INBREEDING DEPRESSION IN JAPANESE QUAIL[1]

K. SITTMANN[2], H. ABPLANALP AND R. A. FRASER

Department of Poultry Husbandry, University of California, Davis

Received February 17, 1966

THE usefulness of Japanese quail (*Coturnix coturnix japonica*) as an experimental animal was first pointed out about eight years ago (WILSON, ABBOTT and ABPLANALP 1959, 1961; PADGETT and IVEY 1959). These birds have steadily gained in popularity since then, but few quantitative genetic studies have been reported to date. The present investigation was designed to assess the response of Japanese quail to rapid inbreeding with the aim, at the outset, of developing viable inbred lines for genetic and biomedical research purposes.

Successive full-sib matings in the present study have led to the complete loss of reproductive fitness by the third generation ($F = .5$) owing to inbreeding depression. Mainly three traits are to be discussed in this connection: hatchability, viability and fertility.

MATERIALS AND METHODS

The population of quail used, line 908, was established in 1960 by crossing three males imported from Taiwan to 15 females of a control line at Davis which in turn had been started with birds of presumably Japanese origin bought from two game farms in California. The new population was propagated by mass matings until generation 4. Thereafter, birds were mated in single pairs and pedigrees kept. Inbred birds from full-sib matings were first produced in generation 6. The observations summarized here cover the period from the hatching of generation 6 (spring 1963) to the hatching of generation 11 (winter 1964).

Population size was restricted especially in the first and also in later generations leading to an estimated average degree of inbreeding of control birds in generation 6 of about 9%. Effective population size of control birds was maintained in subsequent generations at a harmonic mean of about 125 individuals for an average increment in inbreeding of less than 0.5% per generation. Such controls are designated throughout this report as noninbred ($F = 0$) in contrast to individuals deliberately inbred by sib-mating.

The breeding system used in this study is presented schematically in Figure 1, and the total number of matings made per generation is given by type in Table 1. Control birds were mated randomly except that the closest relationship permitted in a pair was that of double second cousins. New inbred lines were started from full-sib matings between control birds in generations 5 to 9. Crosses and sib-matings between previously inbred birds were not represented in as many generations. In addition, a cyclical mating system was started (S^2_1 birds, Figure 1). This system was designed to lead to high levels of homozygosity at a relatively slow rate to permit the elimination of deleterious genes and gene combinations by natural selection. There was no overlap of generations and every parent was mated only once with the exception of generation 9 where controls produced offspring first with a full-sib and then with an unrelated bird. No artificial selection for performance traits was practiced.

---

[1] Supported in part by Public Health Service grant T1GM 701–04/05. Computer facilities were made available through Public Health Service grant FR-0009 at the Computer Center of the University of California at Davis.

[2] Present address: Genetics Department, McGill University, Montreal 2, P.Q., Canada.

Genetics **54**: 371–379 August 1966.

Each generation data from up to five hatches were pooled within mating type without weighting. Generation means of control birds were averaged, also without weighting, in order to combine estimates. For the other mating types, deviations from the controls were obtained within each generation and these deviations weighted by the respective numbers of matings or birds for a pooled estimate. This method was adopted to cope with variation in frequency of matings or birds of a given type and with management differences between generations.

Hatching eggs were collected over two weeks and stored at 13°C prior to incubation. They were candled on the 8th day of incubation and hatched on the 19th day. Fertility of eggs and embryonic mortality were determined macroscopically after candling and after hatching.

