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Research Article

Analysis of Chromosome Karyotype and Banding Patterns of Chicken, Quail, and Their Hybrids

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Abstract

To explore the incompatibility of hybrids between chickens and quails at the chromosome level, in the present study, chickens, quails, and their hybrids were selected and their chromosome karyotype and banding patterns were analyzed. The methods used comprised pre paring chromosomes from air-dried peripheral blood lymphocytes, the embryo method, G-banding, and C-banding techniques. The result revealed that the number of chromosomes (2n) of chicken, quail, and their hybrids was 78, with 10 pairs of macrochromosomes and 29 pairs of microchromosomes; however, there were some remarkable differences in chromosome morphology. There were significant differences in G-banding patterns between chickens, quails, and their hybrids, among which chickens chromosomes were divided into 32 zones with 155 bands, including 71 positive bands. The quails were divided into 28 zones, with 138 bands, including 61 positive bands. C-band analysis showed that the C-band of chickens, quails, and their hybrids were present on all W-sex chromosomes in all female fission phases and were deeply stained. The combined analysis of the karyotypes and different genotypes of chickens, quails, and their hybrids could provide a reference to accelerate the breeding process.

Keywords: *Chicken, Quail, peripheral lymphocyte culture, Karyotype analysis, G-banding C-banding*

Tavuk, Bıldırcın ve Hibritlerinin Kromozom Karyotipleri ve Bantlanma Modeli Analizi

Öz

Tavuk ve bıldırcınlar arasındaki hibritlerin kromozom düzeyinde uyumsuzluğunu araştırmak için, bu çalışmada, tavuklar, bıldırcınlar ve melezleri seçilmiş ve bunların kromozom karyotipi ve bantlanma modelleri incelenmiştir. Çalışmada, havada kurutulmuş perifer kan lenfositlerinden kromozomların hazırlanması, embriyo metodu, G-bantlanma ve C-bantlanma teknikleri kullanıldı. Tavuk, bıldırcın ve hibritlerinin kromozom sayılarının (2n), 10 çift makrokromozom ve 29 çift mikrokromozoma sahip olmak üzere 78 olduğu ancak kromozom morfolojileri bakımından bazı önemli farklılıkların olduğu belirlendi. Tavuk, bıldırcın ve hibritleri arasında G-bantlanma modelinde anlamlı fark olduğu tespit edildi. Tavuk kromozomları 71 pozitif bant içeren toplam 155 banta sahip 23 bölgeye ayrılmaktaydı. Bıldırcınlarda 61 pozitif bant içeren 138 banta sahip 28 bölge bulunmaktaydı. C-bant analizi, tavuk, bıldırcın ve hibritlerinin C-bantlarının tüm dişi füzyon fazında tüm W-seks kromozomlarında mevcut olduğunu ve derinlemesine boyandığını gösterdi. Tavuk, bıldırcın ve hibritlerinin karyotip ve farklı genotiplerin birlikte analizi yetiştiricilik sürecinde referans olarak kullanılabilir.

Anahtar sözcükler: *Tavuk, Bıldırcın, Perifer lenfosit kültürü, Karyotip analizi, G-bantlanma C-bantlanma*

INTRODUCTION

Chickens and quails belong to the family Aves, *Neognathae*, *Galloanserae*, Galliformes, Phasianidae, *Phasianinae*, Gallus and *Coturnix* in the Phasianidae family; which are the same family but different genera. Chickens have a larger body than quails; however, long-term high-intensity breeding has resulted in chicken meat lacking flavor. Quails have a smaller body, and their meat is nutrient-rich, delicious, aromatic, and contains the bioflavonoid rutin, which has some therapeutic value. In our country, quail meat is known as "animal ginseng", and the cholesterol content of quail eggs is lower than that of chicken eggs, which can lower blood pressure. Chicken and quail are different genera, and hybridization between them represents a typical distant hybridization, providing good resources for studies of gene function and comparative genomics. Their hybrids may form a new population showing dominant

