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Chromosome abnormalities in embryos from lines of Japanese quail divergently selected for body weight ⁽¹⁾

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Summary

Karyological analysis was made of 927 embryos from lines of Japanese quail (*coturnix*) divergently selected for body weight at 4 weeks of age, and a randombred control line. Fertility of both selection lines was adversely affected. The sex proportion, calculated as percentage of diploid embryos that was male, was $52.6 \text{ p. } 100 \pm 1.7 \text{ p. } 100$. The line selected for high weight (HW) had significantly more heteroploid embryos than the randombred control (RW) or the low weight line (LW). The frequencies of heteroploid embryos were 8.9 p. 100 in HW, 5.6 p. 100 in RW, and 4.1 p. 100 in LW. The entire difference was accounted for by an increased frequency of haploid/diploid chimeras in HW (4.4 p. 100) compared to RW (1.5 p. 100) and LW (1.4 p. 100).

Key words : Japanese quail, coturnix, embryos, chromosome abnormality, heteroploidy.

Résumé

*Anomalies chromosomiques chez des embryons de caille japonaise
soumise à une sélection divergente sur le poids corporel*

Une analyse caryologique de 927 embryons de caille japonaise a été réalisée dans 2 lignées sélectionnées de façon divergente pour le poids corporel à 4 semaines et dans une lignée témoin. On a observé une réduction de la fertilité dans les 2 lignées sélectionnées. Le sex-ratio, apprécié par le pourcentage d'embryons diploïdes mâles était de $52,6 \text{ p. } 100 \pm 1,7 \text{ p. } 100$. La lignée haute sélectionnée pour un poids élevé (HW) présentait

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significativement plus d'embryons hétéropléides que les lignées basse (LW) et témoin (RW). La fréquence d'embryons hétéropléides était de 8,9 p. 100 ; 5,6 p. 100 et 4,1 p. 100 dans les lignées HW, RW, et LW respectivement. Ces différences s'expliquent entièrement par un accroissement de la fréquence des chimères de type haploïde-diploïde dans la lignée HW (4,4 p. 100) par rapport aux lignées RW (1,5 p. 100) et LW (1,4 p. 100).

Mots clés : Caille japonaise, coturnix, embryons, anomalie chromosomique, hétéropléidie.

I. Introduction

Karyological examination of early embryos of a number of mammalian species and of the domestic chicken has revealed a significant but variable proportion with abnormal chromosomal complements. In man it has been estimated that more than 50 p. 100 of early abortuses are chromosomally abnormal (BOUÉ *et al.*, 1975 ; HASSOLD *et al.*, 1978 ; KAJII *et al.*, 1980 ; LAURISTEN, 1976). The frequency of spontaneously occurring heteroploid embryos in laboratory mammals ranges from 1.5 p. 100 to 6 p. 100 depending upon the strain and experimental conditions (BINKERT & SCHMID, 1977). Samples of sheep and cattle embryos have also been observed. About 6 p. 100 sheep embryos (LONG & WILLIAMS, 1980) and 2 p. 100 of preimplantation cattle embryos (HARE *et al.*, 1980) were heteroploid. Much work has been done to elucidate the etiological factors of importance to the occurrence of various forms of heteroploidy, but such work is hampered by relatively small samples, the low frequency of each type of heteroploidy, and by the difficulty of collecting and analyzing all the embryos contained in the oviducts and uteri of mammalian females.

These difficulties are largely overcome in studies with chicken and other avian embryos. Large samples may be readily collected, all eggs are accounted for, and a high proportion of embryos are successfully analyzed. Work with chicken embryos by BLOOM (1974) and FECHHEIMER (1981) has indicated that many forms of heteroploidy occur and their frequency, ranging from 1.5 p. 100 to 12 p. 100, depends largely on the strain being studied. The mechanisms of origin of the various forms is now established and work is underway to study the various genetic and non-genetic factors that disrupt the reproductive process at various times and in various ways to yield heteroploid zygotes.

