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—Original Article—

Conservation of Migration and Differentiation Circuits in Primordial Germ Cells Between Avian Species

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Abstract. Germ cell differentiation in reverse-sexed reproductive organs and interspecies germ line chimeras provides insight into the mechanism of germ cell development and represents a useful tool for conservation of endangered birds. We investigated the migration and survival capacity of male chicken primordial germ cells (PGCs) in female chicken embryos and in quail and Korean ring-necked pheasant embryos of both sexes. Interestingly, the PGCs were successfully reintroduced in all cases. Furthermore, the cells survived in the recipient gonads until hatching regardless of sex and species of the recipient. In the case of male recipient chickens, PGC-derived offspring were produced. However, the reverse-sexed female chickens, quails and pheasants of both sexes did not generate any male donor PGC-derived progeny. These results suggest that migration and survival circuits in chicken PGCs are conserved in both sexes and between avian species during embryonic development.
Key words: Birds, Germ cell differentiation, Germline chimera, Primordial germ cell, Reverse sex

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In sexual reproduction in animals, offspring are produced by fusion of germ cells, i.e., fertilization between sperm from males and eggs from females. Compared with other somatic cells, germ cells have unique development and differentiation processes during embryonic stages and sexual maturation. In birds, primordial germ cells (PGCs) that first arise in the area pellucida in blastoderm stage X migrate through the blood circulation and then localize to the developing genital ridges [1]. As the chicken has many advantages for industrial application as well as basic research, chicken PGCs have been isolated and characterized for manipulation for various applications, such as germ line chimera production. Wentworth *et al.* [2] were the first to manipulate quail PGCs isolated from the germinal crescent and embryonic blood vessels, and produced germ line chimeras after transplantation into the blood vessels of recipient embryos. In chickens, the PGCs isolated from three different sites, i.e., the germinal crescent, embryonic blood vessels, and gonads, have been identified and utilized to improve the germ line transmission efficiency and transgene introduction into the chicken genome [3–5].

There have been marked advances in techniques for *in vitro* manipulation, and the chicken PGC culture system allows expansion of large numbers of germ cells without loss of germ line-transmissible potential [6–8]. In addition, combined with *in vitro* culture of chicken PGCs, transgenic chickens have been efficiently created utilizing transposable elements [9, 10]. In a previous study, genetically modified chicken PGCs were transferred into recipient embryos, and subsequently, donor PGC-oriented transgenic offspring were generated with high germ line transmission efficiency [6, 10]. However, there have been no previous reports regarding the production of offspring derived from reverse-sexed germ line chimeras after transfer of cultured PGCs. In the study of van de Lavoir *et al.* [6], germ line transmission of cultured male or female chicken PGCs in reverse-sexed putative chimeras was not observed in more than 4300 G1 offspring. Similarly, in the study of Macdonald *et al.* [8], cultured male PGCs did not form functional gametes and appeared to have been lost in the female ovary during sexual maturation.

A germ cell-mediated interspecies germ line chimera production system would be a versatile tool for the conservation of endangered birds [11–13]. Germ cells or PGCs isolated from endangered birds could be transplanted into recipient chicken embryos after *in vitro* expansion. Subsequently, donor germ cell-derived progeny could be generated by mating between male and female germ line chimeric chickens. In the study of Kang *et al.* [11], Korean ring-necked pheasants were produced by mating between interspecies germ line chimeric chickens and wild pheasants. Wernery *et al.* [12] retrieved gonadal cells containing PGCs of wild houbara bustard (*Chlamydotis undulata*) embryos and then transferred them into the embryos of a laying chicken strain. Subsequently, pure-line houbara offspring were generated by artificial insemination between germ line chimeric roosters and female houbara bustards [12]. However, various recipients and developmental stages should be evaluated for efficient adaptation of donor germ cells in recipients. In addition, germ line transmission capacity in the opposite-sexed chimeras should be examined due to the limited numbers of fertilized eggs of endangered birds. Therefore, we examined the migration and survival capacity of chicken donor PGCs in reverse-sexed recipients and different avian species.

Materials and Methods

Experimental animal care

The care and experimental use of birds were approved by the Institute of Laboratory Animal Resources, Seoul National University

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(SNU-070823-5). All experimental birds including White Leghorn (WL) and Korean oge (KO) chickens, Japanese wild quails and Korean ring-necked pheasants were maintained according to a standard management program at the University Animal Farm, Seoul National University, Korea. The procedures for animal management, reproduction and embryo manipulation adhered to the standard operating protocols of our laboratory.

