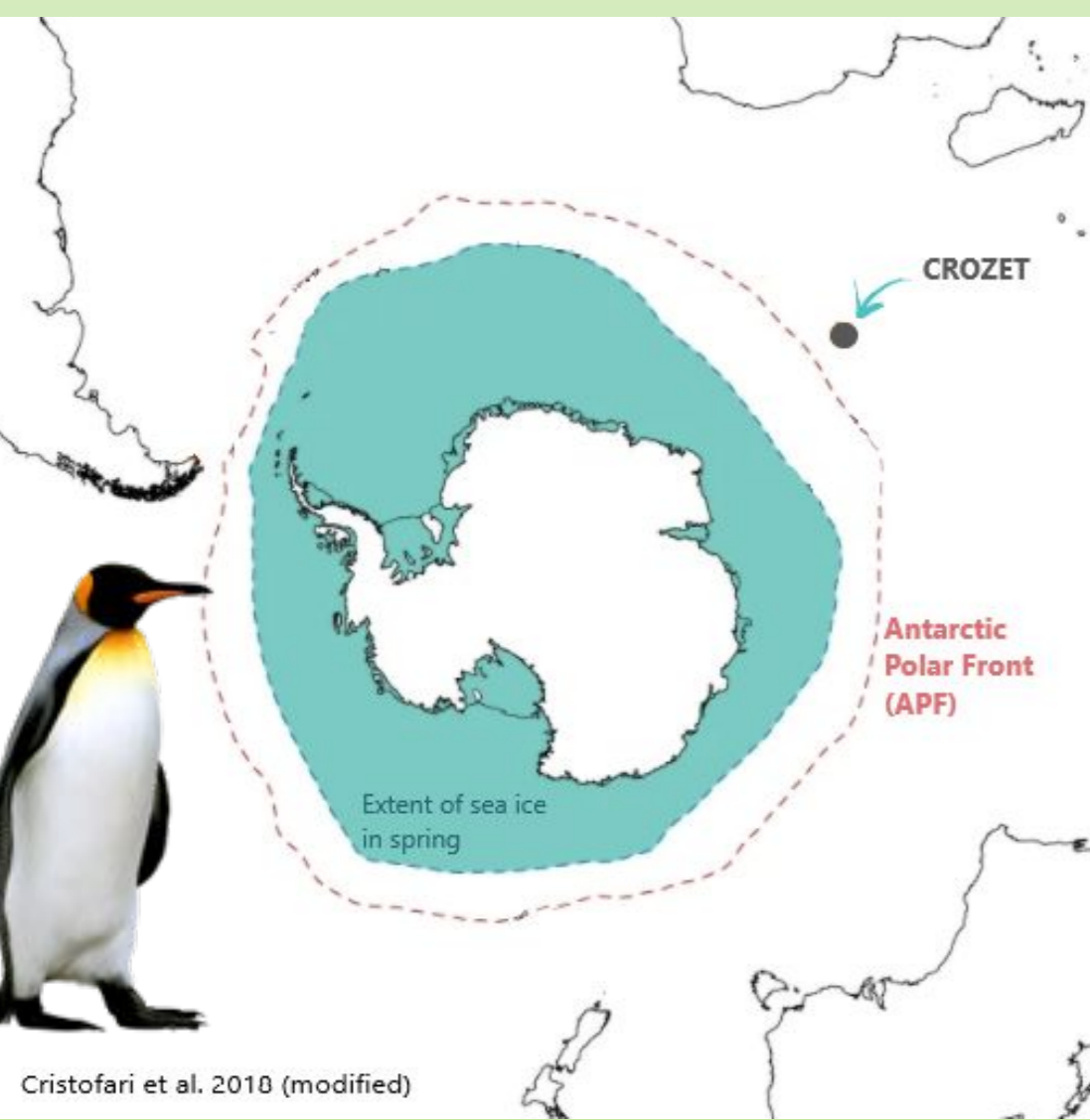


INTRODUCTION

Energetically demanding activities (e.g., reproduction, migration) should be synchronized with peaks in environmental resources for species to thrive in natural systems¹. Consequently, individuals born out of the optimum condition peak are expected to express developmental disadvantages in their early life that may result in lower individual fitness². However, the higher intensity of selection acting upon those individuals could act as a filter against strongly deleterious mutations in the population, especially under increasingly unpredictable climate changes³.



