

Session 2

Biodiversity Conservation



The results of reproductive and genetic research on Okinawa rail (*Gallirallus okinawae*) and its application for a captive breeding program



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Options for wildlife conservation

IN-SITU Conservation

Wild — Sanctuary
in
range — Captivity
in
range

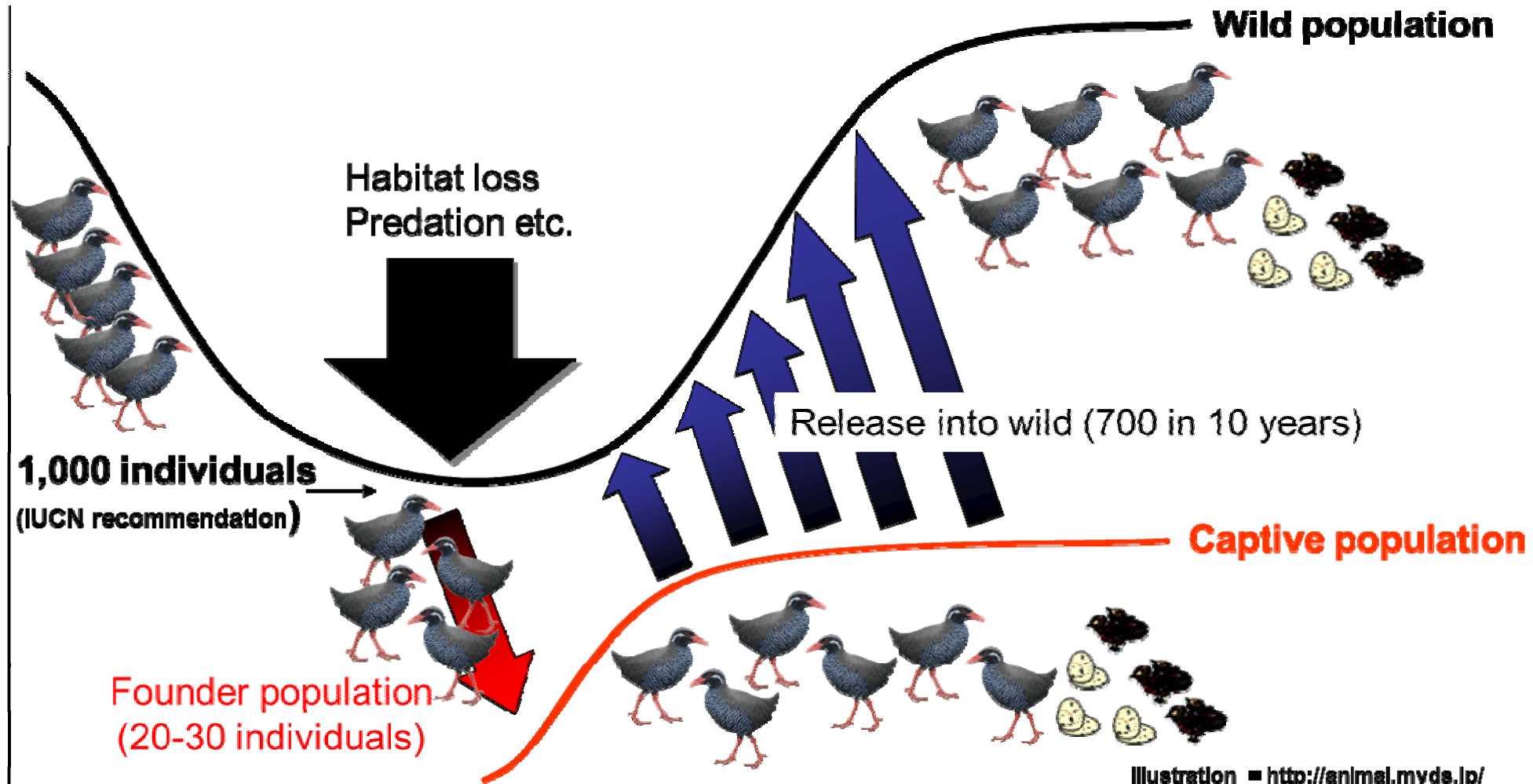
EX-SITU Conservation

Sanctuary
out of
range — Captivity
out of
range — Genetic
resource
banks

Modified from Stanley-Price 1993



Captive breeding program for Okinawa rail

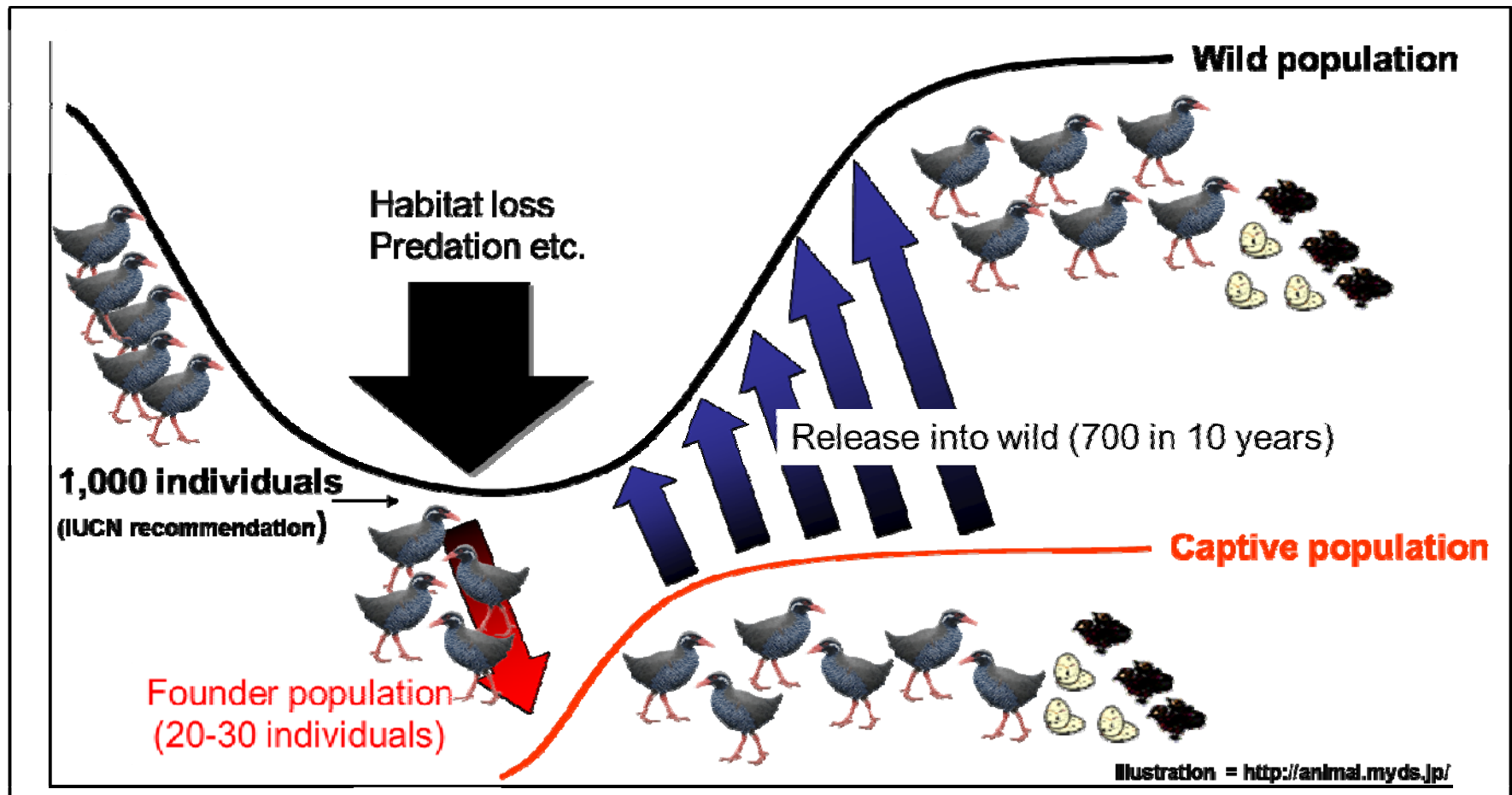


