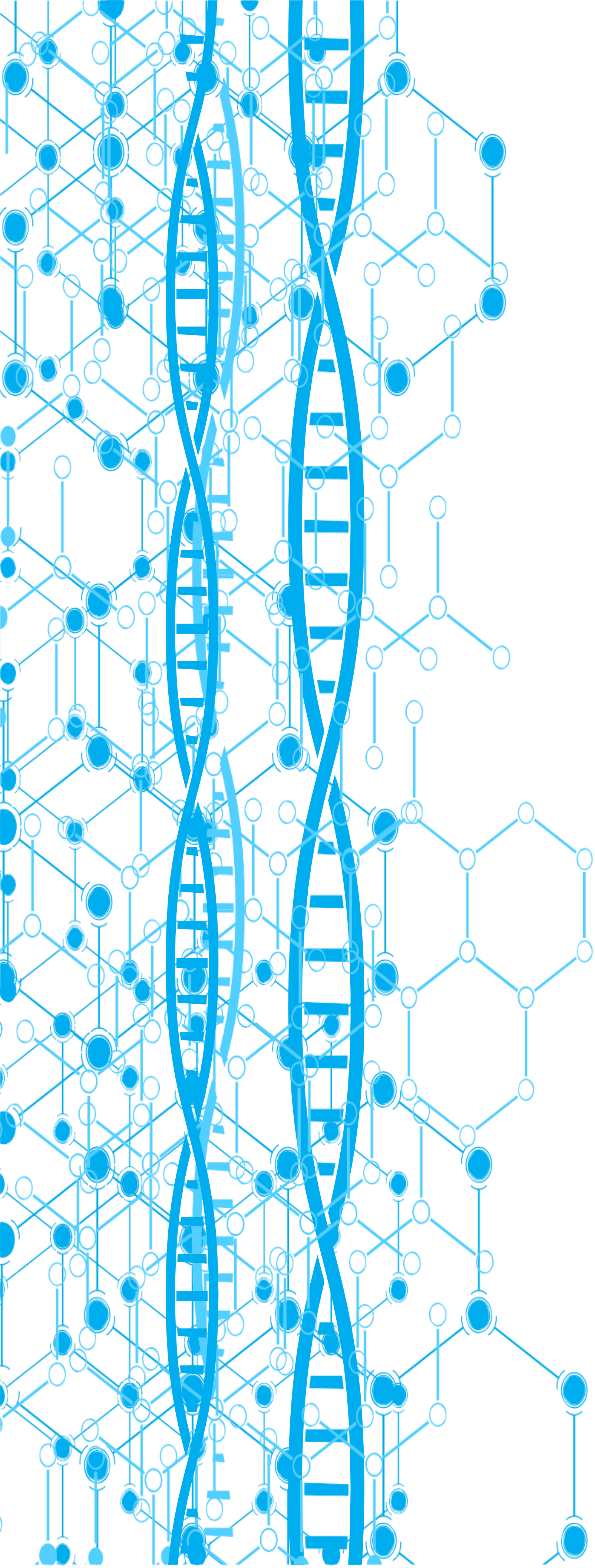
Genetic Preservation Summit: Putting the Pieces Together

PROCEEDINGS

May 24-25, 2017
Alberta Chicken Producers Poultry Technology Centre University of Alberta South Campus
Edmonton, Alberta, Canada

- Balance Breeding
  Derek Emmerson, VP Research and Genetics, Aviagen North America
- Progression of Poultry Welfare
  Teryn Girard, MSc., University of Calgary
- Progress or Perish: Antimicrobial Use and Resistance in Poultry
  Karen Liljebjelke, Ph.D. University of Calgary
- Who's serving who?
  Mary Bailey, editor of the Tomato Food & Drink magazine
- How has genetic progress impacted the realities on the farm and in the barn?
  Scott Wiens , multi-generational Alberta broiler chicken producer
- Progress in Poultry Breeding – Layers and Broilers
  Paul Siegel, Ph.D., Professor Emeritus, Virginia Tech
- **Panel Discussion**

- History of the University of Alberta heritage chicken lines
  Jesse Hunter, MSc., U of Alberta Heritage Chicken Coordinator
- 50 years of Genetic Progress - a Global Perspective
  Martin Zuidhof, Ph.D., University of Alberta
- Survivor from the North: The Finnish Landrace Breed and a Successful Conservation Program in Finland
  Mervi Honkatukia, Ph.D, Natural Resource Institute Finland
- Contributions of Chicken Populations to Science and Society
  Paul Siegel, Ph.D., Professor Emeritus, Virginia Tech
- Preserving and Using Unique Chicken Populations
  Nick Anthony, Ph.D., University of Arkansas
- Insights from Long-term Selection of White Leghorns for High and Low Antibody Production
  Chris Ashwell, Ph.D., North Carolina State University
- Genetic Relationships among Chicken Populations in Conservation Programs
  Dr Mark Berres, Ph. D., Genetics and Biotechnology Center
- Genetic Preservation through Cryopreservation
  Dr Carl Lessard, Agriculture and Agri-Food Canada
- Chicken Major Histocompatibility Complex: Why variation is important
  Dr Janet Fulton, Hy-Line International
- **60 years of Genetic Progress**
  Brenda Reimer, Alberta Agriculture and Forestry



Dedicated to the memory of Derek Emmerson, PhD.

Derek lost his battle with cancer in July 2017.

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The Progression of Poultry Welfare

Teryn Girard, MSc

The Progression of Poultry Welfare

By: Teryn Girard
Genetic Preservation Conference
May 24, 2017

What is animal welfare?

- The state of the animal (OIE, 2012)
  - Encompasses:
    - Biological functioning
      - Health and maintenance
    - Natural behaviour
      - Chickens: nesting, foraging, perching
    - Feelings
  - These three things are not always correlated

Improvements in Poultry Welfare

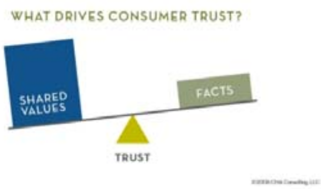
- Disease Awareness and Prevention
  - Vaccines, Pharmaceuticals, Knowledge
- Housing
  - Behavioural needs, Space allowance
- Transportation
  - Transport trucks and crates
- Handling
  - Humane Euthanasia

Why Has Poultry Welfare Improved?

- Science
  - Animal Welfare Research
    - Ethology
  - Food Safety Research
- Animal Medicine
  - Veterinarians and technicians
- Consumer Awareness
  - Interest groups
  - Social License

Social License

- The privilege of working with minimal restrictions based on maintaining the public's trust that you will do what is right



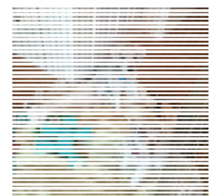
https://www.slideshare.net/GreyBruceFarmersWeek/proactively-addressing-social-license-in-agriculture

Example of Social License

- Housing of Laying Hens
  - Conventional, Enriched, and Free Run/Free range
  - Consumers are adverse to "cage"

Wikipedia.com

www.benandjerry.ca



What does the science say?

- Enriched cages may be a better choice compared to cage-free for commercial poultry
  - Allows for behaviour needs and more sanitary conditions
  - Decreases injuries, competition, and aggression
- Food safety may be decreased in a cage-free system compared to an enriched cages

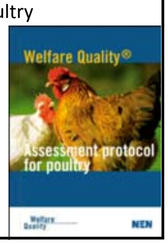
UNIVERSITY of GUELPH

Take home messages

- Bird welfare is constantly evolving
- Consumers shape the industry and science needs to be part of the discussion
  - But who shapes the consumers?
- *How does science play a role with social license?*

Welfare Audits

- There is continuous improvement in livestock welfare
- Audits
  - Third Party Audits for each poultry industry
  - Welfare Quality Assessment Protocol for Poultry
    - Lameness
    - Hock burn
    - Foot Pad Dermatitis
    - Etc.
- What happens if an audit isn't passed?



Example #2 of Social License

- Viral video of a broiler chicken farmer
  - Broilers are not happy and not healthy
    - Why? How do we know this?
  - Broilers don't get fresh air or sunlight
    - What are the risks?
  - Broilers are in pain due to leg injuries
    - How do we know they are in pain?

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Progress or Perish: Antimicrobial Use and Resistance in Poultry

Karen Liljebjelke, PhD
University of Calgary

Progress or Perish

Antimicrobial Use and Resistance in Poultry

Karen Liljebjelke DVM, PhD
University of Calgary
May 24, 2017

Increasing Resistance Threatens Human and Animal Health

- Use of antibiotics in plant and animal agriculture, veterinary and human medicine, and consumer products exerts selection pressure on bacteria for development of antibiotic resistance
- As the world population increases, the volume of antibiotics used increases globally
- Increasing resistance is leading to treatment failure: higher treatment cost, longer hospitalizations, poorer outcomes, and deaths
- The global rise in resistance creates the possibility of untreatable bacterial pandemics

Antimicrobial Resistance - A One-Health Problem

- The problem affects humans, animals, ecosystems worldwide
- Fixing the problem will require buy-in from all stakeholders
- Who are the stakeholders?
- Every person on Earth is affected by the problem, and many individuals contribute to the problem
- Governments
- Health systems
- Agriculture
- Physicians
- Veterinarians
- Food producers – grain crops, fruit, vegetables, meat, dairy, eggs

As Stakeholders, We Have to "Own" Our Piece of the Problem

- Acknowledge contributions to the problem
- Understand how poultry products and consumers can be affected by the problem
- Develop policies, procedures, and protocols to reduce or eliminate contributions to the problem
- By owning a piece of the problem we can protect the reputation of the industry
- Openly owning our piece, and doing our part to reduce the problem creates pressure on other stakeholders to acknowledge their contributions to the problem, and create solutions for their contributions

The Antibiotic Resistome

- All use of antibiotics creates selection pressure for increase in resistance
- This selection pressure on the bacterial antibiotic resistome has increased the absolute number of resistance genes in existence on Earth

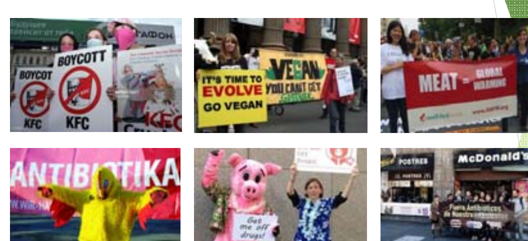
Wright, G. Nature Reviews 2007(3):175

Bothered by Pesky Antimicrobials?

Changing Societal Desires Threaten the Social License to Operate for Producers

- The public give producers and veterinarians their trust to produce a safe product
- Consumers purchase the product which allows producers to operate
- The concerns of consumers are:
- Animal welfare
- Responsible antibiotic usage
- Wholesome product – no food-borne pathogens, antibiotic or hormone residues
- Environment

Consumers are the Drivers of Change



They are not wrong. We can do better.

Slide 8

KL1 Karen Liljebjelke, 5/23/2017

So What Does the Future Look Like?

- Antibiotic – Free Production (No AGP's)
- On-Farm Food Safety Programs
- Quality Assurance Programs
- More expensive production
- More expensive product
- Changes in production practices
- Changes in poultry genetics
- Elimination of the Quota system?
- Integration of the production system
- Consolidation of the industry

Alternatives to Growth-Promoting Antibiotics

- Better and more intensive management of the flock house and hatchery environment
- Enzymes in feed
- Pro-biotics
- Pre-biotics
- Changes in feed formulation
- Competitive exclusion
- Cleaning and disinfection
- Litter treatments
- Biosecurity
- All-in / all-out (end the practice of spiking)
- Quality assurance sourcing
- Changes in bird genetics

Selection for Immune Function Vs Growth

- Selecting for growth may have led to impaired immune function
- Meta-analysis conducted by van der Most, et al. Functional Ecology 2011, 25; 74-80.
- Test for trade-off between growth and immune function on data from lines of poultry divergently selected for either growth or an aspect of immune function
- Found that selection for growth does indeed compromise immune function, but selection for immune function did not consistently affect growth
- Suggests that it may be possible to breed animals for increased growth without loss of immune function

Vital to Preserve Genetic Varieties

- Just as with highly selected grain and vegetable crops, preservation of heritage varieties of poultry is vital
- The living collections of heritage poultry varieties are libraries of alleles for traits including immune function
- Whole genome sequences of heritage varieties are merely card catalogues
- Living individuals are essential for preservation of the genetics

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Who has genetic progress impacted the realities on the farm and in the barn?

Scott Wiens

How has genetic progress impacted the realities on the farm and in the barn?

Scott Wiens – May 24 2017

1980 – 2017 ~ my context

What's changed··?

Fans, heaters, thermostats, ventilation ideas and controls···

CHANGE…

IT'S A THING!!

More like what is still the same..?!

3 things..

Feed

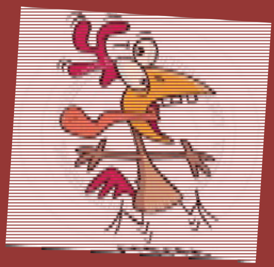
Water

Air

Timeless	Change
Feed	No
Water	Days
Air	Off

Compound Stress Fractures

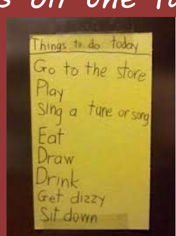
The growing environment needs to be perfect or you lose growth opportunity – errors compound on themselves!



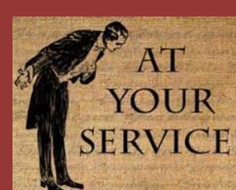
10% off your margin for error!!

As the bird gains efficiencies the margin for error on farm narrows in every respect

How has genetic progress changed realities on the farm and in the barn?



Farmer means servant



More eggs in your basket

There was a time when chickens fit into your life..

Today your life fits into what a chicken wants.

Because farmer means servant

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Progress in Poultry Breeding – Layers/Broilers

Paul Siegel, PhD
Virginia Tech

VirginiaTech
Invent the Future

Progress in Poultry Breeding – Layers - Broilers

Paul B. Siegel 2017

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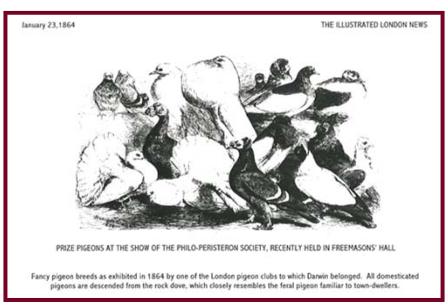
January 23,1864 THE ILLUSTRATED LONDON NEWS

PRIZE PIGEONS AT THE SHOW OF THE PHILO-PERISTERON SOCIETY, RECENTLY HELD IN FREEMASONS' HALL

Fancy pigeon breeds as exhibited in 1864 by one of the London pigeon clubs to which Darwin belonged. All domesticated pigeons are descended from the rock dove, which closely resembles the feral pigeon familiar to town-dwellers.

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Natural Selection

- Relatively slow process
- Selection on fitness for the environment

Artificial Selection

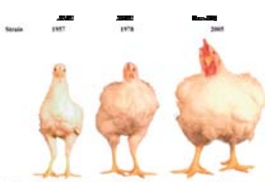
- Directs & accelerates biological changes
- Tailors individuals for specific purposes
- Cannot eliminate forces of natural selection

1950 & Current Chickens

Entries ready for judging in 1947 Chicken of Tomorrow Contest.

Selection: a process that determines which individuals become parents, how many offspring they produce that reproduce

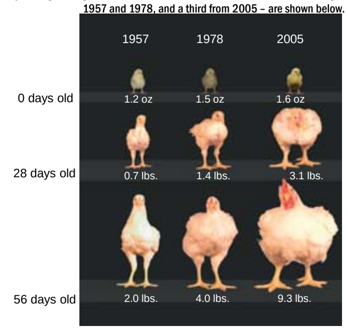
- Selection as a tool
- Meat-type chickens selected for increased BW
- Correlated responses (feed intake, growth, reproduction)



Zuidhof et al., 2014

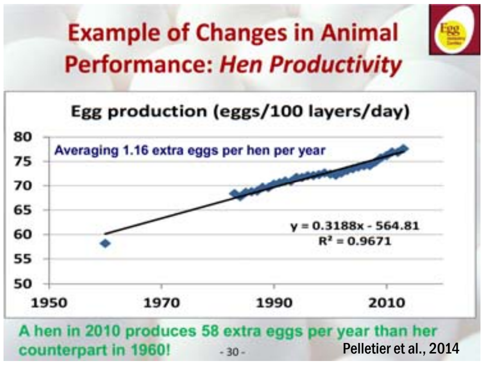
Bulkier Birds

Careful genetic selection has resulted in a much larger commercial broiler chicken compared to those raised nearly 60 years ago. The growth of three strains of broiler chickens – two strains genetically unchanged since 1957 and 1978, and a third from 2005 – are shown below.



Source: Wall Street Journal: Poultry Science, Zuidof, Schneider, Carney, Korver, & Robinson (2014); Univ. of Alberta, Dept. of Ag, Food, & Nutritional Sci.

Development in Egg Production



Examples of % changes in the last 50 years

Egg production	↑	20%
Body weight	↓	20%
Age at 1st egg	↓	15%
Shell strength (nu)	↑	20%
Feed conversion (feed/egg mass)	↓	35%

"Wisdom is knowing you can't be wise"

Paul Engle

"Everything that is there is perfect except the omissions"

Shaw's description of Venus de Milo

Historical is the present tense in telling of past events, whereas the future is that to be.

It is one thing to look back, and from it is there advice for predicting?

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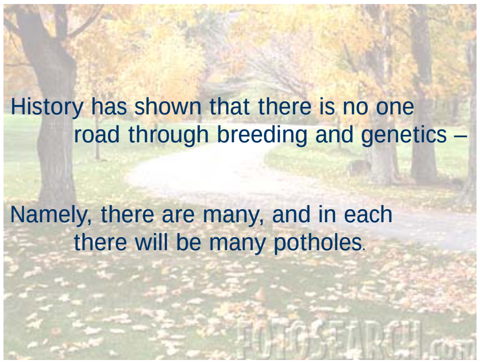

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Once more, I am happy to again be in Edmonton and to be a part of your festivities.

Thank you

Photo by J. McCormick

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The Heritage Chicken Program: Promoting Awareness and Preservation

Jesse Hunter, MSc

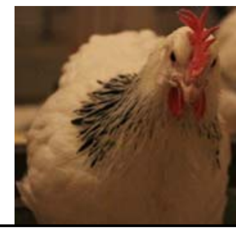
The Heritage Chicken Program: Promoting Awareness and Preservation

Jesse Hunter, Agnes Kulinski
Poultry Research Centre
University of Alberta, Edmonton

Heritage Chicken Program: Development

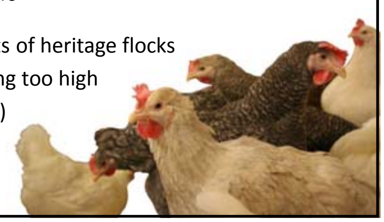
Genetics from

- University of Saskatchewan (5 lines)
  - Broiler lines from 1957 and 1978
- Dr. Don Shaver (2 lines)



Why Heritage Chickens?

