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– GÖTTINGEN CENTRE FOR BIODIVERSITY AND ECOLOGY –

CONCEPT FOR PLANNING CONSERVATION SCHEMES FOR FARM ANIMAL GENETIC DIVERSITY FOCUSED ON GERMAN CHICKEN BREEDS

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Georg-August-Universität Göttingen

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Tamina Pinent

born in Paris (France)

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Referent: Prof. Dr. Henner Simianer

Korreferent: Prof. Dr. Michael Mühlenberg

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ZUSAMMENFASSUNG

Die genetische Vielfalt stellt die Basis für zukünftige Zuchtarbeit in allen Nutztierarten dar und muss daher erhalten werden. Für die Konzeption von Erhaltungsprojekten ist es nötig Kriterien für die Wahl der Rassen zu finden, die mit höchster Priorität erhalten werden sollen. In dieser Studie wurden am Modell der Biodiversität des Huhnes auf der Grundlage des Weitzman'schen Biodiversitätskonzepts solche Kriterien entwickelt. Aufgrund der besonderen Gegebenheiten bei Nutztieren wurde die Methodik um die folgenden Aspekte erweitert: (a) Entwicklung eines Ansatzes zur Berechnung der Weitzman-Diversität innerhalb und zwischen Rassen, (b) Entwicklung approximativer Algorithmen und Programme zur Berechnung der Weitzman'schen Diversitätsstatistik für viele Rassen, und (c) Berücksichtigung sicherer externer Populationen bei der Ableitung von Erhaltungsprioritäten. Es konnte gezeigt werden, dass die Ableitung von Erhaltungsprioritäten nach dem Kriterium Erhaltungspotential im vorliegenden Fall nicht dazu führt, dass besonders stark ingezüchtete Linien die höchste Priorität erlangen.

Für 20 nichtkommerzielle Hühnerrassen und acht kommerzielle Broiler- und Leger-Elternlinien wurden Reynolds Distanzen anhand von 29 Mikrosatelliten geschätzt. Es wurden zwischen 9 und 50 Individuen pro Rasse genotypisiert. Die Diversität zwischen den Rassen, die marginale Diversität und das Erhaltungspotential für alle Rassen wurden mit dem exakten Algorithmus von Weitzman berechnet.

Um größere Datensätze bearbeiten zu können wurde eine Approximation des Weitzman Algorithmus entwickelt. Dies ermöglicht die Diversität zwischen und innerhalb der Rasse in einem Analyseschritt zu ermitteln.

Die Beurteilung der, in verschiedenen Populationen enthaltenen Diversität konnte durch den Relativen Diversität Index (RDI) und durch die Wahrscheinlichkeit normaler Dichten bei Rassehühnern und in kommerziellen Linien erreicht.

Es konnte gezeigt werden, dass ein Großteil der genetischen Diversität innerhalb der Rassen angesiedelt ist, und dass die nicht kommerziell genutzten Rassen einen erheblichen Beitrag zur gesamten Diversität leisten. Die entwickelten Methoden und Programme können für die Ableitung von Erhaltungsprioritäten in anderen Tierarten genutzt werden. Vor allem aber für Geflügel mangelt es an Förderprogrammen, die durch diese Arbeit unterstützt werden und Länderübergreifend Verwendung finden könnten.

SUMMARY

Genetic diversity is the base for future breeding in all farm animal species and therefore needs to be conserved. Criteria for the choice of breeds to be included in conservation programs are essential for the design of conservation strategies. In this study, such criteria were developed based on Weitzman's diversity concept using domestic chicken biodiversity as a model. Taking into account the specific features of farm animal biodiversity, the following extensions of the methodology were developed: (a) an approach to assess the Weitzman diversity within and between breeds, (b) development of an approximate algorithm and software to calculate Weitzman's diversity statistics for many breeds, and (c) an approach to account for safe external (international or commercial) populations in the derivation of conservation priorities.

Based on 29 microsatellites genotyped for 9 to 50 individuals in 20 non-commercial fancy breeds and 8 commercial layer and broiler lines the Reynolds distance was calculated. Diversity between breeds, marginal diversity and conservation potential were calculated for the fancy breeds, with or without accounting for the safe commercial breeds, using Weitzman's exact recursive algorithm.

An approximation was developed to cope with bigger sample sizes. This enabled us to account for diversity between and within breeds in one step analyzing single animal data. Estimation of diversity contained in different populations could be calculated establishing a Relative Diversity Index and looking for the probability of normal densities in fancy breeds and commercial lines.

Several tool s to support decisions in planning and conducting conservation concepts were suggested. Especially for poultry lacking an existing promotion programme this is expected to be reasonable to use an area-wide approach.

1st CHAPTER

INTRODUCTION

1 INTRODUCTION

1.1 Main question

Method and software development to be used for the derivation of conservation priorities in farm animal species was the main intention of this study. How to assess and compute breed information? How to take into account within and between breed diversity? How to define conservation priorities and what kind of support would be appropriate? These are main questions for every conservation project. But additionally there are always practical questions when having large data sets. Especially for calculations in which each possible scenario has to be compared the complexity of a problem increases very fast. If the number of breeds that should be considered is a permutation for each number of elements, the computational and computing dimensions increase by the factor n with each additional element.

1.2 Farm animal genetic resources

1.2.1 Variety of breeds and losing genetic diversity

The definition of a scientific measure to distinguish breeds is not reliable. K. Hammont reportedly stated to J. Woolliams: "A breed is a breed if enough people say it is" (WOOLLIAMS and TORO 2007). There is no clear definition of criteria that has to be fulfilled to call a population a separate breed. This problem occurs for all level of taxa, even for some species but is particularly strong in breed definition. Breeds can be defined in a standard book through only few phenotypic characteristics, or by occurring in some regions, they can and do interbred and are very dynamic. The Food and Agriculture Organization of the United Nations (FAO) argues that a breed is a cultural term and should be respected as such. For chicken breeds in Germany usually the standard defined by Bund deutscher Rassegeflügelzüchter e.V. (BDRG) is used when assuming a chicken population to be a breed.

Genetic diversity provides an additional reference to phenotypic, performance and historical clues to distinguish populations and thus to sort given breeds into clusters of similarity and kinship. For conservation issues it is necessary to define units of

preservation interest. Therefore the terms breed and population have to be defined. Breed similarities, divergences within breeds of different variations and exotic, closely related populations should be taken into account.

The actual problem in loosing breeds is more than a given dynamic of breed development, improvement and migration. Chicken production is a good example for dramatic expansion of few lines. In the last 50 years international breeding companies are dominating nearly the complete commercial chicken market. The high rate of loss of breeds and the limited resources available for conservation require prioritisation and decisions which breeds to care for first. Assessing the degree of endangerment for each population would provide additional information for conservation programmes.

Not only genetic diversity but also production system diversity is shrinking. A Global market and economical development favours a shift from multi-purpose local to few specialized global breeds (TISDELL 2003).

1.2.2 Importance of genetic diversity

The value of a high diversity in the global and local chicken population for commercial use and future breeding work should be strongly considered. In order to access the global market production systems tend to become standardized and uniform allowing the use of specialized, global breeds (TISDELL 2003). Production with global and specialized breeds can not consider the local market and local production conditions. This problem is more prominent if we consider that highly specialized layer and broiler hybrids are sold to developing countries where climate and infrastructural differences in feeding, air-conditioning or housing have to be managed. Economic calculations for breeding programs oftentimes are made on microeconomic scale for a short to medium term perspective (SIMIANER 2005b). Nevertheless future changes in market demand affect not only exotic countries. One example might be the increasing popularity of organic products worldwide. However, genetic diversity is known to be an insurance against future changes (SMITH 1984).

Genetic variation is the basis for any kind of breeding and selection. Farm animal phenotypic characteristics of interest can be divided into production traits and fitness traits.

Neutral genetic diversity is – by definition - not linked to present phenotypic variability but may contain a potential for future adaptations. Neutral genetic variants may mutate, interact with other genes or display measurable phenotypic characteristics only in a specific environment.

The importance of genetic variation is mainly for future challenges but also for present research, market niches, culture, history and ecology.

- Future challenges are changing market demands and production circumstances or outbreaks of diseases or wars. (BROWN and GOLDSTEIN 1984; JATURASITHA et al. 2008; RIKIMARU and TAKAHASHI 2007).

- In research different breeds and crossbreeds are currently used in molecular genetics. If searching for new genes for production or health traits associations between highly polymorphic markers and the quantitative and qualitative trait loci are essential (ATZMON et al. 2008; RUBIN et al. 2007; SCHUTZ et al. 2002; WANG et al. 2007; ZHANG et al. 2008; ZHOU et al. 2006a; ZHOU et al. 2006b; ZHOU et al. 2007a; ZHOU et al. 2007b; ZHOU et al. 2008).

- Local breeds are often used by farmers for special purpose or in special landscapes. These breeds provide income for farmers in developing countries and developed countries with an increasing interest in local products and different qualities (JATURASITHA et al. 2008; WILSON 2008).

- Many breeds are of historical and cultural value. They illustrate a long domestication process and adaptation to special environments such as breeding and housing systems, use by humans and climates (BOOGAARD et al. 2008; SAMBRAUS 1989; SCHIBLER and SCHLUMBAUM 2007).

- Due to these adaptations local breeds are often used in local adapted farm management systems. The awareness is growing for the ecological value of landscapes formed by different multifaceted housing systems (BEINLICH and POSCHLOD 2002).

1.2.3 Genetic data and diversity measures

A number of different genetic markers can help the measurement of genetic diversity. Despite SNP's are becoming more and more popular due to their very high density and high throuphput genotyping techiques, microsatellites provide considerable information within a few genotypes. Microsatellites are typically neutral, co-dominant and widely used for population analysis. The European database AVIANDIV (WEIGEND *et al.* 1999) is building a chicken DNA collection and biodiversity database to evaluate strategic questions relating to the assessment of genetic diversity in chicken using molecular information at DNA level.

Allele frequency is one way of assessing diversity through genetic markers. By defining an allele frequency for each individual as 0, ½ or 1 both, between and within breed diversity, can be assessed. In this approach the breed mean is the estimate of the allele frequency of the breed.

The interpretation of microsatellite data outcome has to take into account that the positioning of the markers used may bias the results. Linkage between markers supposed to be neutral and alleles with significant selective advantage are important since most models assume that the change in allele frequency is only due to genetic drift and not to selection. A given locus may differ in neutrality between different breeds since important alleles may segregate in one breed that are not segregating in another and livestock breeds will be subject to different selection criteria (WOOLLIAMS and TORO 2007).

The problems of biased mutation when using microsatellites to compare species may not be of large importance for our data of close related breeds. A bias in the sense that larger alleles contain more bases and are therefore more likely to be mistranslated in DNA replication becomes primary a problem with increased genetic distance of species. The same is valid for the problem of increased instability during recombination at meiosis if there is a large size difference between individual alleles (JARNE and LAGODA 1996).

1.2.4 Genetic identity and distances

Genetic distance is generally defined as a degree of gene or genome differentiation between individuals or populations measured on a numerical scale. There are

several metrics for genetic similarities and differences between populations. The best known and most frequently used dimensions are the genetic distance D and the genetic identity I of Nei (1972). Basic assumption is an ideal population, having a size where the influence of mutation is larger than that of genetic drift. NEI defines the genetic identity I between two panmictic populations as follows:

$$I_j = \frac{x_i y_i}{\sqrt{x_i^2 y_i^2}} \, ,$$

where x_i and y_i being the frequency of the ith allele at the jth locus of population x and y. For the assessment of genetic differentiation between populations multiple loci are needed to be considered and the genetic identity is then represented as:

$$I = \frac{J_{xy}}{\sqrt{J_x J_y}} \, ,$$

where J_{xy}, J_x and J_y are the arithmetic averages of *gene identities* over all loci. Let x_i and y_i be the frequency if the ith allele at a locus in population X and Y, respectively. The gene identities within and between populations are then computed by $j_x \sum x_i^2$, $j_y \sum y_i^2$ and $j_{xy} \sum x_i y_i$, where $\sum$ stands for the summation for all alleles at the locus. The average gene identities over all loci are the averages of these quantities, that is,

$$J_x = \frac{\sum_{k=1}^{n} j_{xk}}{n} \, , \qquad J_y = \frac{\sum_{k=1}^{n} j_{yk}}{n} \, , \text{ and } \quad J_{xy} = \frac{\sum_{k=1}^{n} j_{xyk}}{n}$$

Where k refers to the kth locus and n is the number of loci examined.

Genetic distance *D* is derived from genetic identity as follows:

$$D = -\ln I$$

Another genetic distance was developed by Reynolds (REYNOLDS *et al.* 1983). While NEI'S distance is based on a classical infinite mutation-drift model REYNOLDS-distance is a pure drift model. Monomorphic loci and mutation is not included, because there is no certainty whether an allele is appearing by mutation or disappearing by genetic drift and/or inbreeding. REYNOLDS distance was developed to analyze micro evolutionary processes as accurately as possible. It is calculated as follows:

$$D^2 = \frac{\sum\limits_{k=1}^{n}\sum\limits_{i=1}^{m}(x_{ki} - y_{ki})^2}{2\sum\limits_{k=1}^{n}(1 - \sum\limits_{i=1}^{m}x_{ki} - y_{ki})}$$

where i is the number of the ith allele of m alleles on locus k and x_{ki} is the frequency of the ith allele on locus k of population x. D^2 is the mathematical value expected to increase linearly with increasing genetic drift (REYNOLDS *et al.* 1983). The accuracy of NEI'S and REYNOLDS genetic distance measurements increases with the number of investigated loci and individuals. Small sample sizes produce large statistical errors and less meaningful genetic distances. Both methods are used for domestic animal breeds and produce very similar results (HILLEL *et al.* 2003).

1.2.5 Graphical illustration

Constructing phylogenetic trees is not a trivial problem. A huge number of possible phylogenetic trees exist for any set of data; for example, only ten species give over two million possible unrooted trees (FELSENSTEIN 1978). These possibilities must be searched to find a tree that best fits the data according to an optimality criterion. However, the data themselves do not lead to a simple, arithmetic solution to the problem. Expecting the distribution of whatever trait (such as alleles of phenotypic traits) to directly follow the branching pattern of evolution, would be an oversimplification. Well understood phenomena of convergent evolution, parallel evolution, and evolutionary reversals (collectively termed *homoplasy*) add an unpleasant wrinkle to the problem of estimating phylogeny. Real phylogenetic data include considerable homoplasy, with different parts of the data suggesting sometimes very different relationships. Methods used to estimate phylogenetic trees are explicitly intended to resolve the conflict within the data by picking the phylogenetic tree that is best fitting to the data overall, accepting that some data simply will not fit.

However data that do not fit a tree are not simply "noise", they can contain relevant information in some parts of a tree, even if they conflict with the tree overall.

Two types of phylogenetic trees will be discussed for microsatellite data: Neighbor-Joining, a general data clustering method and Maximum likelihood where

probabilities for possible phylogenetic trees are inferred (FELSENSTEIN 1980). It evaluates a hypothesis about evolutionary history in terms of the probability that the proposed model and the hypothesised history would give rise to the observed data set. The supposition is that a history with a higher probability of reaching the observed state is preferred to a history with a lower probability. The method searches for the tree with the highest probability or likelihood.

For trees based on phenotype only, different maximum parsimony methods will be used where a tree requiring the smallest number of mutations is build. Different knowledge about the data, mechanisms and criteria can be assumed.

1.3 Gallus gallus as a farm animal

1.3.1 Domestication

The genetic variability in chicken populations evolved during domestication in different climate zones, housing systems, purpose of use and breeding systems. When ever people moved, they took their breeds with them. The symbiosis of *Homo sapiens* and *Gallus gallus* started around 5400 BC (WEST and ZHOU 1988) from different geographic points, all located in Asia. Domestication centres seem to be Yunnan province/China, Southwest China and surroundings (i.e., Vietnam, Burma and Thailand) and the Indian subcontinent as suggested by Liu (LIU *et al.* 2006). Hutt's (1949) assumption that more than one species have contributed to the domestic chicken genome was supported by Eriksson (ERIKSSON *et al.* 2008). He found strong evidence for introgression of different species expressed by yellow leg colour and the candidate gene BCD02. Most commercial layer and broiler lines are homozygote for the BCD02 inhibiting allele but it is not found in the *Gallus gallus* species. The yellow skin allele originates from a different species, most likely *Gallus sonneratii*.

Domesticated chicken arrived Europe with Indo-European clans through Iran and Greece. Finally the Romans spread them out over the whole Roman Empire where cocks were largely used as gamecocks with high cultic value.

Up to our days cock fighting is a popular and wide spread activity in many countries. Different breeds evolved with different fighting techniques and adapted to different environments and human preferences. In Indonesia there are still funerary rituals

where a chicken has to be present as channel for evil spirits and in Confucian Chinese weddings a chicken can even substitute a person that is not available. There are many examples for religious and customs connected with chickens in nearly all cultures (WOOD-GUSH 1956).

Despite all cultural use chickens were mainly kept for eggs and meat production. Depending on local preferences breeds were developed for dual propose, primarily for laying or primarily for meat production. This focus evolved up to the highly specialized layer and broilers lines used today.

1.3.2 Breeds

The FAO has registered 734 chicken breeds (SCHERF 2000; SOW-ANGR 2007) worldwide, 158 of them being transboundary breeds that occur in several countries. In European fancy breed clubs approximately 170 breeds of chickens and bantams are attended to that may display in up to 20 colour and comb variations.

