

## **AUTHOR'S REVIEW OF THE RESEARCH ACHIEVEMENTS AND PUBLICATIONS**

### 1. Name and surname

**Iwona Głazewska**

### 2. Held diplomas, scientific degrees - with the name, place and year of acquisition and the title of the doctoral dissertation

- Master of Science in Animal Sciences, University of Agriculture and Technology in Olsztyn, Faculty of Animal Science, 1980, the title of MS thesis: "The connection between polymorphism of transferrin and ceruloplasmin in cattle and the level of iron and ability to bind it by the plasma transferrin"
- Doctor of Biological Sciences in the field of biology, 2002, the Faculty of Biology, Geography and Oceanology, University of Gdansk, on the basis of the thesis: "Application of the founders contributions analysis to evaluate genetic diversity in the non-random mating population on the example of purebred Arabian horses"

### 3. Information on previous employment in scientific institutions

- assistant in the Department of Animal Genetics, Faculty of Animal Science University of Agriculture and Technology in Olsztyn, in the period: 01.10.1980-15.05.1982
- employee in different unscientific institutions in Gdańsk (Laboratory of Clinical Bacteriology, Regional Agricultural Advisory Centre, Institute of Veterinary Hygiene) in 1982-1990
- specialist in the Centre of Marine Biology, Polish Academy of Sciences, 1.06.1990-31.07.1990
- University of Gdansk, Faculty of Biology (formerly: Department of Biology, Geography and Oceanology) from 01.08.1990, in sequence as follows:
  - science and technology specialist in the Department of Genetics (formerly: Department of Genetics and Cytology) to 28.02.2003
  - assistant professor (research employee) in the Department of Genetics, 01.03.2003 to 30.06.2010
  - assistant professor (research employee) in the Department of Plant Taxonomy and Nature Conservation from 1.07.2010

4. Indication of the scientific achievement, as described in *Art. 16. Section. 2* (Dz. U. nr 65, poz. 595):

A) Title of the scientific achievement:

### **The use of mitochondrial DNA in studies of selected pedigreed populations**

is composed of the series of five publications:

1. **Głażewska I.**, Wysocka A., Gralak B., Prus R., Sell J. 2007. A new view on dam lines in Polish Arabian horses based on mtDNA analysis. *Genetics Selection Evolution* 39, 609-619 (IF=1.735)
2. **Głażewska I.** 2010. Speculations on the origin of the Arabian horse breed. *Livestock Science*, 129, 49-55 (IF=1.295)
3. **Głażewska I.**, Prusak B. 2012. Evaluation of the effectiveness of introducing new alleles into the gene pool of a rare dog breed: Polish Hound as the example. *Czech Journal of Animal Science* 57, 248-254 (IF=0.922)
4. **Głażewska I.**, Zielińska S., Prusak B. 2012. Formation of a new dog population observed by pedigree and mtDNA analyses of the Polish Hovawart. *Archiv Tierzucht* 55, 4, 391-401 (IF=0.463)
5. **Głażewska I.**, Prusak B., Gralak B. 2013. Pedigrees as a source of information in mtDNA studies of dogs and horses. *Animal Genetics* 44, 2, 227-230 (IF=2.584)

My contribution to the multi-author articles generally included: the idea and the plan of research, writing of the publication and pedigree analysis, while the contribution of co-authors consisted of making a laboratory part of the projects. Detailed information on the participation in each of the co-authorships is given in Annexes 4-6.

B) Short description of the scientific / artistic achievement outlining the scientific goal and the results received, together with an indication of their potential use

Multi-generation pedigrees are a valuable source of information about an animal and its ancestors, but their wider use in scientific studies was only enabled by the development of computer technology. From the 1990s, a series of papers devoted to the analysis of different species of domestic animals and wild species kept in captive breeding have been published. The aim of these analyses was to define ancestors of populations studied, to restore population history and to estimate genetic diversity. Pedigree measures were also used to evaluate the way of population managing, to show directions of

its development and to identify genetically important representatives of the population, significant from the point of view of a conservative breeding.

My analyses are a part of this important area of research. The objects of the studies were three populations of pedigreed animals: purebred Arabian horses and Polish Hound and Hovawart dog breeds. A common feature of the populations is their small size, and important advantage is the relatively large amount of pedigree information available, from a few to about 20 ancestral generations. The aim of the study was to evaluate genetic diversity of the populations and to answer the important questions raised by breeders.

