Interspecific hybrids of dwarf hamsters and Phasianidae birds as animal models for studying the genetic and developmental basis of hybrid incompatibility

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Hybrid incompatibility is important in speciation as it prevents gene flow between closely related populations. Reduced fitness from hybrid incompatibility may also reinforce prezygotic reproductive isolation between sympatric populations. However, the genetic and developmental basis of hybrid incompatibility in higher vertebrates remains poorly understood. Mammals and birds, both amniotes, have similar developmental processes, but marked differences in development such as the XY/ZW sex determination systems and the presence or absence of genomic imprinting. Here, we review the sterile phenotype of hybrids between the Phodopus dwarf hamsters P. campbelli and P. sungorus, and the inviable phenotype of hybrids between two birds of the family Phasianidae, chicken (Gallus gallus domesticus) and Japanese quail (Coturnix japonica). We propose hypotheses for developmental defects that are associated with these hybrid incompatibilities. In addition, we discuss the genetic and developmental basis for these defects in conjunction with recent findings from mouse and avian models of genetics, reproductive biology and genomics. We suggest that these hybrids are ideal animal models for studying the genetic and developmental basis of hybrid incompatibility in amniotes.

Key words: birds, hybrid incompatibility, mammals, sexual bias, speciation

INTRODUCTION

Approximately 10% of well-studied groups of animal species are estimated to hybridize with at least one related species (Mallet, 2005). The frequency of species known to hybridize with at least one other species is 9.3% in birds and 6.0% in mammals (Mallet, 2005). Most notably, hybridization occurs mostly between evolutionarily young species.

Hybridization and/or genetic introgression between genetically differentiated populations occasionally contributes to adaptation; more typically, it lowers the fitness of hybrids by causing hybrid incompatibility, manifesting as sterility or inviability (Coyne and Orr, 2004; Mallet, 2005; Maheshwari and Barbash, 2011; Abbot et al., 2013). Hybrid incompatibility is believed to promote speciation by preventing gene flow between populations in sympatry, and may also reinforce prezygotic reproductive isolation between sympatric populations by promoting the evolution of mating behavior or gametic interactions (Coyne and Orr, 2004; Mallet, 2005; Abbot et al., 2013). Another form of postzygotic reproductive isolation is extrinsic reproductive isolation, such as ecological lethality and behavioral sterility, which occurs in interspecific hybrids without apparent developmental defects (Coyne and Orr, 2004).

Hybrid sterility and inviability can be defined as intrinsic postmating, postzygotic reproductive isolation barriers, which are relatively independent of the environment and dependent on developmental defects (Coyne and Orr, 2004). Hybrid sterility is the full or partial sterility caused by developmental problems in reproductive systems or gametes and by neurological or physiological problems that hamper successful courtship. Hybrid inviability is full or partial lethality, which is caused by developmental defects. Sexual bias and parent-of-origin effects are often observed in hybrid sterility and inviability (Haldane, 1922; Coyne and Orr, 2004; Maheshwari...
and Barbash, 2011). Maheshwari and Barbash (2011) suggest that “hybrid incompatibility genes be defined as those that cause a measurable reduction in fitness of hybrids in F1, F2, or BC1 generation interspecific hybrids” (p. 332). The unique feature of hybrid incompatibility genes is that they work effectively in pure species but cause adverse consequences in interspecific hybrids. During the 1930s and early 1940s, Dobzhansky and Muller formulated a model of the evolution of hybrid incompatibility, called the Dobzhansky and Muller (DM) model, using Drosophila species (Dobzhansky, 1936; Muller, 1940, 1942). This model predicts that genetic changes occur at multiple loci in populations originating from the same ancestral population and their interactions cause incompatibility in hybrids between differentiated populations. Recent genetic studies have identified many hybrid incompatibility genes, which conform to the DM model, in interspecies or intersubspecies hybrids of a wide variety of taxa, including Saccharomyces, Arabidopsis, Oryza, Drosophila, Xiphophorus and Mus (Johnson, 2010; Presgraves, 2010; Maheshwari and Barbash, 2011). Furthermore, the large effect of X chromosomes on sexually biased hybrid incompatibility has been shown in interspecies or intersubspecies hybrids of Drosophila, Mus and Gasterosteus (Orr, 1989; Tao et al., 2003; Moehring et al., 2006; Masly and Presgraves, 2007; Good et al., 2008a; Kitano et al., 2009).

