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Avian genetic resources for biological research

ALTHOUGH MOST WIDELY KNOWN for their utility in the production of food (meat and eggs) and other commercial products (feathers, vaccines, fertilizer, etc.), chicken, Japanese quail and other domesticated avian species have long been a preferred experimental organism in applied and basic life sciences. Some features contributing to their popularity are:

- Availability (poultry, especially, are easily raised and readily obtained, with inexpensive common strains providing cost-effective research animals).
- High rate of embryo production compared to mammals.
- Accessibility of the avian embryo (the externally incubated avian embryo is available for experimental manipulation during most stages of embryonic development).
- Excellence as a higher vertebrate animal model.
- Ease of surgical manipulations (surgical procedures on birds are much less likely to result in infections due, perhaps in part, to their high body temperatures).
- Extensive history of use (more than a century of research provides avian scientists with needed information and genetic stocks to ask specific experimental questions).
- Animal welfare considerations are generally less complex for domestic birds than they are for other vertebrates.

Research with a variety of genetic stocks has produced many important discoveries in the areas of genetics, virology, immunology, developmental biology, and animal agriculture. (see CRAWFORD 1990, for a review). These include the pioneering studies on Mendelian inheritance and sex determination of BATESON (1902), and BATESON and PUNNETT (1906, 1908). Not surprisingly, given its history of use in genetic studies, the first linkage map reported for any domestic animal species was that for the chicken published by HUTT (1936). The turkey has provided one of the few known vertebrate models for parthenogenesis (embryo formation in an unfertilized egg) (Box 5), and the wide range of metabolic and developmental mutations in chicken (Boxes 8, 13) and quail have allowed researchers to start investigating the molecular bases of these defects, and, eventually, to determine the normal function of the affected gene. In virology, chicken hosts were used by ROUS (1911) to identify the first tumor-causing virus, Rous sarcoma virus. Later, the genetics of host resistance to RNA viruses was elucidated (CRITTENDEN *et al.* 1963), and the Marek's-like herpes virus found in turkeys was used in the first successful vaccination program against a tumor-causing virus in the chicken (OKAZAKI *et al.* 1970). More recently, BUMSTEAD and PALYGA (1992) described one of the first molecular genetic maps of a domestic animal using DNA markers (RFLPs), and the chicken genome map continues to be among the best developed of those for agricultural animals (LEVIN *et al.* 1994; CHENG *et al.* 1995; CROOIJMANS *et al.* 1996; CHENG 1997). It is by far the best-developed map for any avian species or any species with ZW sex chromosome determination. In particular, the usefulness of the chicken genome map in cross-species comparisons was highlighted at the First International Workshop on Comparative Genome Organization (ANDERSSON

et al. 1996), although workshop participants also expressed concern that the continuing loss of poultry genetic stocks will impair some important comparisons with the human and other genome maps. The chicken gene library was the first one described for an agricultural animal species and, along with the human gene library, was the first constructed for any vertebrate (DODGSON *et al.* 1979). Critical early observations of gene structure, organization, and expression were made using chicken ovalbumin (WOO *et al.* 1978), globin (DODGSON *et al.* 1979), histone (ENGEL and DODGSON 1981), insulin (PERLER *et al.* 1980), and lysozyme (STEINER *et al.* 1987) genes, among others.

Avian reproductive biology

Birds such as the chicken are uniquely adapted for the production of large numbers of offspring, in the form of fertile eggs, over relatively long periods of time. In the wild, birds will usually lay a series of eggs on sequential days until they have as many eggs as they can comfortably incubate (a "clutch"). At that time, they will stop laying eggs and start to incubate or brood the eggs. However, during their long domestication, chickens have been selected for large clutch size and nonbroody behavior, so that hens today will continue to lay at a high rate for at least a year, given proper conditions. In fact, chickens from some commercial stocks that have been selected for egg production may average over 280 eggs in a one-year period, with fertility of greater than 95%. While genetic strains used in research are usually less productive, most produce at least 100 eggs per hen per year, providing researchers with an abundant, dependable source of individually packaged embryos of known genotype.

The functional reproductive system of most female birds, including the chicken, is composed of the left ovary and the oviduct. The ovary contains ova in varying stages of development, with the largest being released at regular intervals. The oviduct is a tubular organ, consisting of several parts: the funnel-shaped infundibulum, where each ovum enters the oviduct and fertilization occurs; the wide, thick-walled, magnum, where the albumen is produced and deposited around each of the ova; the narrower, thinner-walled isthmus, where the shell membranes are made; the large, pouch-like shell gland, where the egg is "plumped" with fluids and electrolytes, before the calcium carbonate shell is deposited on the shell membranes; and, finally, the vagina, with its specialized sperm storage sites at the utero-vaginal junction, where spermatozoa can remain viable for more than a week (VAN KREY 1990; BAKST 1993). Thus, the oviduct is where both the fertilization and the packaging of ovum

Box 13. Inherited muscular dystrophy of the chicken

GENETIC MUSCULAR DYSTROPHY (GMD) of the chicken was first formally described in 1956 in selected New Hampshire strains at the University of California–Davis. Since then, literally hundreds of reports have been published on the abnormality. The disorder is caused by a single gene; but little is known of the molecular nature of the defect (PESSAH and SCHIEDT 1990; WILSON 1990; IANNACCONE *et al.* 1995).