Large bodied strains of chickens, those of broiler type, are characterized by relatively low fertility, high embryonic mortality, and high frequencies of heteroploidy in early embryos (FECHHEIMER, 1981). Lines divergently selected for body weight had a two-fold difference in incidence of heteroploidy (REDDY & SIEGEL, 1977). It would be of great interest to know whether this outcome is the result of a genetic correlation or resulted only from random drift of genes or linkage disequilibrium in the selection lines.

In the present study of Japanese quail embryos from lines selected for large and small body weight and a control line, it was found that the large line has increased frequency of chromosomally abnormal embryos.

II. Materials and methods

A. Animals

A base population of Japanese quail (*Coturnix*) was established and reproduced for 10 generations from more than 100 pairs of parents, selected at random, in each generation. From this base, 3 sublines were commenced. One was a randombred control line, designated RW. Two additional lines were mass selected for high (HW) and low (LW) body weight at 4 weeks of age. Each line was reproduced each generation from 36 males and 36 females, mated at random with the exception that no full-sib matings were made. This study was made in the 10th generation of the sublines. Average body weight at 4 weeks of age was 91 gms., 128 gms., and 49 gms. in RW, HW, and LW respectively. Detailed description of the formation and maintenance of the lines, and of their attributes and performance, is given by NESTOR *et al.* (1982) and NESTOR & BACON (1982).

B. Collection and handling of eggs

Eggs were collected daily, identified by dam and date of collection, and stored at 10 °C for 7-14 days. Batches of 30 eggs were removed from storage and put in an incubator at 39 °C for 16 hours. Following the 16 hour incubation period each egg was injected with an aqueous solution of colchicine. About 0.002 mg colchicine was given for each gm. of egg weight. The eggs ranged in weight from an average of 7 gms. in LW to 13 gms. in HW. The eggs were returned to the incubator for a further 2 hours of incubation.

C. Preparation of slides from embryos

Embryos were removed from the eggs yolks, placed in three-ml tubes in Hanks' balanced salt solution and dissociated by aspiration with a Pastuer pipette. The cell suspension was treated with a hypotonic solution of bovine serum and distilled water (1 : 3) and three changes of acetic acid and methanol fixative (1 : 3). Following the final change of fixative, the cells were concentrated into 4 to 6 drops of fixative and dropped onto cold wet slides. The slides, after drying, were stained with Giemsa stain in Wright's buffer, rinsed with distilled water, allowed to dry and mounted in Permunt. The entire procedure is that of MILLER *et al.* (1971).

D. Examination of slides

Slides were placed and examined in a random order. Each was scanned systematically for presence of cells at a suitable stage for chromosomal analysis. Cells at metaphase that exhibited a round configuration of the chromosomes and in which the chromosome morphology was distinct, were chosen for analysis. Ten such cells

on each slide were analyzed. The analysis consisted for identifying the members of the eight largest pairs of autosomes and the Z and W chromosomes. The microchromosomes, numbering about 60 were disregarded because they cannot be counted accurately nor identified. The eight pairs of macrochromosomes appear morphologically identical to those of domestic chicken (TALLURI & VEGNI, 1965).

If no cells were present on a slide, it was scored as having come from an infertile egg. If cells were present but none was seen at metaphase the embryo was considered to have been dead. A small proportion of slides contained cells at metaphase but they were either too few in number or were not sufficiently clear for analysis. These were scored as live but not analyzed.

E. Analysis of data

Differences between means of the 3 lines were tested for significance using heterogeneity χ^2 analysis.

III. Results

A. Infertility, embryo mortality and other losses of eggs

A total of 1357 eggs was collected of which 927 yielded embryos amenable to analysis (table 1). The remainder were either infertile (9.5 p. 100), were lost during processing (8.8 p. 100), contained dead embryos (5.3 p. 100), or contained live embryos which could not be analyzed (8.1 p. 100).

TABLE 1

Numbers and percentages () of eggs from the 3 lines of Japanese quail that were analyzed or lost for various reasons.

Nombres et pourcentages () d'œufs des 3 lignées ayant été analysés ou perdus pour des raisons diverses.

