Introduction

The CC®P project

At the end of the year 1990 artist Koen Vanmechelen, concerned with genetic diversity, started the Cosmopolitan Chicken Project (CCP). One of the artist’s goals was to create new hybrids of chickens by crossing different chicken breeds. These new “crossbreds” would share many characteristics with all their different ancestors, thus carrying a cosmopolitan genome, as opposed to the primeval chicken - the ‘Red Jungle fowl’ (Gallus gallus). It is believed that this primeval chicken – whose habitat lies at the foot of the Himalayas – is the source of all presently existing breeds, through a process of domestication (i.e. natural selection and inbreeding) during the last 7000 years.
The CCP could also illustrate how the human genome progressively evolves on the planet. Increasing migration and exchange of genetic material, could progressively give rise to a cosmopolitan genome of the human population as well, hence progressively erasing the obvious outward differences between different groups of people. Starting from this interesting concept the artist wanted to expose his CCP to a thorough scientific genetic evaluation under the title CC®P.

Relevance - CC®P research

The project is unique in the world due to the simple fact that there is no on-going CCP that is even remotely comparable to the art project of Koen Vanmechelen. The CC®P, which aims to study the different crossbreds produced by Koen Vanmechelen, is important because the effect of the crossings on the genetic diversity of the chickens is unknown and could generate important insights.

This research project is also potentially applicable to the human population. The exact same process of the CCP namely also takes place in the world population. Genomes are being merged by individuals from different regional groups, not by the entire population simultaneously. Even though individuals have the same genetic roots, over the years mankind has acquired a great deal of genetic diversity.

The chicken is a useful model for a better understanding of the function of the human genome since:

- It is an excellent model for the study of the genetic diversity of both chicken and men, e.g. the difference between the various chicken breeds.
- It offers an exceptional opportunity for the study of the consequences of merging different populations.
- It offers a unique opportunity to identify the genetic foundations of phenotypical characteristics of chickens (and possibly humans).
- Chickens are models for some human diseases such as hypertension, dementia, ovarian carcinoma a.o.

Also, the wider context of the project should not be neglected, and specifically the fact that the CCP can be a helpful representation for a better understanding of the genetic diversity of the human kind and the effects of migration and the merging of various populations on the human genome.

State of the art

Domestication of the jungle fowl: the chicken

Feathers and eggs are characteristics of birds, including poultry. A famous fossil displaying feathers was found in Germany in 1861, just two years after Charles Darwin published “On the Origin of species”. This animal, the Archaeopteryx, lived about 150 million years ago in the Late Jurassic Period. Its features made it a suggestive candidate for a transitional fossil between dinosaurs and birds, although recent fossil evidence seems to cast doubt about it being the direct predecessor of the birds. Chickens belong to the genus Gallus or Jungle fowl. Within this genus, 4 species can be differentiated, namely Red Jungle fowl (Gallus gallus; including different subspecies), Grey Jungle fowl (G. sonnerati), Yellow Jungle fowl (G. lafayetii) and Green Jungle fowl (G. varius) (Delacour, 1977; Sibley & Ahlquist, 1990; Johnsgard, 1999; Nishibori, 2005) (Fig. 1). Although most academics considered that the domestic chicken originated only from the Red Jungle fowl (Darwin, 1868; Hashiguchi et al., 1981; Okada et al., 1984; Fumihito et al., 1996; Nishibori et al., 2005), recent genetic analyses would indicate also minor contributions by the Grey Jungle fowl and Yellow Jungle fowl (Nishibori et al., 2005; Eriksson et al., 2008; Sawai et al., 2010).

There is not much known about the domestication process of chickens. However, everything seems to have happened close to the habitat of their wild ancestors. Archaeological findings in China indicate domestication of poultry as early as between 6,000 and 5,400 BC, although it cannot be excluded that these animals did not contribute to the domesticated chicken of today (West & Zhou, 1988). Findings in the Indus valley also indicate domestication had happened at least between 2,500 and 2,100 BC (Zeuner, 1963). Chickens were however initially not domesticated for their meat and eggs, but for cock fighting and pleasure. Being a religious symbol, chicken was prohibited food in India, a custom later adopted in Mediterranean countries (Crawford, 1990).

The first breeds of chickens can be observed on paintings and drawings of the Renaissance in the 16th century, although earlier inbreeding is most probable. It is only later in the middle of the 19th century that there is a real explosion of creation of breeds accompanied by detailed phenotypic descriptions of these breeds in so-called Standards. All this resulted in more than thousand breeds all over the world, some specifically selected for egg production (layers) or meat (broilers).
At the end of the 19th century and throughout the 20th century, the agricultural revolution resulted in extreme selection for economic breeds of layers and broilers. It is thus not surprising that poultry became the second largest source of meat worldwide and is forecast to surpass pork by 2030 (Fig. 2).

