Ng Lab Research Approaches and Expertise

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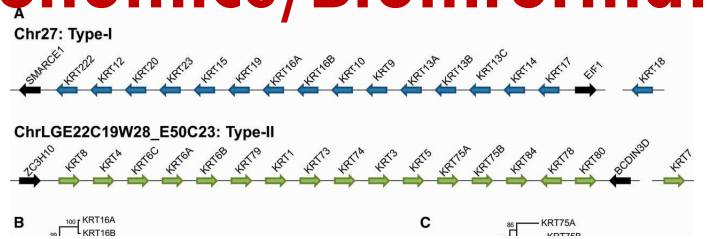
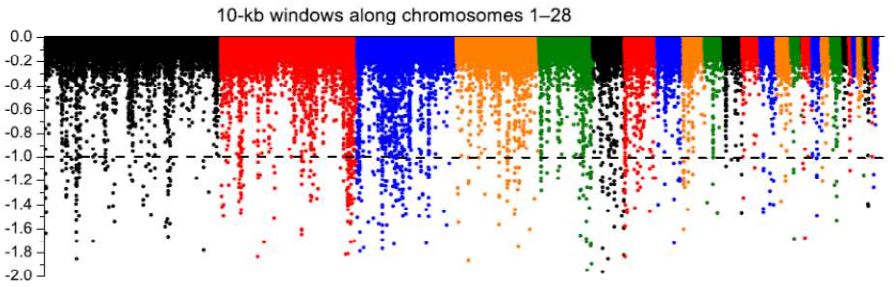
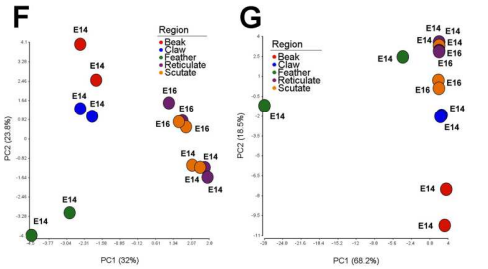
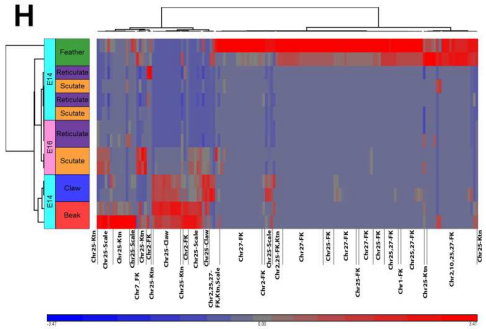
Genomics/Bioinformatics



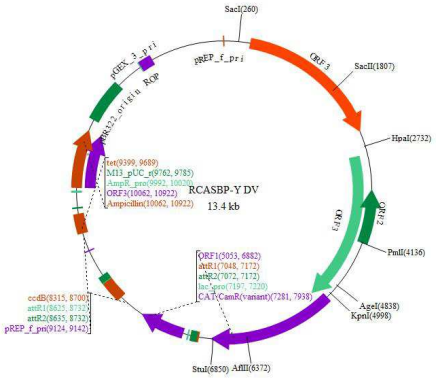


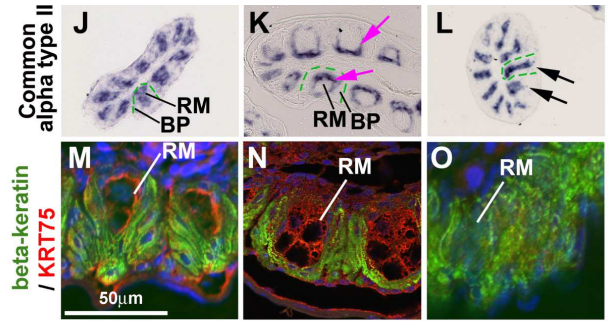
Transcriptomics





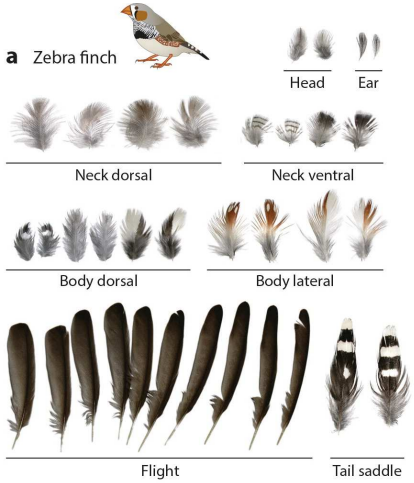
Molecular Genetics

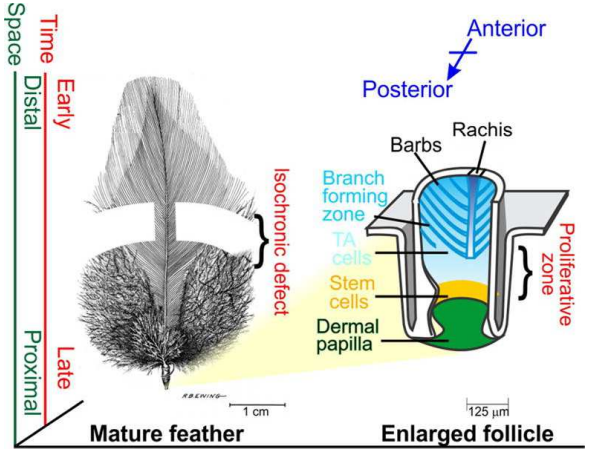




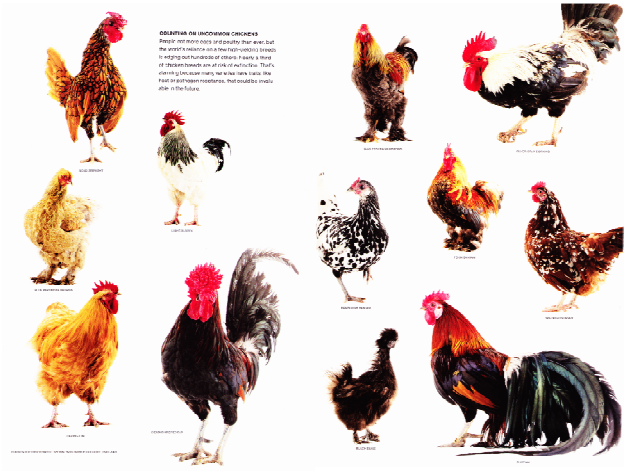
Genomic and Molecular Studies on Evolutionary Innovation and Domestication of Birds