Symptoms of the disorder typically appear during late embryogenesis and neonatal muscle maturation. Muscles with fast twitch, alpha-white muscle fibers such as the superior pectoralis and biceps are most affected. These "white" muscle fibers exhibit irregular-shaped rounded fibers, both larger and smaller than normal. Eventually the muscles atrophy and are replaced by fat and connective tissue. The early appearance of GMD in fast twitch but not in tonic fibers suggests it is expressed in a particular myogenic lineage.

Studies have shown that the problem with dystrophic muscle is not a faulty set of neural instructions. Limb bud transplants demonstrated that dystrophic muscles grafted to a normal host and its nerve were phenotypically dystrophic, and normal muscles transplanted to a dystrophic host were normal, indicating the problem was in the muscle and not directly its innervation. Thus, if neural influences are involved in GMD, it is likely it is the dystrophic muscle that is unable to respond properly to signals from its phenotypically normal nerve.

One of the major uses for the dystrophic chicken has been to test therapies, including an elevated oxygen levels (ASHMORE and SOMES 1966), the drugs D-penicillamine (which affects collagen formation; PARK *et al.* 1979), cyproheptadine, methysergide, and diphenylhydantoin. A Muscular Dystrophy Association-supported drug screening program achieved promising results with steroids such as corticosterone-21-acetate (C21A). The most effective treatments severely reduced the growth rate of the chicks, as if the synthesis of defective proteins play a role in expression of the abnormality.

The discovery of and sequencing of dystrophin, the gene associated with Duchenne muscular dystrophy in the human and the *mdx* dystrophy in the mouse, added a new dimension to the study of muscle abnormalities. The dystrophin protein itself is absent in humans with Duchenne dystrophy and in the *mdx* mouse. This led the Muscular Dystrophy Association to focus its efforts on the human and mouse forms of the disease. But it is still not known whether or not the dystrophin gene product is defective in GMD of the chicken. It is known that dystrophin in normal chickens is closely homologous to the mammalian gene, and that a dystrophin protein is present in GMD chickens. It is not known if this protein is the same as the one in normal chickens. Future research focusing on the role of the dystrophin gene, establishing expression of the abnormality in cell culture, and studying the factors that regulate maturation of normal muscle are worthy of study.

occurs, producing the neat, aseptic association of nutrients and germplasm that will ultimately form the chick. Embryonic development progresses as the egg moves down the oviduct, so that when the egg is laid 24 to 27 hours after fertilization, the embryo is a radial dome of 40,000 to 60,000 cells that is already forming distinct layers (epiblast and hypoblast). At this stage of development, the embryo is comprised of cells that are morphologically undifferentiated (EYAL-GILADI and KOCHAV 1976; WATT *et al.* 1993) and pluripotential, i.e., able to differentiate into a number of different cell types (PETITTE *et al.* 1990).

Numerous studies on avian reproduction have been conducted since the turn of the century, particularly on the control of fertility and egg production (HUTT 1949; LERNER 1950, 1958). Consequently, much is known about genetic and environmental factors that influence or govern reproduction in the male and female chicken, turkey, and Japanese quail. Some of the genetic variants affecting reproductive traits that were studied include: restricted ovulator, multiple ovulator, riboflavin transport deficient, and male infertility associated with the rose-type comb.

Embryology

There is a long and rich history of use of the avian embryo as a model system for study of vertebrate development (ROMANOFF 1960). Beginning with Aristotle (384–322 BC) whose *Historia Animalium* includes the first known fully preserved description of a chicken embryo, researchers and natural science historians have found bird embryos to be a readily available, dependable source of embryos that could be manipulated and observed during the course of development independent of and without harming the female parent (a major issue when studying mammals). Its easy access during organogenesis, relatively inexpensive cost, and bilaterality providing opportunities for contralateral controls for microsurgery and other perturbations have made the avian embryo one of the best characterized vertebrate models, both in classical and molecular terms (MACCABE and NOVEROSKE 1997; MORGAN 1997). Another factor that has made the avian embryo the model of choice for studies of vertebrate development is the respectable catalog of existing mutations (listed in ABBOTT 1967; SOMES 1988; and CRAWFORD 1990). Avian embryos have also proven uniquely adapted to interspecific tissue grafting because of distinct differences between the appearance of chicken and Japanese quail cell nuclei. Selected early embryonic cells from quail have been grafted into chicken blastodiscs, which, after careful incubation, reveal exactly how the donor quail cells are distributed in the later embryos (DIETERLEN-LIEVRE and LEDOUARIN 1993; DIETERLEN-LIEVRE 1997). Such studies have permitted the development of a detailed fate map of the early blastodisc. Additionally, avian embryos have been used extensively in teratological studies, in which their response to chemical, nutritional, hormonal, and environmental challenges were evaluated to provide guidelines for identifying causes of nongenetic developmental defects (LANDAUER 1967, 1973), and, eventually, for identifying the biochemical mechanisms disturbed by such exposures. Avian embryos have also been of value in studies of cell proliferation related to cancer-like cell growth or programmed cell death inherent in certain stages of embryonic development (SANDERS and WRIDE 1997).

