HARNESSING HETEROSIS FOR GROWTH THROUGH INTERGENERIC HYBRID: MOLECULAR CYTOGENETIC STUDIES ON AVIAN HYBRID STERILITY

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ABSTRACT

Harnessing heterosis for growth and reproductive traits is a major target area for improving productivity in livestock and poultry. Intergeneric and/or interspecific hybrids are often used for improving productivity in livestock and poultry. However, hybrids between different species are often inviable or, if they live, they are sterile. Use of sterile hybrids (mule) in human civilization has a long history because mule animals are often performing better than their parents in respect to growth, endurance and draught power. To date, the molecular mechanism of hybrid sterility in avian species is poorly understood. To understand the molecular mechanism of hybrid sterility in mule duck, an intergenic sterile hybrid between domestic duck (*Anas platyrhynchos*) and muscovy duck (*Cairina moschata*), we performed histological and cytogenetic characterization of the sterility by chromosomal morphology, meiotic chromosome configuration, histology of testicular cross sections, and apoptosis of germ cells. We also performed cross species chromosome painting and fluorescence in situ hybridization (FISH) mapping of telomeric repeats. Karyotypic analysis revealed that there are morphological difference in chromosome 1, 2 and Z and size difference in chromosome 5 between two species. Meiotic chromosome configuration revealed high frequency of primary spermatocytes with highly condensed chromosomes at late pachytene to first meiotic metaphase (MI). The spermatocytes with meiotic abnormality were consequently eliminated by apotosis, resulting in the absence of post meiotic cells after MI stage. These results suggest that the failure of meiotic chromosome pairing in primary spermatocytes by mutually incompatible difference in the chromosome morphology, which is followed by meiotic arrest at MI, might be a main cause of male sterility in mule ducks. The findings of these studies in mule duck will improve our knowledge regarding the mechanism of sterility in avian species and assist in the production of mule duck for better meat production.

Keywords: Heterosis, mule duck, FISH, chromosomal incompatibility.

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INTRODUCTION

When hybridization occurs between genetically diverged populations in animals or plants, complete or partial sterility is often observed in the F_1 hybrids or in their descendants. This phenomenon is popularly known as hybrid sterility. Hybrids between different species also show other abnormal phenotypes, such as weakness and inviability. These abnormal hybrid characteristics, collectively called hybrid incompatibility, are assumed to play important roles in speciation by acting as reproductive isolators.[1-2] Hybrid incompatibility is known to us since the time of Aristotle.[3] Since then naturalists and philosophers have been puzzled by its existence and hybrid incompatibilities have been studied in animals and plants over a century.

Nowadays, hybrid incompatibilities are well studied in plants, insects and mammals. In the last decade, a considerable number of genes involved in these hybrid incompatibilities have been identified in mice, fishes, flies, yeast and plants.[4-7] To date, much of our understanding of the genetic mechanism of postzygotic isolation caused by hybrid sterility was produced by the empirical studies of genetic divergence in *Drosophila*. Indeed, several genetic factors of hybrid incompatibility in *Drosophila* have been characterized: (i) hybrid incompatibility alleles are generally recessive;[8] (ii) hybrid male sterility is highly polygenic and complex[8-10] and (iii) hybrid sterility or lethality evolves more readily in males than females.[11-13]

However, all other biological groups do not adhere strictly to the patterns that characterize *Drosophila* systems. In addition, the genetic complexity that typifies Drosophila hybrid male sterility is not necessarily mirrored in other animals. In *Drosophila* and other dipterans, compared with the number of hybrid female sterility or lethality factors, the greater abundance of hybrid male sterility factors are usually attributed to the accelerated evolution of male traits via sexual selection or sexual conflict.[10-11,13-16] Therefore, it is meaningful to study the mechanism of hybrid incompatibilities in diverse species.

Harnessing heterosis for growth and reproductive traits is a major target area for improving productivity in livestock and poultry. Intergeneric and/or interspecific hybrids are often used for improving productivity in livestock and poultry. However, hybrids between different species are often inviable or, if they live, they are sterile. Use of sterile hybrids (mule) in human civilization has a long history because mule animals are often performing better than their parents in respect to growth, endurance and draught power. In Aves, the mule duck, an interspecific hybrid between the Muscovy duck (*Cairina moschata*) and the domestic duck (*Anas platyrhynchos*) is a well-known biological model for studying avian hybrid sterility. When the Muscovy drake is mated to the domestic duck, viable hybrid progenies are produced and they are physically more vigorous than either parental species, but they are completely sterile in both sexes. It is commonly believed that the incompatibility in the development and reproduction of interspecific and intergeneric hybrids is caused by the difference of karyotypes and chromosome structures of parental species.[17-22] The genetic incompatibility between intergeneric hybrid between the Muscovy duck (*Cairina moschata*) and the domestic duck (*Anas platyrhynchos*) is an ideal avian model for studying the hybrid sterility; however, very little is known about the mechanism of the hybrid sterility in mule ducks. To understand the molecular mechanism of hybrid sterility in mule duck, we performed histological and cytogenetic characterization of the sterility by chromosomal morphology, meiotic chromosome configuration, histology of testicular cross sections, and apoptosis of germ cells. We also