Newly hatched chicks were placed in battery brooders where automatic clocks provided for

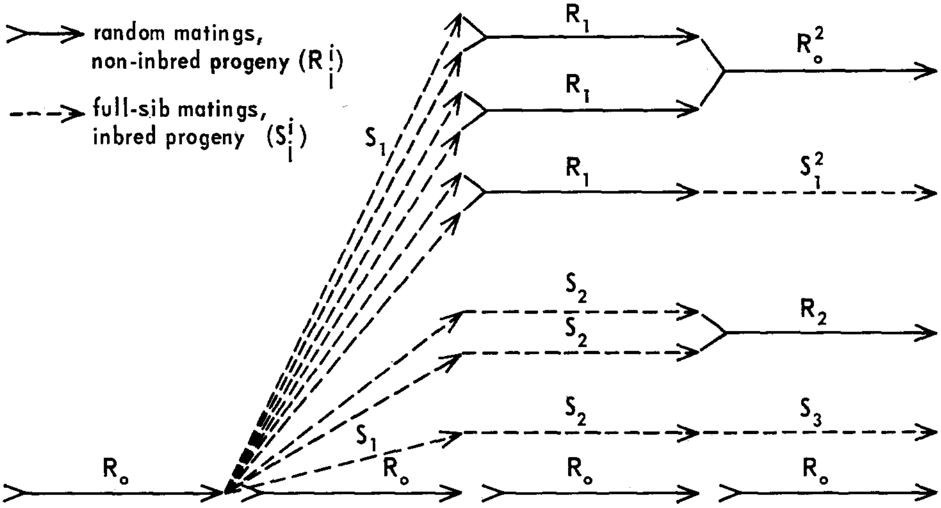

FIGURE 1.—The mating system. Subscripts denote the number of generations of full-sib matings that gave rise to (1) inbred progeny and (2) to the parents of noninbred progeny. The superscript of S_1^2 birds refers to the second cycle of a mating system where in each cycle one generation of full-sib matings alternates with one generation of crosses between inbred lines; R_0^2 birds represent a four-way cross which serves as a control for S_1^2 birds.

TABLE 1

Total number of matings of eight types by generation

	Inbreeding (F, in %)		Number of matings in generation						
Mating type	Parents	Offspring	5	6	7	8	9	10	Total
R_0	0	0	84	35	51	125	138	93	526
R_0^2	0*	0	..	..	17	..	..	..	17
R_1	25	0	..	12	20	..	29	..	61
R_2	37.5	0	..	..	..	..	21	..	21
S_1	0	25	75	39	25	44	155	..	338
S_1^2	0*	31.2	..	..	16	3	..	46	65
S_2	25	37.5	..	19	26	21	..	..	66
S_3	37.5	50	..	..	9	6	..	..	15

* Sire and dam of parents 25% inbred and unrelated.

16 to 24 hours of light per day in four generations and for 9 hours per day in generation 9. At five weeks of age chicks were debeaked and pair-mated in cages where they received 14 hours of light per day. The rooms were equipped with automatic coolers, fans, and electric heaters.

Birds were weighed to the closest gram at 6 weeks of age. Number of eggs laid and egg weight were recorded for each hen individually for a 5-day week: egg number until hens were 16 weeks old and egg weight during the 12th week of age. Heritability estimates of body weight and of egg traits were derived from full-sib correlations in families of control birds of generations 9 and 10. The number of families per estimate ranged between 83 and 113 with an average of 2 to 3 full-sibs each.

RESULTS AND DISCUSSION

Hatchability: This trait is the percentage of chicks hatched from all fertilized eggs. Results for generations 6 to 11 are summarized in Table 2 with the controls in the first column. Chicks that have pierced the shell but have not hatched when incubation is terminated are referred to as pips.

The overall decline in hatchability in matings producing the third full-sib generation (F = 0.5) was about 46%. Parental effects other than direct genetic contributions to the progeny are interpreted here to be a feature mainly of the dam, i.e. maternal effects exerted via egg quality. Several components of quail eggs, especially fat content of the yolk (Lepore and Marks 1965), are known to vary between hens. Maternal inbreeding caused a decline in hatchability of about 3% for each 10% F (Table 2, columns 1 to 3). During embryonic development maternal inbreeding had the strongest effect on early mortality (1 to 8 days) which tended to increase at an accelerating rate with increasing inbreeding of dams. Hatchability percentages shown in the fourth column of Table 2 indicate heterosis of maternal effects; the offspring represented a four-way cross between inbred sib-lines. This four-way cross hatched better than the two-way cross between inbred sib-lines and about as well as the controls.