Hybrid inviability evolves notably faster in mammals than in birds: evolution of hybrid inviability in birds is estimated to take ~21 million years (MY) on average, compared with only 2–4 MY in mammals (Fitzpatrick, 2004). Wilson et al. (1974) proposed that the difference in evolution of hybrid incompatibility is caused by the rapid evolution of the regulatory system of gene expression in mammals. Hybrid incompatibility in animals generally follows Haldane’s rule, which states that hybrid incompatibility is biased towards the heterogametic sex (XY males in mammals and ZW females in birds) (Haldane, 1922; Wu and Davis, 1993; Laurie, 1997; Presgraves, 2008; Ellegren, 2011; Schilthuizen et al., 2011). Sexual bias of hybrid incompatibility has been observed in many different taxa, such as Mammalia, Aves, Amphibia, Reptilia, Teleostei, Diptera and Orthoptera (Schilthuizen et al., 2011). Hybrid sterility evolves faster than inviability in the heterogametic sex in Drosophila, mammals and birds (Wu, 1992; Coyne and Orr, 1997; Price and Bourvier, 2002). The classical model of speciation predicts that hybrid incompatibility is a by-product of adaptation by species to different ecological niches. However, recent findings suggest that this is not necessarily the case for the initial step of the evolution of hybrid incompatibility. More often, this is a by-product of changes in selfish genetic elements (SGEs) and secondary changes in the loci that interact with them, such as molecular arms races between SGEs and host genomes (Johnson, 2010; Presgraves, 2010; Maheshwari and Barbash, 2011; Werren, 2011). Werren et al. (1988) defined SGEs as those elements “having characteristics that enhance their own transmission relative to the rest of an individual’s genome, and that are either neutral or detrimental to the organism as a whole” (p. 297). Werren (2011) listed transposable elements, meiotic drivers, supernumerary B chromosomes, post-segregation killers, and heritable microbes and organelles that distort sex determination as examples of SGEs. A good example of the influence of SGEs on hybrid incompatibility is the Overdrive (Ovd) gene that causes both male sterility and segregation distortion in F1 hybrids between subspecies of D. pseudoobscura (Phadnis and Orr, 2009). F1 hybrid males between D. pseudoobscura bogotana females and D. p. pseudoobscura males are almost completely sterile, with the majority of F1 hybrid males’ progeny being females. The causative Ovd (GA19777) gene is located on the X chromosome and encodes a polypeptide with a single MADF DNA-binding domain near its C terminus. This sex-ratio distortion in progeny is caused by an overrepresentation of X chromosome-bearing sperm among functional gametes, which may be attributable to meiotic drive or to gamete killing or inactivation. In any case, X chromosomes carrying Ovd have a transmission advantage. The Ovd gene exhibited high rates of sequence evolution in the D. p. bogotana lineages, and the mutations in Ovd might have spread in D. p. bogotana because of their own advantage in transmission and not because of their beneficial role in the host organism. Thus, Ovd can be considered an SGE.

Disruption of epigenetic control of transposable elements is also associated with hybrid incompatibility in a wide range of taxa (Rebollo et al., 2010). A well-known case is hybrid dysgenesis in Drosophila species, which is caused by insufficient epigenetic silencing of paternally inherited transposons (Vela et al., 2014). Improper methylation of imprinting genes is associated with developmental defects in interspecific mammalian hybrids (Vrana, 2007; Rebollo et al., 2010; Wolf et al., 2014). Perturbed genomic imprinting is also associated with hybrid abnormalities in plants (Ishikawa and Kinoshita, 2009). Collectively, these findings suggest that a common genetic and molecular basis underpins hybrid incompatibility.

Here, we review the sterile phenotype of interspecific hybrids between two Phodopus species, Campbell’s dwarf hamster (P. campbellii) and the Djungarian hamster (P. sungorus) (Cricetidae, Rodentia), and the inviable phenotype of intergeneric hybrids between two avian species (Phasianidae, Galliformes), the chicken (Gallus gallus domesticus) and Japanese quail (Coturnix japonica). We propose hypotheses for developmental defects that may cause these phenotypes and discuss their genetic and developmental basis. Information on parental species,
including physiology and morphology, has been ade-
quately described in previous literature (e.g., Vojtíšková,
1958; Woodard et al., 1965; Hawkesworth, 1982; Peterson
and Brisbin, 1998; Mizutani, 2003; Sawai et al., 2010), so
we have not included this information in the present
study.