performed cross species chromosome painting, fluorescence in situ hybridization (FISH) mapping of repetitive DNA sequences.

MATERIALS AND METHODS

HISTOLOGICAL ANALYSIS AND THE DETECTION OF APOPTOTIC SPERMATOGENIC CELLS

One adult male each of the domestic duck (*Anas platyrhynchos*), the Muscovy duck (*Cairina moschata*), and their F_1 hybrids, which were obtained by crossing the Muscovy drake with the domestic duck, was used. All individuals were more than six month-old; however, their accurate age was not known. The locations where these samples were collected were as follows: the domestic duck was obtained from a fancier in Ehime prefecture, Japan; the Muscovy duck was obtained from Bangladesh Agricultural University, Mymensingh, Bangladesh; and the mule ducks were purchased from a farmer in Ratchaburi, Thailand. After an intravenous bolus injection of pentobarbital, the testes were taken, fixed overnight in Bouin's solution, and stored in 70% ethanol at room temperature. All experimental procedures using animals conformed to the Guidelines established by the Animal Care Committee, Nagoya University. The fixed testes were sequentially dehydrated in a graded ethanol series: the testes were sequentially immersed in 70%, 80%, 90%, and 95% ethanol and two times in absolute ethanol at room temperature for 30 min each. The testes were immersed in 1:1 ethanol/xylene, two times in xylene at room temperature for 5 min each and three times in paraffin at 58°C for 30 min, and were embedded in paraffin. They were sectioned at a thickness of 6 μm, and the sections were then deparaffinized three times for 3 min in xylene, rehydrated two times in absolute ethanol, one time in 90%, 80%, and 70% ethanol for 3 min each, and were finally stained with hematoxylin and eosin for histological observations.

Apoptosis of spermatogenic cells on testicular cross-sections was examined by the TUNEL (TdT-mediated dUTP Nick-End Labeling) assay using the apoptosis detection kit, ApoMark (Exalpha Biologicals, Shirley, MA, USA). Sections were deparaffinized following the method as described above, and rinsed with 1 × TBS for 5 min. Apoptotic cells were detected following the manufacturer's protocol. Slides were counterstained with methyl green after the reaction.

MEIOTIC CHROMOSOME PREPARATION

One adult male each of the domestic duck and the mule duck was used for meiotic chromosome analysis. The locations where these samples were collected are described in the former section. Meiotic chromosome preparations for light microscopy of meiotic chromosomes were made by the air-drying method as described previously.[23] After hypotonic treatment of the seminiferous tubules in 1% sodium citrate for 20 min at room temperature, they were placed in a fixative solution (1:1 ethanol/acetic acid) for 3−4 min and in 60% fixative solution for 3 min on ice, the cells were collected by filtration using gauze, and were then fixed with 1:1 ethanol/acetic acid. The cells in suspension were dropped onto glass slides and air-dried. Slides were stained with 3% Giemsa in phosphate buffer (pH 6.8) for 10 min.

CELL CULTURE AND SOMATIC CHROMOSOME PREPARATION

One female and two males of one month-old domestic ducks were purchased from a breeding farm in Japan. Their skins and mesenteries were collected and used for fibroblast cell culture. The fertilized eggs of the Muscovy duck were purchased from a breeding farm in Japan, and the fibroblast cells were collected from one female and two male embryos at day 13 after incubation. Small pieces of under-wing skin tissues were collected from two females and one male of the mule duck obtained in Thailand for cell culture. Fibroblast cells were cultured in Dulbecco's Modified Eagle Medium (Invitrogen-GIBCO, Carlsbad, CA, USA) supplemented with 15% bovine serum (Invitrogen-GIBCO), 100 μg/ml kanamycin, and 1% antimycotic (PSA) (Invitrogen-GIBCO). The cultures were incubated at 39°C in a humidified atmosphere of 5% CO_2 in air. Primary cultured fibroblast cells were harvested using trypsin and were then subcultured. After colcemid treatment (0.02 μg/ml) for 45 min, the cells were harvested, suspended in 0.075M KCl for 20 min at room temperature, and fixed with 3:1 methanol/acetic acid following a standard protocol. After centrifugation, the cell suspension was made, dropped on glass slides, and air-dried. For karyotyping, the chromosome slides were stained with 3% Giemsa in phosphate buffer (pH 6.8) for 10 min. We determined the sex of the embryos by a molecular sexing method using PCR analysis of the *CHD1* genes on the Z and W chromosomes,[24] and used the fibroblast cells of one male and one female embryo for chromosome analysis.