Inbreeding of the embryo itself played a more important role than that of the dam as set out in Table 3 on the assumption of independence of maternal inbreeding (data of Table 2). In every one of the four columns, parents with identical

TABLE 2

Embryonic losses (percent) by inbreeding (F) *of parents and offspring*

	Inbreeding (F, in percent)							
Parents:	0	25	38	0*	0	25	38	0*
Offspring:	0	0	0	0	25	38	50	31
Matings, total	526	61	21	17	338	66	15	65
Fertile eggs, total	5301	701	130	208	5257	805	138	645
Mortality $\leq$ 8 days	12	19	26	14	19	29	46	18
Mortality $>$ 8 days	7	9	9	8	15	15	18	14
Pips	8	8	4	7	13	15	10	14
Sum of losses	27	37	39	29	47	60	74	45
Hatchability	73	63	61	71	53	40	26	55

* Sire and dam of parents 25% inbred and unrelated.

TABLE 3

Effects of inbreeding of progeny on embryonic losses (percent). Difference between inbred and noninbred progeny within inbreeding level of parents

	Inbreeding (F, in percent)			
Parents:	0	25	38	0*
Progeny:	25,0	38,0	50,0	31,0
Mortality $\leq$ 8 days	6.8	10.2	20.3	3.9
Mortality $>$ 8 days	7.6	5.7	8.6	6.1
Pip	5.1	7.1	6.4	6.1
Sum of losses	19.5	23.1	35.3	16.0

* Sire and dam of parents 25% inbred and unrelated.

inbreeding coefficients were either sib-mated or crossed. The figures show the excess of losses of inbred over noninbred progeny within inbreeding level of dams. Hatchability was found to decrease by about 7% for each 10% increment in inbreeding of the embryo. As with maternal inbreeding, the greatest losses due to inbreeding of the embryo were incurred in early mortality which increased exponentially with F. It was also in early mortality that heterosis of maternal effects had a disproportionately beneficial effect (Table 3, column 4).

Further, inbreeding accentuated the detrimental effects of storage of fertile eggs prior to incubation. One additional week of storage reduced hatchability markedly when either dam or offspring were inbred, but it did not affect hatchability of controls in this sample (Table 4).

Viability: The unusually high mortality of control birds during the first five weeks after hatching (Table 5) was largely a consequence of the unduly long 19-day incubation period. Many of these control chicks had hatched between the 16th and 18th day and they were then too weak to feed when placed in the brooder on the afternoon of the 19th day of incubation.

Mortality of control chicks is characteristically concentrated in the first three days after hatching; deaths among inbred chicks and also among noninbred chicks from inbred dams are more spread out, mainly over the first two weeks. In addition to recorded deaths, the mortality percentages for the first five weeks (Table 5) include small proportions of chicks unaccounted for at the end of that period. The majority of these chicks lost their wing bands and could not be identi-

TABLE 4

Hatchability (percent) of eggs stored for 8 to 14 days, minus hatchability of eggs stored for 1 to 7 days

	Inbreeding (F, in percent)				
Sire, dam:	0,0	25,0	0,25	0,0	25,25
Progeny:	0	0	0	25	37.5
Fertile eggs, total	1084	362	251	834	384
Hatchability, difference	0.5	—0.8	—9.3	—5.3	—7.4

TABLE 5

Mortality (percent)

	Inbreeding (F, in percent)				
	0	0*	25	37.5	50
Number of chicks banded	2659	499	1391	326	26
0 to 5 weeks	17.2	23.1	25.8	40.9	71.4
5 to 16 weeks					
males	5.9	6.4	7.5	11.5	. . .
females	11.5	5.2	16.1	18.9	. . .

* Both parents 25% inbred.

fied as to mating type; the remainder is presumed to have died unrecorded at rates dependent on mating type.