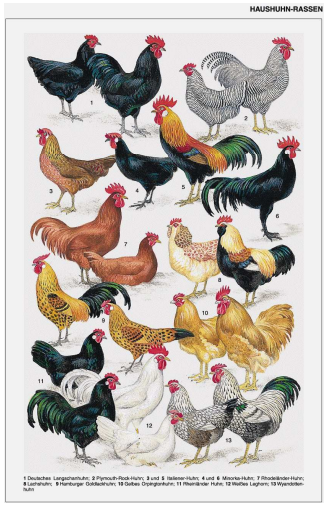
•Molecular and cellular basis of feather differences



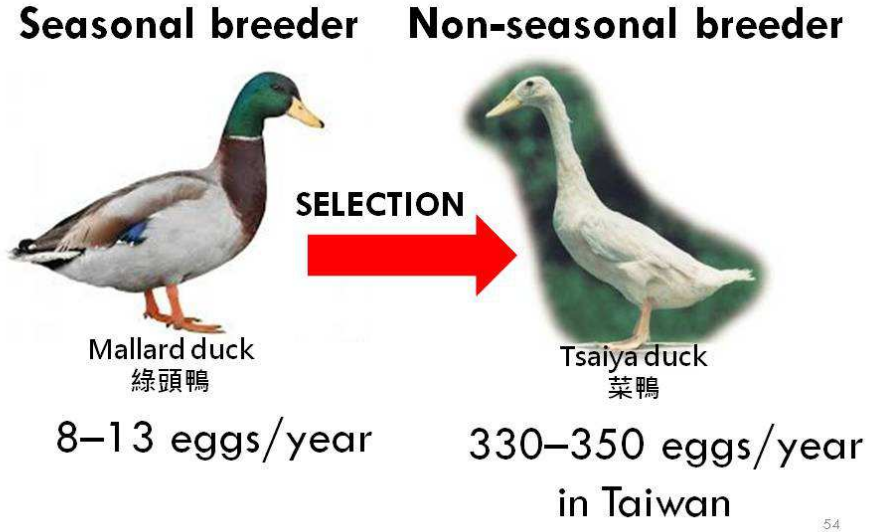


•Genetic basis of interesting morphological traits of birds

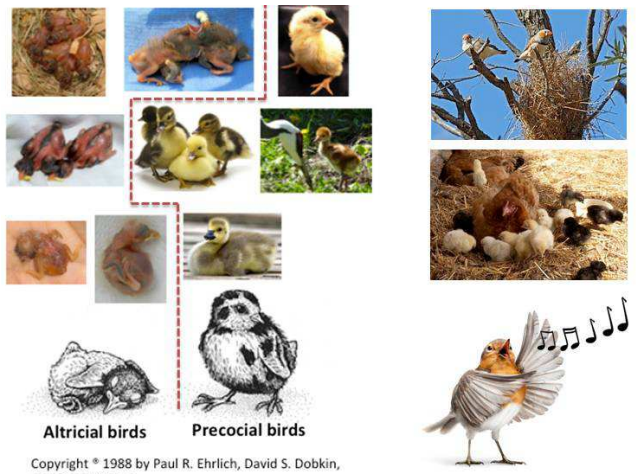




•Genomic changes by strong human selection under domestication



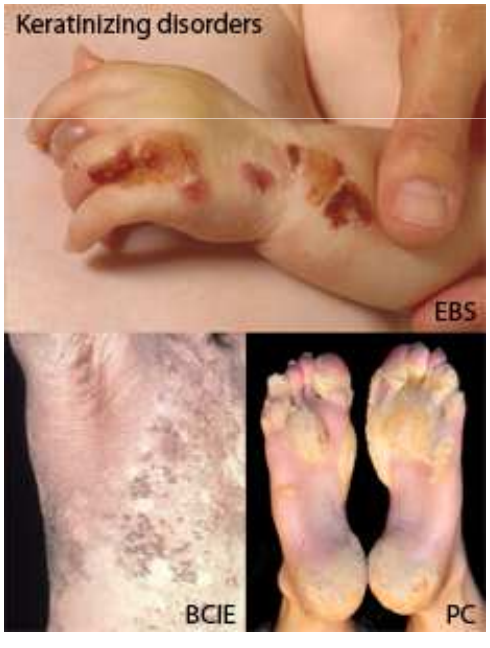
•Genomic and evolutionary analyses of avian features



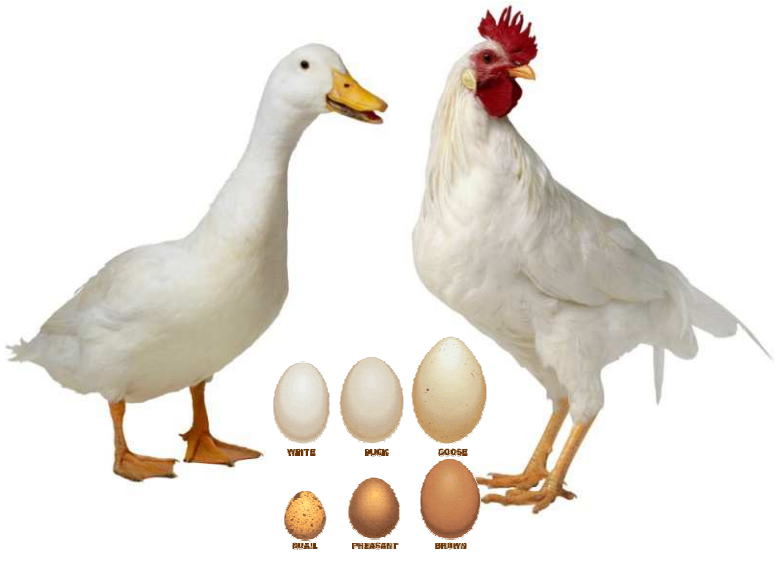
Broader Applications and Impacts

- Conservation of Genetic Resources

- Biomedical Applications



- Agricultural Applications



Future Research Plans:

Genomic and Molecular Studies on Evolutionary Innovation and Domestication of Birds

Explaining how and why the endless forms of life surrounding us evolved has been one of the central issues in biology. Birds have evolved many unique and interesting features, allowing them to adapt and radiate into various ecological niches. The feathers of birds also display a great degree of diversity among species and body parts. Over hundred breeds of domestic birds and related species provide scientists with an enormous source of morphological and behavioral diversity to explore.

The rapidly increasing number of avian genome sequences and the availability of transcriptomic tools provide an excellent opportunity to study evolutionary processes and gene expression patterns that potentially account for morphological characteristics, and to unravel the genetic basis of phenotypic variation. My ultimate goal is to understand the genetic and genomic changes creating the origin and evolution of these novelties. One of the favorite models of my research is the feather: the feather is a complex epidermal integument with highly ordered and hierarchically branched structures that are composed of flexible corneous materials made of α and β keratins. Feathers are also an example of evolutionary innovation: they evolved relatively recently in the avian lineage but have undergone rapid and dramatic diversification.