THE STERILE PHENOTYPE OF PHODOPUS
HYBRIDS

Phodopus campbelli and P. sungorus diverged 0.8–1.0
MY ago (MYA) (Ross, 1995, 1998; Neumann et al., 2006).
These species have the same chromosome number (2n =
28) (Bigger and Savage, 1976; Schmid et al., 1986) and
their chromosomes are similar in terms of size and
morphology. However, several chromosomes show struc-
tural differences in the location and size of C-heterochro-
matin blocks (Romanenko et al., 2007). The two species
do not interbreed in the wild, but they do mate spontane-
ously in captivity and produce F1 progeny (Sokolov and
Vasil’eva, 1993; Safronova and Vasil’eva, 1996). In both
reciprocal crosses, hybrid males are sterile and hybrid
females are fertile but have decreased fertility (Sokolov
and Vasil’eva, 1993; Safronova and Vasil’eva, 1996).
Notably, parent-of-origin effects are observed in the
growth phenotypes of embryos and placentas of the F1
hybrids, which differ between the cross (P. campbelli
females × P. sungorus males) and its reciprocal. In the
cross between P. campbelli females and P. sungorus
males, embryos and placentas grow normally, but in the
reciprocal cross, embryos and placentas exhibit over-
growth (Brekke and Good, 2014; Uno, Y., Ohishi, N.,
Tsuchiya, K., and Matsuda, Y., unpublished data). The
reciprocal cross leads to serious dystocia, which usually
results in embryonic death before birth. Notably, bial-
lelic expression of several genes, which contrasts with
monallelic expression of orthologous mouse genes, is
observed in hybrids. For example, Grb10, Igr2r and
Mash2, whose orthologous mouse genes are expressed
maternally, show biallelic expression in the placentas of
both reciprocal hybrids (Brekke and Good, 2014; Uno, Y.,
Ohishi, N., Tsuchiya, K., and Matsuda, Y., unpublished data).
It is important to note that whether these genes
undergo monoallelic or biallelic expression in parental
species remains unclear.

Here, we summarize our recent findings from histolog-
ical and cytological analysis of spermatogenesis of F1
hybrid males that were obtained by the laboratory cross
between P. sungorus males and P. campbelli females (Fig.
1). Details about the methods and results are described in
our previous literature (Ishishita et al., 2015). Based
on the spermatogenic phenotypes and testes sizes of
hybrids from the cross between P. campbelli females and
P. sungorus males, we classified male hybrids into three
types, Type A, B and C (Fig. 1). Type A testes were
extremely small compared with those of the parent spe-
cies; the relative testis weight (testis weight/body weight)
of Type A males was 4.2% of P. campbelli and 4.7% of P.
sungorus, and testes contained no spermatozoa. Type B
testes were small, compared with those of the parent spe-
cies; the relative testis weight of Type B males was 21.6% of
P. campbelli and 24.4% of P. sungorus. Type B testes
exhibited spermatogenesis that was arrested at meiosis I.
Meiotic arrest occurred frequently at the first meta-
phase I (MI) in Type B testes, which were characterized
by the accumulation of degenerated MI spermatocytes.
Dissociation of the X and Y chromosomes was frequently
observed in the MI spermatocytes (Fig. 1). Furthermore,
a high incidence of univalency was observed in X and Y
sex chromosomes, but not in autosomes, of pachyten-like
spermatocytes (Fig. 1). However, abnormal chromosome
synapsis, such as interlocking and partial synapsis, and
persistent unrepaired double-stranded DNA breaks
(DSBs) were observed at a low frequency in autosomes of
these spermatocytes. Type C testes were smaller than in
the parent species; the relative testis weight of Type C
males was 49.5% of P. campbelli and 55.9% of P.
sungorus. Type C testes also exhibited meiotic abnor-
malities similar to Type B, but contained spermatozoa.
However, most spermatozoa exhibited malformations,
such as abnormal heads with shelving curvature of the
hook, bent hooks, thick hooks and abnormal tails. Their
motility and fertilization ability were not examined. The
XY body is a chromatid domain specifically formed
around the X and Y chromosomes during male meiosis
and involved in meiotic sex chromosome inactivation
(MSCI) (Handel, 2004). XY bodies were of normal form
in Type B and C hybrids, in terms of phosphorylation of
H2AFX, although it remains unknown whether MSCI
was normal in the spermatocytes. Our data concur with
previous studies of F1 hybrids between P. sungorus females and P. campbelli males, and the reciprocal F1
hybrids, with the exception that previous studies
observed a higher frequency of autosomal asynapsis
(Sokolov and Vasil’eva, 1993; Safronova and Vasil’eva,
1996; Safronova et al., 1999). The difference between
these previous studies and ours may be caused by differ-
ences in the genetic background of dwarf hamsters used
for crosses.

