TRANSMISSION OF C-BAND VARIANTS IN JAPANESE QUAIL[1]

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Summary

Heteromorphisms of chromosome banding patterns can be useful markers for gene mapping and other kinds of genetic studies. In Japanese quail, the centromere region of chromosome No. 4 is the site of a heteromorphism. One form of the C-band at this region is relatively small ("a" form); an alternative form is much larger ("b" form). To identify the transmission patterns, all possible matings were made between birds with karyotype a/a, a/b, and b/b. The outcome from all crosses are entirely consistent with the expectation from simple Mendelian transmission. No evidence was found for segregation distortion or gametic selection. This dimorphism, therefore, is a reliable marker.
(**Key Words** : Japanese Quail, Chromosome, C-band, Transmission)

Introduction

Heteromorphisms of chromosome banding patterns can be useful markers for gene mapping (Akeson and Davisson, 1991) and other kinds of genetic studies (e.g., Blazak and Fechheimer, 1980). Their value is increased if they can be easily and reliably identified, are stable, segregate in a regular manner, and do not exert differential effects on gametic or embryonic viability. Variants of C-banding patterns have been identified in man, several domesticated species of animals, as well as the mammals regularity used for laboratory studies (Verma, 1988). Most of them appear to be stable but only a few have been rigorously tested for regularity of transmission, or to detect effects they might have on gametic or zygotic viability.

The normal C-band pattern of Japanese quail has been depicted by Comings and Mattocia (1972), Sasaki et al. (1980) and Stock and Bunch (1982). An idiogram of the most frequently occurring C-banded regions was produced by de la Sena et al. (1991) who also noted C-band variants of chromosomes 4 and Z. The centromere region of the acrocentric chromosome number 4 is the site of a heteromorphism. One form of the C-band at this region is relatively small; an alternative form is much larger and is invariably associated with an elongated short arm. In this paper we report that the transmission of the two forms, by both male and female heteromorphic parents is completely regular.

Materials and Methods

The birds from which samples were obtained for observation of C-band patterns were from a random mating population established from three sources in 1968 and maintained at the Ohio Agricultural Research and Development Center (Nestor et al., 1982). Mature birds to be used as parents in subsequent crosses were selected after their C-band karyotype was observed in lymphocytes cultured according to the method described by Sohn et al. (1990). Birds were screened to detect sufficient numbers that were homokaryotypic for each variant and heterokaryotypic. The small form of the C-band at the centromeric region of chromosome 4 was designated "a" and the large form was designated "b" (figure 1 and 2).

All possible matings, including reciprocals were made between birds with karyotypes a/a, a/b, and b/b, except a/a × b/b. For all matings two pairs of parents were used. The karyotype of progeny was assessed in early

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embryos so that the results would not be distorted by possible differential incidences of embryonic death among the three zygotic types. Eggs were collected daily and stored at 10℃ for a maximum of seven days. Each day 20 -30 eggs were incubated overnight for 16 hours, after which they were injected with colchicine (0.06 ml, 0.2%) and returned to the incubator for two hours. The eggs were then opened, the embryos removed, their cells dispersed, and slides prepared by the method of Wolowodiuk et al. (1985). From each embryo two slides were prepared. One was stained with Giemsa, without further treatment; the second was treated to reveal the presence of C-bands (de la Sena et al., 1991).

A minimum of 10 cells from each embryo was examined. Cells on the slides that had not been treated before staining were examined for the occurrence of various forms of heteroploidy, for sex chromosome complement, and for the presence or absence of an elongated short arm on each chromosome 4; cells on the slides treated to reveal C-bands were also examined to detect heteroploidy and for the occurrence of a small ("a" form) or large ("b" form) prominent C-band located at the centromeric region of each chromosome 4.

Data were analysed by chi-square tests of independence, and goodness-of-fit of observed numbers to those expected on theoretical grounds.