Essential information for successful captive breeding program

1, Efficient and stable reproduction : Breeding season, breeding age

(Onuma et al., 2011)

2, Maintaining genetic diversity : Evaluate genetic diversity



Sample collection

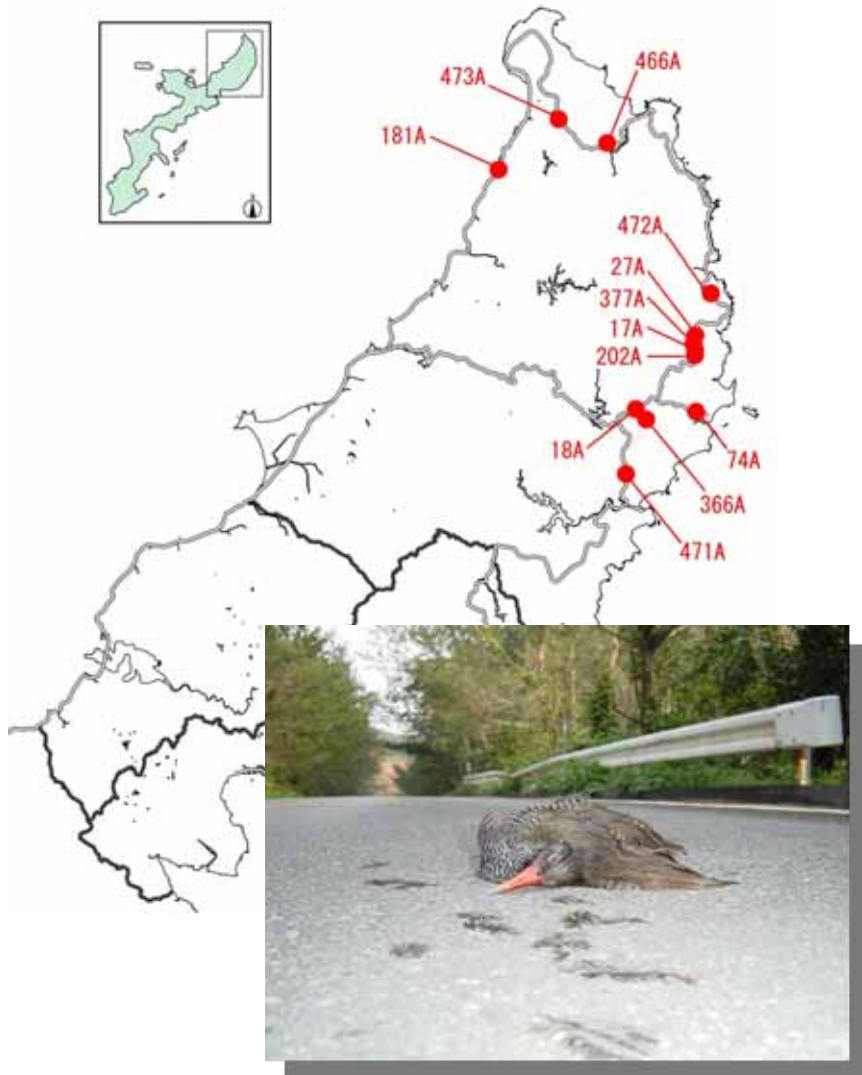


Photo by Ministry of Environment

Time capsule building in NIES



Postmortem examination



Age determination criteria of the Okinawa rail

Jan. Feb. Mar. Apr. May Jun. Jul. Aug. Sep. Oct. Nov. Dec.



Juvenile

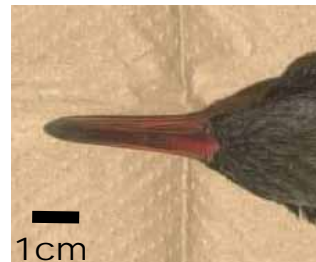


Subadult

Jan. Feb. Mar. Apr. May Jun. Jul. Aug. Sep. Oct. Nov. Dec.