Line	Total number of eggs	Eggs lost in processing (a)	Infertile eggs (a, b, c)	Eggs not analyzed		Eggs analyzed
				Live	Dead (a)	
HW ..	439	41 (9.3) ^a	66 (15.0) ^a	17 (3.9)	22 (5.0) ^a	293 (66.7)
RW ..	478	40 (8.4) ^a	22 (4.6) ^b	46 (9.6)	28 (5.9) ^a	342 (71.5)
LW ..	440	38 (8.6) ^a	41 (9.3) ^c	47 (10.7)	22 (5.0) ^a	292 (66.4)
Totals .	1357	119 (8.8)	129 (9.5)	110 (8.1)	72 (5.3)	927 (68.3)

a, b, c : Within a column, values with different superscripts are significantly different, $P < 0.05$.
Les chiffres suivis de lettres différentes sont significativement différents dans une même colonne, $P < 0,05$.

The lines differed significantly ($P < 0.005$) in the proportion of infertile eggs recovered. RW had least and LW had most. They also differed significantly in the proportion of live embryos that could not be analyzed, but this difference is attributable entirely to a technical error in the laboratory and has no biological importance. Eggs were lost during processing because they had soft shells, because the yolk broke when the embryo was being removed, or because the embryo was not successfully transferred to its tube. The proportion of eggs lost during processing was not different in the 3 lines. The incidence of dead embryos averaged 5.3 p. 100 and the differences among lines were not significant ($P > .975$). Analysis was successfully completed on 89 p. 100 of all slides containing cells from live embryos.

B. Sex proportion

The sex proportion in the 3 lines, expressed as percentage males (ZZ) was 52.8 p. 100 for HW, 50.5 p. 100 for RW, and 55.0 p. 100 for LW. Differences among lines were not significant, so the data may be pooled to yield an estimate of 52.6 p. 100 \pm 1.7 p. 100. This proportion does not differ significantly from an expected proportion of 50 p. 100.

TABLE 2

*Numbers and percentages () of heteroploid embryos
in the 3 lines of Japanese quail.*

Nombres et pourcentages () d'embryons hétéropléides dans les 3 lignées.

Type of heteroploidy										
Line	Number of embryos	1 n and 1 n/2 n chimera (a, b)	Triploid (3 n)	2 n/4 n mosaic	Monosomy (2 n - 1)	Trisomy (2 n + 1)	Aneuploid mosaic (2 n/2 n \pm 1)	Chimera (2 n/2 n)	Structural aberration	Total heteroploid (a, b)
HW ..	293	13 (4.4) ^a	1 (0.3)	3 (1.0)	5 (1.7)	0 (0)	2 (0.7)	1 (0.3)	1 (0.3)	26 (8.9) ^a
RW ..	342	5 (1.5) ^b	1 (0.3)	4 (1.2)	2 (0.5)	2 (0.6)	3 (0.9)	0 (0)	2 (0.6)	19 (5.6) ^b
LW ..	292	4 (1.4) ^b	0 (0)	1 (0.3)	3 (1.0)	0 (0)	3 (1.0)	0 (0)	1 (0.3)	12 (4.1) ^b
Total .	927	22 (2.4)	2 (0.2)	8 (0.9)	10 (1.1)	2 (0.2)	8 (0.9)	1 (0.1)	4 (0.4)	57 (6.2)

a, b : Within a column, values with different superscripts are significantly different, $P < 0.05$.
Les chiffres suivis de lettres différentes sont significativement différents dans une même colonne, $P < 0.05$.

C. Frequency of heteroploidy

The number and frequency of heteroploid embryos in the 3 lines is shown in table 2. A significantly greater frequency was recovered from HW (8.9 p. 100) than from either RW (5.6 p. 100) or LW (4.1 p. 100) ($P < 0.05$). The difference between RW and LW was not significant ($P > 0.10$). Analysis of frequencies of the various types of heteroploidy revealed that the HW line produced 3 times more 1 n and 1 n/2 n chimeric embryos (4.4 p. 100) than did RW (1.5 p. 100) or LW (1.4 p. 100). Frequencies of other types of heteroploidy were relatively low and were too small for reliable tests of significance of differences among the lines. Therefore the primary difference between HW and the other 2 lines is largely accounted for by the increased number of 1 n/2 n chimeric embryos in HW because only 2 embryos, one each in LW and RW, were pure haploid, 1 n.