- Maintain genetics developed by Canadian poultry scientists
- Loss of Heritage genetics throughout Canada and the US in the past 15 years
- Maintenance costs of heritage flocks at PRC were getting too high ($65,000 per year)



Preserving Heritage Chicken Genetics

6 month pilot Heritage Chicken Program

- Developed by Agnes Kulinski, 2013

With support from:

- ALMA Industry & Market Development Program
- Market Development Team, ARD
- Eldesigno –branding, website
- Tomato Magazine, Mary Bailey: review of program
- EFA, CFIA - rules and regulations
- U of A Risk Management Office
- Food Safety and Animal Health Division, ARD
- Poultry Research Centre

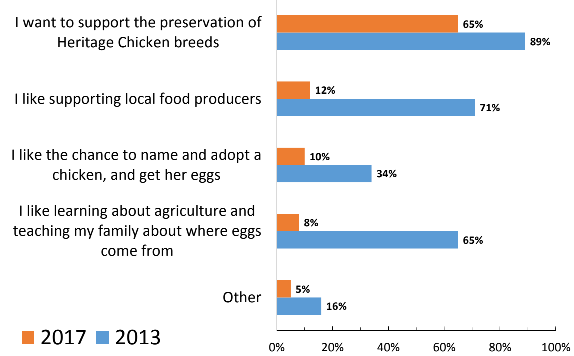
Heritage Chicken Program Developed

- Overwhelming response received from public and media wanting to support genetic preservation of our heritage breeds
- Over 500 people joined the program for 2017, with 400 on the waiting list
- All money raised goes towards maintaining the flocks at the PRC

Heritage Chicken Program: How Does it Work?

- Registrants select a breed online and name their hen
- Registration fee is $150 per hen. All funds go back into maintaining the flock
- Participants receive a certificate of adoption and every two weeks, a dozen eggs produced by the flock

Why do people join the Heritage Chicken Program?



Why do people join the Heritage Program?

- *I like being able to name and adopt my own hen and get the eggs!"*
- *"I want to support the preservation of Heritage Chicken breeds."*
- *"I want to support local food sources."*
- *"I have adopted because all my neighbours are doing it."*
- *"It reminds me when I was a kid seeing all the different colours and shapes of the eggs."*
- *"Now my kids know, their eggs don't just come from a grocery store."*

Why do people join the Heritage Chicken Program?

Key aspects that supporters find valuable:

- Connection to agriculture in an informal setting
- Information sharing: flock updates and photos
- Education: poultry industry and agriculture

Heritage Chicken Program: Spin-off Programs

Stewing Hens

- Each Heritage flock processed at a year and four months of age
- Birds are sold as stewing hens to heritage participants
- Profits go towards maintaining the heritage flock

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Heritage Chicken Program: Spin-off Programs

Help raise funds for breeding program

- 2014: “Heritage Chick Days” pilot program in collaboration with Peavey Mart.
- 2015: Heritage Chick Days - 17 stores across Alberta
- 2017: 20 stores across Alberta

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Heritage Chicken Program: Spin-off Programs

Breeds Available:

- Barred Plymouth Rock
- Brown Leghorn
- 1978 Broiler
- Rhode Island Red
- Light Sussex

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Heritage Chicken Program: Spin-off Programs

- Peavey Mart Collaboration
- Topics include:
  - On-farm biosecurity
  - Housing
  - Nutrition
  - Behaviour
  - Chicken anatomy
  - Disease identification and prevention

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Connecting to Community

Local Urban Community

- Support educational messages developed by EFA, ACP, industry
- Media interviews
- One on one discussions
- Social media, email and website

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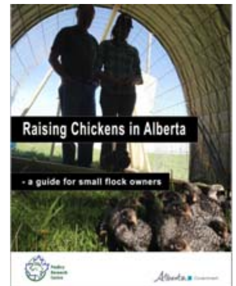
Edmonton, Alberta, Canada

Connecting to Community

Small Flock Owners

- Support educational messages developed by EFA, ACP, industry
- Workshops
- “Raising Chickens in Alberta” manual
- Support the traceability program (PID number)



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Summary

- Preservation of heritage genetics
  - Financial self-sufficiency
- Research projects
  - Heritage lines provide a genetic benchmark
- Connect with consumers
- Educate small flock owners
  - Promote responsible small flock management

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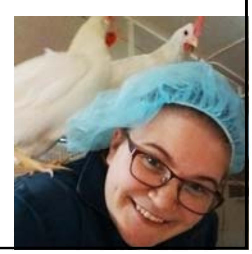
Edmonton, Alberta, Canada

Thank You!

- Poultry Research Centre Staff
- Heritage Chicken Program Participants
- ALMA
- University of Alberta
- ARD
- EFA

More questions?
Email:
uofaheritagechickens@gmail.com



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Fifty Years of Genetic Change: A Global Perspective

Martin Zuidhof, PhD
University of Alberta

Fifty Years of Genetic Change: A Global Perspective

Martin J. Zuidhof, Valerie L. Carney, Brenda L. Reimer, D. R. Korver, and F. E. Robinson
Genetic Preservation Summit
Edmonton, AB
May24-25, 2017

Image source: Zuidhof et al. 2014. Poultry Sci. 93:2970

Overview

- Highlights from "50 year study"
- The reaction
- The dialogue on socially responsible chicken production


Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 2005

Experimental Objective

To assess the impact of 5 decades of quantitative genetic selection on broiler

- Growth
- Efficiency
- Yield

Completely Randomized Design

Source of variation: three genetic lines

- Alberta Meat Control (AMC) genetic benchmark lines
  - AMC-1957 (unselected commercial broiler)
  - AMC-1978 (unselected dam line; strain 30)
- 2005 commercial broiler (Ross 308)

By design, all differences were due to genetics

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The Experiment

- 4 pens per strain
- At placement, n = 180 per strain
- Modern broiler ration
- Weekly BW
- Weekly feed intake
- Semi-weekly dissection: 8 birds/strain
- Sex determined at processing

Solutions for today. Foundation for the future.

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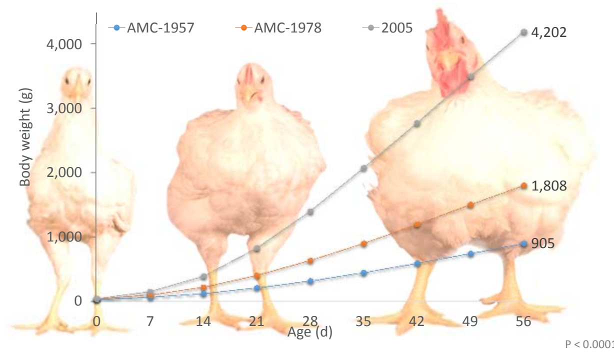
Edmonton, Alberta, Canada

Body Weight



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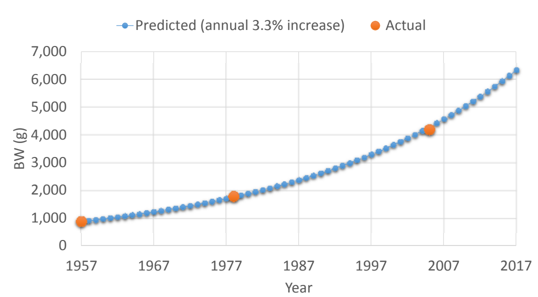
56 d Body Weight



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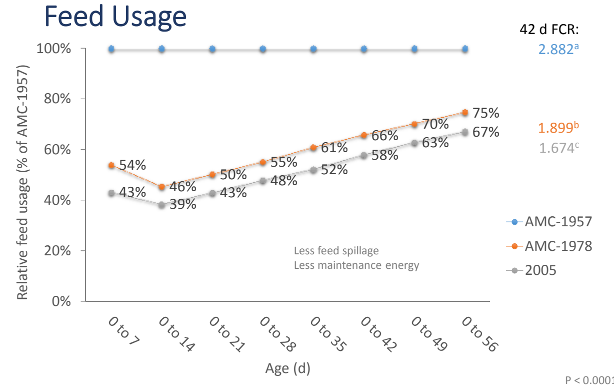
Feed Usage



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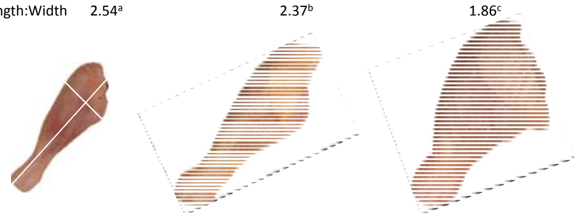
Drum Yield (% live weight)

	AMC-1957	AMC-1977	2005
	$8.6^{c} \pm 0.1$	$9.1^{a} \pm 0.1$	$8.8^{b} \pm 0.1$
Length:Width	2.54^{a}	2.37^{b}	1.86^{c}



AMC-1957 AMC-1977 2005

$P < 0.0001$

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Breast Yield (% live weight)

	AMC-1957	AMC-1977	2005
Yield	$10.7^{c} \pm 0.1$	$11.2^{b} \pm 0.01$	$17.9^{a} \pm 0.01$
BCR (g:g)	$28.2^{c} \pm 0.1$	$17.0^{b} \pm 0.01$	$9.4^{a} \pm 0.01$

AMC-1957 AMC-1977 2005

$P < 0.0001$

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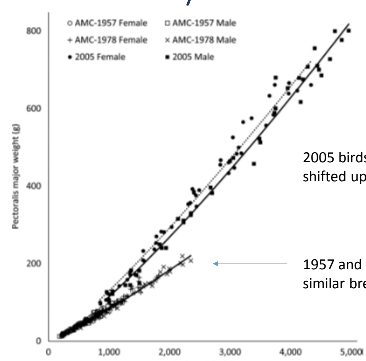
Breast Yield Allometry



2005 birds breast yield was shifted upward

1957 and 1978 birds had similar breast yield pattern

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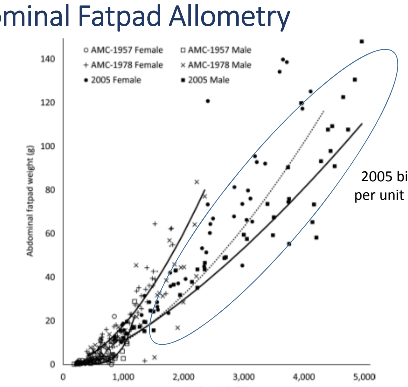
Abdominal Fatpad Allometry



2005 birds had less fat per unit of body weight

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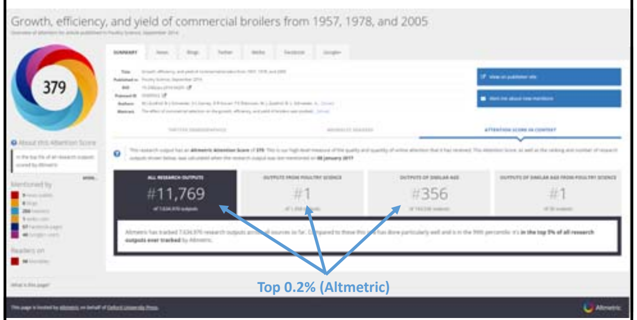
Reaction From the Science Community



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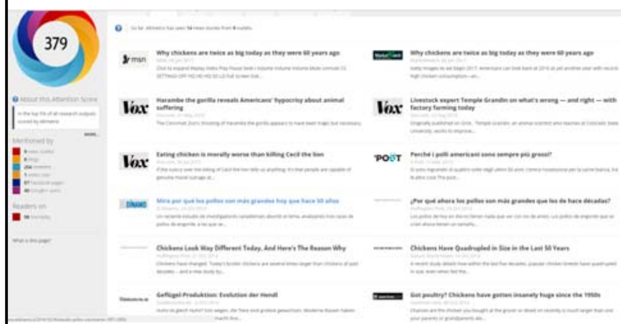
News



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SCIENCE

Chickens Look Way Different Today, And Here's The Reason Why

Chickens have changed. Today's broiler chickens are several times larger than broiler chickens of past decades — and a new study by researchers in Canada offers an explanation for why the birds got so big.

(Story continues below photos.)

1957 1978 2005

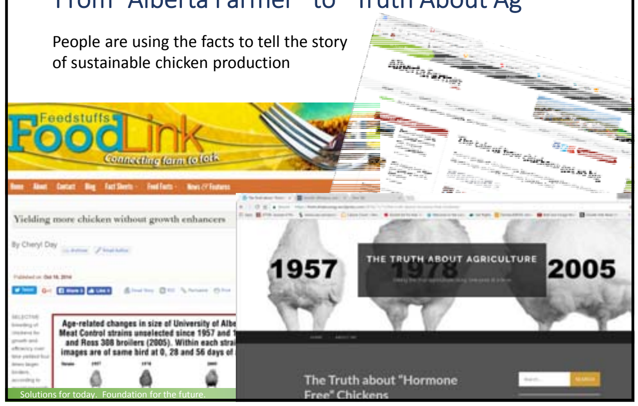
"We fed them exactly the same things, so we did not provide hormones," lead author Dr. Martin Zuidhof, associate professor of agricultural science at the University of Alberta, told the CBC. "The only difference that was part of our study treatments was the genetics." (The U.S. Food and Drug Administration has long banned the use of hormones in poultry production.)

Solutions for today. Foundation for the future.

The Poultry Research Centre
Edmonton, Alberta, Canada

From "Alberta Farmer" to "Truth About Ag"

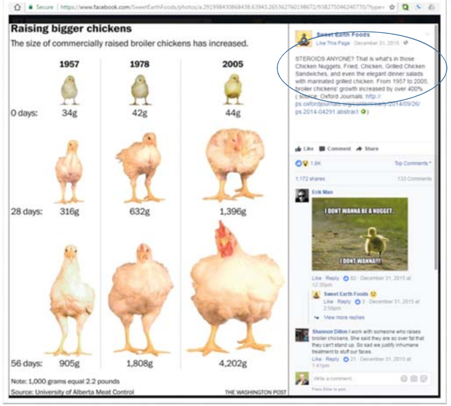
People are using the facts to tell the story of sustainable chicken production



Solutions for today. Foundation for the future.

Alternative Facts

Other people ignore the facts to promote their own version of reality



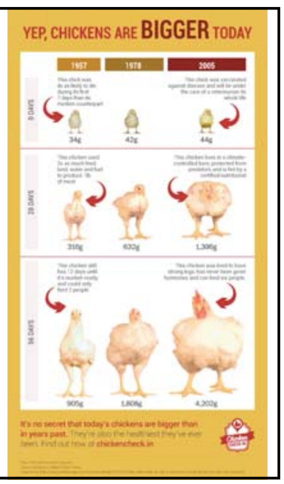
Alternative facts

The Story
is the reason we did the 50 year study

Over almost 50 years, many intended beneficial genetic changes were achieved

- Chicken production is more efficient and sustainable
- Chickens grow 4 times faster
- Chickens needed 1/3 less feed
- 42 d FCR decreased by 2.55% each year
- The pectoralis major (breast) muscle increased by 80%
- Abdominal fat significantly decreased
- Some unintended changes have occurred, and in spite of corrections, created negative perceptions
- Selection programs should continue to support socially responsible production

What parts of the story still need to be told?



Genetic Preservation Summit: Putting the Pieces Together

Survivor from the North: The Finnish Landrace Chicken Breed and a Successful Conservation Program

Mervi Honkatukia, PhD
LUKE, Finland

Survivor from the North: The Finnish Landrace Breed and a Successful Conservation Program in Finland

Mervi Honkatukia
Natural Resource Institute Finland
Jokioinen, Finland

Finnish landrace chicken is classified as a light egg layer. Meat is also consumed subsistence production and it is described as tasteful. There is large variation in phenotypic characteristics between the current family lines inside the Finnish landrace chicken. For example in body size, egg laying performance, broodiness, egg size and color. Usually eggs are small or medium size and the egg color is light brown or beige, but not white. Sometimes even green shaded eggs can been found.

Archaeological evidences are indicating that chicken appeared to Finland almost 1000 years ago. First chickens were kept only by those with elevated socioeconomic status. Over time, chickens proliferated throughout the country and eventually were established for family consumption on almost every farm. Slowly over the time village chickens isolated and formed populations which are founders of current family lines of Finnish landrace chicken.

During its history, several introgression evens occurred, although the breeds crossed were never known. At that time chickens adapted to modest living conditions (cold and poor shelter) and poor nutrition, especially in the winter time. During summer chickens were free ranging.

Large-scale egg production almost totally replaced the native breed in 1950's. Today the Finnish Landrace Chicken remains survived in several remote village population remains. The lines are named either after the place where they were discovered (Hornio, Savitaipale, Kiuruvesi, Piikkiö, Tyrnävä, Luumäki, Ilmajoki, Häme and Iitti) or after the person who found them (Lindell, Jussila or Alho).

First conservation actions were taken already in the 1960s when the 'Kiuruvesi' population or family line was rescued. The conservation programme for the endangered chicken lines was established in 1998 and it maintains today 10 different landrace family lines. The uniqueness of each discovered flock has been evaluated according to their known history and phenotypic characteristics. The programme is based on a network of over 400 hobby breeders and is coordinated by Natural Resource Institute Finland. Currently, the hobby breeders in the network have more than 5 000 Finnish Landrace hens and breeding roosters (adult, older than one year old). The modern trend of raising "city chickens" in urban areas has increased the popularity of the Landrace chicken.

The comprehensive genetic diversity study of the different lines is still in process which will reveal the genetic diversity between the lines and theirs relationships to the European breeds and commercial lines.

Progress in poultry breeding: Layers and broilers

and

Contributions of chicken populations to science and society

Dr. Paul Siegel, Distinguished Professor, Active Emeritus
Virgina Tech., Blacksburg, Virginia, USA

The domestic chicken, which originated from the Jungle Fowl of Southeast Asia, has a long and distinguished history for its contributions to human societies. Domestication may be viewed as a continuous process, and for the chicken, it had an early role in sport and culture. This was followed by its value as a reliable source of food, and in recent centuries for science. Although the initial domestication began during the Neolithic period, it was Darwin who made us conscious of diversity and variation. With the advent of Mendalism at the beginning of the 20th century, science allowed us to understand the "use" of this variation. Because of this variation, chickens could thrive in different environments. Accordingly, today's chickens are found throughout the world. Electricity and the fixed-wing aircraft were two innovations that allowed for the recent rapid global dissemination of the highly adaptable chicken.

Initially, a hen produced a clutch of eggs, incubated them, and brooded the chicks. When they left, she then re-cycled. Human intervention, using technologies and breeding programs, made incubation and brooding behaviors obsolete and thus redundant. Other changes have been dramatic. For example, Jungle Fowl commence egg production when a mere 600 g. Today, the commercial egg layer begins lay at several times that weight, and broiler breeders are feed restricted to maintain reproductive status.

Whereas many changes have occurred during the 8,000 to 10,000 years of domestication, the pace of change accelerated after the rediscovery of Mendalism in 1900 and markedly since the end of World War II – during the life of some of us at this conference! Humans design breeding programs and have a major influence on what occurs after the egg is laid. With this control, however, comes responsibility. Chickens are relatively inexpensive, amenable to experimentation, and well studied across disciplines. An important source of food (eggs and meat) without religious taboos, they are vulnerable, in an evolutionary context, to a narrowing of their genetic base. Once a gene is lost in a population, it is gone. Yes, replacement can occur through introgression if it is identifiable and present in another population. This process is costly, both biologically and economically. The caveat is that there needs to be an available donor population. Genetic variation has been the foundation for today's commercial poultry industries. It is important that this lifeline not be neglected. My presentations will provide examples of changes that have occurred in the chicken meat and egg industries in the feeding of people as well as contributions of the chicken to science.