Replication R-banding for gene mapping by FISH was performed as described previously.[25] Fibroblast cell cultures were treated with 5-bromo-2'-deoxyuridine (BrdU) (25 μg/ml) (Sigma-Aldrich) at the late replication stage and cell culturing was continued for an additional 5 hours, including 45 min of colcemid (0.025 μg/ml) treatment before harvesting. Chromosome slides were made as described above and dried at room temperature for 2–3 days. After staining the slides with Hoechst 33258 (1 μg/ml) for 10 min, R-bands were obtained by heating them at 65°C for 3 min and then exposing them to UV light at 65°C for an additional 6.5 min. The slides were then kept at –80°C until use.

FLUORESCENCE IN SITU HYBRIDIZATION (FISH)

Cross-species chromosome painting with chicken probes was performed as described previously.[26] Chicken (*Gallus gallus*, GGA) chromosome-specific DNA probes for chromosomes 1–9 and Z (GGA1–9 and GGAZ),[27] were used. FISH images of chromosome painting were captured with the 550 CW-QFISH application program of Leica Microsystems Imaging Solution Ltd. (Cambridge, UK) using a cooled CCD camera mounted on a Leica DMRA fluorescence microscope.

The 5.8 kb pHr21Ab and 7.3 kb pHr14E3 fragments of the human ribosomal RNA genes provided by the Japanese Cancer Research Resource Bank (JCRRB), Tokyo, were used for chromosome mapping of the 18S-28S ribosomal RNA genes. A biotin-labeled 42 bpoligonucleotide probe complementary to telomeric (TTAGGG)*n* repeats was used for chromosome mapping of telomeric repeated sequences. After hybridization, the slides were stained with FITC-avidin and subsequently counter-stained with propidium iodide (PI) (0.75 μg/ml) after washing.

A total of 250 µg of the DNA probe was labeled with biotin-16-dUTP and ethanol precipitated with salmon sperm DNA and *Escherichia coli* tRNA for chromosomal localization of functional genes. After hybridization, probe DNA was reacted with the goat anti-biotin antibody (Vector Laboratories), and was then stained with the Alexa Fluor 488 rabbit anti-goat-IgG (H+L) conjugate (Invitrogen-Molecular Probes) and subsequently counter-stained with 0.75 µg/ml PI (25). FISH images were captured using a cooled CCD camera mounted on a Nikon fluorescence microscope.

RESULTS

COMPARISON OF SPERMATOGENESIS AND APOPTOSIS OF TESTICULAR CELLS

Light microscopy of hematoxylin and eosin (HE)-stained testis sections revealed normal development of the seminiferous epithelium in domestic and Muscovy ducks; spermatogonia, spermatocytes, spermatids, and mature spermatozoa were present in the seminiferous tubules (Figure 1).

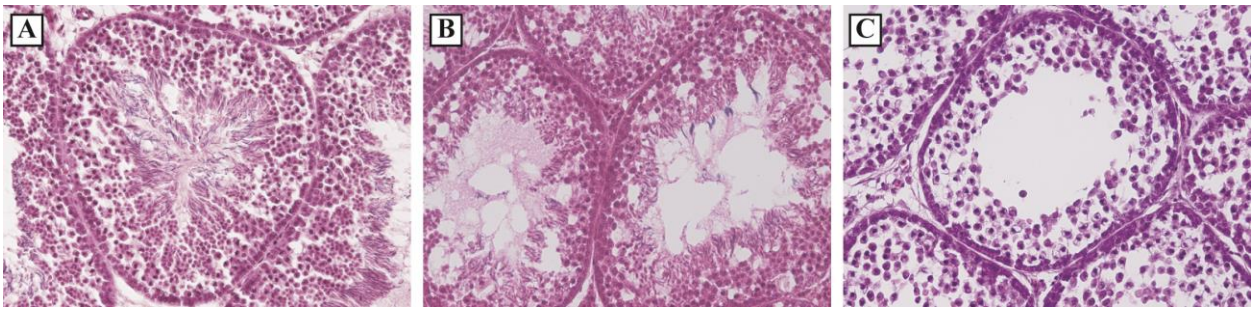

Figure 1. HE-stained testis sections of *A. platyrhynchos* (A), *C. moschata* (B) and F_1 hybrid (C).

