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GENETIC CHARACTERIZATION AND POPULATION STRUCTURE OF LOCAL POLISH AND ITALIAN CHICKEN BREEDS

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Contents

Abstract

The aim of this study was to compare the genetic variation and population structure of five local chicken breeds reared in Poland with six Italian local chicken breeds undergoing *in situ* conservation program. Twenty-three microsatellite markers were investigated in 709 birds belonging to 11 breeds: Miniature Cochin (MCO), Gold Italian (GI), Green Legged Partridge (GLP), Silver Italian (SI), White Leghorn (WL), Ermellinata di Rovigo (ER), Polverara (PL), Padovana (PD), Pèpoi (PP), Robusta Lionata (RL) and Robusta Maculata (RM). Two hundred forty seven alleles were detected in the overall population, with a mean number of 10.74 ± 3.48 alleles per locus. For the local breeds, the observed and expected heterozygosity ranged from a minimum of 0.28 (GI) to a maximum of 0.45 (ER) and from 0.30 (WL) to 0.58 (ER), respectively. The overall population heterozygote deficiency was 0.333, the average Wright's inbreeding coefficient (F_{IS}) was 0.218 and the heterozygote deficiency due to breed subdivision was 0.479. Wright's fixation index was slightly positive for all breeds, the lowest value was detected for GLP ($F_{IS} = 0.11$) and the estimated molecular inbreeding (f_{ij}) within breed ranged from 0.12 (GI) to 0.18 (PP) evidencing limited coancestry. Mean allelic richness, obtained with rarefaction method based on six observations, was 2.255 being the WL the less variable (1.72). Overall, genetic variability was slightly larger for the "Polish" breed compared to Italian ones but still similar to that of other local chicken breeds undergoing *in situ* conservation. Tomiuk and Loeschcke's D_{TL} genetic distance values, as expected, shown that the PD and PL breeds are the most related ($D_{TL} = 0.094$) followed by PD and PP ($D_{TL} = 0.108$) while, when considering the "Polish breed", the two Italian derived GI and SI breeds were the most related ($D_{TL} = 0.307$). The neighbour-net representation of D_{TL} distances evidenced a clear separation between the six Italian chicken breeds and the five 'Polish breed". MCO, as expected, is the more differentiated breed, SI and GI grouped together with WL. Structure analysis clearly confirmed the separation between the Italian and "Polish" breeds and did not evidenced particular substructuring in the analysed breeds. It is worthwhile to note that SI, GI and GLP were not separated by the current analysis. These results represent a starting point for the valorization of these local Polish chicken breeds as an important reservoir of genetic diversity. Moreover, for the Italian chicken breeds, the continuous monitoring of genetic variability parameters is needed to avoid the increase of inbreeding and the loss of biodiversity.

Introduction

Origin and domestication of poultry

The domestic chicken is among the most popular and widely spread domestic animal species. For thousand years, chickens have been used for food, religious activities, decorative arts, and entertainment. The chicken is the only widespread domestic species that apparently did not have origins in the Near or Middle East. The most probable wild progenitor of the domestic chicken belongs to the genus Gallus, however, the progenitor and the location of domestication remain controversial. Archaeological discoveries in the Indus Valley and Hebei Province, China, suggest that chickens were probably domesticated from the red jungle fowl (Gallus gallus), as early as 5400 BC (West and Zhou, 1988). Historically, there have been two hypotheses about chicken domestication: one that defends a monophyletic origin and another that defends multiple origins from several Gallus subspecies (Crawford, 1990, 1995). There are five possible progenitor subspecies of the red jungle fowl: G. g. gallus in Thailand and its adjacent regions, G. g. spadiceus in Burma and Yunnan Province of China, G. g. jabouillei in southern China and Vietnam, G. g. murghi in India, and G. g. bankiva in the Java islands (Crawford, 1990, 1995; Delacour, 1957; Howard and Moore, 1984). It is still not clear how many subspecies have contributed to the origin of chicken. Results of many works revealed that the monophyletic origin conclusions from early studies might result from incomplete sampling of domestic chickens and red jungle fowl. Yi-Ping Liu et al. (2005) demonstrated that chickens have multiple maternal origins and that domestications occurred in at least three regions of South and Southeast Asia.

The review made by Dessie et al. (2011) shows the dispersion of chickens from the putative centres of domestication to different parts of the world. Gene flow in poultry, from developing to developed countries, did play a role in the past, mainly in qualitative terms. All modern commercial chicken breeds trace back to breeds that were already being developed in northern Hemisphere countries one hundred years ago. Many of these breeds were built up from small numbers of imported chickens. These original imports probably date back hundreds of years, and Asian countries are the most important source.

Currently, there are three globally accessible data bases containing information on chickens;

however none of these provide a comprehensive system for systematically classifying domestic chickens in developing countries in terms of their present-day uses, potential for the future and distribution within and across countries. Such a system should be developed to include indigenous chicken genetic resources at the same level of detail as for other farm animals. The data management systems should incorporate all available information at the molecular level. Such information is important not only for discerning the existing diversity but also for making decisions on conservation priorities. Addressing the gaps in information on indigenous chicken genetic resources should primarily be the focus on the Domestic Animal Genetic Resources Information System (DAGRIS, http://dagris.ilri.cgiar.org/). DAGRIS, as a virtual library of indigenous animal genetic resources in developing countries, could play a leading role in delivering systematic information on the diversity, distribution and classification of domestic chicken in the tropics.

The poultry sector in Europe

The development of poultry production reflects the needs of consumers and can be summed up in a short timeline: first the interest was directed to the production traits, then qualitative characteristics, following different approaches have been used to maximize economic profit, while , in more recent years, interest has shifted toward product quality and environmental safety.

Nowadays, the global poultry sector is divided into a commercial sector dominated by international, developed-country based and vertically integrated companies, and a small-scale sector that provides up to 90 percent of the total poultry production in some of the least developed countries.

As incomes rise, people generally prefer to spend a higher share of their food budget on animal protein, so meat and dairy consumption tends to grow faster than that of food crops. As a result, the past three decades have seen buoyant growth in the consumption of livestock products, especially in newly industrializing countries, the so-called Livestock Revolution. Annual meat consumption per person in developing countries more than doubled between 1964-1966 and 1997-1999, from only 10.2 kg per year to 25.5 kg, a rise of 2.8 percent a year. The rise was particularly rapid for poultry, where consumption per person grew more than fivefold. According to FAO studies, world egg production is forecast to increase by 30% by 2015 as compared to 2000, with higher growth rates in developing countries (FAO, 2003). A strong consumer preference on poultry meat over other meat types can be observed. Most of this increase will come from intensive production systems located predominantly in peri-urban areas.

Local breeds are mainly used in the subsistence and small-scale sector of developing coun-

tries, supplying most of the eggs and meat consumed.
Over 6 billion broiler chickens are slaughtered for meat in the EU-27 each year, producing around 9.4 million tonnes of chicken meat, with an average per capita consumption of 17.4 kg/year (Avec, 2011). The top six chicken meat producing countries of the EU-27 are United Kingdom (14,7%), Poland (11,4%), France (11,2%), Germany (11%), Spain (10,9%) and Italy (8,3%) and they account for 67.6% of the total EU-27 Member States production. The UK is the largest producer, with Poland, France, Germany and Spain producing similar amounts.
According to this in Poland are produced about 1.070.000 tons of broilers per year and Italy, instead, produces about 780.000 broilers per year.
Such poultry provide several products and services such as food (meat, eggs), feathers, manure, and serves as dowry, for funerals and sacrifices, and income generation through cock fighting. Indigenous chickens have an inherent scavenging and nesting habit, they are more resistant to diseases, less prone to predator attacks and can survive under harsh nutritional and environmental conditions. Hence, selection in local breeds is targeted at adaptation to harsh environments and resistance to disease rather than enhanced production.
Modern poultry breeding started in the 19th century, and a multitude of breeds emerged, each one adapted to a specific local environment. From these breeds, modern specialised breeds and lines have been developed since the 1950s to produce high output in one major trait. Breeding goals were directed to achieve high performance in a few production traits (e.g. meat, eggs).
A consequence of the uniformity of environmental conditions is the need for fewer breeds, contributing to the reduction of livestock diversity (Tisdell, 2003).

Biodiversity

European Commission defines biodiversity as “the variability of life and its processes. It includes all forms of life, from single cell to complex organisms, processes, pathways and cycles that link living organisms, populations, ecosystems and landscapes” (DG AGRI, 1999). Biodiversity is the life that exists on earth in all its forms, is co-evolution between human beings and the environment, is a set of interdependencies, it is a live and evolve so recicproco. The term ’biodiversity’ is a contraction of “biological diversity” defined by the Biological Diversity Convention (BDC) in Rio de Janeiro in 1992 as the range of variations or differences within the same group of entities, individuals or groups. Therefore Biodiversity refers to the variety of the living world, identified also as “the link with the past at the base of becoming organic” (Matassino, 2010).
Biological diversity can be divided into three sublevels:

- ecosystems diversity (natural environments)
- species diversity (living beings)
- genetic diversity (breeds or varieties)

One more sublevel is represented by the interaction between the first three sublevels since living organisms and ecosystems build complex interconnected networks from which life depends.
The BDC adopted on 5 June 1995 at the World Summit in Rio de Janeiro organized by the United Nations about Environment and Development proposes three objectives:

1. Conservation of biological diversity (at the genetic, population, species, habitats and ecosystems level);
2. Promoting sustainable use of its components;
3. Equitable sharing of benefits arising from the use of genetic resources.

To achieve these goals the Convention requires a more efficient use of knowledges, a deeper understanding of human ecology and environmental dynamics, the application of sustainable technologies and practices and the promotion of international cooperation.
The Convention, finally, stresses the role of local communities and indigenous peoples for the biodiversity conservation.
Genetic diversity is the biological variability more important in agriculture, it is the source and subject of the genes necessary for the evolution and improvement of animals, plants and microorganisms species . This involves the differences that occur within the same species, population, race and varieties. In the opposite direction, when the number of individuals belonging to a population decreases, the genes are kept and this leads to a greater probability of diseases occurrence due to the presence of recessive genes or a general inbreeding depression.
In recent years, that is occurring in the developed countries, with a higher importance in agricultural and zootechnical field due to the exploitation of the few species for food production which leads to an increasing loss of the variability.
The FAO has estimated that on Earth exist 14 million species of which 10 million are animal species (7.616 of zootechnical interest, of which 6.536 local breeds, of which 690 declared extinct), 1.5 million are fungi, and 300,000 are vegetables, the remaining species are composed of algae, bacteria and microorganisms (Panorama on the world's biodiversity, Biological Diversity Convention UNEP, 2002). Speaking about animal diversity, of the 50,000 animal species of mammals and birds, about 30 have been used for agriculture and is estimated that 90% of global production comes from only 15 species of animals. It is estimated also that

currently about 1.350 breeds are at extinction risk. Instead, as regards plant species, only 150 are cultivated, 12 of which contribute approximately 75% of our food requirements, and 4 of them produce more than half of the food that is consumed by humans (FAO 2007). “The protection of biodiversity and ecosystems must be a priority in our attempts to build a world economy stronger, clean and fair” (Gurrìa 2010). Therefore, in recent years the term biodiversity has spread considerably. It has spread, in particular, awareness of the importance of the value of biodiversity (genes, species, ecosystems), believing that their conservation is essential for maintaining life on the planet. Biodiversity is a unique and precious heritage genetic but also cultural, social and economic. Without the variety of life forms, life itself will disappear, because living beings lose their ability to deal with changes, to adapt, and thus to survive. Together with the genetic heritage are lost skills, knowledge, languages. The BDC 1992, defines biodiversity conservation as a ”common interest” of all humanity and as an integral part of the development process, but at the same time contains only a few binding rules giving to any Member State considerable scope in exercise of in the implementation of their rights and their obligations. In 2002, at the World Summit on Sustainable Development in Johannesburg, has set as a goal by 2010 to stop the loss of biodiversity. This target is still very far: the percentage of known species extinct in the last century is more than 100 times higher than the of natural mortality rate calculated on the basis of the fossil record. In December 2006, the General Assembly of the United Nations decided to declared 2010 International Year of Biodiversity. Pushed by the serious concern about the social, economic, ecological and cultural biodiversity loss, the Assembly expressed hope that the 11 the National-States and other actors seize the opportunity to raise awareness on the importance of biological diversity and to carry out local, regional and international actions. On 23 June 2011, the European Union has signed the Nagoya Protocol, an agreement was an important step forward in the biodiversity protection. The protocol does not address only the problem of conservation but supports a more equitable access to genetic resources and equitable sharing of benefits arising from their use. The agreement obliges industries to share the profits, arising from the use of plants and animals genes to develop new products, with local communities, in general indigenous people who live in countries rich in biodiversity which preserve age-old knowledge about their functions and properties.

Since 2010 there is the willingness to popularize the concept of biodiversity through education , because it has a environmental, economic and ethical-cultural value (Fromm, 2000).

The maintenance of the ecological and climate balance of the Planet is one of the most important functions biodiversity performs (Begon et al., 1989; Swingland, 2004).