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traits based on excellent traits such as body size, meat production, and meat quality of the parents, and might add new flavors and delicacies to the human diet. As early as 1964, Mcfarquhar reported the hatching of chicken and quail hybrids [1]. The orthogonal combination of chicken (♂) and quail (♀) was then reported in the United States (1985), Japan (1983), and Malaysia (1989). Chunmei et al.[2], performed chicken and quail hybridization experiments (orthogonal combination) with some success. They used artificial insemination to enhance the hatching rate, and the results revealed that intergeneric hybridization allows the first filial generation to obtain the excellent features of both parents at the same time, including the characteristic of the rapid growth of the male parent, and the genetic characteristic of precocious maturing. However, there has been little study of sex identification in the early development of hybridized poultry embryos. These distant hybridizations can not only enrich the breeding material, but also provide an excellent resource. The products of distant hybridization are incompatible (i.e., hybrid combinations do not produce offspring), and this is true of chicken and quail hybrids, as follows: female hybrids all die during the early embryonic stage, only male individuals survive, and hybrid glands do not show meiotic activity. At present, there is no explanation for the mechanism of incompatibility of distant hybridization in birds, and no detailed cytogenetic studies have been carried out on the hybrids between these two species.

In the present study, karyotype analysis was carried out on chickens, quails, and chicken-quail hybrids using embryo methods and the peripheral lymphocyte culture techniques. G-banded patterns were obtained using trypsin and Giemsa. The karyotypes, G-band, and C-band results for chickens, quails, and their hybrids were compared to determine discuss their similarities and differences. The results provide a valuable reference for research on hybrid incompatibility and hybrid sterility between chickens and quails.

MATERIAL and METHODS

Ethics Statement

This study was approved by the Ethical Committee of Animal Experments, Animal Science and Techonology College, Shihezi University (Number: 2011098). All samples were collected in strict accordance with the committee's guidelines. During the experiment, every effort was made to minimize suffering by the animals.

Test Animals

Fifty adult male chickens and 100 female Korean quails, all with healthy bodies and similar weights, were selected. Wannan three-yellow chicken, which has the characteristics of wild grazing, strong feeding ability, wide adaptability, resistance to rough feeding and strong disease resistance. It can be adapted to various feeding forms (herding chickens, large-scale breeding, etc.) based on grazing, and can be adapted to be raised in most provinces in China. Under various feeding forms, the survival rate of Wannan three-yellow chicken is over 90%, and the production performance is normal. The hybrids of chickens (♂) × quails (♀) were obtained by artificial insemination at five time points in the first seven days of incubation. During the time, the number of alive hybrids was observed and recorded by using an egg light to check the development of hybrid embryos. The fertilized egg embryos develop normally, the blood vessels are radially distributed, the color is bright and red; the dead embryo eggs are light in color, there are irregular blood arcs, blood rings, no radial blood vessels; no sperm eggs are bright, no vascular network, only see the shadow of the yolk.The incubation conditions were 37.8±0.5°C, and humidity control at 60-70% relative humidity (RH). Ninety embryos were harvested and 20 male hybrids were hatched for testing. The animals were tested at the Experimental Station, Academy of Animal Science and Technology, Shihezi University.

Reagents Used

Roswell Park Memorial Institute (RPMI1640, GIBCO, USA); Heparin (Hua Mei, He Bei, China); Colchicine (Tiangen, Beijing, China); Giemsa powder (Hua da, Beijing, China); Inactivated calf serum (Hua Mei).

Chromosome Specimen Preparation Method

The chromosome preparations were treated in 0.2N hydrochloric acid for 30 min and air-dried after rinsing with distilled water. The slides were placed in a 5% barium hydroxide solution at 60°C for 5 to 10 min. The slides were taken out of the barium hydroxide solution and quickly rinsed in 0.1N hydrochloric acid to remove the surface barium hydroxide precipitate and then rinsed with distilled water. The slides were incubated in 55-60°C in 2 × SSC for 45-60 min, rinsed with distilled water, and air-dried. The slides were then incubated in 1:9 Giemsa phosphate buffer (pH 6.8) for 10 min, rinsed with distilled water, air-dried, examined microscopically, and photographed.

Chromosome Analysis Method

Giemsa stained chromosome sections were counted under a microscope. The diploid chromosome number was counted under the microscope using a good chromosome spread and mitotic phase of transparent appearance was determined (50 male and 50 female). The three metakinesis phases of a good transparent chromosome spread was for each poultry and photograph under an immersion objective. The long and short arms of first 10 pairs chromosomes were measured using Photoshop image-processing software to calculate the relative length and arm ratio, the centromere index of each chromosome, and their average value in accordance with the following formula.