Preserving and using unique chicken populations

Dr. Nick Anthony
University of Arkansas
Fayetteville, Arkansas, USA

The focus of this presentation is to provide an overview of the research lines developed over the past 27 years and the challenges I face in order to continue my program. When I first arrived at the University of Arkansas in 1990 I inherited a breeding program established by Dr. Roy Gyles. He had retired years earlier and his research lines were essentially being fed for years with no reproduction. Fortunately, Dr. Gyles was cooperative and interested in continuing the populations so we worked through the reproductive challenges and salvaged the rous sarcoma lines and the line he branded the Giant Jungle Fowl. The lines were further characterized with the help of Dr. Briles (Northern Illinois University). These research lines continue today.

Establishment of a colony of Japanese quail (Coturnix Coturnix Japonica) soon followed after acquiring a sample of the Eastern shore random bred control. This led to the selection program designed to evaluate the timing of selection (Day 10, 17, 28 and 40) and its impact on selection response. In addition, selection for shape of the growth curve was explored. Although these selected lines no longer exist they did provide insight into growth curve characteristics. Quail lines selected for restraint stress response (high, low stress and respective RBC; Fred Silversides, LSU) were transported to Arkansas and are still maintained today. A heavy quail RBC population was established from long term selection studies (Henry Marks, Karl Nestor). The Heavy RBC, acting as a broiler quail, served as a base population in a RFI selection study.

It was important that my research program pursue issues of industry relevance. In 1992 a subline of a pedigree male line was made available the program to serve as the base population for a set of lines to be selected for ascites susceptibility. This line had undergone several generations of relaxed selection prior to selected line initiation. A Broiler RBC was established in 1995 through the generous donation of genetic material from Derek Emmerson while at Campbell Soup Company. This line serves as a snapshot in time of available genetics. The ARB95 serves as a base population for lines divergently selected for muscle color and a second set of lines selected for 4-day breast yield. It is his hope that these research lines would dovetail with the basic and applied programs of colleagues and collaborators. These research lines should serve as a valuable resource for the identification of significant genetic markers for economic traits and metabolic disorders such as ascites and muscle quality. A new RBC was established in 2015 that is more in line with the commercial birds of today. Again a snapshot of the commercial broiler in 2015, it includes some of the warts of the modern broiler including woody breast and white striping.

Selection programs in university settings are dying across the US and Canada. There are many factors that contribute to this loss with the most obvious being cost. My presentation will conclude with a discussion of some of the factors that I have found to be detrimental to the continuing of my program. Although not comprehensive, my list of factors include university, departmental and personal challenges faced every day by researchers maintaining research lines. Research lines and selection programs are lost when your work is only important to you or you can no longer justify the cost of maintaining them. It is so important to have programs like the Genetic Preservation Summit to draw attention to our struggle to maintain a stable genetics program in the face of financial cutbacks and administrative hurdles.

Genetic Relationships among Chicken Populations in Conservation Programs

Dr. Mark Berres
UW-Madison Biotechnology Center
Madison, Wisconsin, USA

Chickens (*Gallus gallus domesticus*) are among the most important domesticated animals, having made significant contributions to human society in both economic and nutritional terms and as models for scientific research. Thought to have initiated at least 6000 years ago, domestication by phenotypic selection transformed ancestral wild junglefowl into a multitude of modern chicken breeds that subsequently spread globally following human demographic and cultural development. Now, a few companies maintain and market world-wide only a few intensively-selected chicken lines for meat and eggs. Compared to their wild ancestors, these activities have reduced genetic diversity in commercial chicken lines considerably, perhaps creating a perceived "selection wall" for specific growth and reproductive traits. Intensive selection may have also increased susceptibility to disease.

Maintaining and improving genetic diversity in chickens is critical for long-term sustainable agriculture. Genetic diversity provides the raw material to improve even strongly-selected industrial breeds and adapt them to changing environments and changing demands. Among the future challenges facing us are climate change, newly emergent disease, pressures on land and water resources, and shifting market demands, which make it more important than ever to ensure animal genetic resources are conserved and used sustainably. The practiced approach has been to passively maintain the extent of genetic variation within and among a limited number of chicken breeds, strains, and lines. Indigenous or heritage breeds (non-commercial lines created historically also through selective breeding and locally maintained) have strong potential to recover genetic variation lost in commercial chicken lines. However, they also appear at far greater risk of declining genetic diversity, including extinction, more so than compared to other domesticated mammal and avian species.

But to conserve genetic diversity, it must first be identified. Using whole-genome, next generation sequencing technology and a variety of computational approaches, we assessed the genetic relatedness of chicken populations in 1. an assemblage of Finnish Landrace chickens, 2. heritage Canadian and US Broilers, and 3. Canadian Standard breeds of chickens. The Finnish Landrace was characterized by 13 distinctive genetic clusters, which corresponded primarily to specific populations. Genetic diversity remains high, but the presence of population admixture is considerable and should serve to inform future management decisions. The population structure of both standard Canadian (including heritage broilers) and selected US lines is highly distinctive. In most cases, historical relationships are identifiable and even source populations of synthetic lines are evident.

Although once thought to be an academic view directed only toward the conservation of endangered wild species, aspects of conservation biology including population genetics and systematics along with established genetic technologies are providing unprecedented opportunities to define and ultimately conserve genetic variation in wild, heritage, and agriculturally important species. Pursuit of this goal has been informative; the average haplotype diversity of the major histocompatibility complex (MHC) between four isolated populations of wild Red Junglefowl sampled in Vietnam and seventeen lines of domestic chickens was 80% and 3%, respectively. The potential to augment commercial poultry genetic management is substantial and both wild junglefowl and heritage breeds should be considered an invaluable genetic reservoir to protect and help maintain a healthy poultry industry.

Chicken Major Histocompatibility Complex: Why variation is important

Dr. Janet E. Fulton
Molecular Geneticist
Hy-Line International

Low pathogen loads in poultry improve overall health and well being of the birds, decrease the levels of food borne pathogens and decrease the overall resource inputs required for poultry production (sustainability). Within the past decade, the ability to control many animal diseases has been greatly reduced as a consequence of the diminished arsenal of allowable veterinary medications. Furthermore there is an increasing emphasis to produce poultry products without the use of any medications, particularly antibiotics.

Genetic resistance to disease has been known in the chicken for more than 50 years. One of the primary genetic regions identified as influencing disease resistance is the major histocompatibility complex (MHC), a cluster of genes that are evolutionarily conserved in all jawed vertebrates. These genes encode proteins that are used by the immune system to identify cells that are different from 'self'. This process is important for tissue rejection and for identification of any cells that are producing foreign proteins (i.e. infected by pathogens). These 'non-self' cells are subsequently destroyed thus limiting pathogen spread within the organism. The MHC proteins are highly variable as they must recognize multiple pathogens within the host. Variation within the chicken MHC (initially called the B blood group) was first shown to influence resistance to Marek's disease virus in 1967 with specific B types being more resistant than others to the disease. Additional studies showed that variation within the MHC also affected resistance to multiple other viruses as well as bacteria (including *Salmonella* and *Escherichia coli*) and both internal (*Eimeria, Ascarids*) and extern al (Northern Fowl mites) parasites.

Chicken MHC variability was initially identified by the use of antiserum that detected the different B blood group types. This early work was done using primarily the White Leghorn breed of chickens, thus little was known about the relationship between MHC variation and disease resistance in other breeds including those utilized for production of brown shell-eggs or meat production. The recent development of DNA-based detection of MHC variation now enables rapid detection of MHC variation in all chicken breeds. Application of DNA-based detection to the heritage breeds maintained at the University of Edmonton has revealed the presence of novel MHC variation within these unique lines. These lines are an excellent resource to gain knowledge on the identification of previously unknown variation within the MHC genes. Understanding the genetic variation within these lines may lead to improved resistance to disease, particularly with those lines of relevance to meat production.

Genetic Preservation through Cryopreservation

Dr. Carl Lessard[1,2], Erl Svendsen[1], Crissandra Auckland[1], Pamela Hind[1]
[1]Agriculture and Agri-Food Canada, Saskatoon, SK; [2]University of Saskatchewan, Saskatoon, SK

With agriculture intensification, genetic diversity of livestock and poultry breeds has significantly declined over the last few decades. To fight against its erosion of animal genetic resources, Agriculture and Agri-Food Canada launched the Canadian Animal Genetic Resources (CAGR). The mission of CAGR is to preserve the genetic diversity of livestock and poultry breeds by preserving germplasm and gonadal tissue. For poultry breeds, CAGR is developing a method to preserve gonadal tissues (by vitrification) from 1-day old donor chicks and then restore them by transferring the gonads into a recipient of same age but with different genetic background. Fertilized eggs from the targeted breed to be preserved were incubated in our facility at Saskatoon. Testes or ovary were harvested from newly hatched chicks and submitted to a standard vitrification procedure (15 min in VS1 (7.5% Ethylene glycol and DMSO); 3 min in VS2 (15% Ethylene glycol and DMSO; plunged in liquid nitrogen). On the day of the transplant (restoration) surgery, vitrified gonads were warmed in a series of solutions of decreasing sucrose concentrations (from 100% to 0%). Recipients (different genetic background from donor breed) were orally administrated with MetaCam to minimize pain after surgery. They were anesthetized with isoflurane gas and the incision area was shaved and cleaned. A small incision (around 2 cm) into the left abdomen was made. The yolk sac was carefully removed to provide space to reach the recipient gonad. Fine forceps of 45º angle tips was used to carefully remove the recipient gonad and to introduce the vitrified-warmed gonad. An orthotopic transfer (put at the same location) was performed for the graft and it was not attached into the recipient. After closing the incision, saline and antibiotic were administrated to the recipients before removing the isoflurane gas. On average, it took 5 minutes for the recipient to wake up after surgery. Post-surgery and until reproductive maturity, recipient birds initially received an immunosuppressant (mycophenolate mofetil, 100 mg/Kg) administrated daily for the first three weeks and then subsequently twice a week. Female recipients laying eggs were inseminated with semen from a rooster with a genetic background identical to the graft and male recipients were sacrificed to recover sperm cells from the graft. In 2015, 22 recipients received grafts (15 ovary and 7 testes); only one male successfully grew its graft to maturity. Matured sperm cells were recovered from this graft. For these experiments, CAGR has used three different recipient lines to test their ability to support the growth of grafts. Only one of them demonstrated ovarian growth; however, folliculogenesis was not observed on the recovered graft. Growing testis graft does not show the same difficulties as ovarian grafts; 50% of male recipients will support the growth of testis grafts. Moving forward, other recipient lines are currently being tested to find the best recipient to support ovarian growth.

Survivor from the North: The Finnish Landrace chicken breed and a successful conservation program

Mervi Honkatukia

Finnish Landrace chicken – an old native and endangered breed

- archaeological evidences indicate that chickens existed in Finland at least 1,000 years ago
- first chickens were kept only by those with high socioeconomic status
- well adapted to cold condition and poor nutrition, especially in the winter time.
- during summer chickens were free ranging
- icon of survivor in harsh northern conditions

Genetic background

- several independent introgressions occurred during the last centuries
- the breeds used for admixture are not known
- evidence from Fulton et al. (2017): mixed with some modern breeds (WL, NH, RIR)
- heterogenous group of animals: multiple populations were maintained in isolated villages

Persistent, longevity and seasonal: special features for the Finnish Landrace chickens

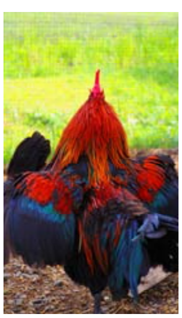
- no artificial selection (except for plumage color in some populations)
- variable in plumage colours: from very light beige to black
- the oldest known chicken 14 years

- average hen weight 1500 gr (varying from 500 to 3500 gr)
- layes egg on average every 2-3 days (production rate 60%)
- the average sexual maturity 22 weeks of age

Follow the seasonal rhythms

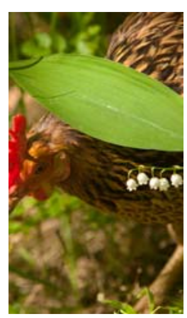
- feather molting in autumn
- a break in egg laying break during the darkest winter
- broodiness in spring: (with exeptions: two populations do not brood; one is is 'an incubator').

Character



- tame and curious
- good capability to 'fly'
- have a habit to hide eggs on the nest under the bushes or in the woods
- males are guarding their flock hardily

The Finnish landrace chicken became rare



- large-scale egg production and commercial hybrids replaced the local native breed starting from the 1920's-30's
- first conservation actions were taken in the 1960s when the 'Kiuruvesi' population was rescued
- another population 'Savitaipale' was found ten years later, by a scientist

- later on, an inventory revealed over 10 populations more in 1990's
- the conservation program was founded 1998
- the conservation program was published also as a BSc thesis at University of Applied Science by Tarja Ojanne

- The conservation program of the Finnish Landrace chicken belongs to the **National Animal genetic Resources Strategy of Finland** which is supervised by the advisory council nominated **by Ministry of Agriculture and Forestry of Finland**

The conservation program aims to maintain

- genetic and phenotypic diversity
- breed purity

In addition to…

- cultural values
- historical values
- scientific values of the breed

The conservation program in practice

- based on the voluntary network of hobby breeders
- coordinated by Natural Resources Institute Finland (Luke) (former MTT)
- practical work is supported by a 4-member advisory group
- open for new breeders
- The Finnish Poultry Association and Finnish Food Safety Authority-EVIRA are contributing by sharing knowledge

Rules of the conservation program

- a new breeder purchases the stock from the network
- breeders are not allowed to keep any other chicken breeds (with few exceptions)
- breeders submit an annual report to the coordinator
- breeders are promoting the conservation program to new candidates

The coordination in Natural Resources Institute Finland (Luke)

- maintains the database and information gathering
- responsible for communicating (website, FB, media)
- publishes the annual newsletter on conservation activities
- organizes annual meetings, courses and provides consultation
- implements research activities
- reports to the advisory group and the Ministry of Agriculture and Forestry

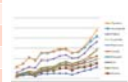
Benefits for a breeder?

- guarantee of the breed purity and traceability of breeding animals
- possibilities to connect with people sharing the same values and interests
- group support from the network members
- assistance given by the coordinators
- eduacation on wished topics (in seminars)
- annual summer meeting
- option to get subsidy for the conservation (EU and national funding for conservation of native breeds)

Current status

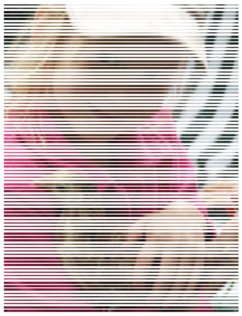
- 10 maintained populations
- 407 breeder members
- over 5000 hens and rooster
- female:male ratio 4:1

	Female	Males	Total*
Alho	899	207	**1107**
Hornio	205	57	**262**
Häme	228	47	**275**
Iitti	320	87	**407**
Ilmajoki	412	110	**522**
Jussila	104	36	**140**
Kiuruvesi	419	107	**526**
Piikkiö	421	115	**536**
Savitaipale	382	99	**481**
Tyrnävä	653	155	**808**
	4013	**1020**	**5157**



Population trends in 1998- 2013

Future? Experiences, opinions and future needs are summarized in a thesis of University of Applied Science by Kaisa Auvinen



- **the main driving forces amongst the breeders are**

1) The appreciation of the native breed as **a valuable genetic resource**, and as an important part of cultural history.

2) native breed as an important part of cultural history

3) the conservation program is regarded valuable

Current trends are favoring and increasing the popularity of native chicken

- “city chickens” in urban areas
- “local food”
- “green care”
- “back to nature”

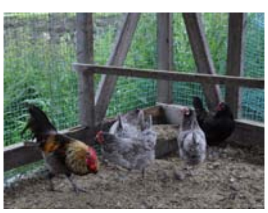
- breeders are very committed to the conservation work
- the conservation program brings together a network of various professionals (such as teacher, priest, metal worker, geologist, gardener, cantor, officer, editor, designer, MD, etc…)
- especially young people are joining in
- social media is useful tool for networking and quick communication

- According to Fulton et al. 2017, Finnish Landrace conservation program has successfully conserved for instance MHC variability (polymorphism in the immune response genes)
- this encourages us to continue our conservation work

Field research activities: cryopreservation & mycoplasma mapping

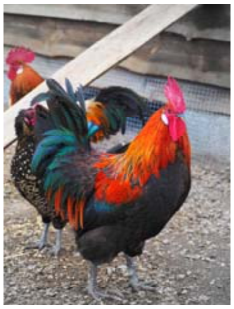
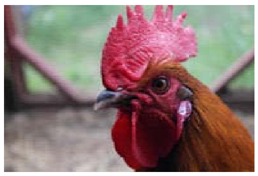
Alho

Häme



Hornio

Iitti

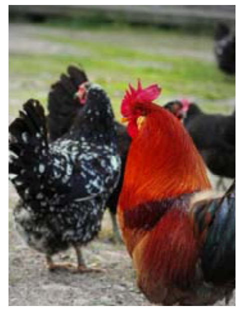
Ilmajoki


Jussila


Kiuruvesi


Piikkiö

Savitaipale



Tyrnävä

Thank you!

Luke's AnGr team

Professor **Juha Kantanen**
Senior Scientist **Mervi Honkatukia**

Pictures © Mia Laitinen, Tiina Herranen, Marjatta Sihvonen, Tapio Eerola, Kirsti Hassinen, Inga Pyykkö, Titta Jönkkäri, Kristiina Salonen, Vuonue ja Viipsinpuu, Mervi Honkatukia

Genetic Preservation Summit: Putting the Pieces Together

Contributions of Chicken Populations to Science and Society

Paul Siegel, PhD
Virginia Tech

Contributions of chicken populations to science and society

Paul B. Siegel 2017

January 23,1864 THE ILLUSTRATED LONDON NEWS

PRIZE PIGEONS AT THE SHOW OF THE PHILO-PERISTERON SOCIETY, RECENTLY HELD IN FREEMASONS' HALL

Fancy pigeon breeds as exhibited in 1864 by one of the London pigeon clubs to which Darwin belonged. All domesticated pigeons are descended from the rock dove, which closely resembles the feral pigeon familiar to town-dwellers.

Barred Plymouth Rock

Black Langshan

White Silkie

Poultry science is an evolutionary process

Where we have been

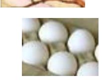
Where we are now

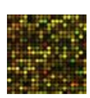
Look to the future --

Poultry Development

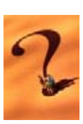
Past:


Present:



Future:



The chicken has been and remains a key in evolutionary biology and food production

- It is common
- It is accessible

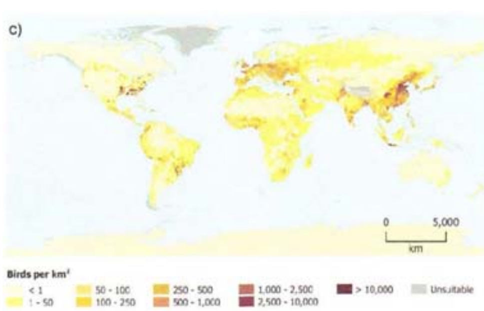
Traits that favored domestication

- Group structure
- Promiscuity
- Precocial young
- General dietary habits
- Adaptability

Although they may cross freely with their jungle fowl ancestor, today chickens have seen much human intervention

– a chicken is not a chick is not a chicken.