Models for human genetic diseases

Animal genetic defects have long been used as model systems in the study of human diseases. A few of the avian genetic disorders that have proven especially useful include the autoimmune forms of avian vitiligo (AUSTIN *et al.* 1992; AUSTIN and BOISSY 1995; ERF *et al.* 1995), scleroderma (GERSHWIN *et al.* 1981; ABPLANALP *et al.* 1990; VAN DE WATER *et al.* 1994; SGONC *et al.* 1995), and thyroiditis (reviewed by ROSE 1994), the polygenic avian scoliosis (RUCKER *et al.* 1986; LIEN *et al.* 1990; MOCHIDA *et al.* 1993), the autosomal recessive form of muscular dystrophy (PESSAH and SCHIEDT 1990; WILSON 1990; IANNACCONE *et al.* 1995) (Box 13), and a polygenic muscle defect in the broiler chicken (Box 14). Other known avian mutations mimicking human disorders include: autosomal and sex-linked dwarfism, lamellar ichthyosis, poly- and hypodactyly, gouty uremia, genetic obesity, several types of micromelia, glaucoma, a non-autoimmune form of vitiligo (BOWERS *et al.* 1994), and a variety of neurological disorders (SOMES 1988; CRAWFORD 1990).

Japanese quail disease models have also attracted the attention of biomedical researchers. For example, at the University of British Columbia, quail serve as a model in the study of atherosclerosis, and in age-related macular

degeneration (AMD). This condition is the most common cause of blindness in humans over the age of 65. Except for primates, the quail is currently the only suitable nonhuman species in which to study this disease.

Despite the proven utility of existing strains of chickens, turkeys, and Japanese quail in biomedical research, fewer and fewer researchers are making use of these unique genetic stocks (WILSON 1990). Contributing to this decline are the increasing costs and the more technical (and costly) nature of many studies that have biased researchers towards smaller vertebrate laboratory species (rats and mice). Whether or not it is the best possible choice, the default condition for medical research continues to be the rodent, ultimately limiting the study of disorders and diseases to those suitable to such animal models. Enhanced use of exceptional avian models depends on the continued accessibility of existing stocks and the development and maintenance of new genetic stocks.

Immunogenetics

Natural genetic variation in the major histocompatibility complex (MHC) has been studied extensively in the chicken, particularly as it relates to disease resistance (GUILLEMOT *et al.* 1989; BACON and DIETERT 1991; HELLER *et al.* 1991; SUNG *et al.* 1993; KEAN *et al.* 1994; SCHAT *et al.* 1994; BACON and WITTER 1995; HEMENDINGER *et al.* 1995). The different MHC haplotypes have been systematically integrated into highly inbred stocks to control or eliminate complications in immune response introduced by subtle differences in the genetic background (ABPLANALP 1992; Box 7). These studies led to a better understanding of the complexities of the vertebrate immune system. They have also provided the framework for the development of disease-resistant poultry stocks (LAMONT 1994) and for understanding the differential effectiveness of certain vaccines in chicken strains with different MHC haplotypes (BACON and WITTER 1992).

In studies which focused on cell-mediated immunity and macrophage function, researchers showed that genetic differences between selected strains can also affect the ability of a given bird to promote or suppress Rous sarcoma tumor formation (QURESHI and TAYLOR 1993).

The relationship between MHC haplotype and immunocompetence is currently attracting the attention of the commercial sector. This is due to a strong negative correlation between characteristics such as improved feed conversion and more rapid growth rates with a weaker antibody response to specific antigen challenges and a decreased effectiveness of cell-mediated immunity, both of which are related to MHC function (GAVORA 1990; HELLER *et al.* 1991). This trend in immune responsiveness of commercial egg-laying Leghorn strains (small, slower-growing birds) is compared with that of commercial broiler strains. In addition, a distinct loss of immunocompetence was shown in a study that compared two broiler-type lines, one descended from commercial stocks developed in Canada in 1957, and the second from modern commercial stocks (QURESHI and HAVENSTEIN 1994). Thus, commercial broiler-chicken breeders are now showing interest in improving immune system function to offset the immune depression that has been bred into their stocks with long-term, intensive selection for meat production traits.

Box 14. Deep pectoral myopathy: One price of intensive selection

DEEP PECTORAL MYOPATHY (DPM) is characterized by death of the mid-region of the supracoracoideus muscle of the large broilers and marketable turkeys. It is also known as "Green Muscle Disease" and "Oregon Muscle Disease".

A series of investigations by Siller, Harper, and their colleagues (WIGHT and SILLER 1980; WIGHT *et al.* 1981; HARPER *et al.* 1983; SILLER 1985; WILSON *et al.* 1990) demonstrated that the myopathy was due to an ischemia caused by swelling of the muscle during exercise. An enlarging muscle, an inelastic muscle fascia, and a rigid sternum create a situation in which circulation is cut off to the mid-region of the muscle. Ultrastructural changes were detectable in as little as 15 minutes when broilers were induced to flap their wings for short periods of time.