Relative length = (The length of the chromosome)/(The total length of chromosomes1-10chromosomes [including w chromosome]) ×100

Arm ratio = (The length of the long arm)/(The length of the short arm)

Centromere index = (Length of the short arm)/(The total length of the chromosome)×100

All the animals were killed by the method of heart oppression. A photograph was taken of a good G-band metakinesis phase of chicken, quail and a hybrid. The number of bands, the relative position, the shade of color, and the width of the chromosomes were observed and recorded under a microscope. The number of bands of the first 10 pairs of chromosomes in each cell were counted; and the frequency statistics for the band mode were determined. After that, the medium-term C band under different alkali treatments was observed under a microscope. Well-processed, well-colored metaphase fission micrographs were selected, and the band characteristics and distribution of the C-band were analyzed. Finally, we observed the morphology and bands of the W chromosome.

Statistical Analysis

The associations of the parameters of the macro-chromosomes among chicken, quail and the hybrid were evaluated using chi-square test. The Data were expressed as the mean±the standard error and all statistical analysis were performed with SPSS for Windows (version 19.0).

RESULTS

Karyotype Analysis of Chicken, Quail, and Their Hybrids

The Number of Chromosomes (2n) of Chickens, Quails, and Their Hybrids: Chromosome sections of chicken, quail and their hybrid were carried out using conventional Giemsa staining of 100 selected samples showing good disintegrated phasing for microscopy to determine the statistics of the diploid chromosomes; the results shown in *Table 1*.

From *Table 1*, the number of somatic chromosomes in cells was 2n=78 for chickens, quails and the hybrid, which was the case for 84, 82, and 81% of the total of cells observed, respectively. This demonstrated that the chromosome number of chickens, quail, and their hybrid was 2n=78. The hybrid embryos were selected at five times points (embryonic day 3-7) to detect the sex of early hybrid embryos. The results showed that there were live 70 embryos among the 90 early embryos *(Table 2)*. The ratio of females to males was compared with the theoretical value ($P<0.05$), and there were significantly more males than females. The mortality rate of early female embryos was significantly higher than that of males ($P<0.05$), which further confirmed that the sex determination methods of hybrids were ZZ (♂) and ZW (♀). As hatching proceeded, the sex ratio of the male and female embryos gradually became more unbalanced.

Karyotype Analysis Among Chickens, Quails and Their Hybrids: *Table 3* shows that there are 10 pairs of macro-chromosomes and 29 pairs of microchromosomes among chicken and quail chromosomes. The microchromosomes are all telocentric chromosomes. Chicken chromosomes 3, 5, 7, and 9 are t-type; chromosomes 1, 2, and 8 are m-type; and chromosomes 4 and 6 are sm-type. In quail, chromosome 1 was sm-type, chromosome 2 was m-type, chromosome 4 was st-type, and all other chromosomes were t-type. The Z chromosomes of chickens and quails were all m-type and all were the fifth macrochromosomes. The chicken W chromosome is m-type, its length was equal to that of chromosome 8, while the quail W chromosome is t-type, with a length between that of chromosome 7 and 8. The results of *Table 4* showed that each chromosome of the hybrid was identical to one from chicken or quail, and the method of gender determination was ZZ (♂) and ZW (♀).

Table 1. *The number of chromosomes (2n) of chicken, quail, and their hybrids*

Variety	Distribution of the Chromosomes (2 n)							Total Cellular Score	2 n Model Number	2 n=78 Frequency (%)
	<75	75	76	77	78	79	80			
Chicken	3	2	3	5	84	2	1	100	78	84%
Quail	3	2	4	4	82	3	2	100	78	82%
Chicken-Quail hybrids	2	5	3	4	81	2	3	100	78	81%

Table 2. *Sex and number of live hybrid embryos at different times*

Sex	Brood Days				
	3 days	4 days	5 days	6 days	7 days
Female	4	4	2	3	1
Male	10	10	12	12	12
c^2-test	3.324 ($P<0.05$)	2.421 ($P<0.05$)	3.142 ($P<0.05$)	2.068 ($P<0.05$)	2.073 ($P<0.05$)