Chicken primordial germ cell (SNUhp26) culture

In our previous report [10], male PGC line SNUhp26 from WL embryonic gonads on day 6 were established and maintained with knockout Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA, USA) supplemented with 20% fetal bovine serum (Invitrogen), 2% chicken serum (Sigma-Aldrich, St. Louis, MO, USA), 1× nucleosides (Millipore, Temecula, CA, USA), 2 mM L-glutamine, 1× nonessential amino acids, β-mercaptoethanol, 10 mM sodium pyruvate and 1× antibiotic–antimycotic (Invitrogen). Human basic fibroblast growth factor (hbFGF, 10 ng/mL; Koma Biotech, Seoul, Korea) was added for PGC self-renewal. The SNUhp26 line was cultured in an incubator at 37 C with an atmosphere of 5% CO_2 and 60–70% relative humidity. The SNUhp26 PGCs were subcultured onto mitomycin-inactivated mouse embryonic fibroblasts in 5- to 6-day intervals by gentle pipetting without enzyme treatment.

For immunostaining of the cultured chicken PGCs with stage-specific embryonic antigen-1 (SSEA-1) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), which is a germ cell-specific surface marker, the cultured PGCs were fixed in 4% paraformaldehyde solution for 10 min, washed three times with phosphate-buffered saline (PBS), and blocked with blocking solution containing 5% (v/v) goat serum for 30 min. After fixation, PGCs were incubated with SSEA-1 primary antibody diluted 1:200 in blocking buffer overnight at 4 C. Following three washes with PBS, PGCs were incubated with secondary antibody labeled with phycoerythrin (PE) (Santa Cruz Biotechnology) for 1 h at room temperature. The stained PGCs were observed under a fluorescence microscope.

Transfection and establishment of GFP-expressing SNUhp26 subline

To establish an SNUhp26 subline expressing green fluorescent protein (GFP), expression vectors containing the GFP gene expressed by cytomegalovirus (CMV) immediate-early enhancer/promoter and neomycin resistance (Neo^R) gene controlled by the Simian virus 40 (SV40) promoter were co-transfected into SNUhp26 PGCs. The *piggyBac* GFP vector and CAGG-PBase (pCyL43) were co-introduced into the established SNUhp26 PGC lines using lipofection with Lipofectamine® reagent (Invitrogen). One day after transfection, 300 μg/ml of G418 was added to the culture media for GFP-expressing PGC selection. The basic vector frames of CAGG-PBase (pCyL43) and *piggyBac* transposon (pCyL50) were gifts from the Sanger Institute (http://www.sanger.ac.uk).

Transplantation and detection of GFP-expressing PGCs in recipients

After GFP transgene transfection and G418 selection, the male SNUhp26 line (p46-p60) was transplanted into recipient embryos from the Korean oge (KO) chicken, quail, and Korean ring-necked pheasant. The developmental stages of the chicken, quail and pheasant embryos were 53 h, 48 h and 60 h of incubation, respectively. A small window was made on the pointed end of the recipient eggs, and a 2 μl aliquot containing more than 1000 GFP-expressing SNUhp26 PGCs was microinjected with a micropipette into the dorsal aorta of the recipient embryos. The egg window of each recipient embryo was sealed with paraffin film, and the egg was incubated with the pointed end down until screening at the developmental stages and hatching. The embryonic gonads or testes/ovaries were dissected, and live images of GFP-positive transplanted PGCs were observed using a confocal laser scanning microscope (LSM 700; Carl Zeiss, Wetzlar, Germany). The transgene in the semen of the putative germ line chimeric quails was detected by genomic polymerase chain reaction (PCR) using Neo^R-specific primers (forward 5'-tgt gct cga cgt tgt cac tg-3' and reverse 5'-cca cca tga tat tcg gca ag-3'). PCR was performed with an initial incubation at 94 C for 5 min, followed by 30 cycles at 94 C for 30 sec, 60 C for 30 sec and 72 C for 30 sec. The reaction was terminated by a final incubation at 72 C for 7 min, and the amplified PCR product size was 359 bp.