1.3.3 Breeding practice

Commercial chicken lines have the most specialized and industrialized production in all animal species showing similarities with plant breeding and highly elaborated breeding schemes and performing tests are used. After the vertical integration of egg and poultry production in the late fifties of the 20ᵗʰ century all parental lines used for commercial hybrid production are managed by few companies, one of them located in Germany (Cuxhaven). In contrast, non-commercial breeds are kept by fancy breeders and small holders without an elaborated central breeding concept. A fancy breeder keeps about 10 animals on average (BDRG 2005). Cocks are traded for new ones in poultry shows but normally there are no recordings about pedigree or performance except for phenotypic characteristics defined in the standard of each breed. German fancy breeders club are in charge of about 200 standardised breeds.

1.3.4 Phenotypes of colour, shape and plumage

The phenotype is a product of genotype and environment (FALCONER and MACKAY 1996). It is an important feature for selection in local populations that are kept by

fancy breeders since at least 50 years as non commercial breeds. These are both quantitative phenotypic traits (measurable on a continuous scale) such as body size and weight, as well as discrete qualitative trait like type of comb shape, plumage colour, naked neck or short legs. Different populations can be discriminated through their breeding history and in particular using phenotypic criteria. Depending on background and complexity of the phenotypic characteristics it can be a more or less reliable indicator for neutral diversity. Phenotypic diversity relates to the expressed genetic diversity and gives a quantitative measure of adaptation of breeds to the environment (HALL 2004).

For many of the phenotypes the genetic background is known at least partially and will be mentioned in chapter 4. Comb shape, incidence of a crest or beard, leg colour, eye colour, feather colour and pattern are used to group the breeds. In this present study ear lobe colour and egg shell colour can not be used because all breeds sampled showed the same variation for this trait. In addition not all colours and plumage patterns and comb shapes are present in the used samples as well as silky plumage, frizzle feathers, long tails, ear tuffs, rumplesness and dark meat. For larger studies with bigger sample size and maybe international breeds involved, these qualitative criteria can be of proper use. White ear lobes are the less common variant in all existing breeds, in the present study, however, the only one represented. This illustrates well the exemplary nature of the study, not claiming to represent the European or global population structure and diversity of chicken breeds. The mentioned phenotypic characteristics in addition to body weight and shape are the main selection criteria used for non-commercial chicken breeds. Disease resistance, behaviour, laying performance, carcass and meat quality are no selection criteria at all or just subordinated. Breed specific occurrence of these characteristics are known and - if possible – included in breeding decisions.

1.3.5 Fanciers' role in development of strains and conservation of unique alleles

A fancier is a person who likes poultry and keeps them out of interest, not out of financial necessity. Some serious fanciers develop very good breeding skills and generate successful stock. From among these fanciers came the earlier commercial producers and professional poultrymen. They developed the stock sense and

practical breeding techniques which formed the basis of poultry husbandry (CAREFOOT 1990). Although aesthetic traits were incorporated early, fanciers realized that their creations had to be economically viable. Indian Game or Cornish breeders always took care of the typical type of body shape. Those standardized characteristics describe a very good table fowl. This was used later in commercial breeding programmes. Local adaptations created a great number of different varieties of breeds. Nonsitting breeds, typically layers are usually standardized as having white earlobes; they are slender in build, active, have a large comb and are laying white eggs. The Mediterranean breeds show yellow skin and no dermal melanin such as Leghorn or white skin and dark legs like Minorka. In colder regions large combs are in danger to freeze. New varieties of comb shapes were developed. Dual purpose breeds, with yellow skin and laying tinted eggs -such as Plymouth Rock, New Hampshire, Rhode Island Red and Wyandotte- were developed in USA due to local preferences for those meat and egg colouration. In continental Europe deep brown eggs and yellow meat have been favoured and breeds like Barnefelders, Welsumers evolved in Holland and Marans in France. Meanwhile a preference for white meat and tinted or brown eggs in England supported breeds like Sussex and Dorking. All those breeds were standardized and selected by fanciers into quite uniformed stocks. They provide the basis for all commercial breeding programmes (CAREFOOT 1990).

1.4 Conservation

1.4.1 Conservation background

Countries having subscribed to the Convention on Biological Diversity (CBD 2005) are obliged to develop strategies for the conservation of biological diversity including farm animals. Available funds for conservation, however, do not cover the costs for preserving the entire variety of breeds. To date, Germany lacks an official conservation and financial promotion concept for poultry. In order to develop a conservation concept, it is necessary to identify the breeds that should be included and to allocate an appropriate amount of monetary and other resources to the breeds of the selected pool. Additionally not only a large number of different breeds has to be maintained expressed as diversity between breeds but also the variability of

breeds itself expressed as diversity within breeds, in some cases even merging different populations to one synthetic breed (BENNEWITZ *et al.* 2008). Since we do not even know what kind of future challenges chicken breeding will be confronted with or what kind of scientific value its diversity will have (DELANY 2003; 2006; HALL 2004), it may be the best strategy to maintain the highest neutral genetic diversity within the whole chicken population.

1.4.2 Conservation programmes

The Central Documentation of Animal Genetic Resources in Germany (TGRDEU) is established on behalf of the German Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) through the Information and Coordination Centre for Biological Diversity (IBV) of the Federal Agency for Agriculture and Food (BLE). TGRDEU is focusing on horse, cattle, sheep, pig and goat, all species that underlie the German animal breeding law. Each federal state has a programme where local breeds are supported and paid different bonuses for keeping and buying breeding animals of endangered breeds, organizing breeding clubs and participating in cryoconservation banks. The breeding animal bonus paying method is an in situ approach. Advantages of in situ conservation –in contrast do ex situ- are opportunities for rural development, maintenance of agro-ecosystem diversity and rural cultural diversity, breed evolution and genetic adaptation and increased knowledge of breed characteristics. Disadvantage compared to cryoconservation is that the conserved populations are not safe against diseases and disasters and may be exposed to genetic drift (OLDENBROECK 2007). Until now there is no financial support for poultry. The main problem in poultry is the lack of herdbook records. Only 5% of privately kept poultry are registered and performance tested (BMELV). One reason for the lack of herdbooks for poultry is the huge effort required monitoring hatching and 'trip' nests, non-commercial poultry keepers can not manage.

In Germany, data for the cryoconservation of semen from poultry are unknown. The Friedrich Löffler Institut (former FAL-TZ) established a blood and DNA bank of around 100 poultry breeds (WEIGEND *et al.* 1999).

The most important non governmental organization in Germany in the field of farm animal conservation is The Society for the Conservation of Old and Endangered Livestock Breeds (GEH) founded 1981. It is member of Rare Breeds International,

Donaulaender alliance for gene conservation and SAVE foundation. Those organisations play a promoting and advising role for interested stakeholders and fancy breeders. To promote in situ conservation they created an Arc Farm Project where animals listed in GEH's Red List of Endangered Stocks of Livestock Breeds are promoted and managed in breeding groups. For Chicken, privately organized breeder circles were initiated for Vorwerkhühner, Deutsche Lachshühner and Ostfriesische Möwen.

Private breeding associations have a long-term interest in conserving a wide array of alleles (KNAP and NEETESON-VAN NIEUWENHOVEN 2005) but only if a utilisation in commercial breeding lines is probable. First the genotype must be of interest (for that it has to be known), it has to be not too hard to measure or of low heritability, the disadvantages in performance have to be sufficiently small to be removed in few rounds of selection, the risk of introducing pathogens by introgression of external breeds into the company's units has to be excluded and the costs for maintenance of new populations may not be too high in comparison with this breed's production level. This leads us -except of financial support and data supply for science from commercial associations- back to governmental and private communities of interests as mentioned above assigned to take care of livestock diversity.

1.4.3 Conventional conservation strategy

For the mammal species where funds are spent for conservation depending on the country and species there are different units supported: Bonus for kept breeding animal, litter bonus, foal bonus, bonus for raising, acquisition bonus, support for breeder clubs, subsidization for extraction and conservation of sperms and embryo or utilisation and conservation of the animals are currently paid (TGRDEU).

1.4.4 Weitzman's approach and genetic diversity

Financial resources are always limited. Therefore this resource has to be spent the most efficient way. In economics optimized models for resource allocation is a well developed field of research. To have a basis for calculations genetic and phenotypic data can serve our purpose.

To estimate neutral genetic diversity it is consensus, that marker loci, as neutral as possible, are an appropriate tool. Microsatellite markers being short repeats of 1 to 4 base pair units in non-coding areas. They are highly polymorphic, abundant and evenly distributed throughout the genome. These properties have made them useful markers for mapping, paternity testing and population genetics (WEIGEND and ROMANOV 2002). Further advantages of microsatellites are their easy detection by using PCR and their co-dominance nature. Being a clone based marker type the sequence of each marker in the genome can be mapped and easily utilises for different genetic applications (SOLLER et al. 2006).

Once having marker information it is crucial how to use this information to compute first the relationship and distances of the populations, second the inner breed variation and third the conservation priorities and fund allocation. For this propose the American economist Martin Weitzmann (1992; 1993; 1998) suggested an approach where extinction probability, costs of altering extinction probabilities and the value of diversity are the main ingredients of a rational overall analysis of diversity-conservation that will be explained and used in this study. The main characteristic of the results obtained by Weitzman's approach is that contradictory to intuition not always the most endangered breed will receive the greatest amount of support. Yet it seems to be much more efficient than other resource allocation methods used for animal conservation programmes (REIST-MARTI et al. 2005; REIST-MARTI et al. 2006; SIMIANER 2005b; SIMIANER et al. 2003).

The exact Weitzman algorithm has a limit of theoretical 36 different objects (populations, breeds or individuals) and in practice 35 cattle breeds (TAPIO 2006) and 28 chicken breeds in this study was the maximum calculated. This limitation exists mainly due to working memory capacity when searching analytically for the best combination and proportion of breeds. Computing complexity is proportional to 2^n with n objects. In this study we developed and tested an approximation to enhance the number of breeds and to make it possible to look at individuals' level. An efficient use of conservation funds encompasses two levels of activity (SIMIANER et al. 2003):

- Individuals' level. Make the breed safe and conserve the diversity within this breed.
- Over all endangered breeds. Distribute the total amount among all breeds and conserve the diversity between breeds.

1.5 Conclusion

The Convention on Biological Diversity (CBD) has recognized "the special nature of agricultural biodiversity, its distinctive features, and problems needing distinctive solutions" (decisions II/15 and V/5).

FAO's Report on Strategic Priorities for Action listed the following steps countries should take:

On national level

- Complete breed inventories and implement monitoring.
- Enhance breed characterization.
- Establish national conservation programmes.
- Maintain traditional knowledge, practices and lifestyles that support conservation efforts.
- Integrate genetic resource management into livestock development planning.
- Improve management, research and institutional capacity for inventory, monitoring and characterization.
- Improve policy development and legal frameworks for animal genetic resources to address the complex driving forces that affect the livestock sector.
- Increase public awareness of the roles and values of animal genetic resources to encourage further investment in this sector.

Action at international level

- Encourage collaborative arrangements to help countries improve their inventories, and better conserve, use and develop their animal genetic resources.
- Improve the process of determining risk status for animal genetic resources.
- Collaborate on research into better methodologies for characterization, economic valuation and improved use of animal genetic resources.
- Bring together recipients and donors to mobilize greater support.
- Establish regional focal points for improved regional collaboration.
- Strengthen the role of international organizations for national programmes.

In this study tools are developed to calculate conservation priorities for endangered breeds taking into account their potential relatedness with external populations and including both, between and within breed diversity.

2nd CHAPTER

Weitzman's approach and conservation of breed diversity considering relations to external populations

2 Weitzman's approach and conservation of breed diversity considering relations to external populations

2.1 Introduction

The genetic variability in chicken populations evolved during domestication in different housing systems, climate zones and breeding systems, represents a cultural value and a reservoir for future breeding work.

Countries having subscribed to the Convention on Biological Diversity (CBD 2005) are obliged to develop strategies for the conservation of biological diversity including farm animals. Available funds for conservation, however, do not cover the costs for preserving the entire variety of breeds. To date, Germany lacks a conservation and promotion concept for poultry, although there is a large number of different breeds and lines, especially in chicken. German fancy breeders keep nearly 200 chicken breeds, half of which are bantam. In order to develop a conservation concept, it is necessary to identify the breeds that should receive funding and to allocate an appropriate amount of monetary and other resources to the breeds of the selected pool.

To provide objective information to support decisions as to which breeds to choose, we made an evaluation of genetic diversity in a set of 20 non-commercial chicken breeds and eight commercial broiler and layer parental lines based on 29 microsatellite markers. These markers are assumed to represent a neutral measurement of the adaptation potential of the breeds. Molecular characterisation is an important complement to the assessment of diversity using phenotypic features as body shape and colour.

For diversity estimation, we made use of an econometric model suggested by Weitzman (1992; 1993). In recent studies it was shown that this approach can be helpful (LAVAL *et al.* 2000; THAON D'ARNOLDI *et al.* 1998). This model was used to evaluate African cattle breeds, and it was found that the efficiency of a conservation scheme could be enhanced by up to 60% (REIST-MARTI *et al.* 2005; REIST-MARTI *et al.* 2006; SIMIANER 2005b; SIMIANER *et al.* 2003).

2.2 Material and Methods

2.2.1 Breeds

In the current project, we chose 20 non-commercial breeds representing European lineages. The European ancestries of these breeds were evaluated with help of both, the breed groups defined in the fancy breeders club BDRG (Bund Deutscher Rasse Geflügelzüchter e.V.) by historical knowledge (SIX and WEIGEND 2002) and microsatellite data. Both methods resulted in groups that broadly coincide. To this set of non-commercial breeds, we added eight commercial lines: one white layer, three brown layers, and four broiler purebred lines. Demographic parameters like population size and number of breeders were collected through the German Poultry breeding Club (BDRG). Attendance to data collection on the breeder's local branches of BDRG increased. Thus all data are corrected for participant rate (69.31 % in 2000 and 94 % in 2005).

20 breeds kept in Germany and used for this study will be introduced in table I and further in the Appendix. Descriptions of origin and breed history are based on traditional knowledge (SCHMIDT 1999; WANDELT and WOLTERS 1996).

Table I: List of breeds

Bergische Kräher	BERKER
Bergische Schlotterkämme black	BSCHL
Brabanter black, gold, chamois	BRABA
Brakel silver	BRAKE
Deutsche Sperber	DSPER
Friesenhühner yellow pencilled	FRIES
Hamburger sprangled	HAMla
Hamburger pencilled	HAMsp
Italiener partridge	ITALp
Italiener black	ITALb
Kastillianer	KASTI
Krüper balck	KRUEP
Lakenfelder	LAKEN
Ostfriesische Möwen silver	OMOEW
Paduaner chamois, black, silver, gold, white	PADOW
Ramelsloher white	RAMEL
Rheinländer black	RHEIN
Thüringer Barthühner black, gold, chamois, silver	THBAR
Vorwerkhühner	VORWE
Westfälische Totleger silver	WETOT
Broiler dam line. A	BROdA
Broiler sire line. A	BROsA
Broiler sire line. B	BROsB
Broiler dam line D	BROdD
Brown layer A	BEGGA
Brown layer C	BEGGC
Brown layer D	BEGGD
White layer A	WEGGA

When possible, full names will be mentioned in tables and figures. Under space restrictions the above listed abbreviations are valid. All considered plumage colours are mentioned to indicate the sample pool. They apply for the whole study and may also not be mentioned under space restrictions. In Germany called Italiener in some countries are known as Leghorn standard type.

German native breeds listed on GEH's red list but not included in this study are: Augsburger, Deutsche Langschan, Deutsches Lachshuhn, Sachsenhuhn, Sundheimer, Deutsches Reichshuhn and the Austrian Breeds Altsteirer and Sulmtaler kept in Germany for decades. Some further 'German' breeds developed recently out of foreign breeds like Bielefelder Kennhuhn (1970) or Niederrheiner are not included either.

2.2.2 Genotyping

Genotyping was realized by the Friedrich Löffler Institute (former FAL) in Marienesee. Blood was sampled from the wing vein of each bird on different German poultry exhibitions. The procedure is described by (HILLEL et al. 2003).

For 28 populations, 29 microsatellite genotypes and, on average, 30 individuals per population (min 9, max 60) were sampled. All markers are FAO recommended by MoDAD project for assessing chicken genetic diversity (FAO 2004). A table with all used markers is listed in Appendix III.

Multiplex polymerase chain reactions (PCR) were carried out according to FAO (FAO 2004) recommendations. Electrophoregram processing on LICOR 4200 and allele-size scoring were performed with RFLPscan software package (Scanalytics, Division of CSP, Billerica, U.S.A.).

2.2.3 Genetic distances and phylogenetic trees

We used two types of distance measurements. First, Reynolds' genetic distance (REYNOLDS et al. 1983) was estimated to detect and analyze micro evolutionary processes and therefore it serves well for breed dimensions of genetic differentiation. For comparison we also looked at NEI's standard distance (NEI 1972) that is also often used in genetic distance analyses on the species and breed level. Second, Weitzman's genetic distance (WEITZMAN 1992) that is part of Weitzman's approach was estimated for further calculations on marginal diversity and conservation potentials.