Adult



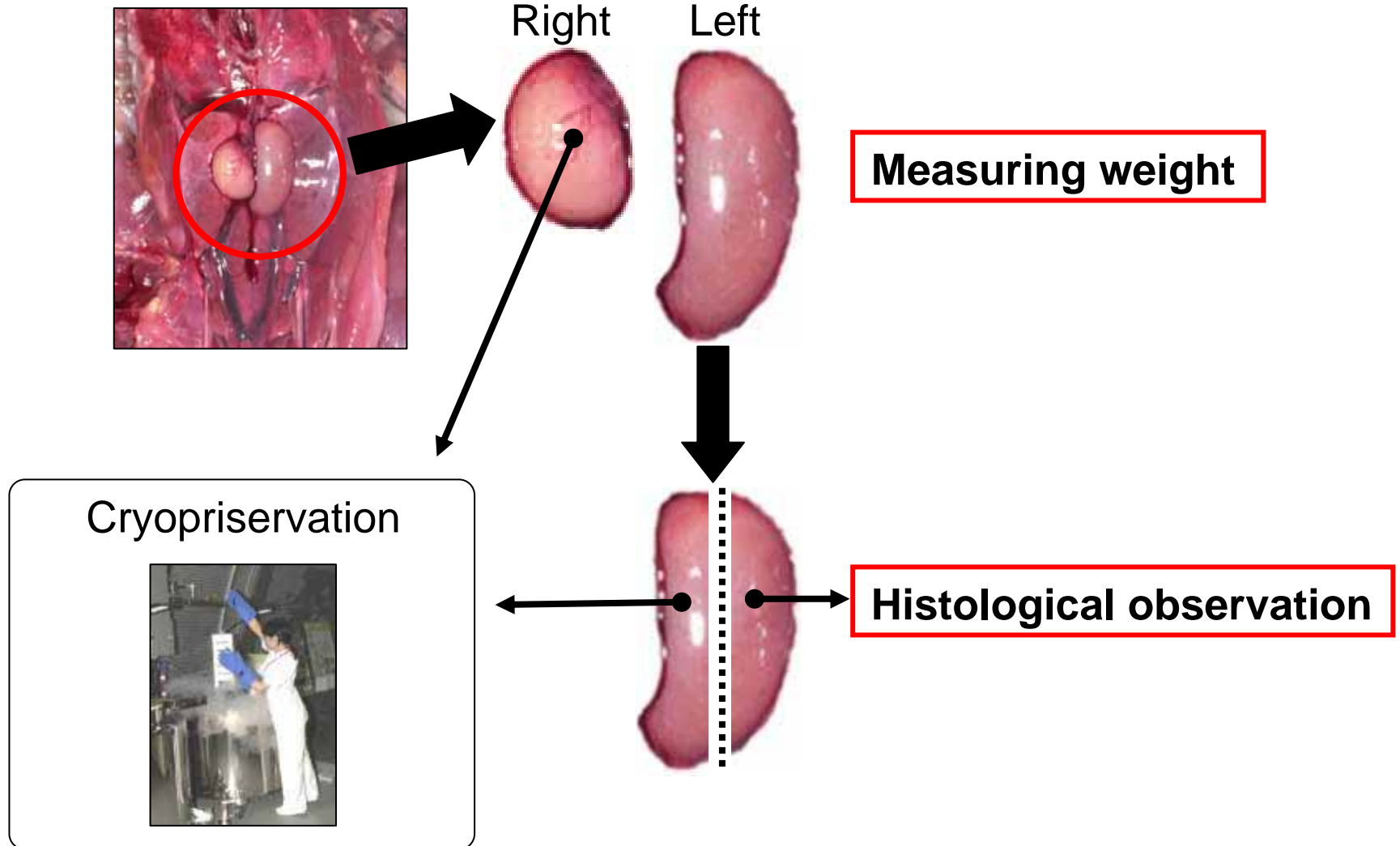
Adult born a year before
(blackish beak)



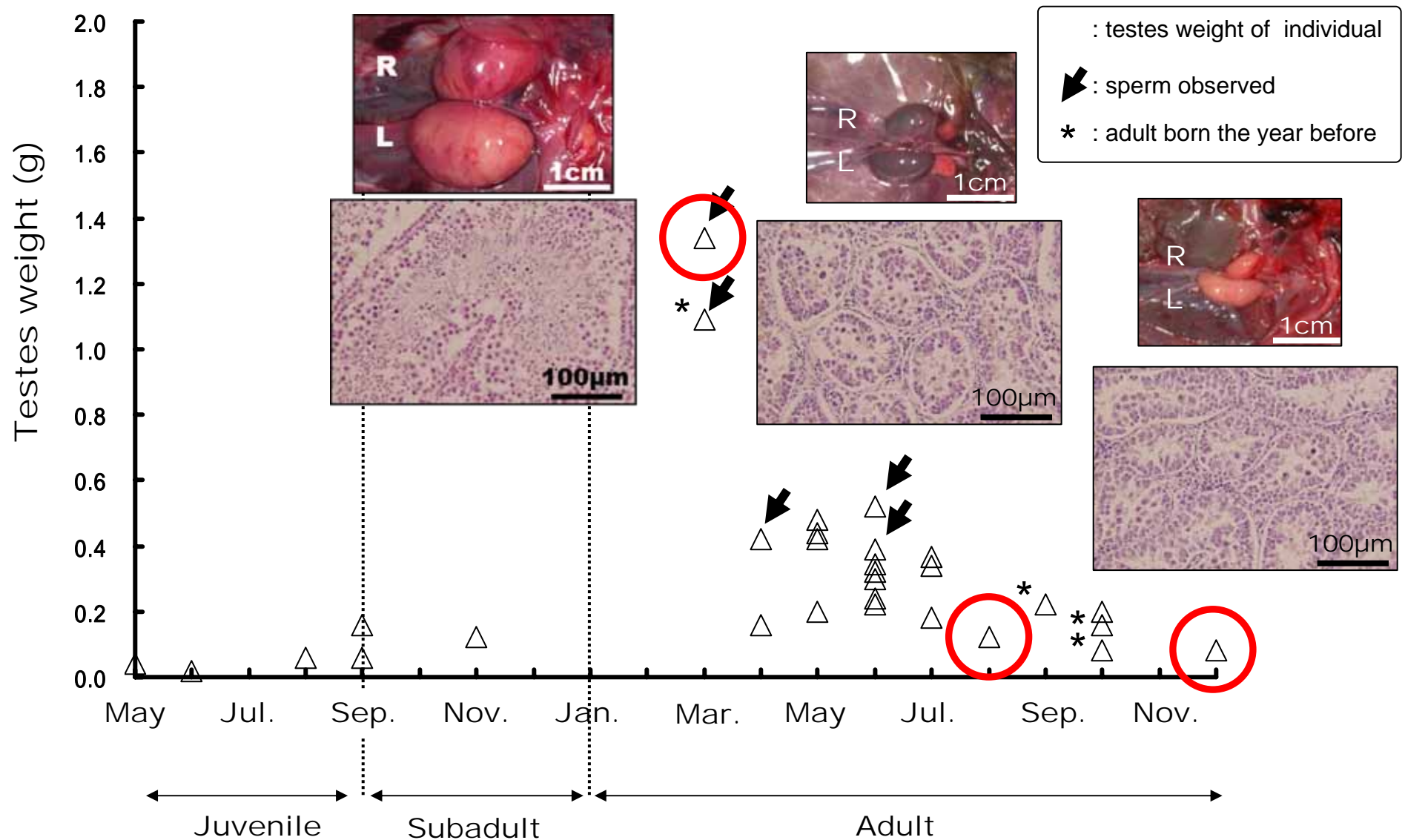
Full adult

Methods (Testis observation)