Polygenic physiological problems are not simply resolved. Food preferences of the public and the economic forces of the poultry industry favor selection for large breasts maintaining a physiological situation in which deep pectoral muscles predisposed to ischemia may continue to be a problem. Indeed, SILLER (1985) said that the disorder was a "penalty of successful selection"; "...wild turkeys and less intensely selected old commercial strains are apparently not susceptible to DPM, it is obvious that this disease is man-made". One solution would be to breed broilers and turkeys with better circulation and a different breast muscle configuration still acceptable to the public which would probably require outcrossing to the unaffected, unimproved genetic stocks.

DPM has its human counterpart. It is one of a class of pathological situations known as "Compartment Syndromes". One of these is anterior tibialis syndrome or "March Gangrene" in which hard exercise by untrained or susceptible individuals (such as military recruits on their first forty-mile hike of basic training) results in permanent muscle damage similar to that found in turkeys and large broilers.

As more is learned about the genetic mechanisms controlling disease resistance and susceptibility, researchers will be able to narrow their focus to specific genes. A few of these genes are now known, but the majority remain to be identified. As with plant genetic stocks, some resistance genes may well be found in stocks not now commercially useful, e.g., wild or feral populations and breeds such as Ancona chicken (Box 15) or other relatively unimproved populations (GAVORA 1990).

Box 15. Ancona chickens give a new angle on disease resistance

AN UNUSUAL GENE CONFERRING disease resistance was recently discovered in the rare Ancona breed of chicken. A unique copy of a gene outside of the known major histocompatibility complex (MHC) was identified that imparts resistance to the commercially troublesome Marek's disease virus. This gene, *Rfp-Y*, now also identified in other chicken breeds, is on the same chromosome as the MHC, and is still being characterized (BRILES *et al.* 1993; WAKENELL *et al.* 1996; MILLER *et al.* 1994, 1996).

Cytogenetics

The chicken has proven to be a good vertebrate cytogenetic model, despite the complexity of its karyotype (the majority of the 78 chromosomes are exceptionally small microchromosomes; see Figure 10). In particular, unique cytogenetic conditions in the chicken have encouraged biologists to use the chicken as a means to study altered chromosome constitutions (e.g., gene dosage effects) on the biology of this and other higher vertebrates. The chicken is exceptional among the higher vertebrate animal group in that several viable genetic strains exist which showcase the effects of chromosomal aneuploidy, polyploidy, or large deletions, several of these occurring in microchromosomes.

The linkage and chromosomal location of the major histocompatibility complex (MHC) with the single nucleolar organizer region (i.e., the rDNA locus) were determined in studies of birds from the Trisomic Line (BLOOM and BACON 1985),

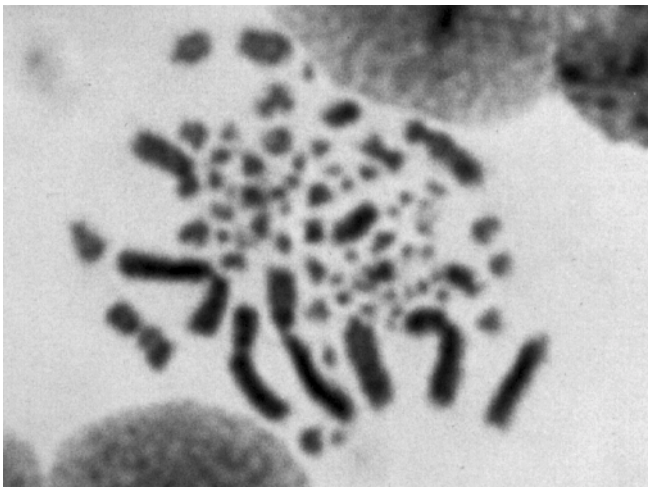

Figure 10. Chromosomes from a Red Jungle Fowl hen from UCD 001 (Photo courtesy of M.E. Delany, University of California–Davis).

which includes individuals with two (normal or disomic), three (trisomic), or four (tetrasomic) copies of chromosome 16 (a microchromosome). The biological consequences of aneuploidy and gene dosage effects on growth, development and immunity have been the subject of many studies (DELANY *et al.* 1988; QURESHI *et al.* 1989; HEMENDINGER *et al.* 1992; LEPAGE *et al.* 1996) and continues to be important in current research. The Trisomic Line has been particularly useful in studying chromosome dosage effects on regulation of MHC expression, with tetrasomics and trisomics expressing distinctly higher levels of MHC-products on their cell surfaces than disomics (MUSCARELLA *et al.* 1987; DELANY *et al.* 1988; HEMENDINGER *et al.* 1992).