In some projects, the results of which I present as a significant scientific achievement, pedigree analysis has been supplemented by the analysis of mitochondrial DNA, used to answer the questions being beyond the scope of the pedigree analysis itself. In general, mtDNA studies in horses and dogs, which mainly concern the phylogenetic, taxonomic and forensic issues, are conducted so far in a relatively small area. For example, according to Verscheure et al. (ZooKeys 2013), only 58 articles presenting results of mtDNA studies in dogs were published in the world between 1995 and 2013. In this niche research area my articles, referring to the origin of a population and verification of pedigrees and genetic diversity of the population, can be found.

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The basic source of information in pedigree analyses are pedigrees, and a necessary condition to obtain a reliable results are their reliability and completeness. Unfortunately, for obvious reasons, the amount of pedigree data are limited and at most cover the period described in studbooks. In turn, the credibility of the data results from a specifics of breeding of a given animal species and a history of a breed. Together with the development of molecular methods, a parentage control, carried out on the basis of genetic marker analysis, has become an essential step in breeding of many breeds of domestic animals to ensure the accuracy of their pedigrees. However, due to a lack of biological samples from further ancestors, nuclear markers can be used exclusively for verification of records from the closest generations in a pedigree. A tool that allows to verify historical records is mitochondrial DNA (mtDNA), which is inherited only in the female line, and thus allows the evaluation of exactly these lines. Samples taken from present-living animals are used in the laboratory analysis, and a research scheme is as follows: assignation of dam lines present in the population on the basis of the whole pedigree data, choosing representatives of particular lines and their branches to mtDNA analysis, and finally laboratory analysis of mtDNA. A verification is possible only if a line has different branches. In practice, the interpretation of the results is based on the basic assumption that in principle pedigrees are true, and the errors are the exception (their effective verification would not be possible while assuming the opposite, i.e. that the pedigrees contain numerous errors). Evaluation of results of the

analysis, which show identical or distinct mtDNA sequences in the different branches of a line, does not require the use of any complicated computer programs, but it is done by a zero-one method. Finding the same sequence in different branches of a given line proves the correctness of pedigree records (there are no grounds to undermine them), and different sequences indicate a pedigree error. A special case is a difference between the sequences of one or two nucleotides, which may suggest either a new mutation or an error in the pedigree. In this case, interpretation of the result requires taking into consideration facts from the breed history favoring the error or excluding it.

The analysis of historical Polish Arabian horse pedigrees, described in the first article [1], is an example of the use of mtDNA in verifying pedigree records. The doubts about one of the dam lines were the starting point of the study (W. Kwiatkowski (*A Guide to the complete pedigrees of Arabian horses from Poland*, 1993). According to Kwiatkowski, one of the two branches of the line of Milordka, born about 1816 in Sławuta stud, in fact originates from unrelated to Milordka the mare Malikarda, born about 1810. On the basis of unpublished stud documents Kwiatkowski suggested that the error referred to one of two Milordka's daughters. These reservations were even more important because while the Polish Arabian breeding started in the end of XVIII century the history of the official Polish stud books goes back only to 1926. In an earlier period, more or less accurate breeding registers were conducted in some of 19<sup>th</sup>-century studs, unfortunately, they suffered complete destruction during the years 1914-1918. We owe our present knowledge of the subject to dr. Edward Skorkowski who recreated nineteenth-century pedigrees on the basis of surviving documentation and oral history, and published them in the first volume of *Polish Arabian Stud Book* (1926) and in the "*Genealogical Charts of Arabian horses*" (1938).

The analysis of samples taken from 44 representatives of dam lines and their branches confirmed the presence of 14 mtDNA haplotypes in 15 lines. Eight haplotypes were each present only in single dam lines. In the case of two haplotypes we stated that they were present in two lines each. The detailed analysis of historical sources did not give any reasons to question pedigree data of these lines. Hence, it should be accepted that the identical haplotype present in two lines is the evidence of origin of their founders from a common ancestor living in the past.

More than one haplotype was found in two lines. In the case of Milordka line, the *D* haplotype was found in the branch established by the Milordka's daughter Zaira 1826, and the *E* haplotype was found in the branch originated from the second Milordka's daughter, Delfina 1825, the origin of which was questioned by Kwiatkowski. The difference between the haplotypes was significant and referred to 13 nucleotides, which excluded the common origin of both branches. The results indicate the presence of two independent lines within the present Milordka line and may confirm the suggestions by Kwiatkowski. At the same time, it should be pointed out that the results cannot be

interpreted as a hundred percent proof that the founder of the second line was actually mare Malikarda because of impossibility of precise dating due to the absence of a greater number of the line's branches.