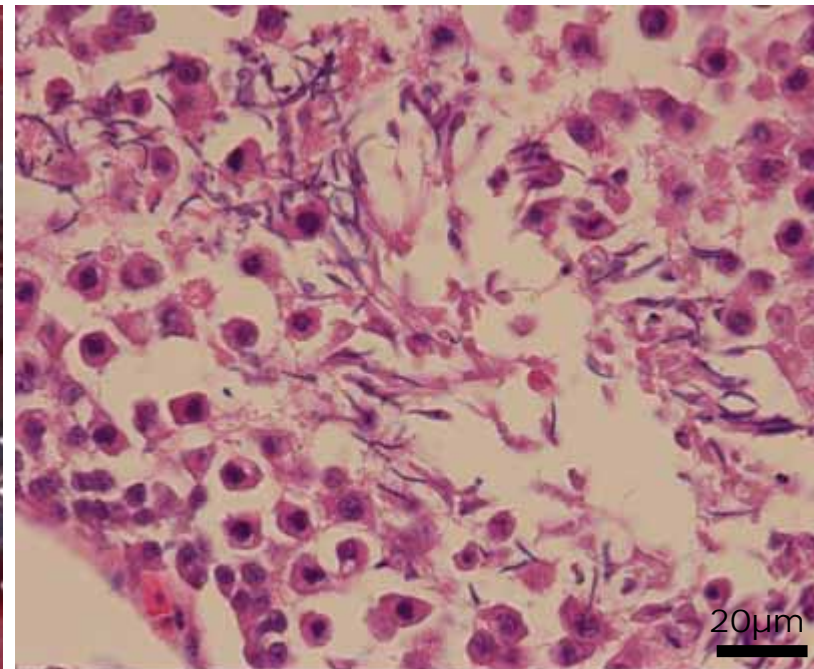
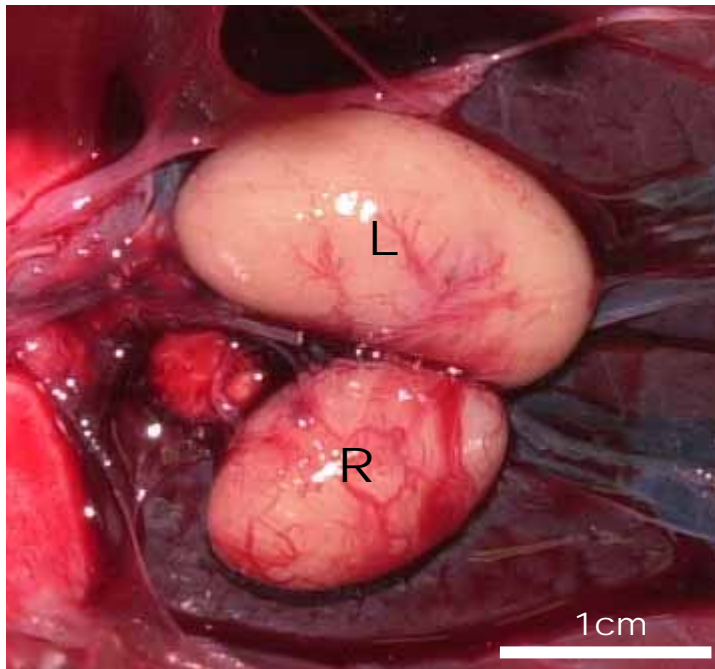
Testis isolation from 30 males
(2005-2010)



Temporal changes in testis weight of Okinawa rail



Gross and histological observation of testis in the first breeding season



Semen collection of Okinawa rail

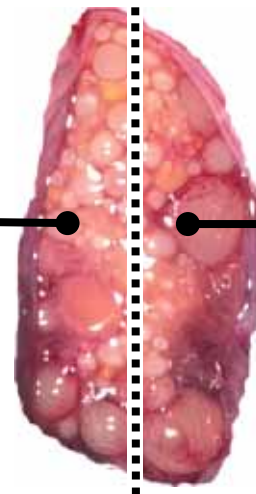


Methods (Ovary observation)

Ovary isolation from 20 females
(2005-2010)