We calculated a maximum likelihood tree using Weitzman's (1992) recursive algorithm. It is not a genealogical representation of breeds but only a one dimensional representation of genetic distances. There are some supposed advantages of maximum likelihood methods over other methods, e.g. lower variance than other methods and thus these methods are least affected by sampling errors, robust to many violations of the assumptions in the model, outperforming of other methods when using short sequences, statistically well founded, they are able to evaluate different tree topologies and to use all sequence information (OLIVERA *et al.* 1998). The biggest disadvantage is to be very CPU intensive, thus having a long runtime. Illustration of the trees was done with TreeView 32 (PAGE 1996). For comparison we computed a neighbor-joining tree using Phylip 3.6.2 (FELSENSTEIN 1980).

2.2.4 Weitzman's Approach

Beyond his distance measurement the economist Martin Weitzman (1992; 1993) has proposed an approach for decision making in what to preserve under resource constraints. The main issue in finding preservation priorities is to find a usable measure for diversity values. By postulating a function that provides a meaningful value of diversity, well defined forms of resource allocation can be applied on those diversity values and costs.

Once having a pairwise-dissimilarity distance values between species or breeds, they have to be converted into an over all measure of the diversity of a set of breeds. This idea of diversity used by Weitzman is a measure of collective dissimilarities. Direct benefits of the breeds such as use value or cultural value can be added but are not reflected in his value of diversity function. The aim is to minimize lost of diversity.

In this study total diversity, extinction probability, contribution and marginal diversity of the breeds is calculated to obtain conservation priorities aiming to consider costs and benefits of conservation of rare breeds.

2.2.5 Total Diversity

The total Weitzman diversity (D) of a set S, $D(S)$ is identical to the ordinates of all nodes in the maximum likelihood tree if the branch lengths are reduced by the total height of the tree (THAON D'ARNOLDI *et al.* 1998). It is defined recursively as:

$$D(S) = \max_{i \in S} \left[D(S_{i \notin S}) + (d(i, S_{i \notin S})) \right]$$

Where the diversity function of any set S of breeds, $S_{i \notin S}$ stands for set S without breed i and $d(i, S_{i \notin S})$ for the distance between breed i and the Set without I *(EDING and BENNEWITZ 2007)*.

2.2.6 Extinction probability z

To make first calculations concerning diversity between breeds, for each breed the degree of endangerment was derived from total population size of the breed in Germany and was quantified as extinction probability. More sophisticated methods can be added and will be mentioned later.

2.2.7 Contribution and marginal diversity of a breed

For further use, marginal diversity was calculated that reflects the change of diversity in the whole population in case of an increase in extinction probability of one breed (SIMIANER *et al.* 2003). Its value depends on the position of the breed in the maximum likelihood tree and on extinction probabilities of the neighbouring breeds in this tree, but is independent of extinction probability of the breed itself. The marginal diversity m_i of breed i is

$$m_i = + \frac{\partial E[D]}{\partial z_i},$$

The contribution, c_i, of breed i to the total diversity population S is calculated as

$$c_i = D(S) - D(S \setminus i),$$

where D(S/i) is the diversity of the total population without breed i. The contribution of an element is proportional to the reduction in tree length caused by its removal from the group.

2.2.8 Conservation potential

Finally, the conservation potential as a product of extinction probability and marginal diversity was calculated, which is a good indicator for the priority of a breed for conservation (SIMIANER *et al.* 2003; WEITZMAN 1993). These calculations were done in two versions: For the subset of 20 non-commercial breeds; for all breeds, including the commercial breeds. Marginal diversities and conservation potentials in both cases were only calculated for the 20 non-commercial breeds.

The conservation potential, CP_i is the product of extinction probability z and marginal diversity m_i of the breed i

$$CP_i = -z_i m_i.$$

2.3 Results

2.3.1 Genetic distances and phylogenetic trees

REYNOLDS' distance and NEI's distance measurements showed no apparent difference in our data, thus Reynolds' was used for further analyses. Comparing the neighbor-joining tree to our maximum likelihood tree computed using Weitzman's approach (*figure 1*) and to a neighbor-joining tree based on Reynolds' distances proposed by (SIX and WEIGEND 2002) similarities appeared. In all tree cases broiler parental lines form a close cluster, as do brown egg layer parental lines. Schlotterkaemme and Krueper representing two variations of the same population always show a close group. Italiener partridge and Italiener black have no close relation to each other. The same occurs with Hamburger sprangled and Hamburger pencilled. Further on Hamburger Pencilled never shows closer relations to other

breeds. Vorwerk chicken is the closest to broilers but was not included in the studies by Six And Weigend (2002), thus only a comparison within our study was possible. On the other hand comparing our results generated by maximum likelihood tree to neighbour-joining tree some differences appeared. In maximum likelihood approach the white layer line clusters to brown layer whereas in neighbor-joining it does not. East European chicken type containing Bergischer Kraeher, Friesian, Hamburger pencilled, Brabanter, Brakel and Paduaner as proposed by Six and Weigend (2002) is a constant cluster regardless of which method was used with exception of Brakel that is not included in maximum likelihood (Figure 1 and 2).
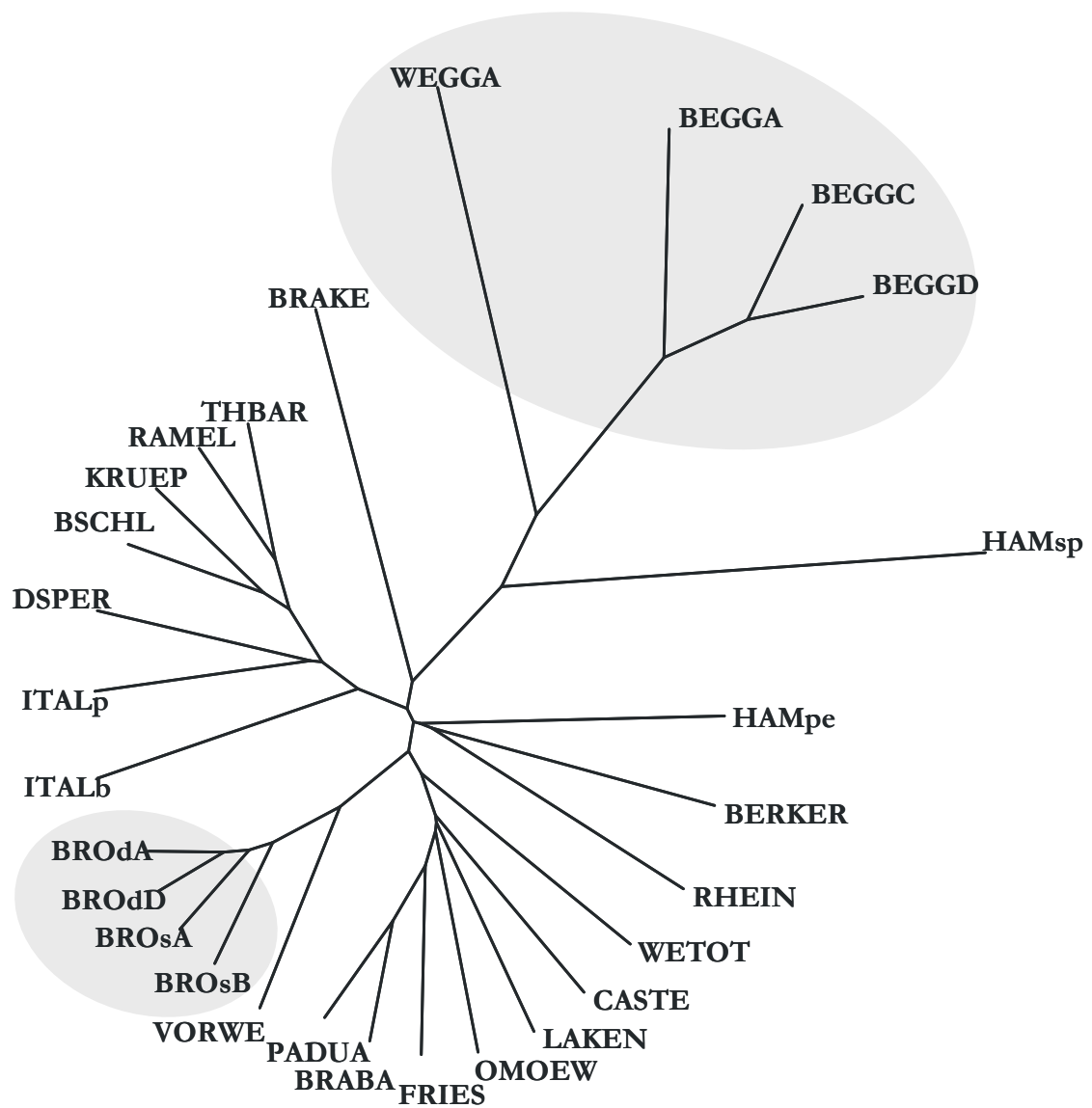
Figure 1: Maximum likelihood tree calculated based on Weitzman's diversity measurement. White and brown egg layers (upper branch) as well as the broiler group (lower branch) are highlighted.
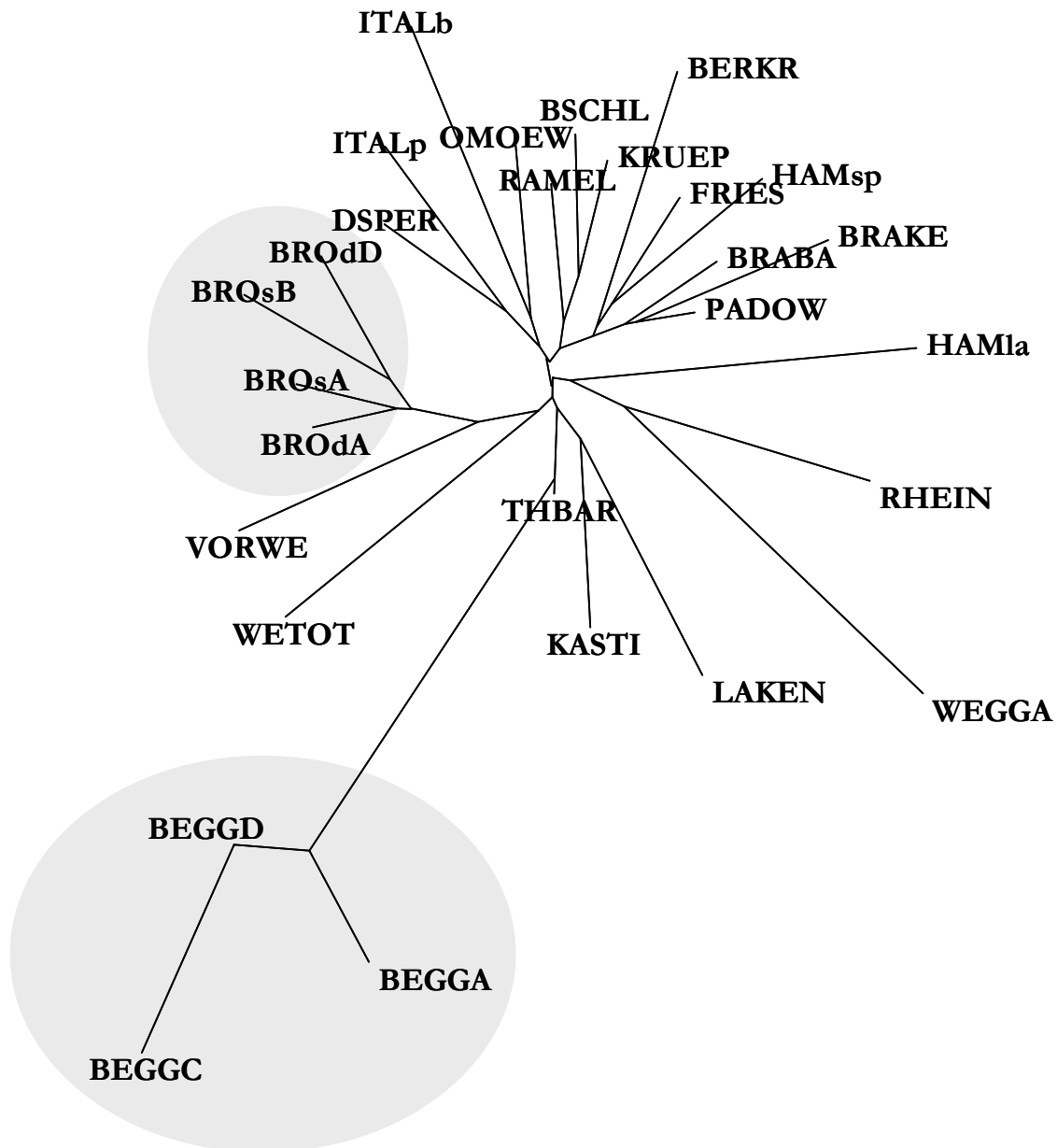
Figure 2: Neighbor-Joining tree calculated on Reynolds's distance. White and brown egg layers (upper branch) as well as the broiler group (lower branch) are highlighted.

Most results fit roughly with historical records and evaluations by the German Fancy Breeders Club. A precise comparison was not possible due to different breed composition.

2.3.2 Extinction probability

Extinction probability was determined mainly by population size (see table II) for first calculations. As population size the number of all animals, male and female, used for breeding and specified by the breeders was used. This is suitable especially for the chosen set of breeds because breeding structure and use are similar. All considered breeds are white egg layers, kept only by fanciers, all are registered by BDRG and none is in a governmental program for conservation. Only Vorwerk chicken has been encouraged by a private initiative. It was the only breed that showed growing population size within the considered years (table II).

Table II: Population size of breeds in 2000 corrected for the proportion of participating breeders to the survey (all specified breeding animals by BDRG)

Breed	total population size 2000
Bergische Kräher	344,7
Bergische Schlotterkämme black	117,0
Brabanter schwarz, gold, chamois	111,7
Brakel silver	918,1
Deutsche Sperber cocoo	517,0
Friesenhühner yellow-white pencilled	743,6
Hamburger silver sprangled	1625,5
Hamburger pencilled	291,5
Italiener partridge	7859,6
Italiener black	3831,9
Kastillianer	264,9
Krüper black	209,6
Lakenfelder	1085,0
Ostfriesische Möwen silver pencilled	1400,0
Paduaner chamois, black, silver, gold, white	458,5
Ramelsloher white	140,4
Rheinländer black	3202,1
Thüringer Barthühner black, gold, chamois, silver	1394,7
Vorwerkhühner	4944,7
Westfälische Totleger silver	1321,3

Extinction probabilities based on population size are shown in table III. Values range from 0.76 as the highest extinction probability for the breed Bergische Schlotterkaemme to 0.15 as lowest extinction probability for the breed Italiener partridge.

Table III: Calculated extinction probability of breeds

Breed	extinction probability
Bergische Kräher	0,529
Bergische Schlotterkämme black	0,763
Brabanter schwarz, gold, chamois	0,500
Brakel silver	0,296
Deutsche Sperber cocoo	0,451
Friesenhühner yellow-white pencilled	0,382
Hamburger silver sprangled	0,274
Hamburger pencilled	0,377
Italiener partridge	0,154
Italiener black	0,199
Kastillianer	0,342
Krüper black	0,419
Lakenfelder	0,444
Ostfriesische Möwen silver pencilled	0,302
Paduaner chamois, black, silver, gold, white	0,510
Ramelsloher white	0,675
Rheinländer black	0,203
Thüringer Barthühner black, gold, chamois, silver	0,276
Vorwerkhühner	0,328
Westfälische Totleger silver	0,243

2.3.3 Current and expected diversity and marginal diversity of a breed

In this study we used diversity, contribution and marginal diversity of a breed only for computing the conservation potential that is our required value.