Global Distribution of Chickens



T. Robinson et al., 2014

Traits that favor genetic analyses

- Can control mating
- Develops outside the mother's body
- Large populations
- Inexpensive
- Mutants
- Amenable to experimentation
- Well studied in other disciplines

Some firsts (science)

1902 – 1st animal species for Mendelian inheritance

Pea comb

2011 – 107 years later SLU scientists showed the mechanism

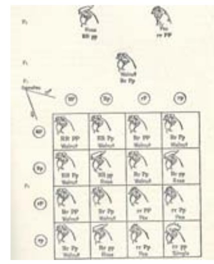
PLoS Genetics

Photo from Poultry Tribune, Mount Morris, IL

Firsts (cont'd)

1905-1908 – Complimentary gene action

Bateson



Graphic from Principles of Genetics (Sinnott, Dunn, & Dobzhansky)

Firsts (cont'd)

1936 – 1st linkage map for farm animals

Hutt

2004 – 1st genome sequence for farm animals

1995 – 1st set of genes for expression of left-right asymmetry

Levine et al. (cell)

Science per se
e.g. Nobel prizes

1929 – Importance of dietary vitamins

Eijkman & Hopkins

1943 – Discovery of vitamin K

Dam & Doisy

1951 – Yellow Fever vaccine

Theiler

1952 – Discovery of streptomycin

Waksman

Science per se e.g. Nobel prizes

1966 – Rous sarcoma
Rous & Huggins

1973 – Social behavior patterns
von Frisch, Lorenz, & Tinbergen

1975 – Reverse transcription & formation of provirus hypothesis
Dulbecco, Temin, & Baltimore

Science per se e.g. Nobel prizes

1989 – Isolation of 1st cellular oncogene
Bishop & Varmus

2015 – Therapies to treat parasitic infection
Campbell, Omura, & Tu

An opinion

Advances by poultry scientists have been preceded by technological changes – the incubator, computer, PCR, amino acid analyzer, microscope.

As Food

- No religious taboos
- Highly efficient converter of plant to animal protein
- Alive (egg)
- Dead (meat)

1950 and current chickens

Entries ready for judging in 1947 Chicken of Tomorrow Contest.

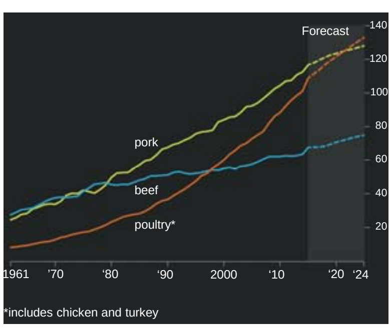
Bulkier Birds

Careful genetic selection has resulted in a much larger commercial broiler chicken compared to those raised nearly 60 years ago. The growth of three strains of broiler chickens – two strains genetically unchanged since 1957 and 1978, and a third from 2005 – are shown below.

Source: Wall Street Journal: Poultry Science, Zuidof, Schneider, Carney, Korver, & Robinson (2014); Univ. of Alberta, Dept. of Ag, Food, & Nutritional Sci.

World Meat Production

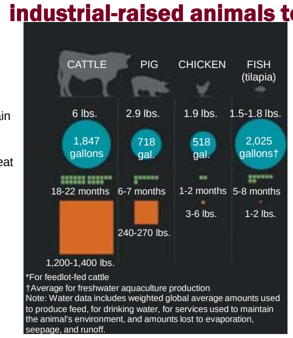
By type, in millions of metric tons



Source: Wall Street Journal: Food and Agriculture Organization of the United Nations; Organization for Economic Cooperation and Development (forecast)

On Their Way To Market

What it takes to bring four typical industrial-raised animals to market



Source: Wall Street Journal: Univ. of Illinois (cattle ratio); Cattlemen's Beef Board & National Cattlemen's Beef Assn. (wt., time); Iowa St. Univ. (pig ratio); USDA (pig wt., time); National Chicken Council (ratio, wt.); LSU Ag. Center (chicken time); Food & Ag. Org. of the United Nations (fish ratio, wt., time); Ecosystems, Mekonnen & Hoekstra, 2012 (water); Water Policy, Verdegem, & Bosma, 2009 (fish water)

Some Givens

When biological, economical, and physiological views clash, knowledge rules over opinion

Some Givens

Poultry science is based on knowledge and science drives technology

Summary

Historical is the present tense in telling of past events, whereas the future is that to be.

It is one thing to look back, and from it is there advice for predicting?

Summary Cont'd

"The only way to predict the future is to have the power to shape the future."

Eric Hoffer

The wheels of a cart are not independent

The phenotype

The genotype

The environment

- Past
- Present

123rf.com

A Final Thought

Once the gene is lost it is gone.

In breeding programs we must always be aware of this caveat.

Genetic Preservation Summit: Putting the Pieces Together

Insights from long term selection of white leghorns for high and low antibody response

Chris Ashwell, PhD
North Carolina State University

Insights from long term selection of white leghorns for high and low antibody response

CHRIS ASHWELL
PRESTAGE DEPARTMENT OF POULTRY SCIENCE
NC STATE UNIVERSITY

NC STATE UNIVERSITY
Prestage Department of Poultry Science

Why do we care about antibody response?

GOAL: Better understand the biology of antibody response…

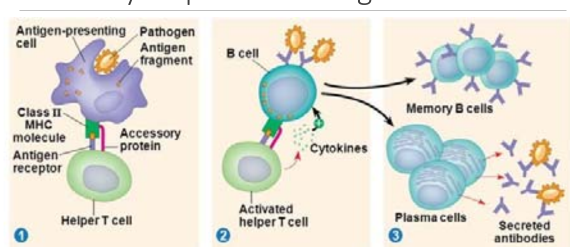
and its role in poultry production.

Vaccine market $20Bil
~62% livestock

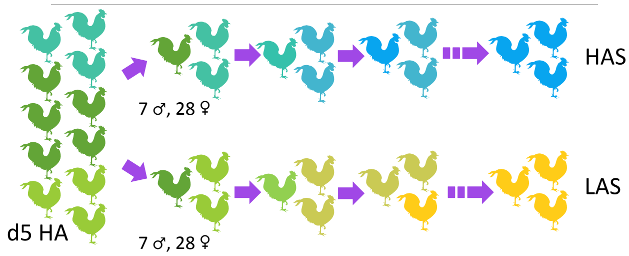
Outline

- Why?
- Selected lines
- Phenotypic data
- Molecular data
- Insights into antibody response

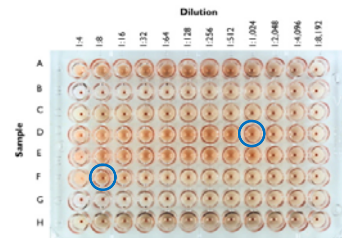
Antibody response to antigen



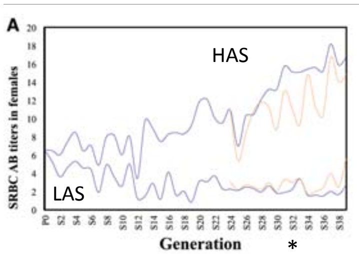
Selection of the lines HAS and LAS
Inject @ d42 with 0.1ml of 0.25% SRBC – IV



SRBC antibody assay – Hemagglutination assay



Changes in SRBC antibody response



- > 6 Std dev difference in d5 Ab production
- Selection relaxed at Gen 24 to produce the HAR & LAR lines
- *Gen 32 used to produce an F2 cross

Similar selection populations

- Va Tech – Selected for SRBC (low) injected IV
- Wageningen - Selected for SRBC and KLH injected IM
- Israel – Selected for E.Coli injected Subcutaneous
- Japan – Selected for Newcastle virus antigen injected IP

Response to selection in HAS and LAS

- Body weight differences LAS > HAS
- Fixation of MHC haplotypes
- Response to disease challenges…
- Differences in genes/loci ?
- Differences in microbiota?

Disease resistance?

	High	Low	Defense mechanism
Antibody	HAS	LAS	**B lymphocytes**
Body Weight	LAS	HAS	**Resources**
	Resistant	**Susceptible**	
Mycoplasma	HAS	LAS	**Antibody**
E. coli	LAS	HAS	**Heterophils**
Avian Adenovirus II	LAS	HAS	**T lymphocytes**
Mycobactrium	LAS	HAS	**Macrophages**
Northern fowl mite	HAS	LAS	**Antibody**
Newcastle	HAS	LAS	

Questions…

What genes (loci) contribute to the differences between HAS and LAS?

To what degree does MHC contribute?

Are resources allocated differently between the lines?

How to the lines respond to vaccine?

Is the difference due to differential antigen recognition? Different immune cell populations?

What environmental factors influence the phenotypic difference?

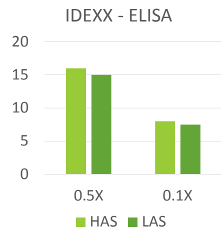
Effect of the MHC B haplotype

- At Gen 16 LAS was fixed for B13, while HAS had both B21 (0.73) and B13 (0.27)
- Gen 32 LAS = B13 and HAS = B21

	HA		LA		Citation
Generation	*freq*(B^{21})	*freq*(B^{13})	*freq*(B^{21})	*freq*(B^{13})	
S10	0.80	0.15	0.01	0.99	Martin et al. 1990 [29]
S13	0.99	0.01	0.02	0.98	Martin et al. 1990 [29]
S32	1	0	0	1	Dorshorst et al. 2011 [48]
R16	0.73	0.27	0	1	Lillie et al. 2017

Response to vaccine

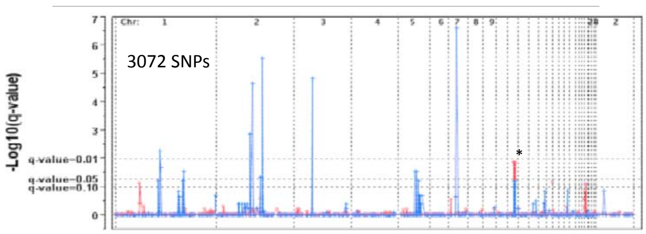
- HAS and LAS birds were vaccinated at 42 days of age with either 0.5X or 0.1X of a standard Newcastle disease virus vaccine (Merial)
- Blood samples were collected on D0 prior to vaccination, and on d5, and d12.
- Sera were evaluated for antibody production by IDEXX ELISA
- No significant differences in HAS vs. LAS Ab titers at either dose.
- HAS is very sensitive to low antigen levels?



F2 cross of HAS x LAS

- 8 HAS males x 32 LAS females & 8 LAS males x 31 HAS females
- 322 F1 progeny
- 8 males and 32 females from each reciprocal cross mated to produce F2
- 513 F2 progeny
- F2 injected at d42 with 0.1ml of 0.25% SRBCs IV, d5 and d12 HA, BW every 2wk, females kept until age of first egg
- Genotyping of 192 F2 from the tails of the d5 distribution

Genome-wide trait association- F2 cross



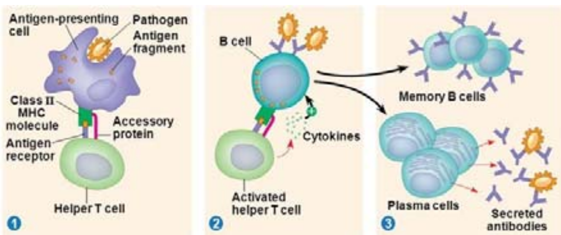
* MHC represents <10% of variation in d5 Ab

Dorshorst et al, 2011

Conclusions of the F2 association study

- MHC contributes ~10% of the phenotypic variance in d5 Ab titer
- Other Loci account for ~30% of phenotypic variance in d5 Ab titer
- Need greater marker density?
- Need more mieoses? Advanced intercross to break up linkage…F8
- Sex effects imply the presence of a contributing locus on the W chromosome, a parent of origin effect on an autosomal locus, or the influence of maternal antibodies in the egg.
- What is the source of the missing ~60% of the variance? Environment?

Antibody response to antigen



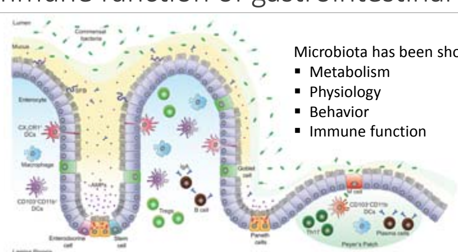
Immune cell populations?

Characterization of B-cell populations in the bursa showed no differences between HAS/LAS.

John Driver at University of Florida looked at blood, spleen, and thymus cell profiles.

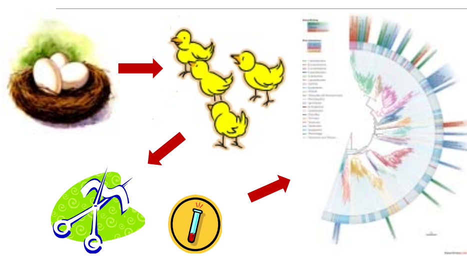
- Significantly higher CD4+ T-cells in HAS compared to LAS

Immune function of gastrointestinal tract



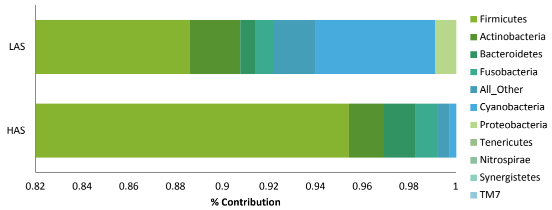
Microbiota has been shown to influence…
- Metabolism
- Physiology
- Behavior
- Immune function

Gastrointestinal Microbiomes of HAS/LAS

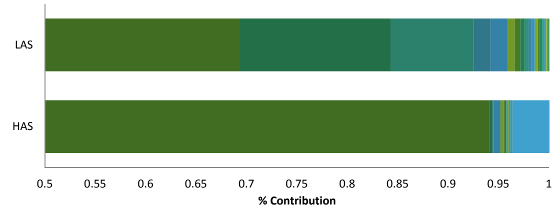


16S rRNA pyrosequencing and taxa analysis using QIIME

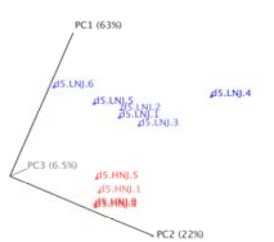
Intestinal microbiome phyla



Firmicute Diversity
– HAS > 94% lactobacillus

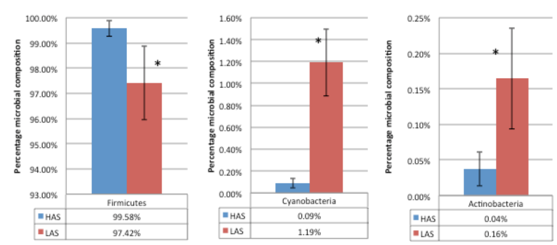


PCA analysis of HAS LAS microbiota



Clear clustering of samples from each line
– jejunum d5 post SRBC challenge

Specific differences in bacterial species - jejunum



Microbiome conclusions

- HAS is very homogeneous - >90% lactobacillus
- LAS is much more heterogeneous
- Interactions with gut microbiota may adjust the sensitivity for the mucosal immune system
- Low microbiota diversity = More sensitive to antigens?
- High Microbiota diversity = Less sensitive to antigens?

Further questions?

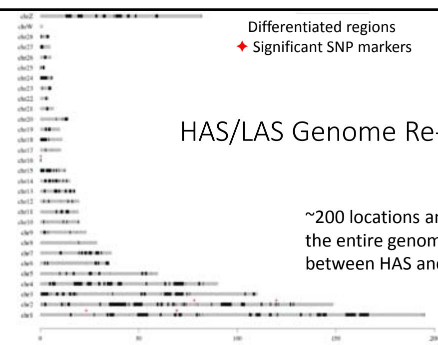
- What gut metabolic mechanisms distinguish the HAS and LAS lines?
- How has selection for primary antibody response remodeled the microbiota?
- Can the difference in gut microbiota explain the differences in growth rates in the HAS and LAS lines?
- Fecal transplant?

Genome (re)sequencing of HAS LAS

- 2010 – 4x coverage of pooled HAS LAS samples generated some sequence variants

Population	N	Day 5 log_2(AB titers)		Genome coverage
		Mean	sd	
HAS39	30	16.7	4.3	32.3
LAS39	30	2.6	1.6	36.7
HAR16	20	15.0	3.2	35.4
LAR16	16	5.3	2.2	34.8

Lille et al, 2017



Differentiated regions
✦ Significant SNP markers

HAS/LAS Genome Re-Sequencing

~200 locations and around 20% of the entire genome is differentiated between HAS and LAS

Lille et al, 2017

Insights…

- Immune response is complex
- Selected lines like HAS and LAS are extremely useful for understanding how important biological functions work – like immunity
- Phenotypic differences between HAS and LAS are caused by…
  - Haplotype differences in many genomic regions ~200
  - Major genes – MHC, TGFBR2, SEMA5A
  - T-cell population differences
  - Gut Microbiota

Acknowledgements

- Paul Siegel – Va Tech
- USDA- Animal Health Funds
- NC Agriculture Foundation
- USDA- NRSP-8 Animal Genome Program

RES and SUS lines

  - Ben Dorshorst
  - Shelly Druyan
  - Candace Smith
  - Shelly Nolin
  - Zack Lowman

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Prestage Department of Poultry Science

Genetic Preservation Summit: Putting the Pieces Together

Genetic Relationships Among Chicken Populations in Conservation Programs

Mark Berres, PhD
University of Wisconsin - Madison

Genetic Relationships among Chicken Populations in Conservation Programs

Genetic Preservation
Summit:
Putting the Pieces Together
May 24-25, 2017

Alberta Chicken Producers Poultry Technology Centre
University of Alberta South Campus

Mark E. Berres
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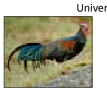
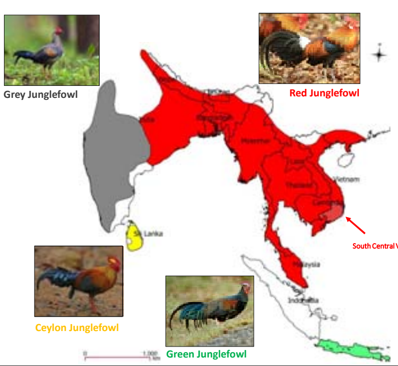
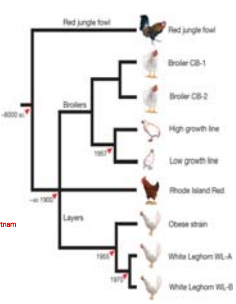
University of Wisconsin Bioinformatics Resource Center
WISCONSIN UNIVERSITY OF WISCONSIN-MADISON



Origins of livestock domestication

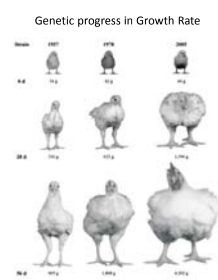
(1) Turkey; (2) Guinea Pig, Llama, Alpaca, Muscovy Duck; (3) Rabbit; (4) Donkey; (5) Taurine Cattle, Pig, Goat, Sheep; (6) Dromedary; (7) Zebu cattle, River Buffalo; (8) Bactrian camel; (9) Horse; (10) Reindeer; (11) Yak; (12) Pig; **(13) Chicken**; (14) Swamp Buffalo; (15) Bali Cattle.





~ 1800 chicken breeds

What have we done?



How did we do it?
Mass phenotype selection, Selection index, BLUP,
QTL, Marker-assisted selection, Genomic selection/prediction

What else happened?