Biodiversity in terms of genetic and cultural variance is the richness of life on the planet being the base of balance and stability and maintaining the ability to adapt to changes in living species. Reduces diversity ecosystem loses his balance, failing be able to restrict the negative phenomena, that can be described by impoverishment of the soil and the resulting erosion, increase in the spread of pathogens and epidemics occurrence, increase of environmental disasters characterized by long periods of drought or the same amount of rain. Maintaining

the balance of nature comes from fauna and flora conservation, but also, from environment. It is necessary to consider the interactions that exist between them and keep them consolidated in order to maintain the ecosystem stability (Swingland, 2004). Over the years, agriculture has evolved focusing on increasing production, which in ecology and environmental field led to standardized productions, with a important and dangerous loss of variability and replacing several local varieties. These new crops, based on a very weak genetic diversity, have low defense ability in adverse climatic and environmental conditions, and in diseases respond. On the contrary, the local varieties are distinguished by their broad-based genetic resource required for the improvement of existing and future generations, are thus the basis for renewing the production techniques and lead to new opportunities for growth and development in humans.

On the contrary, the local varieties are characterized by broad genetic base, resource required to improve existing and future generations; they represent, therefore, the basis to renew production techniques and bring to new opportunities for humans growth and development.

Ethical-cultural dimension of biodiversity refers to all of the ethical, social, religious and symbolic that natural resources represent for different cultures of the world (Negri and Veronesi 2000).

In addition to the environmental and genetic diversity there is also cultural diversity, the culture, in fact, is the interface to our relations with the surrounding world and determines our interrelationships with other species. That includes diversity of language and culture, etc., and is a tool for a better adaptation to different and in continuous mutation environments. The loss of a species or a variety consequently leads to a loss of tradition, knowledge, and thus a lack of use the resource for the future. Biological diversity is perceived as an economic resource ; ecosystems and their species human being produces many necessity such as drinking water, foodstuffs, energy, natural fibers for textile products, building materials or medicinal substances. Genetic resources are essential for the production of new varieties which contribute to diversity of landscapes thus satisfying our aesthetic needs.

These benefits are running low, and the main problem is to quantify the economic value of biodiversity which is an inheritance from thousands of years of ecosystem evolution; determine the economic loss is almost impossible (Clauser, 2002; Santolini, 2004). The human being is the cause of his loss it is his duty find out the most suitable solutions to restore the balance, there is a moral obligation to preserve and improve this heritage, to deliver it to the future generations, because when lost, it will be forever (Alcàzar 2008).

Agro-biodiversity

Agricultural biodiversity is the result of the human beings continuous work, described as the essential resource to satisfy the basic need: feeding, it is also the result of domestication,

adaptation and conservation that generations of farmers have realized from the beginning of agriculture. Agricultural biodiversity is a branch of natural biodiversity, and contribution that gives comes from the human impact on production systems, thus to bring out and diversify the local varieties, themselves genetic base of the development of modern varieties, produced in this century. Agricultural biodiversity is also the outcome of differentiation processes that occur within species as a result of adaptation to the environmental, climatic and ecological conditions, without selection. This branch of biodiversity is of leading importance for the development of animal resources, essential for breeders. Indeed, animal breeds genetically different are source of diverse alternatives to respond to future needs. Animal genetic resources are also crucial for food security, because, they contribute to more than 7 billion people needs, they are the basis of reserves for population survival and welfare and, not least, they contribute to the eradication of hunger (FAO and ONU 2010). The agro-biodiversity must be constantly enhanced and because it is not just source of food sustenance but also provides many alternatives for industrial development, such as textile fibers, as well as, medical principles. It is, therefore, necessary to adopt policy measures to safeguard animal genetic resources.
Despite concerns about the continuous and slow degradation and, knowing its vital importance and agricultural biodiversity is disappearing: if during the history of mankind, it was estimated use of about ten thousand species for feeding and agriculture, currently little more than 120 species of crop plants provide 90% of the feed and only 12 plant species and 5 animal species provide more than 70% of the feed. It is therefore striking and clear the great loss of so-called "principal food species". This is because to deal with a high demand for products such as milk, meat and eggs, it had to respond with a high and uniform output, using specialized breeds and varieties and by modifying the production systems which caused, in particular, an erosion of animal genetic resources. Has been selected the genetic heritage to obtain highly specialized breeds, losing the most of the local and typical breeds , because of their poor productivity, however, representative of a specific culture . By doing so it been lacking also part of cultural diversity.

Causes of biodoversity loss

The United Nations came together 20 years after the meeting in 1992 when they had emanated the Framework Convention on Climate Change (UNFCCC), the Convention on Biological Diversity (BDC) and the Convention to fight desertification (UNCCD). From 20 to 22 June this year took place in Rio de Janeiro the new Earth Summit. After exactly 20 years, climate change is the main cause of biodiversity loss and desertification, which are the issues on which action is needed to save ecosystems.
In addition to climate change, other causes are implicated in the loss of animal genetic

resources:

- Fast socio-economic changes;
- Exploitation of highly productive breeds;
- Occurrence of animal diseases;
- Socio-economic loss in areas with high animal genetic resources.

Alarming data continue to come to light regarding animal genetic resources, in 2007 at the Technical Zoo Genetic resources Conference held in Interlaken (Switzerland), FAO introduced the report: "The State of World's Animal Genetic Resource for Food and Agriculture" with the target to evaluate the global status of animal genetic resources and the capacity of countries to lead this richness in a sustainable manner. According to this report from 2000 to 2007 one animal breed per month has become extinct, in addition, about 20% of the poultry, cattle, horse, sheep and pig breeds are now at risk of extinction.
The FAO describes the main factor, witch regard the animal genetic resources erosion, as the construction of intensive farms arising from the globalization of livestock markets in turn due to changes in food habits of man. The exploitation of a few genetic types has led to the development of highly productive and selected breeds often coming from Northern Europe, with the resulting loss of local and typical breeds, double / triple attitude with great adaptive capacity to environmental changes. Another phenomenon that, according to the FAO, provoke the loss of biodiversity is global warming together with an increase in desertification. Arid or semi-arid ecosystems occupy more than one-third of the lands above sea-level and they are vulnerable to exploitation and degradation, which threat the food security of almost a billion people. In 1991, the process of degradation was about 15 per cent of the mainland, but this percentage has reached 24 per cent in 2008. Is, 'therefore, necessary to preserve the existing genetic resources in agriculture and animals in order to allow adaptation to the continuous climate changes.
A risk is also wild-biodiversity, including flora and fauna, due to fragmentation of the territories, inasmuch the construction of roads, highways, dams, and urbanization projects have encouraged deforestation with loss of habitat and genetic resources.

Animal genetic resources

With Animal Genetic Resources (AGR) are include all animal species, breeds and varieties of economic, cultural and scientific interest in the present and for the future of humanity,

in terms of food and agricultural production. The World Watch List for Domestic Animal Diversity (WWL-DAD), in 2002 at its third edition, is a identification and monitoring system of world domestic breeds wich are at risk of extinction, developed under the Global Strategy for the Management of Farm Animal Genetic Resources Project of FAO. The objective of the WWL-DAD is to communicate the status of animal genetic resources simplify interventions aimed to stop or reduce the negative trend of animal genetic diversity erosion. For each population is given a long list of information, from local names, distribution, status to the main uses of the animals and the presence or absence of conservation plans.
As regards the poultry biodiversity in Italy, a census in 2001 showed the presence of: 9 breeds of domestic and musky duck, 10 of Pharaoh, 53 of chicken, 5 of goose and 12 breeds of turkey all local. Is also important to note that currently only 23% of the Italian biodiversity existed in the '800 and '900 decades has remained almost untouched until today. The preservation of animal genetic resources is implemented through national and international programs that are fundamental as they allow to maintain a high number of the animal population (both in quantity and in terms of genetic variation) in order to permit the preservation of the national livestock. It is crucial as well monitor and safeguard the genetic diversity of poor areas in developing countries, and biological diversity in the broadest sense, as a source of sustenance and survival for local people. Particular attention should be paid to developing countries where are introducing breeds with high productivity that go to supplant the local breeds.

The importance of animal genetic resources protection

According to the FAO classification in "Global Data Bank for Animal Genetic Resources for Food and Agriculture" 7616 breeds have been registered, of which 6536 are Local and 1080 cross-border. Into "The State of World's Animal Genetic Resource for Food and Agriculture" breeds were classified as local (when they are spread in one country) or cross-border (if broadcast in several countries). FAO, furthermore, to protect and promote the genetic resources of domestic animals, and allow the collection of information, set up the Domestic Animal Diversity Information System (DAD-IS, http://dad.fao.org): this system contains information about origin, size and risk status of about 6 379 breeds from 30 different species derived from 170 countries; and it aims to provide guidelines for new conservation activities. In accordance with the FAO report (2007), it required monitoring of genetic resources in developing countries because they have low-spread breeds, so less known than the breeds in developed countries.
The most developed countries, with a production based on a few races, have the highest percentage of breeds at risk of extinction in particular Europe (28% of mammals and 50% poultry) and North America (20% of mammals and 79% poultry) are losing the majority of indigenous genetic resources.

According to the "Report on the Status of Animal Genetic Resources in Italy" (2005) the assets of the ancient local Biodiversity can be defined as a necessity suitable to satisfy the needs socially relevant expressed by a particular social context in a given historical context. An ecological breed or ecotype could identify with a local genetic type (LGT) (Matassino, 1996) or an ancient local genetic type (ALGT) (Matassino, 2001). The first may include genetic types present in a determined environment at least for 50 years, while the second present must go back to more than 50 years, untill centuries (Matassino, 2005).
The protection of animal genetic resource, LGT and ALGT plays a fundamental role for the following reasons:

- socio-economic: mostly bred in marginal areas where intensive production model can not be applied in the absence of economic assumptions that make it convenient only the LGT can play a livestock role considering their production capacity;
- biological: only a broad range gene guarantee the possibility of evolution or change to allow the life of living beings;
- cultural: LGT can be considered as cultural assets because they are an extraordinary biological heritage to hand down to future generations;
- ecological: local breeds and their products are closely linked to the area of origin, thus present the history of that land culture;
- research interests: local breeds have distinctive characteristics and/or unique genetic heritage;
- opportunities for future requests or market changes: a change in the market demand for products leads to a diversification of production or use of different animal genetic resources for hobby activities.

To achieve acceptable goals of development in the local genetic types is essential a strong action of local institutions aimed at training farmers with the purpose of promoting the development of the territory linked to tradition. Already in 1990 the ConSDABI (Consortium for Experimentation, Diffusion and Application of Innovative Biotechniques) has introduced plans for Protection of LGT and/or ALGT on limited diffusion.
At molecular level the efficient management of animal genetic variability is represented mainly by the presence of allelic diversity (the differences in DNA sequences between genes that influence the development and performance of animals). Managing LGT requires human resources (trained staff and technical equipment) that not all countries in the world, and particularly those in developing have access to. In fact, 48% of countries do not have national in vivo conservation programs and 63% of them do not have in vitro programs,

so there is no possibility to preserve embryos, semen or other genetic material that could reconstitute live animals in a following period (FAO 2007).

The selection criteria underpinning the conservation of animal genetic variability

The choice of breeds conservation is based on certain characteristics :

1. *Level of risk of disappearance:* the first and most important criterion. The ideal would be to keep as many breeds as possible. The genetic variability is optimized keeping many pure breeds with an average size of the population rather than a few races with a large size of the population (for example 1000 total individuals , the best strategy is to keep 4 breeds with 250 individuals each). The breed is a fundamental key in the conservation of animal genetic resources. The disappearance risk is high if: the population size decreases, the population level of inbreeding increases and the distribution of farm animals is unbalanced.
   In addition, breeds with a high population effective size are not considered as priority in conservation.
   FAO has outlined the classes of disappearance risk to describe the status of the different species/races, based on the breeding animals number, summed in Table n. 1 (In this study were considered only breed represented by small population).

2. *Adaptability to specific environments:* breeds with adaptability to specific environments should be preferred for conservation compared with other, especially if the environment itself is also to be retained.

3. *Characters of economic importance:* the breeds that are of special interest for conservation are those that present: one or more economically important characters (example: fertility, efficiency of feed conversion, high quality of products, disease resistance) and these character has to be important for the future.

4. *Uniqueness in the characters:* some breeds may be preferred as a choice for conservation according to their physiological, behavioral or phenotypic characteristics; these peculiarities dependent on individual genes or polygenic effects.

5. *Genetic uniqueness of the breed:* the genetic uniqueness of a breed is of value when the same breed possesses combinations of alleles and genes such that the characters they control may be important for future adaptability, environmental production and

research purposes. The conservation of breeds genetically different is an excellent strategy to ensure sufficient genetic variability in the future.

6. *Historical and cultural value:* breeds were developed, in part, by human intervention and thus can be regarded as part of the cultural or historical heritage of a give region or population that has been passed down the generations and thus should be passed on to future generations (Ruane, 2000). It may be appropriate to give higher conservation priority to breeds that have greater cultural importance. In many areas of the world, traditional grazing over many centuries has contributed to the creation and maintenance of agro-ecosystems that have high biodiversity value. Similarly, many landscapes have been shaped over time by traditional farming systems. The results of co-evolutionary processes among locally adapted breeds, traditional farming systems and the natural environment retain their character and richness as long as the breeds and production systems are maintained. A breed's role in maintaining a unique ecosystem may be a reason for giving it a high priority for inclusion in a conservation programme. Methods for estimating the cultural value of a breed are available (Gandini and Villa, 2003; Simianer et al., 2003), but is a criteria very difficult to quantify, depends on the presence history of a specific breed in a given area and for a certain period, and the importance of its products. It can typically generate profit indirectly by tourism activity. The importance of this criteria should still be higher in developed countries than in developing countries. Historical and cultural value is the most important criteria in this study because all the chicken breeds involved are not reared to make profit, they are bred by amateur breeders or they are kept in conservation centers or experimental stations.