Table IV: Calculated conservation potential, marginal diversity and expected diversity of breeds for 20 fancy breeds only and considering 8 commercial lines.

breed	marge20	marge28	expDiv20	expDiv28
Bergische Kräher	0,367	0,370	0,194	0,196
Bergische Schlotterkämme black	0,210	0,211	0,152	0,161
Brabanter schwarz, gold, chamois	0,469	0,553	0,235	0,276
Brakel silver	0,224	0,229	0,066	0,067
Deutsche Sperber cocoo	0,271	0,269	0,122	0,121
Friesenhühner yellow-white pencilled	0,243	0,241	0,100	0,092
Hamburger silver sprangled	0,557	0,594	0,160	0,162
Hamburger pencilled	0,398	0,401	0,150	0,151
Italiener partridge	0,362	0,367	0,055	0,056
Italiener black	0,319	0,320	0,063	0,063
Kastillianer	0,292	0,292	0,110	0,100
Krüper black	0,192	0,191	0,080	0,080
Lakenfelder	0,294	0,290	0,129	0,129
Ostfriesische Möwen silver pencilled	0,268	0,267	0,081	0,080
Paduaner chamois, black, silver, gold, white	0,174	0,177	0,093	0,090
Ramelsloher white	0,191	0,193	0,130	0,130
Rheinländer black	0,362	0,362	0,073	0,074
Thüringer Barthühner black, gold, chamois, silver	0,171	0,168	0,047	0,046
Vorwerkhühner	0,335	0,260	0,089	0,085
Westfälische Totleger silver	0,372	0,363	0,090	0,088
White egg layer A		0,475		
Brown egg layer C		0,275		
Brown egg layer A		0,257		
Brown egg layer D		0,145		
Broiler dam line A		0,101		
Broiler sire A		0,100		
Broiler sire lineB		0,186		
Broiler dam line D		0,093		

2.3.4 Conservation potential

As shown in Figure 4 conservation priorities change, if a reference population of safe breeds (in this case the commercial breeds) is taken into account. Endangered breeds which are genetically similar to a safe breed are downgraded in their conservation priority as it is seen in Kastilianer (from 0,110 to 0,100), Friesian (from 0,100 to 0,092) or Vorwerk chicken (fro 0,089 to 0,085). The other case of being upgraded is most clearly seen for the breed Brakel (from 0,235 to 0,276) or Brabanter (from 0,152 to 0,161). Table V shows the exact values calculated for the shown example. Please notice that the order is changed in figure 3 to fit into the phylogenetic tree.
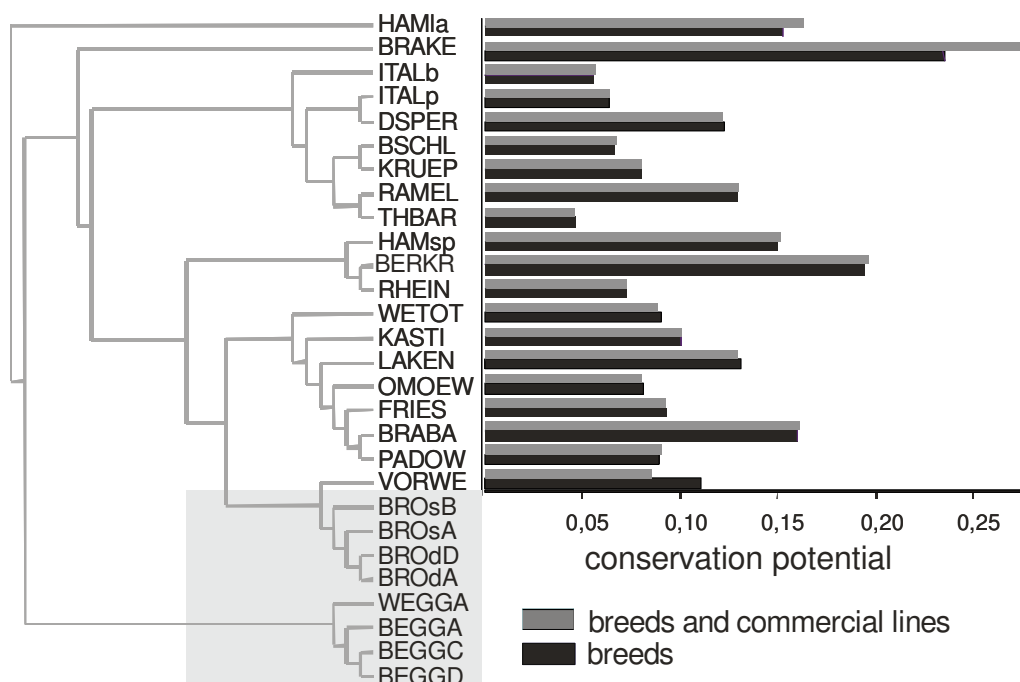
Figure 3: Calculated conservation potential for 20 fancy breeds only and considering 8 commercial lines

Table V shows the exact values of conservation potentials for all 20 breeds and in the last column conservation potentials for the same breeds but considering the presence of 8 commercial lines related to some of the breeds.

Table V: Calculated conservation potential for 20 fancy breeds only and considering 8 commercial lines.

breed	consPot20	consPot28
Bergische Kräher	0,195	0,196
Bergische Schlotterkämme black	0,153	0,161
Brabanter schwarz, gold, chamois	0,235	0,277
Brakel silver	0,066	0,068
Deutsche Sperber cocoo	0,123	0,122
Friesenhühner yellow-white pencilled	0,100	0,092
Hamburger silver sprangled	0,161	0,163
Hamburger pencilled	0,150	0,152
Italiener partridge	0,056	0,057
Italiener black	0,063	0,064
Kastillianer	0,110	0,100
Krüper black	0,081	0,080
Lakenfelder	0,129	0,129
Ostfriesische Möwen silver pencilled	0,081	0,081
Paduaner chamois, black, silver, gold, white	0,093	0,090
Ramelsloher white	0,131	0,130
Rheinländer black	0,074	0,074
Thüringer Barthühner black, gold, chamois, silver	0,047	0,047
Vorwerkhühner	0,089	0,085
Westfälische Totleger silver	0,090	0,088
White egg layer A		4,75E-8
Brown egg layer C		2,76E-08
Brown egg layer A		2,57E-08
Brown egg layer D		1,46E-08
Broiler dam line A		1,02E-08
Broiler sire A		1,01E-08
Broiler sire lineB		1,87E-08
Broiler dam line D		9,34E-09

2.4 Discussion

2.4.1 Genetic distances

Breeds and colour variants that have complex feather patterns, caused by interaction of numerous different genes are less likely to be cross bred. The benefits of mixing foreign blood to these breeds are lower than the loss by disturbing an elaborate interplay of specific and coordinated genes. This is given in Hamburger sprangled which shows the highest divergence to the other breeds. Breeds with uniform colour may be more proper for melioration of colour intensity or body weight by crossbreeding. The same occurs on breeds that are characterized by only one or few features caused by one major gene. They occur for short legged, naked necked or frizzle variants (latter not present in this study). Short legs as in Krueper are homozygous lethal what makes it even impossible to breed short legged animals without the normal leg variant in the same population. This variation to Krueper is Bergische Schlotterkaemme and data show this connection between both breeds very clearly. The same breed structure is known in dog breeds like the dominant type of hairlessness that is lethal for homozygotes in uterus and uses a so called powder puff variant as homozygous wild type (ROBINSON 1985).

The unexpected close relation of Vorwerk chicken and commercial broiler lines in this study may be an example for colour melioration in local breeds. Vorwerk chicken evolved from Lakenfelder but displaying red colour whereas Lakenfelder are white. Asian chicken often have saturated yellow colour and at the same time are one of the founder chicken types for broiler lines. It can be assumed that they were used in the Vorwerk breed for colour and in broiler lines for body size and brought these two breeds closer in the maximum likelihood tree.

However, a difference in trees built based on different algorithms reminds us that one method shows just one aspect of the whole context. Different methods have different assumptions and vantages such as efficiency, power, consistency, robustness or falsifiability (PENNY *et al.* 1982). The diversity measurement of Weitzman is accepted as a good diversity measurement (EDING and BENNEWITZ 2007). It is robust to sampling errors and part of the Weitzman approach used in this study.

2.4.2 Extinction probability

There are many factors influencing the extinction probability and some that surely have an effect like number of breeders, geographical distribution and special traits are documented and assessable. The fact that different known factors have an effect does not mean that it is known how it affects future survival of single breeds. In chapter 3 a score system is described in which different factors assumed to have an effect and are listed and estimated. If the given assumptions are proper future observations will show. We could use only two points in time where population size, number of breeds and geographical distribution could be corrected for the percentage number of participating breeders. To formulate founded predictions the new system of recording all data by BDRG in regular time intervals will be very helpful in future.

2.4.3 Conservation potential

The fact that external populations influence the breeds in different ways can be extended to the problem of national vs. international conservation schemes. If a conservation scheme is only optimized on the national scale, ignoring the fact that there may be closely related and relatively safe breeds in other countries, allocation of funds and conservation efficiency are expected to be sub-optimal (SIMIANER 2005). This fact should be considered when choosing particular breeds that should be conserved by obtaining financial support. FAO's Domestic Animal Diversity Information Service (www.fao.org/dadis/) published this year the additional information to the listed breeds if they are local or transboundary. This facilitates the access to worldwide information about external populations for international conservation schemes.

3rd Chapter

An approximation to Weitzman's approach allowing bigger sample size and calculations considering between and within breed diversity

3 An approximation to Weitzman's approach allowing bigger sample size and calculations considering between and within breed diversity

3.1 Introduction

To be able to cope with larger datasets, we suggest an algorithm to approximate the Weitzman diversity with significantly reduced memory and computing time requirements. The suggested methods are used to derive optimal conservation priorities accounting for within and between breed diversity.

This approximation allowed us to analyse whether maximization of the diversity between breeds leads to less diversity within breeds by favouring inbred lineages, because of their supposed differentiation, like EDING (2002) and CABALLERO AND TORO (2002) have supposed.

To answer the question whether maximization of diversity based on between-breeds diversity leads to a loss of within-breed diversity it was necessary to develop an approximation of the Weitzman algorithm. By approximating instead of calculating analytically, larger sample sizes can be computed. The diversity measure D proposed by WEITZMAN (1992) can be calculated exactly for a limited number of objects. While Weitzman indicated an exact calculation of D should be possible for 35 objects, the maximum number applied objects so far were 28 chicken breeds in this study and 35 cattle breeds in a study done by TAPIO et al. (2006).

Approximations were proposed by THAON D' ARNOLDI et al. (1998) or GARCIA *et al.* (2005) and are based on a restriction in the combination possibilities, done in each step of the recursive algorithm proposed by WEITZMAN (1992). First consisting of randomly sampling trees among the 2^{n-1} trees generated by the algorithm and taking the maximum of these values. While the exact algorithm can be implemented recursively this approximation can not leading to a point where the approximation takes more time than the exact one (GARCIA *et al.* 2005). Second consisting in splitting in each step into two sub-matrices, checking their dispersion, and if all the values in one sub-matrix are close enough together around a mean value, to use this value, multiplied by the remaining number of steps, as the diversity value for that submatrix, and if not, then proceed with the exact computation in the usual way.

These approximations only allow a moderate increase of the number of objects to include. Besides running time primarily memory capacity is a limiting factor.

3.2 Material and Methods

3.2.1 Exact algorithm

Weitzman's (1992) total diversity is recursively defined as:

$$D(S) = \max_{i \in S}\left[D(S_{i \notin S}) + (d(i, S_{i \notin S}))\right]$$

Where the diversity function of any set S of breeds, $S_{i \notin S}$ stands for set S without breed i and $d(i, S_{i \notin S})$ for the distance between breed i and the Set without I.
The marginal diversity m_i of breed i is

$$m_i = +\frac{\partial E[D]}{\partial z_i},$$

The contribution, c_i, of breed i to the total diversity population S is calculated as

$$c_i = D(S) - D(S \backslash i),$$

where D(S/i) is the diversity of the total population without breed i. The contribution of an element is proportional to the reduction in tree length caused by its removal from the group.
The conservation potential, CP_i is the product of extinction probability z and marginal diversity m_i of the breed i

$$CP_i = -z_i m_i.$$

3.2.2 Approximation

The approach proposed in this study is based on a numerical procedure. Looking at the diversity contribution B_i in each step of the Weitzman algorithm shown by THAON D' ARNOLDI (1998), it is shown that in each round a new link element is considered. B_i is the shortest distance of this element to all other, "unconsumed" link elements. The object y with the shortest distance d_{xy} to the observed object x is called the representative. It can appear several times in different steps whereas the link appears just once. One condition is that contribution has to be monotonically increasing, thus $B_{i+1} \geq B_i$. With N breeds, the Weitzman diversity is the sum of all contributions

$$D = \sum_{i=1}^{N-1} B_i$$

The calculation scheme is symbolized by $\mathbf{L}$, being the vector of sequence of all link elements. However there are many $\mathbf{L}$ vectors permitted, leading to different diversities $D|\mathbf{L}$. The Weitzman diversity is the maximum of these diversities. The aim is to find the link vector for which $D|\mathbf{L}$ is maximal. Given N objects there are N! possible $\mathbf{L}$ vectors. We suggest to estimate the maximization of this vectors by a two step procedure:

We generate M_1 random $\mathbf{L}$ vectors and verify them for validity. The valid vector with the biggest diversity value is saved.

Following the vector found in the first step is transformed in the way that two randomly chosen elements i and j are interchanged. If the obtained vector is valid and has a higher diversity value, this link vector is saved instead of the first. This procedure is done M_2 times.

For our data set this algorithm converged well using $M_1 = 500'000$ and $M_2 = 10'000$. The whole procedure was carried out 5 times with different random number seeds and the highest value was accepted. The four other values that were not chosen deviated less then 1% from the maximum value. Notice that a bad convergence still would always lead to an underestimation because the true value is the maximum of all diversities.

The accuracy of this approximation can be controlled only for data sizes that are small enough to be calculated exactly by Weitzman algorithm. In our case, dealing

with 25 animals the approximation was always correct. As the approximation algorithm needs a very small memory capacity and runtime increases linear with M_1 and M_2 increasing, it can be used for much larger data sets, if necessary with larger values for M_1 and M_2 and more replicates.

3.2.3 Extinction probability

The probability that a breed will become effectively extinct in a given time frame can only be estimated due to future is not predictable. In his crane example WEITZMAN (1993) used the opinion of current crane specialists based on current population sizes and their likely future trend but not by any underlying consistent demographic methodology. He argues that there was no larger disagreement among the specialists concerning the ranking from most to least endangered species. REIST-MARTI *et al.* (2005) developed a score system where different criteria could be taken into account.

In this study extinction probabilities were scored due to five criteria that seemed to be sufficiently precise since the study aims to develop a method that can be adapted for further use in different kind of conservation programmes.

The chosen criteria are: total population size, change in population size, geographical distribution, risk of indiscriminate cross and special traits as shown in table VI. Special traits were assumed to reduce the risk of extinction because of fanciers being aware of the peculiarity of the respective breed. All demographic information were provided by records of the German poultry breeders club (BDRG 2000; 2005).

Table VI: Variables and criteria for estimation of the relative extinction probability in German chicken populations.

Variable	Criterion and value
total population size	0.3 <250
	0.2 = 251 to 1000
	0.1 = 1001 to 3000
	0.0>100 000
	missing value = 0.1
change in Population size in the last 5 years	0.1 = decreasing (>20%)
	0.0 = increasing or stable
geographical distribution	0.1 = local (few breeders) <150
	0.05 = wide geographic distribution of breeders or exotic breed
	0.0 = international incidence
	missing value = 0.0
risk of indiscriminate cross	0.1 = high
	0.0 = marginal
special trades	0.1 = no
	0.0 = yes
	missing value = 0.1

These values were rescaled to avoid the assumptions of completely save (z_i = 0) or entirely doomed (z_i = 1.0) breeds. Commercial lines were considered not to be endangered, thus their extinction probability was set to zero.

$$z_i = \frac{0.8}{0.7} * \sum_{a0i}^{5} z_i a + 0.1$$

The number of breeders and their distribution over the German territory as well as the local origin in contrast to exotic origins from other European countries were determined and added in a score system to integrate demographic and cultural aspects in our approach.

3.2.4 Relative diversity index (RDI)

Because of different sample sizes due to difficult access to very rare breeds, the calculated diversities cannot be compared directly, as larger samples are more likely to display a higher diversity. The expected diversity given a certain sample size, not considering the membership of a given animal to a certain breed, was estimated. For that purpose samples were generated by sampling N = 10, 20, 40, 60, to 80 or 100 animals at random from all breeds and lines mixed. A linear regression with intercept 0 was used to quantify the functional relationship between sample size and between individual diversity. A second regression was fitted for the diversities obtained within the different breeds with given sample sizes. In this case, the between-breed diversity was not taken into account. The difference of the regression coefficients of these two lines reflects the proportion of within-breed diversity relative to the overall diversity.

For each breed we measured the relative diversity index (RDI) as the deviation of its diversity from the expected value conditional on sample size N, $E(D|N)$

$$RDI = 100 \times D / E(D|N).$$

To answer the question if maximization of the diversity between breeds leads to less diversity within breeds by favouring inbred lineages, the conservation potential and the RDI were compared. The first represents an optimisation based on diversity between breeds, the other represents diversity within breeds. If they were negatively correlated, this would support the assumption that using conservation potential selects for inbred lineages.

3.4 Results

3.4.1 Approximation

The suggested approximation was applied on 724 single animals. Computing time was in the range of seconds. To compare the results to the exact algorithm the breeds which sample size did not exceed 25 animals were calculated with the exact Weitzman algorithm and compared to the values obtained by approximating with the mentioned procedure. The regression $r = 0.999$ and $p = 0.0001$, $sd = 0.039$ with $n = 11$, shown in table VII and figure 4.

Figure 4:   Approximated and exact diversity values for a subset of breeds.

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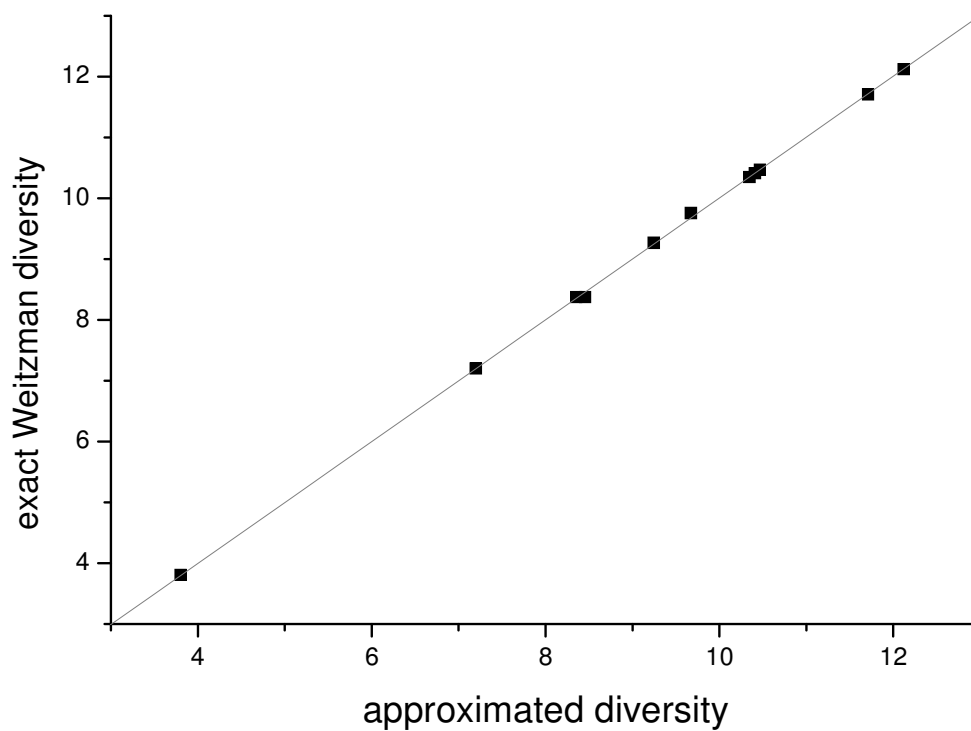
Figure 4:   Approximated and exact diversity values for a subset of breeds.