However, marked accumulation of primary spermatocytes was observed in the seminiferous epithelium of the F_1 hybrid and theses were extensively exfoliated into the lumina of seminiferous tubules, while no spermatids or spermatozoa were contained in the seminiferous tubules. These results indicate that spermatogenesis in the F_1 hybrid is arrested at meiotic nuclear division I.

Apoptosis of testicular cells were assessed by the TUNEL method, which detects DNA fragmentation resulting from apoptotic signaling cascades. Apoptotic cells were rarely observed in the seminiferous tubules of the parental species (Figure 2).

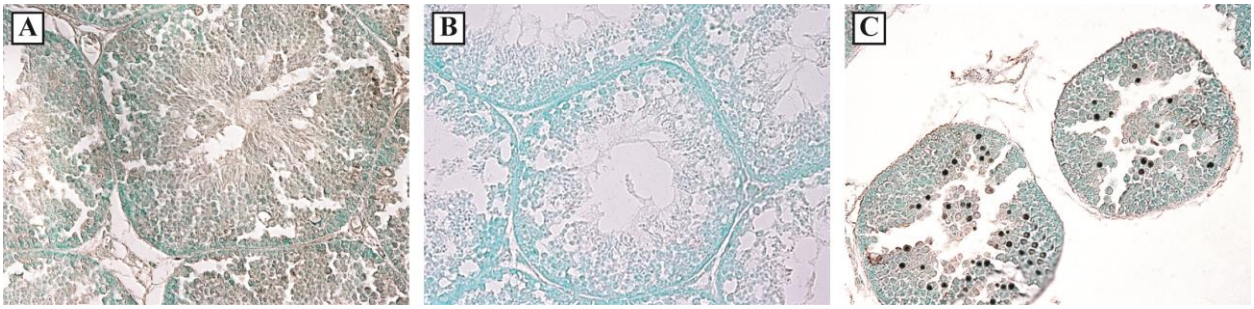

Figure 2. TUNEL-stained testis sections of *A. platyrhynchos* (A), *C. moschata* (B) and F_1 hybrid (C). Dark staining shows TUNEL positive apoptotic cells. Tissue sections were counter-stained with methyl-green.

In contrast, a high frequency of TUNEL-positive cells was observed primarily in the interior side of the seminiferous tubules of the F_1 hybrid, suggesting the occurrence of apoptotic cell death in the accumulated primary spermatocytes of the F_1 hybrid.

MEIOTIC STATUS OF SPERMATOGENIC CELLS

To determine the meiotic status of spermatocytes, Giemsa-stained preparations of testicular cells and nuclei from adult males of the domestic duck and the F_1 hybrid were examined. In the domestic duck testis, early and elongating spermatids and mature spermatozoa, as well as early pachytene, pachytene, metaphase I (MI), and metaphase II (MII) spermatocytes were observed (Figure 3).