The mPNU line segregates for a large deletion in the nucleolar organizer region of chromosome 16, giving it a reduced number of rRNA genes (DELANY *et al.* 1995). This line was used to establish the developmental threshold (i.e., lethal limit) for rRNA gene copy number for the first time in a higher vertebrate (DELANY *et al.* 1994, 1995). The Trisomic and mPNU lines were instrumental in establishing the location of a new locus, *Rfp-Y* (Box 15) on this microchromosome (MILLER *et al.* 1994, 1996).

The CSIRO Triploid line, maintained in Australia, is an important higher vertebrate polyploid model. Studies of the line have contributed information regarding errors of meiosis, genetic basis for inheritance of meiotic errors, sex determination, gonadal differentiation, sex reversal, and polyploidy effects on growth and development (LIN *et al.* 1986; THORNE *et al.* 1987, 1988, 1991, 1997; SOLARI *et al.* 1991; THORNE and SHELDON 1991).

Genomics

Genomics is a rapidly evolving field of science that emphasizes the study of complete genomes and chromosomes (Box 16). Genomic studies have also changed in focus, from the general organization of the genome to the identification and localization of functional genes. Genomic research is well advanced with the chicken.

Genome map development involves coordinated approaches (BURT *et al.* 1995): 1) expanding and improving the genetic linkage maps of chickens and other poultry, 2) using markers, such as microsatellites, that have high utility, improving the cytogenetic maps available for birds, and 3) making new efforts towards integrated physical (recombinant DNA clone-based) maps. Candidate gene research involves a variety of genes of interest, including those encoding the major histocompatibility complex genes, other immune-response genes, ribosomal RNA genes, cytokine and other hormone-encoding genes, and genes related to muscle and morphology. Finally, several efforts are underway to map and identify anonymous genes associated with disease resistance, growth and reproduction, and general viability of poultry.

The interdependence of genome research and genetic resource conservation can hardly be overstated. Inherent to all genetic experimentation and breeding is the fundamental requirement for allelic diversity. It is preferable that diverse alleles be preserved in well-characterized, inbred (homozygous) lines. As a concrete example, it is widely agreed that one of the reasons that the laboratory mouse has become the most important model system for biomedicine has been the availability of diverse inbred lines (pioneered most notably by the Jackson Laboratory). COPELAND and JENKINS (1991) used such inbred mouse strains in their intraspecific backcross reference family approach to gene mapping. A similar strategy was followed in developing the East Lansing chicken mapping reference population, derived from the segregating progeny of a cross between two inbred lines: Red Jungle Fowl (UCD 001) and White Leghorn (UCD 003), genetic stocks developed by H. Abplanalp at the University of California, Davis (CRITTENDEN *et al.* 1993). The two lines are highly divergent, displaying a high degree of interline polymorphism. The diverse and inbred character of these lines has been a major contributor to their subsequent utility. In contrast, BUMSTEAD and PALYGA (1992) used animals from two divergent Leghorn lines as the parents for their reference backcross panel. Cheng and Bacon (Avian Disease and Oncology Laboratory, personal communication) have used inbred lines (RPRL 6I2 and 7I3) for mapping of non-MHC genes which are responsible for resistance/susceptibility to Marek's disease virus. More generally, BACON's (1987) congenic MHC lines have been critical for a variety of genetic analyses of the avian immune system. Again, the speed with which this work was accomplished was enhanced and it could only have been accomplished using the available well-characterized genetic lines.

One long-term objective of avian genomics is to develop an understanding of genes responsible for economically important traits in poultry. These are variously known as quantitative trait loci (QTLs) (VERRINDER GIBBINS 1993) or economic trait loci (ETLs). The ETLs are analogous to, and sometimes models of, complex genetic traits of humans and other species. In some cases, the protein product of these genes is already known. In other cases, researchers seek to identify and further characterize previously unknown ETLs (anonymous genes). Among the genes under study are those encoding disease resistance traits. These include major histocompatibility genes, cytokine genes, and anonymous genes encoding Marek's disease virus resistance. Other genes of interest include those which regulate growth, reproduction and morphology (e.g., endocrine genes, collagen and other bone and connective tissue genes, anonymous ETLs), and genes which relate to general viability and cellular function (e.g., ribosomal RNA genes,

Box 16. Government involvement in genome research

THE US GOVERNMENT IS HEAVILY invested in genome research. In response to the call for a USDA National Genetic Resources Program in the 1990 Farm Bill, the USDA created the National Animal Genome Research Program (NAGRP), administered by Cooperative State Research, Education, and Extension Service (CSREES) with Richard Frahm as Direcctor. The NAGRP was developed as an equal companion to the National Animal Germplasm Program (NAGP), administered by Agricultural Research Service (ARS) with Roger Gerrits as director. The essential interrelationship of genome research and germplasm resources is emphasized by the common origin of and close ties between the NABRP and the NAGP. Suppport for agricultural animal genomics comes primarily from the USDA as direct support to ARS laboratories, such as the Avian Disease and Oncology Laboratory (ADOL) in East Lansing, the National Research Iniative Competitive Grants Program, and limited funding through CSREES to Regional Research Programs (e.g., NC-168) and a National Research Support Program, NRSP-8. NRSP-8 provides for coordination of animal genome research via the appointment of Species (Poultry, Cattle, Sheep, Swine) Technical Committees and Coordinators. Parallel developments in animal agriculture genomics have occurred world-wide, particularly in Europe (e.g., Institute for Animal Health, Compton, England; Roslin Institute, Edinburgh, Scotland; University of Wageningen, The Netherlands). One of the major goals of the NAGRP is to cooperate with international efforts to enhance progress in animal genetics.

anonymous viability genes, and neuropeptide/neurotransmitter genes).