Desappearance class risk	Number of females	Number of males
Extinct	–	–
Critique	≤ 100	≤ 5
Critical-Safeguard	≤ 100	≤ 5
Danger	$100 - 1000$	$5 - 20$
Danger-Safeguard	$100 - 1000$	$5 - 20$
Not at risk	> 1000	> 20
Unknown	?	?

Table 1: Disappearance classes of risk based on FAO classification (2007)

Conservation methods

Conservation strategies to preserve the breeds that have already been adequately characterized, identified and classified be at risk are aimed to promote activities that allow to the breeds to become self-sufficient in the future. These strategies are:

- In situ-*alive conservation:* keeping the breeds in the production system in which are grown, adding value to their productive characteristics, without severely impacting on sustenance opportunities, particularly for the poorest farmers. It includes the protection and management of alive animals of local breeds but it can also regards the In vitro conservation method.
  Animals are used to allow land and vegetation management and/or organic farming.
  Sometimes actions are required at economic level of subsidies from public or private funds to allow this type of storage.
  Even today, not much is known, about how to improve production systems and infrastructure in order to ensure the survival and food security of the local population and in the same time preserving local genetic resources.
  This type of preservation can be defined as conservation of genetic resource through the continuous use of the various local genetic types by "breeders keepers" in agro-system which they are rooted. The advantage is that it allows to use an ideal rearing environment for all local genetic types at the same time, however, it has the disadvantage of having an unbalance relationship between male end female number, compared to the "Effective Number" of the population (use of one or few males only).

- Ex situ-*alive conservation:* the conservation can also be ex situ thus the alive population is not maintained in territory of origin but somewhere else. This also provides the opportunity to study different type of adaptation, production and behavior at temporal and spatial level, parameters useful for the estimation of fitness. But it even involves the In vitro conservation, in fact, the most efficient method is to match the vivo with the in vitro in order to maintain an accessible source of genetic material of the local breeds in a different environment than the original.

- In vitro-*conservation:* when storing in vivo can not be performed (such as due to the occurrence of an epidemic). This method refers to the collection and cryopreservation of genetic material for reproduction in the so-called gene banks, it regards semen, embryos and several type of tissues samples. Clearly this conservation approach does not require bond with the breeds place of origin, so it can be carried out in situ or ex situ mode.

A working sample of in situ conservation plane is the CO.VA project. The CO.VA project (Conservation and Value of venetian chicken breeds), created in 2000 thanks to the Veneto Region, has the aim of safeguarding some of Venetian chicken breeds (chickens: Ermellinata of Rovigo, Paduan, Pépoi, Polverara, Robusta Maculata and Robusta Lionata; ducks: Mignon Duck and Germanata Veneta; turkeys: Ermellinato of Rovigo and the Bronzato Comune and finally the Camosciata Fowl), were judged as historical and socio-cultural interesting, so, deserving protection and enhancement. The six chicken breeds included in

CO.VA project were been analyzed in this work as Italian local breed and more detailed information about them will be available in the following pages. Over the years the market has had to respond to demands of increasingly standardized products and this has led to the spread of crossbreeds able to achieve high output resulting in a reduction of the local breeds. The project regards preserving Venetian poultry micro-production chain that were getting lost, valorizing in parallel hardiness and resistance to diseases of these breeds compared to commercial hybrids.
The project want to keep the genetic resource pure, maximizing genetic variability and, in the same time, valorizing their products.
The in situ conservation stations from which the Italian local breeds came breeds are: "Antonio Della Lucia Institute", Feltre (BL); "C. Cavour Institute", Castelfranco Veneto and Montebelluna, (TV); "Sasse Rami Experimental Farm", (RO) and " Duca degli Abruzzi Institute", Padua, (PD). Livestock farming activities and conservation plane are developed in the same way and time in all stations: the conservation stations are divided into three zones: zone of hatching, zones of adult boxes and zones of reassembles animals boxes. The breeding area, which has a hatchery and the rooms of weaning is active from February to May, in the adults boxes the houses for rest have access to a large outdoor paddock, they are usually two, one for females and one for males. Usually the box for females are bigger than those for males. The boxes of the reassembles animals, used from April to October, are similar to the adults ones but divided into two zones, each one per one family, with a large number of trees to ensure animal welfare and protected them from predators attack. In each station for each race there are about 34 females and 20 males. Usually in a randomly selected population, the rate of inbreeding per generation should not exceed 1% (risk threshold), for that reason it is estimated that the minimum number of individuals should not be less than 50 (Meuwissen and Wolliams, 1994).
The male breeders are divided in two families based on genetic relationships identified by molecular markers analyses in the first two years of project, females are instead kept in a single group. The animals chosen for breeding must first submit the phenotypic characters that fit within the standard of a race and must come from both families. The choose of the breeding animals is an important step, which influence the success of the conservation plan. To complete this phase is also provided collection and analysis of the production and reproduction data, such as maturity weight and laying and incubation of eggs. The reassembles is constituted by two separate groups arising from the shifts of the two groups of breeding males.
The breeding season begins in February by using an artificial lighting program.
The first group of breeding animals is placed for about three weeks with the females. After removing the first males the collection of eggs is interrupted for two weeks to allow females to be ready for the second group of males for another three weeks period. The breeding period ends when is reached the target of 90 chicks per family (180 chicks per race). At hatching, the chicks are marked with wing brand and at the end of the breeding season (end of April)

are put in a box. Between October and November is also performed the selection of young breeding males and females that will be used in the next season. The conservation program is based on a complete biennial replacement of breeding animals in order to increase the generation interval, the effective population size and reduce genetic drift. Each year, then, for each race, 50% of males (about 10 male) and 50% of females (about 17 females) are replaced.
The indexes used for the selection of breeding stock are four: original group, morphological standard of race, production performance and reproductive performance. The first index is the most important, it is necessary that 50% of males and females come from the two groups (families). The young animals must also comply with the requirements of the breed standard as the color of the plumage and tarsus, the overall morphology and size. At this time, productive and reproductive parameters are evaluated. In December, all breeding selected, are weighed and were taken from them a blood sample of each one for DNA analysis. Finally, in January, all breeding males of all races are exchanged between the conservation stations according to a defined pattern.
Until 2005, the genotyping of individuals was performed using the AFLP technique (De Marchi et al., 2006). Later, microsatellite molecular markers have been used. These markers are distributed throughout all the genome, have a high polymorphism and are easy to detect by standard and automated methods (Cheng et al., 1995).
As the example above-mentioned, in developed countries, a large number of breeders contribute to conserving their traditional and local breeds. There is an effort to maintain in vivo the reserve lines from companies or university research lines that are no longer needed. These breeds represent an important cultural value because they are directly linked to territory, traditions and people. They are an essential resource that must be preserved for future generations.
The conservation of biodiversity is fundamental in the management of local and less diffused breeds but before taking any protective measure, data covering molecular genetic diversity of breeds is an absolute requirement. Microsatellite markers have been proven to be efficient in evaluating genetic diversity and relationships of farm animals and in particular local chicken breeds (Hillel et al. 2003, Dávila et al. 2009, Tadano et al. 2007, Zanetti et al. 2011).

Population genetics methods

Population genetics refers to the branch of genetics that analyzes the establishment of the populations in quantitative terms, such as allele and genotype frequencies, and evaluates the population in temporal and spatial terms, respectively the ways which genetic characteristics are transmitted to offspring and their value in relation to the territory.
The genetics of the population is represented by a theoretical part which uses statistical

methods and one, more empirical, which detects and quantifies the gene diversity to knowledge objectives about the natural history of the populations or the level of biodiversity to conservation purposes as in this the project.

Genetic structure of populations

In natural populations is important to know the genetic structure in order to study the variability and to form correct hypotheses and approaches to maintenance/preservation of biodiversity in populations. In populations of the majority of species there are different levels of genetic differentiation. To describe the genetic structure of populations are commonly used two approaches:

- *Direct methods:*based on the observations of animals movements, gametes or molecular markers. This approach, however, provides restricted visions of gene flow in time and space, given that occasional wide-ranging migrations are extremely difficult to observe, and movements observed of animals, gametes and markers may not make sense from an evolutionary point of view if individuals observed do not reproduce in the new place.
- *Indirect methods:*based on the observed spatial distribution of the alleles, genotypes or phenotypes, which allow to make hypothesis about historical levels or on the flow pattern of gene that gave rise to the current pattern of genetic variability.
  These methodologies are in other words, sensitive to the rare past events that have influenced the current structure of populations. The methodology for indirect estimation of gene flow are ground on equilibrium expectations from theoretical models of population structure.

Hardy-Weinberg principle

The Hardy-Weinberg principle (also known as the Hardy-Weinberg "Equilibrium", "Model", "Theorem", or "Law") states that allele and genotype frequencies in a population will remain constant from generation to generation in the absence of other evolutionary influences.
H. Hardy and W. Weinberg called in 1908 "population equilibrium" a population in which neither allele frequencies and distribution of genotypes change with the ensue of several generations. These evolutionary influences include non-random mating, mutation events, natural selection events, genetic drift, gene flow and meiotic drive. Since one or more of these influences are typically present in real populations, the Hardy-Weinberg principle describes an ideal condition against which the effects of these influences can be analyzed. According to this principle a population remains in equilibrium only if occur some restrictive conditions, such as:

- no mutations event;
- no gene flow between populations;
- the population is infinitely large;
- no natural selection, all genotypes must have the same reproductive and adaptive capacity.

The occurrence of these conditions within a population causes a stable status of allele frequencies for an indefinite period of time. In contrast, the absence of these conditions causes a change of allele frequencies or evolution. There are very few populations who reflect this law and then are perfectly in equilibrium, in effect an opportune choice of matings makes it possible to increase or decrease the level of population homozygosity or, conversely, leave it unchanged.
In the last years it is increasingly evident as farmers are trying to improve the characteristics of their animals using as breeders the "best" of each generation with programmed mating so as to obtain, after a certain number of generations, a "genetic pool" of animals with the desired characteristics. To achieve a standard of "type" the breeders use inbreeding animals. This method causes, at the genetic level, an average increase of homozygosity and a simultaneous reduction of heterozygosity.

Molecular Markers

Molecular markers (the so-called II class) are defined as a polymorphic sequence of DNA located in a specific genomic locus that marks, in typically and unequivocally way, the chromosome section in which is identified. Molecular markers can be detected with probes and/or specific primers, they allow to obtain very detailed information, analyzing, in addition to the stretches of DNA coding sequences also of no coding DNA.
The application of molecular markers in recent years has taken on a important role in the research on genome of many species. They identify easily mutations that have been accumulated during the evolutionary history of the population under investigation and may allow the study of phylogenetic relationships. The level of polymorphism of the single marker is directly proportional to its ability to discriminate and therefore higher is the number of polymorphisms larger is the informativeness of the marker.
Molecular markers have several important features such as:

- *Spread:* they are spread on all the genome;

- *Polymorphism:* they are characterized by a high polymorphism, it is also possible to detect polymorphism between individuals genetically similar and phenotypically identical;
- *Universal;*
- *Inheritable;*
- *Not influenced:* by the environment, sex, or age; they are stable
- *Neutral;*
- *Co-dominant:* generally (there are exceptions) are co-dominant, then allow to distinguish homozygous from heterozygous;
- *Present in any tissue:* can be analyzed from any tissue samples (blood, hair, meat, milk, feathers,...);
- They don't present, unlike morphological markers, epistatic or pleiotropic effects;
- *Easy to monitor;*
- *Analysis automatizable.*

The goals of the use of molecular markers are several:

- *Product Traceability:* thank to the simultaneous use of more molecular markers are obtained allelic profiles witch are able to uniquely identify individuals (fingerprinting). Given the limited probability that two individuals share the same genetic profile for an adequate number of polymorphic markers, there is an effective opportunity to identify with certainty the animals or products originating from them along the entire chain;
- *Studies of phylogeny, genetic variability, structure and dynamics of species and populations:* molecular markers can be used to evaluate the genetic variability present in the species and breeds and to provide information on the evolutionary history of a population;
- *Estimation of inbreeding;*
- *Analysis of paternity/maternity and forensic genetics:* important for selection, as it can contribute to the reconstruction of pedigree (Vignal and coll.2002), or in cases of legal disputes;

- *Mapping of QTL (Quantitative Trait Locus):* the characters in animal science are primarily quantitative, the manifestation of these characters is due to the expression of multiple genes simultaneously. The genomic loci that contain these genes are defined QTLs. Identify them is interesting in order to understand the molecular mechanisms which determine the expression of the character as well as the potential application of a process of genetic selection based directly on the genes that determine the productive characteristic concerned;

- *Mapping and isolation of useful genes.*

In order to investigate the genetic variability through the use of molecular markers is essential to obtain information such as:

- *Observed and expected heterozygosity:* the heterozygosity is the percentage of loci (markers) on total heterozygous loci analyzed. In particular, if there is a deficit of heterozygosity for which the observed heterozygosity was less than expected (compared to expected values if the population were in Hardy-Weinberg equilibrium) may be due to the existence of semi-isolated units in the population (the population is structured in itself) or the presence of a high inbreeding level;

- *Allelic diversity:* is the average number of alleles on all loci in a population;

- *Linkage disequilibrium:* indicates the presence of associations between specific alleles related to two or more loci, the phenomenon of association may also occur between loci not necessarily physically close to each other, if within the population there is a large group of individuals originated from a common ancestor and there is not a big flow, at the genetic level, such as to ensure an effective recombination.