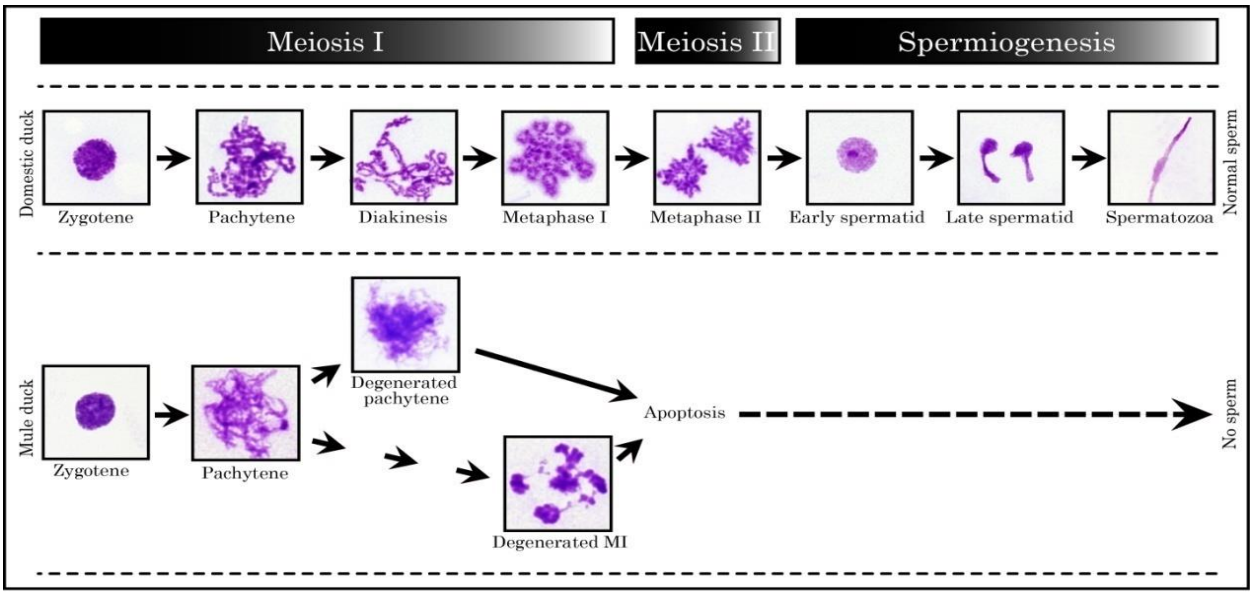

Figure 3. Schematic representation of the spermatogenic status of the domestic duck and the F_1 hybrid (mule duck).

In the F_1 hybrid testis, neither secondary spermatocytes nor postmeiotic cells were found, although early pachytene and pachytene spermatocytes and a number of degenerated spermatocytes with abnormally condensed chromosomes were observed (Figure 3). Most of the degenerated spermatocytes in the F_1 hybrid testis were pachytene cells and the others were diakinesis to MI cells. Few or no such degenerated spermatocytes were observed in the domestic duck testis. These results suggest that meiosis is mainly arrested at pachytene in F_1 hybrid spermatocytes, and that a small number of meiotic cells are allowed to progress beyond pachytene and are then arrested at the diakinesis-MI stage. Such spermatocytes arrested at the pachytene and diakinesis-MI stages may be subsequently eliminated by apoptosis, leading to sterility in the F_1 hybrid male.

CHROMOSOME MORPHOLOGY OF DOMESTIC AND MUSCOVY DUCK

To examine morphological differences in the chromosomes of the domestic duck and Muscovy duck, Giemsa-stained karyotypes of the two species and their F_1 hybrids were examined (Figure 4). Chromosome numbers were counted for seven metaphase spreads of each individual. Two species and their F_1 hybrid showed the same diploid chromosome number (2n=80). However, significant morphological differences were found in the location of the centromere of chromosome 1 and the morphology of the Z chromosome between the two species. The Z chromosome of the domestic duck was subtelocentric, whereas the Muscovy Z chromosome was acrocentric.

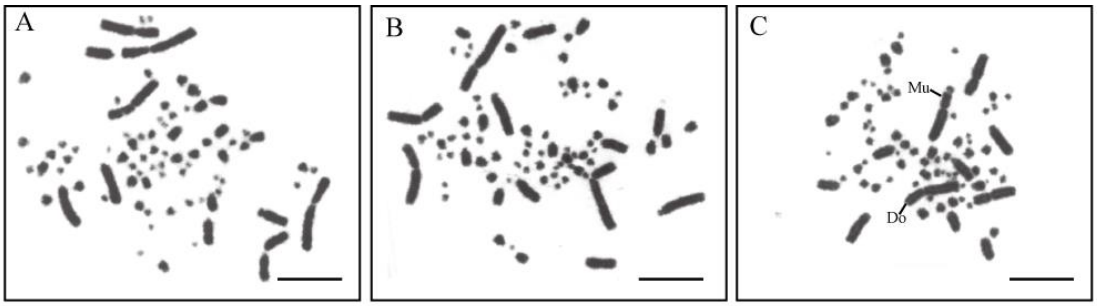

Figure 4. Giemsa-stained metaphase spreads of *A. platyrhynchos* (A), *C. moschata* (B) and F_1 hybrid (C).

COMPARATIVE CHROMOSOME PAINTING

Chromosome painting with chicken chromosomes 1–9 and Z probes was performed for *A. platyrhynchos* and *C. moschata* (Table 1).

Table 1. Summary of comparative chromosome painting with the chicken chromosome 1-9 and Z specific DNA probe on the PI-stained metaphase chromosome spreads of *A. platyrhynchos* and *C. moschata*.

