level to partially override the controlling effects the IMC has upon it.

The genetics of mating frequency was examined using replicated lines of Japanese quail that had been selected for high and for low mating frequency. Analyses involving pureline, F_1, F_2 and backcross generation progeny indicate that additive genetic effects are the primary source of heritable variation for mating frequency. The relationship between mating frequency, cloacal gland size and relative aggressiveness suggests that common underlying factor(s) influence these traits.

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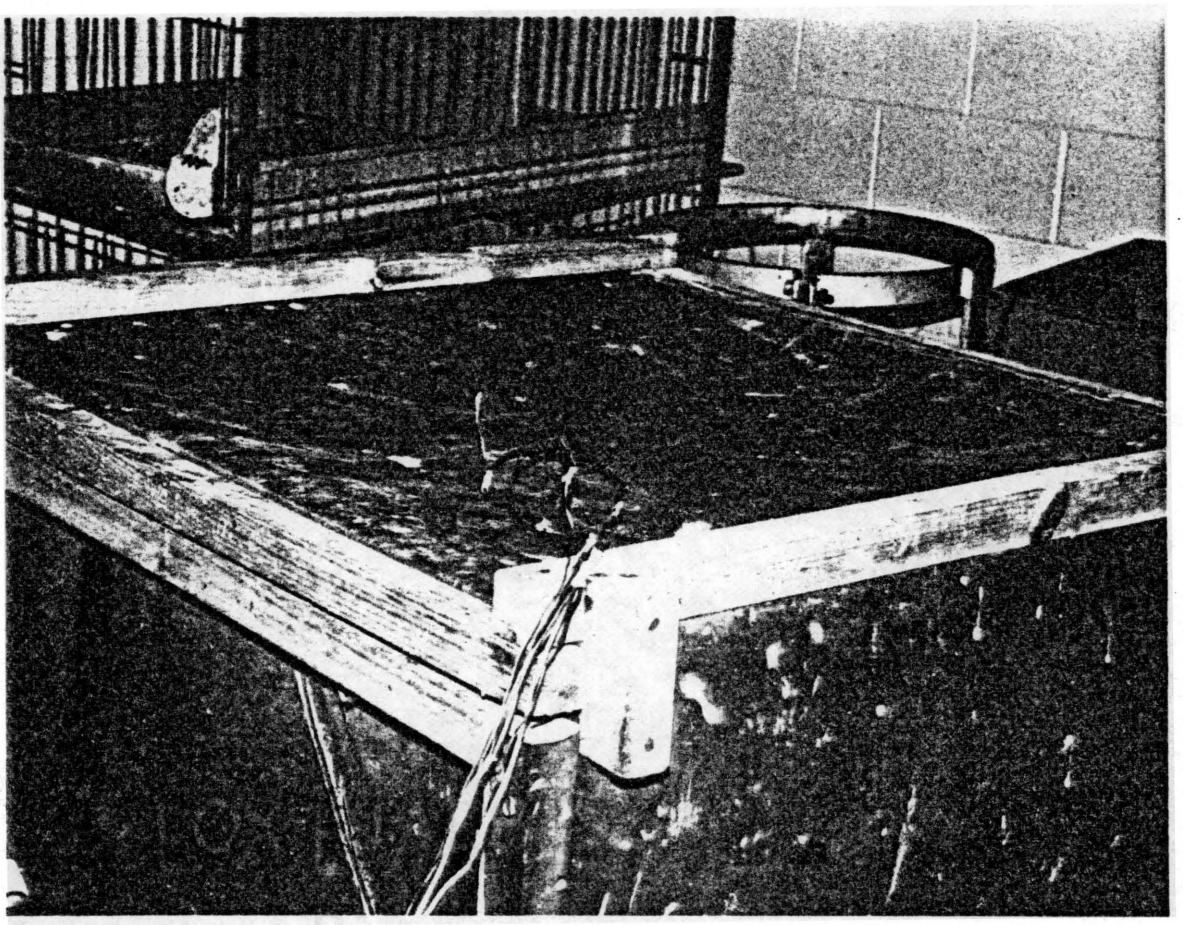
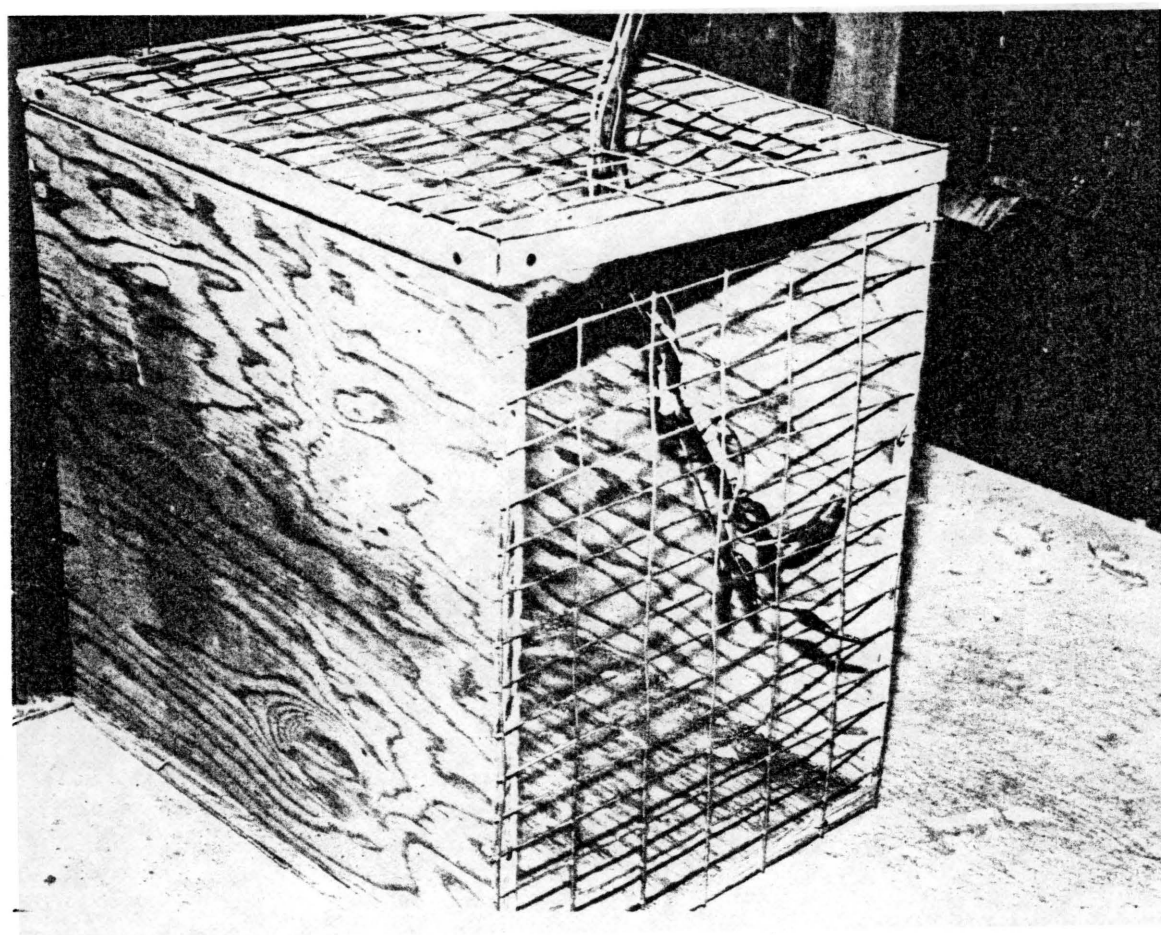
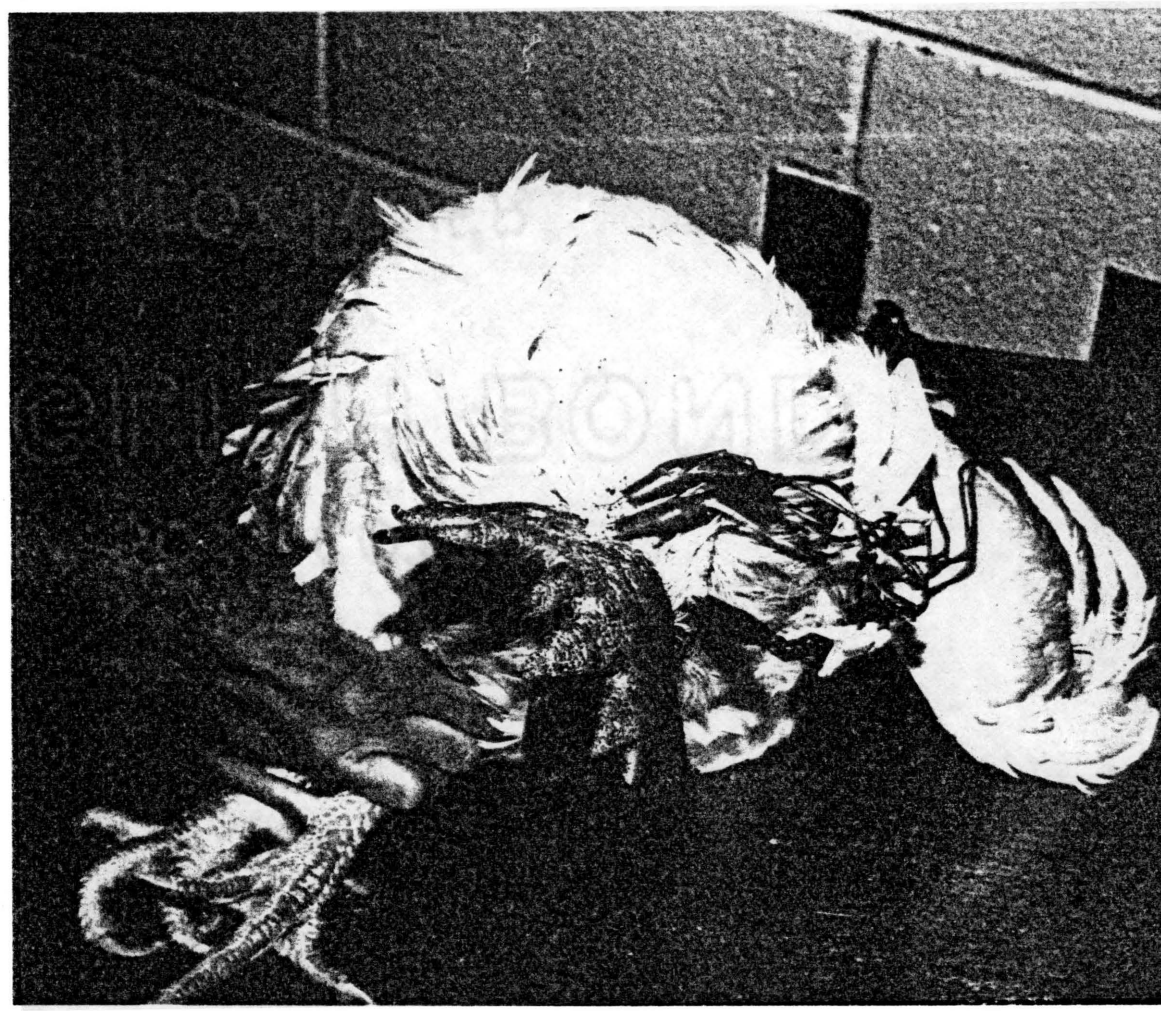