In this project, we investigate the evolutionary contribution of individuals born out of the species phenological optimum in contrast to the detrimental costs of nonoptimal conditions to their individual development and fitness using a subantarctic species, the King penguin (*Aptenodytes patagonicus*) as model.

Figure 1. Sampling location, Crozet Archipelago, Indian Ocean

- ### OBJECTIVES
- I) Compare the differences in survival and gene expression at early development of individuals born inside and out of the peak of seasonal resources in a natural population;
 - II) Explore the evolutionary advantages, at the genomic level, of regularly generating individuals under unfavorable conditions to the species persistence under unpredictable environmental conditions.

METHODS

Blood samples were collected from early and late chicks born in the breeding seasons of 2020 and 2021 in Possession Island (Crozet Archipelago, 46°25'S, 51°45'E, Figure 1), at hatching and at fledging. We performed DNA and RNA extractions on these samples for subsequent whole genome sequencing and 3'end RNA sequencing, respectively (Figure 2).

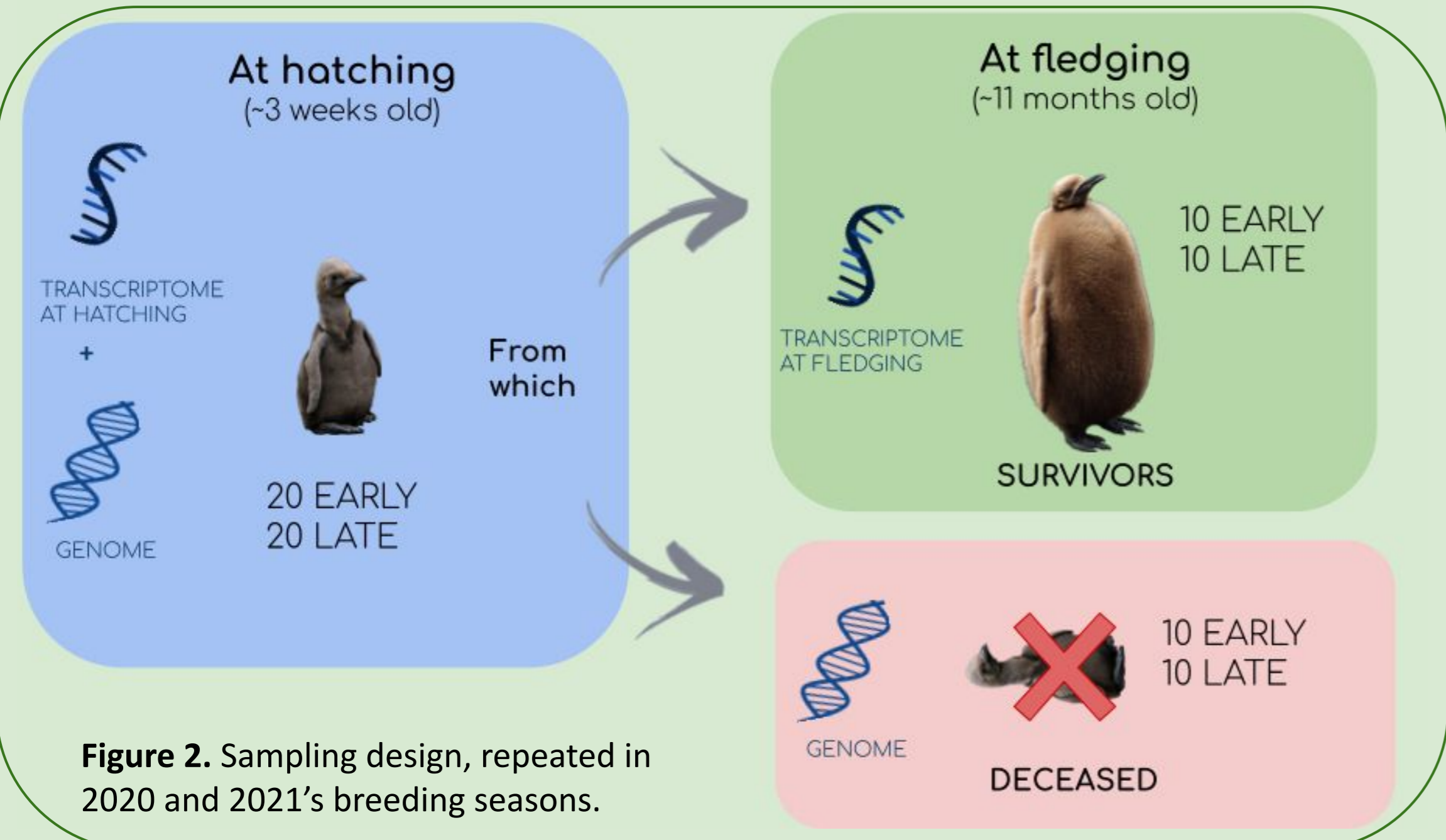


Figure 2. Sampling design, repeated in 2020 and 2021's breeding seasons.