Chromosome	Mallard	Muscovy
1	Full	Full
2	Full	Full
3	Full	Full
4	Full+ 1 pair micro	Full + 1 pair micro
5	Full	Full
6	Euchromatin	Euchromatin
7	Euchromatin	Euchromatin
8	Euchromatin	Euchromatin
9	Euchromatin	Euchromatin
Z	Full	Full

Each probe painted a single pair of chromosomes, except for GGA4, in the two species. The GGA4 probe hybridized to the fourth largest acrocentric chromosome and additionally to a single pair of microchromosomes (Figure 5).

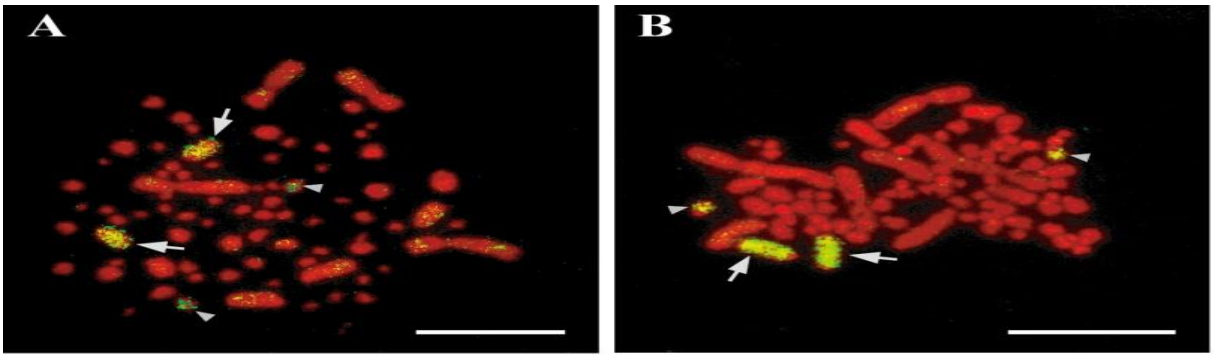

Figure 5. Comparative chromosome painting with the chicken chromosome 4-specific DNA probes (GGA4) on the PI-stained metaphase chromosome spreads of *A. platyrhynchos* (A) and *C. moschata* (B).

*CHROMOSOMAL LOCATIONS OF THE 18S-28S RRNA GENES AND TELOMERIC (TTAGGG)*N REPEATS

The 18S-28S rRNA genes were localized to four pairs of microchromosomes in both *A. platyrhynchos* and *C. moschata* (Figure 6). The (TTAGGG)*n* repeats were localized to both telomeric ends of all chromosomes in the two species and additionally in the interstitial region of the short arm of *A. platyrhynchos* chromosome 1 and in the centromeric region of *C. moschata* chromosome 1 (Figure 6 B). The hybridization signals of (TTA*G*GG)*n* repeats were small on the telomeric ends of macrochromosomes, whereas the intense amplification of the (TTA*G*GG)*n* repeats was observed on almost all microchromosomes.