Among the markers currently most commonly used to investigate the variability and biodiversity should be mentioned the microsatellite (STR, simple tandem repeats) and SNPs (single nucleotide polymorphisms).

Microsatellites or STR (Short Tandem Repeats)

Microsatellite markers have been proven to be efficient in evaluating genetic diversity and relationships of farm animals and in particular local chicken breeds (Hillel et al. 2003, Dávila et al. 2009, Tadano et al. 2007, Zanetti et al. 2011).
Microsatellites are regions di- DNA characterized by a tandem repeat di- short sequences of nucleotides, such repetition. (AG) n repetition. The repetition (AT) n is the most frequent

in plants, while in mammals, the most frequent is (AC) n repetition. The repetitions dinucleotide-AC / TG occur with greater frequency and are highly polymorphic, they are rather rare repetitions CG / GC because the cytosine residues flanked on 3' by guanine (CpG) are subject to methylation and subsequent phenomena deamination convert cytosine to thymine, and then the CpG change progressively in TpG.

Microsatellites were detected in large numbers in both prokaryotic organisms and eukaryotes, and are present in the coding regions of the genome and non-coding (Zane et al., 2002). The high variability found in the microsatellite loci is due to the high mutation rate, which was estimated to be 10-2 to 10-3 events per locus per generation, very high compared to the percentage of variation that occur at genetic level 10-5 / locus / generation (Li et al., 2002). The high variability observed in microsatellites is manifested in the different number of repetitions of the repeating pattern. This event most likely occurs because of the "slidin" of the polymerase during DNA replication. Precisely because they have highly repeated sequences, the DNA polymerase enzyme, during the DNA replication, may incur in pairing errors. If repeated over time cause an increase or a decrease of the repeats inducing the onset of different variants depending on the individual (Cunningham et al., 2001). Moreover, a further reason for to the high polymorphism can be attributed to unequal recombination processes or gene conversion. The regions that flank the repeated pattern are conserved within the species and often also at the interspecific level. Through these conserved regions is possible to construct probes or primers which allow to detect the repeated regions. The technique used for their detection is the PCR (polymerase chain reaction) followed by capillary electrophoresis. PCR allows to synthesize repeatedly by enzymatic via one or more DNA segments located between two known nucleotide sequences, producing a high number of copies through a series of DNA denaturation reactions, hybridization with the primers and synthesis of new strands. The products obtained from the PCR reaction are subjected to high resolution electrophoretic analysis in order to determine their size and to identify the allelic variants present.

The primers, short DNA fragments of 18-20 bases, are designed to be complementary to the adjacent sequences respect to repeated one. They are also marked with different fluorophores; these molecules absorb the energy provided by a laser and then re-emit at characteristic wavelengths for each fluorophore. The graphic result of the computerized processing of the fluorescence signal is said electropherogram. It is a graphical representation in which the area and the height of the peaks indicates the intensity of the signal that depends on the amount of amplicon present and the size of the fragment according to the time electrophoresis requires comparing the products concerned with the standards of known size.

Currently for most domestic species investigated (cattle, sheep, goats, poultry) are available hundreds of microsatellite markers already "mapped" of which are known all the necessary information (sequence, chromosomal location, etc..) for their analysis.

Biodiversity analysis in poultry

Recently, analysis were conducted on a large scale that have included dozens of chicken populations sampled in geographic regions around the world (Hillel et al., 2007; Granevotze et al., 2007; Granevotze et al., 2009). The populations analyzed were separated into three groups at the macroscopic level, corresponding to the regions of Asia, Africa and Europe, with different levels of polymorphism for each region. In European breeds was found a low degree of polymorphism, mainly due to standardization, selection and limited numerosity of populations. In contrast, the native populations of Asian and African non-selected show high levels of genetic variation and show no structured populations. High levels of differentiation can be measured only between areas and countries very far (Muchadeyi et al., 2007; Mwacharo et al., 2007; Berthouly et al., 2008; Chen et al., 2008).
Smaller-scale studies carried out at the national level on local breeds have confirmed that they are genetically distinct, with well-defined genetic structures, but show low levels of genetic diversity, especially regarding variability within breeds (Zanetti et al., 2010 Marelli and coll., 2006; Strillacci et al., 2009; Tadano et al., 2007).
Some genetic data collected regarding the chickens were deposited in the AVIANDIV database (European project that promotes collaboration between different European research centers to study biodiversity) with the aim to compare the results between the different units research. This database contains information on gene pools of 52 commercial breeds genotyped at 20 microsatellites loci and 20 more breeds genotyped at 27 loci, there are also data concerning the markers SNP genotyping but only for a limited number of races and individuals.

Chapter 1

Aim

The objective of this study was to estimate and compare intra and inter genetic variability and population structure of five old local chicken populations reared in Poland (Miniature Cochin, Gold Italian, Silver Italian, Green Legged Partridge and Leghorn) and six from Italy (Robusta Maculata, Robusta Lionata, Ermallinata di Rovigo, Pèpoi, Polverara, Padovana) using microsatellite markers.

Chapter 2

Materials and methods

2.1 History and characteristics of involved breeds

This work includes five old breeds kept in Poland: Miniature Cochin, Gold Italian, Silver Italian, Green-Legged Partridge and Leghorn; and six Italian (Venetian) chicken breeds: Robusta Lionata, Robusta Maculata, Ermallinata di Rovigo, Pèpoi, Polverara, Padovana.
Most of the breeds considered in this work are bred at an amateur level in Poland and Italy. More information is available about the breed Green-legged partridge reared in Poland because is under conservation plan while the others are not. This also applies to the Italian breeds mentioned in this work, there are no genealogic books that allow to have sure data on the numerosity of these populations, the figures cited were obtained thanks to the work of the stations where all the six breeds are kept under conservation plan. Shown below a brief description of the breeds in terms of historical and functional features.

2.1.1 Breeds kept in Poland

Miniature Cochin

Miniature Cochin is a variety derived from the original Cochin Chinese breed, exported to Britain and America in the middle of 19th century. The most distinctive feature of the Cochin is the excessive plumage that covers leg and foot. The Cochin is a hardy, friendly, and docile chicken. Cochins also will adapt very easily to confined spaces or open range. Cochin hens are fairly broody and good mothers, and are known to be good surrogate incubating birds in even falcon breeding. They can lay many eggs, but usually not for extended periods of time.

However, they are slow to mature. This breed was admitted into the APA in 1874. There are 18 colors of the cochin chicken, including: partridge, birchen, splash, blue, buff, gold laced, silver laced, barred, black, red, and white. The skin beneath the feathers is yellow and the egg colour is brown, more detailed informations are given in Table 2.1. Cochins also come in a variety called frizzled, in which the feathers are turned outward.

Figure 2.1: Miniature Cochin individuals from experimental stations (Poland)

Gold Italian

The breed were formed from countryside Italian chickens
in Italy in the 19th century.In a short time became a very popular breed due to combination of beauty and performance and it became widespread in many European countries. Gold Italians were brought in America, then in England and next in Germany, the first time, of which there are records, in Germany was in 1870 . There are 22 recognized colors, in addition to the variety of color and harmonious lines this breed has virtues such as early maturity, and high performance for many years. The impact of color is very distinctive and allows to distinguish the gender with safety, even in 1 day of life chicks.
The Italian are medium in size with elongated neck, especially in hens, the tail is strong, broad and long, carried horizontally. In cocks is slightly bent backwards, relatively long and full, with a large clump. Wings are relatively long, but brought closed. The chest should be broad and full.
The main colors allowed are: partridge, gold, silver, black, black with white spots, yellow, stripes, partridge-blue, partridge-gold, gold-blue, orange, red, blue, white, columbia black and white, golden brown porcelain and gold with white spots. These animals have a strong and proud attitude. Sexual maturity is reached in 5.5-6 months, it's not an exigent breed so easy to breed and profitable, considering the costs and performance. As regarding gold italian, head and neck are golden in color in both gender, the tail is dark in cocks and brown in hens and the skin is white in color. More details are given in table2.1.

Figure 2.2: Gold Italian individuals from experimental stations (Poland)

Silver Italian

The breed were formed from countryside Italian chickens in Italy in the 19th century. In a short time became a very popular breed due to combination of beauty and performance and it became widespread in many European countries. Silver italians were brought in England and then in Germany. The Italian are medium in size with elongated neck, especially in hens, the tail is strong, broad and long, carried horizontally. In cocks is slightly bent backwards, relatively long and full, with a large clump. Wings are relatively long, but brought closed. The chest should be broad and full. The characteristics of this variety in cocks are: white head, white-silver neck with black strips and black wings with green reflexes ; in hens: silver-gray head, silver-white neck with dark strips and silver-gray wings. More details are given in Table n. 2.1.

Figure 2.3: Silver Italian individuals from experimental stations (Poland)

Green Legged Partridge

Green-legged Partridge hens were recognized as a breed in the late nineteenth century and previously known as Galician breed. At first, the improvement and popularization of Green-legged Partridge hens faced many problems resulting from the fact that there was no standard for the breed. The most important promoters of the breed were: Klementyna Stasiewiczówna, Stanislaw Kwiecinski, Henryk Mankowski and Bronislaw Obfitowicz. In 1906, the latter described Green-legged Partridge in the Hodowca Drobiu (Poultry Breeder) magazine. In 1921, the standard for the Green-legged Partridge breed was elaborated by Maurycy Trybulski, the President of the Principal Committee for Poultry Breeding and a well-known author of poultry books. The requirements of the time were that the hen´s head and neck should be yellow with a dark shade that turns into brown; every feather on the neck should have a stripe running along the shaft; the breast part should have salmon-pink feathers; the underbelly feathers and wings should be brown-black; and the shanks “reseda” green. It should be stressed that in 1930s almost 70 percent of Poland´s area was designated for Green-legged Partridge breeding. In the 1950s and 1960s, Green-legged Partridge hens were bred mainly in southern and eastern Poland. Over the years, the population was considerably decreased

(from 11.4 percent in 1961 to only 1-2 percent in 1973). The poor laying performance and the difficulties of selling birds with dark skin and shanks limited interest in this breed and reduced the number of large flocks. In the 1970s, to protect the dwindling native breeds from extinction, the National Research Institute of Animal Production and the Central Poultry Research and Development (COBRD) developed programs for the conservation of native breeds of chickens, geese and ducks including a unique ex situ alive conservation and inventory. Green-legged Partridge hens were kept in two distinct closed populations, which created two lines: Z-11, kept since 1972 in a poultry farm in Zyczyn, and moved in 1995 to the Experimental Station of the National Research Institute of Animal Production in Chorzelów; and Zk, kept since 1945 in Felin in a farm belonging to the Agricultural University in Lublin. Currently, approximately 660 birds (60 males and 600 females) from lines Z-11 and Zk are included in the "Laying Hen Genetic Resources Conservation Programme" (J. Calik and J. Krawczyk). Green-legged partridge hen is well adapted to free-range. More detailed informations are given in table2.1. Cock's feathers are shiny, metallic-green and black (tail), golden-red (head and neck), brownish-red (back), and hen' s are partridge-colour and pattern, with beige-and-brown back. skin is darkish in color, and legs are greenish. Green-legged partridge hens are disease-resistant. They are very independent, are able to wander on her own over the pastures, feeding on greenery and invertebrates, and find their way to the poultry house when the night is coming. Their brooding abilities are especially well developed. Eggs of green-legged partridge have lower content of yolk cholesterol, and this trait is genetically determined. They have a very pleasant flavor, and the yolk is proportionally bigger than in eggs of other breeds.