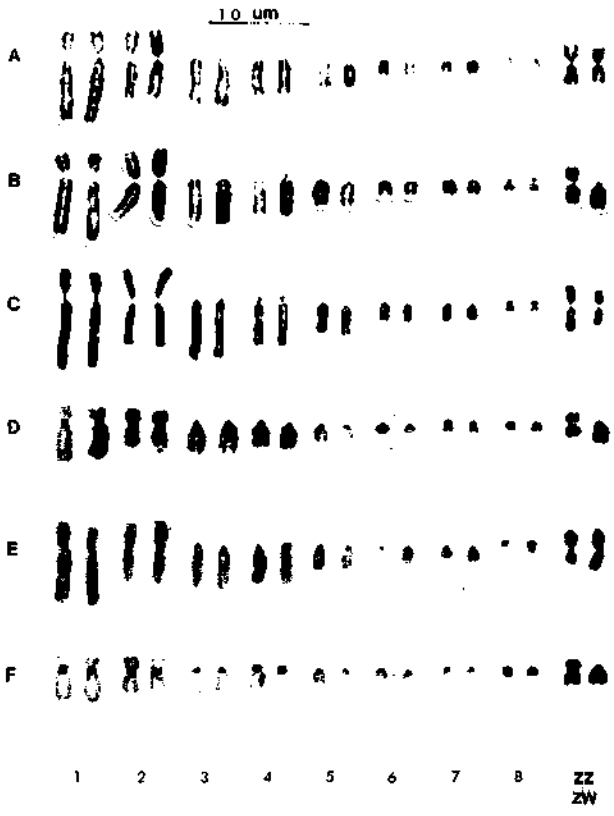

Figure 1. The partial karyotypes of Japanese quail (*Coturnix coturnix japonica*). A, B, C: Partial karyotypes with Giemsa staining. D: C-banded karyotype of A type. E: C-banded karyotype of B type. F: C-banded karyotype of C type.

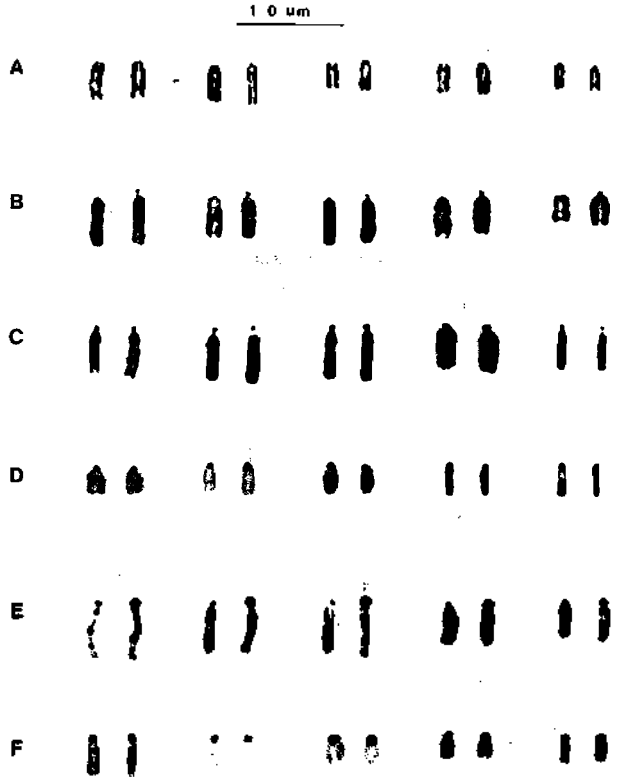

Figure 2. The arrangement of variants of chromosome 4. A, B, C: Arrangements of chromosome 4 with Giemsa staining. D: C-banded chromosome 4 of A type (a / a). E: C-banded chromosome 4 of B type (a / b). F: C-banded chromosome 4 of C type (b / b).

Results and Discussion

From a total of 844 eggs that were incubated, 491 (58.2%) embryos were successfully examined. Of the remaining eggs 148 (17.5%) were infertile, 74 (8.7%) were broken during processing, 131 (15.5%) contained embryos some of which had presumably died, because only a few cells at metaphase were seen on the slides (table 1).