Measuring weight

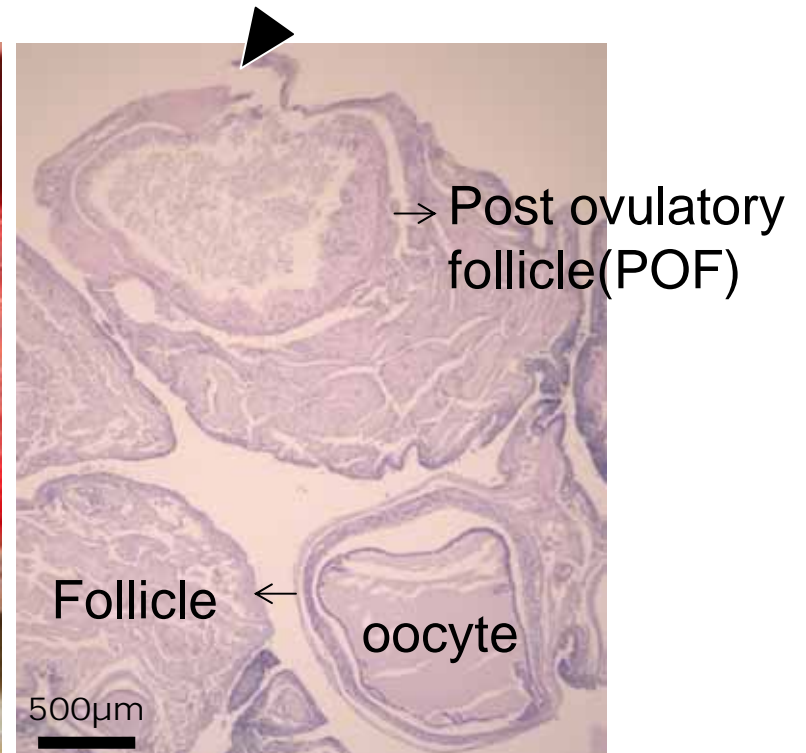
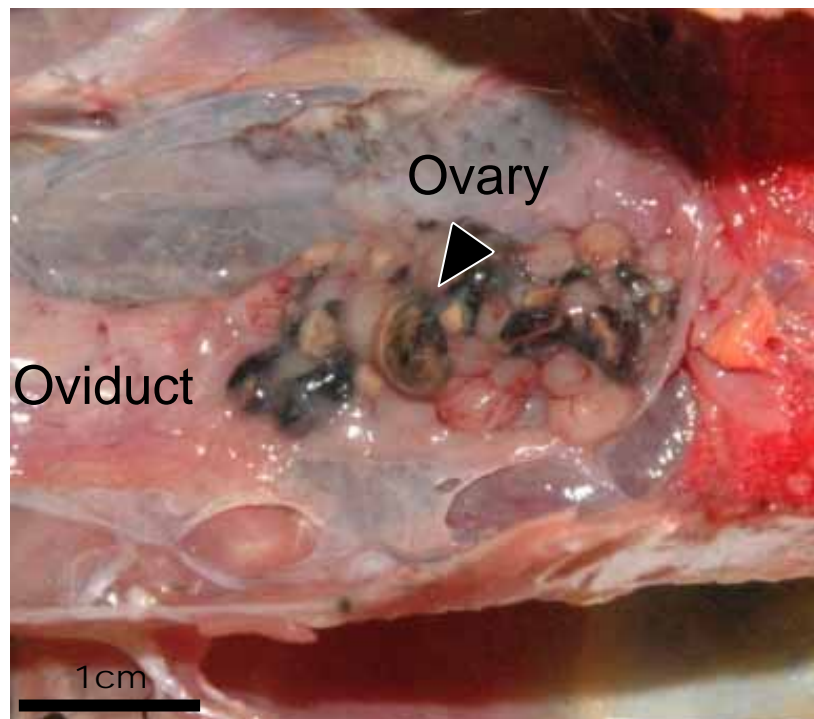