SIMILARITY OF PHODOPUS STERILE HYBRIDS
TO MUS STERILE HYBRIDS

Similar sterile phenotypes to Phodopus hybrids are also
observed in intersubspecific and interspecific hybrids in
Mus. A well-studied example is male sterility in hybrids
between M. musculus musculus and M. m. domesticus
(Good et al., 2008a, 2008b; Teeter et al., 2008). Mus m.
musculus and M. m. domesticus diverged less than 1 MYA
(Suzuki et al., 2004; Lecompte et al., 2008; Gerald et al.,

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Fig. 1. Sterile phenotypes of Phodopus hybrids. (A) Representative images of males of two Phodopus species and their hybrids: From left to right, P. campbelli, P. sungorus, P. campbelli × P. sungorus F1 hybrid (CS) and P. sungorus × P. campbelli F1 hybrid. (B) Gross appearance of testes of P. campbelli (left panel) and CS (middle and right panels). The image in the right panel represents Type A hybrid testes with very small sizes. The image in the middle panel represents Type B and Type C hybrid testes, which are smaller than those of parental species and larger than Type A testes. (C–F) Hematoxylin and eosin-stained testicular cross-sections of P. sungorus (C) and CS (D–F). Seminiferous tubules are thin in Type A hybrid testes, and a small number of cells are present in these tubules (D). Arrows indicate primary spermatocytes. Primary spermatocytes accumulate in seminiferous tubules of Type B hybrid testes, and few or no secondary spermatocytes and postmeiotic cells are present in these seminiferous tubules (E). A small number of spermatocytes are present in seminiferous tubules of Type C hybrid testes (F). (G, H) Representative images of terminal association of X and Y chromosomes (G, arrow) and dissociation of X and Y chromosomes (H) in MI primary spermatocytes of hybrids. Meiotic chromosomes of MI primary spermatocytes of CS were visualized using Giemsa staining. (I, J) Representative images of synaptic X and Y chromosomes and asynaptic X and Y chromosomes in pachytene or pachytene-like spermatocytes of hybrids. Immunostaining of spermatocyte nuclei of CS was performed with antibodies to the synaptonemal complex proteins SYCP1 and SYCP3, which are structural components of the transverse filaments and the axial/lateral elements, respectively, of the synaptonemal complex. Green and red colors represent localization of SYCP1 and SYCP3, respectively. Yellow color represents colocalization sites of the two proteins. Blue color represents 4′,6-diamidino-2-phenylindole (DAPI)-stained nuclei. All autosomal pairs and X and Y chromosomes undergo synapsis in the pachytene spermatocyte (I, arrow). All autosomal pairs complete synapsis in the pachytene-like spermatocyte; however, the X and Y chromosome pair remains unsynapsed (J). Scale bars, 100 mm (A, B), 50 μm (C–F), 20 μm (G, H) and 10 μm (I, J). Panels (C–J) are adapted from Ishishita et al. (2015).
cross between female mice of the C57BL/6 (B6) strain (derived mainly from *M. m. domesticus*) and *M. spreptus* males exhibit meiotic arrest at MI, which is accompanied by small testes, a high incidence of abnormal chromosome synopsis at prophase I, and dissociation of X and Y chromosomes at MI (Matsuda et al., 1991; Hale et al., 1993). F1 hybrids between the B6 strain and the *M. m. molossinus*-derived MSM/Ms strain are fertile. However, a consomic strain, B6-ChrX<sup>SM</sup>, exhibits male sterility with arrest of spermatogenesis at the pachytene and meiotic prophase I stages, a reduced sperm number, and sperm malformation (Oka et al., 2004, 2007, 2010). These findings imply a common basis of hybrid sterility in *Phodopus* and *Mus* hybrids.

**POTENTIAL DEVELOPMENTAL DEFECTS THAT CAUSE MALE STERILITY IN PHODOPUS HYBRIDS**

We now discuss potential developmental defects that underlie the sterile phenotypes of *Phodopus* hybrids and the genetic basis of male-specific sterility. Synaptic failure (asynapsis) of chromosomes is a subcellular defect that potentially causes male sterility of the *Phodopus* hybrids. Burgoyne et al. (2009) proposed a model for the consequences of asynapsis as follows. Partial asynapsis, including non-homologous synopsis due to chromosome rearrangement, and chromosome univalency may often arrest spermatogenesis either at the pachytene stage, due to transcriptional silencing of critical genes in the unsynapsed chromatin (meiotic silencing of unsynapsed chromatin, MSUC), or at the postmeiotic stage, due to postmeiotic repression of critical genes as a consequence of MSUC. In addition, in spermatocytes with limited autosomal asynapsis, premature chromosome dissociation causes arrest of meiosis at MI. In spermatocytes with extensive autosomal asynapsis in numerous chromosome pairs, meiosis may often be arrested at the pachytene stage due to MSCI failure or the checkpoint response to unrepaired DSBs. The pachytene and/or MI arrests induce apoptosis in spermatocytes, and postmeiotic transcriptional repression of critical genes induces apoptosis in postmeiotic cells. Therefore, in *Phodopus* male hybrids, limited autosomal asynapsis, which occurs at a low frequency, would induce a low frequency of meiotic arrest in pachytene spermatocytes or postmeiotic cells via MSUC. Conversely, X-Y univalency, which occurs at a higher frequency than autosomal asynapsis, would induce a high frequency of meiotic arrest in MI spermatocytes.