3.4.2 Extinction probability

According to the criteria listed in table VI a score was calculated for each breed. Calculated scores for all traits were summed for each breed Brabanter chicken having the highest and Vorwerk the lowest score (table VII).

Table VII: Estimated values for the extinction probability of breeds.

breed	total population size	change in population size	distribution	risk of crossing	special traits	Σ	scaled ext. prob.
Bergische Kräher	0.20	0.00	0.10	0.00	0.10	0.40	0,56
Bergische Schlotterkämme	0.30	0.00	0.10	0.10	0.10	0.60	0,78
Brabanter	0.30	0.10	0.10	0.10	0.10	0.70	0,90
Brakel	0.20	0.10	0.10	0.00	0.10	0.50	0,67
Deutsche Sperber	0.20	0.00	0.10	0.10	0.10	0.50	0,67
Friesenhühner	0.20	0.00	0.10	0.00	0.10	0.40	0,56
Hamburger sprangled	0.10	0.10	0.10	0.00	0.10	0.40	0,56
Hamburger pencilled	0.20	0.10	0.10	0.00	0.10	0.60	0,79
Italiener partridge	0.00	0.10	0.00	0.10	0.10	0.30	0,44
Italiener black	0.00	0.10	0.00	0.10	0.10	0.30	0,44
Kastillianer	0.20	0.10	0.05	0.10	0.10	0.55	0,73
Krüper	0.30	0.00	0.10	0.00	0.10	0.50	0,67
Lakenfelder	0.10	0.00	0.10	0.00	0.10	0.30	0,44
Ostfriesische Möwen	0.10	0.00	0.10	0.00	0.10	0.30	0,44
Paduaner	0.20	0.00	0.05	0.00	0.10	0.35	0,50
Ramelsloher	0.30	0.00	0.05	0.10	0.10	0.55	0,73
Rheinländer	0.00	0.10	0.05	0.10	0.10	0.35	0,50
Thüringer Barthühner	0.10	0.10	0.10	0.00	0.10	0.40	0,56
Vorwerkhühner	0.00	0.00	0.05	0.10	0.10	0.25	0,39
Westfälische Totleger	0.10	0.00	0.10	0.00	0.10	0.30	0,44

3.4.3 Relative diversity index (RDI)

For all breeds a relative diversity index could be determined, describing the diversity contained in each breed in relation to the diversity expected in a population of the respective size (Table VIII).

Table VIII: Relative Diversity Index calculated for all breeds.

breed	RDI
Bergische Kräher	108,92
Bergische Schlotterkämme	92,24
Brabanter	102,52
Brakel	99,34
Deutsche Sperber	107,17
Friesenhühner	91,44
Hamburger sprangled	108,20
Hamburger pencilled	115,05
Italiener partridge	101,76
Italiener black	106,35
Kastillianer	101,16
Krüper	92,43
Lakenfelder	104,80
Ostfriesische Möwen	101,60
Paduaner	105,39
Ramelsloher	102,25
Rheinländer	111,67
Thüringer Barthühner	104,87
Vorwerkhühner	82,53
Westfälische Totleger	108,75
Broiler dam line A	98,20
Broiler sire line A	95,98
Broiler sire line B	103,29
Broiler dam line D	100,93
Brown layer A	97,16
Brown layer C	89,78
Brown layer D	98,15
White layer A	95,99

The same data plotted relatively to the expected diversity value in the over all breeds population given the sample size of each breed. The ranking of the single breeds are adapted to fit into a maximum likelihood tree calculated in chapter 2.
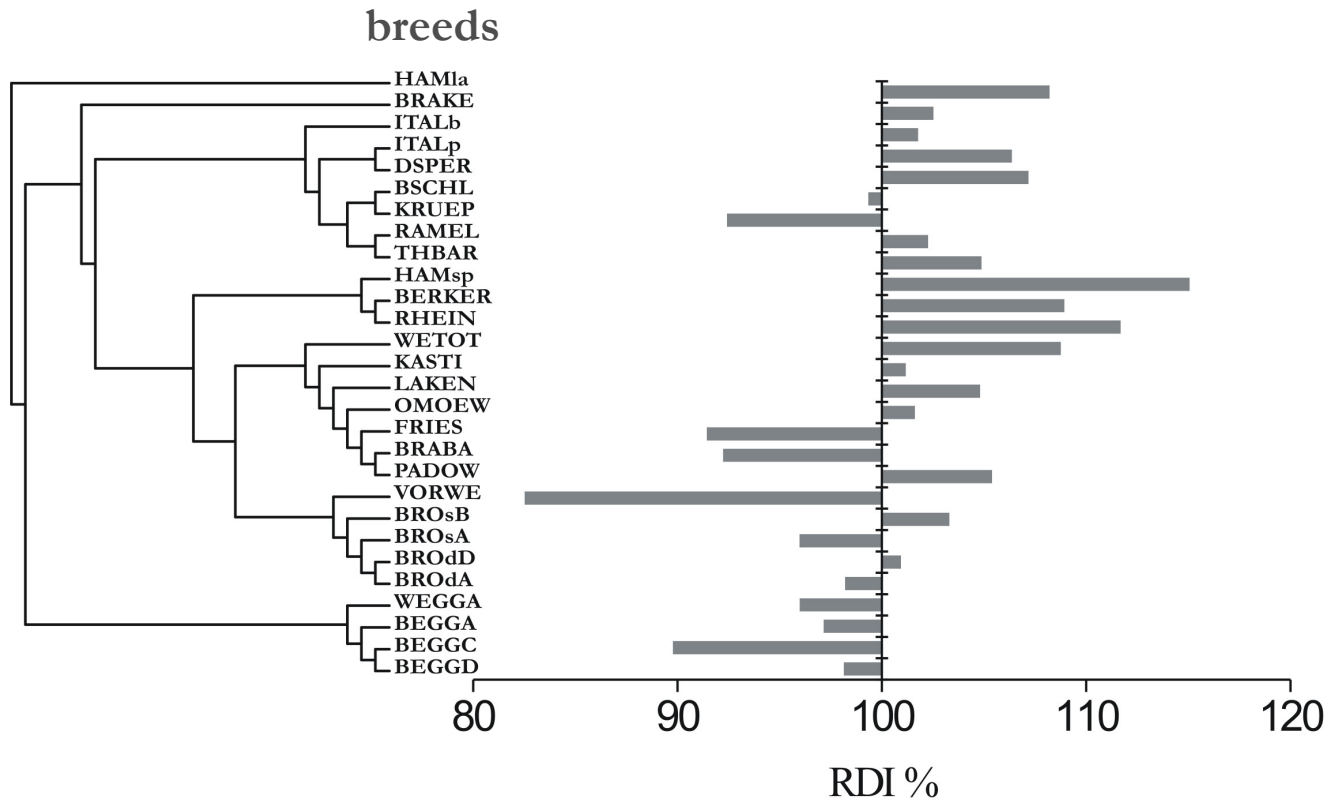
breeds

Figure 5:   Relative Diversity Index of the breeds. 100% represents the expected diversity for the sample size of each breed.

When applying diversity estimations on single animal data over several populations different sample sizes have to be taken into account.

In Figure 6, the linear regression of sample size on estimated diversity is displayed for the within-breed analysis (r^2=0.97) and for the samples across breeds (r^2=0.88), respectively. The slope of the latter exceeds the slope of the within-breed regression by 7.8 per cent, indicating that the within-breed diversity accounts for 92.7 per cent of the total variance, which is in line with similar findings in the literature (HALL 2004). This seems even more interesting since most conservation programs are based on between-breed diversity, maybe wasting the major part of possibilities.
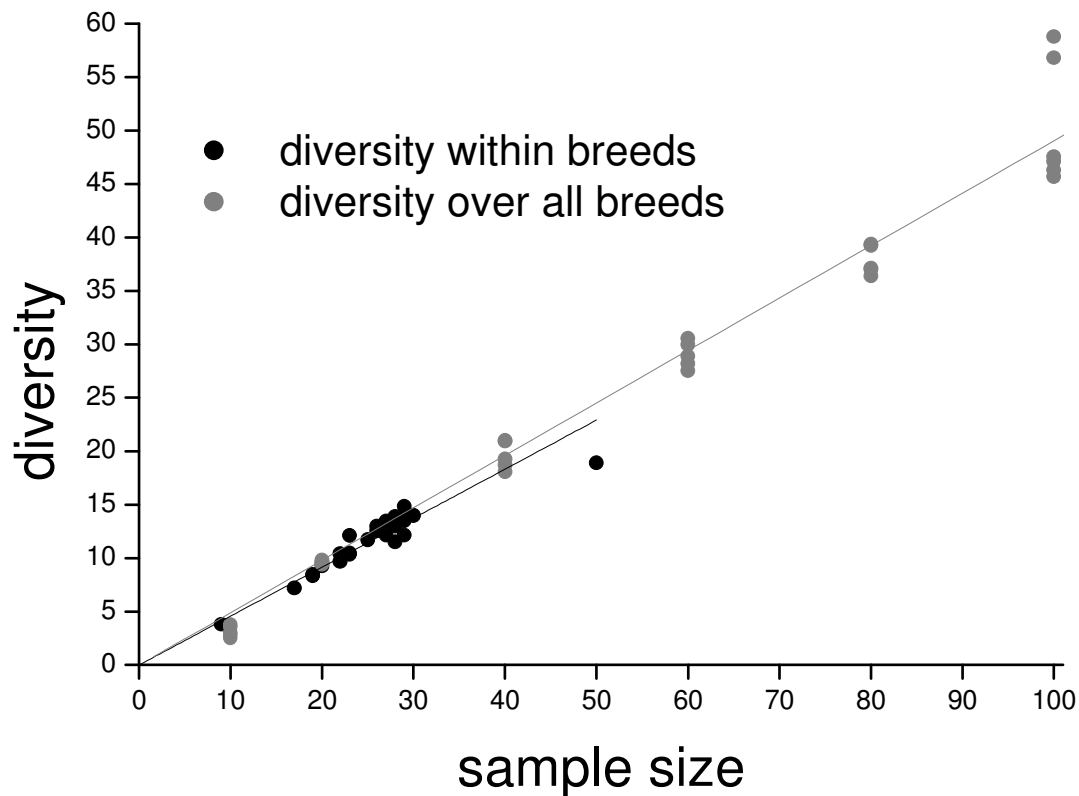
Figure 6: Regression of expected diversity for different sample size if including both, within and between breed diversity (grey line) and for samples out of each specific breed having a given sample size due to accessibility of individuals for the study (black line).

The utilization of conservation potential based on between-breed diversity for estimating conservation priorities and within-breed diversity represented by RDI was compared to conservation potential values calculated in chapter 2. This comparison of two approaches, one based on between breed diversity but taking the extinction probability into account, the other taking both, between and within breed diversity is not significantly divergent from zero, the tendency is even slightly positive (r^2=0,02, p= 0,5) as shown in figure 7 and not negatively as it might be expected if selecting for conservation potential would favour inbred lines and RDI taking within breed diversity into account.

Estimating the Weitzman approach on individual level the effect of sample size had to be taken into account.
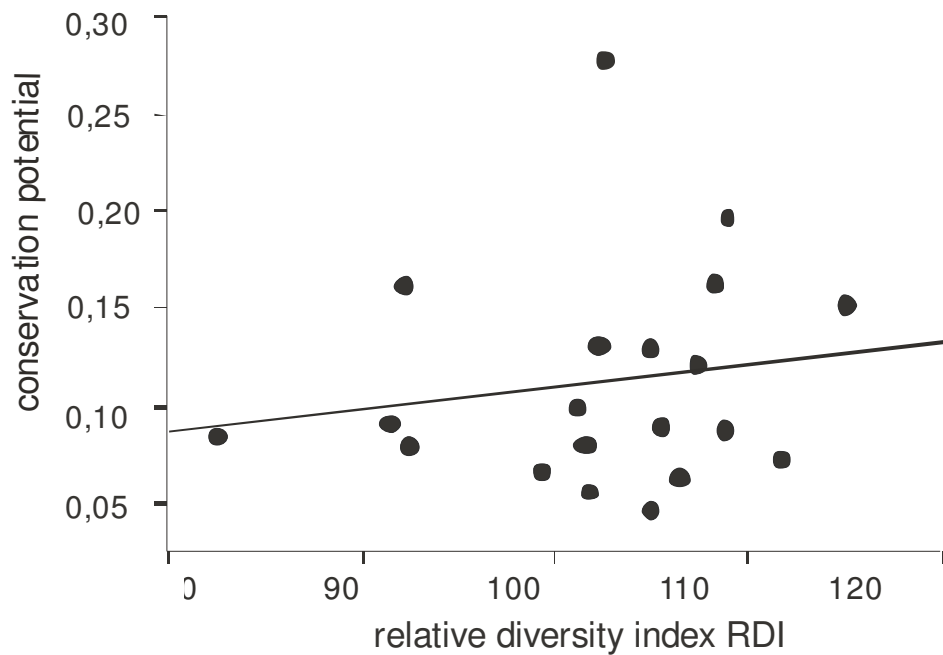
Figure 7: Relative diversity index RDI (representing the diversity within breeds) plotted against the conservation potential obtained using between breed diversity.

3.5 Discussion

3.5.1 Approximation

EDING'S *et al.* (2002) and CABALLERO and TORO'S (2002) claim that selecting breeds for conservation using phylogenetic criteria will reduce the total diversity conserved was an additional motivation to develop a method that is able to answer this kind of questions. To answer to what extend selection methods used up to now leads to reduction of total diversity because the within-breed diversity was not taken into account we compared the results generated by the described approximation with Weitzman's conservation potential. The fact that the results were not contrary to each other is because conservation potential is precisely not only maximizing diversity between breeds. It depends on the extinction probability and that avoids selecting for very small and thereby inbred lines. This result indicates that breeds with very small population size are not favoured by the Weitzman's approach. Not favouring small populations in optimizing the allocation of conservation funds revealed to have a side-effect of indirectly considerer within population diversity that correlates with

population size. Nevertheless the presented approximation enables to consider directly the diversity within breeds that is not only due to population size and will open new possibilities for maximizing genetic diversity.

3.5.2 Relative diversity index RDI

The RDI value contains between and within-breed diversity in equal parts. This simplifies diversity studies and enhances the results by including both sources for diversity.

For within breed diversity widely used measurements are allelic diversity and gene diversity (KREMER 1998; TORO and CABALLERO 2005), inbreeding coefficient (BALLOUX *et al.* 2002) or marker estimated kinship (FALCONER and MACKAY 1996) whereas for between breed diversity Wright's fixation index (WRIGHT 1969) or models with biological assumptions (NEI 1972) are used. To take both, within and between breed diversity into account it is necessary to combine two methods.

The number of alleles is considered the most relevant for within breed diversity (BARKER 2001; FOULLEY and OLLIVIER 2006; RÉMY J. PETIT 1998). Due to its sensitivity to bottlenecks it can be used to consider fluctuations in population size. One problem is its sensitivity to sample size and due to not being sensitive to allele frequencies the inflated figure of rare alleles.

The second widely used index measuring expected heterozygosity or probability that two variants taken at random in the population are different. It depends mostly on the frequency of the most frequent allele. This leads to the problem of rare alleles not contributing much to gene diversity(KREMER 1998).

Marker estimated Kinship (MEK) is also used for within breed diversity estimation even if pedigree data are missing like it would be the case for non-commercial chicken breeds. It is possible to estimate the kinship based on microsatellite markers (EDING and BENNEWITZ 2007; EDING and MEUWISSEN 2003). This approach gives a measure of similarities within and between breed diversity. If corrected for the fact, that identical alleles may not always be due to kinship (identical by decent) but also

through mutations (identical by state) the core set method is, beside Weitzman's approach, a suitable way of assessing conservation priorities(OLDENBROECK 2007).

Wrights Fixation index F_{ST} gives a over all comparison of population structure where certain alleles get fixed when subpopulations are isolated (EDING 1999). The main criticism is that populations should differ only slightly since F_{ST} never exceeds 1. When using microsatellites that are known for high mutation rates, divergent population may have low values due to not detected alleles (BALLOUX *et al.* 2002). A further disadvantage is that F_{ST} assesses only pairwise distances simultaneously.

Methods with biological assumptions used for between breed diversity accounts for drift and mutation. One classical model is Nei's (1972) standard genetic distance but the short divergence time of domestic animals makes it less reliable (EDING and BENNEWITZ 2007). No admixture is a major assumption that is mostly not true for domestic animals (FELSENSTEIN 1982).