Testcross analysis for germ line transmission

The male SNUhp26 line derived from WL embryo has a dominant pigmentation inhibitor gene (*I/I*). In contrast, KO as a recipient has a recessive pigmentation inhibitor gene (*i/i*). Through testcross analysis by mating with regular KO chickens (*i/i*), the germ line chimeras were identified by the phenotype of their offspring. Endogenous germ cells in the KO recipient chickens (*i/i*) produced only black KO due to the recessive pigmentation inhibitor gene (*i/i*), whereas WL donor-derived germ cells (*I/I*) produced white hybrids with *I/i*.

Results

Chicken primordial germ cell (SNUhp26) culture

The male chicken SNUhp26 PGCs were used in a transplantation study after GFP transgene transfection and G418 selection. After subpassage, the SNUhp26 PGCs maintained the unique morphological properties of germ cells and were also positive for SSEA-1 antibody staining, which is a germ cell-specific marker in chicken (Fig. 1). Generally, the cultured PGCs were larger than somatic cells. Furthermore, similar to PGCs in developing chicken embryos, the cultured chicken PGCs had clear cytoplasm, an eccentric nucleus, and many granules in the cytoplasm (Fig. 1A). The SSEA-1 epitope is a carbohydrate antigen associated with core glycolipids and is expressed on the cell surface. Obviously, anti-SSEA-1 antibody stained the cell membrane of cultured chicken PGCs (Fig. 1B). The cultured PGCs did not attach to the bottom of the culture dish and grew in suspension (Fig. 1).

Transfection and establishment of a GFP-expressing SNUhp26 subline

The G418-selected SNUhp26 subline grew stably and constantly expressed GFP (Fig. 1B). Chicken PGCs were killed by a concentration of 300 μg/ml G418 without transfection of the neomycin resistance gene, but transgene-transfected and G418-selected SNUhp26 PGCs grew normally under the selective conditions and still showed the morphological properties of germ cells (Fig. 1B). After G418

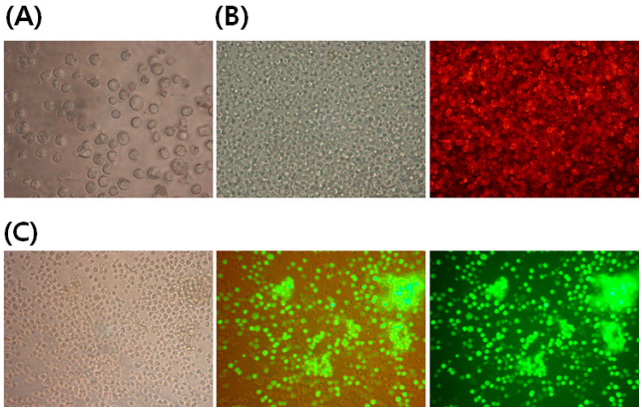

Fig. 1. (A) Cultured male chicken primordial germ cells (PGCs, SNUhp26 line) (magnification: 400×). (B) Staining with anti-stage-specific embryonic antigen-1 (SSEA-1) antibody, which is a chicken germ cell-specific marker (left panel, bright field). Phycoerythrin (PE)-conjugated secondary antibody was used to detect SSEA-1 primary antibody (right panel, fluorescent field) (magnification: 100×). (C) GFP-expressing chicken PGCs after transfection and G418 selection. Most individual chicken PGCs and colonies showed strong expression of GFP (magnification: 100×).