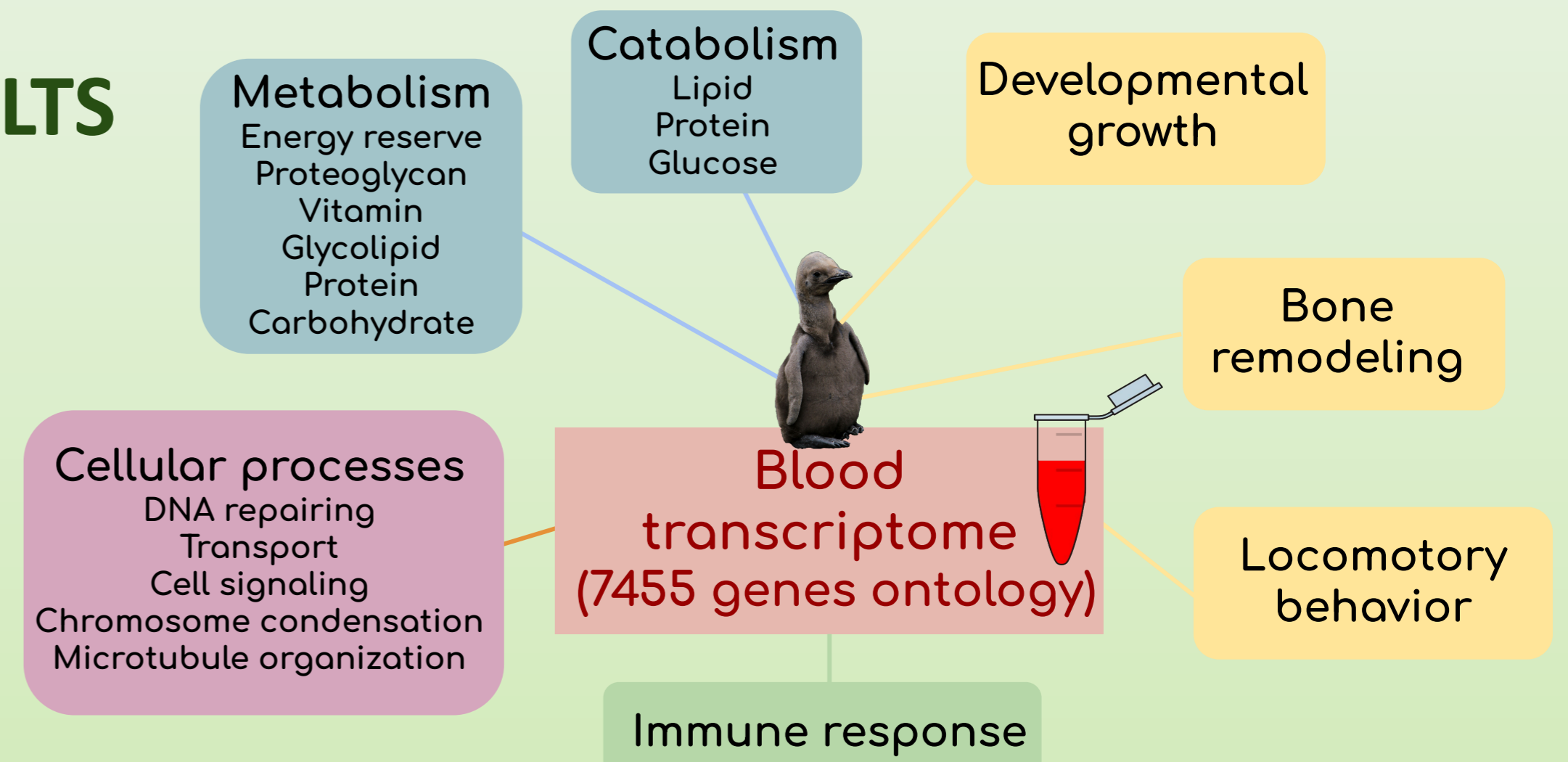
TRANSCRIPTOMIC ANALYSIS:

1. Characterization of the king penguin's blood transcriptome;
2. Differential gene expression analyses between early and late survivors at hatching and at fledging.