Calculating within and between diversity in one step both are equally valued because for the algorithm there is no difference between comparing individuals out of one breed or of different breeds. Weighting for conservation of specific traditional breeds or special trades is not included. This can be taken into account later due to a scoring system mentioned above.

4th Chapter

Fancy breeds as a source of genetic diversity

4 Fancy breeds as a source of genetic diversity

4.1 Introduction

Breeding companies tend to affirm that by taking care of inbreeding issues and keeping several parental lines they established a broad diversity among commercial lines. Some comments on congresses suggested that there is even more diversity within commercial lines than in fancy breeds (Internationale Legehennentagung 2004). TADANO *et al.*(2007) shows that commercial lines have high polymorphism, heterozygosity and differentiation levels among each other. There poultry breeding industry is concerned about genetic loss. Some commercial and experimental lines are kept as insurance but cost cutting measures mean that lines are eliminated (CAREFOOT 1990).

Fancy breeders on the other hand refer to the great variety in phenotype that may reflect genetic as well as cultural variety and researchers claim for unknown value of local breeds to science (DELANY 2006). In this chapter we applied the approximation introduced in Chapter 3 to compare the total diversity within and between breeds for a pool of commercial lines and on the other side of fancy breeds.

In a second part, we built phylogenetic trees based only on the phenotypic data recorded for the 20 fancy breeds used in this study to estimate whether phenotypic records and genetic markers data lead to similar kinship results. Because of lacking production traits we used phenotypes concerning the beauty standardized for each breed.

4.2 Material and Methods

4.2.1 Probability of normal densities

To assess if commercial breeds harbour a major part of the genetic diversity, the following strategy was used. From the 28 fancy and commercial breeds, 8 breeds were chosen at random and from each of these 8 breeds, 10 animals were sampled. For this sample of 80 animals, the approximate Weitzman diversity was computed using the approach described in chapter 3, and the whole procedure was repeated 1000 times. Then, the same procedure was done, but instead of sampling 8 breeds at random, the sampling was restricted to the 8 commercial breeds only. The comparison of the two results reveals, how much diversity was lost if only the commercial strains were maintained.

4.2.2 Contribution

Using the same procedure as mentioned above, sampling always 10 animals out of the total pool of animals, each time excluding one breed, the contribution of this specific breed can be estimated.

4.2.3 Shape and colour phenotypes

Only characteristics and colours used in this study will be mentioned (Table IX). Body weight, egg number or egg weight was not included because of lacking performance recordings. All breeds in this list have white ear lobes and lay white eggs. Some breeds were divided in two populations by feather colour or pattern. All traits were weighted equally although breeders have different rigor in prosecuting failure in different traits. The last column in table IX indicates the status given by GEH (Gesellschaft zur Erhaltung alter und gefährdeter Haustierrassen) a German NGO taking care of endangered native breeds. This status can be extreme endangered (I), very endangered (II), endangered (III) or under observation (obs). The Criteria for this categorization differs between species and is defined by an expert committee each year.

Table IX: Phenotypic and cultural characteristics used in the study.

Breed	cultural value	plumage colour	comb shape	eye colour	leg colour	additional trait	status by GEH
Bergische Kräher	native	duck wing	single	orange	blue grey	long crower	II
Bergische Schlotterkämme	native	black	single	red brown	grey		I
Brabanter		black, gold, chamois	v-shape	orange	-	crest	
Brakel	native	silver pencilled	single	dark brown	grey with white nails		obs
Deutsche Sperber	native	barred	single	red	flesh		II
Friesenhühner	native	yellow pencilled	single, rose	red	blue grey		
Hamburger	native	silver sprangled	rose	red	grey		
Hamburger	native	pencilled	rose	red	grey		
Italiener		partridge	single	red	yellow		
Italiener		black	single	red	yellow		
Kastillianer		black	single	brown	grey		
Krüper	native	black	single	brown	black	short legs	I
Lakenfelder	native	lakenfelder	single	red	grey		III
Ostfriesische Möwen	native	silver pencilled	single	brown	grey		II
Paduaner		chamois, black, silver, gold, white	v-shape	red brown	blue grey	crest	
Ramelsloher	native	white	single	dark brown	blue		I
Rheinländer		black	rose	dark brown	grey, black		
Thüringer Barthühner	native	black, gold, chamois, silver	single	brown	blue, grey	beard	obs
Vorwerkhühner	native	vorwerk	single	orange, yellow	grey		obs
Westfälische Totleger	native	silver pencilled	rose	dark brown	blue grey		obs

As described in the book Poultry Breeding and Genetics by R.D. Crawford each mentioned trait will be explained shortly as follows:

White ear lobes are a phylogenetic trait based on pigmentation due to purine accumulation. This purine causes an opaque white pigmentation sometimes with greenish or yellowish glimmer. It overlays the red colour of the skin caused by strong blood circulation of the head attachments. Various genes are related with this polygenic trait and not sufficiently known yet.

For Brown Layers there are currently 13 genes discussed for the different variations of egg shell surface colour caused by oopryphrin. A greenish colouration caused by oocyanin within the shell is the other factor. Combinations of both deposits lead to a large number of brown, green, blue and red coloured chicken eggs.

A dark coloration of the skin is caused by pigmentation of the connective tissue. Again several genes are involved: inhibitors of dermal melanin, pigment cell activators and gender specific melanin inhibitors, which also affect plumage colour.

Different comb shapes are caused by two genes on two different chromosomes determining rose comb or pea comb. If none of these genes is active single comb (wild), if both are active a walnut comb will be developed.

Leg colours are a combination of dermis and epidermis colouring and other modifying genes such as gender specific cuckoo genes. The colours of the upper and lower layers of the skin are determined mainly by major genes such as W +, id + and e + and than pronounced or diluted by modifying genes.

Eye colour varies too and is determined for most breed standards. It is the result of pigmentation of different structures like iris, retina, uvea and ciliary body. The underlying genetic has not yet been finally resolved. Reddish deviations from the wild-brown are caused by carotenoid pigments or increased blood circulation. Darker brown coloration is caused by eumelanin deposits in the entire eye. About the so called pearl eye little is known. (SMYTH 1990) assumed the same eumelanin display like in brown eyes but without carotinoides. Additionally the eye colour is affected by a variety colour improving and colour inhibiting modifying genes.

Plumage colour can be split in the base colour with their modifications, and the primary and secondary pattern. 79 standardized plumage colours were listed by JEFFREY (1977) in the USA and many of these could vary in leg colour or in shade of pigmentation. The basic colours are black and red (gold). They can be increased, diluted or masked. White can be achieved in different ways through inhibition of black

and red by different gene combinations. In addition to the pigment colours perceived plumage colours can appear by reflections on specific surface structures of the feather without any pigmentation. Purple and emerald coloured animals owe it to the reflections on their feathers. Primary plumage patterns refer to the whole body, such as the Columbia, Lakenfelder and Vorwerk colour where black colouration is restricted to the neck, tail and point of shoulder. The secondary pattern refers to the individual feathers for example the simple and double laced speckles and other (SMYTH 1990).

Short legs like in Krueper display their heterozygosity for the lethal creeper (cp) gene when homozygous. Interactions with other genes may shift the degree of leg shortness and mortability of chicks (LANDAUER 1944; SOMES 1990a).

Crests and beards are autosomal incomplete dominant with variable peculiarity (SOMES 1990b).

The graphical illustration of phenotypic similarities was done based on different maximum parsimony methods using the PHYLIP package (FELSENSTEIN 1980). Maximum parsimony is a widely-used character-based tree estimation method for morphological data. It operates by evaluating candidate phylogenetic trees according to an explicit optimality criterion. The PHYLIP package contains different programs based on maximum parsimony that differ in assumptions made about the evolutionary model, knowledge about the data and optimality criterion. We used four different models to compare phenotype based results to the genetic based done in antecedent chapters.

PARS is a general parsimony program which carries out the Wagner parsimony method with multiple states. Wagner parsimony allows changes among all states. The criterion is to find the tree which requires the minimum number of changes. The Wagner method was originally developed by (ECK and DAYHOFF 1966) and by (KLUGE and FARRIS 1969). Following assumptions are made: Ancestral states are unknown; different characters evolve independently; different lineages evolve independently; changes to all other states are equally probable; these changes are a priori improbable over the evolutionary time spans involved in the differentiation of the group in question; other kinds of evolutionary event such as retention of polymorphism are far less probable than these state changes and rates of evolution

in different lineages are sufficiently low that two changes in a long segment of the tree are far less probable than one change in a short segment.

MIX is a general parsimony program which carries out the Wagner and Camin-Sokal parsimony methods in mixture, where each character can have its method specified separately. We used Camin-Sokal (CAMIN and SOKAL 1965) parsimony where it is known which state is the ancestral one and, for that assumes that changes $0 \rightarrow 1$ are allowed but not changes $1 \rightarrow 0$. The criterion is to find the tree which requires the minimum number of changes. Following assumptions are made: Ancestral states are known; different characters evolve independently; different lineages evolve independently; changes $0 \rightarrow 1$ are much more probable than changes $1 \rightarrow 0$; both of these kinds of changes are a priori improbable over the evolutionary time spans involved in the differentiation of the group in question; other kinds of evolutionary event such as retention of polymorphism are far less probable than $0 \rightarrow 1$ changes and rates of evolution in different lineages are sufficiently low that two changes in a long segment of the tree are far less probable than one change in a short segment.

PENNY is a program that will find all of the most parsimonious trees implied by data. It does so not by examining all possible trees, but by using the more sophisticated algorithm. There is, however, a price to be paid for the certainty that one has found all members of the set of most parsimonious trees: this program, despite its algorithmic sophistication, is very slow. The search strategy used by Penny is a modified backtracking depth first search algorithm, a branch and bound algorithm, a standard computer science search strategy first applied to phylogenetic inference by (PENNY *et al.* 1982). The counting of the length of trees is done by an algorithm nearly identical to the corresponding algorithms in Mix, again we used Camin-Sokal.

DOLLPENNY, like penny, is a branch and bound algorithm but the counting of length of trees is done by a Dollo and polymorphism parsimony method. The Dollo parsimony method was first suggested by (LE QUESNE 1974) and was first well-specified by (FARRIS 1977). The method is named after Louis Dollo since he was one of the first to assert that in evolution it is harder to gain a complex feature than to lose it. The algorithm explains the presence of the state 1 by allowing up to one forward change $0 \rightarrow 1$ and as many reversions $1 \rightarrow 0$ as are necessary to explain the pattern of states seen. The program attempts to minimize the number of $1 \rightarrow 0$ reversions necessary. Following assumptions are made: We know which state is the ancestral one (state 0); the characters evolve independently; different lineages evolve

independently; the probability of a forward change (0→1) is small over the evolutionary times involved; the probability of a reversion (1→0) is also small, but still far larger than the probability of a forward change, so that many reversions are easier to envisage than even one extra forward change; retention of polymorphism for both states (0 and 1) is highly improbable; the lengths of the segments of the true tree are not so unequal that two changes in a long segment are as probable as one in a short segment.

4.3 Results

4.3.1 Probability of normal densities

Figure 9 shows the ordered values of diversity obtained from 1000 replicates from 10 animals sampled from either 8 randomly sampled breeds including commercial lines or from only out of 8 commercial lines. The resulting curves represent two probability functions of the corresponding normal densities. Both distributions differ by 7.05 per cent (41.22 vs. 44.13 for the midst value). Having in mind the above mentioned result that between-breed component accounts for only 7.8 per cent of the total diversity, this result shows that diversity within commercial breeds is of similar size as the diversity within a single randomly chosen breed.
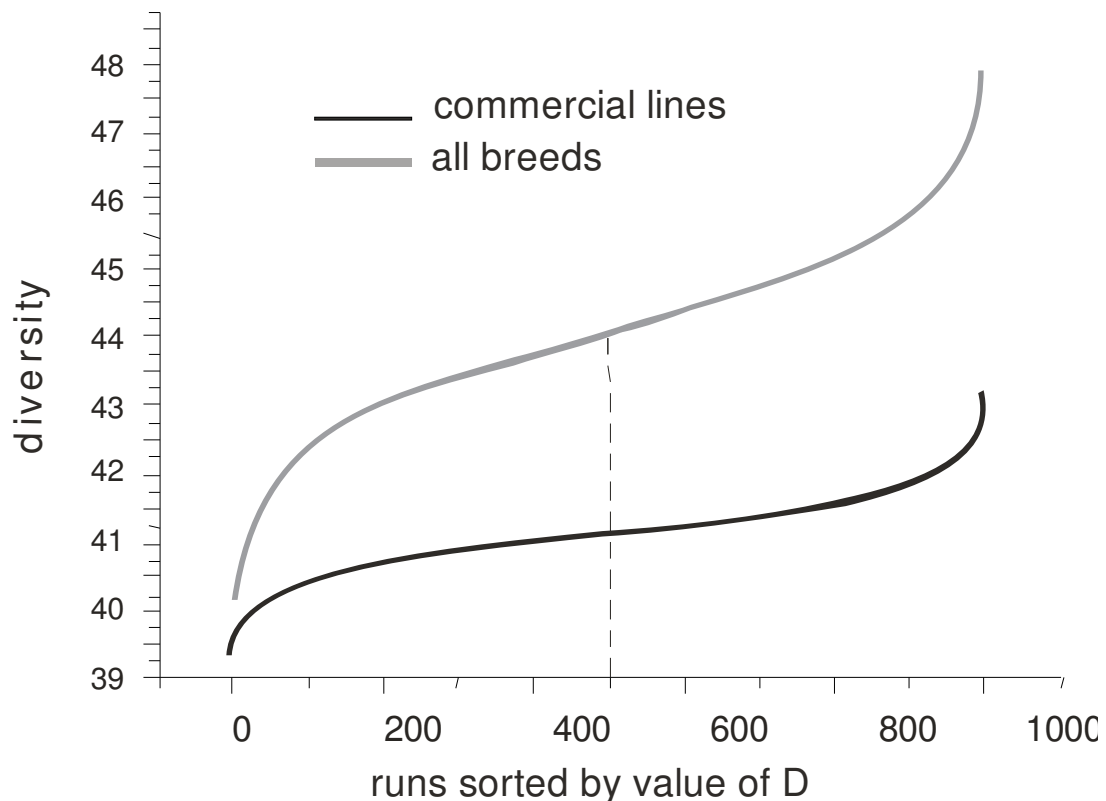
Figure 8:   Sorted results of diversity estimations based on 1000 replicates from 10 animals, respectively, sampled from either 8 randomly sampled breeds or out of 8 commercial lines.

4.3.2 Contribution

The effect of single breeds to the total diversity measured when only a restricted number of breeds are allowed to be maintained is shown in figure 8. If some breeds were included to be possibly chosen for conservation by displacement they lower the chance for other breeds to be chosen. In the shown case where 10 animals from 8 breeds were allowed to be chosen out of the 28 breeds described in chapter 2 Paduaner and Brown layer D line have the lowest value. Their diversity values on the left site of the vertical indicates that including this breeds to be considered for conservation activities would cost other breeds the possibility that would add more additional diversity to the total pool. Thüringer Barthühner and Brakel would increase average diversity if 8 breeds with 10 representatives each would be chosen out of our 28 possibilities. In figure 9 the breeds are arranged in order to fit the maximum likelihood tree calculated in chapter 2 to give a over all context.
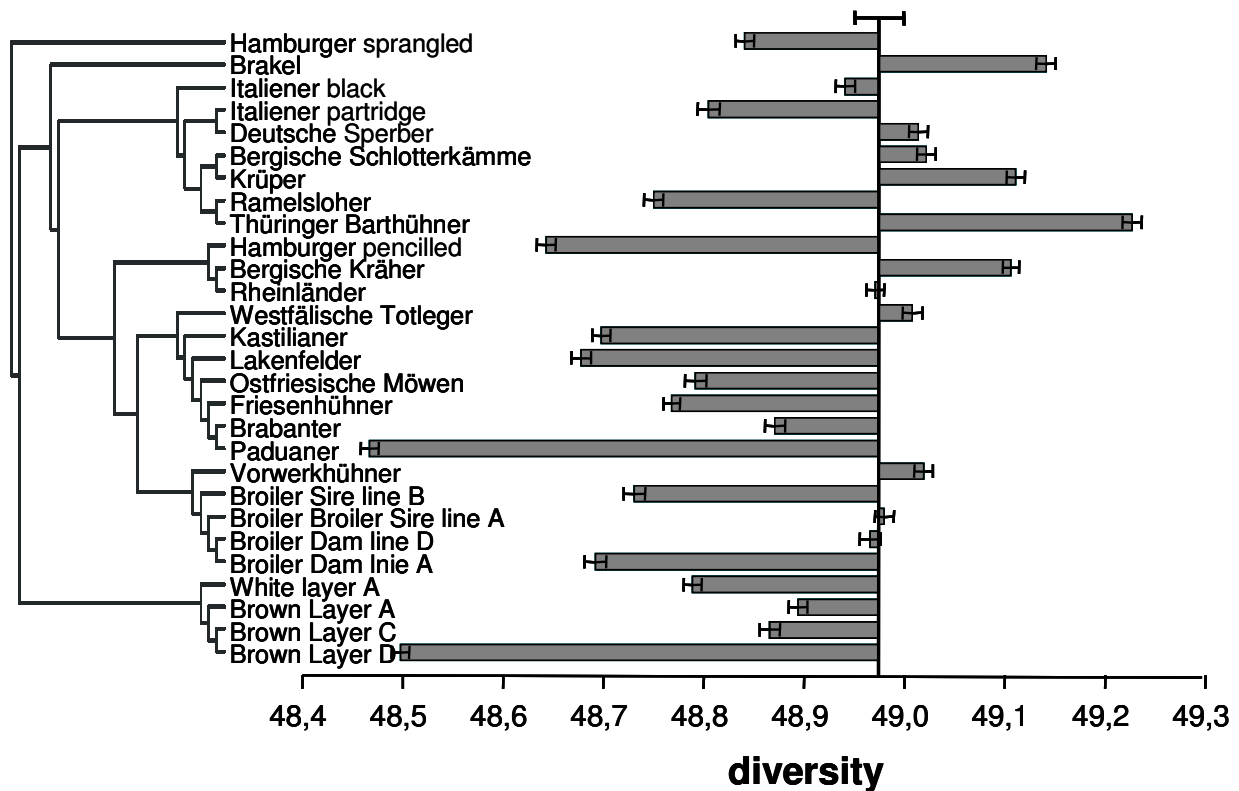
Figure 9: Contribution of breeds to total diversity. Zero line representing diversity obtained when all breeds are mixed and equal probably chosen by random.