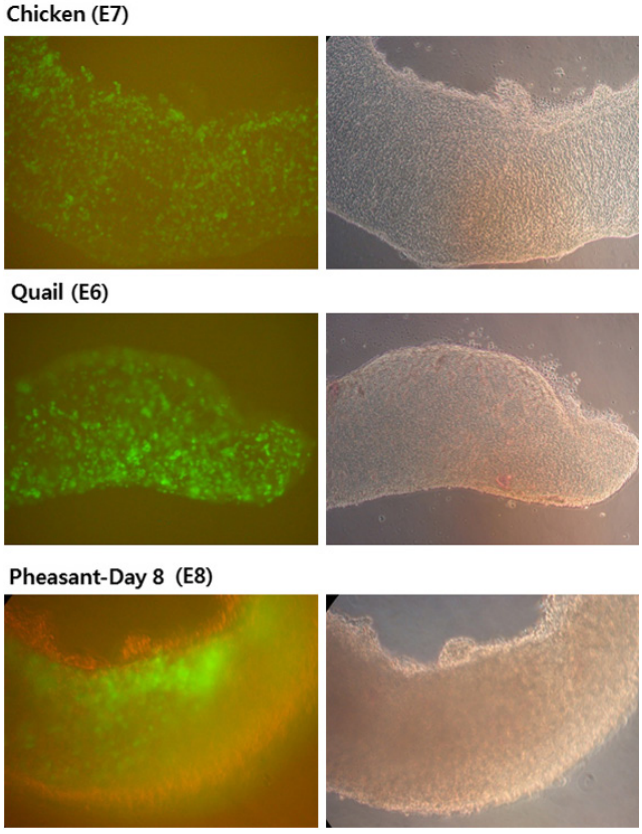

Fig. 2. Detection of transplanted GFP-positive chicken primordial germ cells (PGCs) in the embryonic gonads of recipients. The embryonic gonads of chickens, quails and pheasants were screened at 7, 6 and 8 days, respectively. GFP signals were observed by fluorescence microscopy (magnification: 200×).

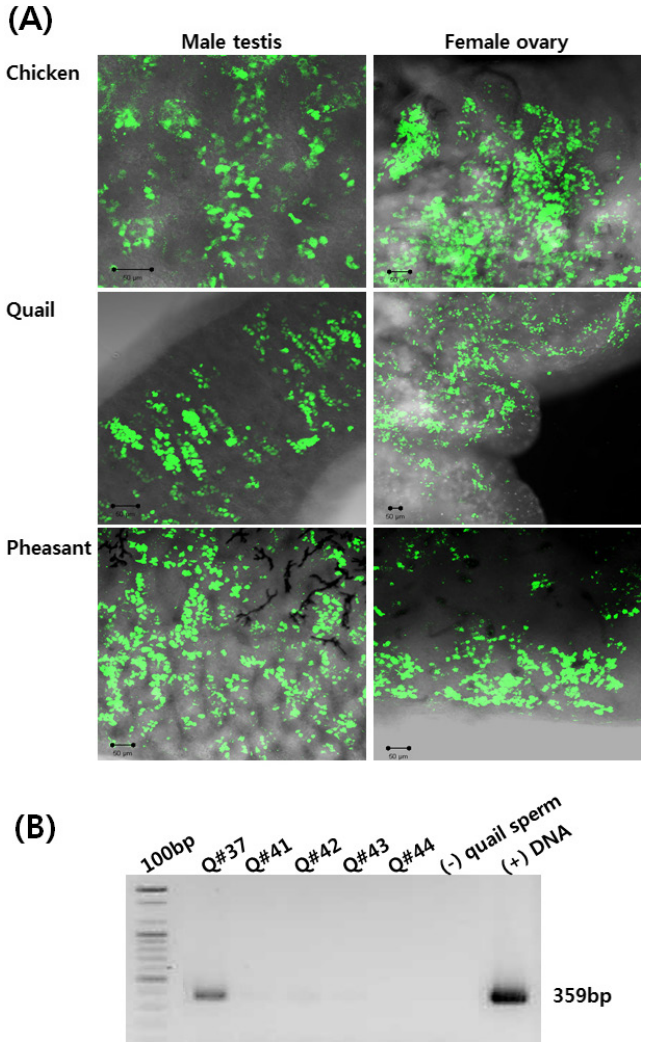

Fig. 3. (A) Detection of transplanted GFP-positive chicken germ cells in the testis or ovary of recipients after hatching. The testes or ovaries of each avian species (i.e., chicken, quail and pheasant) were observed by confocal microscopy. GFP-expressing donor germ cells were localized to the seminiferous tubules in the testis of three avian recipients or colonized in the cortex area in the ovary of the recipients (magnification: indicated as a scale bar). (B) Detection of transgene in the semen of five putative germ line chimeric quails by PCR. The amplified PCR product was 359 bp.

selection, most chicken PGCs showed strong expression of the GFP transgene and could be easily detected by fluorescence microscopy (Fig. 1B). Highly expressed GFP did not affect cell growth or survival of chicken PGCs. The G418-selected chicken PGCs were subpassaged onto inactivated mouse embryonic fibroblast feeder layers in intervals of 5–6 days.