Red Junglefowl (Vietnam)

3% MHC diversity in commercial lines

80% in Red Junglefowl

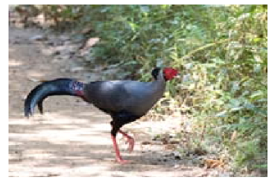
MHC haplotypes of Red Junglefowl vs domestic chickens

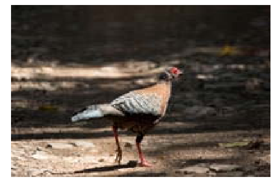
Line type	No. Samples	No. Haplotypes	Nucleotide diversity π	Haplotype diversity H_d	Haplotype percentage
Red Junglefowl	199	310			
CTN	**46**	**92**	**0.29260**	**100.00%**	**100.00%**
HBA	**56**	**82**	**0.28460**	**98.97%**	**73.21%**
LGO	**39**	**48**	**0.27900**	**97.86%**	**61.54%**
YDN	**58**	**91**	**0.28780**	**99.39%**	**78.45%**
Broiler-UAB-AMC-1957	71	8	0.25347	80.42%	5.63%
Broiler-UAB-AMC-1978S	64	5	0.19893	63.78%	3.91%
Broiler-UAB-AMC-1978D	78	10	0.25537	80.98%	6.41%
Broiler-UGA-ACRB	100	11	0.25796	80.32%	5.50%
Broiler-UGA-ARB	71	4	0.21043	70.95%	2.82%
Broiler-UAR-RB	54	7	0.26625	75.86%	6.48%
Standard-UAB-BPR	76	4	0.24627	73.50%	2.63%
Standard-UAB-SBPR	80	4	0.25612	70.57%	2.50%
Standard-USK-BPR	96	2	0.04281	17.13%	1.04%
Standard-UAB-SRIR	80	4	0.11339	40.58%	2.50%
Standard-UAB-WL	72	3	0.10629	33.41%	2.08%
Standard-UAB-LS	77	3	0.18736	57.92%	1.95%
Standard-UAB-NH	73	4	0.05272	55.93%	2.74%
Standard-Ill-NH	94	3	0.05874	33.23%	1.60%
Standard-UAB-BL	76	1	0.00000	0.00%	0.66%
Synthetic-Ill-PC	92	3	0.13741	52.47%	1.63%
Synthetic-USK-EPI	97	9	0.23621	66.90%	4.64%

Poultry Science. 2016 Feb;95(2):400-11

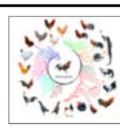
Conservation genetics

"With the accelerating destruction of the world's wildlife and wildlands, gene pools are becoming diminished and fragmented into gene puddles" Thomas Foose (1983)





Genetic diversity



1. Fundamental Theorem of Natural Selection
   "Natural selection is a mechanism for generating an exceedingly high degree of improbability." R.A. Fisher
2. Heterozygosity is often positively correlated with fitness
3. The global pool of genetic diversity represents all information for all biological processes.

Sulmtaler

Ayam cemani

Is conservation genetics needed in poultry?

GENETICS

POLICY FORUM

Poultry Genetic Resources—Operation Rescue Needed

Janet E. Fulton and Mary E. Delany

Dating from the early genetic studies of William Bateson on comb inheritance, which demonstrated both that Mendel's Laws applied to animals and the phenomenon of epistasis (*1*), poultry have been a consistent part of the genetics research landscape. Specialized experimental populations have been developed, characterized, and studied during the previous 60 years that have contributed to advances in biology (e.g., vertebrate limb, skeletal, and craniofacial development), biomedicine (e.g., viral oncogenesis, scleroderma, vitelligo, muscular dystrophy, autoimmune thyroiditis), and agriculture (e.g., genetic basis for resistance and susceptibility to disease) (*2–8*).

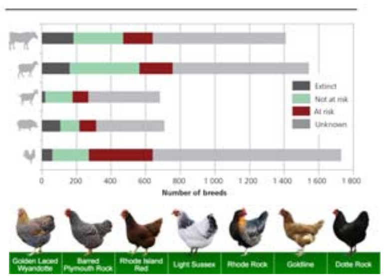
well as individual stocks within collections. Lines were dropped which had no counterpart, no secondary backup location, and typically with little advance warning to the research community. In 1998, the survey reported 323 living stocks in the United States and 45 in Canada, and there have been further losses since then.

An example of an entire collection on the brink of elimination is that held by the University of British Columbia. The Japanese quail (*Coturnix japonica*) is an excellent avian laboratory model because of its small size, prolificacy, and early onset of sexual maturity. Japanese quail are the only nonprimate model for the study of age-related macular degeneration, because they age rapidly and have a cone-dominant retina (*15*). In 1996, the University of British Columbia in Canada had the largest collection of mutations and

Science 13 June 2003: Vol. 300, Issue 5626, pp. 1667-1668

Status of the world's livestock breeds

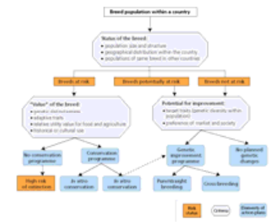
...



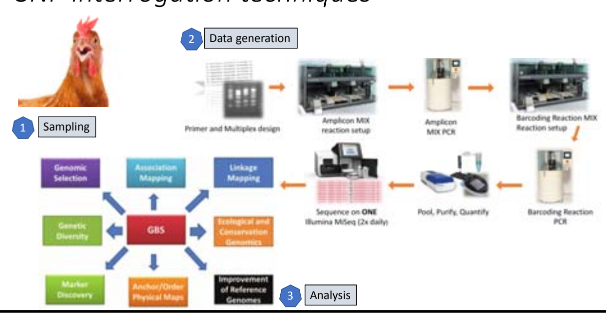
...

How is conservation genetics done?

- Identification, Inventory
  - "Field work"
- Analysis and Interpretation
  - "Laboratory work"
  - "Thinking work"
    - This is really the difficult part
- Management
  - "Doing work"
    - Also very difficult



SNP interrogation techniques

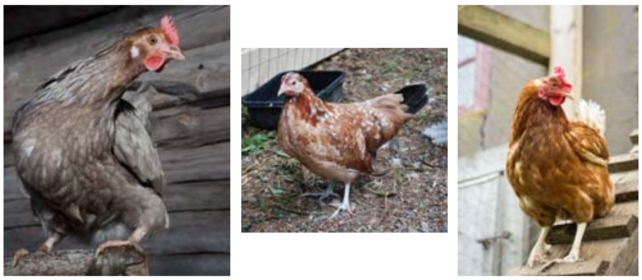


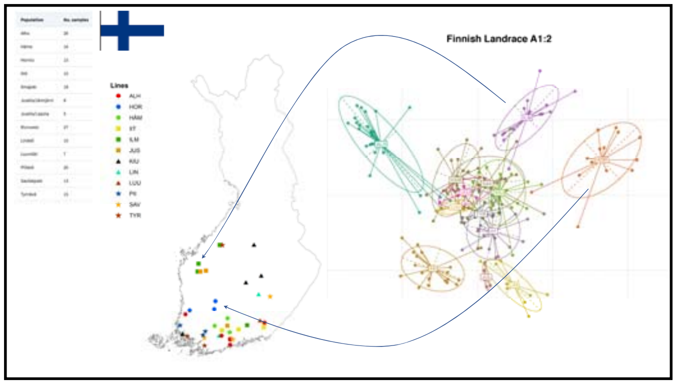
SNP interrogation techniques

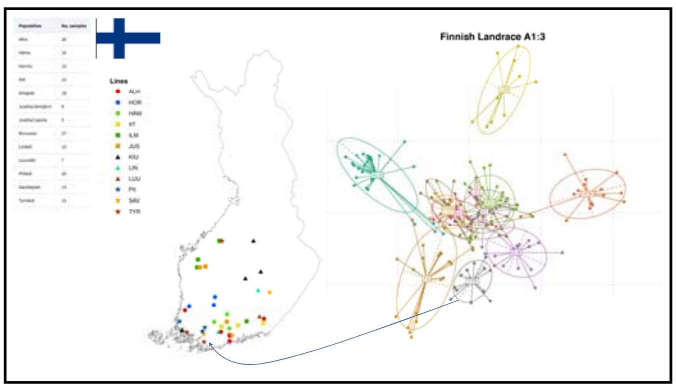
- 97% successful SNP design with a 98% sample call rate
- Quantitative genotype result based on allele frequency of NGS reads is critical for analysis of polyploid samples
- High call rate with crude extraction methods
- Only 4 ng DNA used per multiplex reaction
- Highly flexible to accommodate varying sample numbers and SNPs

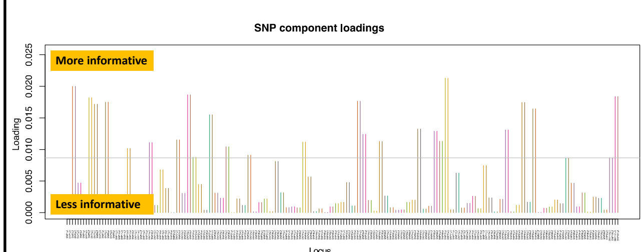
SNP FAILme	SNP_001	SNP_002	SNP_003	SNP_004	SNP_005	SNP_006	SNP_007	SNP_008	SNP_009	SNP_010	SNP_011	SNP_012	SNP_013
SNP ID	rs13827582	rs13845815	rs13895651	rs13918857	rs13929720	rs13777960	rs13624986	rs13960246	rs13989181	rs14139664	rs14153518	rs14161225	rs14164722
Kracken ID	X-PAT-01-0179	X-PAT-01-0310	X-PAT-01-0850	X-PAT-01-1080	X-PAT-01-1201	X-PAT-01-1224	X-PAT-01-1329	X-PAT-01-1620	X-PAT-01-1854	X-PAT-02-0126	X-PAT-02-0245	X-PAT-02-0324	X-PAT-02-0363
Allele_1	G	C	G	A	C	C	A	A	A	A	G	A	C
Allele_2	A	T	T	G	T	T	G	C	G	G	T	G	T
FIN-TYR	A	C / T	T	A / G	C / T	T	G	A / C	A / G	G	G / T	A	T
FIN-TYR	G / A	C / T	T	G	C / T	T	G	C	G	G	G	A	T
FIN-TYR	G / A	C	T	G	C / T	T	G	C	G	G	G	A / G	T
FIN-TYR	G / A	C / T	G / T	G	C / T	T	G	C	A / G	G	G / T	A / G	T

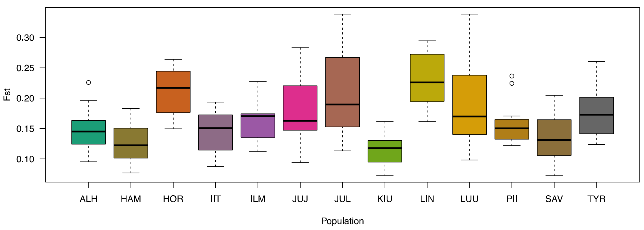
Maatiaiskana (Finnish Landrace)



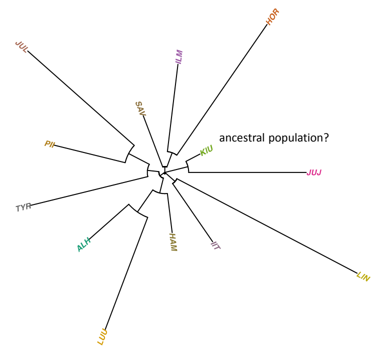


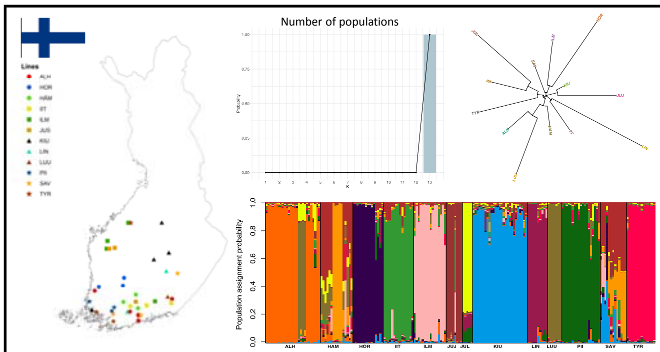






Population	No. samples
Alho	26
Häme	16
Hornio	15
Iitti	15
Ilmajoki	18
Jussila/Jämijärvi	8
Jussila/Lepola	5
Kiuruvesi	27
Lindell	10
Luumäki	7
Piikkiö	20
Savitaipale	13
Tyrnävä	15

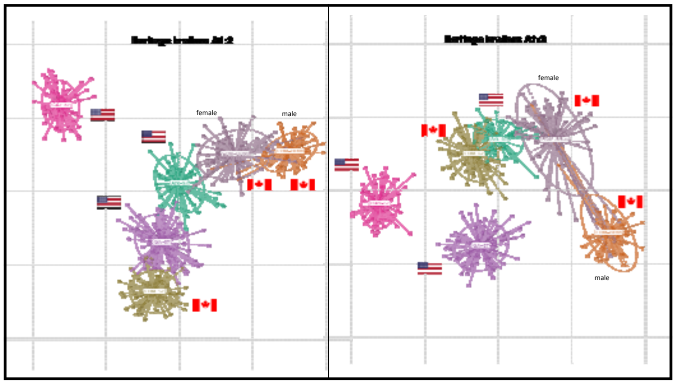




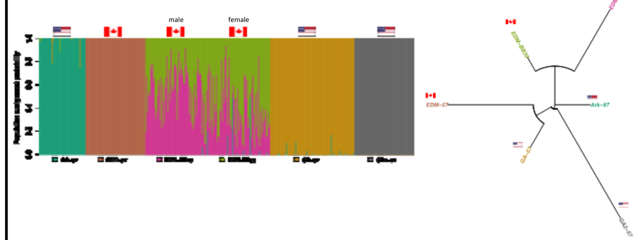
Heritage broilers – Canada and US

- EDM-57: developed in 1957 @ Ag Canada.
  - Housed now at University of Alberta
- EDM-BB20: male line developed in 1978
- EDM-BB30: female line developed in 1978

- ARK-97: University of Arkansas
- GA-57: derived from EDM-57 in 1957
  - Synonym: ACRB (Athens Canadian Random Bred population).
- GA2-57: NOT derived from EDM-57
  - Both GA- and GA2-57 @ University of Georgia

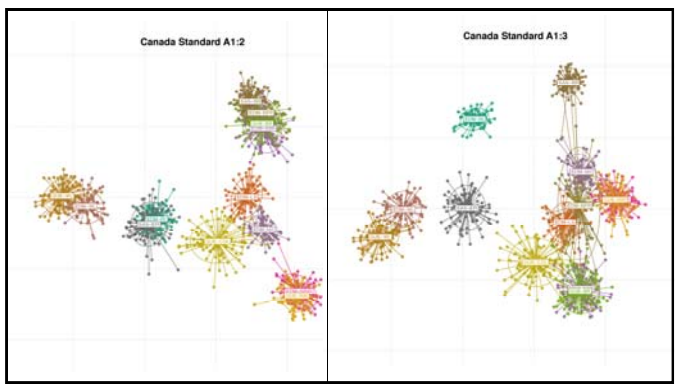


Heritage Broilers – Canada and US



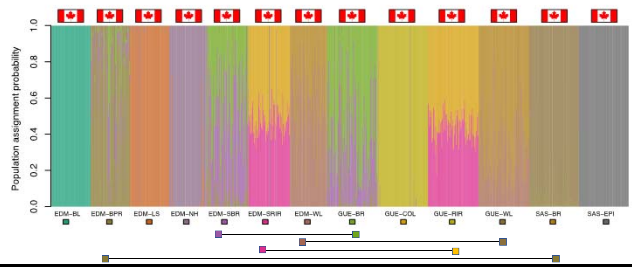
Standard breeds – Canada

- EDM-BPR: sourced from SAS-BR, 25 years ago
- EDM-SBR: Shaver, Barred Rock, 15 years ago
- EDM-BL: Brown Leghorn (heritage)
- EDM-LS: Light Sussex (heritage)
- EDM-SRIR: Rhode Island Red, Shaver, 15 years ago
- EDM-WL, White Leghorn
- EDM-NH, New Hampshire
- GUE-BR: Guelph BR; Shaver
- GUE-Col: Guelph Columbian Rock; Shaver
- GUE-RIR: Guelph RIR; Shaver
- GUE-WL: Guelph WL
- SAS-EPI: Composite cross
- SAS-BR: bottleneck @ 1990s



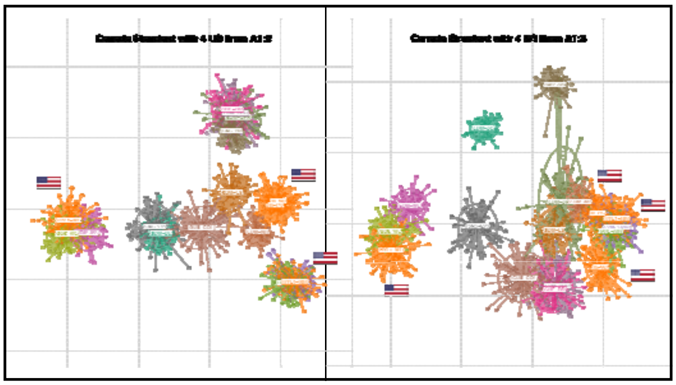
Standard lines– Canada

Standard lines– Canada

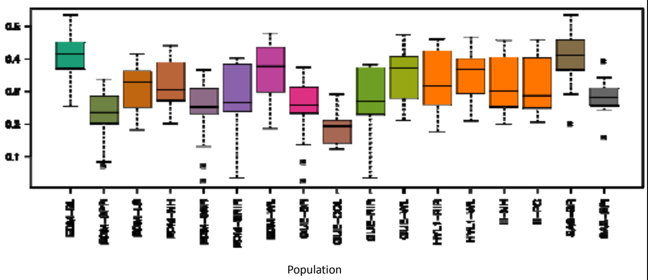


Standard lines – Canada + 4 US lines

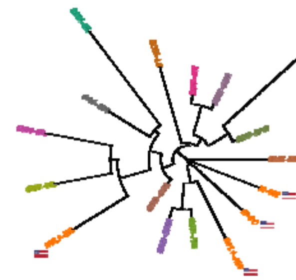
- Chets NH: NH breed from University of IL (slow broiler)
- Chets PC: Breed from University of IL (synthetic, slow broiler)
- HYL1-WL: Hyline White Leghorn
- HYL1-RIR: Hyline Rhode Island Red



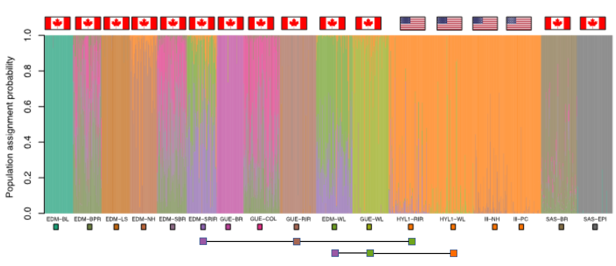
Standard lines– Canada + 4 US lines



Standard lines– Canada + 4 US lines



Standard lines– Canada + 4 US lines



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Biotechnology Center

Bioinformatics
Resource
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Genetic Preservation Summit: Putting the Pieces Together

Genetic Preservation through Cryopreservation

Carl Lessard, PhD
Agriculture & Agri-Food Canada

Genetic Preservation through Cryopreservation

Dr. Carl Lessard[1,2], Erl Svendsen[1], Crissandra Auckland[1], Pamela Hind[1]
[1]Agriculture and Agri-Food Canada, Saskatoon, SK; [2]University of Saskatchewan, Saskatoon, SK

With agriculture intensification, genetic diversity of livestock and poultry breeds has significantly declined over the last few decades. To fight against its erosion of animal genetic resources, Agriculture and Agri-Food Canada launched the Canadian Animal Genetic Resources (CAGR). The mission of CAGR is to preserve the genetic diversity of livestock and poultry breeds by preserving germplasm and gonadal tissue. For poultry breeds, CAGR is developing a method to preserve gonadal tissues (by vitrification) from 1-day old donor chicks and then restore them by transferring the gonads into a recipient of same age but with different genetic background. Fertilized eggs from the targeted breed to be preserved were incubated in our facility at Saskatoon. Testes or ovary were harvested from newly hatched chicks and submitted to a standard vitrification procedure (15 min in VS1 (7.5% Ethylene glycol and DMSO); 3 min in VS2 (15% Ethylene glycol and DMSO; plunged in liquid nitrogen). On the day of the transplant (restoration) surgery, vitrified gonads were warmed in a series of solutions of decreasing sucrose concentrations (from 100% to 0%). Recipients (different genetic background from donor breed) were orally administrated with MetaCam to minimize pain after surgery. They were anesthetized with isoflurane gas and the incision area was shaved and cleaned. A small incision (around 2 cm) into the left abdomen was made. The yolk sac was carefully removed to provide space to reach the recipient gonad. Fine forceps of 45º angle tips was used to carefully remove the recipient gonad and to introduce the vitrified-warmed gonad. An orthotopic transfer (put at the same location) was performed for the graft and it was not attached into the recipient. After closing the incision, saline and antibiotic were administrated to the recipients before removing the isoflurane gas. On average, it took 5 minutes for the recipient to wake up after surgery. Post-surgery and until reproductive maturity, recipient birds initially received an immunosuppressant (mycophenolate mofetil, 100 mg/Kg) administrated daily for the first three weeks and then subsequently twice a week. Female recipients laying eggs were inseminated with semen from a rooster with a genetic background identical to the graft and male recipients were sacrificed to recover sperm cells from the graft. In 2015, 22 recipients received grafts (15 ovary and 7 testes); only one male successfully grew its graft to maturity. Matured sperm cells were recovered from this graft. For these experiments, CAGR has used three different recipient lines to test their ability to support the growth of grafts. Only one of them demonstrated ovarian growth; however, folliculogenesis was not observed on the recovered graft. Growing testis graft does not show the same difficulties as ovarian grafts; 50% of male recipients will support the growth of testis grafts. Moving forward, other recipient lines are currently being tested to find the best recipient to support ovarian growth.