IV. Discussion

A. Comparison of Japanese quail with chicken

The HW line yielded significantly more heteroploid embryos than RW or LW. The frequency of 1 n/2 n chimeras was especially high in HW. A similar result was reported by REDDY & SIEGEL (1977) in lines of chickens divergently selected for body weight for 17 generations. In other karyological observations of chicken embryos it was found that one large bodied (broiler) line produced significantly more heteroploid embryos of several types, including 1 n/2 n chimeras, than a small egg-laying line (FECHHEIMER, 1981). Furthermore crosses between the 2 lines and *inter se* matings of F1 parents yielded intermediate frequencies of heteroploid embryos. Taken as whole, the 3 studies indicate that a genetic correlation exists between body weight and production of heteroploidy embryos in gallinaceous birds. In all 3 studies, the frequency of 1 n/2 n chimerism was markedly higher in the heavy lines.

It has been established in the chicken that the occurrence of haploid cell lines in embryos, either pure haploidy (1 n) or haploid/diploid chimerism is of androgenetic origin (FECHHEIMER & JAAP, 1978; 1980). The haploid cell line is derived from a spermatozoon that enters the egg, does not engage in syngamy, but proceeds to proliferate by mitosis. Thus 1 n/2 n chimeras result from dispermy. Two sperm enter the egg, one fuses with the maternal pronucleus to yield the normal zygotic 2 n cell line and the second acts as the origin for the 1 n cell line. The occurrence of haploidy in chicken eggs, and presumably also in quail, is the result of an error of fertilization, i.e. dispermy, and the propensity for dispermy to result in 1 n/2 n chimerism rather than in triploidy (3 n). The high rate of dispermy in chicken and quail of high body weight is apparently attributable to the ovulation of eggs in which the normal mechanisms for blockage of polyspermy are not effectively operating. Eggs that are ovulated either prematurely, or whose ovulation is delayed, might lack the capacity to block polyspermy.

Lines of chickens (JAAP & MUIR, 1968), turkeys (NESTOR & BACON, 1972), and quail (BACON *et al.*, 1973) selected for rapid growth typically exhibit erratic oviposition,

a high proportion of defective eggs, and other signs of irregularly timed ovulation. The apparent genetic correlation between rapid growth and incidence of chimerism might be indirect, mediated by the propensity for irregular ovulatory cycles in rapid growth lines of birds.

The array of types of heteroploidy observed in the quail is similar to that seen in various lines of chickens (BLOOM, 1972 ; FECHHEIMER, 1981). Furthermore their relative frequencies are roughly similar, $1 n/2 n$ chimerism being the most frequent in both species. More lines of quail need to be examined before it can be established that interline variability is as great as in chickens. However if the RW line is representative of domesticated quail it would appear that they might well be an excellent model system for the study of etiology of heteroploidy in domesticated birds.

B. Estimates of heteroploid frequencies

Only 10 cells from each embryo were analyzed initially and only 9 pairs of chromosomes, of a total of about 39, were accounted for in each cell. Accordingly the frequencies of some types of heteroploid will be underestimated. Mosaic and chimeric embryos in which one cell line is represented by a small proportion of cells might well not have been detected. Likewise only $1/2$ of the $2 n/2 n$ chimeras could have been detected because no autosomal markers were used ; determination of the chimeric state was made from the gonosomes only. The frequency of aneuploid embryos represents aneuploidy for only one-fourth of the pairs of chromosomes if aneuploidy for the microchromosomes, which were not scored, occurs at about the same rate as that of the larger pairs. Even though the frequencies of various types of heteroploidy are minimal estimates, the same estimates were made of the 3 lines so that comparisons among the lines is perfectly valid.

C. Sex proportion

The sex proportion, pooled over the 3 lines was $52.6 \text{ p. } 100 \pm 1.7 \text{ p. } 100$ male. This is not significantly different from the theoretical expectation of $50 \text{ p. } 100$. In the strict sense the estimate is not of the primary sex ratio. The embryos were in only the first day of incubation however, and the frequency of embryonic death had been low, about $5 \text{ p. } 100$. Therefore, unless the differential rate of mortality had been very great, the estimate made from embryos at 16 hours of incubation must be a close approximation of the primary sex proportion.

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