Two major strategies are being used to locate and characterize genes of interest. In the candidate gene approach, potential ETL-associated genes are chosen based on the physiological or genetic effects of the proteins or RNAs they encode as studied either in poultry or other species. The candidate gene is then isolated by recombinant DNA techniques, further characterized, and used to generate markers to test its usefulness as an ETL indicator in appropriate populations. In the map-based approach, anonymous ETLs are mapped in a test or resource population. Then a gene that is closely associated with a particular ETL can be isolated based on its location in the map, or by a combination of candidate and map-based techniques. A fundamental tool required for the latter approach (and also useful for the candidate-gene approach) is a high quality, integrated genome map. High quality means densely spaced markers that are useful in many populations; integrated implies that the poultry genetic, cytogenetic, and physical maps are also correlated with genome maps of other animal species. The identification of conserved syntenic groups between poultry genomes and those of humans and mice will facilitate the use of genetic and physiological data from these more highly studied species.

A critical point with regard to genome research is the availability and suitability of poultry genetic resources. While commercial breeders maintain populations that presumably contain a wide variety of alleles, these populations are often not useful for research purposes if these breeders are unwilling to make populations (or data) available because of trade secrecy (proprietary) concerns, or the commercial lines are so outbred and therefore so heterogeneous at economic trait loci that it becomes impossible to measure specific effects at one or a reasonable number of candidate loci. Therefore, genetic mapping experiments require the availability of diverse populations of well-characterized, inbred, or partially inbred (or carefully random-bred) germplasm. Public access to genetic stocks and information is essential for the advancement of science. Thus, the growing number of patent requests on such material by private corporations is a concern, as markers identified by researchers supported by funding from private organizations, using stocks that are privately owned (as with recent gene mapping studies by the Groenen group (CROOIJMANS *et al.* 1996) may be restricted in their availability.

Developmental genetics

Chicken developmental mutations played a significant role in the early days of the science of genetics. Bateson's work at the turn of the century showed that different comb shapes in the chicken followed Mendelian inheritance patterns. This proved that Mendel's principles, though discovered in plants, also applied to animals (BATESON 1902; BATESON and PUNNETT 1906, 1908). Today, there is increasing interest in the molecular genetic analysis of such developmental or pattern mutants to determine the basis of the developmental defects that produce these changes. The defined physical defects associated with the avian developmental mutations, exemplified in Figures 11 and 12 and Box 17, contrast strongly with many of the synthetic (knock-out) mutations in the mouse, whose phenotype is often simply characterized by the timing of early embryonic death.

Currently, developmental genetics is one of the most rapidly advancing areas in the biologi-

Figure 11. *Wingless-2* chicken embryo from UCD Wingless-2 X 331 (Photo courtesy of J.M. Pisenti, University of California–Davis).

cal sciences (RIDDLE *et al.* 1993; FALLON *et al.* 1994; MORGAN 1997; SANDERS and WRIDE 1997; CHUONG 1998). Questions that have intrigued and puzzled embryologists and geneticists for decades can finally be addressed. The award of the 1995 Nobel Prize in Medicine/Physiology to E.B. Lewis, C. Nusslein-Volhard, and E.F. Wieschaus reflects the worldwide recognition of the fundamental importance of research in developmental genetics. Their work in identifying fruit fly (*Drosophila melanogaster*) developmental mutants and determining the molecular basis for some of these mutations provided key information for identifying a whole class of pattern-altering genes (the homeobox-containing genes) found in such diverse organisms as the fruit fly, nematode, echinoderm, fish, chicken, mouse, and human.

Avian developmental geneticists interested in pattern formation have studied an array of morphological mutants, most of which are recognizable early in development. These mutants typically alter the beak, head, trunk (including the tail), limbs, and integument (skin, feathers, and scales) (ABBOTT 1967; ROMANOFF 1972; LANDAUER 1967, 1973; SAWYER and GOETINCK 1988). In many instances, as exemplified by the *wingless-2* mutation in Figure 11, the mutations have a pleiotropic effect (alter more than one embryonic structure), with combinations of defects involving the limbs, trunk, beak, feathers, heart, kidneys, and so on. Another tantalizing finding, much investigated by LANDAUER (1967, 1973), reflected the close similarity of many known mutant syndromes with those produced by treatments with a variety of chemicals or hormones, or caused by nutritional deficiencies or excesses

Figure 12. *Eudiplopodia* foot from UCD Eudiplopodia X 003 (Photo courtesy of J.M. Pisenti, University of California–Davis).