During my postdoctoral research, I and my collaborators have successfully located a candidate gene and functionally validated the causative mutation using molecular and developmental biology techniques. I also collected the whole-genome wide variation in two domestic chicken breeds using Illumina sequencing. Moreover, I am working on the gene expression profiles of developing feathers using RNA-seq. In addition, I successfully used RCAS retrovirus to express mutated genes and antisense RNA to reveal the functions of some α- and β-keratin genes. Combining the model, approaches, techniques, and resources I developed, I will be able to study the genetic, developmental, and molecular bases of morphological variations in birds (Fig. 1). My lab will investigate the roles of new candidate genes which were identified by my transcriptomic analyses and were not previously known to involve in feather development as well as study the domestication of other birds. In the next several years, I shall be pursuing the following topics:

Topic 1) Studying the molecular and cellular basis of feather differences

My study of the transcriptomes of regenerating adult feathers in chicken has significantly increased our understanding of the expression profiles of feather related genes. I examined the expression profiles of genes associated with the development of feather structure and compared the gene expression patterns in different types of feathers and different portions of a feather to advance our understanding of the molecular mechanisms of feather growth and the molecular basis of variation in feather structure. The results are a valuable resource for understanding the molecular mechanisms of avian feather development. My study produced abundant data for the analysis of gene expression during feather morphogenesis. Morphotype-specifically expressed genes were identified from five zones of feather filament epithelia (Fig. 2). Some identified genes may be associated with the growth control during feather regeneration, the formation of special branching structures, or barb differentiation themselves. It provides a basis for future study of the complex molecular and cellular events during feather development. Many candidate genes were identified for growth control, morphogenesis, or the differentiation of specific structures of different feather types. I will mainly focus on cytoskeletons, cell junctions, and extracellular matrix that are differentially expressed among different types of feathers.

I will start a new study to increase our understanding of the complex molecular and cellular events in feather development processes by investigating the roles of the candidate genes of cytoskeletons, cell junctions, and extracellular matrix I identified from the transcriptomes. To reveal cell type-specific functions as well as the mechanisms of feather genes by which mutations lead to diseases, it will be necessary to use conditional gene-targeting strategies and the introduction of mutated gene copies. The RCAS (Replication-Competent ASLV long terminal repeat with a Splice acceptor) retroviral vector has been shown to be an efficient technique for functional study in the avian system (Fig. 3). I have previously shown that the chicken feather is an excellent model to validate the cytological phenotypes of mutated human α-keratin genes. I also made antisense constructs to knockdown the gene expression of β-keratin genes in embryonic feathers. **As far as we know, we are among the first to express antisense RNA reliably and stably using the RCAS vector.**

To study the function of candidate genes comprehensively, I propose to transfect RCAS vectors each carrying a specific α-keratin gene (cDNA) into chicken cell lines and collect the high titer virus for transgenesis. I will introduce the RCAS retrovirus in embryonic and adult feather follicles of various types of feathers. I propose to generate major mutations with dominant-negative phenotypes causing severe human diseases in our cloned genes and study the function of each gene in feather development and validate the potential effect of the mutations. Our preliminary result shows that by introducing mutated α-keratin genes, we not only can investigate the specific role of an α-keratin in

feather development, but also can test if the mutations found in humans are able to generate mutant phenotypes using the feather as a model.

Furthermore, alternative splicing (AS) has been proposed as an important source of genetic diversity. Birds possess diverse and distinct features, yet they share similar repertoires of coding genes with other reptiles and mammals. Feather evolved only in the lineage of feathered dinosaurs that led to birds, and the development of feather also employs many genes similar to the development of other organs and skin appendages. Does the development of feather purely utilize existing gene networks? Or new isoforms of proteins are also involved? Transcriptomes of chicken internal organs have been compared to those of mammals previously, providing a good database. My preliminary analysis of feather transcriptomes suggests that different AS isoforms of cytoskeleton, cell junction, and extracellular matrix genes are expressed in different types of feathers. Thus, it is interesting to find out how much AS contributes to feather protein diversity.

Topic 2A) Studying the genetic basis of interesting feather traits

The Silkie has been subjected to human selection leading to remarkable phenotypic changes in morphology, physiology and behavior. Identifying the genetic changes underlying these developments will provide new insight into general mechanisms by which genetic variation shapes phenotypic diversity. Genome-wide single nucleotide polymorphism (SNP)-trait association analysis has been used to detect genomic regions showing significant association with hyperpigmentation (*Fm*), crest (*Cr*), polydactyly (*Po*), silky feathering or hookless (*h*), feathered legs (*Pti*), vulture hock (*V*), rose comb (*R*), and duplex comb (*D*).

The crest phenotype is characterized by a tuft of elongated feathers atop the head. A similar phenotype is also seen in several wild bird species. Homozygosity for Crest has been associated with cerebral hernia that causes malformation of the cranium. Moreover, ptilopody is a phenotype characterized by feathers present on the shank, feet, and toes. Identification of this developmental mutation may facilitate the examination of the cellular and genetic mechanisms underlying feather origin and evolution. In addition, silkiness is also referred to as hookless, which is known to be directed by a lack of barbicel formation on the highly branched structure of the feather. It is inherited as an autosomal recessive trait. Identification of this mutation may help untangle the cellular mechanisms in the development of feathers. Candidate genes of crest, ptilopody, and silkieness have been identified as *HOXC8*, *PITX1*, and *SOBP*, but their functions in feather formation and morphogenesis as well as molecular and cellular mechanisms of mutations are still unknown (Fig. 4). Given the genetic tools I have, I have cloned the genes and prepared for functional validations. The results from my studies will significantly increase our understanding of the complex molecular and cellular events in feather

development processes and provided a foundation for future studies on the functions of these genes in other animals.

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Topic 2B) genomic changes by strong human selection under domestication

Domestic animals are excellent models for genetic studies of phenotypic evolution. They have evolved genetic adaptations to a new environment, the farm, and have been subjected to strong human-driven selection leading to remarkable phenotypic changes in morphology, physiology and behaviour. Identifying the genetic changes underlying these developments provides new insight into general mechanisms by which genetic variation shapes phenotypic diversity. Genomic technologies for domestic animal species have revolutionized the study of animal domestication, allowing an increasingly detailed description of the genetic changes accompanying domestication and breed development.

Advances in sequencing technologies allow whole genomes to be sequenced more economically and efficiently than ever before, providing an excellent opportunity to discover numerous genetic polymorphisms in a genome and for quantitative trait locus (QTL) analysis and marker-assisted selection. Therefore, I will conduct a whole-genome analysis to examine the genetic and genomic features of the selection line for high feed efficiency and control line for Tsaiya duck (菜鴨) (Fig. 5). This study aims to identify genes that show evidence of recent artificial selection on high feed efficiency during duck domestication. I will apply population genomic approaches to the study of genetic changes in the Tsaiya duck by providing a comprehensive genome map of directional selection by detecting selective sweeps using an F_{ST}-based approach that detects directional selection in lineages leading to the selection line and using a haplotype-based test that detects ongoing selective sweeps within the line.