Figure 2.4: Green Legged Partridge individuals from experimental stations (Poland)

Leghorn "old type"

Leghorn is from Central Italy, takes its name from the small town from which they were exported, but called "Italiana" and also widely known as "Poulette d'Italie". In the first half of 1800s, Italian chickens left the harbor of Leghorn (Tuscany) to reach the United States. These birds, all with a white livery, were initially called "White Spaniards" or "White Italians". In 1868 the White Leghorn was introduced to England and in 1876 the

English Leghorn Club was founded. The first hens of this breed were brought to Poland before World War I. Over many decades the breed was object of strong genetic improvement, however the White Leghorn chickens analyzed in the present study can be perceived as "old type" Leghorn without intensive selection pressure. The "old" Leghorn is currently bred in white variety, generally for sport and only rarely for production purposes. Originally six varieties were known: partridge, silver partridge, cuckoo, buff, back and white.
The native Livorno is a leaner and taller breed compared to the Italiener selected in Germany. The neck is carried upright and slightly arched, which confers to the bird a lively and alert appearance. The tail is carried with an angle of 40 to 45° in the male and 30 to 35° in the female. The body has the shape of a cylinder, of average length, slightly sloping towards the rump. The shanks are fine-boned, of a beautiful deep yellow, the abdomen is well developed, especially in the female. Comb single, five-pointed, of medium size, carried erected in the male, and folded after the second point in the female. The comb blade follows the neckline without touching it. Wattles oval-shaped, of medium length, earlobes white, stretched and smooth, with no trace of red. The Leghorn is an excellent layer, the shell of which is invariably brilliant white.
Consumers generally prefer buying eggs with a pigmented shell, which is probably the main obstacle preventing a return to more consistent and substantial breeding of the Leghorn in Italy. The currently revived interest for typical and country-specific products, however, could be the answer to the lack of massive demand from the marketplace.
Leghorn is an early maturing breed and of lively temperament. Its brooding abilities are not well developed, it has good feed conversion ratio, and long standing high laying ability, so its economic value is high. Their meat is also of good quality.
These birds had a single comb, white earlobes, yellow skin and shanks; prolific and fast growing.
These chickens are superior for their excellent productivity, fast growth and ability to adapt to the most different environmental conditions.
More detailed informations are given in Table n. 2.1.

Figure 2.5: White Leghorn "old type" individuals from experimental stations (Poland)

2.1.2 Italian (Venetian) chicken breeds

Robusta Lionata

This breed was formed in 1965 in an experimental station of poultry in Rovigo (Veneto, Italy) by crossing two breeds: Tawny Orpington and White American.
The population size now it is about 3000-5000 individuals.

There are two varieties gold and light brown.
It is an hardy breed, with a good attitude to eggs and meat production and it has a fairly rapid growth. This medium- hardy breed is no very widespread but now increasingly used on organic farms.
The chicks at birth have a fawn-color duvet and dark dots on the head. The adults have a fawn-base color with black tail and green hues, the dark extends to the wings. They have yellow skin and legs. Females of this breed have a strong aptitude in brooding and in taking natural care of chicks.
Sexual maturity is reached at 6-7 months in males and 6-5 in females.

Figure 2.6: Robusta Lionata individuals from conservation centre (Italy)

Robusta Maculata

This breed was formed in 1965 in an experimental station of poultry in Rovigo (Veneto, Italy) by crossing the same two breeds of the Robusta lionata. The population size now it is about 3000-5000 individuals.
The breed has been growing strongly in the last years also thanks to its elegant appearance. It is characterized by good rusticity and is suitable for organic farming. It is a dual purpose breed with good attitude to eggs and meat production. The chicks have a dark color with light speckling, the duvet of the belly is light and on the head there is a dark spot.
Adults are characterized by a white plumage with black irregular spots all over the body, hackle feathers are silver. Skin and legs are yellow.
Sexual maturity is reached at 6-7 months in males and 6-5 in females.

Figure 2.7: Robusta Maculata individuals from conservation centre (Italy)

Ermellinata di Rovigo

The constitution of this breed began in 1959 in the experimental poultry station of Rovigo (Veneto, Italy) with the aim of obtaining poultry with high-quality of meat but still classified among the dual purpose breeds, indeed it continues to be a good producer of eggs.
The population size now it is about 3000-5000 individuals.
Sussex and Rhode Island breeds contributed to its creation.
The chicks have a yellow duvet with a wingspan light-gray. However, adults have ermine color with white-base color and dark feathers tail.
Skin and legs are yellow.
Sexual maturity is reached at 6-7 months in males and 6-5 in females.

Figure 2.8: Ermellinata di Rovigo individuals from conservation centre (Italy)

Pèpoi

This breed is especially widespread in the north-east of Veneto and Friuli, is one of the few small size-breeds on the market. They have good muscles of the chest and provide meat very tasty. The population size now it is about 3000-5000 individuals.
The chicks are light-brown with darker streaks on the back and on the head. Adults are usually gold. Skin and legs are yellow.
Sexual maturity is reached at 6 months in males and 5 in females. Females of this breed have a strong aptitude in brooding and in taking natural care of chicks. The average percentage of born alive compared to the total of incubated is 70%.

Figure 2.9: Pèpoi individual from conservation centre (Italy)

Polverara

Ancient breed which takes its name from a small town in the south of Padua, some people that it would be the parent of Paduan chickens, instead, others argue the opposite.

Figure 2.10: Polverara individual from conservation centre (Italy)

It is characterized by the presence of the tuft, which leads straight on the head but it is smaller than the Padovana one.This breed is raised because its meat is very tasty and has a good egg laying.
It had almost disappeared and is now under preservation. This local breed is included in the traditional products list by the Italian Agriculture and Forestry Ministry and officially recognized in Italy.
The population size now it is about 3000-5000 individuals. There are two colors white and black and both of them have completely monochrome and brilliant plumage. The white has pink-yellow bill and the black has black bill. Sexual maturity is reached at 6-7 months in males and 6-5 in females.

Padovana

The "Padovana dal gran ciuffo" is a very old breed, described in many works since 1500 in the area of Padua and famous because of its high productivity. Actually the origin is in Poland, was brought to Italy in 1300, probably by Giovanni Dondi Dell'orologio, noble Paduan, who was impressed by the beauty and elegance of these chickens.
The origin of this breed is still debated, appears to be linked not only to the Polish but also to the Dutch that has the tuft but not the beard. Many publications between XIX and XX century about raising chickens report detailed descriptions of Paduan, unfortunately, in the course of 1900 its number was gradually lowered due to the reduced number of breeders dedicated to this breed. The population size now it is about 3000-5000 individuals. It can be divided in straight-feathered and curly- feathered. There are several colors like silver edged black, white edged blue, chamois edged white, gray, black, white, gold edged black, hod, three-color. The main characteristics of the breed are a massive tuft with the presence of cerebral hernia and beard well developed, it is an ornamental breed.
Sexual maturity is reached at 6-7 months in males and 6-5 in females.

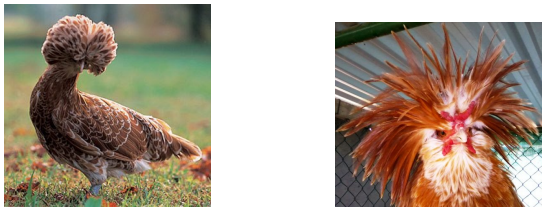


Figure 2.11: Padovana individuals from conservation centre (Italy)

Breed	BW (kg) roosters	BW (kg) hens	LA (eggs/year)	EW (g)
MCO	0.85	0.75	80	30
GI	2.50-3.00	1.75-2.00	180-200	55
LP	1.70-2.20	1.50-1.80	180-190	55-58
SI	2.50-3.00	1.75-2.00	180-200	55
WL	2.00-2.70	1.70-2.20	250	55
RL	4.20	3.00	160-170	55-60
RM	4.20	3.00	150-160	55-60
ER	3.20	2.40	150-160	55-60
PP	1.50-3.00	1.00	160-180	40-45
PL	2.60	2.00	150-160	50-55
PD	2.00	1.60	160-170	50-60

Table 2.1: Mean values of some performance traits of analysed chicken breeds. BW: body weight; LA laying ability; EW: egg weight. Breed names: MCO, Cochin; GI, Gold Italian; GLP, Green Legged Partridge; SI, Silver Italian; WL, White Leghorn; RL, Robusta Lionata; RM, Robusta Maculata;
ER, Ermellinata di Rovigo; PP, Pèpoi; PL, Polverara; PD, Padovana.

2.2 F-statistics: genetic differentiation between populations

In 1951, Wright introduced a method to describe the population genetics of diploid organisms using three coefficients F (also called Fixation indeces). The different F-statistics look at different levels of population structure:

- F_{IT} is the inbreeding coefficient of an individual (I) relative to the total (T) population;
- F_{IS} is the inbreeding coefficient of an individual (I) relative to the subpopulation (S) using the above for subpopulations and averaging them;
- FST is the effect of subpopulations (S) compared to the total population (T), and is calculated by solving the equation:

$$(1 - F_{IS})(1 - F_ST) = 1 - F_{IT}.$$

F_{IS} (inbreeding coefficient) is a measure of the deviation from the Hardy-Weinberg subpopulation equilibrium, also identified as inbreeding coefficient, describes the genetic variance within individuals in relation to the subpopulation (or population) of belonging; in other words the excess (or defect) of homozygotes in the subpopulations. The range of F_{IS} coefficient is from -1 to +1, if the values are ¡0 there is an excess of homozygotes and with values ¿0 there is a defect of homozygotes in the subpopulation.
F_{IT} (overall population heterozygote deficiency) is a measure of the deviation from equilibrium similar to the previous one, which evaluates the individual relative to the total

population, and represents the general excess (or defect) of homozygotes of total population. The range of F_{IS} coefficient is from -1 to +1, if the values are ¿ 0 there is an excess of homozygotes and with values < 0 there is a defect of homozygotes in the population.
Simplifying: for both coefficients, F_{IS} and F_{IT}, positive values indicate a deficiency of heterozygotes and conversely an excess of homozygotes.
F_ST (heterozygote deficiency due to breed subdivision) is a measure of genetic differentiation between subpopulations, it takes values in the range from 0 to 1 and can be interpreted as a measure of the standardized variance of allele frequencies between subpopulations independently from the number of individuals considered.
These three coefficients are related by:

$$F_ST = \frac{F_{IT} - F_{IS}}{1 - F_{IS}}$$

To calculate F- are required heterosis measures such as:

- H_o, observed heterozygosity (proportion of heterozygous) in a population;
- H_e, expected heterozygosity in a subpopulation according to the H-W Equilibrium;
- H_t, expected heterozygosity assuming "random mating" within the entire population.

The index of heterozygote deficiency due to breed subdivision allows to evaluate how different are the subpopulations. Formally, it measures the existence of a deficit of heterozygotes in the meta-population due to differentiation among subpopulations. The index of heterozygote deficiency due to breed subdivision is used to measure the differentiation between a group of subpopulations but also as a measure of the genetic distance between pairs of populations.

$$F_ST = \frac{H_t - H_e}{H_t}$$

F_{IS} shows the presence of inbreeding in subpopulations by comparing oH and eH (as average values among the subpopulations in question):

$$F_{IS} = 1 - \frac{H_o}{H_e}$$

Therefore, F_{IS} extent, if it exists, a deficiency or an excess of heterozygotes in the subpopulation. The F_{IT}, instead, indicates how the population structure has influenced the average heterozygosity in the total population:

$$F_{IS} = \frac{H_t - H_o}{H_e}$$

2.3 Molecular analysis

In the present work a total of 709 individuals were analyzed, 138 of them were randomly selected from experimental flocks in Poland: Miniature Cochin (MCO) n = 23; Gold Italian (GI) n = 16; Green Legged Partridge (GLP) n = 22; Silver Italian (SI) n = 39; White Leghorn (WL) n = 38. 571 individuals belonging to six Italian local chicken breeds were also included in the analysis for comparison purposes. Samples were taken from conservation flocks: Ermellinata di Rovigo (ER) n = 92; Polverara (PL) n = 30; Padovana (PD) n = 135; Pèpoi (PP) = 115; Robusta Lionata (RL) n = 99; Robusta Maculata (RM) n = 100.
The collection of samples was performed in collaboration with the Poznan University of Life Sciences, one blood sample from each individual was taken from the ulnar vein, in the wing region, using Vacutainer tubes and using sodium citrate as anticoagulant.

2.3.1 Genomic DNA isolation

Genomic DNA isolation was performed from 10 μl of blood using the modified DNA purification kit (Gentra System Puregene DNA, QIAGEN, Hilden, Germany). The blood was mixed with 300μl of Cell Lysis Solution (50 mM Tris-HCl pH 8.0, 20 mM EDTA, 2% SDS) and incubated at 37°C for one hour to allow the lysis of the erythrocytes. After incubation time the samples were treated with RNase (0.04$ng/\mu l$) at 37°C for 30 minutes. Subsequently the protein precipitation was performed using 100μl of PPS solution (10M ammonium acetate) through centrifugation at 14.000 xg for 5 minutes. The supernatant containing the DNA in solution was transferred to a new tube containing 300μl of Isopropanol. Precipitation of DNA occurred by centrifugation at 14.000 xg for 5 minutes. The DNA pellet was washed using 300μl of EtOH 70%. Purified DNA was resuspended in 100μl of ddH_2O.

2.3.2 Genomic DNA sizing

The purified DNA was quantified by electrophoresis using 1% Agarose gel and was quantified using DNA form λ phage with known concentrations as reference (10$ng/\mu l$, 20$ng/\mu l$, 40$ng/\mu l$, 80$ng/\mu l$). The purified DNA was then normalized to 50$ng/\mu l$ in ddH_2O.