4.3.3 Shape and colour phenotypes

Except of all Algorithms have clustered both Italiener (Leghorn Standard Type) colour variant and grouped both Hamburger (pencilled and sprangled) and the Rheinländer chicken in one group no further similarities could be found when using different maximum parsimony methods to the phenotypic data recorded in this study.
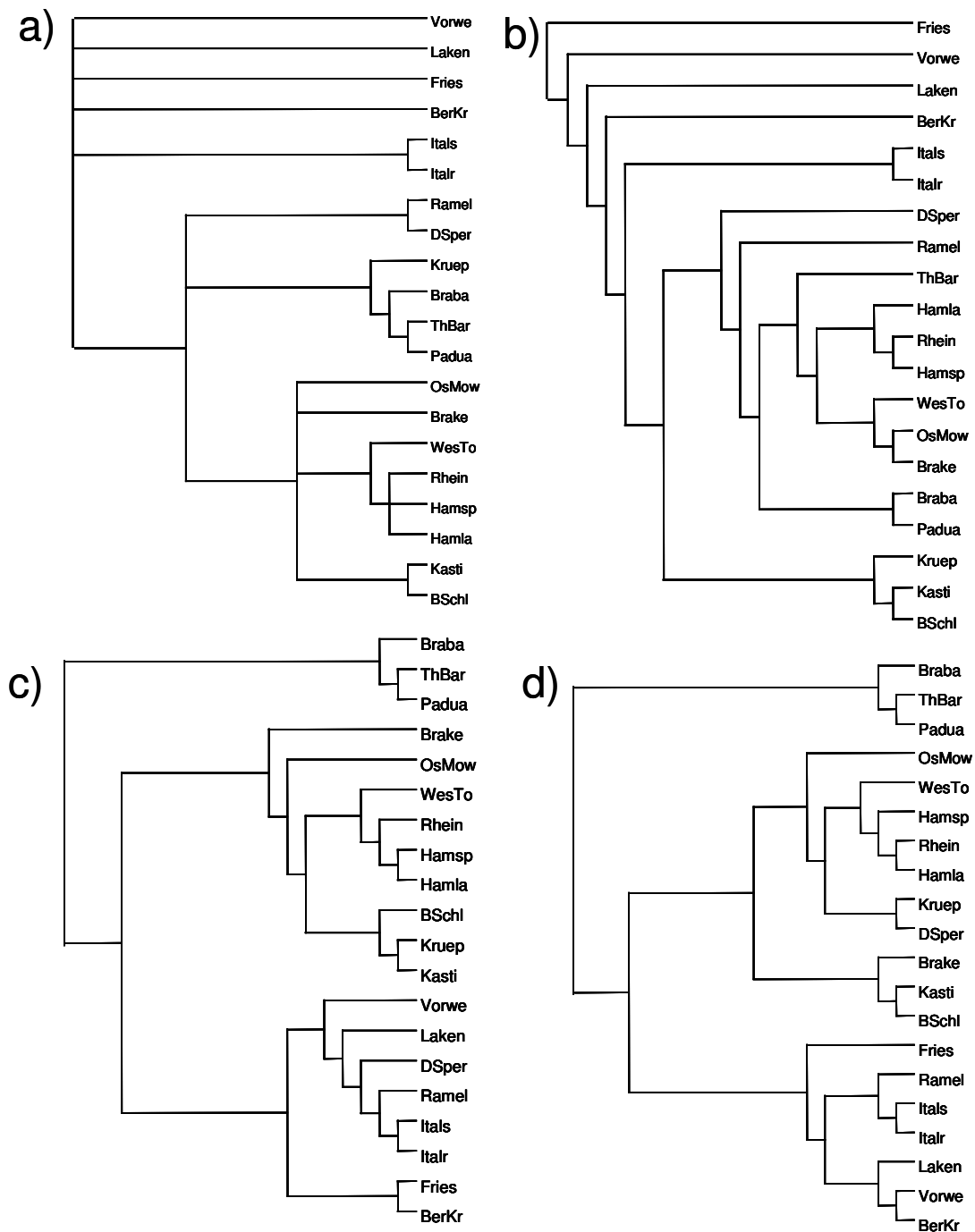
Figure 10: Phylogenetic tree based on phenotypic characteristics using a) PARS general parsimony b) DOLPENNY branch and bound program with Dollo algorithm. c) MIX general parsimony program with Camin-Sokal algorithm. d) PENNY branch and bound program with Camin-Sokal algorithm.

The PARS Algorithm clustered, similar to DOLPENNY Vorwerk, Lakenfelder, Friesenhühner Bergische Kräher and both Italiener (Leghorn Standard type) as most distant to all others (figure 9).

Despite some similarities to PARS mentioned above, DOLLPENNY not grouping Thüringer Barthühner to Paduaner and Brabanter even though they are all crested breeds and that for known to be related (figure 9 a and b). DOLLPENNY and MIX were the only one to show the relatedness of Krüper and Bergische Schlotterkämme They are more or less the same breed but Krüper having a heterozygous short leg gene. By breed standard leg colours of this breeds are defined differently (Bergische Schlotterkämme: black and Krüper: grey). This was apparently not weighted too much by those algorithms. The similarity of grey and black compared to fleshy was not defined in the data used but would certainly improve the results. For that purpose a clear definition and if possible physiological background would have to be defined. The definition in the breed standard varies between the single breeds and plumage colours.

The MIX algorithm using Carmin-Sokal has similarities to PENNY using Carmin-Sokal, too. Both group Brabanter, Thüringer Barthühner and Paduaner not only together but as most distant to all other breeds (figure 9 c and d).

Figure 9 d shows a three build with PENNY algorithm using Camin-Sokal. Despite some similarities with MIX the group Friesenhühner, Ramelsloher, both Italiener (Leghorn Standard type), Lakenfelder. Vorwerk and Bergischer Kräher do not contain Deutscher Sperber like in MIX (figure 9 c and d).

4.4 Discussion

4.4.1 Probability of normal densities

Genetic diversity contained in commercial lines is smaller than in the same number of equal sized fancy breed populations. In our example it was possible to choose eight breeds from each group, commercial line ore fancy breed. The number of fancy breeds being kept in Germany is very large and it can be assumed that they contain a major part of the diversity among all chickens. Therefore commercial lines alone cannot be considered as a sufficient repository of neutral genetic diversity. This is an

additional incentive for preservation of the numerous breeds existing in small populations kept by fanciers but containing a unique gene reservoir.

If conservation capacities are restricted however, considering too many breeds foe conservation would not achieve the best results. Breeds having a lower contribution would displace other breeds with higher contribution.

4.4.2 Phenotypes

Most noticeable are the very different relations between populations by using slightly different algorithms. This may have various reasons. Firstly there are few traits taken into account. Only six phenotype traits were used in contrast to 29 microsatellite markers and the chosen breeds are similar in various phenotypic characteristics. This leads to no clear grouping among close related breeds, all central European white layer type with dark or fleshy shanks. Secondly all models suppose a one directional evolution of the analyzed populations. This is not always true for species and it is never true for breeds, like in our case. Breeds are not mainly similar because they have common ancestors but because they are crossed with each other. Thirdly many variations in traits occur in various breeds even if this special variation was not included in this study. For example Italiener (Leghorn Standard Type) was sampled in two populations both having single comb, one population with black plumage and the other with partridge plumage. However, Italiener exists with rose comb, too and in 22 plumage colour variations having for each of these a bantam type. This reflects that eye-catching phenotypes are not necessarily a distinguishing mark for different breeds. It is more a combination of different phenotypic characters such as shape, colour, weight, type, performance and behaviour plus a historic background. This is hard to put into calculable units and would require a study focused on that issue. For our purpose of calculating similarities of German or European breeds for conservation priorities it is not suitable if used alone. Nevertheless these traits can be included into the Weitzman model described in the chapters 3 as an addition to genetic data.

Of course, since not every possible phenotypic characteristic could be measured and encoded for analysis, the selection of features to measure is a major inherent obstacle to this method of using only phenotypes. The decision of which traits to use

as a basis for the matrix necessarily represents a hypothesis about which traits are important.

5th Chapter

GENERAL DISCUSSION

5 GENERAL DISCUSSION

The overall goal of this study was to enhance tools for calculating conservation priorities in farm animals for instance in German chicken breeds. This is especially necessary for poultry because no governmental conservation and promotion program exists. In poultry, especially in chicken the confusing large number of breeds and varieties makes decisions about conservation priorities, uniqueness and similarities difficult. This study developed a way of calculating neutral genetic values within and between a large number of populations and individuals and combined it with phenotypic particularities and cultural importance to make decisions about conservation priorities.

When using microsatellites as it is described in chapter 1, the main problem that remains even when using them for breeds is the question about neutrality in respect to selected traits. Using FAO recommended markers we used established and preferably uncomplicated markers developed by specialised working groups but always keeping in mind possible linkages of markers to traits that are directly or indirectly selected by breeders or environment. Breeds that experience a similar environment or utilization may be assigned to be more similar due to microsatellite analysis as they would be if the whole genome was known. This could be corrected by including knowledge of factors that might influence selection and include more than one breed of each selection type.

As shown in Chapter 2, external populations influence priority rating for the breeds considered for conservation activities. This influence leads to the necessity of coordinating national and international conservation projects. If conservation activities are concentrated and optimized on local scale, ignoring the fact that there may be closely related breeds in other regions, allocation of funds and conservation efficiency are expected to be sub-optimal (SIMIANER 2005). FAO's Domestic Animal Diversity Information Service (www.fao.org/dadis/) newly published the additional information to the listed breeds if they are local or transboundary. This facilitates the access to worldwide information about external populations for conservation schemes. Problems in considering populations for governmental supported programmes beyond narrow regions of responsibility begin even inside Germany.

Each federal state accounts for its territory to formulate animal breeding laws at least for mammals (TierZG §9). Cooperation between federal states and countries is especially supported by FAO and EU within member countries. One organization that suggests risk states on international level is the American Lifestock Breeds Consevancy (ALBC). SIMIANER (2005a) concluded in his review that since the major species are globally distributed, conservation of farm animal diversity is a global task. Since in contrary to this, conservation decisions are mostly made on the national level he claims that creating an internationally co-ordinated conservation policy is of crucial importance. The results shown in Chapter 2 underline this aspect.

Differences to other conservation programmes compared to Weitzman's approach are its iterative character designating the contribution to total diversity for each breed in dependence of all other breeds and calculating its conservation potential not only based on population size but on the gain of saved diversity per amount spent on that specific breed, including extinction probabilities, special traits and cultural values. The result may differ from intuitively tendency of always putting the biggest effort in the rarest breed. Political preferences for certain breeds can be included by setting a high cultural value for preferred breeds. Cooperation with other country's conservation programmes can easily be included as shown in chapter 2.

The main objective of Chapter 3 was to develop a possibility to calculate Weitzman's approach for bigger sample sizes and this allowed for inner breed diversity to be taken into account. This was done by an approximation that estimates Weitzman diversity very accurately and was used to calculate diversity within and between breeds in one step and determine a new measurement that we called Relative Diversity Index (RDI). In contrast to the core set proposed by EDING *et al* (2002) that was developed to consider between and within breed diversity based on kinship, no estimator is needed in Weitzman's approach. Weitzman's mathematically well-founded approach (WEITZMAN 1992; 1993; 1998) used in several studies (CAÑÓN *et al.* 2001; GARCIA *et al.* 2005; LAVAL *et al.* 2000; REIST-MARTI *et al.* 2005; 2005a; SIMIANER 2005b; 2003; THAON D'ARNOLDI *et al.* 1998) can be utilized for more extensive sample sizes and for consideration of within population diversity.

Chapter 4 presents a further possibility of application for the above mentioned approximation by calculating the probability of normal densities of diversity contained in small subsets of the whole chicken population or fancy breeds only. This simulation of small conservation units compared with the whole existing diversity underlines the importance of fancy breeds. Even though only a small number of 20 breeds out of about 200 breeds existing in Germany and these all being of a similar, white egg laying central European type they contained about the same diversity that was found in eight commercial lines representing broiler, white layer and brown layer types.

This great variance in fancy breeds that are selected mainly by appearance leaded us to have a look at how they would be arranged within a phylogenetic tree if considering only very obvious phenotypic traits. Poor precise information, similarities between the studied breeds, overlapping of traits and no increased emphasis in this issue led us to conclude that using only phenotypic traits described in the breeding standard is not applicable for our need of planning conservation schemes for genetic diversity. Even though plumage colour has been used for identification of chicken populations (YAMI 1995) because its easy assess, phenotypic variation is influenced by the environment (CROOIJMANS *et al.* 1996) and will not reflect true genetic diversity (EDING 1999). For standardized breeds however, especially if displaying complex patterns, it may be an indicator for being isolated populations kept by concerned breeders.

In Europe fanciers keep a great number of chicken breeds out of passion spending time, money and locality for keeping different breeds without economic interests. Not only in Europe but in nearly every country there are people keeping and breeding poultry for fun, game, ornament, tradition or other cultural reasons. This fact and the result of chapter 4 regarding the amount of genetic diversity contained in non-commercial breeds leads inevitably to a conservation concept using these resources to promote in situ programmes encouraging smallholders and fanciers. In-situ conservation copes with conservation of cultural aspects of farm animals and also maintains a breed dynamic to adapt to the environment and develop its characteristics. Breeds that live in a real word remain known at least by local people, it is adapted to different conditions and experience continuous breeding progress (HALL and BRADLEY 1995; HENSON 1992). A combination between in situ and

cryoconservation seem to be a desirable way for effective conservation of genetic backups of livestock (HALL 2004).

The scientific approach suggested in this thesis can be used as a tool to create and monitor conservation priorities what breeds should be supported and spend given funds on, the most efficient way in means of maximizing diversity. Funds can be spend in different ways of support such as developing local or niche markets, establishing herdbooks, promoting "forgotten" breeds and explore specialities of breeds by quantifying different traits in performance tests can be taken into account. SIMIANER (2005a) listed six major conservation activities that can be implemented in different ways:

- a premium paid to balance loss of income compared to keepers of more competitive breeds
- training courses to improve genetic management
- support of marketing activities
- a kryoconserve of embryos and semen
- organisation of exchange of male animals between herds
- invest in AIDS research because of the destruction of social structures by this disease which may put high risk on the breeds kept in those countries

In addition non-negligible costs for design and co-ordination of conservation projects should be taken into account (SIMIANER 2005a).

The questions that remain in breed conservation are more a matter of principle. Do we want to conserve mainly neutral diversity and are microsatellites really neutral? To what extend do we want to include known phenotypic traits and cultural values? How important are aesthetics and the recreational benefits? How to appoint the financial benefits in case of future use of conserved gene pools? How much money do we want to invest overall species and breeds? These questions, especially the latter cannot be derived from a scientific point of view, since this is rather a political decision. The difficulties in assigning cost and benefits of conservation as well as the imbalance of local and present conservation but global and future gain affect the outcome of such general decisions (SIMIANER 2005a).

Germany lacks a conservation and diversity promoting programme for chicken. In contrast to other species such as cattle, pigs, sheep, goats and horses, poultry is not included in the German animal breeding law (TierZG) that regulates governmental conservation efforts for each country. This apparent disadvantage enables us to develop a transboundary concept at least for all German countries.

The breed samples utilized in this study are an example set to model a usable method. It contains 20 breeds all kept and sampled in Germany but not all being original German breeds in the sense that the first herd book was established in Germany nor are all German breeds included in the set of breeds as it is described in chapter 1. The Italiener chicken (also called Leghorn standard type), was sampled in two plumage colour variants and they were treated as two populations whereas other breeds were treated as one population even though different colours were included. This was done to reveal possible effects of plumage colour selection within breeds. This is arguable for our purpose of testing the method but of course leads to no statement about conservation priorities for German chicken breeds. Further on all utilized breeds were of a south European white egg laying type whereas the commercial lines were one white layer, tree brown layers and four broilers. This leads to distorted conclusions when looking at relationships and influences between local breads and commercial lines and even when looking at the amount of diversity in each of these groups when comparing them. Again, for showing that there is an influence and to some extend in which direction this influence acts, these samples were good enough but not for any kind of quantitative statement.

Implications and recommendations

First sampling of all German and other relevant breeds is necessary. Then political decisions on what the goals of conservation are. Third fields have to be weighted: future use, present use in niche markets and preservation of national treasures. Thus economy, agriculture and cultural tradition are the mainly divisions to involve.