Topic 3) Genomic and evolutionary analyses of Anseriformes features

Seasonal breeding in the Anseriformes (雁科) presents an outstanding opportunity to study the evolution of seasonal breeding in animals. Seasonal breeders are divided into "long day" breeders and "short day" breeders based on when they become reproductively active. Ducks are long day breeders in natural environment: their gonads enlarge and begin to produce gametes when the day gets longer (Spring); but they are dormant in Fall and Winter (Fig. 6). On the other hand, for short day breeders, such as geese, their gonads behave in a reversed fashion --- active in Fall and Winter, but dormant in Spring and Summer. Seasonal reproduction occurs in natural environment, but artificial selection has established Leghorn chicken (來亨雞) and Tsaiya duck (菜鴨) as two major strains for year-round egg

production. However, for other domestic fowls, such as Taiwan Country chicken (台灣土雞), Muscovy duck (番鴨), and White Roman goose (白羅曼鵝), their seasonality remains and their reproduction is limited to their specific seasons. The Muscovy duck is a long day breeder, whereas the White Roman goose is a short day breeder.

Muscovy duck and White Roman goose meats are commonly consumed in Taiwan. Despite the agricultural and biological importance of Muscovy ducks and White Roman goose, breeding and genetics studies have been hindered by the lack of reference genome sequences. We have just completed the whole genome sequencing of Mandarin duck, Muscovy duck, and White Roman goose (Fig. 7), and I will conduct transcriptomic analyses of hypothalamus, pituitary and gonad gene expression profiles in Muscovy duck and White Roman goose and characterize the reversed seasonality by comparative hormonal and transcriptomic analyses between male Roman geese and male Muscovy ducks. Identifying the genes that control the seasonality of these birds will by my first priority. These genome sequences will also be useful for mapping reads obtained by resequencing more breeds of duck and geese, which will facilitate the identification of SNP markers for genomic marker–assisted breeding. It will also be useful for improving the utility of the domesticated birds as a biomedical model. In addition, the genes we will identify are related to seasonal breeding and may be used as markers for breeding longer laying duration to increase egg production.

Long-term plans

In my long-term plan, I will apply a comprehensive approach, ranging from transcriptomics and genetic mapping to *in vivo* functional experiments, to uncover the genomic regions, and ultimately the genes and mutations (and their effects on function), involved in phenotypic variation of feather (Fig .1). First, I will use different chicken breeds to uncover the genetic and developmental mechanisms underlying specific feather morphological changes in which genetic analyses will be applied to identify the genomic regions and specific mutations responsible for generating morphological changes in feathers. Second, I will apply developmental genetic and genomic approaches to gain a deeper understanding of the molecular pathways that control feather development. Third, I will apply genetic and molecular tools to examine and manipulate the molecular and cellular pathways that generate the diversity. Fourth, I will apply evolutionary and comparative genomic approaches to understand how these pathways have evolved, and what changes in these pathways were responsible for the origin and diversification of new structures and processes.

Broader Application and Impacts of My Research

My research studies not only can make advancements in my field, but can also provide models, techniques, and resources for boarder applications to other scientific communities. The experimental models and techniques can also be shared by developmental biologists, immunologists, and neurobiologists in the College of Life Science. I can also make significant contributions and impacts to biomedical sciences, animal sciences, and conservation biology in Taiwan (Fig. 8).

Humans have at least 54 functional keratin genes, which are divided into type I and type II α keratins. Most of the type I keratin genes, designated *KRT9* through *KRT20*, are located in a cluster on chromosome 17. The type II keratin genes, designated *KRT1* through *KRT8*, are found in a cluster on chromosome 12. Mutations in at least 20 *KRT* genes have been found to cause human diseases affecting the skin, hair, nails, and related tissues. I have used the chicken model to study the functions of keratins by introducing mutated genes and antisense RNA. Some dermatologists in Taiwan and Europe already showed their interests in collaborating with me to investigate the basic function of some keratins as well as cytoskeletons, using the chicken model and molecular techniques I developed.

Bird domestication involves marked morphological and genetic changes that occurred in a relatively short window of time in evolution. Identifying the mutations that drove the behavioral and physiological transformation of wild red jungle fowls into domesticated chickens through artificial selection constitutes a formidable challenge that can only be tackled by an interdisciplinary approach. The sequenced genomes of domesticated birds provide critical resources for understanding the genetic basis of domestication. My genomic analyses will shed new light on the mechanism of artificial selection and will allow the mapping of genes involved in important domestication traits. My findings for domestication traits based on results from genome-wide association studies and selective-sweep scans for artificially selected genomic regions will help animal scientists to improve the methods for marker-assisted selection and selective breeding.

The vast diversity of phenotypes among breeds that has been created by selective breeding of domesticated animals can compare to those observed among wild species in nature. In addition to agricultural applications, domesticated animals have also contributed to basic and medical biology, as they provide excellent opportunities for unraveling the genetic and molecular basis of phenotypic variations. The chicken is the most variable bird, because there are hundreds of chicken breeds, integrating various mutations that affect body size, reproduction, growth rate, posture, color, feather structure and distribution, comb shape, or/and behavior. Domestic animals have some advantages over traditional model organisms. Extreme phenotypes that may not be found in the wild can be maintained by artificial selection for aesthetic or economic demands. Mutations of biologically important traits

have a greater chance of appearing and of being selected due to a large population size and higher longevity in domestic animals, providing us an exceptional opportunity to identify novel functions for specific genes. The identification of genetic variants controlling morphology in the chicken population has helped us understand the genetics and genomics of both simple and complex traits of birds.