2.3.3 Microsatellites amplification

A set of 23 microsatellite markers (table n.2.2), included in the list of recommended microsatellites for chicken analysis by the ISAG/FAO Standing Committee, were amplified using three multiplex fluorescent PCR reactions (Table n. 2.2). For each microsatellite locus the forward primer was labeled at the 5' end with a fluorophore (D2, D3 or D4, table n.2.2). Amplification was performed in a GeneAmp 9700 thermal cycler (Life Technologies, USA) with the Type-IT Microsatellite PCR Kit (Qiagen, Hilden, Germany) using 50 ng of purified DNA, 6.25μl of MasterMix (2X), 1.5μl of Primer Mix (0.2 mM for each primer) and 3.75μl of ddH_2O. The 23 microsatellites were amplified using the following conditions: initial denaturation step of 5 min at 95°C, followed by 35 cycles including:

- *denaturation* at 95° C for 30s;
- *annealing* at 61° C for 1 min 30s;
- *extension* at 72° C for 30s.

Finally a further extension cycle at 60° C for 30 min was carried out.

LOCUS	FWD sequence	REV sequence
ADL0268	CTCCACCCCTCTCTCAGAACTA	CAACTTCCCATCTACCTACT
MCW0216	GGGTTTTACAGGATGGGACG	AGTTTCACTCCCAGGGCTCG
MCW0248	TTGCATTAACTGGGCACTTTC	GTTGTTCAAAAGAAGATGCATG
ADL0278	CCAGCAGTCTACCTTCCTAT	TGTCATCCCAAGAACAGTGTG
MCW0081	GTTGCGAGAGCCTGGTGCAG	CCTGTATGTGGAATTACTTCTC
MCW0069	GCACTCGAGAAAACTTCCTGCG	ATTGCTTCAGCAAGCATGGGAGGA
MCW0222	GCAGTTACATTGAAAATGATTCC	TTCTCAAAACACCTAGAAGAC
MCW0034	TGCACGCACTTACATACTTAGAGA	TGTCCTTAATTACATTCATGGG
LEI0166	AAGCAAGTGCTGGCTGTGCTC	TCCTGCCCTTAGCTACGCAC
LEI0094	GATCTCACCAGTATGAGCTGC	TCTCACACTGTAACACAGTGC
MCW0111	GCTCCATGTGAAGTGGTTTA	ATGTCCACTTGTCAATGATG
MCW0103	TTTCCTAACTGGATGCTTCTG	AACTGCGTTGAGAGTGAATGC
MCW0295	ATCACTACAGAACACCCTCTC	TATGTATGCACGCAGATATCC
MCW0037	ACCGGTGCCATCAATTACCTATTA	GAAAGCTCACATGACACTGCGAAA
MCW0016	ATGGCGCAGAAGGCAAAGCGATAT	TGGCTTCTGAAGCAGTTGCTATGG
MCW0014	AAAATATTGGCTCTAGGAACTGTC	ACCGGAAATGAAGGTAAGACTAGC
MCW0183	ATCCCAGTGTCGAGTATCCGA	TGAGATTTACTGGAGCCTGCC
MCW0123	GGAACCACTAGAAAAGAACATCC	AATGTATTTCCACCCCCAAAG
MCW0020	TCTTCTTTGACATGAATTGGCA	GCAAGGAAGATTTTGTACAAAATC
MCW0165	CAGACATGCATGCCCAGATGA	GATCCAGTCCTGCAGGCTGC
MCW0078	CCACACGGAGAGGAGAAGGTCT	TAGCATATGAGTGTACTGAGCTTC
MCW0067	GAGATGTAGTGCCACATTCCGAC	GCACTACTGTGTGCTGCAGTTT
MCW0080	CCGTGCCATTCTTAATTGACAG	GAAATGGTACAGTGCAGTTGG

Table 2.2: Microsatellite locus and details of forward (FWD) and reverse (REV) sequence

Microsatellite sizes, dyes, pool and annealing temperature used for the multiplex amplification are shown in table 2.3

LOCUS	SIZE	POOL	DYE	T °C
ADL0268	93-119	A	D3	61
MCW0216	141-151	A	D3	61
MCW0248	195-243	A	D3	61
ADL0278	110-126	A	64	61
MCW0081	111-139	A	D2	61
MCW0069	150-178	A	D4	61
MCW0222	217-229	A	D4	61
MCW0034	222-246	A	D2	61
LEI0166	251-263	A	D2	61
LEI0094	249-285	A	D4	61
MCW0111	90-118	B	D3	61
MCW0103	260-274	B	D3	61
MCW0295	82-118	B	D4	61
MCW0037	147-159	B	D2	61
MCW0016	130-152	B	D4	61
MCW0014	130-188	B	D4	61
MCW0183	295-339	B	D4	61
MCW0123	136-154	C	D3	61
MCW0020	181-199	C	D3	61
MCW0165	108-126	C	D4	61
MCW0078	130-160	C	D4	61
MCW0067	168-182	C	D4	61
MCW0080	265-295	C	D2	61

Table 2.3: Lists of the loci examined in this study; size of fragment; subdivision in pool; fluorophore used and annealing temperature

2.3.4 Microsatellite Genotyping

Genotyping of microsatellites amplified in multiplex PCR was performed using an automatic capillary sequencer (CEQ 8000 Genetic Analysis System, Beckman Coulter, Fullerton, CA, USA): $2\mu l$ of PCR reaction solution were mixed with $18\mu l$ of ddH_2O and denatured at 95 ° C for 5 min. $19.5\mu l$ of ddH_2O and $0.5\mu l$ of Size Standard-400 were finally added before loading into the instrument. Analysis of the electropherograms and the sizing of fragments was performed using the software V.9.0 Genetic Analysis System (BeckmanCoulter).
In figure 2.12 an example of an electropherogram representing some microsatellite markers with their intensity and size.

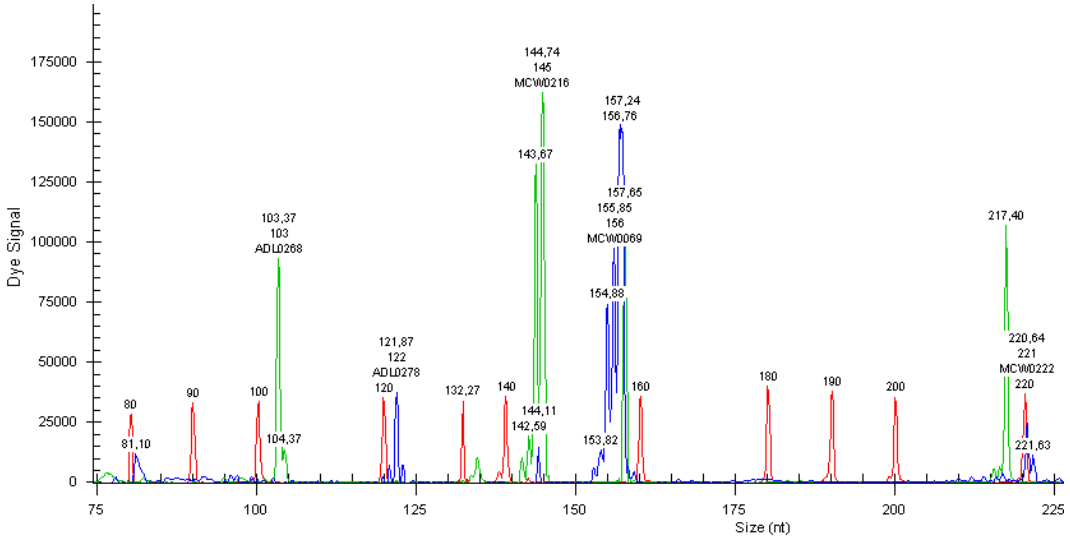


Figure 2.12: Example of electropherogram

2.4 Statistical Analysis

MSA v4.05 (Dieringer and Schlötterer, 2003) was used to calculate the total number of alleles per locus (TNA), allelic frequencies, observed (H_o) and expected (H_e) heterozygosity, allelic richness (AR, mean number of alleles per locus corrected by sample size), gene diversity (GD), and Wright's fixation index (F_{IS}). Exact tests for deviation from Hardy-Weinberg equilibrium (HWE) were applied using a Markov Chain Monte Carlo simulation (100 batches, 5.000 iterations per batch, and a dememorization number of 10.000) as implemented in GENEPOP v4.0 (Rousset et al. 2007). Allelic richness and private alleles per breed were also calculated using rarefaction method to adjust for different population sizes using ADZE (Szpiech et al. 2008). Molecular coancestry coefficients (fij) and Tomiuk and Loeschcke's (1995) genetic distances (DTL) were measured using MOLKIN 3.0 (Gutiérrez et al., 2009).

DTL distances among populations were represented by a neighbor-net tree using SplitsTree4 (Huson and Bryant, 2006). The significance of the differences between the parameters of NMA, H_o, H_e, F_{IS} and fij between breeds were calculated using the multcomp library and Holm correction in R (R Development Core Team, 2012). The test compares the difference between the average of the subpopulations, considering the dispersion of the data (expressed as standard deviation). Genetic structure, breed assignment percentages and the degree of admixture, if any, for all the analyzed breeds were investigated using the Bayesian clustering approach implemented in Structure v2.2.3 (Pritchard et al. 2000). The most likely number of populations (K) given the observed genotypic data was estimated by running 10 independent runs for each K ($1 = K = 14$). The admixture model with correlated allele frequencies was used with a burn-in length of 50 000 followed by 500 000 MCMC iterations for data collection. The most likely number of K clusters fitting the observed data was established by plotting the $lnPr(G \mid K)$ values obtained in the 10 independent runs for each K, as suggested by Pritchard et al. (2000), and by estimating delta K (ΔK) statistics, as proposed by Evanno et al. (2005). The output obtained from STRUCTURE was used directly as input by the cluster visualization program DISTRUCT.

Chapter 3

Results and discussion

3.1 Markers polymorphism

The total number of alleles detected across the 23 microsatellite markers was 247. All the microsatellite markers were polymorphic with an average of 10.74 ± 3.48 alleles per locus (Table n. 3.1). The most polymorphic locus was MCW0014 with 19 different alleles. The least number of alleles were detected for MCW0216, LEI0166, MCW0103 and MCW0037 and was equal to 6.

Allelic richness values ranged from 2.53 (MCW0103) to 9.30 (MCW0034), with an average of 5.43 ± 1.86 across all markers. The polymorphic information content represent the informativeness of each microsatellite marker, values of PIC from 0.25 to 0.5 indicates a good informativeness, if higher than this range the microsatellite is considered highly informative. The microsatellite markers analyzed in this work were highly informative with an average value of PIC larger than 0.5 (0.833 ± 0.064). The largest PIC was observed for MCW0034 (0.914) and the lowest for MCW0103 (0.643). The gene diversity (GD) over all the loci showed a mean value of 0.689 ± 0.111, ranging from 0.390 to 0.870 in MCW0103 and MCW0034, respectively. The F_{IS} coefficient showed an excess of homozygotes among all microsatellite markers with an average of 0.238 ± 0.087 ($P < 0.01$), the minimum value was 0.074 (MCW0034) and the maximum was 0.483 (MCW0165); as showed in table n. 3.2.

LOCUS	SIZE	TNA
ADL0268	93 - 119	10
MCW0216	141 - 151	6
MCW0248	195 - 243	10
ADL0278	110 - 126	9
MCW0081	111 - 139	13
MCW0069	150 - 178	14
MCW0222	217 - 229	7
MCW0034	222 - 246	13
LEI0166	251 - 263	6
LEI0094	249 - 285	15
MCW0111	90 - 118	13
MCW0103	260 - 274	6
MCW0295	82 - 118	12
MCW0037	147 - 159	6
MCW0016	130 - 152	12
MCW0014	130 - 188	19
MCW0183	295 - 339	15
MCW0123	136 - 154	10
MCW0020	181 - 199	10
MCW0165	108 - 126	9
MCW0078	130 - 160	14
MCW0067	168 - 182	8
MCW0080	265 - 295	10
Average ($\pm SD$)		$10,74 \pm 3,48$

Table 3.1: Analyzed loci, chromosome (chr), fragment size (bp), total number of detected alleles

LOCUS	CHR	FRAG. SIZE	AR	PIC	GD	$F_{IS}{}^{***}$
ADL0268	1	93 - 119	7.16	0.891	0.795	0.217
MCW0216	13	141 - 151	4.60	0.838	0.714	0.302
MCW0248	1	195 - 243	3.66	0.726	0.525	0.286
ADL0278	8	110 - 126	6.04	0.870	0.769	0.325
MCW0081	5	111 - 139	7.74	0.912	0.836	0.237
MCW0069	26	150 - 178	4.30	0.788	0.571	0.226
MCW0222	3	217 - 229	2.73	0.786	0.593	0.224
MCW0034	2	222 - 246	9.30	0.914	0.870	0.074
LEI0166	3	251 - 263	4.62	0.830	0.713	0.242
LEI0094	4	249 - 285	7.17	0.835	0.675	0.122
MCW0111	1	90 - 118	5.42	0.830	0.690	0.258
MCW0103	3	260 - 274	2.53	0.643	0.390	0.222
MCW0295	4	82 - 118	6.91	0.859	0.711	0.259
MCW0037	3	147 - 159	3.22	0.724	0.514	0.265
MCW0016	3	130 - 152	6.60	0.881	0.780	0.346
MCW0014	6	130 - 188	7.66	0.863	0.787	0.169
MCW0183	7	295 - 339	7.77	0.853	0.719	0.138
MCW0123	14	136 - 154	5.89	0.849	0.734	0.292
MCW0020	1	181 - 199	4.27	0.865	0.752	0.176
MCW0165	23	108 - 126	3.91	0.828	0.687	0.483
MCW0078	5	130 - 160	5.07	0.828	0.683	0.194
MCW0067	10	168 - 182	3.39	0.897	0.660	0.131
MCW0080	15	265 - 295	4.95	0.855	0.680	0.269
Average±SD			5.43 ± 1.860	0.833 ±0.064	0.689 ±0.111	0.238 ±0.087