Genetic Preservation Summit: Putting the Pieces Together

Chicken Major Histocompatibility Complex: Why variation is important

Janet Fulton, PhD
Hy-Line International

Hy-Line 80 Genetic Excellence®

Chicken Major Histocompatibility Complex: Why variation is important

Dr. Janet Fulton, Hy-Line International

Genetic Conservation Summit, Edmonton , 2017

Importance of Disease Resistance

- **Decreasing availability of medications to treat poultry**
- **Salmonella**
  - Enrofloxacin (Baytril), for gram negative bacteria
- **Mycoplasma**
  - Tylan (Tylosin)
- **Blackhead (histomoniasis) Turkeys**
  - Nitarsone, removed Dec 2015
- **Decreased pathogen load in animals has 3 positive impacts**
  - Improves health and well being of animals (animal welfare)
  - Decreases levels of food borne pathogens (food safety)
  - Healthy animals require less resources to produce (environmental benefit)
- **Improved response to pathogen challenge is another route to decreasing the pathogen load**

Genetic Resistance to Disease

- **The chicken is the poster child for genetic resistance to disease**
- **1967, Hansen (Hy-Line) first showed that resistance to Marek's Disease had a genetic component**
- **Marek's Disease is a viral tumor-causing disease**
  - Virus can cause cancer
  - Genetic resistance to cancer
- **Resistance is due to variation within the MHC**

What is the MHC?

- **Major Histocompatibility Complex**
- **Cluster of genes found in all jawed vertebrates (evolutionarily conserved)**
- **These genes encode proteins essential for the immune system to recognize foreign molecules**
  - Responsible for graft rejection
  - Identify cells with foreign proteins (invading pathogens) and enables them to be destroyed.
- **MHC-B and MHC-Y; on the same chromosome, but genetically unlinked**
- **Excellent recent review, Miller and Taylor, Poultry Science 2016**

Hy-Line Genetic Excellence

Some Chicken MHC History

- **Briles (1950)**
  - Identified multiple blood group systems in the chicken
    - (A, B, C, D etc) (think human; ABO, RH blood types)
- **Schierman and Nordskog (1961)**
  - B blood group is the major histocompatibility locus (MHC)
- **Hansen (1967); Briles et al (1977)**
  - B blood group variation associated with resistance or susceptibility to Marek's disease in commercial chickens and experimental lines

Hy-Line Genetic Excellence

MHC Detection

- **Serological Detection**
  - Detected by alloantisera, produced following blood cell immunizations between birds of different B types
  - Different B types called B1, B2, B3, (~30 different B types)
  - Limitations
    - Requires fresh blood samples and biological reagents (antisera) (importation limitations)
    - Biological antisera is limited, difficult to replicate exactly
    - Reagent cross-reactions
    - Fine for inbred lines, or within specific lines
    - Not for outbred lines, complex cross reactions, presence of other non-B blood groups, multiple MHC types
    - Limited information for brown egg lines and broilers
  - Thus, most work has been done in WL breed, and little known about B types in other breeds

Hy-Line Genetic Excellence

Genetic Resources - definitions

- **Random bred lines**
  - Limited selection for specific traits
  - Much variability
- **Pure bred lines**
  - Standard breeds (mostly developed in late 1800's)
  - Selected for specific physical characteristics
  - Breed performance, feather color, body shape
- **Elite lines**
  - Commercially utilized
  - Intensively selected for specific traits
    - Broilers (meat)
    - Layers (egg production)
- **MHC-congenic chicken lines**
  - are identical except for the MHC
  - Can study difference related to MHC variation only
- **Inbred lines**
  - developed by inbreeding,
  - have minimal genetic variation
  - Individuals are very similar, repeatable results
- **Divergently selected lines (high vs low trait)**
  - Can study genetic differences related to the trait that was selected

Hy-Line Genetic Excellence

MHC and Marek's Disease

- **Hansen et al 1967**
  - Elite lines at Hy-Line
  - B19/B19 = 29% MD mortality
  - B21/B21 = 16% MD mortality
- **Cole 1968**
  - Divergently selected for resistance (line N) or susceptibility (line P) to MD
  - Base population had 52% mortality
  - After 2 generations of selection
  - Line N = 13% MD mortality
  - Line P = 91% MD mortality
- **Briles et al 1977**
- **MHC-B types in the Cole resistant and susceptible lines (after 4 generations of divergent selection)**
  - Resistant line N was 100% B21
  - Susceptible line P was 97% B19, and 3% B13
- **Bacon and Witter, 1994 and 1994**
  - MHC congenic lines
  - Level of vaccinal protection is different for different haplotypes and different MDV serotypes.

Other viruses

- **MHC associations found for other viruses including**
  - Rous sarcoma virus
  - Newcastle disease virus
  - Infectious bursal disease
  - Infectious bronchitis disease
  - Fowl pox

Bacterial Diseases

- **Staph. aureus (Cotter et al 1992)**
  - MHC congenics (% mortality)
    - BQ/BQ = 0 B3/B3 = 6 B18/B18 = 5
    - B15/B15 = 17 B24/B24 = 20
    - B2/B2 = 30 B19/B19 = 35 B21/B21 = 45
  - Low, moderate or higher mortality
- **Other bacterial diseases with MHC associations**
  - Salmonella spp.
  - Pasteurella multocida
  - Escherichia coli
  - Clostridium perfringens

Northern Fowl Mites

- **Owen et al 2008**
  - Mite Density
  - MHC congenic lines
  - B2/B2 = 150 mites
  - B15/B15 = 270 mites
  - B18/B18 = 110 mites
  - B21/B21 = 130 mites
- **Commercial Chickens**
  - B2/B15 = 450 mites
  - B2/B21 = 250 mites

Coccidiosis

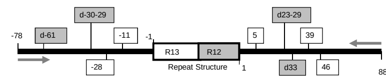
- **Three species of protozoa Eimeria cause intestinal lesions in poultry**
- **Eimeria acervulina infects the upper third of the small intestine**
- **Lillehoj et al 1989**
  - MHC congenic birds were challenged, then oocyte levels were measured
- **200 fold difference between the lowest (B13) and the highest (B15)**

MHC Type	No. Oocysts
B2	400,000
B13	12,500
B15	2,500,000
B19	1,250,000
B21	25,000

DNA based detection

- **DNA based detection eliminates many of the limitations of serological detection**
  - does not need fresh blood samples
  - Easier to transport, less concern about biosecurity
  - Can use stored samples,
  - Easier to repeat and compare between labs
- **Multiple methods**
  - Southern blots, AFLP-PCR, SSCP, sequence
  - Each has advantages and disadvantages, but none are amenable to high throughput due to costs, time and technical expertise required
- **Microsatellite marker LEI0258**

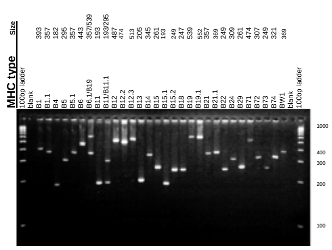
Microsatellite Marker



- **LEI0258**
- **Within the MHC**
- **Complex VNTR (Variable Number of Tandem Repeats)**
  - Variation within two tandem repeats (R13 and R12)
    - R13 = (CTATGTCTTCTTT) n=1-28
    - R12 = (CTTTCCTTCTTT) n=2-20
  - Plus small indels in the flanks
  - Detect size differences with DNA sequencer (expensive) OR
  - Size differences easily visualized on agarose gel (cheap, low tech)

From Fulton et al 2006 Immunogenetics

- **Agarose gel**
- 12-13 bp differences in alleles
- Range from 182-552bp
- Mutation rate 1/1000
- Has been extensively used to identify MHC variation in indigenous chicken populations in multiple countries
- 80 populations from Asia, Africa, Europe found 79 alleles (Chazara et al 2013)



From Fulton et al 2006 Immunogenetics

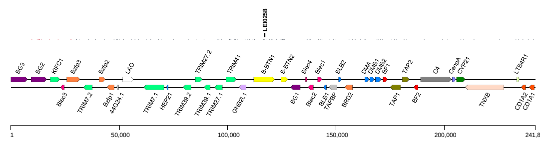
LEI0258 Limitations

- **Different MHC serological-defined types can have the same LEI0258 allele**
  - B2 and B15 are both 261
  - B13 and B17 are both 205
  - B21 and B23 are both 357
  - LEI0258 allele 357 shared in 9 haplotypes
- **Thus LEI0258 can underestimate MHC diversity**
- **Same MHC type can have different LEI0258 alleles**
  - B12 can have 461, 474 or 487
  - B19 can have 539 or 552
  - B1 can have 393 or 405
  - All are 12 or 13 bp different, reflecting change in the repeat number for either the 12 or 13 bp repeat (mutation rate)

Genomic Information for MHC

- **Chicken MHC is located on chr 16 (Bloom and Bacon 1985)**
  - Determined using trisomic chicken population
  - 3 copies of chr 16
- **Reference genome (2004), poor sequence, wrong orientation**
  - Multiple gene families, high GC content, gene duplication
- **Improved sequence builds**
  - Build 4 (Nov 2011)
  - Build 5 (Dec 2015)
  - Build 6 (soon) * should be accurate
- **Bac clones of same reference bird; Shiina et al (2007)**
- **242 Kb of sequence, 7.5x coverage**
  - Will be used to align for Build 6

MHC-B Shiina et al 2007

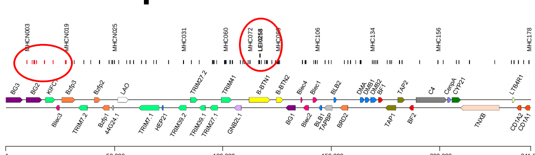


- **242,000 bp**
- **45 genes**

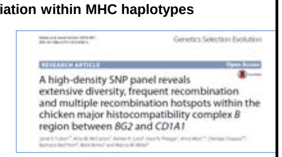
MHC-B SNP panel

- **Practical, application oriented detection system**
- **Single SNP testing (KASP)**
- **Fluorescence-based, end-point read detection**
- **$2.50 per sample (100 SNP)**
- **Moderate throughput**
- **SNP alleles can be detected by other methods, including genotyping by sequencing (GBS) or allele specific gel electrophoresis**

MHC SNP panel



- **101 SNP, encompass MHC from BG2 to CD1A1 (210,000 bp)**
- **Allows comprehensive examination of variation within MHC haplotypes**
- **Manuscript published (GSE 2016)**

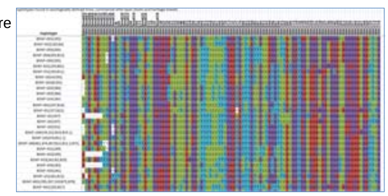


Diverse sample sets

- **LEI0258 sample set (2006)**
  - Commercially-utilized WL, RIR and WPR breeds (Hy-Line)
    - Multiple lines selected for egg production
  - MHC defined populations (NIU, ISU, UCD, ADOL, UNH)
    - Inbred lines, MHC-congenic lines
- **Serologically-identified MHC recombinants**
- **Heritage broilers (UGa, UArk, UEdm, UIll),**
  - broiler genetics of the 1950's, 1970's and 1990's
- **University populations (UEdm, USask)**
  - Specific breeds (RIR, NH, White and BPR, Light Sussex, BL and WL)

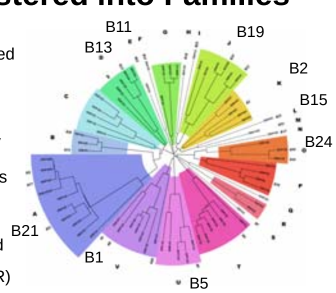
MHC-B SNP panel detected diversity

- **78 unique MHC-B types**
- Needed new nomenclature to define each haplotype because most had no serological information
  - Eg. BSNP-A04(357:B21)
- Some haplotypes are similar, with stretches of identical SNP
  - Clustered into families
- Much variability in the upstream 'BG' region
- Some SNP gave strange results in some haplotypes in the BG region



MHC-B types clustered into Families

- Clustered into 22 families based on proportion of identical SNP (70%) (A through V)
- Some families have multiple MHC types (eg A, D)
- Some are represented by only MHC type (eg I, L, S)
- Serological-defined MHC types are very different (ie found in different families)
- Some families have no MHC types that have been identified serologically (thus no disease resistance studies) (eg C, G, R)

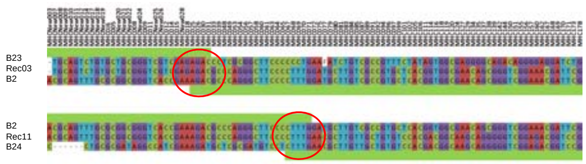


Recombination Rate

- **Detected by serology 1970's, 1980's**
  - Specific matings, with defined parental MHC types, large numbers of chicks
  - Rate of 4 per 10,000 or .0004
- **Using the SNP panel**
  - Confirmed 11 historical serological recombinants
  - Identified 33 more (must have both parental haplotypes found in the same population)
  - Genotyped 1200 chicks and parents, found 7 novel recombinants
  - Rate of 7 per 1200, or .006. **10 fold increase**

MHC Recombinants

- 11 Serologically-identified recombinants,
- confirmed with SNP panel,
  - Rec03 = B23 and B2
  - Rec11= B2 and B24



Canada Results

- Edmonton and Saskatchewan heritage lines are unique resource of untapped MHC variability
- Heritage Broilers
  - UAB-AMC-1957 8 (1R)
  - UAB-AMC-1978S 5
  - UAB-AMC-1978D 10 (1R)
- Saskatchewan
  - USK-BPR 2
  - USK-EPI 9 (1R)
- Standard Breeds
  - UAB-BPR 4
  - UAB-SBPR 4 (1R)
  - UAB-NH 4
  - UAB-RIR 4 (1R)
  - UAB-LS 3
  - UAB-WL 3
  - UAB-BL 1

1. 11 lines have more than one MHC type
   - BL had only one, (unique)
   - LS has 3 (unique)
2. 5 novel recombinants
3. Over all lines there are 33 MHC types
4. 22 are found in only 1 of the 12 lines
5. Only 9 of the 22 have any serological information (B2, B13, B15, B22, B24, B73, B76, B77, BQ)

Summary

- MHC variation is involved with resistance to disease and thus is important to identify and understand
- Previous rapid detection methods (serology, VNTR) are limited in detecting all MHC types
- MHC SNP panel can be used to detect different MHC types including recombinants
- Disease studies have been done with a very limited number of MHC types
- Well-studied MHC types (serologically-defined) are not common in other populations

Summary (2)

- GOLD MINE!
- Heritage lines at the University of Alberta are a unique and valuable source of novel MHC types
- The heritage broiler lines represent genetics (and MHC) of commercially utilized broilers
- Nothing is known about the relative disease status conferred by these novel MHC types
- The unique recombinants can be used to narrow down genetic regions involved with resistance
- Potential application to improve disease resistance for free range production

Future

- **Examine role of MHC variation in heritage broilers and disease resistance**
- **Trials to investigate differences in immune response**
  - Specific diseases
    - Mites
    - Ab response following vaccination
  - Immunology based tests
    - Sheep RBC
    - Wattle response
    - etc

Acknowledgements

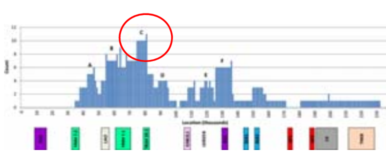
- **Samples**
  - Henry Hunt (USDA)
  - Sue Lamont (ISU)
  - Mary Delany (UCD)
  - Rene Kopulos (NIU)
  - Sammy Aggrey (UGA)
  - Chet Utterback (UIll)
  - Nick Anthony (UArk)
  - Hank Classen (USask)
  - Doug Corver (UEdm)
  - Gregoy Bedecarrats (UGue)
  - Alison Martin/Jeanette Beranger (Livestock Conservancy)
  - Steffen Weigend (Germany)
  - Mervi Honkatukia (NRI, Finland)
- **Mark Berres (UWis)**
- **Marcia Miller (COH)**
- **Bob Taylor (WVU)**
- **Hy-Line**
  - Amy McCarron
  - Ashlee Lund
  - Kara Pinegar
  - Grant Liebe

Genetic Excellence ®

Thank you for your attention!