Histological observation

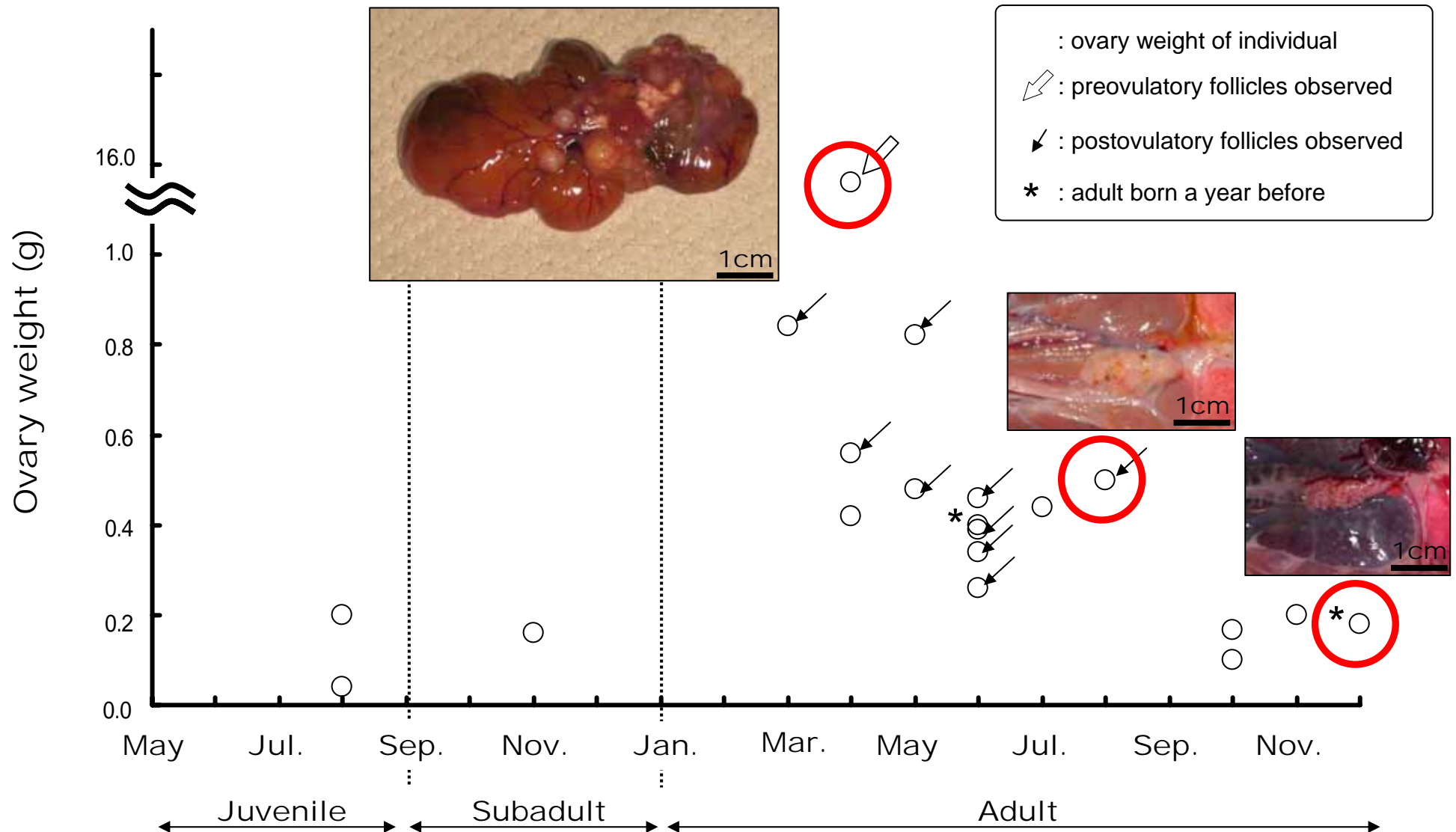
Cryopreservation



Gross and histological observation of ovary



Temporal changes in ovary weight of Okinawa rail

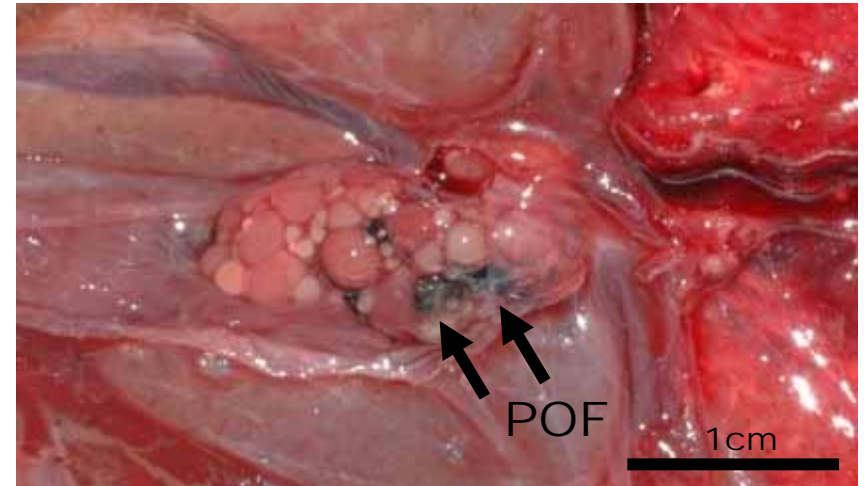


Gross observation of ovary in June

Adult born a year before



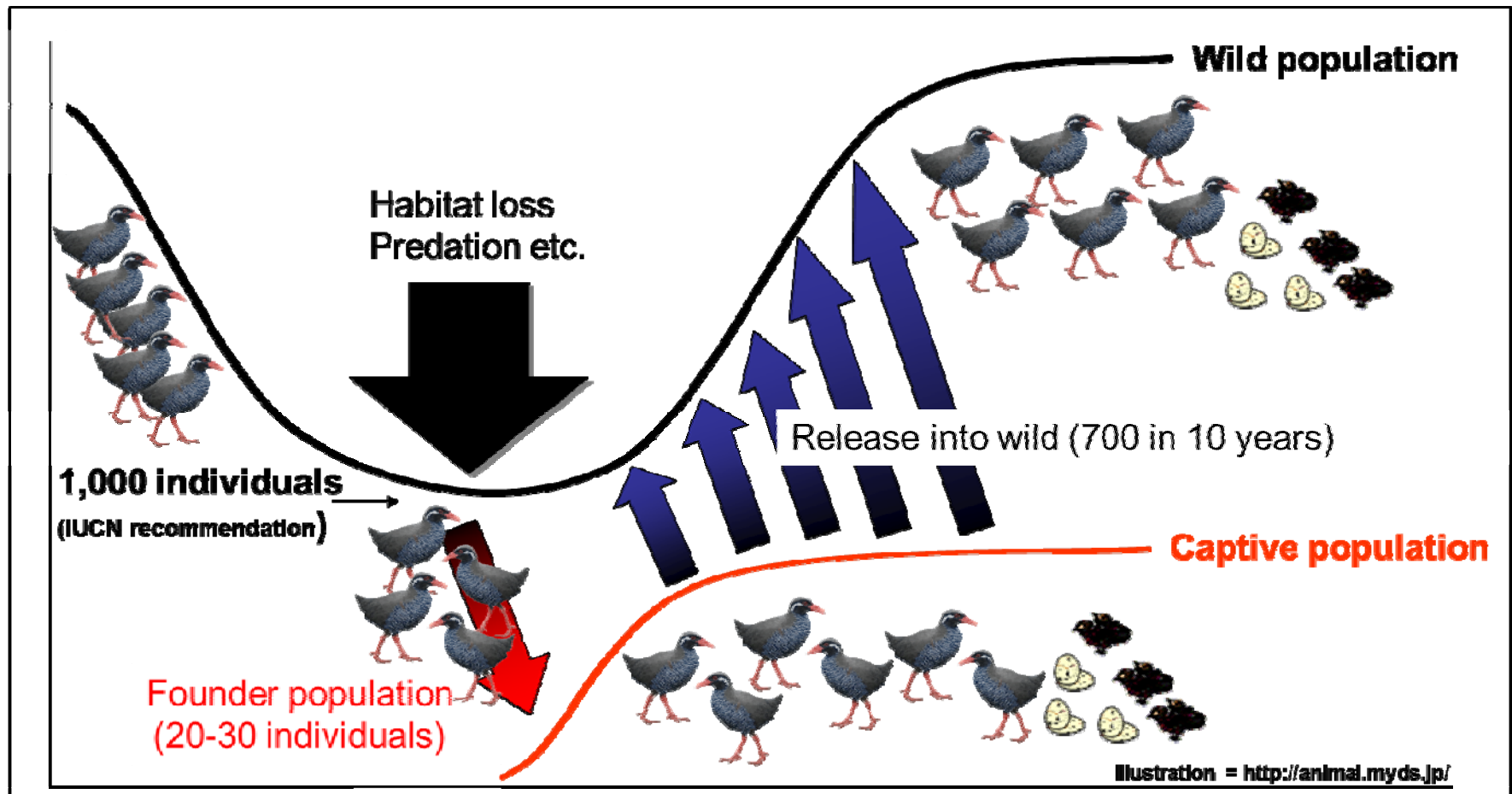
