Chromosome analysis of chicken and quail

Chicken, quail and Chicken-Quail hybrids were used as objects of study, in order to investigate the crossing incompatibility between Chicken-Quail in the level of chromosome. Chromosome specimens are prepared by the peripheral blood lymphocyte cultivation, airing and embryonic, with karyotype, G and C band analysis. The results showed that the number of chromosomes of chicken and quail are 2n = 78, including 10 pairs macrochromosomesand 29 minute chromosome, but apparented differences are existed in the morphous of chromosomes, there are comparatively large difference between the number of fringe and the width of fringe of G band stripe of chicken and quail. It can be divided into 32 zones, with 155 straps and 71 positive bands for chickens. And it can be divided into 28 zones, with 138 straps and 61 positive bands for quails. Through the analysis of C zonation, it indicated that W chromosome of fission phase in all the female always show C-band and the whole band anachromasis, easily identified, which further confirmed the C-band analysis is an effective way of birds sexing.

Key words: Chicken, Quail, Hybrid Species, Karyotype

According to the statistics, 56 percent of kinds of birds can be used to species hybridization and 44 percent of kinds birds can be used to intergeneric cross, epecially between family or subfamily[1]. These phenomenon of distance hybridization can make breeding materials richer and provide superordinary goods group, however, the problems accompanied by distant hybridization are cross incompatibility (hybridized combination can not obtain the offspring), and hybrid sterility. There are same matters between the crossing of chickens and quails, such as the early

death of female individuals of hybrid species, only the maleness ones survivaled but there are no activity of genitical gland of hybrid species. Now the mechanism of distance cross-incompatibility of birds are imperfect, and there are no detailed cellule genetics research of generic cross. Chickens, quails and intergeneric hybrid species were used as object in this study, Chromosome specimens are prepared by the peripheral blood lymphocyte cultivation, airing and embryonic, with karyotype. With the comparison among the objects, G-band and C band, many valuable references for cross incompatibility and the hybrid sterility are provided between chicken and quail.

Test materials

Test animal

Gallus gallus domestica tested 20 (10 â™‚, 10 â™€), quail 20 (10 â™‚, 10 â™€), the ripe Chicken-Quail hybrids 20 and Chicken-Quail hybrids embryos 90 adopted artificial insemination method by hybridization of chicken (â™‚) and quail (â™€), were taken from experimental station Animal Science and Technology Institute of Shihezi University.

Reagent

RPMI1640 (GIBCO); Heparin (Hua Mei biological engineering company); colchicine (BIB subpackage); Giemsa powder (Sigma subpackage); calf serum (Hua Mei biological engineering company), inactivated , cryopreservation; PHA (Institute of Pharmaceutical Industry in Guangzhou).

Preparation of conventional chromosome specimen

Chromosome specimens slice of chicken, quail and ripe Chicken-Quail hybrids prepared[2] by the peripheral blood lymphocyte cultivation, airing. Chromosome specimens slice of Chicken-Quail hybrids prepared by embryonic. Referring the methods of preparing the chromosome sample of G-zonation of Chen guo-hong[3] and so on(2003), then preparing the chromosome sample of C-zonation for using the regulation of BSG[4].

Chromosome analysis method

Chromosome sections with Giemsa stained can be counted under a microscope. The diploid chromosome number are counted under the microscope with selecting of good chromosome spread and fission phasing of limpid appearance (50 male and 50 female). The 3 metakinesis phasing and good chromosome spread and limpid appearance selected in each poultry were carried out microphotograph under the immersion objective choice. The long and short arms of first 10 pairs chromosomes were measured by Photoshop image-processing software, then according to the following formula, calculated the relative length of each chromosomes, arm ratio and Centromere index. According to the standards of Leven, regular karyogram were got for the size, the location of centromere of chromosomes. For using the mean value of karyotype parameter, we can draw a ideograph of karyotype.

Relative length=-100

Arm ratio= Centromere index=-100

The good metaphase G-band ideograph for division of zone of chicken, quail and a hybrid were shooted. With the survey of microscope, the number of stripe, the relative location of stripe, the shade of colouration and the width of stripe were all sure. The number of stripe for the first 10 pairs chromosomes of each cellule were calculated, and counted the frequency of modal number of stripe. Partited[5] the zone for referencing the pattern of the G-zonation of Gallus gallus domestica, then drawed the mode of the G-zonation of intermediate stage for chicken, quails and hybrid species. The condition of metaphase phasing C zonation with alkali treatment was observed under the microscope, selecting complete limpid metakinesis phasing of good disposal, chromatosis and disintegration for micrograph, magnification, cutting out, paring, with the analysis of the C-band characteristics, location show, and other laws, focusing on observation of the W chromosome morphous and banding circumstances.

