Mendelian Units of Inheritance Control Color Preferences in Quail Chicks (*Coturnix coturnix japonica*)

Abstract. *Genetic analysis of approach preferences for blue and red colors in 1-day-old, experientially naïve quail, at 12th through 14th generations of bidirectional genetic selection, implicate four to eight segregating units of inheritance. Because the quails' initial approach choices are also readily modifiable by experience, these results point the way to studying the mediation of gene effects, environment effects, and gene-environment interactions in visually guided behaviors.*

Newly hatched quail chicks tend to approach conspicuous visual stimuli and exhibit distinct preferences between stimulus colors. Initial manifestation of these behaviors is not conditional on prior learning, and the quail responds well to their genetic selection. At the same time, these behaviors change with age and are subject to modification by experience. Such attributes, plus the exceptionally short generation time of the quail, allow us to study how gene effects, environment effects, and gene-environment interactions are mediated and gain phenotypic expression in vertebrate behavior (*1*). This report describes an experiment in which quail were bidirectionally selected for approach preferences between a blue and a red stimulus for 14 generations, during which genetic crosses were made. Results implicate four to eight segregating units of inheritance.

Color preferences were mass-screened in 1-day-old, dark-reared quail chicks (*1, 2*). The apparatus is best visualized as a Galton board, wherein each point of the same binary alternatives was represented by a discrimination compartment offering the choice between a pair of stimuli, one blue and another red. Cascading progression of subjects through these compartments depended on choices made per subject per compartment. As in a Galton board, arrival in a particular collection box in the bottom row indicated the number of times one stimulus was chosen over another by each subject. Repeatedly testing an individual that exhibits a stable probability of choosing one stimulus in this apparatus, or mass-screening a group of individuals, each of which exhibits the same choice probability, were expected to and do result (*3, 4*) in choice scores distributed according to the binomial probability function $P_n(k) = (n\|k)\, p^k(1 - p)^{n-k}$. Correspondingly, a behavioral phenotype assessed in this apparatus is definable only by the probability (p) of choosing one over another stimulus. The expected mean of choice scores in a phenotypically homogeneous population is $\bar{x} = np$, and the variance $s_x^2 = np(1 - p)$ reflects the random variations of the phenotype. Mass-screening phenotypically different individuals in a single group results in a mixture of binomial distributions, with population mean $\mu_x = n\bar{p}$ and population variance $\sigma_x^2 = n\bar{p}(1 - \bar{p}) + \sigma_p^2 n(n - 1)$, where $\bar{p}$ is the mean and σ_p^2 is the variance of represented probability values (that is, of nonrandom individual variations). Estimates of phenotypic mean and variance may thus be made from an empirical choice distribution of a sufficiently large sample, by $\bar{p} = \bar{x}/n$ and $s_p^2 = [s_x^2 - n\bar{p}(1 - \bar{p})]/n(n - 1)$, respectively; where $\bar{x}$ is the mean of scores, n is the number of trials, and s_x^2 is the calculated variance of manifest scores. Unlike $\bar{x}$ and s_x^2, $\bar{p}$ and s_p^2 meet the criterion of independence needed for estimating the number of genes from variance increases of segregating over nonsegregating genetic populations.

Figure 1 illustrates the progress of bidirectional selection (*3*) of quail for preferences of red versus blue, for which the above behavior-assessment procedures were used. Means of progressive generations of the unselected control line fluctuated well within half a score above and below the foundation mean, which was nearly eight blue over six red choices ($\bar{x} = 7.96$). Selection changed average choices by large margins, resulting in a nearly 13:1 ratio of choosing blue over red and better than 12:2 ratio of choosing red over blue, in the respective lines. Individual variations in selected lines first increased then decreased with selection, suggesting initial breakages of linked genetic material and subsequent elimination of a portion of additive genetic variations.