Adult



Essential information for successful captive breeding program

1, Efficient and stable reproduction : Breeding season, breeding age

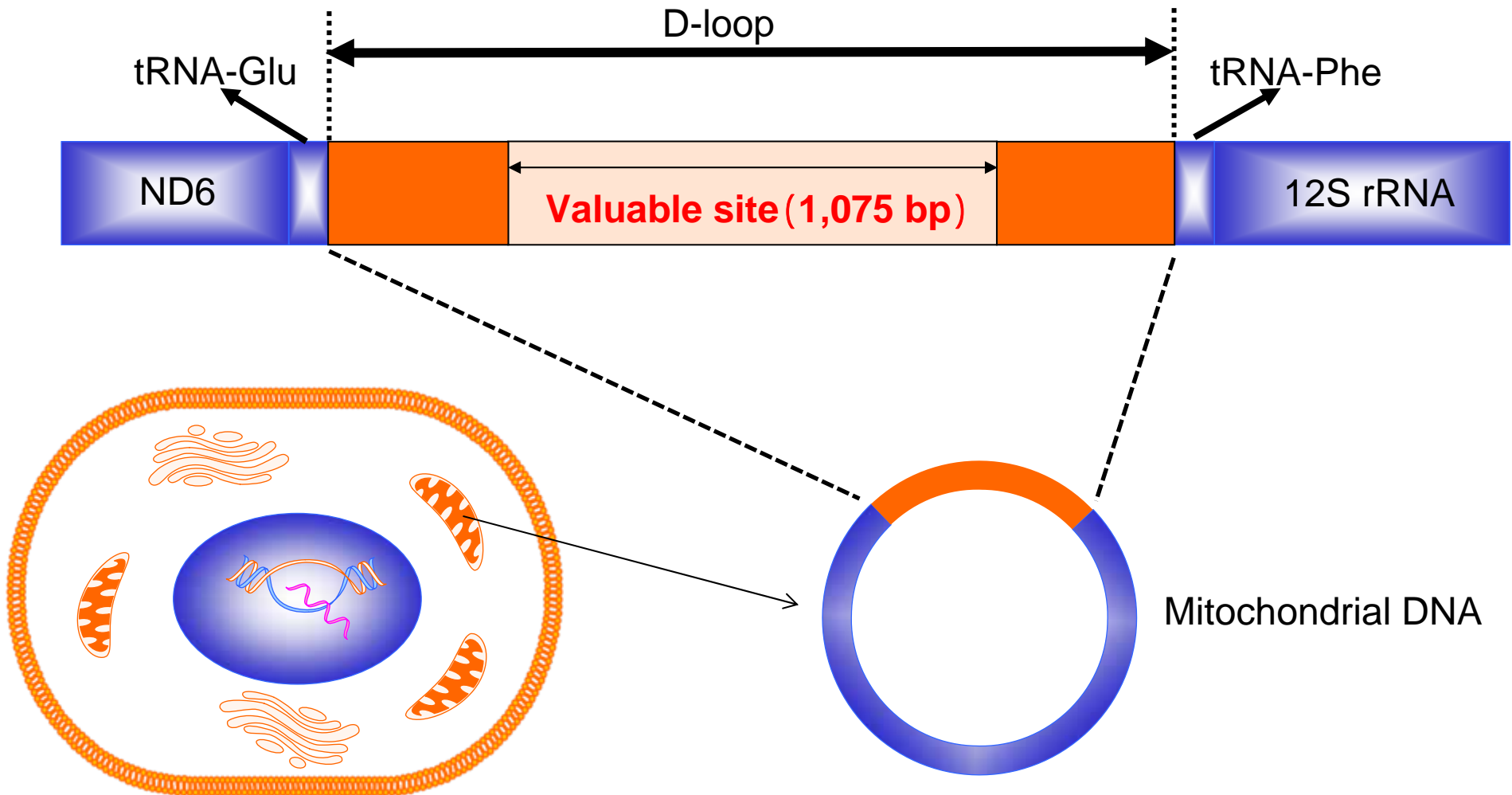
2, Maintaining genetic diversity : Evaluate genetic diversity



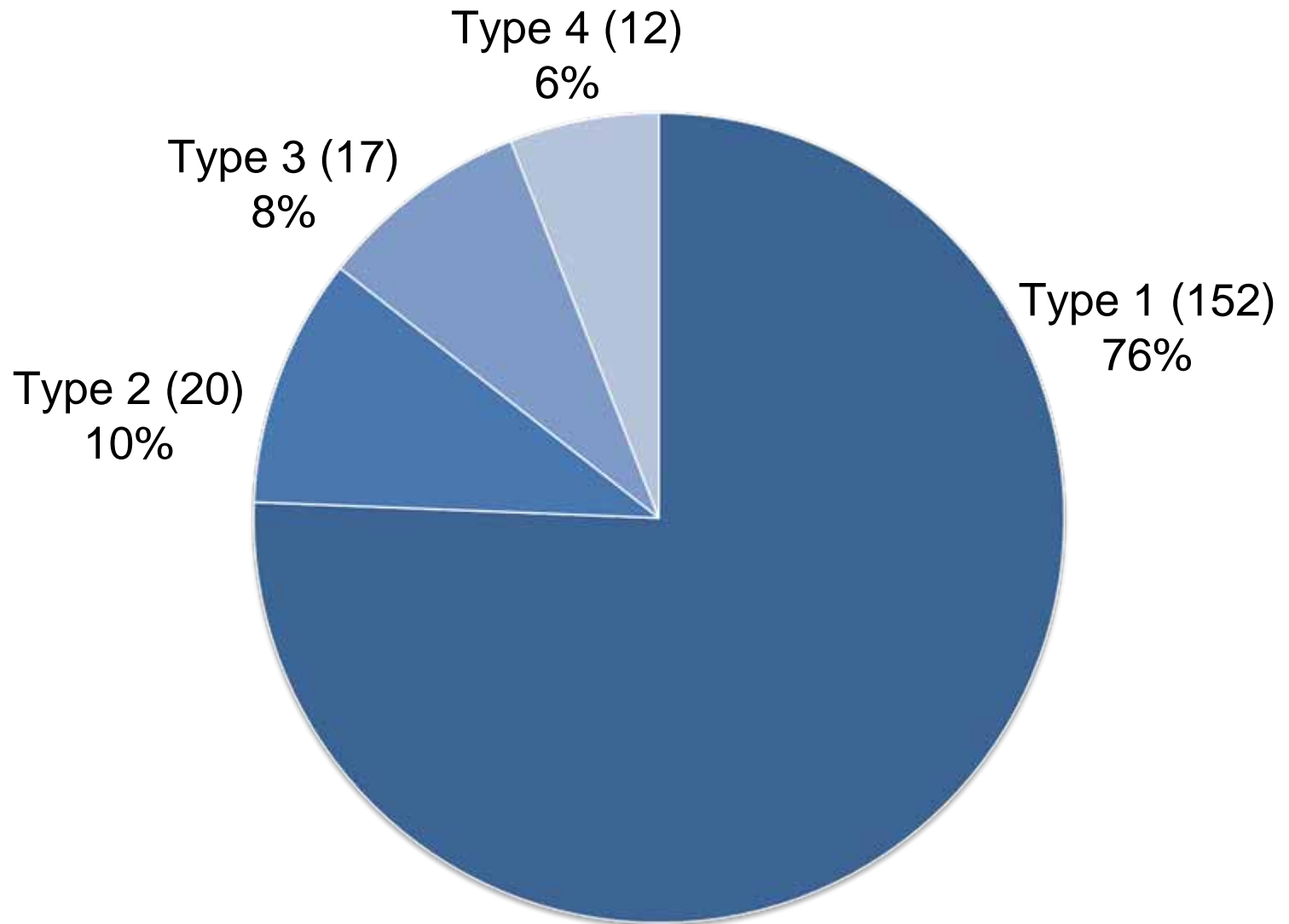
Method (Genetic diversity evaluation)