Table 3.2: Analyzed loci, chromosome (chr), fragment size (bp), allelic richness (AR), polymorphic information content (PIC), gene diversity (GD) and fixation index (F_{IS}) according to Weir & Cockerham (1984).
$^{***}P < 0.001$

3.2 Inter - breed variability

Considering all populations, the fixation indexes were $F_{IS} = 0.218$, $F_{IT} = 0.479$ and $F_{ST} = 0.333$ indicating that almost 33% of the observed variability was attributable to among breed variation. The results underline a high degree of breed differentiation that is in accordance with the values reported by Tadano et al. (2008) and Zanetti et al. (2011) for Japanese and Italian local chicken breeds. Moreover the high F_{IS} value indicate a significant excess of homozygotes throughout the whole population.
The genetic variability in each population was studied in terms of mean number of alleles (Na), allelic richness obtained with rarefaction method (N_{AR}), private allelic richness (P_{AR}), inbreeding coefficient (F_{IS}) and molecular inbreeding (f_{ij}) (Table n. 3.3). The White Leghorn breed showed the least number of alleles (2.39) while the largest Na values were found in two Italian breeds, Padovana and Ermellinata di Rovigo (6.09 and 5.96 respectively). Unfortunately, due to unequal sample sizes of breeds the use of rarefaction method was necessary (Szpiech et al. 2008) in order to adjust allelic richness and private alleles values per population. Results confirmed the lowest variability of White Leghorn ($N_{AR} = 1.72$) and a larger variability present in Ermellinata di Rovigo ($N_{AR} = 2.71$).
P_{AR} values were lower than one in all breeds with the lowest value found in the Silver Italian breed ($P_{AR} = 0.07$) while the largest was found in the Ermellinata di Rovigo ($P_{AR} = 0.31$).
F_{IS} values were positive for all breeds indicating a significative excess of homozygotes. The Gold Italian showed the largest excess of homozygotes ($F_{IS} = 0.36$) while the Green Legged Partridge evidenced a smaller excess of homozygotes ($F_{IS} = 0.11$).
Estimation of molecular coancestry coefficients (f_{ij}) is another method by which within-breed diversity can be measured. The values of f_{ij} were similar in all the breeds but slightly larger in Pepòi and Padovana breeds with 0.18 and 0.17, respectively indicating a limited identity by state (IBS) at the analysed loci.

BREED	N	$Na \pm SD$	$N_{AR}(6)^1$	$P_{AR}(6)^1$
MCO	23	3.00 ± 0.95	2.04	0.19
GI	16	2.96 ± 1.19	2.05	0.15
GLP	22	3.22 ± 1.35	2.29	0.20
SI	39	3.13 ± 1.36	2.14	0.07
WL	38	2.39 ± 0.89	1.72	0.10
ER	92	5.96 ± 2.38	2.71	0.31
PL	30	4.22 ± 1.41	2.55	0.10
PD	135	6.09 ± 2.41	2.50	0.14
PP	115	4.83 ± 1.34	2.12	0.14
RL	99	5.00 ± 1.86	2.28	0.16
RM	100	5.13 ± 2.42	2.41	0.27

Table 3.3: Sample sizes (N); mean number of alleles (Na); allelic richness obtained with rarefaction method (N_{AR}), private allelic richness (P_{AR}); inbreeding coefficient (F_{IS}); molecular inbreeding (f_{ij}). Breed names: MCO, Miniature Cochin; GI, Gold Italian; GLP, Green Legged Partridge; SI, Silver Italian; WL, White Leghorn; ER, Ermellinata di Rovigo; PL, Polverara; PD, Padovana; PP, Pepòi; RL, Robusta lionata; RM, Robusta maculata.
[1]Number of observations in each breed

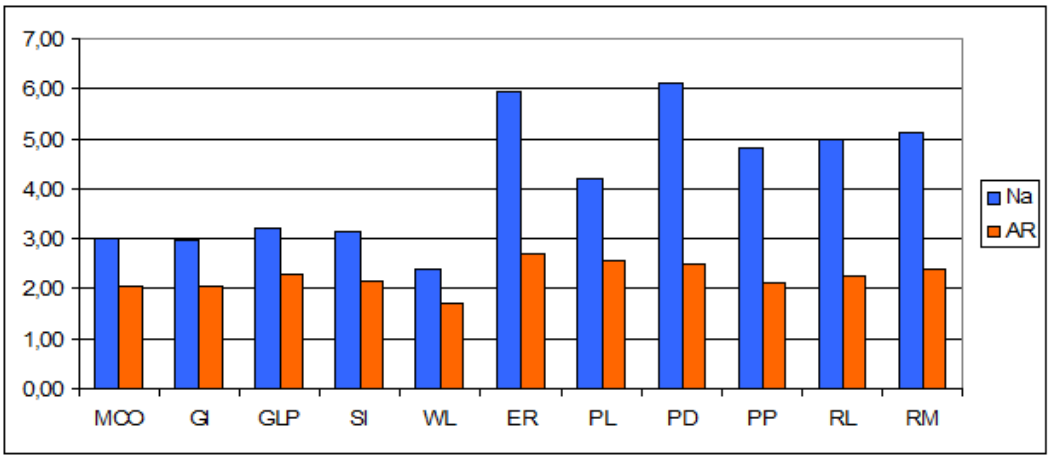


Figure 3.1: Number of allele (Na) and Allelic richness (AR) for each breed

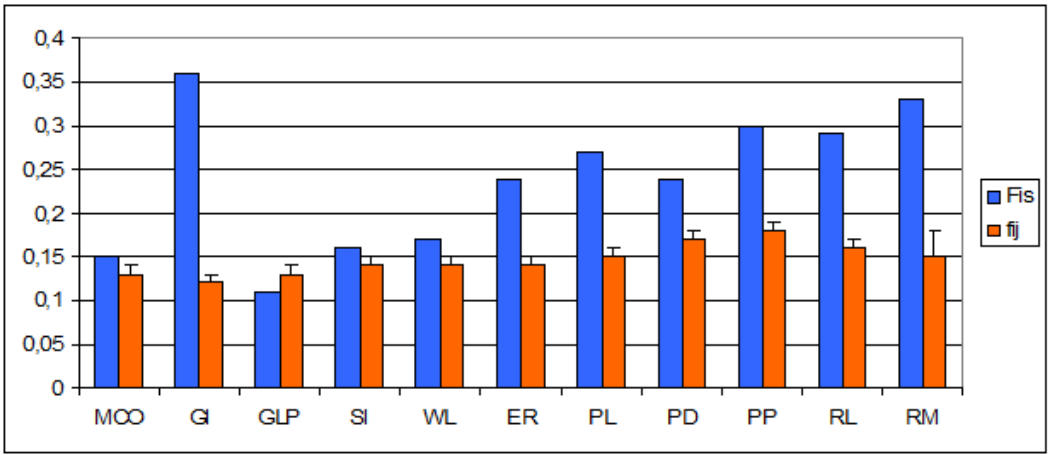


Figure 3.2: Number of allele (AR) and Allelic richness (AR) for each breed

The expected heterozygosity is an index of natural genetic variability of a locus. It is uniquely defined from the allele frequencies.
The expected heterozygosity can be described as the probability that a genotype randomly selected in a population under the Hardy-Weimberg equilibrium (HWE) is heterozygote. Expected and observed heterozygosity together with F_{IS} and f_{ij} values for each breed are presented in table 3.4 and figures 3.3. Observed heterozygosity (H_o) was similar to H_e and it was in the range of 0.28 ± 0.23 (Gold Italian) to 0.45 ± 0.23 (Ermellinata di Rovigo), these limited values are in agreement with those reported by other authors for other local chicken breeds (Hillel et al. 2003, Dávila et al. 2009, Tadano et al. 2007, Zanetti et al. 2011). On average, Italian breeds showed a slightly larger expected heterozygosity compared to that of breeds kept in Poland, these differences are not reflected in the observed heterozygosity. F_{IS} indexes also reflect, on average, the larger excess of homozygotes in Italian breeds compared to "Polish" breeds. The larger H_e values for the Italian breeds could be mainly due to the larger sample size rather than to a real larger gene diversity. Moreover, values of f_{ij}, which are also similar among all analysed breeds, thus indicating the limited identity by state (IBS) present in all breeds.

The between populations F_{ST} and D_{TL} genetic distances are given in Table n. 3.5. The F_{ST} values were rather high when compared to breed differentiation values in other species such as cattle (Maretto et al., 2012) but also when compared to Italian and Japanese chicken breeds (Zanetti et al., 2010 and Tadano et al. 2007) or Indonesian local chicken breeds analysed with SNP markers (Riztyan et al. 2011). Pairwise F_{ST} values ranged from 0.122 (PD-PL) to 0.562 (WL-MCO). In general, Italian breeds tend to be more related while WL and MCO are the most differentiated breeds.

The D_{TL} distance was preferred because it simultaneously accounts for the impact of mutation and genetic drift on gene frequencies (Tomiuk and Loeschcke 1995). In addition, D_{TL} is

BREED	N	$H_o \pm$ SD	$H_e \pm$ SD	F_{IS}	$f_{ij} \pm$ SD
MCO	23	0.36 ± 0.26	0.39 ± 0.21	0.15	0.13 ± 0.01
GI	16	0.28 ± 0.23	0.39 ± 0.21	0.36	0.12 ± 0.01
GLP	22	0.44 ± 0.23	0.49 ± 0.20	0.11	0.13 ± 0.01
SI	39	0.43 ± 0.29	0.44 ± 0.23	0.16	0.14 ± 0.01
WL	38	0.30 ± 0.23	0.30 ± 0.21	0.17	0.14 ± 0.01
ER	92	0.45 ± 0.23	0.58 ± 0.17	0.24	0.14 ± 0.01
PL	30	0.41 ± 0.18	0.56 ± 0.15	0.27	0.15 ± 0.01
PD	135	0.40 ± 0.21	0.52 ± 0.17	0.24	0.17 ± 0.01
PP	115	0.32 ± 0.24	0.43 ± 0.21	0.30	0.18 ± 0.01
RL	99	0.36 ± 0.25	0.46 ± 0.21	0.29	0.16 ± 0.01
RM	100	0.35 ± 0.21	0.50 ± 0.20	0.33	0.15 ± 0.03

Table 3.4: Sample sizes (N), expected heterozygosity (H_e) ± standard deviation, observed heterozygosity (H_o) ± standard deviation, F_{IS} and molecular inbreeding (f_{ij}) for analysed breeds.

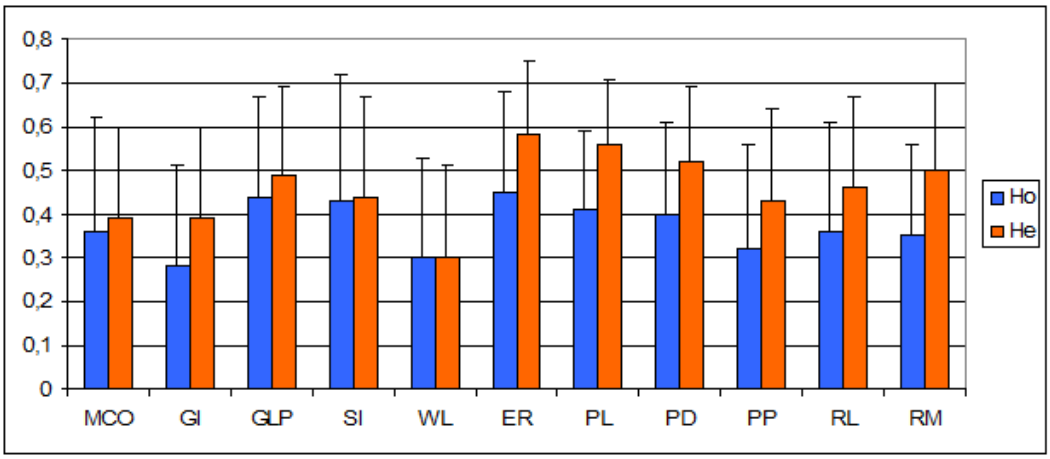


Figure 3.3: Observed and Expected Heterozygosity ± standard deviation for each breed

more robust to deviations in the assumptions concerning mutation and genetic drift (Tapio et al. 2003). According to D_{TL} the PD and PL breeds are the most related ($D_{TL} = 0.094$) followed by PD and PP ($D_{TL} = 0.108$) while, when considering the "Polish breed", the two Italian derived GI and SI breeds were the most related (DTL = 0.307). The neighbour-net representation of D_{TL} distances shown in figure n.3.4 also evidences a clear separation between the six Italian chicken breeds and the five "Polish breeds". MCO, as expected, is the more differentiated breed sitting at the end of the longest branch of the network due to its exotic origins. SI and GI grouped together with WL even tough the latter shows a D_{TL} distance of 0.450 and 0.434 with GI and SI, respectively.