Initial hybrid data, collected at S_8, indicated dominance effects and raised the possibility of segregating Mendelian units. However, the difference between F_1 and F_2 variances at S_8 was only marginally significant. This and the lack of regression in the F_2 mean suggested caution against attributing undue significance to these results. Therefore, it was decided to repeat hybrid crosses at a more advanced stage of selection, to test larger samples, and to undertake backcrosses to controls (Table 1).

Backcrossing selected males to control females at generation 12 indicated additive genetic interaction and confirmed the small directional dominance of factors responsible for blue preference. Hybrid data at S_{12} similarly confirmed segregation, by highly significant increases in F_2 over F_1 variances. Numbers of segregating units in F_2 were estimated at 4 to 8 by the formula $k = (2a^2 + d^2)/4(s_{F_2}^2 - s_{F_1}^2)$, where a is half the difference between parental phenotypic values, d is dominance effect estimated from the deviation of the F_1 mean from the midpoint between parental values, and variances refer to the phenotypic values (s_p^2).

The use of this procedure for estimating k assumes that (i) all "blue" genes were in one and all "red" genes were in the other parental line, (ii) parental lines were selected long enough so that genes segregating in F_2 were at frequencies of one-half, (iii) there was no linkage, and (iv) genes interacted additively with equal magnitudes of effects on the trait. There was no independent proof for these assumptions. But, even if none were met, obtained estimates of k define the minimum number of segregating units (*5*), and the identifiable maximum cannot be far from estimated upper limit. This is so because, with $s_{F_2}^2 - s_{F_1}^2 = (2a^2 + d^2)/4k$, even a small increase of k over 8 could not be reliably

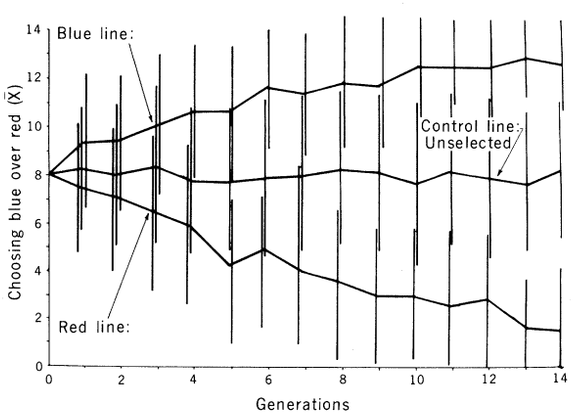

Fig. 1. The quails' response to 14 generations of bidirectional genetic selection for approach choices between a blue and a red stimulus. The blue line was selected for choosing blue over red, the red line for choosing red over blue, and controls were maintained without selection. Mean scores refer to choosing blue over red in 14 trials, in all lines. Vertical bars indicate standard deviations.

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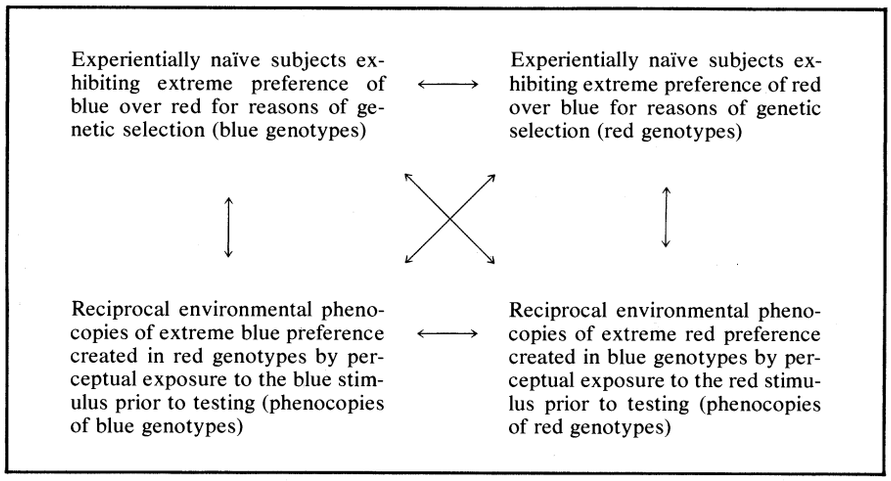