Of the 491 diploid embryos 46% (S.E. = 2.3%) were male (ZZ). The data were combined with those from previous studies of Japanese quail embryos at the same stage of development (Wolowodiuk et al., 1985; de la

TABLE 1. NUMBER OF EGGS IN CHROMOSOME ANALYSIS

	(a/a) ×(a/a)	(a/a) ×(a/b)	(b/b) ×(a/b)	(a/b) ×(a/b)	(b/b) ×(b/b)	Total
Total No. of eggs						
	84	285	217	134	124	844
Infertile eggs (%)						
	11	55	44	24	14	148
	(11.3)	(19.3)	(20.3)	(17.9)	(11.3)	(17.5)
Eggs not analyzed (%)						
Live	12	26	20	12	4	74
Dead	10	48	30	9	34	131
	(11.9)	(16.8)	(13.8)	(6.7)	(27.4)	(15.5)
Eggs anlyzed (%)						
	51	156	123	89	72	491
	(60.7)	(54.7)	(56.7)	(66.4)	(58.1)	(58.2)
Sex ratio (♂:♀)						
	1:1.5	1:1.2	1:1.3	1:0.8	1:1.3	1:1.2
♂	20	71	54	49	32	32
♀	31	85	69	40	40	40

TABLE 2. NUMBERS OF PROGENY FROM RECIPROCAL CROSSES OF PARENTS HOMOMORPHIC AND HETEROMORPHIC FOR A C-BAND VARIANT ON CHROMOSOME 4

Mating	Number of embryos			χ^2-test of independence	
♂ × ♀	(a/a)	(a/b)	(b/b)		
(a/a)×(a/b)[1)]	43	42		0.29	p>0.5
(a/b)×(a/a)	32	39			
(a/b)×(b/b)		28	28	0.06	p>0.75
(b/b)×(a/b)		35	32		

[1)] "a" signifies chromosome bearing small C-band.
"b" signifies chromosome bearing large C-band.

TABLE 3. NUMBER OF EMBRYOS RECOVERED FROM PARENTS HOMOMORPHIC OR HETEROMORPHIC FOR A C-BAND VARIANT OF CHROMOSOME 4

Mating[1)]	Number of embryos and (expectation)			Total	χ^2-test for goodness-of-fit
	(a/a)	(a/b)	(b/b)		
(a/a)×(a/a)[2)]	51	0	0	51	—
	(51)				
(a/a)×(a/b)	75	81	0	156	0.23, p>0.5
	(78)	(78)	(0)		
(b/b)×(a/b)	0	63	60	123	0.07, p>0.75
	(0)	(61.5)	(61.5)		
(a/b)×(a/b)	22	52	15	89	3.66, p>0.1
	(22.3)	(44.5)	(22.3)		
(b/b)×(b/b)	0	0	72	72	—
	(0)	(0)	(72)		

[1)] Replicated matings and reciprocal crosses combined.
[2)] "a" signifies chromosome bearing small terminal C-band.
"b" signifies chromosome bearing large terminal C-band.

Sena, et al., 1992). The number of embryos in the pooled sample totaled 3,456, of which 50.7% (S.E. = 0.7%) were male.

Complete concordance between observations of cells on the two slides from the same embryo was noted. If one or both homologues of chromosome 4 had an elongated short arm in the cells on slides not treated for display of C-bands, the same number had the "b" form of the C-band in cells from the same embryo on slides that were treated to display C-bands. Futhermore, no intercellular variations of the C-bands on chromosome 4 were observed.

The results from replicate matings within each cross were not significantly different ($p > 0.1$ for each cross). Likewise, the results from reciprocal matings of the crosses (a/a × a/b) and (b/b × a/b) did not differ significantly ($p > 0.5$ for each cross) (table 2). Therefore the data from replicate matings and reciprocal crosses were pooled to yield the data shown in table 3.

The crosses between like homomorphic parents, i.e (a/a × a/a) and (b/b × b/b), served as controls to estimate the accuracy with which the two forms of chromosome 4 were detected. No errors were made in scoring the 51 and 72 embryos derived from the two crosses, respectively (table 3). The numbers of embryos of each type derived from each of the segregating crosses are also shown in table 3. From both crosses between homomorphic and heteromorphic parents, i.e (a/a × a/b) and (b/b × a/b) the numbers of embryos of the two expected types that were recovered did not differ significantly from expectation ($p > 0.5$ for each). From the cross of two heteromorphic parents the ratio of recovered embryos was not significantly different from the expected 1:2:1 ($p > 0.1$). The outcome from all crosses are entirely consistent with the expectation from simple Mendelian transmission of the two forms of the C-band on chromosome 4. No evidence was found for segregation distortion or gametic selection. This dimorphism, therefore, is a reliable marker for the centromeric region of chromosome 4, provided that the three karyotypic forms do not differentially affect zygotic mortality subsequent to 16 hours of incubation.

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