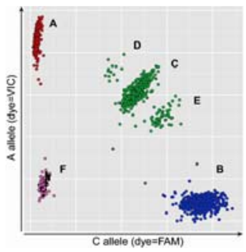
Recombination Hotspots

- Utilized all 44 recombinants
- Identified region of recombination for each recombinant (1,000 bp segments)
- Aligned all and counted how many times each segment was involved
- Identified hotspots of recombination



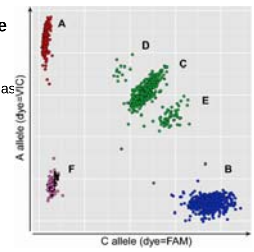
SNP Genotype Results

- **Fluorescence detection results (KASP cluster figure)**
  - Normal 4 clusters
    - Cluster A = A/A = Red
    - Cluster B =C/C = Blue
    - Cluster C = A/C = Green
    - Cluster F = no DNA (Black)
  - For this SNP there are additional clusters
    - Cluster F = failed (Pink)
    - Hets that don't fall into the expected het cluster



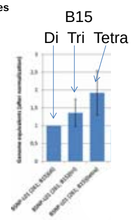
Gene Duplication/Deletion

- **SNP in BG regions can give strange results**
  - B21 = always het (AC/AC)
    - (has 2 copies of the gene, each copy has different allele)
  - B24 = always negative (gene deletion)
  - Normal 4 clusters
    - Cluster A = A/A
    - Cluster B =C/C
    - Cluster C = A/C
    - Cluster F = no DNA (black)
  - Plus we see additional clusters
    - Cluster D = het with B21= A/AC
    - Cluster E = het with B21 = AC/C



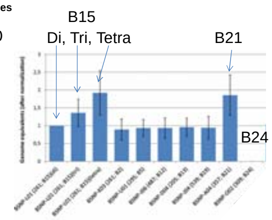
qPCR Results

- **Chr 16 trisomic chicken line (3 copies of chr 16) (B15)**
- **Disomic = 2n (1 genome equivalent)**
- **Trisomic = 3n**
- **Tetrasomic = 4n**



qPCR Results

- **Chr 16 trisomic chicken line (3 copies of chr 16) (B15)**
- **Disomic = 2n (1 genome equivalent)**
- **Trisomic = 3n**
- **Tetrasomic = 4n**
- **B2, B5, B12, B13 and B19 have 1 genome equivalent**
- **B21 has 2 genome equivalents (duplication)**
- **B24 has 0 genome equivalents (deletion)**

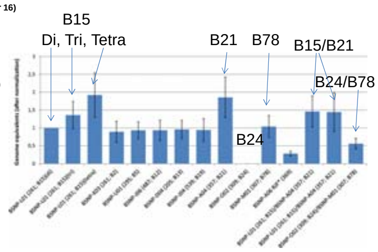


Hy-Line 80 Genetic Excellence ®

qPCR Results

- Chr 16 trisomic chicken line (3 copies of chr 16) (B15)
- Disomic = 2n (1 genome equivalent)
- Trisomic = 3n
- Tetrasomic = 4n
- B2, B5, B12, B13 and B19 have 1 genome equivalent
- B21 has 2 genome equivalents (duplication)
- B24 has 0 genome equivalents (deletion)
- B78 has 1 genome equivalent
- B15/B21 has 1.5 genome equivalents
- B24/B78 has 0.5 genome equivalents

- **B21 has duplication**
- **B24 has deletion**



Genetic Preservation Summit: Putting the Pieces Together

Measuring Sixty Years of Genetic Progress

Brenda Reimer, MSc
Alberta Agriculture & Forestry

MEASURING 60 YEARS OF PROGRESS

Brenda Reimer, Valerie Carney, Martin Zuidhof, Doug Korver, Nick Anthony & Frank Robinson

Inspiration

Carcass Composition and Yield of 1991 vs 1957 Broilers When Fed "Typical" 1957 and 1991 Broiler Diets[1]

G. B. HAVENSTEIN,[2] P. R. FERKET, S. E. SCHEIDELER,[3] and D. V. RIVES

Department of Poultry Science, North Carolina State University, Raleigh, North Carolina 27695-7608

ABSTRACT Whole carcass yield and the yield of parts (i.e., wings, saddle and legs, Pectoralis major, Pectoralis minor, breast skin, rack, abdominal fat pad, heart, and lungs), as well as whole carcass analysis for fat, moisture, and ash, were measured in the 1957 Athens-Canadian Randombred Control (ACRBC) and in the 1991 Arbor Acres (AA) feather-sexable strain, when fed "typical" 1957 and 1991 diets.

A Comparison of the Immune Performance of a 1991 Commercial Broiler with a 1957 Randombred Strain When Fed "Typical" 1957 and 1991 Broiler Diets[1]

M. A. QURESHI and G. B. HAVENSTEIN

Department of Poultry Science, North Carolina State University, Raleigh, North Carolina 27695-7608

ABSTRACT The general objective of the present study was to assess the contribution that changes in genetic selection and dietary regimen have made on the immune performance of broilers. Chicks were hatched from 1991 and 1957 strains and placed on diets thought to be typical of those fed during 1957 and 1991. Immune responses were measured as total, IgM, and IgG antibody production, macrophage, and natural killer (NK) cell functions. Significant differences were observed between strains in antibody production. For exam-

Growth, Livability, and Feed Conversion of 1957 vs 1991 Broilers When Fed "Typical" 1957 and 1991 Broiler Diets[1]

G. B. HAVENSTEIN,[2] P. R. FERKET, S. E. SCHEIDELER,[3] and B. T. LARSON[4]

Department of Poultry Science, North Carolina State University, Raleigh, North Carolina 27695-7608

ABSTRACT The relative contributions of genetic selection and dietary regimen on the performance of broilers was assessed. Body weight, feed consumption, mortality (M), and the degree of tibial dyschondroplasia (TD) were measured in the 1957 Athens-Canadian Randombred Control (ACRBC) strain of broilers and in the 1991 Arbor Acres (AA) feather-sexable strain when fed "typical" 1957 and 1991 diets. Energy and protein levels, vitamin and mineral packs, and the coccidiostats used in the two dietary regimens were chosen to be representative of those in use by the industry for the two time periods. Eight treatment groups, i.e., two strains, two sexes, and two dietary

Inspiration

Carcass Composition and Yield of 1991 vs 1957 Broilers When Fed "Typical" 1957 and 1991 Broiler Diets

A Comparison of the Immune Performance of a 1991 Commercial Broiler with a 1957 Randombred Strain When Fed "Typical" 1957 and 1991 Broiler Diets

Growth, Livability, and Feed Conversion of 1957 vs 1991 Broilers When Fed "Typical" 1957 and 1991 Broiler Diets

Carcass Composition and Yield of 1957 Versus 2001 Broilers When Fed Representative 1957 and 2001 Broiler Diets[1]

A Comparison of the Immune Response of a 2001 Commercial Broiler with a 1957 Randombred Broiler Strain When Fed Representative 1957 and 2001 Broiler Diets[1]

Growth, Livability, and Feed Conversion of 1957 Versus 2001 Broilers When Fed Representative 1957 and 2001 Broiler Diets[1]

Inspiration

Carcass Composition and Yield of 1991 vs 1957 Broilers When Fed "Typical" 1957 and 1991 Broiler Diets

A Comparison of the Immune Performance of a 1991 Commercial Broiler with a 1957 Randombred Strain When Fed "Typical" 1957 and 1991 Broiler Diets

Growth, Livability, and Feed Conversion of 1957 vs 1991 Broilers When Fed "Typical" 1957 and 1991 Broiler Diets

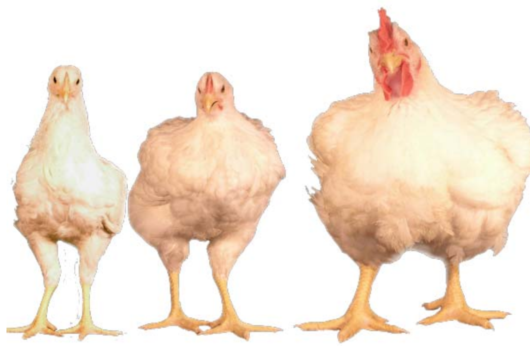
Carcass Composition and Yield of 1957 Versus 2001 Broilers When Fed Representative 1957 and 2001 Broiler Diets

A Comparison of the Immune Response of a 2001 Commercial Broiler with a 1957 Randombred Broiler Strain When Fed Representative 1957 and 2001 Broiler Diets

Growth, Livability, and Feed Conversion of 1957 Versus 2001 Broilers When Fed Representative 1957 and 2001 Broiler Diets

Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 2005[1]

Inspiration



Design

- Havenstein series – 12 weeks, sex-separate, representative diets
- 2005 –8 weeks, mixed sex, 2005 diet
  - All were broiler trials
- We were very interested in the breeder side of the equation
- 2017 was the time for a 60 year trial…

Design

- 4 strains
  - 1957 strain – University of Alberta
  - 1978 strain – University of Alberta (male line)
  - 1995 strain – Arkansas Random Bred 95
  - 2015 strain – Arkansas Random Bred 15
- Chicks were vent sexed at hatch
- 2 trials
  - Male broiler trial – grown to 12 weeks
  - Animal Science 471 class project

Design

- Breeder Female Trial
- Feeding Treatments
  - Restricted to 2015 curve (average of Cobb and Ross breeder targets)
  - Full Fed
- 2 pens per strain*treatment combination
- ~25 pullets per pen

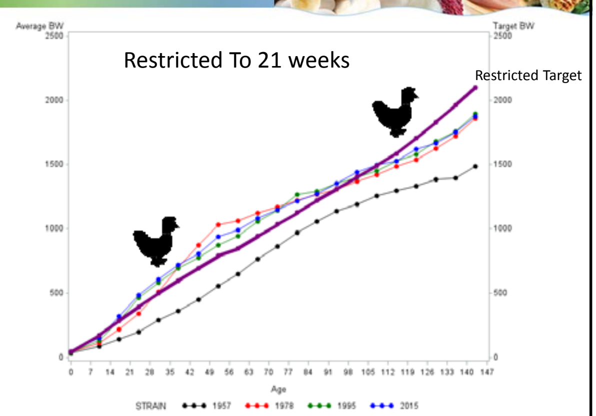
Measurements

- To 19 weeks of age:
  - Weekly Individual BW
  - Weekly Feed Intake (pen basis)
  - Photographed every 4 weeks
  - Chest width & shank length every 4 weeks

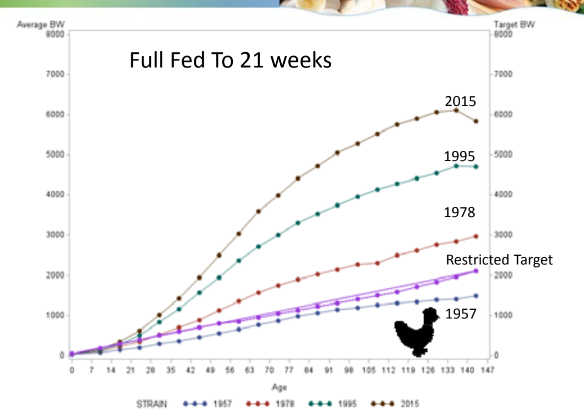
Measurements

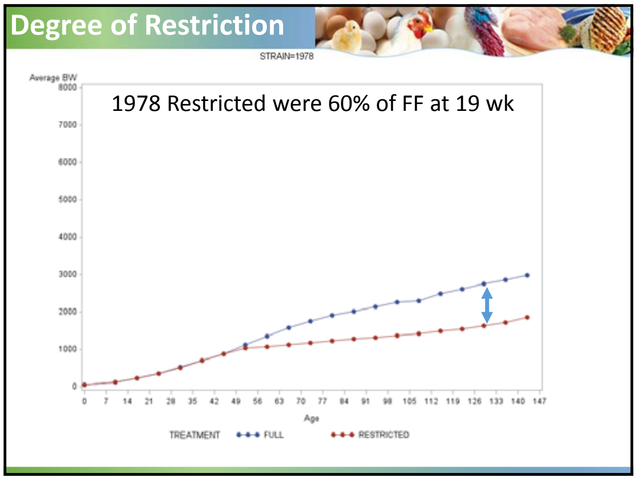
- Planned – move hens to individual cages at 18 weeks
- Decided to wait til photostimulation to limit time in cages for full fed birds
- At ~19 weeks of age we had eggs!
  - 288 pullets moved to individual cages
  - Individual feed intake weekly
  - Individual BW weekly
- Photostimulated at 21 weeks
  - A birds dissected at photostimulation
  - B birds dissected after 2nd egg

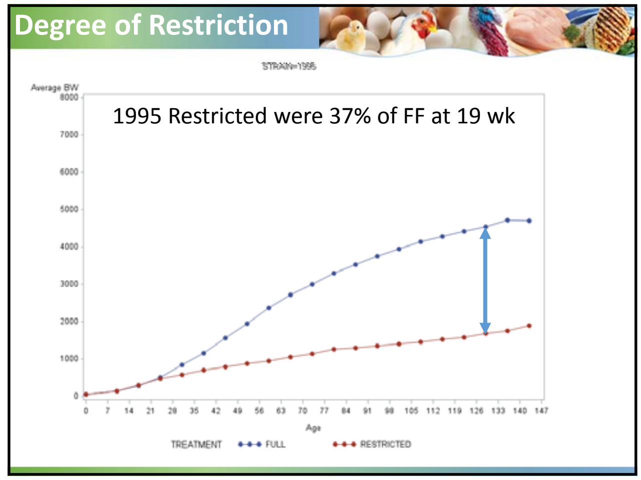
BW strain difference

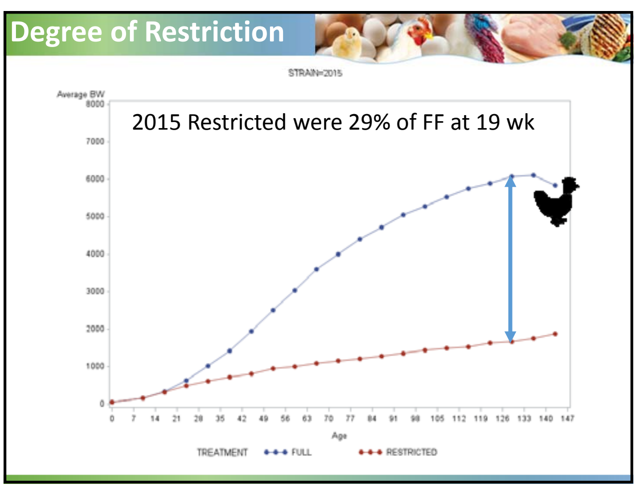


BW strain difference




Degree of Restriction
STRAIN=1978
Average BW
1978 Restricted were 60% of FF at 19 wk
Age
TREATMENT
FULL
RESTRICTED


Degree of Restriction
STRAIN=1995
Average BW
1995 Restricted were 37% of FF at 19 wk
Age
TREATMENT
FULL
RESTRICTED

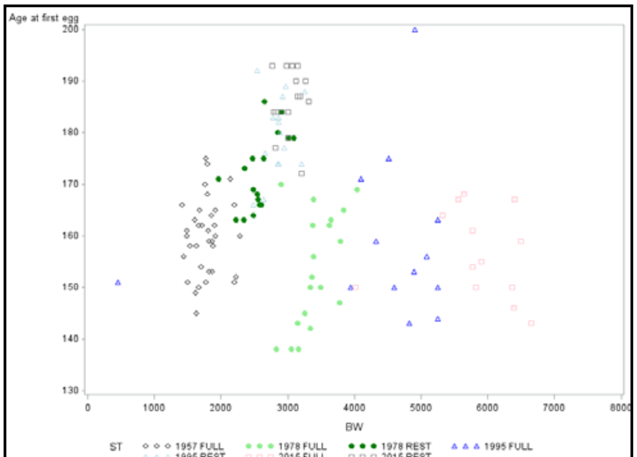

Degree of Restriction
STRAIN=2015
Average BW
2015 Restricted were 29% of FF at 19 wk
Age
TREATMENT
FULL
RESTRICTED


Feed Restriction
57%
29%
25%

Feed Efficiency

Lay

- By 21 weeks 15% of the flock (full & restricted) had laid at least one egg
  - 31% of the 1957
  - 16% in the 1978
  - 3% in the 1995
  - 14% in the 2015
- 89% were Full Fed



Challenges

- $$$$$$$$$$$$$
  - User fees ($8500),
  - Feed costs ($2000),
  - Equipment – new drinkers $3500
  - Labour (?)
    - Labour – US, 471 students and grad students, one hired helper and family coercion ☺
- Ultimately funded largely through Alberta Agriculture & Forestry (same as 50 year trial)

Challenges

- LOGISTICS
  - Getting eggs across the border
  - Vent sexing
  - Labour arrangements
  - Weighing full fed 2015 birds was…. A challenge

Collaboration

- LOGISTICS
  - Getting eggs across the border – the 4-5 day tour!
  - Multiple import/export processes to follow
  - Fedex couldn't guarantee climate control
  - Nick Anthony's technician drove eggs hours from the university to join a shipment of Aviagen eggs headed to Abbotsford, British Columbia (11-12 h from Edmonton)
  - A call to the transporter to confirm when the eggs would arrive in Abbotsford revealed that the truck was also stopping in Lethbridge (5 h from Edmonton)

Collaboration

- LOGISTICS
  - We burned a lot of favours!
    - Aviagen – support with import process, shipping eggs
    - Lethbridge Sunrise Hatchery – received eggs and included our eggs in their import process
- Without strong connections throughout the industry and within our group – the study never would have happened

Future

- Nutrient digestibility
- Immune function
  - inflammation LPS
  - Antibody responses to vaccination
- Motivation to feed
- Bone development/porosity
- Posture – pelvic tilt
- Meat quality
  - Functional properties
  - Taste
- Characterize activity level
- Pain – self medication

Future

- Embryo development
- Embryo metabolism
- Shell temperatures
- Residual yolk
- Embryonic heart rate
- Shell quality
- Photo refractoriness
- Egg size relative to BW/age

Genetic Preservation Summit: Putting the Pieces Together

Sixty Years of Genetic Change: A Preliminary Perspective

Martin Zuidhof, PhD
University of Alberta

Sixty Years of Genetic Change: A Preliminary Perspective

Martin J. Zuidhof, Valerie L. Carney, Brenda L. Reimer, F. E. Robinson, and Nicholas B. Anthony
Genetic Preservation Summit
Edmonton, AB
May24-25, 2017