Unfavorable effects of maternal inbreeding were evident in the first period after hatching from a comparison of the two types of noninbred chicks and also from the curvilinear increase in mortality with increasing F (Table 5). Mortality up to the 5th week was increased by about 2% and 4% for each additional 10% inbreeding of dam and offspring, respectively. The same amount of inbreeding decreased viability by about 0.8% and 1.9% in males and females, respectively, between the ages of 5 and 16 weeks.

Surviving chicks were sexed at five weeks of age. The sex ratio fluctuated considerably within mating types over hatches and generations, but there was generally a slight preponderance of females implying absence of sex-linked lethals at appreciable frequencies. The sex difference in adult mortality was in part due to young hens that did not survive the onset of laying.

Infertility of females: The number of hens that had failed to lay their first egg when 12 or 16 weeks old is given in Table 6 as a proportion of the total number of females surviving to these ages. The average generation interval in these data was about 18 weeks. The ages of 12 and 16 weeks thus approximate the beginning and the midpoint of an average reproductive period. The percentages in Table 6 show clearly that age at sexual maturity was delayed in inbred hens and that

TABLE 6

Proportions of non-layers among survivors

	Inbreeding (F, in percent)			
	0	0*	25	37.5
≤ *12 weeks of age*				
Total survivors	836	146	376	77
Percent infertile	9.0	8.4	17.0	26.4
≤ *16 weeks of age*				
Total survivors	793	128	350	61
Percent infertile	5.0	5.0	8.7	14.6

* Both parents 25% inbred.

TABLE 7

Fertility of incubated eggs

	Inbreeding of birds mated (F, in percent)			
	0	0*	25	37.5
Matings with eggs	567	82	127	40
Total eggs set	10863	1241	2775	741
Eggs set/mating/hatch	10.0	10.8	8.9	8.4
Fertility, all matings	79.5	76.4	50.3	33.0
Fertility, fertile matings	88.2	89.1	72.4	52.2

* Both parents 25% inbred.

maternal inbreeding was no longer effective in this trait when the daughters were 12 weeks old.

Fertility: Three criteria of fertility observed in the hatchery are set out in Table 7. "Fertility in all matings" includes those matings in which the male was completely infertile whereas "fertility in fertile matings" pertains to matings that produced at least one fertile egg. The difference between the two traits indicates the magnitude of complete male infertility. For 10% inbreeding, "fertility in all matings" was depressed by approximately 11% of which about 4 were due to complete male infertility.

The "number of eggs set per mating per hatch" is an attempt to place egg production on a percentage scale. The upper limit of 100% equals 14 eggs, i.e. one egg laid per day and set at the end of the standard 14-day saving period preceding incubation. For each 10% F, an average of 0.4 fewer eggs were set per mating and hatch.

Genetic load: Following the concept of MORTON, CROW and MULLER (1956), provisional estimates of the damage expressed in the controls (A), of the hidden genetic damage (B), and of the B/A ratio were calculated from average probabilities pertaining to $F = 0$ and $F = .25$ (Table 8). Mortality was averaged over sex for estimates concerning survival from 5 to 16 weeks. The total number of lethal equivalents was thus estimated at 8.7 plus an unknown proportion of the damage expressed in the controls (A) that is due to genetic causes. All estimates

TABLE 8

Probability of "survival," and genetic load

Trait	F=0	F=.25	*A*	*B*	*B/A*
Hatchability	.727	.531	0.319	1.257	3.9
Survival, 0–5 weeks	.828	.742	0.189	0.439	2.3
Survival, 5–16 weeks	.913	.867	0.091	0.207	2.3
Fertile females, 16 weeks	.950	.913	0.051	0.159	3.1
Eggs set/mating/hatch	.714	.636	0.337	0.463	1.4
Fertility, all matings	.795	.503	0.229	1.831	8.0
Total	.296	.100	1.216	4.355	3.6

of the B/A ratio were relatively low. Known simple recessives were infrequent and they accounted for a very minor portion of the genetic load.