Fig. 2. Six-way comparison of genetically and environmentally labeled elements of approach preferences.

detected with present sample sizes, large though they are. Furthermore, statistically significant increases were found in F_1 over foundation and contemporaneously tested control variances. This indicates that significant heterozygosities had remained, or, more likely, were selected for in the parental lines. Such heterozygosities should counteract detection of segregation in F_2 hybrids. Together these considerations suggest most strongly that the actual number of segregating units in F_2 is within the identified limits and is closer to 4 than 8.

There is considerably less ground for certainty in interpreting observed differences between means of the F_1 distributions of reciprocal crosses (Table 1, $t = 14.29$, $p < .001$), which suggested

Table 1. Selection data for generations 12 through 14 and results of genetic crosses. Performance indicators refer under all conditions to choosing blue over red in 14 trials. Performances of foundation and control populations and responses to selection from the 1st through 11th generations are described elsewhere (*1*). N.S., not significant.

Condition	Parental families (No.)	Subjects tested	Subjects completing 14 trials (%)	Mean scores	Blue preference (p)	Variances of scores	Phenotypic variances	Bartlett's test χ^2	Bartlett's test d.f.	Bartlett's test $P\leq$	Estimates of segregating units (k)
				Generation 8							
Control line	100	1551	98.0	8.29	.59	9.92	.036				
Blue line	138	1791	98.0	11.77	.84	7.07	.029				
Red line	141	1726	98.0	3.63	.26	9.25	.036				
F_1 hybrids	120	222	100.0	8.57	.61	11.50	.045	4.76	1	.05	4.49
F_2 hybrids	90	501	98.0	8.77	.63	13.90	.058				
				Generation 12							
Control line	140	1746	97.0	7.89	.56	10.62	.039				
Blue line	152	2465	98.0	12.49	.89	4.14	.015				
Red line	150	2555	95.0	2.90	.21	7.43	.028				
				Generation 13							
Control line	129	1806	82.0	7.78	.56	8.86	.030				
Blue line	147	2681	97.0	12.97	.93	2.51	.009				
Red line	140	2975	92.0	1.69	.12	4.36	.016				
				Generation 14							
Control line	122	2197	75.0	8.29	.59	9.28	.032				
Blue line	136	1976	99.0	12.90	.92	3.34	.013				
Red line	135	2194	97.0	1.63	.12	4.43	.016				
				Backcrosses to controls at S_{12}							
Blue line males crossed with control females											
F_1	15	272	94.0	10.90	.78	8.89	.036	1.37	1	N.S.	
F_2	15	637	82.0	10.43	.75	8.47	.032				
Red line males crossed with control females											
F_1	15	297	97.0	5.25	.38	9.57	.035	4.94	1	.05	
F_2	15	562	73.0	6.64	.47	11.53	.044				
				Hybrid crosses at S_{12}							
Blue line males crossed with red line females for F_1											
F_1	33	802	96.0	8.92	.64	12.34	.050	28.28	1	.001	4.35
F_2	39	1472	95.0	8.04	.57	16.25	.070				
Red line males crossed with blue line females for F_1											
F_1	33	882	97.0	7.60	.54	15.14	.064	8.17	1	.005	7.26
F_2	39	1526	97.0	7.79	.56	17.33	.076				

sex linkages or maternal cytoplasmic effects. Yet, in the light of the relatively simple genetic determination indicated by F_2 data, they may reflect nothing more than random error in drawing the small parental groups. This issue, together with the task of specifying the source of segregation in F_1 and isolating genes that segregate in F_2, remain matters for further investigation.