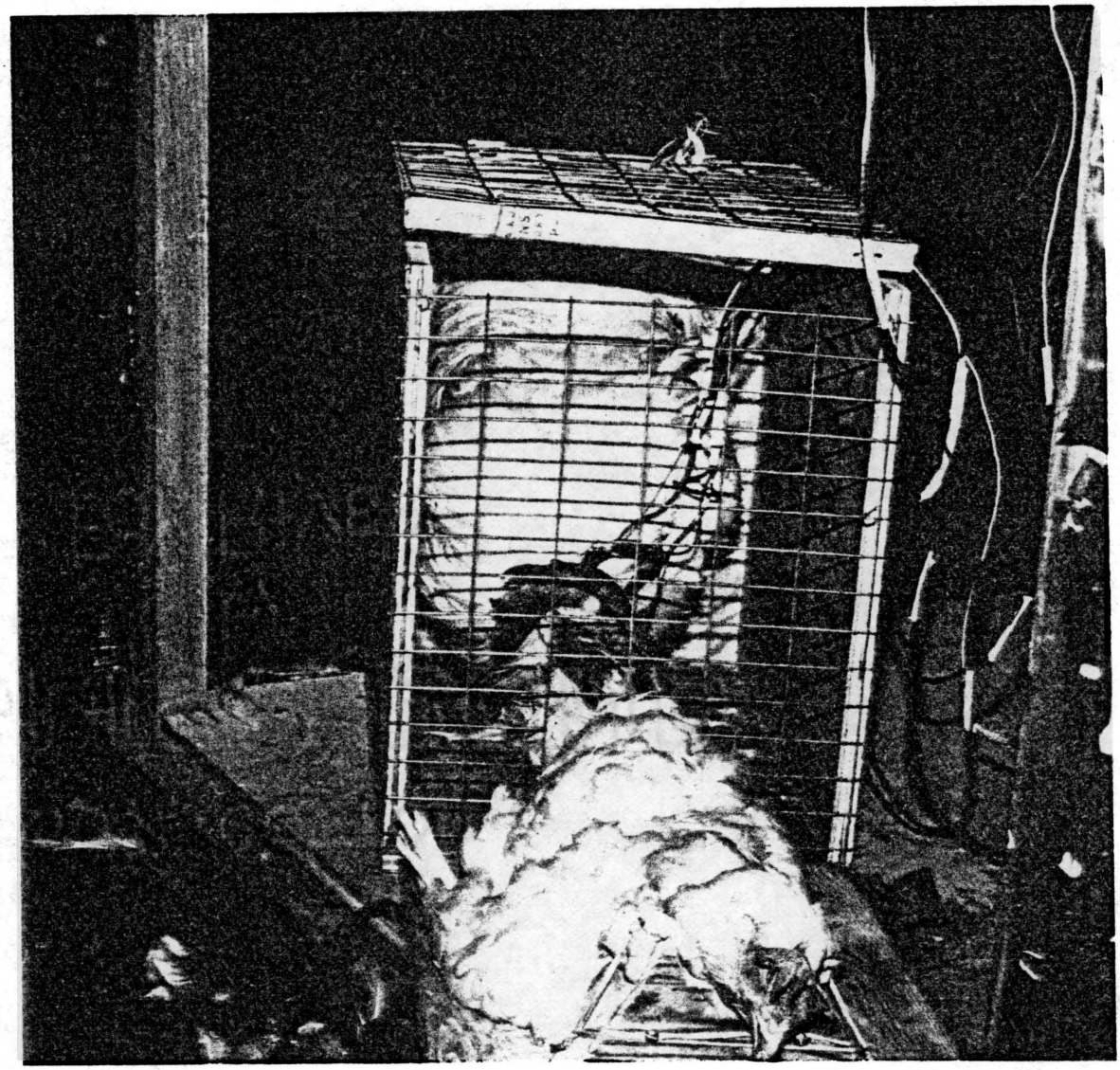
Appendix Figure 1. Copper shielded container used for EEG measurements