Production traits: On the average, 10% inbreeding of the progeny reduced body weight at six weeks by 2 grams in males and by 4 grams in females (Table 9). The sex dimorphism in body weight at six weeks can be attributed largely to the greater weight of the maturing reproductive organs of females. Inbreeding delays sexual maturity and thus depresses six week body weight of females more than that of males. By six weeks, control males and females attained 92% and 78% of their respective mature weights at 20 weeks of age (generation 9). Inbred males and females grew at slightly slower rates between 6 and 20 weeks of age than did the controls.

Sexual maturity of females was delayed by slightly more than one day for each 10% of their own inbreeding. The egg numbers in Table 9 pertain only to those hens that laid at least one egg and survived to 12 and 16 weeks, respectively. The total number of eggs was reduced by about 1.5 eggs for each 10% inbreeding. The 16.7 eggs laid by control birds between the ages of 12 and 16 weeks represented observations for 20 out of the 28 days in that period for an average rate of production of 83.4%. The difference between the sum of the two partial records and the number of eggs for the entire laying period indicates that survival of the more intensive layers was generally lower, especially in the most highly inbred group. The inbreeding depression in egg production during the period in which eggs were saved for the next generation (12 to 16 weeks) was slightly less than the proportionate decrease in the "number of eggs set per mating and hatch" in Table 7, namely —3.5% *vs.* —4.3% per 10% increment in F. The difference may reflect increased breakage of hatching eggs from inbred hens.

Genetic and environmental variability: The heritability estimates given in Table 10 are based on full-sib correlations and they contain, therefore, contributions from nonadditive genetic sources of variation and from variance due to genetic and environmental differences between dams. Most of these estimates are larger than expected by inference from relevant observations in chickens (LERNER

TABLE 9

Body weight at six weeks and egg traits

F (percent)	Body weight (g)		Age at first egg (days)	Number of eggs			Egg weight (g)
	Male	Female		≤12 wk.	12–16 wk.	≤16 wk.	
Mean, by level of inbreeding							
0	107.9	121.1	56.7	18.1	16.7	34.1	10.26
0*	104.3	112.7	57.3	17.6	17.0	35.1	10.27
25	102.7	110.0	59.6	15.4	15.3	30.3	9.83
38	98.9	108.2	65.1	13.5	13.4	25.1	9.56
Percent change per 10% inbreeding, relative to performance of controls							
dam	—1.3	—2.8	+0.4	—1.1	...	...	...
progeny	—1.9	—3.4	+2.2	—6.0	—3.5	—4.7	—1.7

* Both parents 25% inbred and unrelated.

TABLE 10

Heritability estimates (percent) of body weight at six weeks, and of egg traits

Generation	Body weight (g) Male	Body weight (g) Female	Age at first egg (days)	Number of eggs ≤12 wk.	Number of eggs 12–16 wk.	Number of eggs ≤16 wk.	Egg weight (g)
9	68	38	54	20	6	40	65
10	72	70	11	19	12	32	. .

1958) and several exceed those derived to date for Japanese quail. In contrast to a mean heritability estimate in the present data of .6 for body weight at weeks, response to selection for this trait gave a heritability estimate in the narrow sense of .2 (Collins, Abplanalp and Hill 1966). Also, Marks and Kinney (1964) obtained from sire components a heritability estimate of .36 for body weight at an age beyond the influence of maternal effects (19 weeks). Although the heritability estimates of Marks and Kinney for age at first egg (.36) and for total egg number (.39) agree closely with the respective mean estimates of .32 and .36 in the present data, estimates from both sources are too high as judged from response to selection for both traits (Abplanalp, unpublished).

Repeated observations of egg weight within hen permitted a demonstration of an increase in phenotypic variability due to inbreeding (Table 11). Individual variances of egg weight of all inbred hens ($F \geq .25$) exceeded those of all noninbreds ($F = 0$) in four of the five generations for an average difference of 10% over the entire period. Part of this difference in variability of egg weight may have been due to the erratic rate of lay of the inbred hens.