Also in the Gazella line, imported to Jarczowice in 1845, we found two haplotypes, which differ from each other by nine nucleotides. The *F* haplotype is present in the branch represented by Gazella II, born in 1914 in the Jezupol stud, and her four daughters. The second haplotype, identical to haplotype *H* found the Sahara line, is present in the branch established by Fryga, born in 1914 in the Pelkinie stud, and her two daughters. This result undermines the official pedigree records of Gazella line. The analysis of historical sources indicated some possible reasons of the error, but the most likely seems to be a mistake as to the identity of Fryga during the war. In 1914, when almost all the horses from Pelkinie stud were confiscated by the Russian army, only a few foals were left in the stud, together with Fryga. Unfortunately, owing to the lack of stud data a precise indication of potential identity of Fryga is not possible, although the results of the mtDNA analysis indicate her affiliation to the Sahara dam line, which was also bred in this stud.

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The reason for the next research issue, described in the article [1], were doubts about the origin of the oldest Polish Arabian dam lines and their breed purity. Different opinions on the origin of foundation stocks of the oldest Polish studs were presented in historical sources. According to them, the stocks were originated from local breeding Tarpan-type mares mated by oriental stallions or from mares defined as Polish, Persian and Cherkesian. In the comparative analysis, conducted using mtDNA sequences of American Arabian horses (Bowling et al. *Animal Genetics*, 2000), we stated that the sequences of two out of the five questioned lines are identical to those found in lines established by mares considered to be original Arabians (or.ar.). The occurrence of identical haplotypes in dam lines of the mares recognized as or.ar. and of mares of unknown origin is a strong argument for the Arabian origin of the latter.

A more detailed analysis of issues related to the origin of the Arabian horse breed was presented in the next publication [2]. The origin of Arabian horses is the subject of many myths and legends, combining the beginning of the breed with the historical characters of King Solomon and Muhammad. Although the origin of the Arabian horses dates back to the distant times, history of the modern population of the breed, described in pedigree records, is no longer than 200 years. The founding material of the European and American studs were mainly horses imported in the nineteenth century and in the first decade of the twentieth century from the Near East, representing different dam lines (strains) of Arabs, and horses of unknown origin. The problem of the purity of the breed and strains is one of the most frequent topics in Arabian horse publications. According to the common opinions a

desert breeding was conducted in purity, and Arab breeders strictly kept the separate character of particular dam lines.

In order to check the validity of these opinions I performed a comparative analysis of mtDNA sequences derived from American and Polish Arabian horses with known affiliation to a dam line, and sequences from different groups of horses potentially unrelated to Arabian horses, which were deposited in the GenBank database. The analysis confirmed the high level of mtDNA diversity within the Arabian breed, in which 38 different sequences in 43 dam lines have been found. The clear concordance between mtDNA sequences and a founder affiliation to a given strain was not found. Haplotypes characteristic of different strains occurred in particular haplogroups and, at the same time, haplotypes of the same strains belonged to different haplogroups.

A number of identical sequences were noted in the comparison of Arabian sequences and those from other breeds. Besides the identical sequences, similar sequences with differences of one or two nucleotides, which might be a result of new mutations, were found for most of the lines. Particularly significant was the similarity between Arabian sequences and sequences found in horse remains at archaeological sites. Six of the eight haplotypes found in 13 horses from the Scythian grave at the Berel site (Kazakhstan, 3th century BC) were identical to those of the Arabian lines. This refers to three Polish lines established by mares of unknown origin and five lines of mares recognized as purebred Arabian horses. Identical sequences were found also among the sequences found in the horse remains from China, Ireland and Europe. Worth noting is also the similarity of the Arabian sequences to modern sequences of Akhal-Teke horses which are recognized as one of the oldest breeds of horses, Polish primitive horses, and heavy horses from Poland and Germany.

Identical sequences widespread on both continents at a very early period of history justify the hypothesis that significant mixing of originally endemic mtDNA sequences took place in the vast area from China to Western Europe and North Africa during the early stages of domestic horse history. The reason for this “genetic mixing” might have been the intense trade exchange on the Silk Road, and the migration of Great Steppe peoples. The movement of horses from local populations caused that presently it is impossible to precisely determine where ancestors of particular breeds come from, and the more detailed interpretation of the results requires accessing results of studies of the history of human populations. The findings permitted to formulate the general conclusion that the Arabian horse breed was created on the basis of many different horse breeds and populations, and the concept of breed purity might refer, at most, to the present population with a history that does not exceed two hundred years.