Table 3. *The parameters of the macrochromosomes of chicken and quail (X ± SD)*

NO	Chicken				Quail			
	Relative Length	Leverage	Kinomere Index	Kinetochore Location	Relative Length	Leverage	Kinomere INDEX	Kinetochore Location
1	22.09±1.54	1.52±0.11	38.95±0.02	m	21.98±0.79	2.24±0.08	32.04±1.24	sm
2	18.19±1.64	1.59±0.18	38.29±0.06	m	18.21±0.49	1.42±0.06	41.53±1.24	m
3	12.76±0.49	∞	0	t	11.58±0.91	∞	0	t
4	11.07±0.38	2.78±0.42	25.98±0.12	sm	11.14±0.52	5.68±0.16	14.78±0.75	st
5	7.93±0.59	∞	0	t	7.51±0.42	∞	0	t
6	6.29±0.49	1.88±0.37	34.22±0.03	sm	6.14±0.41	∞	0	t
7	5.23±0.39	∞	0	t	4.98±0.58	∞	0	t
8	4.88±0.32	1.09±0.26	46.96±0.12	m	4.42±0.36	∞	0	t
9	3.99±0.31	∞	0	t	3.58±0.26	∞	0	t
Z	9.78±0.81	1.15±0.11	47.49±0.06	m	9.93±0.59	1.14±0.08	47.95±0.41	m
W	4.81±0.21	1.97±0.07	48.49±0.05	m	4.58±0.28	∞	0	t

Table 4. *The parameters of the macrochromosomes of hybrids (X ± SD)*

No	Moiety Chromosome Same as Chicken Karyotype				Moiety Chromosome Same as Quail Karyotype			
	Relative Length	Leverage	Kinomere Index	Kinetochore Location	Relative Length	Leverage	Kinomere Index	Kinetochore Location
1	24.06±1.76	1.56±0.13	39.06±0.04	m	22.24±0.56	2.16±0.03	31.64±1.34	m
2	19.76±1.64	1.59±0.18	38.29±0.06	m	18.21±0.37	1.42±0.01	41.53±1.32	m
3	12.99±0.49	∞	0	t	11.58±0.88	∞	0	t
4	10.91±0.38	2.86±0.42	25.79±0.12	sm	11.14±0.59	5.68±0.19	14.84±0.75	st
5	7.63±0.59	∞	0	t	7.51±0.26	∞	0	t
6	6.54±0.49	1.81±0.37	35.19±0.03	sm	6.14±0.38	∞	0	t
7	5.32±0.39	∞	0	t	4.98±0.47	∞	0	t
8	4.89±0.32	1.23±0.26	46.49±0.12	m	4.42±0.41	∞	0	t
9	3.97±0.31	∞	0	t	3.58±0.31	∞	0	t
Z	9.91±0.81	1.11±0.11	48.11±0.06	m	9.93±0.61	1.14±0.11	47.95±0.38	m
W					4.58±0.29	∞	0	t

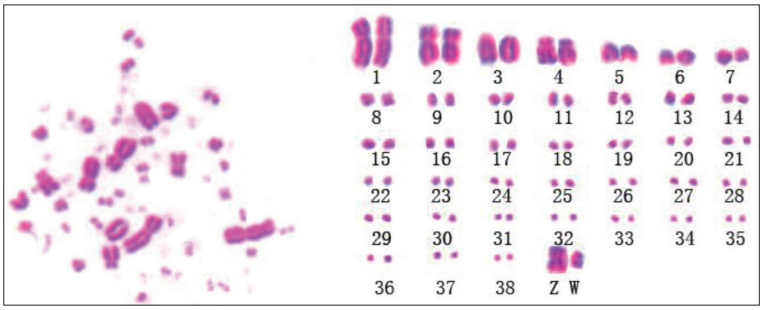

Fig 1. Metaphase chromosomes and idiogram of chicken (♀)

Sex Distribution of Early Embryos: According to the statistical results in *Table 3*, the karyotype maps of the chickens, quails, interspecific hybrids and intergeneric hybrids were drawn *(Fig. 1-4)*. In *Fig. 3* and *Fig. 4*, the chromosome on the left of each pair of chromosomes comes from the chicken, and that on the right comes from the quail

Analysis of Chromosome G-banded Patterns and C-banded Patterns of Chicken, Quail and Their Hybrids