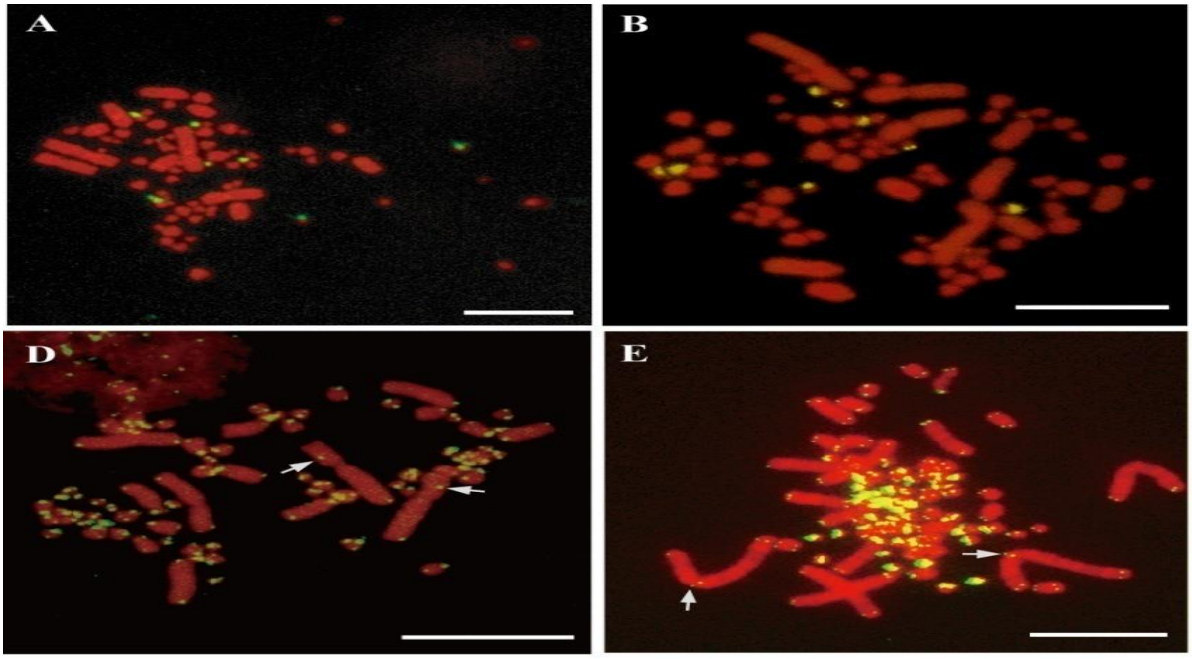

Figure 6. Fluorescence in situ hybridization pattern of the 18S-28S ribosomal genes (A-B) and telomeric (TTAGGG)n repeats (C-D) on PI-stained metaphase chromosome spreads of *A. platyrhynchos* (A, C) and *C. moschata* (B, D).

DISCUSSION

In this study, we have found marked accumulation of primary spermatocytes, high frequency of apoptotic testicular cells, failure of meiosis to progress beyond MI, and a number of degenerated spermatocytes in the testis of the F_1 hybrid between the Muscovy drake and the domestic duck, and revealed that pachytene cells were dominant in the degenerated spermatocytes and diakinesis to MI cells were few. These results suggest that meiosis normally advances to the chromosome synaptic stage (zygotene-to-pachytene) but is arrested primarily at pachytene in the F_1 hybrid spermatocytes, and that a small number of meiotic cells are allowed to progress beyond pachytene and are then arrested at the diakinesis-MI stage. Such spermatocytes arrested at the pachytene and diakinesis-MI stages may undergo degeneration and subsequent elimination by apoptosis, leading to spermatogenic breakdown in the F_1 hybrid male. We have shown morphological differences in chromosome 1 and the Z chromosome between the parental species. Our present findings on the meiotic phenotype and karyotype of the F_1 hybrid differ from the findings in previous studies on the mule duck[28-30] in several points. Yamashina[28-30] described that meiotic arrest occurred at diakinesis-MI. Morphological or size differences in chromosomes were found for chromosome 1 and the sixth largest chromosome in,[28-29] whereas a difference was shown in chromosome 1 and the Z chromosome in,[31] which was the same result as that of our present study. The reason for these discrepancies needs to be clarified in the future.

The sterility of hybrids or their progeny is more common than inviability due to the postzygotic reproductive isolation mechanism in vertebrates.[32] Among the primary factors for hybrid sterility, chromosomal incompatibility, which is a consequence of karyotypic evolution during the process of speciation, is the main cause of spermatogenic breakdown. Structural differences in chromosomes often cause disturbances in homologous chromosome pairing and subsequent meiotic recombination in primary spermatocytes, leading to meiotic arrest during meiosis I.[19-22,33] The F_1 hybrid between the horse (*Equus asinus*) and the donkey (*E. caballus*) can be used as an appropriate example here. The chromosome numbers of the horse (2n = 64) and donkey (2n = 62) differ only by one pair of chromosomes; however, comparative cytogenetic analysis by cross-species chromosome painting revealed that the syntenies of six horse chromosomes have each broken into two segments, which form separate donkey chromosomes or part of the donkey chromosome.[34-35] Such structural differences in chromosomes cause the failure of meiotic chromosome pairing in F_1 hybrids, resulting in spermatogenic breakdown in primary spermatocytes.[36] Our present study also suggests the possibility that meiotic arrest in the mule duck is associated with impaired meiotic chromosome pairing in primary spermatocytes due to chromosomal incompatibility between the parental species.[28-29]

In the sterile interspecific hybrid between the laboratory mouse (*Mus musculus*) and *Mus speretus*, both of which have the same diploid chromosome number (2n = 40) consisting of all acrocentric chromosomes, meiotic breakdown occurred at two different stages (pachytene and MI).[19-20,33] Asynapsis of the X and Y chromosomes during pachytene due to the genetic divergence of the pseudoautosomal region between two species caused an arrest at the MI stage. However, the failure of autosomal chromosome pairing is more serious for the progression of meiosis, which results in meiotic arrest at pachytene, whereas primary spermatocytes with X-Y asynapsis but normal autosomal pairing advance to the MI stage. Since the sex chromosomal constitution of the male mule duck is homozygotic ZZ, meiotic arrest at pachytene may be mainly caused by the impaired meiotic pairing of morphologically different chromosomes (chromosome 1 and the Z chromosome). However, a small number of spermatocytes proceeded to diakinesis-MI, which succeeded in passing the synaptic stage of the first meiotic prophase.