Transplantation and detection of GFP-expressing PGCs in recipients

GFP-expressing chicken PGCs between passages 46 and 60 were utilized for transplantation into recipient embryos. Passage number did not affect the properties of cultured chicken PGCs, such as

migration and proliferation after transfer into the recipient embryos. As embryo developmental processes are different between avian species, the transplanted donor PGCs were detected in chicken, quail, and pheasant embryos at 7, 6, and 8 days, respectively, at which both the endogenous and exogenous PGCs had completed their migration into the developing genital ridges. At 7 days in chicken embryos, the transplanted GFP-positive cells were detected in the recipient embryonic gonads (Fig. 2).

In the next step, to examine the species-specific migration capacity of male chicken PGCs, we extended the recipients to different avian species, i.e., quail and pheasant embryos. After transfer, we sacrificed 6-day-old quail and 8-day-old pheasant embryos and identified the transferred GFP-positive PGCs in the gonads of the recipients (Fig. 2). Interestingly, the GFP-expressing chicken PGCs circulated through the blood vessels and migrated into the genital ridges of both quail and pheasant embryos. Subsequently, all of the transplanted recipients, including chickens, quails, and pheasants, were screened after hatching. At this stage, we used confocal microscopy to identify the GFP-expressing cells because the testes and ovaries of hatched chicks were too thick to detect the transplanted PGCs. GFP signals were clearly observed by confocal microscopy without sectioning (Fig. 3A). Regression of the right ovary is well known to occur during embryonic development in female birds, and therefore the sex of the recipients could be morphologically determined using a microscope. In the testes of all recipients, the seminiferous tubules were well developed, and the transferred GFP-positive germ cells were localized to the seminiferous tubules regardless of the recipient species (Fig. 3A). In contrast, the transplanted chicken PGCs were mainly colonized in the cortex area in the ovary of hatched chicks (Fig. 3A). At hatching, the transplanted GFP-expressing donor germ cells survived and showed colonization in all recipients regardless of sex or species.

Testcross analysis for germ line transmission

In a previous study [10], we confirmed that transfer of male chicken PGCs into male chicken recipients produced donor PGC-derived offspring from germ line chimeras with high efficiency. In the present study, the germ line transmission efficiency of male donor PGCs in male recipients was 93.8% on average (Table 1). GFP transgene was detected in the putative quail semen by PCR (Fig. 3B). One (#37) of five quails showed relatively strong PCR amplification of transgene in the semen (Fig. 3B), but unfortunately, no germ line chimeras were identified in other experimental stocks of five transplantation combinations, i.e., female chicken recipients, and quail and pheasant recipients of both sexes. In the former case, 1943 progeny were produced from 84 female chicken recipients, but no transplanted donor PGC-derived chicks were obtained (Table 1). The donor chicken PGC-transferred male quails were mated with hens by artificial insemination, but we did not even obtain fertilized eggs. From testcrosses between putative germ line chimeric male and female quails, 254 progeny hatched, but all of these were quails derived from endogenous gametes of the host quails (Table 2). Of 69 eggs from the putative germ line chimeric female pheasants after artificial insemination with rooster semen, 10 hybrids hatched, but there were no pure chicks derived from the donor chicken PGC-derived oocytes (Table 2). There were also no donor germ cell-derived offspring in the case of male pheasants.

Discussion

A comprehensive understanding of the mechanisms that regulate germ cell development and differentiation is crucial to animal production and conservation of endangered species. In birds, germ cell identification and manipulation techniques, such as purification protocols with specific markers, *in vitro* culture and germ line chimera production systems by transfer of germ cells into recipients, are well established [2–8, 14]. However, the mechanisms of migration and differentiation in avian germ cells remain to be elucidated. Particularly, signaling and interaction between germ cells and stromal cells in sex-reversed reproductive organs should be further examined. In this study, we investigated the migration, survival, and differentiation capacities of chicken PGCs in opposite-sexed and interspecies gonads.

PGCs are the precursor germ cells of sperm and oocytes and can be isolated at different developmental stages in chickens because they migrate into the genital ridges through the blood vessels [15]. However, the critical receptor(s) and ligand(s) for attraction of chicken PGCs to embryonic gonads are still unknown, and it is not clear whether the regulatory circuits are or are not conserved between male and female embryos and between different avian species. In the present study, introduced male chicken PGCs migrated into quail and pheasant embryonic gonads of both sexes as well as the genital ridges of female chicken embryos (Fig. 2). These results indicate that the pathway and signaling mechanisms for avian PGC migration into embryonic gonads are conserved, although we did not identify the pivotal receptor-ligand interaction between PGCs and epithelial cells near the developing genital ridges.