The stringency of cell-cycle checkpoint systems in meiosis I is lower in females than in males (Hunt and Hassold, 2002; Cohen et al., 2006; Burgoyne et al., 2009; Rojo et al., 2010; Kurahashi et al., 2012). For example, X chromosome univalency does not interfere with female meiotic progression in mice (Burgoyne et al., 2009). Therefore, if autosomal asynapsis occurs at the same rate in male and female hybrids, meiotic arrest would be more serious for males than females owing to the sex difference in checkpoint systems. There are no data regarding chromosome asynapsis in female hybrids of *Phodopus*; however, we suggest that X-Y univalency accounts for the higher frequency of meiotic arrest in male hybrids.

The DNA sequence in the pseudoautosomal region (PAR) is considered to evolve rapidly (Raudsepp et al., 2012); therefore, sequence homology at the PAR is predictably low, even between closely related species. This sequence dissimilarity may disturb homologous pairing and recombination, which may result in a higher incidence of X-Y asynapsis at pachytene and X-Y univalency at MI.

Male hybrids from *M. m. musculus × M. m. domesticus* exhibit a higher incidence of asynapsis (Forejt, 1996; Gregorová and Forejt, 2000). Spermatocytes of the *Mus* hybrids display improper gene expression due to MSCI failure (Good et al., 2010; Bhattacharyya et al., 2013; Campbell et al., 2013). Several genetic loci, including *Hat1 (Prdm9)*, which is involved in determining recombination hotspot locations, are associated with this hybrid male sterility; however, the molecular mechanisms have not yet been fully elucidated (Forejt, 1996; Mihola et al., 2009; Davies et al., 2016). Our data show that autosomal asynapsis in *Phodopus* hybrids is not greatly affected, and that the XY body appears to form normally in hybrids (Ishishita et al., 2015). This differs considerably from the phenotype of *Mus* hybrids, although it is possible that overexpression of X-linked genes due to MSCI failure is involved in male sterility in *Phodopus* hybrids.

Proteins involved in spermatogenesis evolve rapidly in mammals (Torgerson et al., 2002; Good and Nachman, 2005); therefore, malformation and reduction of sperm in Type C testes are likely to involve incompatible protein-protein interactions during spermiogenesis. In addition, MSUC-induced postmeiotic transcriptional repression, due to MSCI failure-induced disrupted postmeiotic sex chromatin repression, is an alternative cause of the malformation and reduction of sperm (Burgoyne et al., 2009; Campbell et al., 2013). A reduction in the number of postmeiotic cells due to the arrest of meiosis I may cause a reduction in sperm, leading to reduced fertility.

Previous studies suggested that misregulated gene expression due to incompatibilities between *cis* and *trans*-regulatory elements (*cis-trans* incompatibilities) contributes to hybrid male sterility in *Drosophila* and *Mus* (Landry et al., 2005; Haerty and Singh, 2006; Oka et al., 2014; Oka and Shiroishi, 2014). Thus, misregulated gene expression owing to divergence of the regulatory system of gene expression is a possible causal factor of hybrid sterility.
Extremely small testes with no spermatozoa in *Phodopus* hybrids may result from the arrest of meiosis at the pachytene and MI stages and from other defects that disturb proliferation and differentiation of germ cells and/or somatic cells in the testes. Elucidation of the genetic basis for Type A testes would contribute to a better understanding of hybrid male sterility.