Analysis of Chromosome G-banding: We observed the G-band split phase between chicken, quail, and their hybrids. After treatment with the trypsin-Giemsa method, macrochromosomes showed a relatively clear G band pattern and were rich in bands, homologous chromosome banding patterns are basically the same *(Fig. 5-7)*; most of the microchromosomes had fewer bands, generally only 1-2, and some had no bands and were difficult to identify. There were marked differences between the G banding of chickens and quail chromosomes, which was mainly reflected in the number of bands and the width of the banding pattern. For chromosomes 1 and 2, in chicken, there were seven deep streaks on the q arm and nine deep streaks on arm q, while in quail, there were 5 deep streaks on the p arm

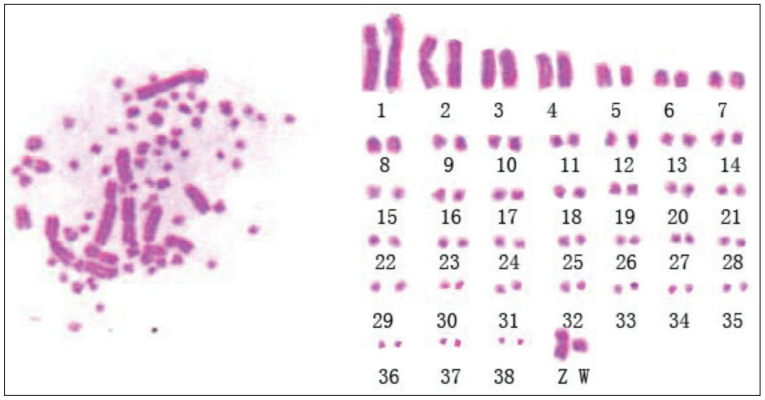

Fig 2. Metaphase chromosomes and idiogram of quail (♀)

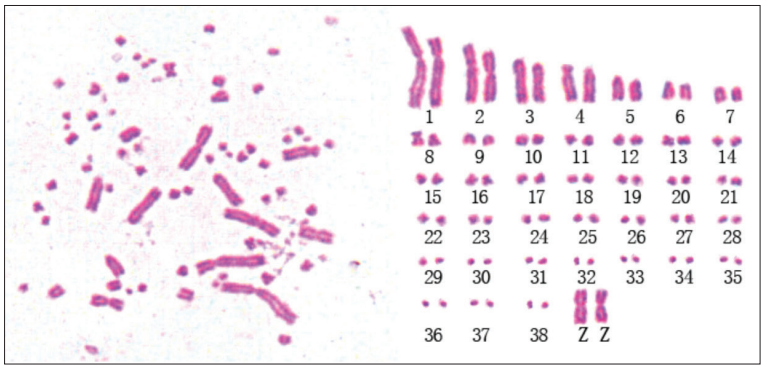

Fig 3. Metaphase chromosomes and idiogram of hybrid (♂)

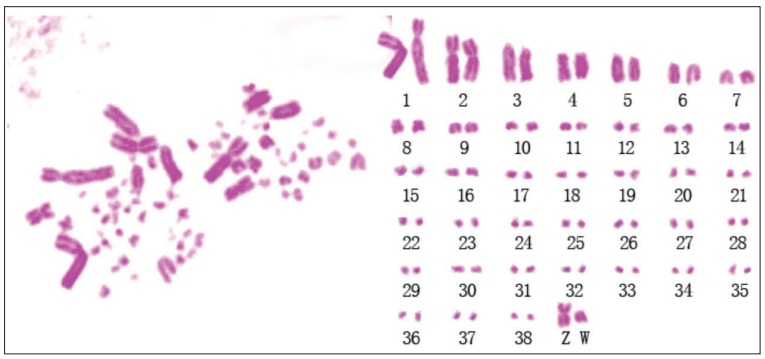

Fig 4. Metaphase chromosomes and idiogram of hybridization embryos (♀)

and 10 deep streaks on the q arm. In chicken, there are two arms on the p arm of chromosome 2 with wider deep-dyed ribbons, while the quail p- and q-arms have a wide deep-dyed band. Chromosomes of the hybrids comprised chromosomes derived from chickens and quails, each of which has the G band characteristic of chicken or of quail. In accordance with the G band pattern, the chicken could be divided into 32 zones, with a total of 155 bands, of which 71 were positive; quail could be divided into 28 zones, with a total of 138 bands, of which 61 were positive. After G-banding, homologous chromosomes showed the same light and dark stripes, and with the help of these stripes, each pair of chromosomes could be accurately paired. Meanwhile, in addition to gene mapping, chromosomal disorders can be diagnosed using G-bands of the disease-associated regions and can be used to explore the correlation between G-bands and production performance on a cytogenetic basis.

