Regeneration of a Pure Hinai-dori Chicken (Japanese native Chicken) via Germline Chimeras

***Rikimaru, K.*[1,4], *Takahashi, D.*[1], *Ito, N.*[2], *Komatsu, M.*[1], *Nakamura, Y.*[3], *and K. Matsubara*[2]**

[1]*Akita Prefectural Livestock Experiment Station, Akita, Japan E-mail: Rikimaru-Kazuhiro@pref.akita.lg.jp*

[2] *Graduate School of Agriculture, Iwate University, Iwate, japan*

[3] *Division of Germ Cell Biology, National Institute for Basic Biology, Aichi, Japan*

[4] *Graduate School of Agricultural Science, Tohoku University, Miyagi, Japan*

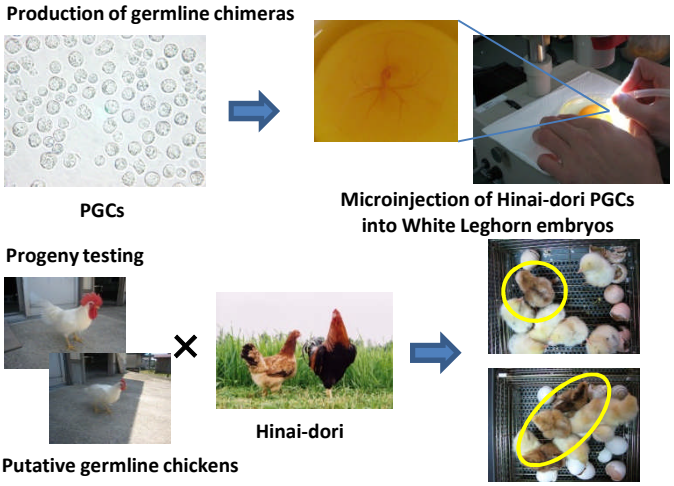
INTRODUCTION

Production of germline chimeras by transferring primordial germ cells (PGCs) is useful for genetic conservation in chicken. To identify germline chimerism, the progeny test has been the most widely used. In addition to this, currently available molecular identification method provides more accurate results. In this study, we attempted to regenerate live offspring using eggs and spermatozoa derived from the PGCs of Hinai-dori , which is a native chicken breed in Japan, and to molecularly identify these offspring using the Hinai-dori-specific markers, with a view toward future conservation of the Hinai-dori breed at the cellular level.

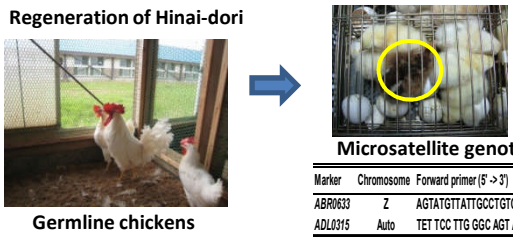
MATERIAL AND METHODS

The Hinai-dori PGCs were intravascularly micro-injected into the White Leghorn (WL) embryos to produce germline chimeras.

Putative germline chimeras that survived to sexual maturity were crossed with Hinai-dori chickens by artificial insemination and the feather color of their offspring was examined.



After identification of germline chimerism, they were mated to regenerate live offspring of Hinai-dori using eggs and spermatozoa derived from Hinai-dori PGCs. For molecularl identification, two Hinai-dori-specific microsatellite markers that can distinguish Hinai-dori from WL were used, one is located on the Z chromosome (*ABR0633*) and the other is located on an autosomal chromosome (*ADL0315*).



Marker	Chromosome	Forward primer (5' -> 3')	Reverse primer (5' -> 3')
ABR0633	Z	AGTATGTTATTGCCTGTGGC	TTTGGGAGAAGGAATGTTGT
ADL0315	Auto	TET TCC TTG GGC AGT AGT TTC AA CTC CCA TGT TGC TTC TTT AG	

RESULTS

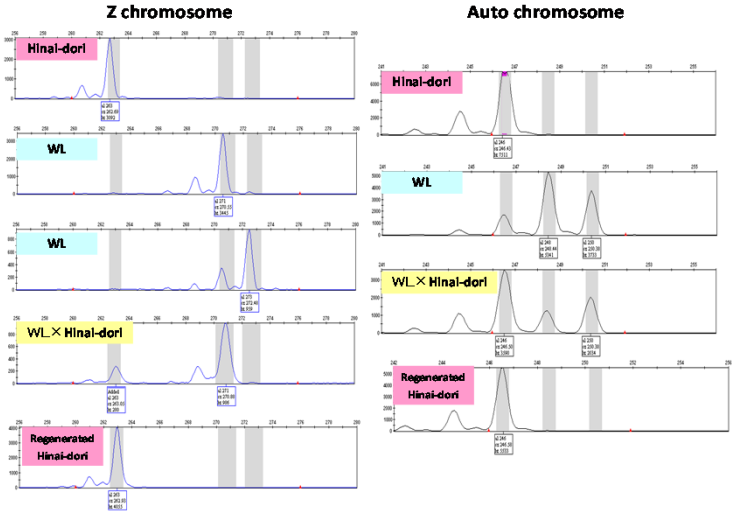
One male and two female manipulated chickens were identified as germline chimeras by test-cross anlysis. We obtained one phenotypically Hinai-dori hatching from total of 105 hatching by crossing these germline chimeras.

In the progeny genotyping, only donor-derived Hinai-dori allele was detected in the genotyping of microsatellite markers on both the Z and autosomal chromosomes of this progeny (Table 1).

Based on these results, this regenerated chick was comfirmed as the Hinai-dori breed.

Table 1. Genotype of the progeny produced by mating the germline chimeras that had Hinai-dori PGCs

Chicken	No. of chicks hatched	Markers			
		ADL0315 (Autosomal chromosome)		*ABR0633* (Z chromosome)	
Hinai-dori	–	246		263	
White Leghorn	–	248	250	271	273
Donor (Hinai-dori)-derived progeny	1	246		263	
Recipient (WL)-derived progeny	104	248	250	271	273
Donor and recipient-derived progeny		246	248 or 250	263	271 or 273



CONCLUTION

We succeeded in regenerating one live, pure Hinai-dori progeny by crossing germline chimeras.