The target of genetically selecting quail for color preferences is not peripheral but central mediation (*1*, *6*). Therefore, these genetic preparations may facilitate the search for physiological events of processing visual information by the vertebrate brain. Because the quails' early approach preferences are readily modified by exposure to stimuli, gene-environment interactions may be tested in choices between composite discriminanda that combine genetically and environmentally labeled elements of this behavior (*7*). Particularly promising is a six-way comparison (Fig. 2).

These comparisons are based on the assumption that individuals exhibiting a behavior solely for reasons of genotype and individuals of different genotypes exhibiting the same behavior for reasons of prior experience should exhibit differences in the mediation of that behavior. Examining anticipated behavioral and neurophysiological differences (such as, for example, in stimulus generalization or in neuroelectrical and neurochemical indicators) may lead to inferences on how gene effects, environment effects, and gene-environment interactions are mediated in behavior.

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References and Notes

1. J. K. Kovach, *Appl Anim. Ethol.* **1**, 77 (1974); *J. Comp. Physiol. Psychol.* **87**, 1040 (1974); _____, G. Wilson, T. O'Connor, *ibid.* **90**, 1144 (1976); J. K. Kovach, *Behaviour* **64**, 173 (1978); *ibid.* **65**, 263 (1978); *ibid.* **68**, 31 (1979); _____ and G. Wilson, *Anim. Behav.* **23**, 357 (1975).
2. Mass-screening procedures were first applied to geotactic and phototactic responses in *Drosophila* [J. Hirsch and R. C. Tryon, *Psychol. Bull.* **53**, 402 (1956); N. M. Hadler, *Biol. Bull. (Woods Hole, Mass.)* **126**, 264 (1964); Th. Dobzhansky and B. Spassky, *Proc. R. Soc. London Ser. B* **168**, 27 (1967)]. The mass-screening apparatus developed for the present study of the quail was built of 28 discrimination compartments, arranged in the manner of a Galton board so that a single compartment was on top, two compartments were in the second row, three in the third row, and so on through eight collection boxes on the ground floor. The target end of each compartment offered choice between two stimuli, one blue and another red. Subjects could proceed from one to the next compartment through two trapdoors in each compartment, one in front of each stimulus, which opened under the weight of the subject as it approached within 7.5 cm of the stimulus. Arrival in a collection box indicated the number of times one over another stimulus was chosen in seven trials. Stimuli backlighted with fluorescent light sources flashed at the rate of 3 Hz of equal light-dark cycles. Size (2.5 cm^2) and luminance of stimuli (10.1 lux) were kept identical; only colors were different. Stimulus-on intensities were measured at the source with a photometer (Tektronix J16) and probe (Tektronix J6501). Spectral characteristics corresponded to Wratten gelatin filters (No. 45 for blue and No. 29 for red). Subjects were light-adapted to background illumination of 1- to 2-lux scattered light before testing. They were incubated and reared in the dark, received no prior experiences with colors, and were placed into the starting compartment of the apparatus in successive groups of 25, accumulating to not more than 250 in a testing session. Although tested in groups, each subject was individually identified and scored for two consecutive runs through the apparatus. Average age at testing was 27 hours after hatching, with 1 standard deviation of age variation being approximately 5 hours.
3. J. K. Kovach, *J. Comp. Physiol. Psychol.* **41**, 851 (1977).
4. Parental subjects used in this selection experiment were mated and housed in individual pairs. Because the quail is unusually sensitive to inbreeding depression [H. Abplanalp, *Zuecht. Z. Theor. Angen. Gen.* **37**, 99 (1967); L. E. Iton, *Poultry Sci.* **46**, 1275 (1967); A. W. Kulenkamp, thesis, Washington State University (1967)], only subjects that shared no ancestors within three preceding generations were mated. Approximately 20 percent of subjects were selected from appropriate ends of preference distributions. A genetic control line was maintained without selection. Selected lines were hybridized for F_1 and F_2 at S_8 and again at S_{12}. Data were examined by reciprocal crosses at S_{12}. At this generation, backcrosses of selected subjects to genetic controls were also made.
5. D. S. Falconer, in *Methodology in Mammalian Genetics*, W. J. Burdett, Ed. (Holden-Day, San Francisco, 1963), p. 193.
6. F. R. Yeatman and J. K. Kovach, paper presented at a meeting of the Animal Behavior Society, New Orleans, 10 to 15 June 1979; in preparation.
7. J. K. Kovach, paper presented at a meeting of the Animal Behavior Society, New Orleans, 10 to 15 June 1979.
8. I thank L. Coyne for help with statistical analysis and G. Wilson, L. Baker, and C. Neal for assistance in conducting this experiment. Supported by NICHD grant HD-06770-07, NIH Research Career Development award 5-K02-MH-20140-10, and The Menninger Foundation. I would be glad to provide subjects from the described genetic populations to investigators who could profitably use them in related research.