	MCO	GI	GLP	SI	WL	ER	PL	PD	PP	RL	RM
MCO	-	0.421	0.336	0.412	0.562	0.339	0.352	0.361	0.370	0.408	0.390
GI	0.543	-	0.312	0.286	0.479	0.364	0.345	0.338	0.410	0.487	0.450
GLP	0.392	0.330	-	0.331	0.441	0.299	0.290	0.290	0.392	0.403	0.376
SI	0.504	0.307	0.419	-	0.416	0.335	0.292	0.297	0.365	0.425	0.390
WL	0.755	0.450	0.505	0.434	-	0.412	0.446	0.402	0.473	0.481	0.472
ER	0.505	0.537	0.479	0.537	0.635	-	0.250	0.264	0.327	0.290	0.311
PL	0.479	0.516	0.435	0.408	0.626	0.244	-	0.122	0.283	0.291	0.283
PD	0.450	0.395	0.364	0.373	0.512	0.137	0.094	-	0.241	0.293	0.265
PP	0.434	0.426	0.474	0.381	0.588	0.196	0.188	0.108	-	0.293	0.265
RL	0.495	0.597	0.450	0.540	0.527	0.221	0.174	0.124	0.159	-	0.254
RM	0.494	0.642	0.535	0.539	0.721	0.169	0.206	0.152	0.214	0.140	0

Table 3.5: Genetic distances for 11 chicken breeds using F_{ST} (P<0.001, above the diagonal) and D_{TL} (below the diagonal). Breed names: MCO, Miniature Cochin; GI, Gold Italian; GLP, Green Legged Partridge; SI, Silver Italian; WL, White Leghorn; ER, Ermellinata di Rovigo; PL, Polverara; PD, Padovana; PP, Pepòi; RL, Robusta lionata; RM, Robusta Maculata

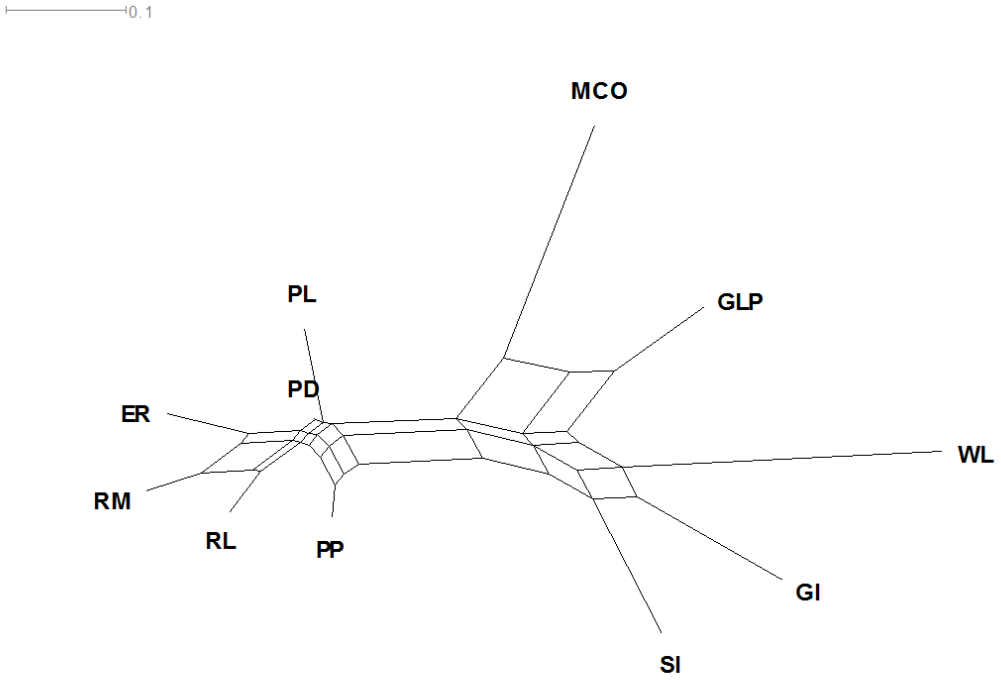
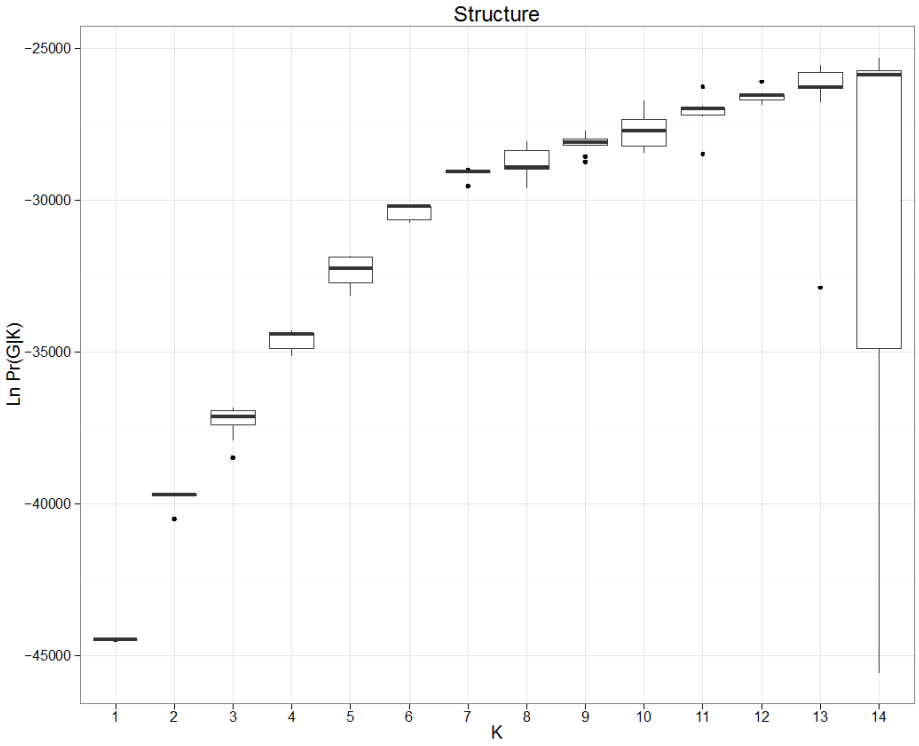


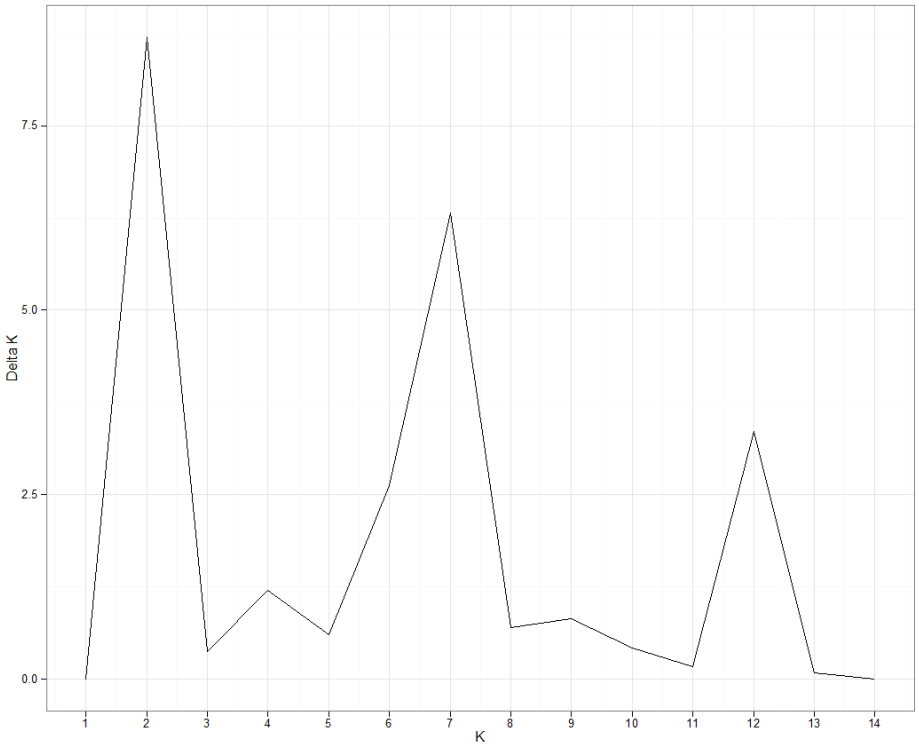
Figure 3.4: Neighbor-Net network of polish and italian chicken breeds using D_{TL} genetic distances (Tomiuk and Loeschcke,1995). Breed names: MCO, Miniature Cochin; GI, Gold Italian; GLP, Green Legged Partridge; SI, Silver Italian; WL, White Leghorn; ER, Ermellinata di Rovigo; PL, Polverara; PD, Padovana; PP, Pepòi; RL, Robusta lionata; RM, Robusta Maculata

3.3 Population Structure

The population structure of the 11 analyzed breeds was investigated using the Bayesian approach implemented in the software STRUCTURE v.2.3.3. The cluster analysis performed without prior information evidenced a meaningful pattern of mean $lnPr(G|K)$ values from K = 2 to K = 12 (Figure 3.5 (a)). According to Delta K (Δ K) statistics, following Evanno et al. (2005), a mean peak at K = 2 and secondary peaks at K = 7 and K = 12 were found (Figure 3.5 (b)).
According to Structure analysis (Figure 3.6) at K=2 a cluster made of ER, RL and RM differentiated from all the other breeds grouped together in the second cluster. With the increasing in the number of K (data not shown) the other italian breeds differentiated from the "Polish breeds". At K=7 the "Polish breeds" were clearly differentiated from the Italian breeds even tough only two clusters were identified (WL and the group made of GI, SI, MCO and GLP). Regarding the Italian breeds at K=7 PD and PL were still found in the same cluster. At K=12, the more likely number of K describing our dataset, only SI, GI and GLP were still grouped together while presence of subgroups in RM, PD and ER was found. The subgroup found in the PD breed correspond to two ecotypes belonging to the PD breed: the Padovana Camosciata and Padovana Dorata, while for the ER and RM group the subdivisions could be due to a limited sampling in only one conservation flock during the first year of monitoring. Limited admixture of some individuals was found mainly in Italian breeds and in particular in the PD breed.



(a) $lnPr(G \mid K)$ values are presented as a function of the number of clusters among 50 runs.



(b) ΔK values calculated following Evanno et al. (2005).

Figure 3.5: Estimated posterior probabilities of $lnPr(G \mid K)$.

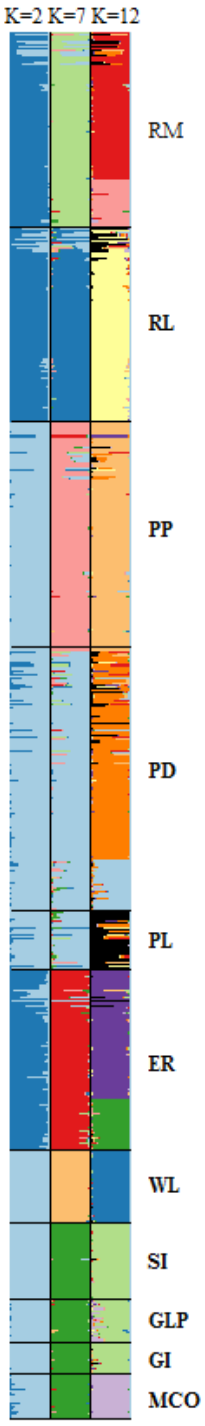


Figure 3.6: Estimated group assignment percentages for the whole population divided in breeds based on a Bayesian clustering approach as implemented in Structure v.2.2.3. Values of runs with the largest $lnPr(G \mid K)$ for K=2, K=7 and K=12, among the 10 independent runs, are shown. Each individual is represented by a single vertical line broken into K colour segments, with lengths proportional to the estimated membership to the inferred cluster.

Chapter 4

Conclusions

In this study we estimated the inter- and within breed genetic variability of five old local chicken populations reared in Poland and six Italian local chicken breeds undergoing a conservation program. According to their recent breeding history the five breeds from Poland were clearly separated in terms of genetic identity. Gold Italian and Silver Italian breeds grouped together in the same cluster according to their similar origins. Cochin and White Leghorn breeds were the two most separated breeds while the Green Legged Partidge, even if clustered with the two Italian derived breeds, was at the end of the longest branch of the network. The Italian breeds were divided in two main groups: the first was composed of Padovana and Polverara breeds and the second included Pepòi, Robusta Maculata, Robusta Lionata and Ermellinata di Rovigo breeds. Structure analysis clearly separated the Italian and "Polish" breeds and did not evidenced particular substructuring in the analysed breeds. It is worthwhile to note that SI, GI and GLP were not separated by the current analysis indicating that an increase in the number of K clusters is needed to differentiate these breeds based on genotypic frequencies of the analysed microsatellites. Overall, genetic variability was slightly larger for the "Polish" breed compared to Italian ones but still similar to that of other local chicken breeds undergoing in situ conservation. Nevertheless, and in particular for the Italian chicken breeds the continuous monitoring of genetic variability parameters is needed to avoid the increase of inbreeding and the loss of biodiversity. These results also represent a starting point for the valorization of these local Polish chicken breeds as an important reservoir of genetic diversity.

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