A comparative genomic approach with chickens has helped to delineate the process of genomic and chromosomal rearrangements that resulted in species-specific differences in avian karyotypes. Hybridization with chicken paints (GGA1–9 and Z) showed that each chicken probe hybridized to a single pair of chromosomes for all three anserid species with the exception that GGA4 hybridized to the fourth largest acrocentric chromosome and a single pair of microchromosomes. The same results have also been reported in palaeognathous birds and many galliform species (including pheasants, turkeys, capercaillie, New World quails, and plain chachalaca),[26,37-39] suggesting that the fusion of a microchromosome to ancestral acrocentric chromosome 4 occurred in the lineages of the chicken and other several phasianid species (including Old World quails, peafowls, and partridges) after Galliformes split from the common ancestor of Galloanserae. However, in the related species of *A. cygnoides*, the greylag goose (*Anser anser*), GGA4 paint hybridized to a single pair of submetacentric chromosome 4,[40] suggesting that the fusion between ancestral acrocentric chromosome 4 and a microchromosome occurred in *A. anser* after the genus *Anser* appeared.

The 18S-28S rRNA genes were localized to four pairs of microchromosomes in two duck species (*A. platyrhynchos* and *C. moschata*) and eight pairs of microchromosomes in *A. cygnoides*. Considering that

the 18S-28S rRNA genes are supposed to have been located in a single pair of microchromosomes in the ancestral avian karyotype, which were found in palaeognathous birds and the chicken,[26,41] this result suggests that rRNA genes were dispersed in different microchromosomes during the process of homogenization of centromeric heterochromatin between different microchromosomes.

Interstitial telomeric sequences (ITSs) have been reported in many avian species as well as mammals and lower vertebrates.[42-43] These non-telomeric sites of (TTAGGG)*n* repeats and the co-localization of telomeric repeats and satellite DNA sequences are most likely the result of chromosome rearrangements involving the chromosome ends (including inversions, centric fusion, and telomere-to-telomere fusion).[44-49] In addition, sequence analysis of ITSs revealed that the interstitial arrays of (TTAGGG)*n* repeats could be inserted at intrachromosomal sites through double-strand breaks in ancient chromosomes.[50-51] ITSs, in addition to the regular telomeric sites, were found in the short arm of *A. platyrhynchos* chromosome 1 and the centromeric region of *C. moschata* chromosome 1, which was confirmed by Nanda *et al*.[43] However, the ITS signal in chromosome 1q, which was found in the graylag goose (*A. anser*),[43] was not observed in *A. cygnoides*, indicating species differences in the chromosomal distribution of non-telomeric sites in the genus *Anser*. No supporting cytogenetic evidence for telomere-to-telomere fusion or inversion containing telomeric ends at these sites was obtained in the present study. In contrast, no hybridization signals of ITSs were detected in Falconidae and Accipitridae of Falconiformes by FISH mapping of telomeric (TTAGGG)*n* repeats, although telomere-to-telomere fusions frequently occurred between macro- and microchromosomes and between microchromosomes in these species.[52-53] Nucleotide sequence-based analysis is needed to understand the structures of ITSs and their origins in these avian species.

Comparative cytogenetic analysis using FISH revealed the chromosome rearrangements of macrochromosomes between the chicken and Japanese quail (three pericentric inversions and one centromere repositioning)[54] and between the chicken and pheasant (two interchromosomal rearrangements).[38] However, a reduction in the number of spermatogonia and meiotic arrest at early prophase before the completion of chromosome synapsis have been commonly observed in F_1 hybrids between the chicken, Japanese quail, and pheasant,[55-56] which suggests that the mechanism controlling sterility in these F_1 hybrids is due to genetic, rather than chromosomal incompatibilities between the parental species. In contrast, the effect of chromosomal dissimilarities between the domestic duck and Muscovy duck is not so serious that meiosis progresses beyond the zygote to the early pachytene of meiosis I in their F_1 hybrids. Therefore, the mule duck could be a useful avian model for studying the molecular basis of hybrid sterility caused by a chromosome incompatibility. Chromosome structural differences at the molecular level between two species and the status of chromosomal synapsis at zygote-to-pachytene in their F_1 hybrids needs to be examined for a better understanding of the genetic basis for avian hybrid sterility.

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