Results and analysis

Karyotype analysis of Chicken, Quail and Their Hybrids

Diploid (2n) cell chromosome number

Chromosome sections of Chicken, quail and a hybrid were carried out conventional Giemsa staining with the selection of 100 good desintegrate phasing respectively for micrograph and the statistics of diploid chromosomes, the results shown in Table 1.

Table 1 The diploid chromosomes of Chicken, Quail and Their Hybrids

From table 2, 10 pairs macrochromosome and 29 pairs minute chromosome are included in the chromosomes of chickens and quails, and minute

chromosome are telocentric chromosome with sex determination style of ZZ (â™‚) and ZW (â™€). No. 3, No. 5, No. 7, No. 9 chromosome in chicken autosome are t-type, No. 1, No. 2, No. 8 chromosome are m-type; No. 4, No. 6 chromosome are sm-type; except the quail autosome No. 1 chromosome for sm-type, No. 2 chromosome for the m-type, No. 4 chromosome for st-type; other chromosomes are t-type. The Z chromosome of chicken and quails are m-type and the fifth macrochromosome; for chicken, the W chromosome is m-type, with the length the same as No. 8 chromosome; for quail, the W chromosome is t-type, with the length between No. 7 chromosome and No. 8 chromosome.

Chromosome karyotype of Chicken-Quail hybrids

Table 3 The parameter of macrochromosomes of Chicken-Quail hybridsï¼ˆ±SEï¼‰

The chromosome parameters of Chicken-Quail hybrids in Table 3, compared with chromosome karyotype parameters of chicken, quail in Table 2 found that each chromosome of every chromosome of the hybrid is basically same chromosome karyotype of chicken, quail with sex determination style for ZZ (â™‚) and ZW (â™€).

Based on the result of Table 2 and Table 3, the ideograph of first 10 pairs of chickens, quails and hybrid species were drawed(see figure 5 and 6) , and reviewed the appearance of each chromosomes. The karyogram of chickens, quails, adult hybrid and majority hybrid species and their embryo were showed from figure 2 to figure 5. The matched-pairs of chromosomes in

figure 3 and figure 4, in which the left of paired chromosomes is from chicken and the right is from quails.

Disposition of Chicken-Quail hybrids' early embryo sex proportion

According to the chromosome karyotype and heterosome differences combing with the C-band banding pattern figure, the early sexuality identification of crossing progeny is carried out for 5 selected time. 90 embryos at early age are choosen, of which 24 female and 66 male are included. With the comparison of the theoretical, female and male ratio take on a significant difference (P <0. 05). The male is significantly more than the female, the ratio of the female at early stage is higher than the male. For the further step, the way which the sex determination of hybrid is ZZ (â™‚) and ZW (â™€).

With the time running, the ratio of the male and the female is disbalanced, and the female is gradually died. The results in table 4.

Table 4 Early embryo hybrids'sex in different times

Chromosome G-banded patterns

Characters of chicken and quail chromosome G-banded

To the G band of Chicken, quail and the hybrid, more than 50 cells were observed. By the treatment of trypsin and Giemsa, the macrochromosome has shown that clearly G band and abundant bandings with same homologous chromosome bandings; (see Figure 7 to 9); generally speaking, the number of most bandings of the minute chromosome is only 1 ~ 2 straps. Because there is no clearly strap, so it is difficult to identificate and

match-pairs. There are big differences on chicken and quail chromosome G-banding pattern, mainly in the banding numbers and banding width. The most major difference is reflected on the No. 1, No. 2 chromosome, which displayed that, there are 7 anachromasis straps on the p arms and 9 anachromasis straps on the q arms of No. 1 chromosomes of chicken, but 5 on the p arms of quails and 10 on the q arms of quails. The No. 2 chromosomes showed 2 widly anachromasis straps on the p arms of chicken and 1 widly anachromasis straps on the p and q arms of quails, respectively. The chromosomes of cross hybrids is originally from chickens and quails. Each pair of chromosome has a characteristic of chicken chromosome with the G band and a characteristic quail chromosome with the G band.

Ideograph draw of chicken and quail G-banded

In accordance with the regulation of the Paris meeting and the relative length, arm ratio and G-banding pattern of the first 10 macrochromosomes(including the Z, W chromosome) of chickens and quail, G band ideograph is drawed (see Figure 10, 11). For chicken, it is divided into 32 zones, 155 bands, of which 71 bands are positive; for quail, it is divided into 28 zones, 138 bands, of which 61 bands are positive.