**THE INViable PHenOTYPE OF CHICKEN × QUAIL HYBRIDS**

Chickens and Japanese quail (hereafter quail) diverged approximately 35 MYA (van Tuinen and Hedges, 2001; van Tuinen and Dyke, 2004); however, intergeneric hybrids can be produced between male chickens and female quail using artificial insemination (Mitumoto and Nishida, 1958; Wilcox and Clark, 1961) (Fig. 2). To date, no hybrid has been obtained by the reciprocal cross. Previous studies on hybridization of chickens and quail showed that the fertilization and hatching rates were low and that only sterile male hybrids hatched (Wilcox and Clark, 1961; McFarquhar and Lake, 1964; Bammi et al., 1966; Watanabe and Amano, 1967; Takashima and Mizuma, 1982; Khosravinia et al., 2005). Furthermore, a high incidence of embryonic lethality occurred during the early stages of development, and the sex ratio of living embryos was biased toward males (Takashima and Mizuma, 1981; Khosravinia et al., 2005). Chickens and quail have an identical number of chromosomes (2n = 78), and the structures of chromosomes are very similar between the two species, even though there are large inversions and/or centromere repositioning in several chromosomes (Shibusawa et al., 2001; Kayang et al., 2006). The number of chromosomes in chicken × quail hybrids is 78, and chromosomal abnormalities, such as loss or duplication of specific chromosomes, have not been reported (Bammi et al., 1966; Okamoto et al., 1991; Ishishita, S., Tatsumoto, S., Kinoshita, K., Go, Y., and Matsuda, Y., unpublished data).

Here, we briefly describe our recent findings on fertilization rate and hatching rate in the cross between male chickens and female quail, and also on survival rate and developmental status of chicken × quail hybrid embryos at various incubation periods (Ishishita et al., 2016). This study shows that fertilization rate was 25%–30%; hatching rate was only 1% of incubated eggs, and only 26% of fertilized eggs reached 2 days of development. Furthermore, the survival rate of hybrid embryos decreased gradually thereafter: 21% embryos survived at 5 days, 15% at 10 days, and 14% at 12 days. There was large variation in fertilization and survival rates between individual female quail, which concurs with previous studies (Haley et al., 1966; Takashima and Mizuma, 1981). Therefore, fertilization and survival rates are likely to fluctuate across studies.

Compared with the parent species, early development of chicken × quail hybrid embryos tended to be delayed between the blastoderm at Eyal-Giladi and Kochav Stage 10 and at least 7 days after hatching. Embryonic lethality occurred at various stages during 0–2 days of incubation, including the early blastoderm, pre-primitive streak, streak, somite and pre-circulation stages (Fig. 2). However, hybrid embryos whose development was arrested during the early cleavage stages may have been counted as unfertilized eggs. All hatched hybrids were males, conforming to Haldane’s rule. In a previous study, the sexual bias of living hybrids was observed from 3 days of incubation (Takashima and Mizuma, 1981). However, our data indicate that female-biased lethality begins at 7–10 days of incubation. This difference may be caused by differences in the genetic background of chickens and quail used in the two studies.

Testes of male hybrids at 9–12 months of age were much smaller than those of the parent species, and contained only basal spermatogonia in the seminiferous tubules (Takashima and Mizuma, 1981). The authors suggested that the sterility of F1 hybrids was caused by a specific endocrine disturbance. We also observed small testes in the male hybrids that we generated (Fig. 2).

**COMPARISON OF CHICKEN × QUAIL HYBRID PHENOTYPES WITH OTHER PHASIANIDAE SPECIES**

Interspecific hybrids can arise from crosses between chickens and other Phasianidae species, such as pheasant, turkey, peafowl, capercaillie and guinea fowl (Shaklee and Knox, 1954; Asmundson and Lorenz, 1957; Sandnes, 1957; Gray, 1958; Olsen, 1960; Hanebrink, 1976; Skjervold and Mjelstad, 1992; Price and Bouvier, 2002; Arrieta et al., 2013; Haru et al., 2013). Fertilization and survival rates during embryogenesis have been well studied in chicken × ring-necked pheasant (*Phasianus colchicus*, Phasianidae) and chicken × turkey (*Meleagris gallopavo*, Phasianidae) hybrids (Sandnes, 1957; Asmundson and Lorenz, 1957; Olsen, 1960). We compared these data with those of chicken × quail hybrids. Chickens and ring-necked pheasants diverged 20 MYA (Helm-Bychowski and Wilson, 1986). Chicken × ring-necked pheasant hybrids can be produced by reciprocal crosses; however, both sexes are sterile (Sandnes, 1957; Asmundson and Lorenz, 1957). Fertilization rate of the cross between male chickens and female ring-necked pheasants was 34%, which is comparable with chicken × quail hybrids. Survival rates were 60% of fertilized eggs at 7 days of incubation, and 57% at 17 days of incubation, with 39% of all fertilized eggs hatching (Sandnes, 1957). Asmundson and Lorenz (1957) showed that, in chicken × ring-necked pheasant hybrids, fertilization rate was 20% and 21% of fertilized eggs hatched. The incubation period of hybrids was 23–
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24 days for chicken eggs and 22–24 days for pheasant eggs. Thus, hatching rate is much higher than that of chicken × quail hybrids. Embryonic lethality occurs frequently during the first 7 days in chicken × ring-necked pheasant hybrids, although the survival rate at 7 days of incubation is higher in chicken × pheasant than in chicken × quail hybrids. In crosses between chicken females and pheasant males, the sex ratio was biased towards males; however, no bias was observed in the reciprocal cross (Shaklee and Knox, 1954). However, Asmundson and Lorenz (1957) reported that, in both crosses between chickens and ring-necked pheasants, no apparent bias was observed in the sex ratio of hybrids.