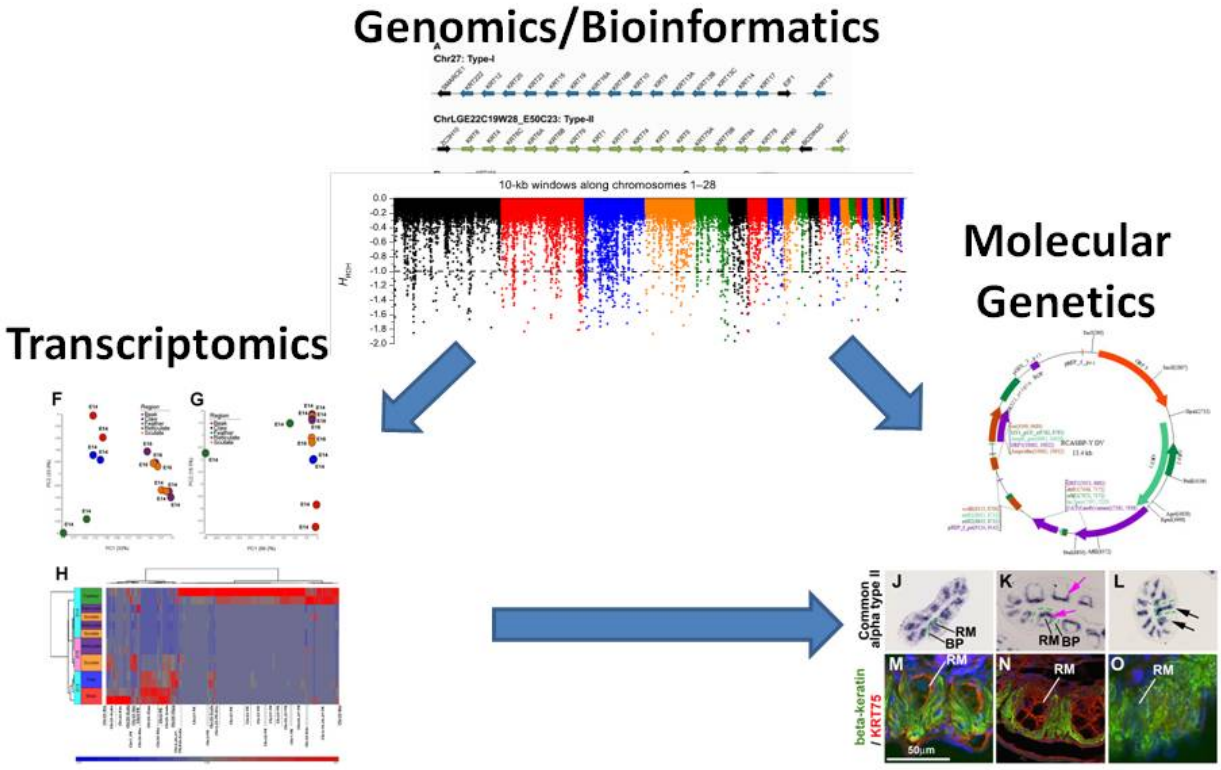

Figure 1. My research approach.

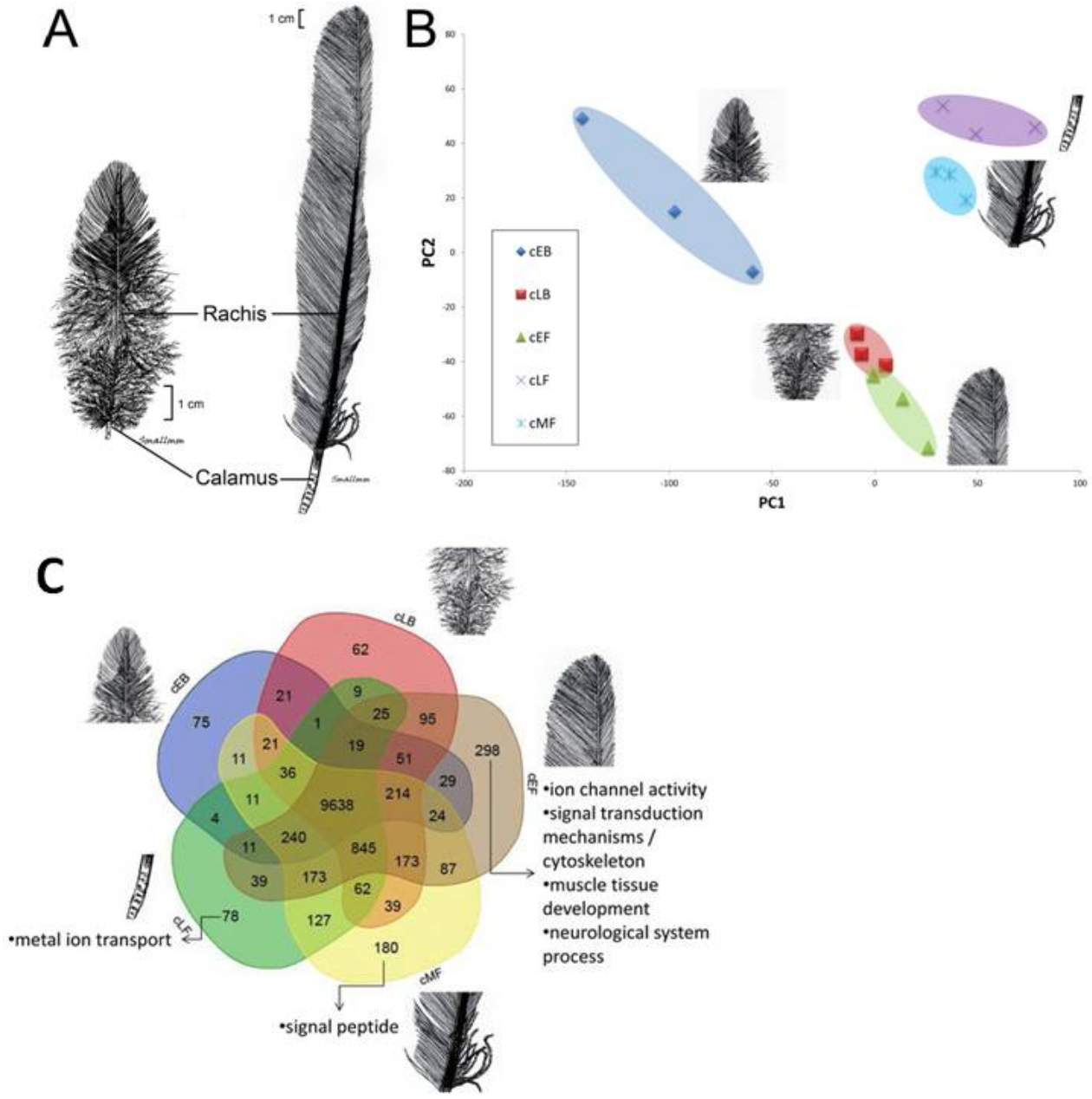

Figure 2. Transcriptomic analyses of regenerating adult feathers in chicken. A. Morphology of body (left) and flight feathers (right). **B.** Principal Component Analysis (PCA) of gene expression profile. **C.** Venn diagram showing the genes expressed in each of the five feather tissue types.