Banding pattern of chromosome C band

50 C-band metakinesis were observed in this study, it infered that most minute chromosome have anachromasised with C band, but the macrochromosome has little appearance of C-band on the centromere. All of the W chromosomes is anachromasis, as the same specification with Liuli[2, 7, 8] of better repeatability and recognizatible. We also found that the display of C-band has comparatively large influence by the alkali treatment.

The shorter time of alkali treatment than 1 or 2 minutes, the worse C-band of chromosomes shows. With the increasing time of the treatment, the W chromosome is the first to show up the C-band, then the the centromere of macrochromosome, and the macrochromosome telomere and minute chromosome at last. The C-band of hybrid chromosome in Figure 12.

Discussions

Chromosome number of Chicken, Quail and Chicken-Quail hybrid

Because large amount of minute chromosomes of karyotype in chicken, so it is not convenient to count and shape description. It indicated that the incomplete karyotype made of the first 10 pairs chromosomes of birds may be the representative of the specificity of genus[7]. So we analysised only the first 10 pairs chromosomes.

Generally speaking, the ascertainment standard of diploid chromosome number is that the modal number cells accounted for above 75% of the cells observed[8]. The cells that chromosome number is of 2n = 78 in chickens, quails and hybrids were separately accounted for 82%, 81%, 80% of total cellular score observed in the experiment. And of this three, the number of chromosome 2n were all determined for 78. As for the 2n ≠ 78, It may be the large amount of , or the Robertsonian translocation the minute chromosome has happened. And when dropping slices, the minute chromosome was easily to drift following fluid and lost, but also the colorant particle and the dopant were easily to be confused with the minute chromosome for causing.

Compairation on chromosome karyotype between Chicken and Quail

In this studyï¼Œthe description of chicken macrochromosome appearance were basically consistent with the findings which were got in recent years.

The findings of the quail and the results by Chen aikui, Xu Qi were almost the same, only in individual chromosomes with slightly differences, mainly in the No. 2, No. 4, No. 6 Chromosomes. Chen aikui thought that No. 2 chromosome was the sm-chromosome, and No. 6 chromosome was the st- chromosome. But XuQi thought that No. 4chromosome was the t-chromosome[9, 10]. Besides, XuQi thought that the Z chromosome was the 4th macrochromosome of quail , for the description of the length of Z chromosome in the quail. The result of our research is the same as the findings of Chen aikui which think that the Z chromosome is the fifth macrochromosome.

It is generally believed that the evolution rate of birds karyotype is slow, and the chromosome number are very conservative. Related species with the same or similar karyotype, chicken and quails separately belongs to Aves, Galliformes, Phasianidae, the family pheasant, chickens, quails, and the relative length of the first 10 pairs of macrochromosome of quails and chickens is almost the same, but there are some differences in chromosome appearances, mainly in the No. 1, No. 4, No. 6, No. 8 and W Chromosomes. These differences have made it clear that the category diversity is between chicken and quail, and provide the evidences of cell geneticsis for appreciation of quail and chicken[11, 12]. Generally speaking, the evolution trends of karyotype is that the minute chromosome are fewer than ever, and the macrochromosome are gradually more, for the types of centromere, which are from the end to the central. It may be relatively primitive species that the birds karyotype with more st / t-chromosomes, and species with more sm / m-chromosome have relatively specialized [13]. The t-

chromosome of quail is significantly more than the chicken’s. According to the modern point of evolutionary biology of birds, Galliformes and ratite are birds of primitive archikaryon [14]. It is one of the main form of birds karyotype evolution that single headed chromosome developed two headed chromosome through pericentric inversion[15]. It can be inferred that the evolution extent of quail might be lower than the chicken’s. Now, it is generally believed that evolution degree of the quail is lower than the chicken.

G banding technique of chicken, quail and Chicken-Quail hybrid

The manufacture skill of the bird G-band chromosomes is difficult, up to now, there is little succeeded domestic records with clearly G-band. Xu Qi , etal think that the main reason for unsuccessful G-band map may be that birds metaphase chromosomes have higher spiralization, so the bandings are often with combination and strongly dye with vague characteristics. Then the reason may be the big differences with the length of chromosomes. In late prophase and early metaphaseis, the zonation results may be affected for the long No. 1, 2, 3 chromosomes , while the other shorter chromosome are easy to winding and overlap then affect are easy to extently digest, which bring more production difficulties. The appearance of zonary displacement on the No. 1, No. 2 chromosomes of chicken and quails may be leaded by pericentric inversion, which are almost the same with research of reports of Sasaki and so on[16-19].