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MtDNA studies in dogs were a part of a wider analysis on genetic diversity in populations of two breeds of dogs. The starting point for the studies on Polish Hounds were signals from breeders about health problems in the breed. One of the possible reasons for this could be an excessive level of relationship of the breed, bred almost exclusively in Poland. The first stage of my research consisted of a standard pedigree analysis, which was described in an article presented in the second part of the review, and which revealed dramatically low level of genetic diversity of the population. Significant impoverishment of the gene pool of Polish Hounds and its incorrect structure draws into question the future of the breed and its chances of survival. In such cases, the recommended way of enriching the gene pool of a dog breed can be either introducing dogs of other breeds or of unknown origin that are phenotypically similar to a given breed. In the case of Polish hounds, since the 1970-ties, dogs registered in the Introductory Book (KW) were used in breeding. The origin of the KW founders is unclear; they might be either purebred dogs bred without formal Polish Kennel Club documentation or dogs descended from other breeds. Depending on the origin of these dogs, there were two possible scenarios: in the first case, genes, which were already present in the gene pool, were introduced to the pool, while in the second case they were genes, about which it is not known whether they were identical or different from the Polish Hound's genes, and whether their presence in the gene pool will have positive effects in the future or not. Due to critical opinions of the quality of KW dogs, the Polish Kennel Club decided to close the Introductory Book in November 2010. This decision led me to evaluate the breeding importance of Polish Hounds registered in KW and to check their origin with the help of mtDNA analysis [3].

Pedigree analysis indicated the very limited breeding success of KW dogs. In the years 1960-2008, the population of Polish Hounds descended from 21 founders of the breed, including four dogs and 13 bitches registered in KW. Among the established dam lines the line of the first founder of the breed, Czita, dominated. The proportion of litters in lines founded by the KW bitches was only 16.5% of the litters, and also a disproportionately small number of dogs and bitches born in KW dam lines were used in breeding. Although the number of KW individuals was 80.9% of the total number of breed founders, but the proportions of KW dams and sires were equal at just 14.3 and 4.7 %, respectively, of the total number of breeding dogs. A lack of interest from the breeders in the KW dogs resulted in their limited genetic importance, which was reflected in the structure of the gene pool of the population. The contribution of the KW founders to the gene pool of the population increased until the period 1990-1994, when it reached the value of 18.2%, and then decreased to 12.3% in 2005-2008.

In light of these results, evaluating breeding affiliation of the founders registered in KW, based on the results of mtDNA from samples taken from living representatives of six dam lines, had a significant importance. In the context of the history of the Polish Hounds, the old breed, which

practically became extinct on Polish lands as the result of the World War II and the shifting of national borders, the study seemed particularly appropriate, since it is doubtful that it was possible at present to find purebred Hounds bred out of registered kennels. The expected result was to find different mtDNA sequences in the line of Czita, imported from Belarus in 1958, and those of Yuma Strapczyna, imported from Lithuania in 1990 due to different origin of both founders. Regarding the remaining four lines, two different results were possible: either the analysis would indicate sequences identical to the Czita haplotype, which may be recognized as the basic haplotype in the breed, or distinct sequences. Qualifying KW founders for breeding based exclusively on their phenotypic similarity to the breed standard does not guarantee their purebred origin nor exclude origin from other dog breeds. The results of mtDNA analysis confirmed the initial hypothesis. The sequences in Czita nad Yuma dam lines (*PH1* and *PH2* haplotypes, respectively) were not only different, but also showed a very high degree of genetic distinctiveness. Regarding the remaining lines, the *PH1* haplotype was found in the two lines, and the new *PH3* haplotype was found in the next two lines.

A comparison with sequences of other dog breeds from the GenBank database indicated that the *PH1* and *PH3* sequences are identical to sequences of several breeds representing different utility types of dogs. No sequences identical to *PH2* were found, and the most similar sequences, differing by one nucleotide from *PH2*, were those of the Walker Hounds and the shepherd's Schipperke breed. Different mtDNA haplotypes confirmed distinct origin of at least a part of the KW dogs. The breeding use of such individuals had to result in introducing new variants of the genes into the gene pool of the breed, and the obvious result of this was an increase in phenotypic diversity in the population, which unfortunately was not acceptable to the breeders. The situation of the Polish Hound illustrates a "trap of genetic impossibility": the breed has a very limited gene pool which cannot be enriched without dogs of distinct origin, but their breeding use is unacceptable by breeders because of a worse quality of their descendents.

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The conclusions of the history of Polish Hounds led me to perform the next project, the goal of which was to determine conditions which decide about the structure of gene pool of a newly created dog population [4]. The object of the study was a Polish population of Hovawart dogs, which similarly to the Polish Hounds, is small but is a part of a relatively large world population. The basic step in the study was the pedigree analysis, and the mtDNA analysis was used here to determine the origin of the population's female founders and evaluate their relationship, as well as to verify pedigrees.