Analysis of Chromosome C-banding

We observed the metaphase C-banding of chicken, quail, and their hybrid chromosomes *(Fig. 8)*. Many of the microchromosomes showed deep-staining C-bands, whereas the macrochromosomes did not show C banding in the centromere region. All W chromosomes were darky stained, with strong repeatability, making them easy to identify. Meanwhile, we observed that the C banding was most affected by alkali treatment; when the alkali treatment time was too short (1-2 min), none of the chromosomes showed a C band. As the alkali treatment time increased, the C bands appeared on the W chromosome first, whereas the centromere of the macrochromosome showed a weaker C band, followed by the C band of the macrochromosome telomere, and the minute chromosomes. Analysis of the chromosomal C bands among chicken, quail and their hybrid represented a feasible method to identify the gender of a bird.

DISCUSSION

Chromosomal karyotype not only reflects the germplasm characteristics of a species, but also is useful for breeding studies. Chickens have a large number of microchromosomes; therefore, it is inconvenient to count and describe the chromosomal morphology. Some studies have demonstrated that the incomplete karyotypes of the first 10 chromosomes in birds can represent a species-specific karyotype [3]. Consequently, in the present study, the first 10 pairs of macrochromosomes were analyzed. Thorneycroft et al.[4] performed karyotype analysis of sparrows distributed in the Mudanjiang region and found that their top 10 pairs of chromosomes had the same morphological structure, and the number of chromosomes with 2n=78 accounted for 93.28% of the total observed cells. The criteria for having a diploid chromosome number is that more than 75% of the observed cells should have that chromosome number. Kuchta et al.[5] studied of chicken chromosome karyotypes and found that the chicken chromosome number 2n=78 cells accounted for 85% of the total observed cells. Clagett et al.[6] conducted a chromosomal karyotype analysis of Nick Red Chicken and found that the number of cells with