(the so-called phenocopy effect). A field with great potential then involved studies of the basis of phenocopies and of means of overcoming them. Later, studies with pesticides provoked additional interest, as many of these environmental toxins could also produce phenocopies of different mutant syndromes.

Currently, the genes affecting patterning in the avian embryo are increasingly important in developmental studies (RIDDLE *et al.* 1993;

Box 17. Patterns in vertebrate limb development

LIMBLESS IS AN AUTOSOMAL recessive mutation that causes the complete absence of limbs in homozygotes; heterozygotes have normal limbs (PRAHLAD *et al.* 1979). The earliest stages of limb development appear to occur normally in the mutant homozygotes, but shortly after the limb bud forms, it ceases to develop and soon regresses. Two recent studies of this mutation (GRIESHAMMER *et al.* 1996 and NORAMLY *et al.* 1996) have provided evidence that although the phenotype of the mutant is a lack of limb outgrowth, the primary defect in *limbless* embryos is a lack of normal dorsal-ventral patterning at a very early stage of limb development. This apparently results in an inability of the embryonic cells in the prospective limb-forming territory to respond appropriately to the normal limb-inducing signal, and as a consequence the apical ectodermal ridge does not form. In the absence of this important signaling center, limb outgrowth does not occur. The importance of these studies is that they have revealed a previously unknown link between patterning along the dorsal-ventral axis and the initiation of limb bud formation.

This breakthrough in understanding the fundamental mechanism of limb development in vertebrates would not have been made were it not for the availability of chicken stocks carrying the *limbless* mutation, because similar mutations in other species such as mice and humans are not known. However, it is extremely important to bear in mind that the discovery that *limbless* encodes a gene playing a critical role in limb development could not have been made before the molecular markers for dorsal and ventral cell identities became available. Other mutations in avian stocks may be equally valuable, but their importance cannot be assessed at present because of limitations in our knowledge about limb development or because the appropriate markers for assaying gene function are not available. If we discard stocks because we do not appreciate their value, we may be discarding the key to a more profound understanding of a particular developmental or disease process.

RODRIGUEZ *et al.* 1996). Specifically, these mutations can be used to study signaling, biological clocks, interactions between different developmental controls, and the relationship of the defective patterns to similar syndromes produced by chemical or other experimental means. Importantly, these mutants can be used to investigate directly various hypothetical models of gene action developed by other means.

Limb development

For more than 50 years, vertebrate limb development has been a major focus of developmental biology (SAUNDERS 1948; ABBOTT 1967; SAWYER and GOETINCK 1988; FALLON *et al.* 1993; LAUFER *et al.* 1997). Between 1972 and 1998, advances in this field have been highlighted at six International Conferences on Limb Development and Regeneration. The limb bud has been of interest because it provides an experimental paradigm for exploring fundamental mechanisms of vertebrate tissue outgrowth and patterning (SAUNDERS 1972; EDE *et al.* 1977; HINCHLIFFE and JOHNSON 1980; CONNELLY *et al.* 1981), and for identifying the molecules that mediate these processes. It also provides a model for studying the mechanism of embryonic induction (CONNELLY *et al.* 1981). Most of what is presently known about vertebrate limb development has come from studies performed in avian species, primarily the chicken, but it is now clear that the mechanism of limb development has been highly conserved throughout evolution, and what has been learned about limb development in the chick is equally informative about limb formation in mammals (FALLON *et al.* 1993). Indeed, it appears that the early steps in limb development and the molecules that perform them are virtually identical in all vertebrate species (MORGAN 1997), and it is only at relatively late stages that species-specific differences become significant. A number of researchers have used developmental mutations to test hypothetical control mechanisms governing limb pattern at the tissue level. More recently, still other researchers have returned to these mutations to study perturbations in the expression of developmentally regulated molecules that could explain the pattern abnormalities characteristic of such mutations (GRIESCHAMMER *et al.* 1996; NORAMLY *et al.* 1996; RODRIGUEZ *et al.* 1996; LAUFER *et al.* 1997). Thus, there is still much to be gained from the analysis of the mutations that have occurred in avian stocks and which have been maintained in university collections. Two particularly useful chicken mutations are the *limbless* mutation, which has recently been used to gain a remarkable insight into the fundamental mechanism of limb initiation (Box 17), and the *eudiplopodia* mutation (Figure 12), which has showcased the critical nature of timing and location of signaling molcules in early limb bud on the final patterning of the limb (LAUFER *et al.* 1997).

Feather and scale development

The morphogenesis of cutaneous appendages, such as feathers, scales, and hair, depends on a series of reciprocal interactions between the epithelial and mesenchymal layers of the embryonic skin (SENGEL 1976). Such epithelial mesenchymal interactions also take place in the development of the limb (RIDDLE *et al.* 1993; FALLON *et al.* 1994; NISWANDER *et al.* 1994), tooth (VAINIO *et al.* 1993), kidney (PATTERSON and DRESSLER 1994), lung (PETERS *et al.* 1994), and mammary gland (CUNHA 1994). Evidence is accumulating which indicates that the epithelial-mesenchymal signaling that occurs during the early morphogenesis of these various organs relies on common molecular mechanisms (Box 18 and CHUONG 1998). Thus, information gained on the mechanisms for any one of these organs systems is very likely to be applicable to our understanding of patterning events in other systems. A future challenge is to understand how different embryonic structures acquire their identity while relying on common signaling mechanisms.