Between 1988 and 2008, a total of 192 litters were born in Poland, by 93 dams and 115 sires. The founding stock consisted of twenty-three imported bitches. The majority of sires was of foreign breeding (79.1%). Constant use of foreign dogs had a positive impact on the level of pedigree



measures. The constant increase of the total number of founders was accompanied by increasing number of founders corresponding for  $\geq 50\%$  of the gene pool, and the inbreeding coefficient ( $F$ ) showed the constant decreasing trend. These results are an obvious consequence of the open character of the population and almost unlimited choice of unrelated sires. Nevertheless, it is noteworthy the Polish breeders care about maintaining a high level of genetic diversity of the population. Phenomena typical for breeding dogs, which lead to decrease of genetic diversity of the population, did not occur in a wider scale. Particular attention is paid to low average number of litters per one father, and a significant percentage of fathers with one litter (76.5%). Breeders willingly reached for new sires, which resulted in the ratio of 0.81:1 of the number of mothers to fathers. This is one of the most interesting results, as generally in dogs, due to the so-called "popular sire effect", the number of bitches used in breeding is greater than the number of males, such as in Polish Hounds, in which the ratio was 1.6:1, or in breeds studied by Leroy and Baumung (*Animal Genetics*, 2011) (the ratio of 1.12:1- 2.33:1). The positive aspects of the breeding policy was to avoid mating between relatives. This approach is not typical of dog breeding. According to Leroy et al. (*Revue Méd. Vét. Toulouse* 2007), 24% of French breeders declared using close-breeding, and a similar tendency can be probably found in the breeding of the majority of dog breeds.

The second stage of the research concerned mtDNA diversity in the population. MtDNA analysis in descendents of 23 founder bitches revealed two mtDNA haplotypes, respectively Ho1 and Ho2, differing by 18 nucleotides and belonging to different haplogroups. The comparative analysis showed that the Ho1 haplotype is identical to haplotypes of several breeds representing different utility types, and an haplotype identical to Ho2 was so far found only in the Labrador retriever breed. The analysis confirmed the opinion that modern breeds of domestic animal are derived from different and genetically distinct ancestors. The important conclusion from the analysis of mtDNA was to determine the consistency of pedigree and molecular data, which is an interesting result, given that similar studies in horses has always led to the undermining some of the records.

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The last publication of the presented series is a summary of my experiences gained from the pedigree analyses of the dog and horse populations. In the previously discussed projects the mtDNA analysis served as an auxiliary tool, used to clarify issues beyond the scope of pedigree information. The opposite situation, however, is also possible when pedigree data are used as a source of additional knowledge when such knowledge is not available to the researcher who has exclusively pure results of laboratory analysis of mtDNA. In the article [5] three examples of such use of pedigree analysis are presented.

The first example concerned the use of pedigree analysis to detect all carriers of potentially different mtDNA sequences. Realistically speaking, finding mtDNA haplotypes present in a population of animals of unknown origin requires testing the whole population. With known animals origin, and assuming that pedigrees are reliable and nucleotide mutations can be excluded from consideration because of the very low mutation rate, it is possible to limit the scale of the analysis to representatives of particular dam lines that potentially possess different mtDNA haplotypes. For example, samples from 11 hounds and 23 Hovawarts permitted finding mtDNA sequences present in the whole populations, and samples from only 44 Arabian horses allowed to find mtDNA present in 98.4% of Polish broodmares.

The second example concerned the frequency of mtDNA haplotypes in a population. For obvious reasons, the laboratory analysis does not permit observing changes in the frequencies of particular haplotypes over longer period. Recognition of the history of haplotypes is possible if we calculate haplotype frequencies in an indirect way, by calculating the number of representatives of particular dam lines. The graphs presented in the article show the changes in the haplotype frequency in Polish Hounds and Arabian horses over the years. Significant differences in the frequency of particular haplotypes in the long term and significant differences between two Arabian horse studs are both noteworthy. Another interesting observation was the high stability of the haplotype number in the Arabian horse population and the loss of haplotypes present in extinct dam lines in the Polish Hounds. The results allow to express the conclusion that the number of haplotypes arises mainly from the history of population and breeding policy regarding dam lines (the breeders attach great importance to their continuation, as in Arabian horses, or do not).