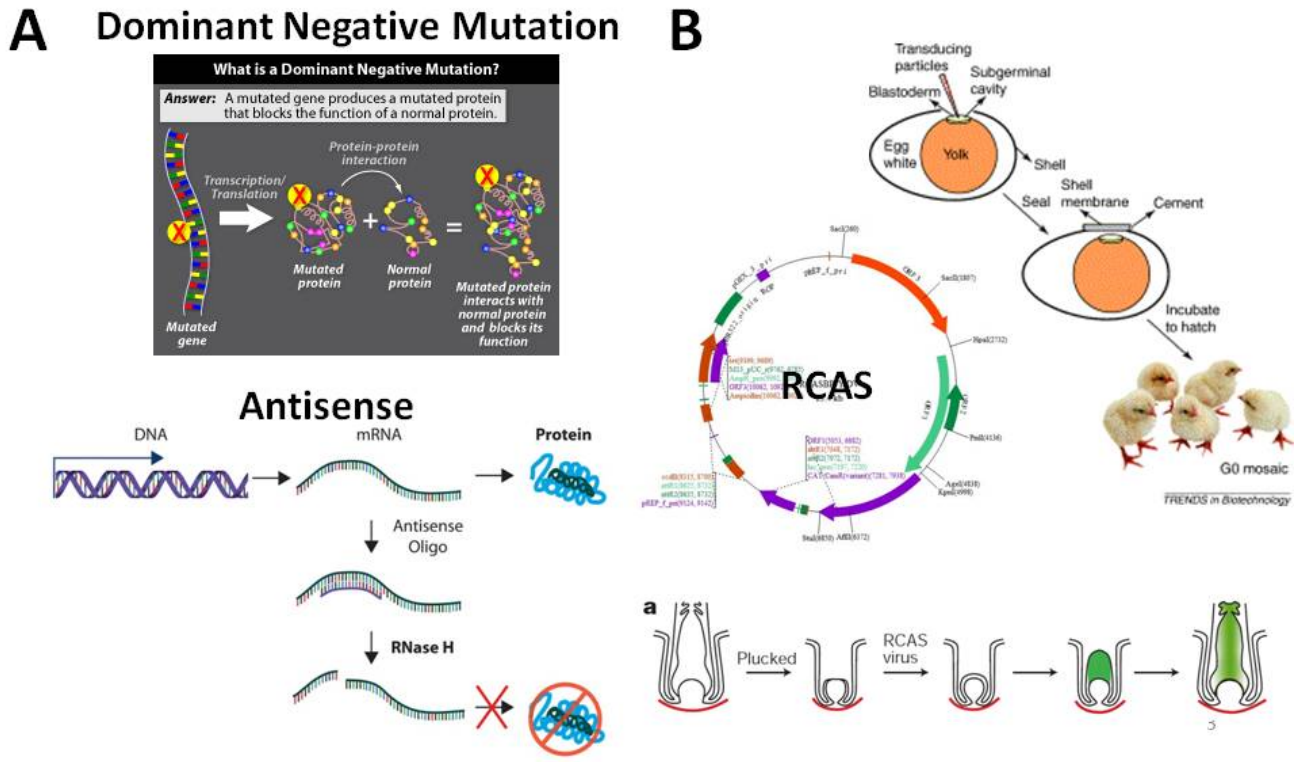

Figure 3. Molecular approaches for functional studies. A. I will expressed the gene with dominant negative mutation and/or antisense RNA to study the functions. **B.** The candidate genes are expressed using a RCAS retrovirus transgenic system in adult and/or embryonic feathers.

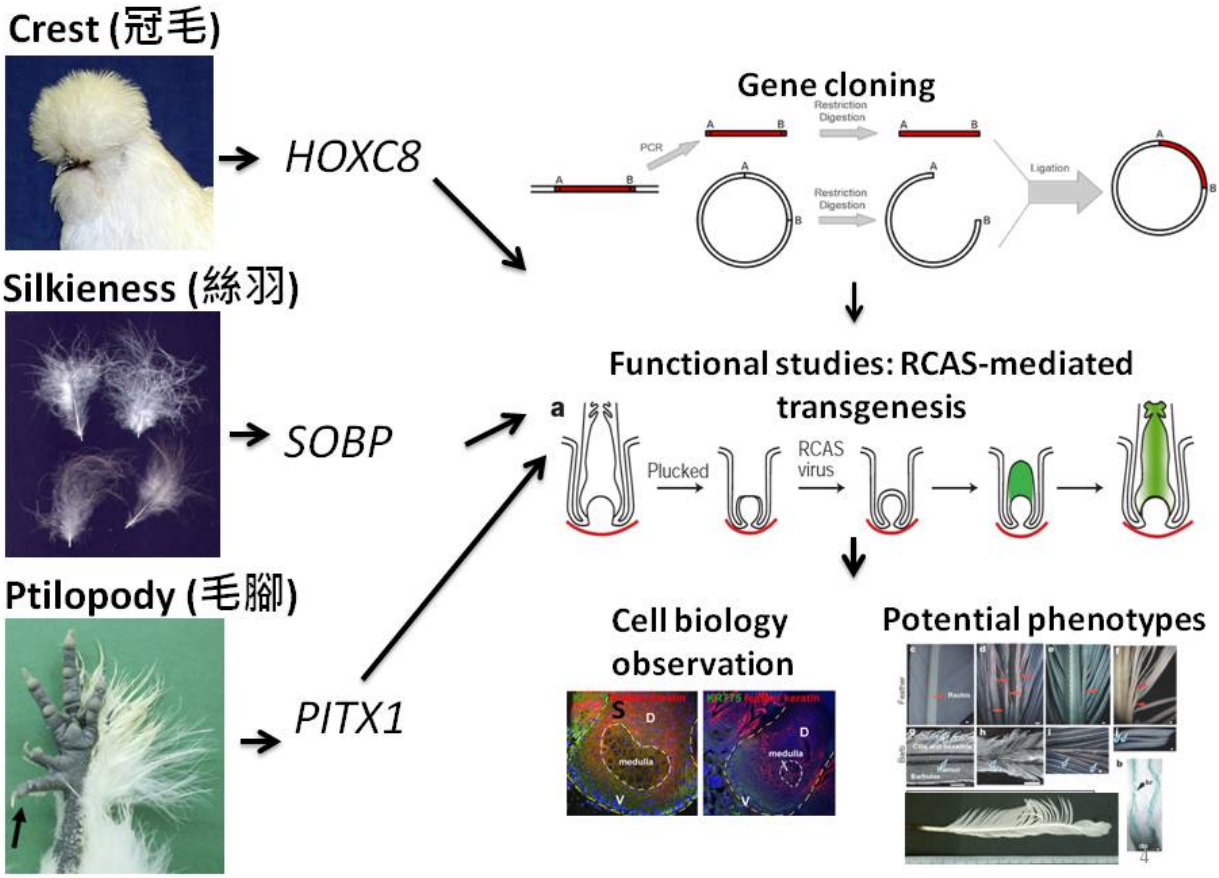

Figure 4. Genetic basis of morphological diversity in the Silkie.