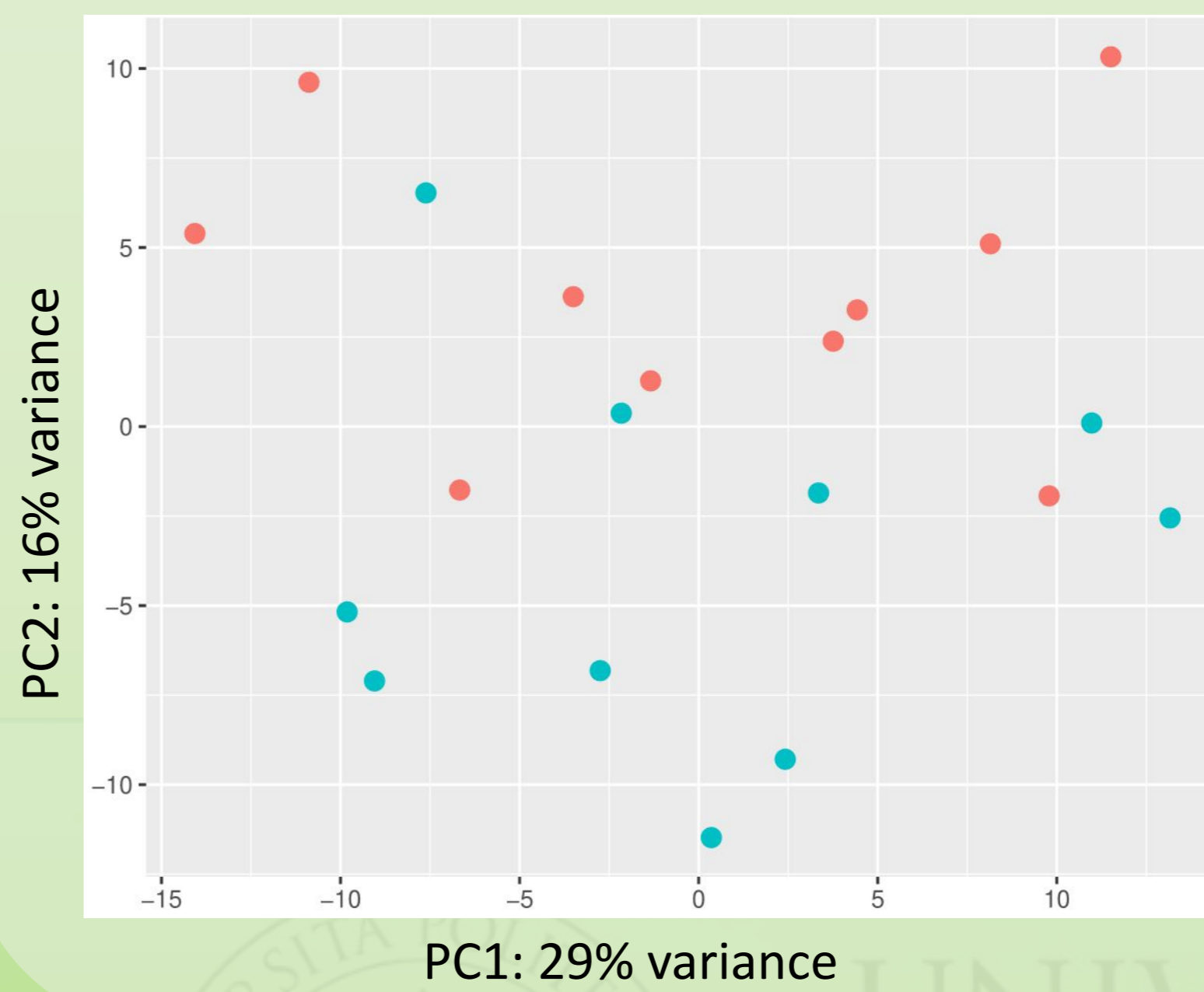
GENOMIC ANALYSIS:

1. Checking for signals of genetic differentiation between early and late, survivors and deceased in the first year (e.g., F_{ST} , clustering analysis);
2. Searching for signals of purifying selection on the late survivor group (e.g., unexpected allele frequency spectrum shifts).