Naito *et al.* [16] reported the germ line transmission of transplanted donor PGCs in opposite-sex recipients, but the rates of transmission were less than 1%. However, in more recent studies [6, 8], no germ line transmission of chicken PGCs was observed in reverse-sexed putative germ line chimeras. Similarly, in the present study, we could not identify germ line transmission in reverse-sexed recipients of any of the avian species (Table 1). Tagami *et al.* [17] reported that female PGCs settled and survived in the male testis. However, hardly any W-bearing spermatozoa were observed in the recipient testis during the spermatogenesis process, and ultimately, the putative germ line chimeric roosters failed to generate female donor PGC-derived offspring [17]. They concluded that female germ cells in the male testis could not produce fertile germ cells due to their incomplete spermiogenesis. Similarly, our data indicated an incomplete differentiation process for production of fertile gametes in the reverse-sexed and interspecies hosts, although the transgene could be detected in semen of recipient males. Thus, the interaction between PGCs and gonadal stromal cells during embryonic stages and sexual maturation would be crucial to germ cell differentiation and the subsequent production of fertile gametes.

With regard to interspecies germ line chimeras mediated by PGCs in birds, Kang *et al.* [11] were the first to report production of pheasant offspring from germ line chimeric chickens after transplantation of pheasant PGCs during the early embryonic stages. However, germ line chimeric roosters were mated with female pheasants to generate

Table 1. The efficiency of germ line transmission in male and female putative germ line chimeric chickens after transplantation of male chicken PGCs into embryonic blood vessels

Chicken ID	Number of incubated eggs	Number of hatched chicks (%)	Number of donor-derived chicks (%)
Male[1)]			
M#1	55	43 (78.2)	43 (100.0)
M#2	68	40 (58.8)	32 (80.0)
M#3	64	26 (40.6)	26 (100.0)
M#4	34	14 (41.2)	13 (92.9)
M#5	54	26 (48.1)	25 (96.2)
M#6	36	12 (33.3)	12 (100.0)
Subtotal	311	161 (51.8)	151 (93.8)
Female[2)]			
F-group#1	369	239 (64.8)	0 (0.0)
F-group#2	441	261 (59.2)	0 (0.0)
F-group#3	453	262 (57.8)	0 (0.0)
F-group#4	524	326 (62.2)	0 (0.0)
F-group#5	459	213 (46.4)	0 (0.0)
F-group#6	453	257 (56.7)	0 (0.0)
F-group#7	694	385 (55.5)	0 (0.0)
Subtotal	3,393	1,943 (57.3)	0 (0.0)

[1)] Individual roosters were used to artificially inseminate three hens. [2)] Each group consisted of 12 female putative germ line chimeric chickens (total of 84 chickens in 7 groups). After artificial insemination, the fertilized eggs of each group were collected and incubated to hatch.

Table 2. Summary of testcross analysis for putative germ line chimeric quails and pheasants after transplantation of male chicken PGCs into embryonic blood vessels

ID		Number of incubated eggs	Number of hatched progenies (%)	Number of donor-derived progenies (%)
Quail[1)]				
Male	Female			
#37	#45	31	20 (71.4)	0 (0.0)
	#39	28	17 (53.1)	0 (0.0)
#41	#40	32	14 (45.2)	0 (0.0)
#42	#46	23	10 (43.5)	0 (0.0)
#43	#47	16	13 (81.3)	0 (0.0)
	#48	32	26 (81.3)	0 (0.0)
#44	#49	33	22 (66.7)	0 (0.0)
	#58	29	14 (53.8)	0 (0.0)
#51	#72	26	0 (0.0)	0 (0.0)
#70	#74	26	25 (65.8)	0 (0.0)
#73	#75	38	33 (84.6)	0 (0.0)
#81	#77	39	19 (67.9)	0 (0.0)
	#79	28	25 (62.5)	0 (0.0)
#82	#84	40	16 (55.2)	0 (0.0)
Subtotal		421	254 (60.0)	0 (0.0)
Pheasant[2)]				
fPh#1		10	1 (10.0)	0 (0.0)
fPh#2		59	9 (15.3)	0 (0.0)
Subtotal		69	10 (14.5)	0 (0.0)

[1)] The putative germ line quails were individually mated between male and female that were transferred chicken male PGCs. [2)] Only female pheasants reached sexual maturity and were mated with a chicken rooster by artificial insemination.