The third issue related to mtDNA studies is a question of knowledge, which is provided by these studies. MtDNA analysis of modern populations does not detect sequences carried by female founders of extinct dam lines or male founders. Therefore, the important question remains to identify what part of the gene pool sources of a given population is defined by mtDNA analysis. This is particularly important in phylogenetic studies, where we are interested in the origin of the whole gene pool and not just part of it seen through the mtDNA analysis. Evaluating this through laboratory analysis is impossible, but this is possible with the help of the analysis of founder contributions to a gene pool. Of the three populations analyzed, low contributions of the founders with known mtDNA were found in two populations with the constant inflow of genes of foreign males, i.e. in the Arabian horses (with the maximum at about 10%) and Hovawarts (up to 26%), and higher contributions (about 40%) were observed in the close population of the Polish Hounds. The above figures show a possible source of differences in results of phylogenetic studies depending on what genetic marker, mitochondrial or nuclear, was used in studies. The degree of discrepancies of the two estimations probably also

depends on the history of a population (e.g., local or foreign origin of sires); in the first case the relatively high concordance between mtDNA and nuclear markers estimations might be expected, whereas in the second case the fundamental divergences between results of both analyses are highly probable.

### Summary

All publications in this series met with great interest among scientists around the world, seen by numerous requests for copies of the articles and citations in articles published in important genetic journals, such as *Animal Genetics*, *BMC Genetics*, *J. Heredity* and *PNAS*. Particularly important was the interest in my work from the Arabian horse breeders, the Office of the World Arabian Horse Organization and Arabian horse fans gathered at international internet forums. The article on dogs met also with a positive reception in the kennel community. Worthy of notice is also the use of the Polish Arabian horse sequences, examined in our study, by other scientists in their analyses that were later on published in the prestigious journals: *PNAS* (Orlando et al . 2009; Achilli et al. 2012) and *Plos One* (Cieslak et al . 2010) .

### C) Description of other scientific publications and achievements

The beginning of my interest in pedigree analysis, which is crowned by the publications discussed above, dates back to the times of high school and my participation in the Biological Olympics, my studies at the Faculty of Animal Science of the University of Agriculture and Technology (ART) in Olsztyn and the period of my work as an assistant at the Department of Animal Genetics of the ART. After returning to the Tri-City in 1982, I worked in a variety of non-scientific institutions in the field of human and veterinary medicine and agricultural advisory services. The work in diagnostic laboratories gave me a lot of experience in the field of laboratory analyses, and the work as an agriculture adviser attracted my attention again to the crucial role of a breeder and his decisions on the composition of a population gene pool. The experience gained during this period had an essential impact on my scientific interests and choices.

In the first years of my work at the Department of Genetics of the University of Gdansk, my professional duties consisted in laboratory work of the research projects conducted in the Department, which referred to genetic markers polymorphism in aquatic Crustaceans. In the case of four projects, my participation in the studies was reported as the co-author of the publications which are described in the further part of the text. In 2002, this stage of my working life was terminated because of health reasons; in subsequent years, my research interests focused on the issues related to pedigree analysis.

In 1997, I decided to begin a doctorate compatible with my interest in this field, and with Arabian horses as a model population. The study regarded the founder contribution analysis, a new research

area, which was launched by researchers of the wild species in conservation breeding. The PhD thesis was defended in February 2002, and the two articles describing the preliminary and final results and its publishing effect were as follows:

- **Głażewska I.** 2000. The founder contribution analysis in currently living Polish Arabian brood mares. *Animal Science Papers and Reports* 18, 19-31
- **Głażewska I., Jezierski T.** 2004. Pedigree analysis of Polish Arabian horses based on founder contributions. *Livestock Production Science* 90, 293-298 (IF=1.363).

The analysis presented new insights into the Polish population of Arabian horses bred in the state studs in the 1948-1997 period. Pedigree database, which was the basis for the calculations, reached 1793, when a first known ancestor of the modern population was purchased. The founders of the post-war Polish Arabian population were 213 horses, significantly differed in their contributions to the population gene pool (<0.005% to 7.96%). The almost closed nature of the population contributed to a steady increase in the level of relationship and inbreeding of the population, accompanied by a growing percentage of the founders present in all pedigrees (from 0% to 38.4%).

Taking into account the crucial importance of Polish Arabian horses in the world breeding of this breed and innovative nature of the research, the results of my work have been noticed in foreign research centres, as evidenced by citations of the latter article in publications from around the world.

The next three positions of the publications related to my research on inbreeding in Arabian horses are as follows:

- **Głażewska I.** 2004. Mating and selection in national Arabian horse breeding: inbreeding coefficients analysis. *Electronic Journal of Polish Agricultural Universities, vol. 7,1, ser. Animal Husbandry*
- **Głażewska I., Gralak B., Niemczewski C.** 2004. Is inbreeding coefficient a credible measure of autozygosity in Polish Arabian horses? *Animal Science Papers and Reports* 22 (4), 621-629
- **Głażewska I., Gralak B.** 2006. Balancing selection in Polish Arabian horses. *Livestock Science* 105, 1-3, 272-276 (IF=1.131)

The first publication referred to evaluation of breeder decisions on mating and selection in the Arabian state studs, observed through inbreeding coefficients analysis. A noteworthy observation concerned the avoidance of matings between close relatives by the breeders, which resulted in a lower level of inbreeding than would occur with random matings. The analysis also showed that sires were characterized by significantly lower values of inbreeding coefficient than non-breeding stallions. Since the breeders reported that the value of inbreeding coefficient was not taken into account when choosing horses to breeding stock, it may be assumed that selection favouring individuals with higher levels of heterozygosity took place in the population independently of breeders' will and knowledge.