RESULTS



Differential Gene Expression

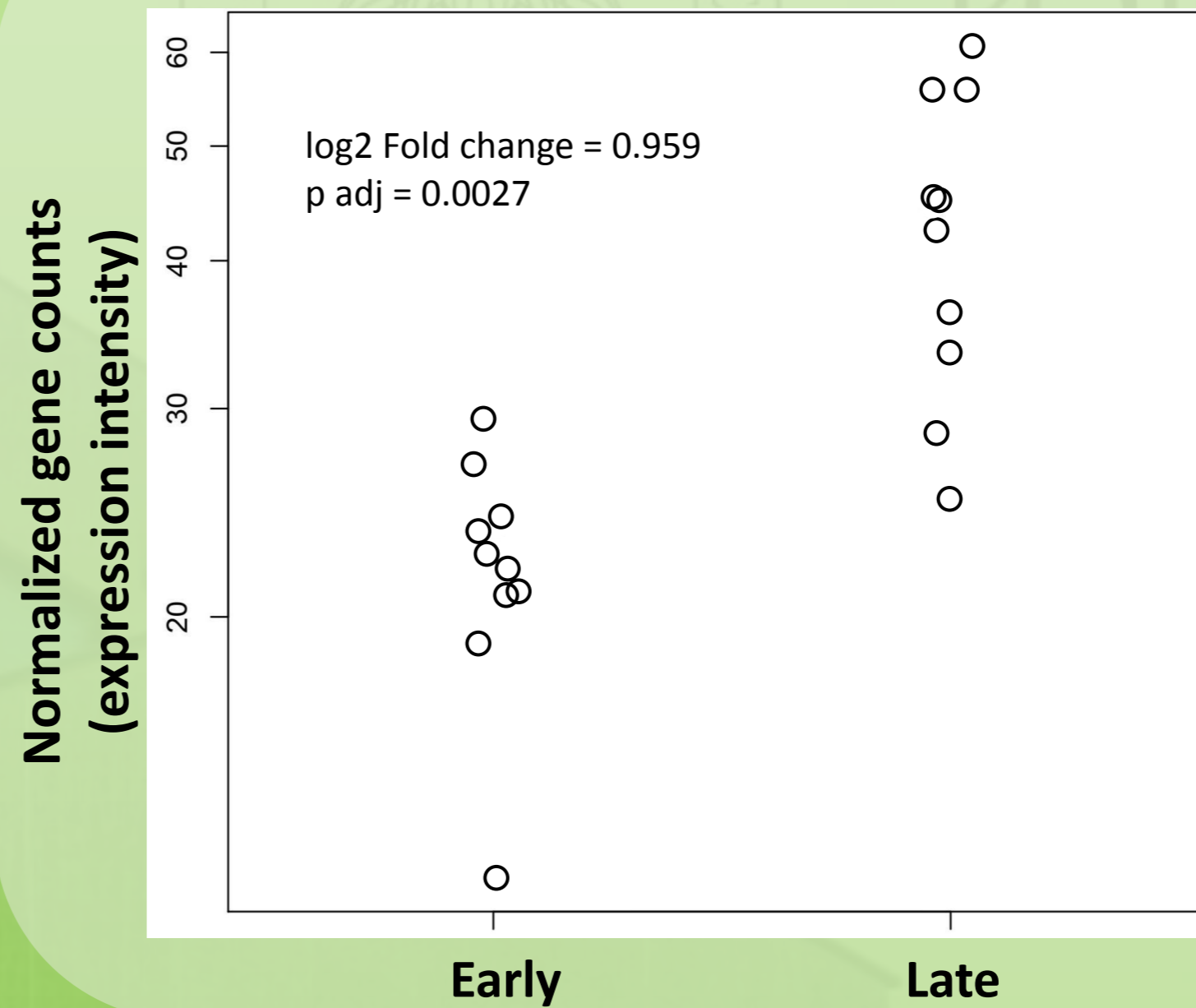


→ Slight differentiation between the expression profiles of both groups at hatching (Figure 3).

- **Early chicks:** 33 upregulated genes
- **Late chicks:** 31 upregulated genes

Figure 3. Principal Component Analysis (PCA) of gene counts of early and late king penguin chicks born in 2020 at hatching (DESeq²).