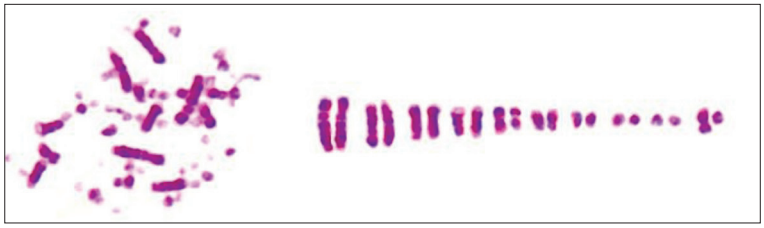

Fig 5. Chromosome G-banding of chicken

Fig 6. Chromosome G-banding of quail

Fig 7. Chromosome G-banding of hybrid

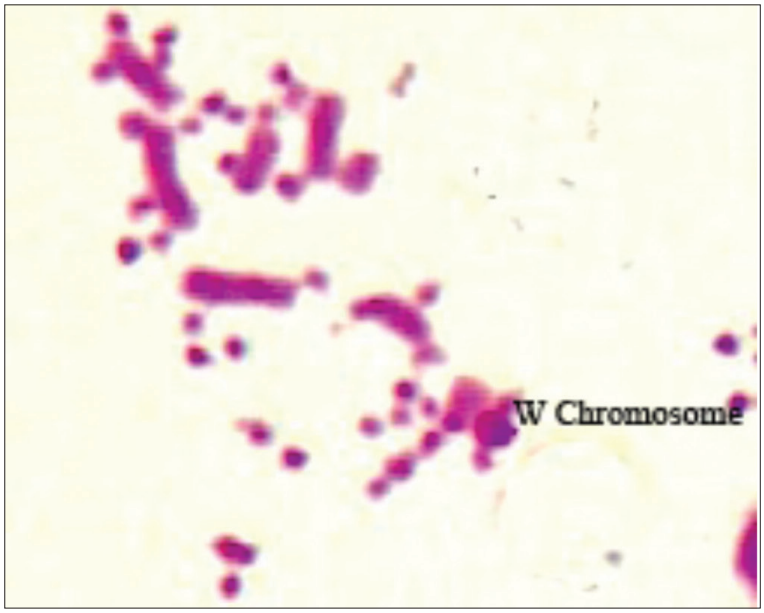

Fig 8. Chromosome C-banding of hybrid

2n=78 chromosomes accounted for 78% of the total observed cells. In the present study, 84%, 82%, and 81% of the total number of observed cells had 2n=78 chromosomes in chickens, quails, and their hybrids, respectively, which was consistent with previous studies. In the cases where 2n≠78, this may reflect the small number of chromosomes in the poultry karyotype or the occurrence of Robertsonian translocations in microchromosomes [7]. Shi et al.[8] compared the karyotype of quail and chickens, and showed that the number of chromosomes were 2n=78; however, the shape and relative length of the chicken chromosomes were significantly different from those of quail ($P<0.05$). Xu et al.[9] analyzed the chromosome karyotype of quail, and found that 2n=78 accounted for 78% of the total number of cells analyzed. In quail, the number of copies of chromosomes 2, 4, and 6 are slightly different, Which was similar to the results of Shi at al.[8] and Xu et al.[9].

It is generally believed that the rate of karyotype evolution in birds is very slow, the number of chromosomes is well conserved, and closely related species have basically the same or similar karyotypes. Chickens and quails are two organisms of the same family but different genera. Their chromosomal relative length is almost the same, but there are marked differences in chromosome morphology, mainly for chromosomes 1, 4, 6, 8 and the W chromosome, indicating a cytogenetic basis for their assignment to different genera [10,11]. The results revealed that the evolutionary trend of karyotype is, from more to less for the small chromosomes, from less to more for the large chromosomes, and from the end to the middle in the development of the centromere type [12]. Species with more subterminal/terminal (st/t) chromosomes in bird karyotypes may be relatively primitive species, whereas species with more sumedian/median (sm/m) type chromosomes are relatively specialized. Quail has significantly more t-chromosomes than chickens. According to the modern theory of bird evolution, the *Galliformes* and the chest type are preserved more than the original karyotype of birds [13], and single arm chromosome inversion through arm evolution to a two-armed chromosome is one of the major forms of bird karyotype evolution [14]. Taking these observations in to account, we hypothesized that quails may have evolved to a lesser extent than chickens.

G-banding is a chromosomal banding technique

in which metaphase chromosomes are treated with trypsin and then stained with Giemsa, which produces patterns of light and dark phases. To date, there have been few successful reports in the domestic literature of clear G-band maps, and the G-banding technique for bird chromosomes is more difficult compared with that for mammals. Xu et al.[9] mapped the G-band pattern of the top 10 quail chromosomes (including the Z and W chromosomes) and found that the main reason for the unsuccessful G-band pattern might the higher degree of spiraling of the metaphase chromosomes, such that the stripes are often combined with thick, fuzzy features. In birds, there are large length differences between the chromosomes; an early metaphase split phase; chromosomes 1, 2 and 3 are too long and easy to wrap around one another or overlap, which affects the zonation effect, and other shorter chromosomes are easily over-digested, all of which create further difficulties in the interpretation of G banding results [9]. Albertson et al.[15] analyzed the first 10 pairs of chromosomes (including the Z and W chromosomes) in chicken, and indicating that there was a band like displacement on chromosomes 1 and 2, which might have been caused by inversion between the arms. In the present study, we found that the macrochromosomes from chickens, quails, and their hybrids showed a clear G band pattern with abundant bands. The bands on the homologous chromosomes were basically the same [16]. A few lacked obvious stripes, making it difficult to identify the pairs. In addition, chromosomes 1 and 2 showed band displacement, which was consistent with the data of Albertson et al.[15].