The goal of the second study was to evaluate heterozygosity of genetic markers in the selected group of Arabian horses, carried out with the direct method based on the number of heterozygous loci in the offspring, as well as indirectly, using SIM2 similarity index based on genotypes of their parents. The results were very striking: a statistically significant correlation between parents' and offspring's heterozygosities was found, but the analysis did not confirm the expected negative correlation between the level of inbreeding and heterozygosity of genetic markers in descendants. The results led to the general conclusion that inbreeding coefficients do not reflect the real level of heterozygosity of descendants, which might suggest that the inbreeding coefficient is not a reliable measure of autozygosity in the population analysed.

The goal of the third publication was to evaluate the process of choosing breeding mares to the stud. Heterozygosity of ten markers of breeding and non-breeding mares from four state studs was taken into account. In the three out of the four studs, breeding mares showed a higher level of heterozygosity than non-breeding mares of the same year, and a similar trend was found in breeding and non-breeding daughters of twelve out of eighteen the most important fathers. What is more, breeding mares were more heterozygous than non-breeding mares in particular inbreeding ranges, too. The results confirmed previous assumptions about the presence of balancing selection favouring heterozygotes. This selection, independent of breeders knowledge, could have arisen from the qualification criteria for the Arabian breeding horses which include the beauty, soundness and racing stamina, i.e. the features which are considered to be connected to heterozygosity. Due to the stable breeding policy over the years, it can be supposed that the balancing selection affected in the long term and, therefore, could lead to the discrepancy between the real level of homozygosity and that expected on the basis of pedigree data.

A very important item in my scientific achievements is the first publication on dogs, describing the results of pedigree analysis in Polish Hounds:

- **Głażewska I.** 2008. Genetic diversity in Polish hounds estimated by pedigree analysis *Livestock Science* 2-3, 296-301 (IF=1,091)

The analysis of Polish Hounds included pedigree information from the years 1960-2004 about 247 litters born in 147 kennels by 88 fathers and 141 mothers. The first founders of the post-war population were two dogs and two bitches. In subsequent years, three males and twelve females of unknown origin, registered in the Introductory Book (KW), were used in breeding. The analysis of contributions of 19 breed founders to the gene pool showed strong imbalance and a dominant position of just two founders, whose total contributions to the pool ranged from 61.43% to 78.58%. A consequence of the low number of the founders and a strong imbalance in their genetic significance was the steady increase in the inbreeding level of the population, calculated on the basis of the whole

pedigree information, which reached a level of 37% in 2000-2004. Due to the novelty of the topic and the importance of the problems, the article was noticed in the world, as evidenced by the numerous citations and its being recorded in a famous report of the Royal Society for the Prevention of Cruelty to Animals (RSPCA): "*Pedigree dog breeding in the UK: a major welfare concern?*" (2008), concerning breeding and welfare of dogs, as well as in the report "*The welfare of pedigree dogs*" (2009) published on the veterinary website of the University of Sydney. The article was received with great interest also by breeders of Polish Hounds, at the request of whom I published on a website its popular version.

A special place on my accomplishments list has "*The breeding book of Hovawarts. Pedigree database 1988-2004*", published by the Polish Hovawart Club in 2006. The description consists of 140 pages and contains the basic pedigree information of first years of Hovawart breeding in Poland, presented in a list of litters in order of date of birth, fathers and kennels. Additionally, pedigrees of imported dogs and foreign sires are placed in the "Book", together with an index of names of dogs present in the pedigrees. "The breeding book of Hovawarts", which is an unique study in Polish cynology, was very well received by the Polish breeders as well as by breeders from national organizations belonging to the International Hovawart Federation (IHF), including the German Rassezuchtverein für Hovawart-Hunde. "The Book" was also one of the arguments taken into account when adopting the Polish Hovawart Club to the IHF.