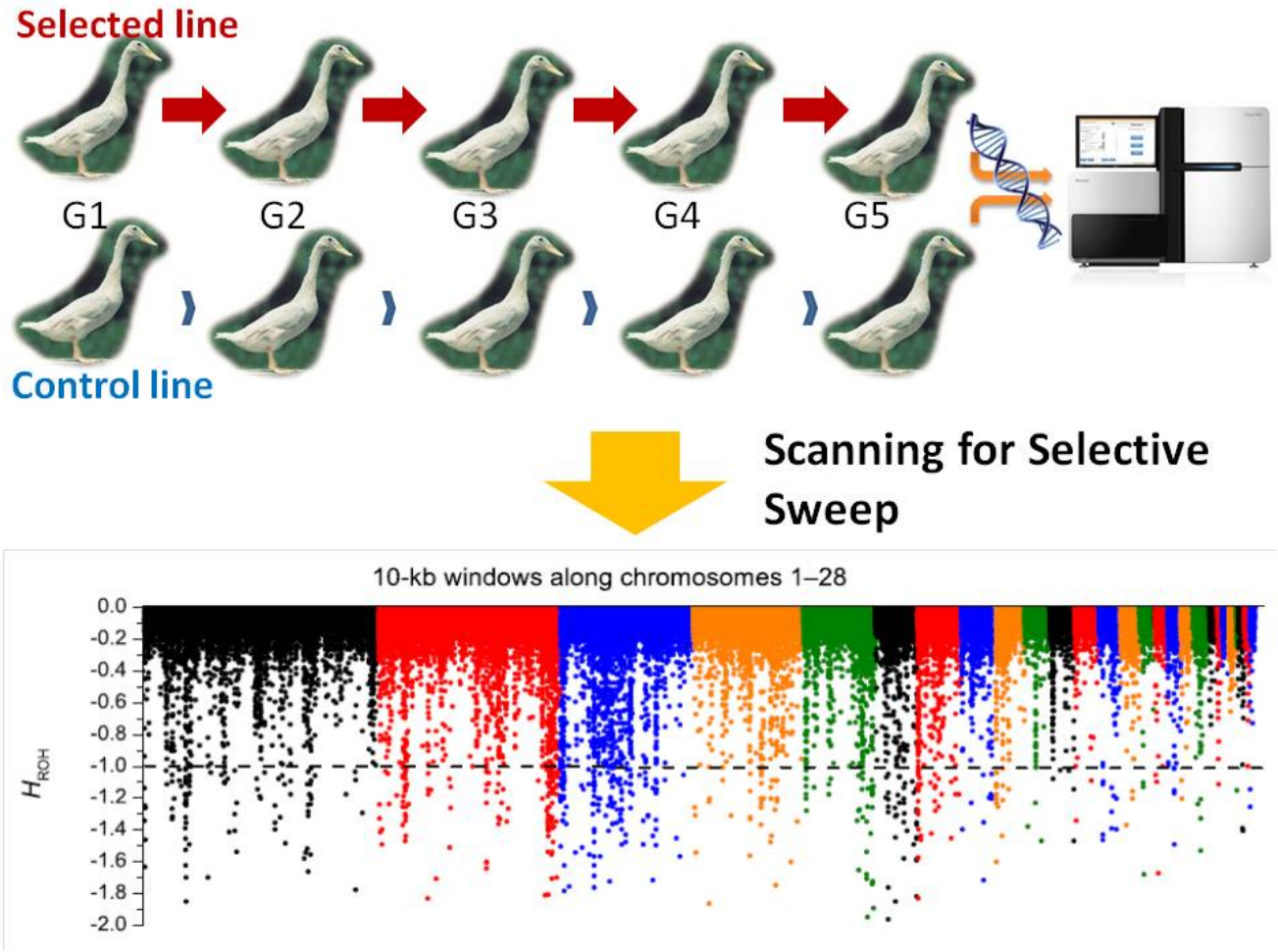

Figure 5. Genome-wide association study on high feed efficiency in brown Tsaiya ducks (菜鴨).

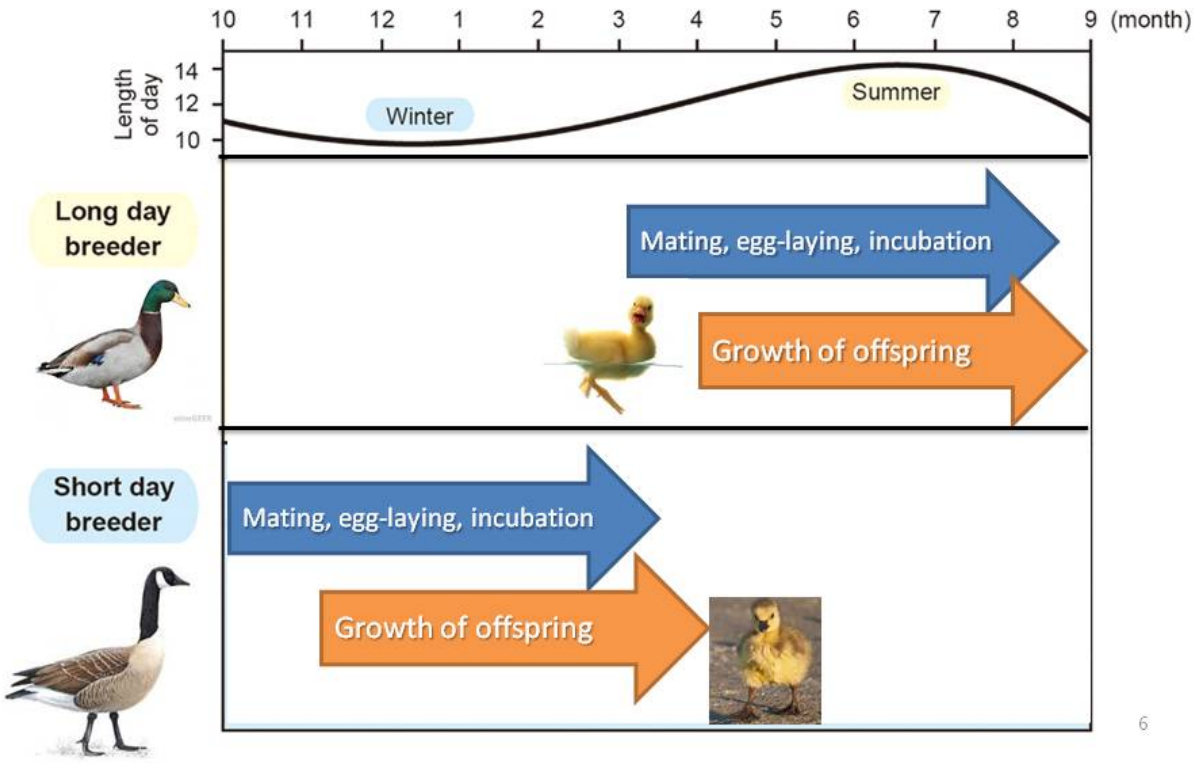

Figure 6. Seasonal breeding of ducks and geese.