Animal: 201 individuals (male:107, female:94)

Genetic marker: Mitochondrial DNA D-loop region



D-loop variations of Okinawa rail



Conclusions

- **Efficient and stable reproduction**

Breeding season: March to June (Seasonal breeder)

Male: mature in the first breeding season

Female: mature in the second breeding season

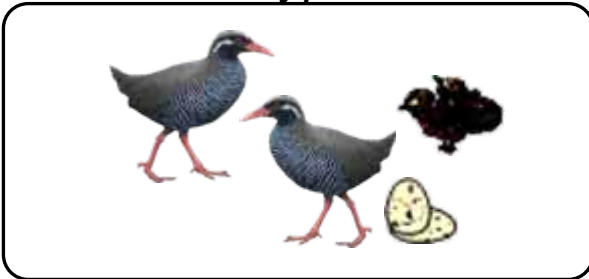
- **Maintaining genetic diversity**

Maintaining 4 haplotypes

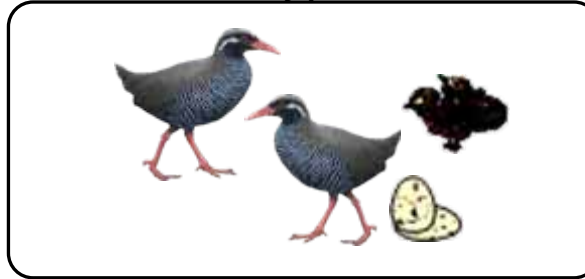


Photo by
Conservation & Animal Welfare Trust

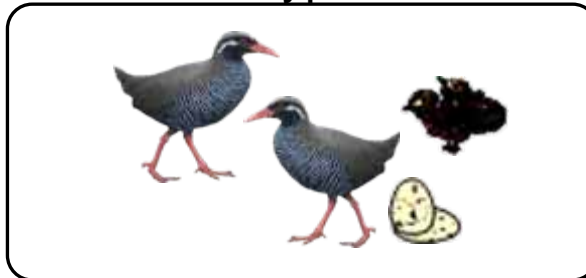
Type1



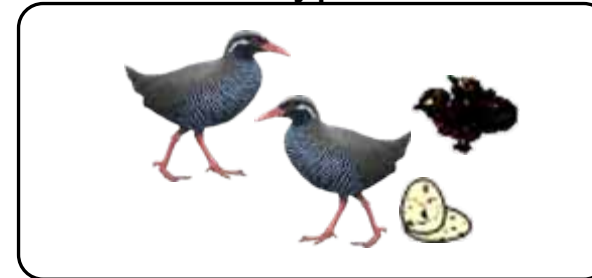
Type2



Type3



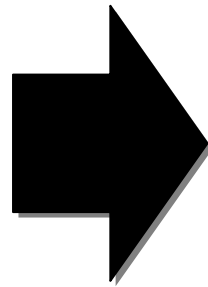
Type4



Whole genome sequencing of Okinawa rail in progress

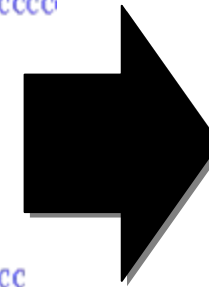


High-throughput sequencing
(32GB)



```
TCCTAAGAACATACCCCTACTCGGTACCCCCCTACCCCC  
TCCTAAGAACATACCCCTACTCGGTACCC  
TCCTAAGAACATACCCC  
TCCTAAGAACATAA  
TCCTAAGAACATACCCCTACTCGG  
TCCTAAGAACAA  
TCCTAAGA  
TCCTAAGAACATACCCCTAC  
TCCT  
TCCTAAGAACATACCCCTACTCGGTACCCCCCTACCCC
```

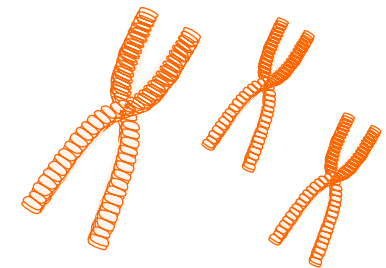
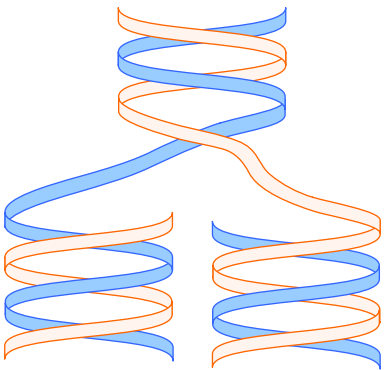
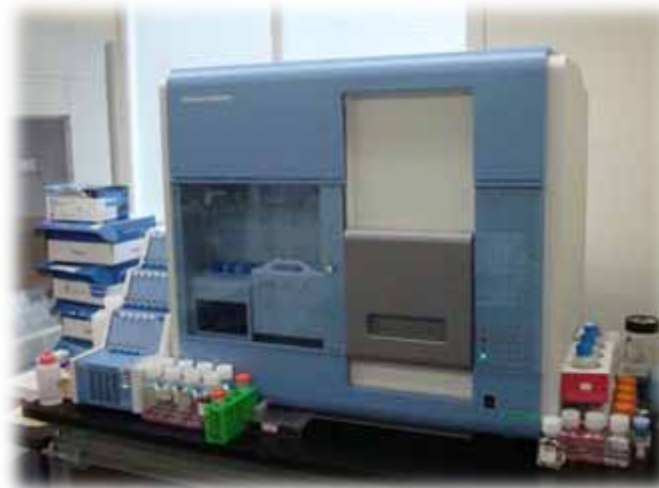
Sequence assembly



```
AAGGACAAGTATTGAGATGATGC  
TGTATTGATATAAGGACAAGTAT  
TGAATGCGTGGATAAACTGTAA  
AATGATGCGTGGATAAACTGT  
CAAGTATTGAAATGATGCGTGGGA  
TTCTAAGACATTAAATTGTAATG  
TGAATGCGTGGATAAACTGTAA  
TATGATGATAATGATAATGATAATG
```

1,000 gene sequences
will be identified by the
end of 2011.

Draft genome sequence
and chromosome mapping
will complete by 2015.



ACKNOWLEDGMENTS

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