What is a genome and how does it work

In each generation information is passed from parents to offspring. The genome refers to the entire hereditary information of an organism. In higher organisms, such as men and chicken, this information is stored in one set of molecules, collectively called the DNA, that is present in the nucleus of each cell. A single DNA molecule represents one chromosome and is a double stranded linear molecule that contains genes. The human genome contains 23 pairs of chromosomes. For each pair, one chromosome is inherited from the father and the other from the mother. Genes consist of coding (exons) and non-coding (introns) regions, and code for proteins, which have a function in the cell and are responsible for normal functioning of the organism.

As an organism grows, in each somatic cell division, called mitosis, the two DNA strands are separated and duplicated to yield two progeny cells with an identical amount of DNA as the progenitor cell. As such each somatic cell in the body contains an identical number of chromosomes. This is called the diploid number (2n) since all chromosomes appear in homologous pairs in somatic cells. In the meiosis, two gametes (sperm cells or egg cells) are produced that each have half of the genetic information of the progenitor cell. Each gamete receives one chromosome of each homologous chromosome pair and is haploid (n).

The building blocks of DNA, the nucleotides, contain three essential components: a phosphate group, a pentose sugar and a nitrogenous base. There are four different nucleotides in DNA each differing in the base: adenine, cytosine, guanine, and thymine, often abbreviated as A, C, G and T. The length of DNA is expressed as the number of bases (Kilobases (Kb), Megabases (Mb), Gigabases

Fig. 1. – Geographical distribution of the different wild chicken species and subspecies

Red Jungle fowl (Gallus gallus):
- Cochin-Chinese (gallus)
- Tonkinese (jabouillei)
- Burmese (spadiceus)
- Javan (bankiva)
- Indian (murgi)

Yellow Jungle fowl (Gallus larvatus):
- Grey Jungle fowl (Gallus sonnerati)
- Green Jungle fowl (Gallus varius)

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order to perform a high quality genetically analysis, a large number (typically sixty thousand to several million) of SNP have to be determined. For this, SNP chips were developed. These SNP chips are glass slides about half the size of a bankcard on which thousands of SNPs can be identified. They are a very powerful tool in genetic analysis. In this study a 60,000 SNP chip specific for the chicken genome was used.

While one gene codes for one protein, these proteins usually interact producing very complex phenotypes. For example the single comb, pea comb, rose comb and walnut comb in chicken are four different phenotypes caused by the interaction of two different genes.

In many organisms, sex of the individual is genetically determined by the presence or absence of particular sex chromosomes. If the sex chromosomes are the same this is called the homogametic sex and in all mammals, including men, this is the female (XX) while the heterogametic sex is the male (XY). In chickens this is the opposite: the heterogametic sex is the female (ZW) and the homogametic sex is the male (ZZ). The sex chromosomes in chickens are referred to as Z and W to make a clear distinction with the X and Y of mammals.

Inbreeding and hybrid vigour

Inbreeding is defined as the mating of relatives. The inbreeding coefficient is a measure of the degree at which pairs of alleles on randomly chosen loci in an individual are identical (homozygous) by descent. Because an individual inherits one allele from the father and one allele from the mother, this identity by descent can only occur if both parents have a common ancestor. Inbreeding leads to a higher degree of homozygosity and is commonly used to create breeds with the purpose to make the animals homozygous for the alleles underlying desired traits. It results in more uniform populations with fixed characteristics but with less genetic diversity. If the level of inbreeding in a certain population is very high some disadvantages will occur, called inbreeding depression. Since all individuals are more alike, the genetic diversity is decreased which results in an impaired ability to adapt to a changed environment. Moreover genetic defects may occur and survival rate and fertility may be hampered.

In case of crossing different inbred strains there is a good chance that the two parent populations are homozygous for different alleles, resulting in a higher degree of heterozygosity in the crossbreeds. This will have a positive effect on the survival rate

Fig. 2. — Economic importance of chicken (adapted from WATT Executive Guide 2011). The global meat market for chicken has surpassed that for beef at the beginning of the century and is expected to surpass pork in the next few years.
Another distinctive difference between macro- and micro chromosomes is gene density. Analyses of the whole genome sequence have indicated that a chicken has between 20,000 and 23,000 genes. Although the macro chromosomes make up almost 70% of the complete genome sequence, it is estimated that they only contain about 50% of all chicken genes and that micro chromosomes are therefore twice as gene dense than macro chromosomes.