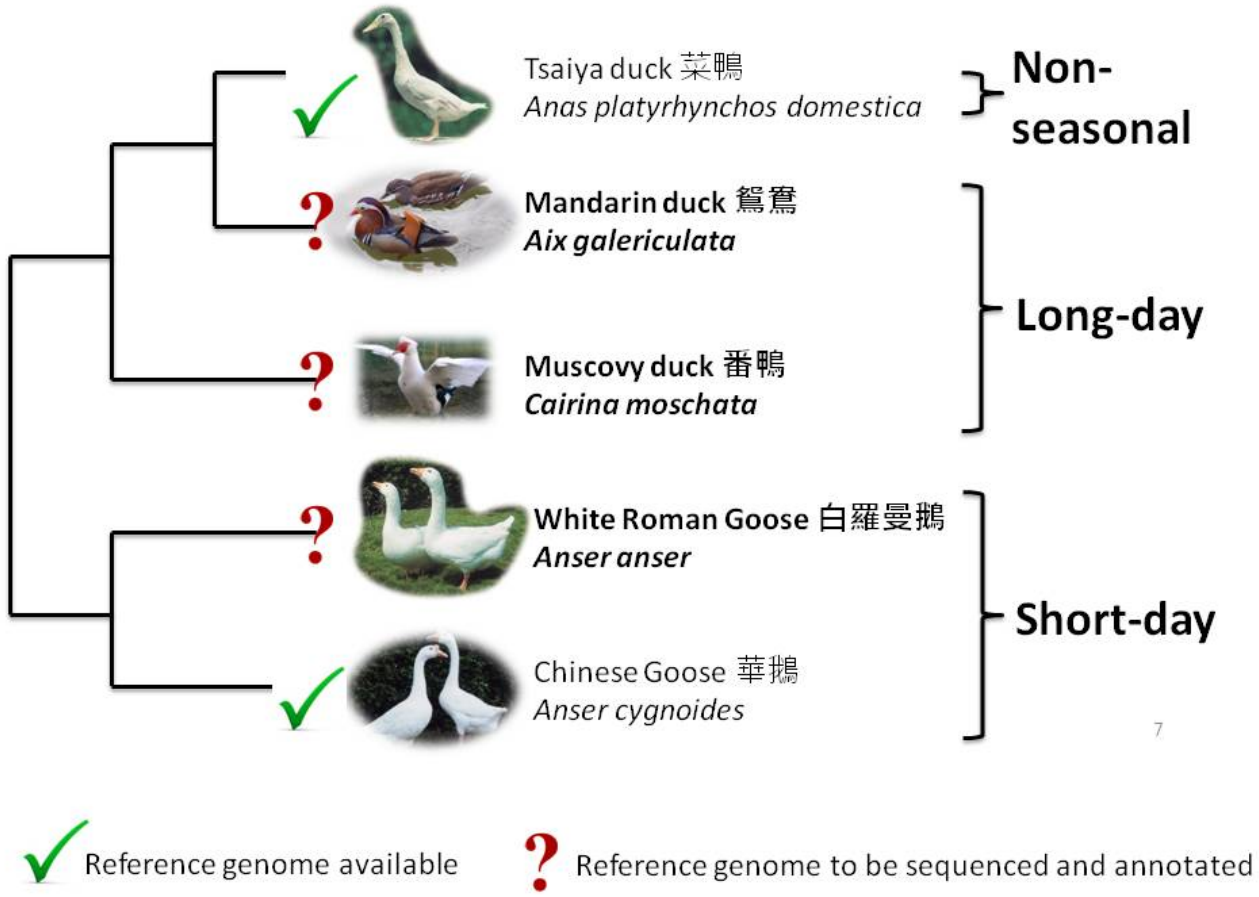

Figure 7. We are sequencing, assembling, and annotating the genome sequences of several species of ducks and geese.

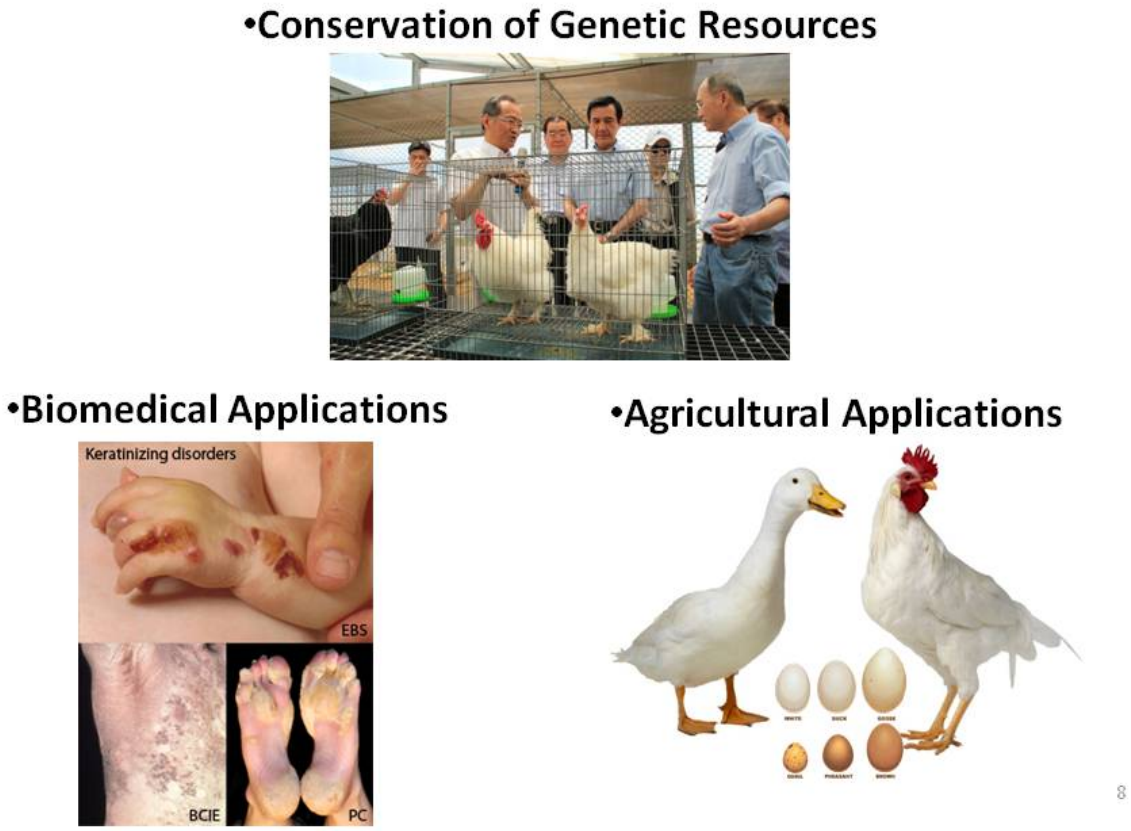

Figure 8. Broader applications and impacts.