Objective

To evaluate the impact of 6 decades of quantitative genetic selection on broiler

- Growth
- Efficiency
- Yield

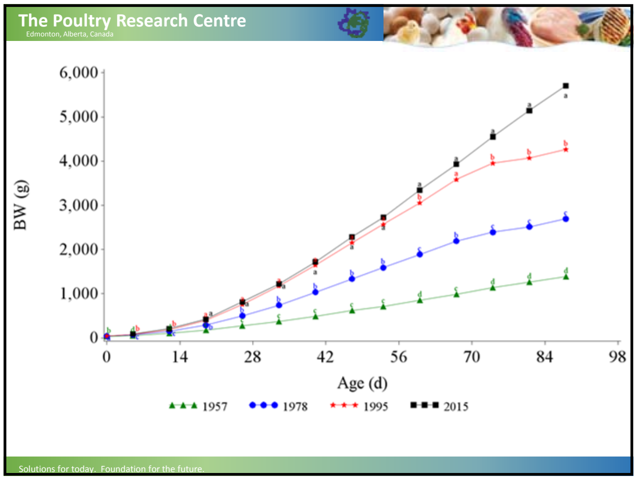
Completely Randomized Design

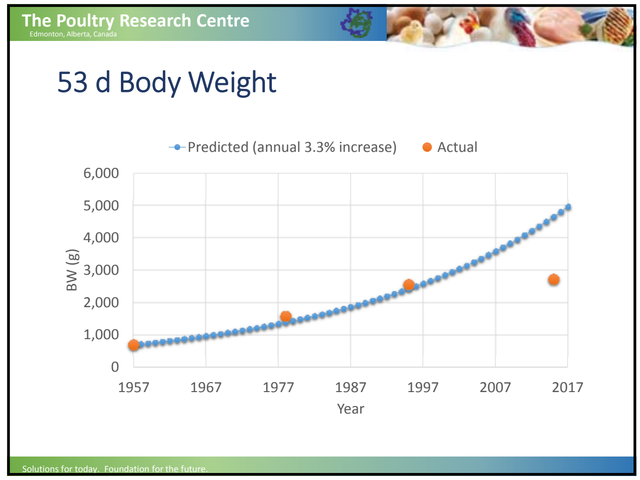
Source of variation: genetic benchmarks

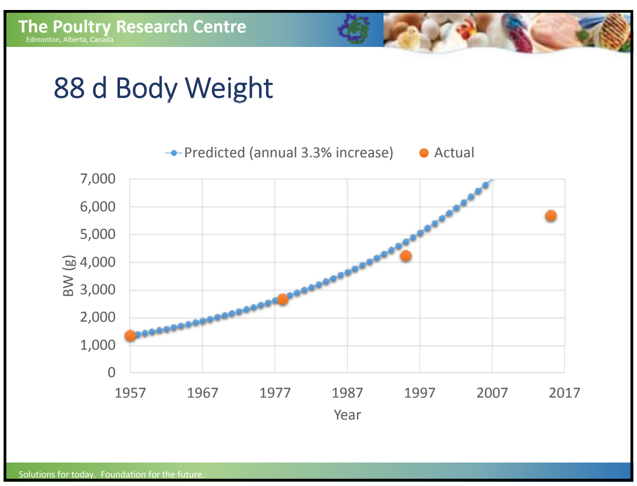
- Four genetic lines
  - Alberta Meat Control (AMC) lines
    - AMC-1957
    - AMC-1978 (dam line; strain 30)
  - Nick Anthony's lines (Arkansas)
    - 1995 commercial broiler
    - 2015 commercial broiler

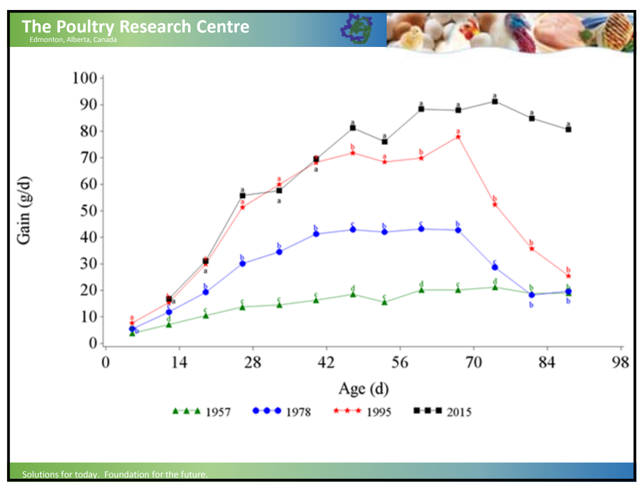
The Experiment

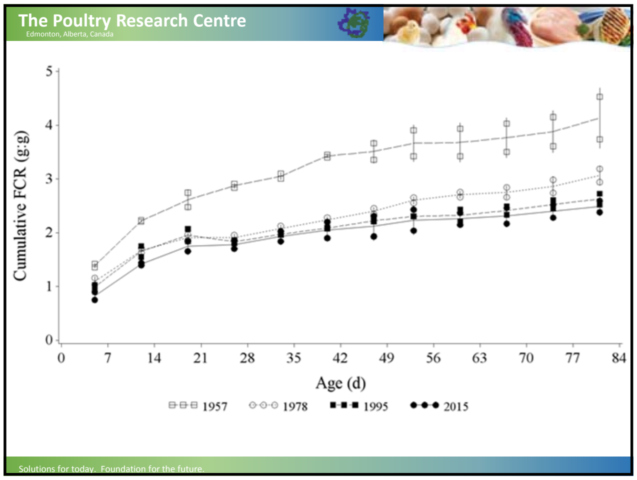
- 2 pens per strain
- Set 300 fertile eggs per strain
- Vent sexed at hatch
  - n=145, 144, 75, and 106 males per strain
- Low intensity broiler ration
  - Starter (0 to 28 d) 2,725 kcal/kg; 21% CP
  - Grower (>28 d) 2,718 kcal/kg; 16% CP
- Weekly BW
- Weekly feed intake
- Bi-weekly dissection: 10 birds/strain
- Sex verified at processing

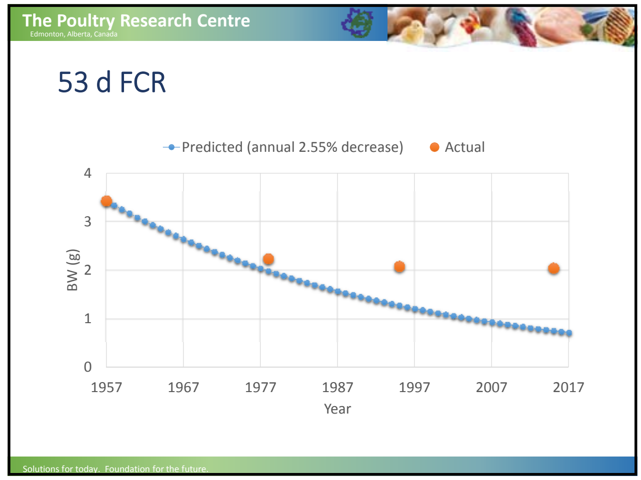

The Poultry Research Centre
Edmonton, Alberta, Canada
BW (g)
6,000
5,000
4,000
3,000
2,000
1,000
0
0
14
28
42
56
70
84
98
Age (d)
1957
1978
1995
2015
Solutions for today. Foundation for the future.

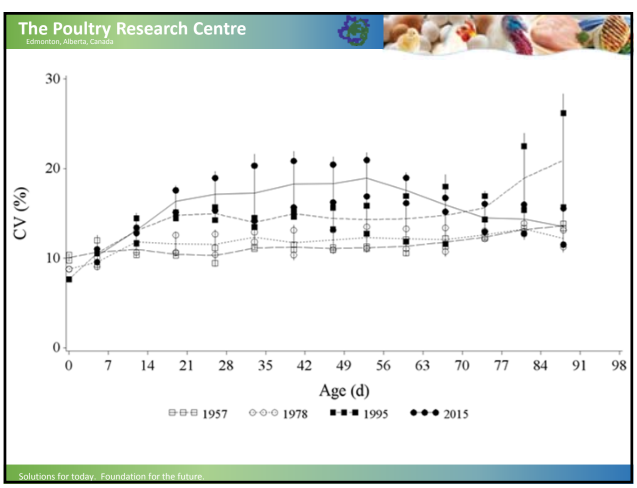

The Poultry Research Centre
Edmonton, Alberta, Canada
53 d Body Weight
Predicted (annual 3.3% increase)
Actual
BW (g)
6,000
5,000
4,000
3,000
2,000
1,000
0
1957
1967
1977
1987
1997
2007
2017
Year
Solutions for today. Foundation for the future.

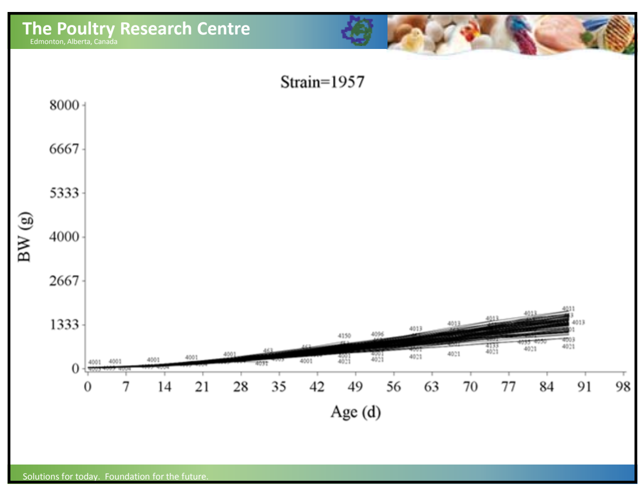

The Poultry Research Centre
Edmonton, Alberta, Canada
88 d Body Weight
Predicted (annual 3.3% increase)
Actual
BW (g)
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
1957
1967
1977
1987
1997
2007
2017
Year
Solutions for today. Foundation for the future.

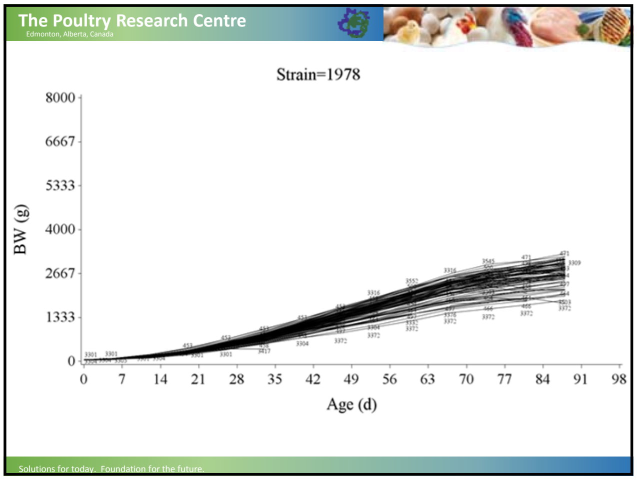

The Poultry Research Centre
Edmonton, Alberta, Canada
Gain (g/d)
100
90
80
70
60
50
40
30
20
10
0
0
14
28
42
56
70
84
98
Age (d)
1957
1978
1995
2015
Solutions for today. Foundation for the future.

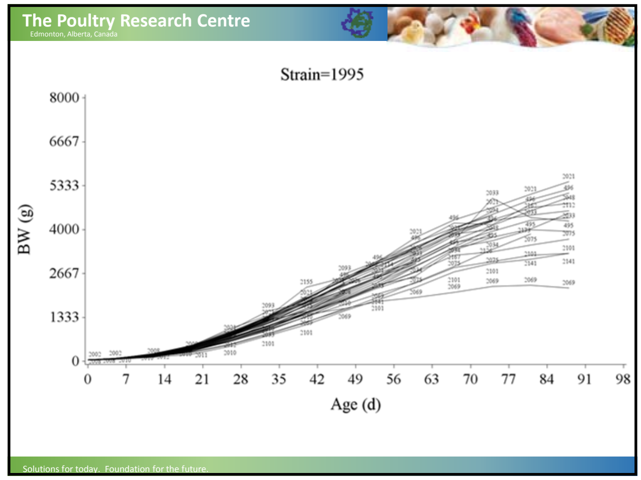

The Poultry Research Centre
Edmonton, Alberta, Canada
Cumulative FCR (g:g)
Age (d)
1957 1978 1995 2015
Solutions for today. Foundation for the future.

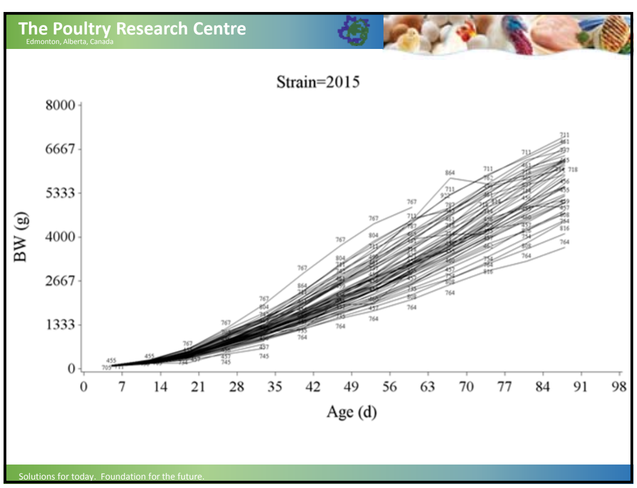

The Poultry Research Centre
Edmonton, Alberta, Canada
53 d FCR
Predicted (annual 2.55% decrease)
Actual
BW (g)
Year
Solutions for today. Foundation for the future.

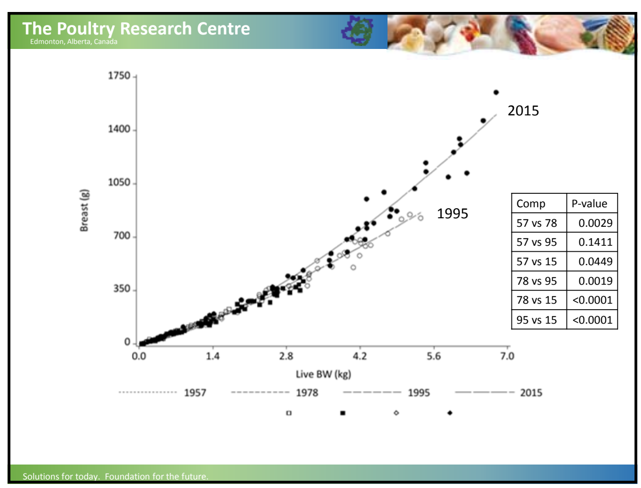

The Poultry Research Centre
Edmonton, Alberta, Canada
CV (%)
Age (d)
1957 1978 1995 2015
Solutions for today. Foundation for the future.


The Poultry Research Centre
Edmonton, Alberta, Canada
Strain=1957
BW (g)
Age (d)
Solutions for today. Foundation for the future.



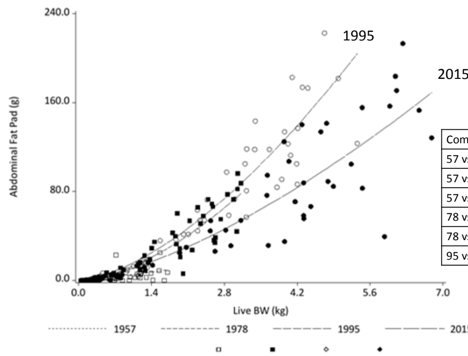






Comp	P-value
57 vs 78	0.0029
57 vs 95	0.1411
57 vs 15	0.0449
78 vs 95	0.0019
78 vs 15	<0.0001
95 vs 15	<0.0001

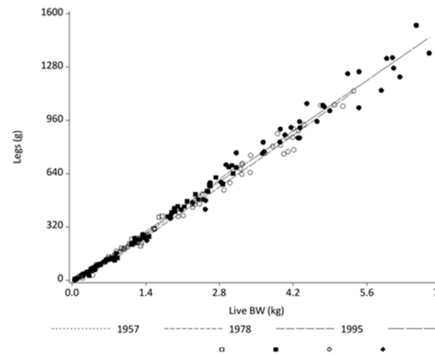
The Poultry Research Centre
Edmonton, Alberta, Canada



Comp	P-value
57 vs 78	<0.0001
57 vs 95	0.0023
57 vs 15	0.2217
78 vs 95	0.3299
78 vs 15	0.0137
95 vs 15	<0.0001

Solutions for today. Foundation for the future.

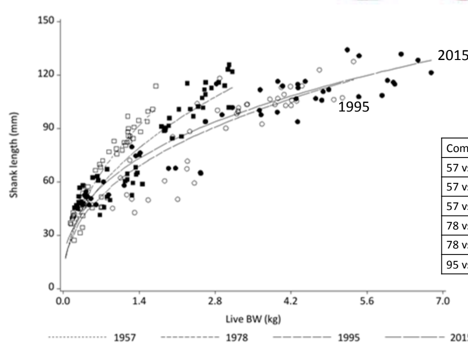
The Poultry Research Centre
Edmonton, Alberta, Canada



Comp	P-value
57 vs 78	0.2825
57 vs 95	0.0235
57 vs 15	0.2542
78 vs 95	<0.0001
78 vs 15	0.0554
95 vs 15	0.0246

Solutions for today. Foundation for the future.

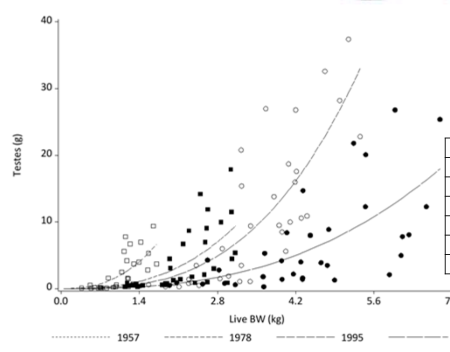
The Poultry Research Centre
Edmonton, Alberta, Canada



Comp	P-value
57 vs 78	<0.0001
57 vs 95	<0.0001
57 vs 15	<0.0001
78 vs 95	<0.0001
78 vs 15	<0.0001
95 vs 15	0.4259

Solutions for today. Foundation for the future.

The Poultry Research Centre
Edmonton, Alberta, Canada



Comp	P-value
57 vs 78	0.0077
57 vs 95	0.0252
57 vs 15	0.0043
78 vs 95	0.4189
78 vs 15	0.0031
95 vs 15	<0.0001

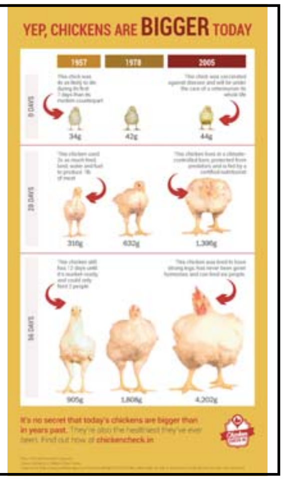
Solutions for today. Foundation for the future.

The Story
60 year study

Over almost 60 years, many intended beneficial genetic changes were achieved

- Chicken production is more efficient and sustainable
- Chickens still grew 4 times faster
- Chickens needed 40% less feed
- 42 d FCR has not decreased a lot in the last 40 years (could be nutrition related)
- The pectoralis major (breast) muscle continues to increase
- Abdominal fat continues to decrease
- Broilers are getting 'stockier' legs

What parts of the story still need to be told?



Future – Parameters of Interest

Nutrition
- Nutrition vs. Genetics
- Nutrient digestibility

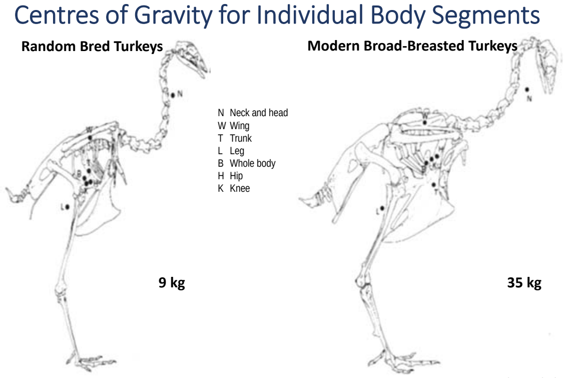
Immune function
- Inflammation
- Antibody responses to vaccination
- Energy cost
- Antibiotic free production

Behaviour
- Motivation to feed
- Characterization of behaviour budget
- Activity levels
- Pain – self medication

Growth and development
- Conformation, center of gravity
- Bone development / porosity
- Posture / pelvic tilt

Meat quality
- Functional properties
- Taste

Centres of Gravity for Individual Body Segments



Abourachid, 1993

Future – Parameters of Interest

- Breeders / Reproduction
  - Egg production
  - Onset of lay thresholds
    - BW
    - Age
    - Fat
    - Feed intake
    - Light / Photorefractoriness
  - Conventional vs. Precision Feeding
    - Carcass conformation
- Shell quality
- Bone density
- Egg size
  - BW
  - Age
  - Nutrient intake
- Embryo development
  - Embryo metabolism
  - Shell temperature
  - Residual yolk
  - Embryo heart rate