Box 18. Patterns in skin development

THE *SCALELESS* MUTATION has contributed significantly to our understanding of both the cellular and molecular mechanisms that control the formation of cutaneous appendages, particularly the feathers and scales in birds. Chickens homozygous for the *scaleless* mutation (Figure 13), an autosomal recessive trait, lack most feathers and scales (ABBOTT and ASMUNDSON 1957) as well as the scleral ossicles (the precursors to the bony eye ring) (PALMOSKI and GOETINCK 1970). These defects have made this mutant an ideal model system for studying the early development of cutaneous appendages, and, by extrapolation, other organ systems depending on epithelial-mesenchymal interactions. In some of the earliest studies in which normal and *scaleless* ectoderm and mesoderm were recombined, researchers showed that the *scaleless* mutation specifically affects the ectodermal epithelium (GOETINCK and ABBOTT 1963; SENGEL and ABBOTT 1963). This ectodermal defect prevents the formation of the ectodermal placodes in the skin of *scaleless* embryos (GOETINCK and SEKELLICK 1972), thus interrupting the normal sequence of tissue interactions. Despite the ectodermal defect, the mutant mesenchyme is fully capable of participating in feather and scale development when recombined with genetically normal ectoderm (GOETINCK and ABBOTT 1963; SENGEL and ABBOTT 1963; SONG and SAWYER 1996).

Interestingly, the mutant phenotype can be greatly modified through selection for increased or decreased number of feathers. The accumulated modifier genes for increased feathering alter the expression of the *scaleless* gene by altering the response of the mesodermal signals (BROTMAN 1977a,b). A number of studies also addressed the cellular and subcellular (histological) differences in the development of the feathers and scales (SAWYER and ABBOTT 1972; SAWYER *et al.* 1974; SAWYER 1979). These classical studies in the genetics, histology, and tissue and cell biology of this mutation laid essential groundwork for current molecular studies that were designed to explore molecular control mechanisms governing feather and scale development.

In a recent study, the role of fibroblast growth factor (FGF) signaling was examined in the epithelial-mesenchymal interactions during the initiation of feather germ formation in genetically normal and *scaleless* embryos. A spatially and temporally restricted pattern of transcription was seen for the genes that encode FGF-2 and FGFR-1 in developing feather germs of the normal chick embryo. FGF-2 expression is restricted to the early feather epidermal placodes, whereas, FGFR-1 expression is limited to the dermal condensations underlying the placodes. Transcription of these genes could not be detected in skins of *scaleless* embryos that failed to develop feathers as a result of their ectodermal defect. However, when *scaleless* skin was treated with FGF-2, feathers would form at the site of application of the growth factor, and the induced feathers express FGFR-1 in their dermal condensations . However, the response was restricted to a narrow window of time, strongest around day seven and eight, and gone by day 11 (SONG and SAWYER 1996). Based on these observations, FGF-2 was hypothesized to be an active molecular component of the early signaling between the dermal mesenchyme and the overlying epithelium during feather morphogenesis. The observation that FGF-2 can rescue the mutant phenotype of *scaleless* embryos suggests that FGF-2 either is, or is downstream from, the signal that the ectoderm of the *scaleless* mutant fails to generate.

The results obtained with the *scaleless* skins indicate that this mutant is an excellent system to elucidate signaling pathways by FGF and other signaling molecules in the morphogenesis of cutaneous appendages. Mechanisms identified from this system may be applicable to the understanding of developmental mechanisms in other organs in which epithelial-mesenchymal interactions play a role (PETERS *et al.* 1994).

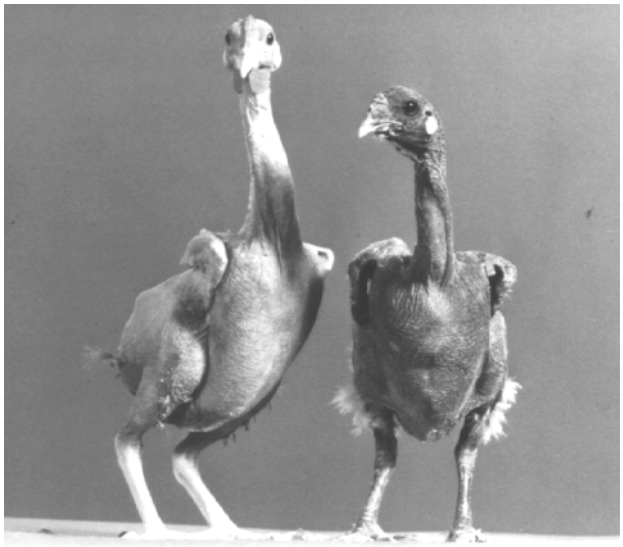

Figure 13. Hens from UCD Scaleless-Low (Photo courtesy of J. Clark, University of California–Davis).