Appendix Figure 2. Crate for holding male during EEG measurements



Appendix Figure 3. Recording wires attached to males for EEG measurements



Appendix Figure 4. Arrangement of male and female during exposure

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GENETIC ANALYSES OF REPRODUCTIVE BEHAVIOR IN THE DOMESTIC FOWL AND THE JAPANESE QUAIL

by

Douglas Emile Bernon

(ABSTRACT)

Four experiments were conducted in an effort to explain further the genetics and physiology of sexual behavior in chickens and Japanese quail. The populations used in this research included lines that had undergone over 20 generations of divergent selection for high and low mating frequency and the randombred control population from which the selected lines originated.

In the first experiment, fertility comparisons under natural and artificial mating situations were made over time among male chickens from the selected and control lines. Although there were highly significant differences among the lines for mating frequency, total fertility was similar among lines when mating was by either natural or artificial means. Significantly more days were needed to reach peak fertility when mating was by natural rather than artificial means. This difference may be attributed to male-female interactions whereby all females in a flock would not be fertilized on the same day in natural mating situations. This same reasoning could explain the significant differences noted between mating situations for duration of fertility.

The second experiment examined the mode of inheritance of mating behavior and testosterone levels in chickens using the selected lines plus reciprocal crosses among these lines. No differences among mating combinations were found for circulating testosterone levels. In all cases, androgen titers appeared to be of a sufficient magnitude to influence mating behavior. Heterotic effects were found for mating behaviors in cross-pureline comparisons suggesting that nonadditive genetic variation influences the thresholds for mating in the fowl. Electroencephalographic effects of mating behavior of the selected lines were studied in the third experiment. There was an inverse relationship between number of peaks and voltages per peak, with differences in the number of peaks being significant among lines, implant locations and behavioral situations. Highly significant differences were found among lines for all voltage measurements with the control line having the highest voltage and the low mating line having the lowest voltage. These observations were discussed in the context of their effects on inhibitory and stimulatory mating centers.

The genetics of mating frequency in male Japanese quail was studied in the fourth experiment utilizing replicated lines selected for high or low mating frequency and the randombred control line that served as the base population for the selected lines. Comparisons involved the purelines, F_1, F_2 and backcross generation progeny. The results indicate that the primary heritable variation for mating frequency in this species is primarily additive. Correlated responses of cloacal

gland size and relative aggressiveness to selection for mating frequency are discussed in the context of alterations in physiological and behavioral responses.