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Cigarette Smoking Associated with Sleep Difficulty

Abstract. *A group of 50 smokers experienced greater sleep difficulty than a group of 50 nonsmokers matched by age and sex. The two groups did not differ in personality patterns or drug consumption. Also, sleep patterns significantly improved in a group of eight chronic smokers when they abstained from cigarette smoking. These findings are consistent with reports on the stimulant effects of nicotine.*

The findings of two studies in this report suggest that chronic cigarette smoking is associated with sleep difficulty and that the abrupt withdrawal of cigarette smoking in chronic smokers results in improved sleep. In the first study we used sleep laboratory recordings and data from a questionnaire given to 50 cigarette smokers (31 males and 19 females, with a mean age of 39.8 years) and 50 nonsmokers matched by sex and age. All of the smokers had been smoking for more than 3 years, and at the time of the study they smoked a mean of 1.25 packs of cigarettes per day. Each of the 100 subjects was evaluated in the sleep laboratory for four consecutive nights, the first night allowing for adaptation of the subjects to the laboratory. Standardized methodology was used for recording electroencephalograms (EEG), electromyograms, and electrooculograms. The records were scored independent of any knowledge of the experimental conditions (*1*). In addition, each subject completed a sleep history questionnaire and the Minnesota Multiphasic Personality Inventory (MMPI).

Table 1. Comparison of sleep efficiency and sleep stage variables between smokers and nonsmokers monitored for four consecutive nights in a sleep laboratory. The values are means and standard errors of nights 2 to 4 for both groups. The length of the recordings each night was 8 hours. The Student's *t*-test was used for all comparisons. Sleep efficiency is measured in minutes; stages are in percentages.

Parameter	Smokers	Nonsmokers
Sleep efficiency		
Sleep latency	43.7 ± 5.5	29.8 ± 2.8*
Time awake after sleep onset	49.0 ± 4.2	44.1 ± 3.3
Total time awake	92.7 ± 6.8	73.9 ± 4.5*
Sleep stage		
1	5.7 ± 0.4	5.1 ± 0.4
2	64.4 ± 1.0	63.3 ± 0.9
3	4.8 ± 0.8	6.0 ± 0.7
4	0.7 ± 0.3	1.1 ± 0.4
REM	24.4 ± 0.6	24.5 ± 0.5

*$P < .05$.

The results of the sleep laboratory recordings (on nights 2 to 4) comparing smokers with nonsmokers suggest that cigarette smoking contributes to sleep difficulty (Table 1). The smokers were awake for a significantly longer time than the nonsmokers (92.7 versus 73.9 minutes, $P < .05$), primarily because they had greater difficulty in falling asleep (43.7 versus 29.8 minutes, $P < .05$). The two groups did not differ significantly in sleep stage parameters.

Analyses of the MMPI revealed no significant differences between the smokers and nonsmokers on any of the eight major clinical scales, and the two groups showed no differences in the use of

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