MAX Dimerization Protein 4 (MXD4) protein coding gene



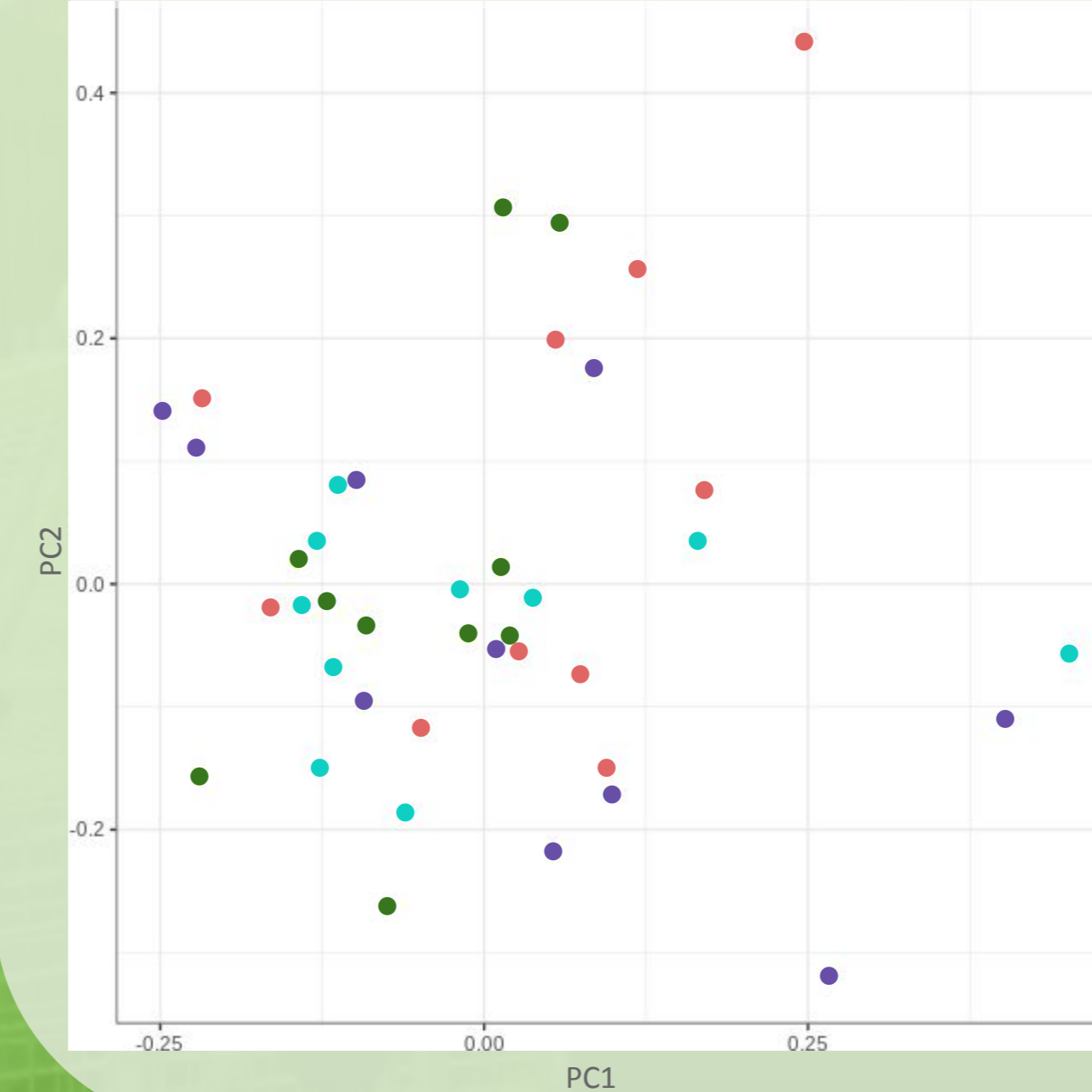
The most differentially expressed gene between early and late chicks

Low expression of MXD4
→ Proper differentiation of blood cells

Figure 4. Normalized counts of the MXD4 gene in early and late chicks.

Genomic differentiation

PCA of 658 High Effect SNPs



→ 719 autosomal scaffolds

Predicted effect of SNPs (SnpEff⁵)

	HIGH	MODERATE	LOW
Number SNPs	658	53,611	95,097

→ Likely deleterious

No global genetic differentiation was detected between the early and late, survivors and deceased groups (Figure 5).

Figure 5. PCA of the high effect SNPs in PCadapt⁶. Legend: Early Survivor, Early Dead, Late Survivor, Late Dead

Partial conclusions: Plasticity (gene expression) likely overcomes inherited adaptations to unfavorable birth conditions in this species.

Perspectives:

- Transcriptomes of year 2021 chicks → confirmation of characteristic genes at each phenological state.
- Compare the allele frequency spectrums of the high effect SNPs and neutral SNPs → detection of purifying selection.