pure-line pheasant progeny. Wernery *et al.* [12] reported that houbara bustards, which are wild seasonally breeding birds, were produced from germ line chimeric chickens after transferring PGC-containing gonadal cells of houbara embryos. In both studies, germ line chimeric roosters were mated with wild-type female pheasants or female houbara bustards. There are no previous reports on the generation of pure-line donor-derived offspring by mating between germ line chimeric males and females. Furthermore, the efficiencies of germ line transmission were still low (ranging from 4.4 to 17.5%) in both studies [11, 12]. Thus, the development of a culture system for the establishment of germ line-competent PGC lines from various avian species is necessary due to the limited number of fertilized eggs of wild birds. In addition, donor PGC lines individually isolated from the embryos of each sex should be transplanted into same-sexed recipients and then mated between male and female putative germ line chimeric recipients. Recently, van de Lavoir *et al.* [13] transplanted cultured chicken PGCs into guinea fowl (*Numida meleagris*) and produced chicken PGC-derived progeny from the germ line chimeric guinea fowl. In that study, chicken hens were artificially inseminated with semen from chicken PGC-transferred putative germ line chimeric guinea fowl, and male and female putative germ line chimeric guinea fowl were not mated. However, they reported that approximately 50% of the offspring had the donor-PGC phenotype, indicating that most of the progeny were derived from the fertilized chicken eggs with chicken sperm [13]. In the present study, no germ line chimeras were identified in either quail or pheasant after transfer of chicken PGCs. Compared with the germ line-compatible guinea fowl as a recipient [13], the quail and pheasant may be less adoptable as recipient hosts for complete differentiation of chicken germ cells. The mechanism and process of germ cell differentiation in the reverse-sexed and interspecies chimeras should be further investigated with regard to the different developmental stages. Alternatively, the failure of germ line transmission may have been due to the small number of putative germ line chimeric quails and pheasants used in testcrosses. In particular, the putative germ line chimeric pheasants, which are seasonally breeding birds, were not analyzed with sufficient numbers of progeny to evaluate the production of donor-derived chickens.

The most successful cases of donor-derived pure-line offspring production from interspecies germ line chimeras have been in fishes [18, 19]. Takeuchi *et al.* [18] isolated GFP-expressing PGCs from the genital ridges of newly hatched rainbow trout (*Oncorhynchus mykiss*) and transplanted them into the peritoneal cavities of newly hatched masu salmon (*Oncorhynchus masou*) embryos. Phylogenetically, these two species have been separated for at least 8 million years. Nevertheless, the recipient male salmon produced fertile rainbow trout sperm [18]. Okutsu *et al.* [19] advanced the xenotransplantation technique using sterile triploid salmon, which were unable to produce fertile sperm or eggs. Subsequently, all of the offspring produced from germ line chimeric salmon parents in which the exogenous rainbow trout germ cells dominated the testis or ovary were donor-derived rainbow trout [19].

For the production of reverse-sexed germ line chimeras in fish, Okutsu *et al.* [20] transplanted testicular germ cells containing spermatogonial stem cells (SSCs) isolated from adult male rainbow trout into the newly hatched embryos of both sexes. Surprisingly, the testicular germ cells migrated into the developing genital ridges and also differentiated not only into spermatozoa in male recipients but also into fully functional eggs in female recipients [20]. Furthermore, the donor-derived spermatozoa and eggs from the recipient fishes produced normal offspring. Reversely, Yoshizaki *et al.* [21] transferred ovarian germ cells retrieved from female rainbow trout and examined colonization in the ovary or testis. Eventually, the transplanted ovarian germ cells in recipient gonads proliferated and differentiated into sperm in male recipients and into eggs in female recipients. Similarly, the donor-derived eggs and sperm were fertile and generated normal offspring [21]. These observations indicate that the undifferentiated germ cells of fish, such as SSCs in males and oogonia stem cells in females, have a high level of sexual bipotency. However, in avian species, such sexual plasticity has not yet been confirmed. Thus, germ cells should be transplanted into same-sex recipients for successful germ line transmission, and other obstacles including optimization of species-specific germ cell culture techniques, egg shell size of the recipients and differences in sexual maturation periods between the donor germ cells and the recipients, should be resolved. In the present study, we demonstrated that migration and survival circuits in chicken PGCs were conserved between males and females and between different avian species.

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