Materials and methods

Animals and samples

The setup of the CC®P is quite different from traditional breeding experiments. Briefly, starting from the crossbreed of two purebred chickens (Mechelse Koekoek x Poule de Bresse), every generation is then crossed with a few animals from another breed. The setup is shown schematically in Figure 3.

In the total CC®P study, 652 chickens of 36 breeds were included. The experimental population consisted of purebred animals, crossbreds and Red, Green, Yellow and Grey Jungle fowl (Red: G. gallus gallus, G. gallus jabouillei, G. gallus spadiceus; green: G. varius; Yellow: G. sonneratii; Grey: G. lafayetti). In this experiment however, only the genotypes of 13 purebred and 13 crossbred populations were used (Fig. 3).

All animals were kindly provided by Koen Vanmechelen and several hobby breeders. Of each animal, feathers, muscle or blood were collected for DNA extraction. Each animal was photographed. Ancestry of individual animals was not available. The latter precludes traditional approaches such as linkage analysis.

Genotyping

DNA was extracted from feathers and whole blood with a protocol adapted from Sambrook & Russell (2001). The amount of extracted DNA or RNA was quantified by measuring the absorbance at 260 nm with a UV-spectrophotometer.

The genotyping of the samples was performed with the commercially available Illumina chicken 60k SNP chip (Groenen et al., 2011). The SNP chip contained approximately 58,000 SNPs and SNPs were equally divided across the genome, taking into consideration that recombinations are in general more frequent on the micro chromosomes. This had as result that most SNPs were located on the macro chromosomes but the SNP density (SNP/kb) was higher on the micro chromosomes.
Fig. 3. — Experimental setup of the CCRP, depicting the sequence of pure chicken lines that were subsequently crossed into the subsequent hybrids.
crossbreeding experiment. Informative SNPs typically segregate within a given population and therefore they are a good indication for genetic diversity within a population. The number of expected informative SNPs can be defined as an estimation of the number of informative SNP in a generation given that the population is of sufficient size to maintain all informative SNPs.

However, as the set-up of the CCP was to generate new generations by crossing the former generation with a limited number of pure bred cocks or hens of a new, purebred population, the condition of sufficient population size cannot be met. Therefore, the number of effective informative SNPs is also calculated, meaning the actual number of informative SNPs present in the different CCP generations. Results are indicated in Table 1 and Figure 4.

Course of expected informative SNP over several generations shows the arise of a cosmopolitan chicken genome

Expected informative SNPs show a steady increase in number over the consecutive generations (Fig. 4B). In the purebred Mechelse Koekoek

Statistical analysis

For every breed or crossbred it was studied how many SNPs had 2 alleles segregating in the population. This was done by creating “breed” specific data sets that were analysed using Plink (Purcell et al., 2007). Those two alleles could occur within one individual when it is heterozygous or between two individuals when both are homozygous for one of the two alleles. When crossing two breeds the total number of expected informative SNPs is at least equal to the maximum number if there is a complete overlap but probably more because in both breeds different SNPs are informative. The number of effective informative SNPs is the number found in this specific population and is probably less than the expected number because heterozygosity is lost due to random sampling or sometimes a small number (drift).

Results and discussion

Based on the 60K SNP genotypes obtained with the Illumina chicken 60K SNP chip, the number of expected and effective informative SNPs was calculated for the first 13 generations of the CCP.
relatively unknown, this is an important result that generates important insights.

Furthermore, when the results in the chicken genome are extrapolated to the human genome, increasing migration and exchange of genetic material could progressively give rise to a cosmopolitan genome of the human population as well.

However, important to keep in mind is that these results imply that, both in chicken and men, populations that admix are sufficiently large to maintain all informative SNPs from previous generations. When, like in the Cosmopolitan Chicken Project, small generations are crossed with one male or female of another, purebred population, results are completely different.

An erratic course

For the effective informative SNPs, no steady increase is witnessed, in fact these SNPs show a rather erratic course (Fig. 4C). In 11 out of 13 generations, the numbers of effective informative SNPs fluctuate between 40173 and 50273. In generation 3 (Mechelse English Redcap) and 4 (Mechelse Jersey Giant) however, number of effective informative SNPs is 25393 and 10392 respectively (Table 1) due to low number of individuals sampled.