A separate chapter of my research activity is my participation in studies of protein markers polymorphism conducted in the Department of Genetics, the result of which were four articles:

- Sywula T., **Głażewska I.**, Koszteyn J. Kwaśniewski S., Sell J. 1993. An analysis of the population structure of *Calanus cf. finmarchicus* (Copepoda) from the Hornsund Fiord Region, Spitsbergen. *Variability and Evolution*, 2/3, 113-119
- Sywula T., **Głażewska I.**, Whatley R. C., Moguilevsky A. 1995. Genetic differentiation in the brackish-water ostracod *Cyprideis torosa* (Jones). *Marine Biology*, 121, 647-653 (IF=1.288)
- Sywula T., Waife G., Sell J., **Głażewska I.** 2002. Genetic subdivision of the upwelling copepod *Calanoides carinatus* (Krøyer, 1849) off the continental shelf of Ghana. *Journal of Plankton Research*, 24, 5, 523-525 (IF=1.444)
- Chibani M., **Głażewska I.**, Rokicki J. 2004. The use of isozymes to identify specimens of *Pomphorhynchus* (Acanthocephala) in flounder, *Platichthys flesus* from the Baltic Sea. *Journal of the Marine Biological Association of the United Kingdom*, 84, 277 – 279 (IF=0.781).

The first publication of this series presented the results of studies carried out during our expeditions to Spitsbergen and concerned genetic polymorphism of local populations of copepods of the *Calanus* genus. Analysis of the markers was carried out by electrophoresis on cellulose acetate.

This was a new method that was chosen for our study because of its speed and a small amount of biological material needed for analysis. My role was to implement the method to the conditions of our laboratory, and then adapt it to specificity of each species analysed. My participation in the mentioned and subsequent projects involved making laboratory tests and analysis of the results.

The second project concerned the analysis of populations of *Cyprideis torosa* from the freshwater places in the UK, the Netherlands and Poland. This was one of the first studies of Ostracoda performed by electrophoresis methods, and therefore caught the attention of researchers in this field, resulting in citations recorded by WoS and Scopus databases.

The third publication presented the results of the study on *Calanoides carinatus* species living in marine waters of Ghana, and the last of the work - species identification of parasites from the Baltic flounders. In this case my participation in the publication included also co-authorship of the genetic part of the article.

On the list of my publications are also four scientific articles in Polish in the field of pedigree and mtDNA analyses. The next position are mtDNA sequences published in GenBank database. They are 14 mtDNA sequences of Arabian horses and 5 dogs sequences that have been received in the projects described in the articles [1, 3, 4]. The final manifestation of my research activity are presentations of preliminary or final study results at scientific conferences. This included four oral presentations at Polish conferences, and 11 poster presentations on Polish and international conferences.

The fact that my scientific activity has been noticed in a narrow area of pedigree and mtDNA studies in domestic animals has resulted in invitations from the editor of seven foreign and Polish journals to review research articles on the horses, dogs and other animal species; so far my achievements include thirteen reviews.

An interest in my scientific activity by fans and breeders of Arabian horses led me to the regular presentation of research results also in popular articles. The first ones were articles on Arabian horses, published in the "Koń Polski" magazine in 2002-2004. In subsequent years, I published articles on dogs in bulletins edited by Polish Hounds and Hovawart Clubs, and on their websites. Due to the closing of these websites, I decided to create my own website for the presentation of scientific and popular articles, dedicated owners and breeders of the dog breeds studied. "Project Sicz Orłowska" website ([www.siczorlowska.republika.pl](http://www.siczorlowska.republika.pl)) debuted on the internet in autumn 2013; nine articles and links to two scientific articles available on an open access basis, together with two pedigree databases, published in six files. Among my popularizing activities there is also my participation in a series of lectures of the Faculty of Biology for high school students entitled "Invite a scientist to School", in which I present a lecture on pedigrees and their verification, and my participation in subsequent editions of the Baltic Festival of Science.

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**Summary of scientific and popular achievements :**

Scientific and popular achievements after obtaining a doctoral degree (2002-2014):

- 5 articles in the JCR journals, in the series being the “scientific achievement” (IF= 6.999, 150 MNiSW points according to the 2013 list)
- 10 articles, including six in the JCR journals (IF=5.81; 184 points according to the 2013 MNiSW list)
- 1 book (pedigree database)
- pedigree databases published online -six data files of two dog breeds
- 14 mtDNA sequences of Arabian horses and 5 mtDNA sequences of dogs deposited in GenBank database (NCBI)
- 3 oral presentations at national conferences
- 10 poster presentations, including five presented at international conferences
- 16 popular articles
- 13 reviews for Polish and foreign journals, including 12 reviews for JCR journal

Scientific and popular achievements before obtaining a doctoral degree:

- 4 scientific articles, including 2 articles in the journals of the JCR list (IF=1.288; 64 points according to the 2013 MNiSW list)
- 1 oral presentation presented at a national conference
- 1 poster presentations at a national conference

*Justyna Glawnska*