Conclusions: Rapid loss of heterozygosity by successive full-sib matings led to considerable inbreeding depression in all traits considered. As expected, traits directly related to fitness and characterized by relatively small proportions of additive genetic variance were affected most. Estimates of the depression in hatchability and in post-hatch mortality in these data are conservative, in that the long incubation period disproportionately raised hatchability of inbreds and post-hatch mortality of noninbreds.

Inbreeding depression is a linear function of F when it is due entirely to

TABLE 11

Variance components for egg weight within hens

Generation	Noninbred (F=0) Number of hens	Noninbred (F=0) Mean weight (g)	Noninbred (F=0) Variance component	Inbred (F=.25) Number of hens	Inbred (F=.25) Mean weight (g)	Inbred (F=.25) Variance component	Ratio of variances
6	64	10.6	.1112	23	9.9	.1182	0.94
7	144	10.4	.3398	90	9.7	.2718	1.25
8	213	9.5	.5145	38	8.9	.5545	0.93
9	220	10.5	.4584	58	10.0	.6046	0.76
10	191	10.5	.0818	112	10.2	.1301	0.63
Average			.3011			.3358	0.90

increased homozygosity of deleterious recessive genes. However, the slope of the depression deviates downward if epistasis, or unfavorable interactions are present. Mean performances of most traits examined indicated an accelerating decline at the higher levels of inbreeding. This pattern was most apparent in early embryonic mortality. It is suggested that nonlinearity in this trait was caused mainly by an interaction between impaired egg quality of inbred dams and low vitality of their inbred progeny. Nonlinear depression in other traits that are likewise influenced by maternal environment may have been caused by a similar mechanism. The importance of nonadditive genetic variation is reflected by the unexpectedly high heritability estimates calculated from full-sib families.

The development of viable inbred lines of Japanese quail seems predicated on the investigation of mating systems that approach high levels of inbreeding slowly and thus permit the building up of more compatible homozygous gene complexes than is possible with successive sibbing. A cyclical mating system (Figure 1) was initiated in which such favorable rearrangements within the genome can be selected and maintained more effectively. The upper limit of 33% inbreeding for a two-generation cycle in this system can easily be raised by increasing the number of sib-matings per cycle whenever warranted by adequate reproductive performance of inbreds.

SUMMARY

The probability for a zygote to reach maturity and to leave offspring was reduced from 0.3 in control birds to 0.1 by one generation of full-sib matings and to practically zero by three generations. Hatchability declined by 7% and fertility by 11% for each 10% increment in inbreeding of the progeny. Other fitness and production traits were less affected.—Nonlinear depression, and estimates of heritability indicated presence of epistatic gene action and of an interaction between inbreeding level of dams with that of their progeny. Maternal effects were important in traits observed in embryos and in young birds.

LITERATURE CITED

COLLINS, W. M., H. ABPLANALP, and W. G. HILL, 1966 Mass selection for body weight in quail. (In preparation).

LEPORE, P. D., and H. L. MARKS, 1965 Genetic variation of some chemical components of Coturnix quail egg yolk. Poultry Sci. **44**: 184–186.

LERNER, I. M., 1958 *The Genetic Basis of Selection.* Wiley, New York.

MARKS, H. L., and T. B. KINNEY, JR., 1964 Estimates of some genetic parameters in Coturnix quail. (Abstr.) Poultry Sci. **43**: 1338.

MORTON, N. E., J. F. CROW, and H. J. MULLER, 1956 An estimate of the mutational damage in man from data on consanguineous marriages. Proc. Natl. Acad. Sci. U.S. **42**: 855–863.

PADGETT, C. ANN, and W. D. IVEY, 1959 Coturnix quail as a laboratory research animal. Science **129**: 267–268.

WILSON, W. O., U. K. ABBOTT, and H. ABPLANALP, 1959 Developmental and physiological studies with a new pilot animal for poultry—Coturnix quail. (Abstr.) Poultry Sci. **38**: 1260–1261. —— 1961 Evaluation of Coturnix (Japanese quail) as pilot animal for poultry. Poultry Sci. **40**: 651–657.