The fluctuating pattern, but also the sharp decrease in effective informative SNPs in generation 3 and 4 are also due to the set-up of the CCP. By

Table I. — Overview of expected and effective number of informative SNP in 13 CCP generations. N°: number. 

| Generation n° | Crossbreed                  | N° of animals | Expected n° of informative SNP | Effective n° of informative SNP |
|---------------|-----------------------------|---------------|-------------------------------|-------------------------------|
| 1             | Mechelse Koekoek            | 43            | 47245                         | 47245                         |
| 2             | Mechelse Bresse             | 6             | 51933                         | 43647                         |
| 3             | Mechelse English Redcap     | 2             | 52240                         | 25393                         |
| 4             | Mechelse Jersey Giant       | 1             | 52888                         | 10392                         |
| 5             | Mechelse Dresdner Huhn      | 3             | 53056                         | 40173                         |
| 6             | Mechelse Owlbeard           | 13            | 53216                         | 47115                         |
| 7             | Mechelse Louisiana          | 11            | 53343                         | 44909                         |
| 8             | Mechelse Thai Fighter       | 11            | 53343**                       | 50273                         |
| 9             | Mechelse Araucana           | 11            | 55584                         | 50178                         |
| 10            | Mechelse Denzili Longrower  | 11            | 53627                         | 46042                         |
| 11            | Mechelse Cubalaya           | 10            | 53717                         | 46975                         |
| 12            | Mechelse Ancona             | 10            | 53799                         | 42424                         |
| 13            | Mechelse Orloff             | 10            | 53878                         | 40841                         |

** No genotypes for Thai Fighter were available.

(stinckens-indd 170)
crossing only a few inbred animals with the previous generation, half the genomic information of these animals will be contributed to the existing genomic information. In some generations, this leads to an increase in genetic diversity, but in others by chance the opposite effect can be witnessed as a consequence of allowing only a few animals to contribute to the next generation of hybrids.

Furthermore, when we examine generations 3 and 4 more closely, it can be shown that, on top of the fact that only a few males were used to create these populations, the number of animals analysed was also very small (2 and 1 animals, respectively).

In conclusion, it can be witnessed that, when we exclude generations 3 and 4, instead of creating a Cosmopolitan Chicken Genome, with the set-up of the CCP the genetic diversity in the different generations fluctuates within more or less fixed boundaries. However, although this means that the overall genetic diversity remains more or less constant, this does not mean that the informative SNPs for the different generations are the same. More probable is that informative SNPs in one generation get replaced by other informative SNPs in a consecutive generation.

Although this result does not reflect the initial set-up of the CCP, this is a very interesting result when it comes to applicability to the human population. The exact same process of the CCP namely also takes place in the world population. Most of the time, genomes are being merged by individuals from different regional groups, not by the entire population simultaneously. Therefore, when we extrapolate the CCP results to mankind, it is very likely that genetic diversity in different individuals also fluctuates within given boundaries. A reflection on this might be that this way, an evolutionary optimum is reached between the versatility of being heterozygous and the positive effects of being homozygous for several desired traits.

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Addendum

Sociological Importance of The Cosmopolitan Chicken Research Project

A suddenly chickenless world would spell instant disaster because the chicken is the universal animal, the world’s most omnipresent bird. Only one country, Vatican City, and one continent, Antarctica, are chicken-free. For every man, there are three chickens. Out of fifteen thousand species of mammals and birds, the chicken emerged 8,000 years ago as man’s most valuable animal companion. It allowed humanity to travel the oceans, conquer the wilderness and explore uncharted territory. Humanity has forgotten this.

The Cosmopolitan Chicken Project wants to restore the lost dignity of humanity’s most staunch and varied co-species. At the same time, it resists the temptation to diminish the animal to a multipurpose beast that provides man with everything he wants. The chicken is also a spiritual companion and a guide. It became a crucial player in the evolution of human cultures.

Leaving the Southeast Asian jungle as a magical creature, the chicken spread around the globe and became a holy messenger of light and resurrection. It entertained people, served as a medicinal cure, and inspired many. As Andrew Lawler writes in his book ‘Why did the chicken cross the World?’, “no other animal has attracted so many legends, superstitions, and beliefs across so many societies and eras”. No animal conquered such an important place in many languages.

KoenVanmechelen reintroduces the bird in the human arena. Its potential, spiritual, metaphysical and scientific, has not been fully explored yet. Hidden in its genes and memes lies the future of the world. As it once helped Charles Darwin to make his theory of evolution, and Louis Pasteur to create the first modern vaccine, it will continue to inspire future scientists and philosophers. Its egg will remain the first model organism of science for a long time.

However, the bird needs to regain its original power, which can only be achieved by rewilding and by mixing its genes and generating new memes. To achieve that end, instead of an industrial chicken Vanmechelen constructs a cosmopolitan chicken, a companion and guide animal for the new generations of humanity, living in the mega cities of the near future and far beyond.

P. Dupont, K. Vanmechelen.

Reference

Lawler A. Why Did the Chicken Cross the World?. New York, Atria Books, 2014.