C-banding is created by treatment with strong acids and bases, followed by visualization with Giemsa. Currently, barium hydroxide treatment is commonly used to visualize the location of structural heterochromatin [17]. There have been few reports about Chromosome C banding or the analysis of the chromosomes of livestock and birds. Christensen et al.[18] analyzed C-band patterns in pigs and found that in cells undergoing mitosis, at the centromere and its vicinity, the amount of structural heterochromatin is constant. In addition, the mid-term chromosome C band size was highly reproducible, and in the same individual during different periods, the C-band sizes were similar. Takuma et al.[2] showed that in Taihe Silky Fowl, chromosome C banding treatment revealed that all sex chromosomes in the hen's split phase had C bands, and were deeply stained and easy to identify. The results of the present study showed that the vast majority of microscopic chromosomes showed deep C-bands, whereas large chromosomes had single shallow C bands or none at all. Reproducibly, C-banding of the W-chromosome appeared first, with the entire chromosome being stained. This result is similar to those reported by Liu et al.[2]. Our results further confirmed that the centromeric region of chicken miniature chromosomes contains more heterochromatin, whereas the large chromosomes contain little or no heterochromatin [5,7,8,19]. Wojcik et al.[7] showed that a large number of microstructural heterochromatic chromosomes might be more prone to Robertson's translocation. There are many microchromosomes in the poultry genome, and the W chromosome is just 1/5–1/2 of the size of Z chromosome, and is usually difficult to identify accurately. Thus, in non-banding specimens, identifying the W chromosome of birds is always harder than in mammals, making chromosome identification much more difficult. Wang et al.[20] used C-banding technology to successfully identify the male and female *Nipponia nippon*. In that experiment, C-banding was performed using the peripheral blood lymphocyte division phase, and the W chromosome was successfully identified, allowing the sex of the early embryos of intergeneric hybrids to be determined. In the present study, we used C-banding to identify W chromosomes, in combination with the morphological identification of Z chromosomes, which greatly improved the accuracy of sex determination of birds, providing a safe and reliable sex identification test for certain rare avian species [21,22].

Karyotype analysis illustrated that males and females were present in the hybrid embryos at 3-5 days after inoculation, while the adult hybrids were all male, indicating that all female hybrids died at the embryonic stage and only male individuals survived. Why did this occur? The mechanism of early death of female embryos in distant hybrids of poultry remains unclear. Chromosome analysis suggests that early embryonic death might be caused by variations in the number of chromosomes and structural abnormalities. The sex of the silkworm is decided in the same way as that of the poultry, and all of them are male ZZ or female ZW. A recessive lethal gene in the Z chromosome causes all the embryonic female individuals to die [23,24]. If the Z chromosome has the recessive lethal gene, when the sex chromosomes are of ZW type, gene expression from the unpaired lethal gene leads to the death of female embryos. However, interspecific hybrid infertility and cell chromosomes are linked: If the two parents differ greatly in terms of their chromosomal characteristics, meiosis will be blocked such that the hybrid cannot produce normal germ cells and cannot reproduce [25]. Du et al.[26] analyzed the somatic chromosome karyotypes of Muscovy duck, Strabian duck, and their interspecific F1 hybrid. Compared with their parents, the F1 hybrids had the same number of chromosomes, but a different number of arms. The first and second pairs of autosomes had a centromere index and arm ratio with intermediate values between those of the two parents, the two sister siblings were homologous to the paternal and maternal preference respectively. Sex chromosome centromeres with double characteristics can be used for identification of the authenticity of hybrids. Mank et al.[25] found that the karyotypes of chromosomes 1 and 2 in Muscovy duck and domestic duck were the main causes of F1 sterility in the F1 hybrid. In the present study, hybrid embryos were selected for embryo

development for 3-7 days to detect the sex of early hybrid embryos. The results found that there were among the 90 early embryos, 70 were viable. The ratio of females to males diverged significantly from the theoretical value ($P<0.05$), and there were more males than females. The mortality rate of early female embryos was significantly higher than that of males ($P<0.05$), which further confirmed the sex determination of hybrids as ZZ (♂) and ZW (♀). As hatching progressed, the gender ratio became gradually more unbalanced.

In summary, chromosomes of chickens, quails, and their hybrids were selected and analyzed. Chickens and quails had the same chromosome number (2n=78), but there were significant differences in chromosomal morphology, mainly for chromosomes 1, 4, 6, 8, and the W chromosome. Their chromosome karyotype parameters showed certain differences, and there were differences in the number of bands and the width of the bands in the G-banding experiment. These differences disrupted the inherent balance between the hybrid chromosomes, thereby undermining the meiotic process and increasing the frequency of reproductive failure. The problem of sterility after distant hybridization is a complex biological issue, and comprehensive studies from the aspects of cell biology, morphology, physiology, molecular biology, and immunology are required to determine the mechanisms.

Competing Interest

The authors declare that they have no competing interests.

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