Chickens and turkeys diverged 40 MYA (Dalloul et al.,...
Fertilization rate of crosses between chickens and turkeys was 52%, and 73% of embryos died during 0–2 days of incubation, at the point where extraembryonic membranes and blood islands were formed but well-formed embryonic structures were absent in hybrid embryos (Olsen, 1960). Survival rates of hybrids were 27% at 2 days of incubation, 13% at 8 days of incubation, 11% at 15 days of incubation, and 8% at 22 days of incubation. Only 2% of the embryos hatched, at 22–25 days. Hybrid embryos at 15 days or later, including hatched chicks, were all male. Thus, a substantial proportion of hybrid embryos died during the first few days, after which the survival rate gradually decreased through subsequent developmental stages. Such high incidence of early embryonic lethality was also observed in chicken × quail hybrids and in chicken × pheasant hybrids. These findings suggest that developmental arrest during the early embryonic stages is a common feature of interspecific hybrids of Phasianidae.

POSSIBLE EXPLANATIONS FOR ABNORMAL DEVELOPMENTAL PROCESSES THAT CAUSE EMBRYONIC LEATHALITY IN CHICKEN × QUAIL HYBRIDS AND THEIR GENETIC AND DEVELOPMENTAL BASIS

First, we propose that incompatibility of genetic interactions between different species causes hybrid incompatibility in chicken × quail hybrids, concordant with the DM model (Dobzhansky, 1936; Muller, 1940, 1942). Multiple genetic interactions may be affected in this hybrid, and the stage where developmental arrest occurs may depend on the genetic background of the parents. Genes associated with hybrid incompatibility often show higher rates of sequence divergence (Presgraves, 2010). It is likely that protein-coding genes exhibiting high evolution rates between chicken and quail orthologs, which can be characterized by high ratios of the number of nonsynonymous substitutions per nonsynonymous site (Ka) to the number of synonymous substitutions per synonymous site (Ks) (Ka/Ks ratios), are fundamentally associated with hybrid incompatibility. Importantly, genes related to the immune response and reproduction undergo rapid evolution in various species (Swanson and Vacquier, 2002), which suggests that developmental arrest of hybrid embryos results from incompatibility of rapidly evolving genes involved not only in embryonic development but also in other biological processes. In addition, as reported in Drosophila hybrids (Johnson, 2010; Presgraves, 2010; Maheshwari and Barbash, 2011; Werren, 2011), inviability of chicken × quail hybrids may be caused by the divergence of SGEs and their genetic/epigenetic regulatory mechanisms between the parent species.

An alternative possibility is the failure of egg activation after fertilization. In birds, multiple (20–60) sperm enter the egg at fertilization and then form pronuclei, although only one of these male pronuclei fuses with a female pronucleus (Fofanova, 1965; Nakanishi et al., 1990; Wishart, 1997). The role of sperm that are not involved in nuclear fusion is to transfer paternal factors that are required for successful egg activation and embryonic development (Mizushima et al., 2014; Hemmings and Birkhead, 2015). The number of sperm that enter the egg in chicken × quail hybrids may be not sufficient for egg activation and subsequent embryonic development. Immunological responses of female quail to chicken sperm are a possible cause of the low fertilization rate in the interspecific cross (Haley and Abplanalp, 1970).

We propose two possibilities for the genetic basis of female-biased embryonic lethality in chicken × quail hybrids. There are several representative theories of Haldane’s rule, including the dominance theory, male-faster theory and meiotic drive theory (Coyne and Orr, 2004). In the dominance theory, recessive incompatibility genes on the X or Z chromosome are expressed in hybrids of the heterogametic sex, causing selective inviability or sterility (Turelli and Orr, 1995). According to this theory, we propose as the first possibility that Z chromosome-linked recessive incompatibility genes underlie female-biased inviability in chicken × quail hybrids.