Chromosome C banding of chicken, quail and Chicken-Quail hybrid

There is few report on the C zonation of chromosomes of Gallus gallus domestica, but more on the chromosomes of domestic animal and birds. The

analysis of C-band of Susscrota domestica by Xu yin-xue showed that the amount of structuredness heterochromatin near the centromere during the mitosis of cells is permanent. The C-band size of metaphase chromosome have high repeatability and the size with C-band of the same individual at different stages have similarity. The finding about study of amphibians and mammals showed that the constitutive heterochromatin mainly distributed in some sectors of the centromere district, the telomere district, and the chromosome arm. The results of this study show that deeply C band is displayed in the majority minute chromosome, the macrochromosomes showed the shallow C band, the W chromosome showed always the first to appear the C-band with the whole deep-dyed and strong repetitivity, which were similar to reports of predecessor Liu Li, which comfirmed that the kinetochore domain of chicken minute chromosome contained a larger of heterochromatin further, and macrochromosomes contained very few or non-heterochromatin[2, 618]. Guo chao-wen, and so on conclude that it may be easily proceeded to Robertson translocation for the existing of large number of heterochromatin minute chromosomes.

The minute chromosome are multitude in birds chromosome. But the size of W chromosome is only 1 / 5 to 1 / 2 of the Z chromosome size and usually can not exactly be identified. In samples of non-banding, the W chromosome of birds is always much more difficult to identify than the Y chromosome of mammals. It is blind that Karyotype analysis (particularly slices are not good at a time) depend on the sex of poultry only. Liu ling-yun , and so on[19] had successfully identified the sex of crested ibis with C-band technology. In this experiment, the phasings of peripheral blood lymphocytes were treated with

C-band, and the W chromosome was successfully identifed, the sex of early embryo of hybrids is identified clealy. If the W chromosome would be identifed with C banding technique, and combined with morphous identification of the Z chromosome in conventional film, the accuracy of sex determination of birds would be greatly improve, which provided a safe and reliable way for sex determinatio of some rare birds of which external sex characteristics were not obsolete obvious[20, 21].

Discussions on the early embryonic death of Chicken-Quail hybrid

Through the analysis of karyotype, we found that the hybrid embryo had male and female brooding in 3-5 days, but the adult hybrids were all male, which explained the female of hybrids were all died in their embryonic period, only the male ones did survival. It has no well-explain on the mechanism about the early death of female hybrid embryos in distance hybridization of birds. With the analysis of chromosome, the early death of embryosmay be leaded by the abnormality of chromosome numbers and structures. The ways of sex determination of bombyx mori are the same as birds', always the male is ZZ, the female is ZW. In bombyx mori, the recessive lethal gene in the Z chromosome leads to all female deaths in the embryonic period , that is, the lethality is induced by linkage equilibrium. Assumed the recessive lethal gene is existed in the Z chromosome of the hybrids, then it is possible that the expression of lethal gene leads to the death of female embryos while the heterosome are ZW. Currently, there is no sufficient evidence on this hypothesis, so the mechanism on the regulation of gene expression is needed in deeply research.

Discussions on the sterility mechanism for Chicken-Quail hybrid

The sterility of inter-species hybrid has great relations to cell chromosome. It happened to the hindering of meiosis with the result that the abnormal reproductive germ cells and breed. On the research of the Muscovy duck, City Link duck and the mule duck , Cheng guanchao et[22] found that the number of basical arms for the hybrid duck have big differences with the parental of hybrid. The centromere index and the arm ratio of the autosomal No. 1, 2 chromosomes are between the parents. The centromere position of sex chromosome of hybrid duck have the characteristics of the two parents. Song jianjie found that the sterility of F1 hybrid is cause by the difference of karyotype between No. 1 and 2 chromosomes of Muscovy duck.

Although the number chromosome of chickens is the same as quails (2n = 78) in this research, there are obviously differences of chromosome appearance, mainly in the chromosomes No. 1, No. 4, No. 6, No. 8 and the W. There are differences between the parameters of karyotype, the numbers and width of G-band. They disturbed the inherently balance of chromosomes on the hybrid and destroy the procedure of mitosis, so the sterility was led for the bad pairing of chromosomes. It is common that of Robertson translocation and close interspecific karyotype inversion in birds chromosomes always happens. Stock [17], and so on think that the inversion could prevent extensive inter-species hybrid and increase dysgenesia, which may be led to hybrid sterility. Distant hybridization infertility is a complex biological problem, with the exception of cell genetics, comprehensive

studies of the morphology, physiology, molecular biology, immunology, and other points of view.