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APPENDIX

Involved organizations

BLE

On behalf of the German ministry for food, agriculture and consumer protection (BMELV) the federal institute for agriculture and food (BLE) oversees scientific projects. Among other they have two subject areas where biological diversity is the main topic:

- Inventories and surveys in the area of biological diversity.
- Pilot and demonstration projects in the field of innovative conservation and sustainable use of biological diversity. The BLE was giving financial support to this study.

Friedrich-Loeffler Institut (former FAL)

In close work with this facility of the German ministry for food, agriculture and consumer protection all lab work was done in the Institute for Animal Health in Mariensee.

BDRG

The German fancy poultry breeders club (BDRG) cares for the standard of local and exotic poultry breeds. About 300.000 people are joining. The BDRG supported blood sampling activities on poultry shows.

ILRI

The International Livestock Research Institute (ILRI) works at the crossroads of livestock and poverty, bringing high-quality science and capacity-building to bear on poverty reduction and sustainable development. The ILRI provided parts of the software used in this study.

Breeds and traits used in this study

Bergische Kräher

Cultural value: A legend about this German breed says that the Earl Eberhard von Berg (died 1152) was retrieved from a lost ballet by the crow of a cock. He bought the cock from a charburner and a convent owned by the earl continued breeding this chickens. They crow five times longer than other breeds and in contrast to others they keep on walking while crowing and lower the head slowly until reaching the ground. Because of a later enthusiasm of people for betting on crow competition, laws were implemented to regularize these activities. Like other long crower breeds of Japan and China they have an arched back.

Colour: The typical colour is rare in other breeds but the only they display in.

Comb: single

Legs: grey-blue

Eyes: orange or light red

It is listed as strongly endangered German breed by the GEH.

Bergische Schlotterkämme Black

Cultural value: In the same area as the Bergischer Kräher for centuries people kept this other German breed. Due to geographical nearness and the fact that this breed

can display in the same rare colour pattern as the Bergische Kräher there surely occurred crossbreeding. Krüper and Castellana were also crossed in.

Colour: In this study only the black coloured were sampled

Comb: single

Legs: In this case dark grey

Eyes: brown red

It is listed as extremely endangered German breed by the GEH.

Brabanter Black, Gold, Chamois

Cultural value: A Dutch breed documented first in a painting of Melchior d'Hondecoeter (1636 – 1694). In France and Germany they crossed La Flèche to obtain a "proud" body posture. *Colour:* In this study only the black, gold and chamois where sampled

Comb: two v-shaped horns

Legs: blue grey

Eyes: orange or red

Additional trait: sharp crest and beard

Brakel Silver

Cultural value: Brakel is a Dutch rooted breed that was modified by Belgians and than spread in Germany since 1895. It is assigned as German breed.

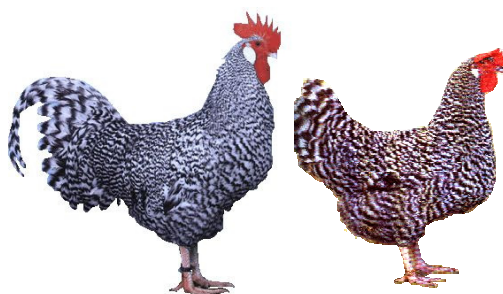*Colour:* It has a feather pattern similar to pencilled, like Totleger, but it should be stripier than those.

Comb: single

Eyes: dark brown surrounded by black eye lid

Legs: bluish with white toe tips.

It is listed as to be observed in the German red list for breed by the GEH.

Deutsche Sperber

Cultural value: This German breed is a result of crossing Italiener (Leghorn standard type) with Plymouth Rocks and later Minorca and Schlotterkaemme since 1900. It is known to be a calm breed that does not tend to overtake fences.
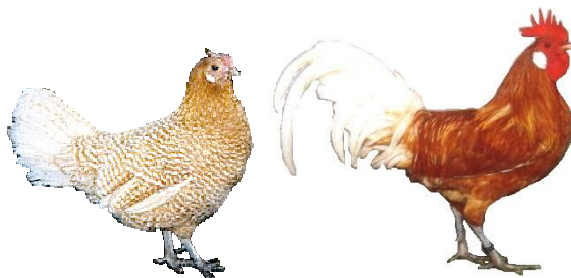
Colour: cuckoo

Comb: single

Eyes: red brown

Legs: fleshy

It is listed as strongly endangered German breed by the GEH.

Friesenhühner Yellow Pencilled

Cultural value: A primordial Dutch breed. They are supposed to immigrate with the Frisian tribe into the Netherlands. It is a delicate and agile animal. They are known to overtake high fences.

Colour: Their feather pattern is similar to pencilled and was described in 1880 as the same of and rough-textured tissue. In German it is called "geflockt" in contrast to "sprenkel" and "gebaendert". Though in English all are called pencilled the pattern of single feathers and its distribution over the body is different. In this study only the yellow pencilled original colouration was sampled.

Comb: single

Eyes: Big dark orange red eyes

Legs: grey blue

Hamburger Silver Sprangled

Cultural value: This old German breed was painted by the English artist Albin in 1740. It was subtitled "Hamburghs" indicating its origins. They originate from old German pencilled breeds, Paduans and English Mooney (Redcap) breed. Later, back in Germany it was crossbred with Sumatra, Moewen, Westfaelische Totleger, Spanish and Minorca for body shape, feather density, sheen and colouration. It is a gracile but not to small animal.

Colour: only sprangled was sampled for one population in this study

Comb: rose comb that narrows to a spur as a typical characteristic of Hamburger chickens. *Eyes:*dark red brown

Legs: grey blue or black

Hamburger Pencilled

Colour: only pencilled was sampled for one population in this study

Italiener (Leghorn standard type) Partridge and black

Cultural value: Since 1870 the Romans from Lombardy spread their chickens from Italy and England to the whole continent. There are some assumptions that these chickens arrived Germanic areas when colonised by the Romans. This partridge coloured animals were known as brown Leghorn. It is a genuine layer.

Colour: only partridge and black were sampled for one population in this study

Comb: single

Eyes: orange red

Legs: yellow

Castelliana

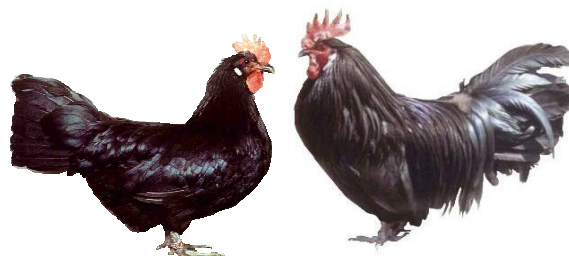*Cultural value:* A Spanish breed that is supposed to be described in the year 800 in Spain.

Colour: black shining like a beetle

Comb: single

Eyes: brown

Legs: grey

Krüper Black

Colour: In this study only the black coloured were sampled

Comb: single

Eyes: brown

Legs: grey

Additional trait The main characteristic of this German breed is its short legs. It is due to a heterozygote occurrence of a lethal gene cp that makes it necessary to keep a non-short legged line. This mutation is supposed to have occurred independently several times.

It is listed as extremely endangered German breed by the GEH.

Lakenfelder

Cultural value: A German breed developed from the pencilled chicken type of the north. First descriptions of the breed are from in 1835.

Colour: white with black head, neck and tail. It occurs exclusively in this breed.
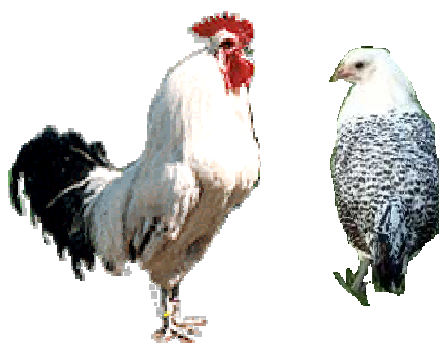
Comb: single

Eyes: dark red

Legs: grey blue

It is listed as endangered German breed by the GEH.

Ostfriesische Möwen Silver pencilled

Cultural value: A German breed descendant of pencilled north type chickens.

Colour: a typical rough textured colour pattern next to sprangled. All black spots must have a green sheen.

Comb: single

Eyes: brown or dark red

Legs: grey blue

It is listed as strongly endangered German breed by the GEH.

Paduans Chamois, Black, Silver, Gold, White

Cultural value: It is known to be the oldest crested breed. Assumedly ancestors from Russia it is known since the 15th century. The breed was developed in Poland, Italy and England. First documentation in Germany was a painting of Frisch in 1763.

Colour: For this study only Chamois, gold, silver, black and white animals were sampled.

Comb: no comb

Eyes: brown or orange red depending on the feather colour

Legs: blue grey

Additional trait The main feature is its crest caused by a protuberance of the brain and cranium. It is necessary to comb the crest feathers because the animals are not able to remove pinfeathers and parasites. Because of restrictions of the visual field due to a big crest it is an issue discussed in animal welfare topics. Breeders have to care for a free sight.

Ramelsloher White
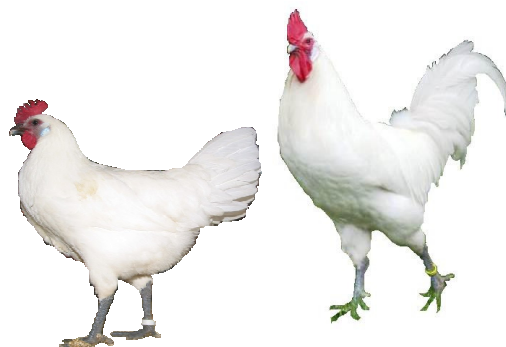
Cultural value: A German breed developed in the convent of Ramelsloh 1870.

Colour: Only white coloured were sampled in this study

Comb: single

Eyes: dark with black eyelid

Legs: blue with white toes

It is listed as extremely endangered German breed by the GEH.

Rheinländer Black

Cultural value: A German breeder created this chicken and wrote 1894 that his main interest was egg production. They were influenced by Bergische Kraeher and Le Mans.

Colour: only black coloured were sampled in this study

Comb: rose

Eyes: dark brown

Legs: black

Thüringer Barthühner Black, Gold, Chamois, Silver
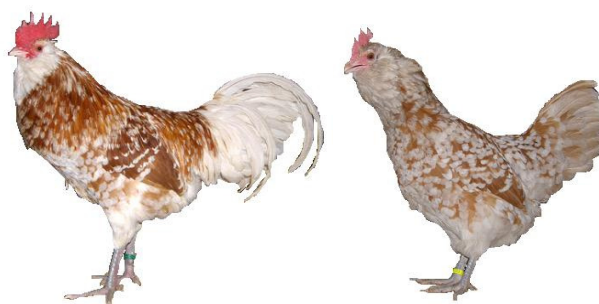
Cultural value: A German breed known since early 18th century originates from native "Otterkoepfe" and influenced by Paduans. It always displayed in several colours. In 1880 12 different colours were known.

Colour: only Black, Gold, Chamois and Silver coloured were sampled in this study

Comb: single

Eyes: different

Legs: blue grey

Additional trait: bearded

It listed as to be observed in the German red list for breed by the GEH.

Vorwerkhühner

Cultural value: Is a German breed developed from the *Lakenfelder* around 1900. It was bred as a dual propose breed in yellow plumage, a favoured colour that times. The yellow colour was introduced through Sussex, Orpington and Andalusian.

Colour: black markings like only *Lakenfelder* have but yellow instead of white.

Comb: single

Eyes: yellow-red

Legs: grey-blue

It is listed as to be observed in the German red list for breed by the GEH.

Westfälische Totleger Silver

Cultural value: In contrast to single combed pencilled chickens from Belgium and north Germany this rose combed breed was kept in central Germany, in Westphalia in beginnings of the 19th century. Its name indicates that it was mainly kept for egg production and "lays until it dies".

Colour: In this study only silver pencilled were sampled

Eyes: dark brown

Legs: grey

It is listed as to be observed in the German red list for breed by the GEH.

Microsatellite markers

Table X: Used microsatellite markers

| Marker | Ort | | foreward-Primer | reverse-Primer | Annealing Temperature |
	Chromosom	Position			
MCW0034	2	230	TGCACGCACTTA CATACTTAGAGA	TGTCCTTCCAATT ACATTCATGGG	60°C
ADL0268	1	288	CTCCACCCCTCT CAGAACTA	CAACTTCCCATCT ACCTACT	60°C
MCW0069	E60C04 W23	23	GCACTCGAGAAA ACTTCCTGCG	ATTGCTTCAGCAA GCATGGGAGGA	60°C
MCW0183	7	79	ATCCCAGTGTCG AGTATCCGA	TGAGATTTACTGG AGCCTGCC	67°C
ADL0112	10	0	GGCTTAAGCTGA CCCATTAT	ATCTCAAATGTAA TGCGTGC	58°C
MCW0295	4	75	ATCACTACAGAAC ACCCTCTC	TATGTATGCACGC AGATATCC	58°C
MCW0206	2	104	ACATCTAGAATTG ATGTTCAC	CTTGACAGTGAT GCATTAAATG	60°C
MCW0111	1	118	GCTCCATGTGAA GTGGTTTA	ATGTCCACTTGTC AATGATG	62°C
MCW0016	3	96	ATGGCGCAGAAG GCAAAGCGATAT	TGGCTTCTGAAG CAGTTGCTATGG	55°C
ADL0278	8	87	CCAGCAGTCTAC CTTCCTAT	TGTCATCCAAGAA CAGTGTG	62°C
MCW0078	8	87	CCACACGGAGAG GAGAAGGTCT	TAGCATATGAGTG TACTGAGCTTC	60°C
MCW0037	3	317	ACCGGTGCCATC AATTACCTATTA	GAAAGCTCACAT GACACTGCGAAA	66°C
MCW0067	10	61	GCACTACTGTGT GCTGCAGTTT	GAGATGTAGTTG CCACATTCCGAC	60°C
MCW0081	5	123	GTTGCTGAGAGC CTGGTGCAG	CCTGTATGTGGAA TTACTTCTC	60°C
MCW0222	3	86	GCAGTTACATTGA AATGATTCC	TTCTCAAAACACC TAGAAGAC	60°C
MCW0216	13	28	GGGTTTTACAGG ATGGGACG	AGTTTCACTCCCA GGGCTCG	58°C

MCW0103	3	210	AACTGCGTTGAG AGTGAATGC	TTTCCTAACTGGA TGCTTCTG	64℃
MCW0248	1	20	GTTGTTCAAAAGA AGATGCATG	TTGCATTAACTGG GCACTTTC	60℃
MCW0098	4	217	GGCTGCTTTGTG CTCTTCTCG	CGATGGTCGTAAT TCTCACGT	60℃
LEI0234	2	50	ATGCATCAGATTG GTATTCAA	CGTGGCTGTGAA CAAATATG	55℃
LEI0094	4	153	GATCTCACCAGTA TGAGCTGC	TCTCACACTGTAA CACAGTGC	62℃
LEI0166	3	300	CTCCTGCCCTTA GCTACGCA	TATCCCCTGGCT GGGAGTTT	62℃
MCW0014	6	96	(AAAA)TATTGGCT CTAGGAACTGTC	(ACCG)GAAATGA AGGTAAGACTAG C	55℃
MCW0020	1	460	TCTTCTTTGACAT GAATTGGCA	GCAAGGAAGATT TTGTACAAAATC	60℃
MCW0080	15	49	GAAATGGTACAG TGCAGTTGG	CCGTGCATTCTTA ATTGACAG	60℃
MCW0104	13		TAGCACAACTCAA GCTGTGAG	AGACTTGCACAG CTGTGTACC	60℃
MCW0165	23		CAGACATGCATG CCCAGATGA	CAGACATGCATG CCCAGATGA	60℃
MCW0123	14	45	CCACTAGAAAAG AACATCCTC	GGCTGATGTAAG AAGGGATGA	60℃
MCW0330	17	41	TGGACCTCATCA GTCTGACAG	AATGTTCTCATAG AGTTCCTGC	60℃

How to use the programmes

To use Weitzman programmes in combination with the Phylip package following sequence has to be exercised. ⋮ means that the file has to be renamed.

program name	**file name**	**data type**
	eingabe	array length
erzPhylipEingabe		
	ausgabe	
	infile	
Phylip / Gendist		
	outfile	
	dmatN.out	array length
WeitzmanN.f		
	weitzN.out	
	weitzN.var	
marginalN.f		
	margeN.out	marginal diversity
	exproN.txt	extinction probability

CURRICUKUM VITAE

Family name: Pinent

Name: Tamina

Date of Birth: 01 September 1976

Place of Birth: Paris, France

Gender: Female

Nationality: German / Brazilian

Education Background

2004-2008: PhD fellow, Institute of Animal Breeding and Genetics, Georg-August Universität, Göttingen, Germany

1996-2004: Diploma in Biology (Theoretical Biology and Cybernetics, "Sehr Gut"), University of Bielefeld, Germany

1995-1996: Advanced Certificate of Secondary Education (Abitur, 2,2), Marianne-Weber-Gymnasium, Lemgo, Germany

1994-1995: General Certificate of Secondary Education, Bürgermeister-Gräfer-Realschule, Lemgo, Germany

1992-1994: Colégio Cruzeiro do Sul, Porto Alegre, RS, Brazil

1991-1992 Colégio Bom Conselho, Porto Alegre, RS, Brazil

1988-1989 Realschule Ascheberg, Ascheberg, Germany

1985-1988 Grundschule Davensberg, Germany

1984-1985 Grundschule Loevelingloh, Germany