The second possibility for female-biased lethality is inadequate gene dosage of Z-linked genes in female hybrids. Unlike mammals, global dosage compensation is absent in the sex chromosomes of birds, and dosage compensation of individual genes appears to be regulated in a gene-by-gene manner (Itoh et al., 2007, 2010; Mank and Ellegren, 2009; Wang et al., 2014). Recent transcriptome analyses of various bird species show that the expression levels of Z-linked genes have diversified more than those of autosomal-linked genes, particularly in females (Dean et al., 2015). These findings imply that the systems regulating Z-linked gene expression have diversified more than those regulating autosomal-linked gene expression, at least in female birds. Thus, the adjustment of Z-linked gene expression may be more difficult in female than in male hybrids, leading to inadequate gene dosage of Z-linked gene(s) and female-biased lethality. The diversification of the system regulating Z-linked gene expression may also cause cis-trans incompatibilities, leading to misregulated Z-linked gene expression in female hybrids. Gonads undergo sex-based differentiation from 6 to 10 days in chicken embryos (Smith et al., 2009), which would affect the sexual differentiation of somatic tissues. Therefore, inadequate Z-linked gene dosage in sexually differentiated somatic cells may have adverse effects on embryogenesis, resulting in a female-biased lethality during the late embryonic stage.
IMPLICATION FOR COMMONALITY OF GENETIC BASIS BETWEEN MALE-BIASED STERILITY IN MAMMALIAN HYBRIDS AND FEMALE-BIASED INVIAIBILITY IN BIRD HYBRIDS

Finally, we discuss the commonality of genetic mechanisms between male-biased hybrid sterility and female-biased hybrid inviability. Recent studies have suggested that hybrid male sterility in Mus arises from global mis-regulation of X-linked genes in testes, which is caused by incompatibility between X-chromosome-linked genes and autosomal genes (Good et al., 2010; Bhattacharyya et al., 2013; Oka et al., 2014; Turner et al., 2014). The incompatibility of Z-linked genes and autosomal genes may also be the principal factor for hybrid inviability in avian hybrids. Protein-coding sequences of Z-linked genes evolve faster than those of autosomal genes in birds (Mank et al., 2007, 2010; Ellekren, 2009; Wang et al., 2014) and snakes (Vicoso et al., 2013), as do protein-coding sequences of X-linked genes in Drosophila (Musters et al., 2006; Baines et al., 2008; Singh et al., 2008; Vicoso et al., 2008; Bachtrog et al., 2009; Langley et al., 2012; Llopart, 2015), primates (Khaitovich et al., 2005; Lu and Wu, 2005; Nielsen et al., 2005; Hvislson et al., 2012; Veeramah et al., 2014) and mice (Baines and Harr, 2007; Kousathanas et al., 2014). This may lead to a high divergence of trans-acting elements on Z chromosomes between parent species. In addition, the expression levels of Z-linked genes are more highly diverged than those of autosomal genes in birds (Dean et al., 2015), which is similar to X-linked genes in primates (Khaitovich et al., 2005) and Drosophila (Kayserili et al., 2012; Llopart, 2012; Meisel et al., 2012). Divergence of Z-linked proteins and/or Z-linked genes’ expression may affect genetic interactions between Z chromosomes and autosomes in bird hybrids. This Z-autosome incompatibility would be more prominent in female hybrids because only single alleles of Z-linked genes are present in female hybrids, and Z-linked gene expression may evolve faster in female than in male birds. Thus, the Z-autosome incompatibility may cause female-biased dysfunction, leading to female-biased hybrid inviability. Therefore, X- or Z-autosome incompatibility may be a common genetic basis of male-biased sterility in mammalian hybrids and female-biased inviability in bird hybrids. This possibility should be validated empirically.

PERSPECTIVES

Interspecific hybrids of Phodopus species are models for the study of reproductive isolation between different populations that are genetically similar. Female hybrids of Phodopus species are fertile, as are hybrids of Mus species, which allows a genetic analysis of sex-specific hybrid sterility to investigate a common genetic basis for hybrid sterility. Spermatogenesis-related genes with high levels of sequence divergence between two Phodopus species may help to elucidate the genetic basis of various abnormal spermatogenic phenotypes in interspecific vertebrate hybrids.

The genetic basis of hybrid inviability is anticipated to be partially shared between hybrids of evolutionarily young bird species and chicken × quail hybrids. Therefore, chicken × quail hybrids are a good model for the study of postmating pre- and postzygotic reproductive isolation in birds. Characterization of the inviable phenotype of chicken × quail hybrids at the molecular level will provide an understanding of the developmental basis of hybrid inviability. Furthermore, comparisons of gene expression between hybrids and the parent species will provide an understanding of the genetic cause of female-biased developmental arrest in hybrids.

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