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CURRENT RESEARCH DIRECTIONS IN THE GENETIC BASIS OF FLIGHT PERFORMANCE IN RACING PIGEONS

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Abstract

Pigeon breeding in Poland, just behind the Belgian and Dutch, is unquestionably one of the leading in Europe. Furthermore, the sport of bird flying is a highly profitable industry in both the service, product and amateur breeding markets, with elite birds achieving outstanding sporting results being valued in the thousands of euros. In recent years, one of the rapidly growing areas of research in animal improvement has been the search for the genetic basis of traits related to broad utilisation. The paper summarises and presents the achievements to date and the current directions of research into the genetic basis of flight performance in racing pigeons.

Keywords: racing pigeons, flight performance, flying

Introduction

Pigeon breeding in Poland, just behind the Belgian and Dutch, is unquestionably one of the leading in Europe. The tradition of sport pigeon flying dates back to the beginning of the 20th century, the first association of pigeon breeders was founded as early as 1905 in Zabrze. After Poland regained its independence in 1918, more than a dozen such associations were established with the approval of the Ministry of Military Affairs. Although the breeding talents and results of Polish breeders were recognised by the international community, the Second World War caused irreparable damage. Due to the possibility of these birds sending information, the German occupying forces requisitioned all flocks of pigeons, as well as forbidding their breeding and flying on pain of loss of life. Shortly after the war, however, Polish breeding began to revive again. In Krakow in 1946, a national breeders' organisation was set up and a breeding programme established. The headquarters of the Association of Racing Pigeon Breeders became Poznań, where the trade publication "Hodowca Gołębi Pocztowych" was also relaunched with its pre-WWII traditions. Two years later, the first all-Poland exhibition and the first international flight with a launch on the "Blyskawica" ship were organised in Katowice,

with 20,000 pigeons (www.pzhg.pl). Currently, there are around 40 000 breeders in the Polish Pigeon Breeders' Association, with an estimated pigeon population of 5 700 000, while nearly two million birds take part in flights every year (www.pzhg.pl). In addition, the breeding and sport associated with the homing pigeon is a highly profitable industry in both the service, product and amateur breeding markets. Elite birds achieving outstanding sporting results are valued in thousands of euros. In 2012, the record price for the bird reached €207,000 (Shapiro i Domyan, 2013) and in 2013 the Bolt pigeon was sold for a price of €310,000, which is one of the records. In the case of the sale of entire farms, on the other hand, prices run into millions of euros.

Pigeon sport classification

Sport pigeons take part in flying competitions at different distances and in different categories (Cat.) appropriate to the rank of the flights organised: Cat. A - flying with distance: 100-400 km (short distance); Cat. B - flying with distance: 300-600 km (medium distance); Cat. C - flights over 500 km (long distance); Cat. M - flights over 700 km (marathon). They start their career in the year of birth in junior pigeon competitions. The final rankings of the birds' performance during a given flying season are based on the so-called ACE points. These points are awarded to pigeons that have completed a flight (in Poland, 20% of the pigeons in a rate win prizes), with the maximum number of points going to the first pigeon at the finish line. ACE points for individual pigeons are calculated according to the formula (Dybus i in. 2021):

$$AP = \left[\frac{a - b + 1}{a}\right] * 100$$

where:

AP – ACE points scored;

a – number of starting pigeons subject to an average of 20% of the starting list (or less than 20% if less than 20% of the starting pigeons have completed the flight);

b – place on the flight completion list.

Review of potential molecular markers used to aid selection in racing pigeons

The modern homing pigeon is the result of the crossbreeding of many lines of pigeons and a sharp one-sided selection for flight traits targeting speed, endurance and spatial orientation in the field, with the aim of obtaining a bird that will travel specific distances to the pigeon house in the shortest possible time under different weather conditions at an average speed of more than 70 km/h (Caspermeyer, 2018). Improvement of breeding animals, including racing pigeons, using basic methods based on phenotypic observation of parental specimens, pedigree analysis and compilation of flight results has resulted in unquestionable improvements in many important traits from the point of view of the birds' sporting performance. Properly conducted breeding work forms the basis of breeding programmes, the overriding aim of which is to accelerate breeding progress while maintaining maximum genetic variability. In recent years, one of the rapidly growing areas of research in animal improvement has been the search for the genetic basis of traits related to broad utilisation. The use of genetic markers in sport is popular in humans (Ahmetov i Fedotovskaya, 2015), horses (Hill i in., 2010), (Ropka-Molik i in., 2019) or dogs (Huson i in., 2011), among others . Compared to other animals used in sport, the search for the genetic basis of extreme performance in racing pigeons remains niche. There is a lack of studies considering large groups of individuals to associate genomic sites previously selected using the tools of modern molecular genetics, such as whole genome or transcriptome sequencing. However, on a smaller scale, using cheaper and traditionally available techniques,

studies are being undertaken to look for associations between potential markers and racing performance in pigeons. To date, single nucleotide changes in the following genes have been selected: *LDHA*, *LDHB*, *DRD4*, *MSTN*, *AGLOB* et al. encoding proteins responsible for the regulation of important metabolic processes related to muscle exercise or mental predisposition (Dybus i in., 2018). Current markers on the market are selected on the basis of a prediction between physiological processes determining bird flight and phenotypic traits*(LDHA*, *DRD4*, *CRY1*). Some of the earliest studies on identifying polymorphisms in candidate genes involved DNA isolation, cDNA amplification of protein-coding sites and restriction enzyme cutting (Dybus and Kmieć, 2002; Dybus, 2007).

Polymorphisms in the gene *LDHA*

The first markers selected for racing pigeons were two variants in the *LDHA* gene, which encodes an enzyme, lactate dehydrogenase, that catalyses the conversion of pyruvate to lactate, an important energy source for working mammalian muscles (Van Hall, 2000). In the first studies in the early 2000s, a fragment of the LDHA gene including parts of exon 1, 2 and 7, flanking sequences and a fragment of the 3'UTR region were analysed using random restriction enzyme cutting (*HaeIII* and *NlaIV*). Potential polymorphic sites were identified in this region on a group of 45 pigeons, which were named A:B; C:D alleles (Dybus and Kmieć, 2002). In order to molecularly identify these polymorphic sites and to determine allele frequency and allele frequency in the racing pigeon population, the *LDHA* gene fragments analysed were subjected to Sanger sequencing. This method identified two polymorphic changes in intron 6 of the *LDHA* gene, identified then as variants A(AGCC, TTAAT) and B(GGCC, TGAAT). Due to their proximity to the donor site of gene folding, the so-called *splicing* site, they can influence the expression of different variants of the *LDHA* gene, its transcript level, as well as the enzymatic activity of this protein. According to work by Dybus et al. (2006) the most favourable genotype in racing pigeons is AA, but also, depending on the populations studied, AB. In pigeons selected for flight performance, a significantly higher frequency of the *LDHA_A*allele was shown in racing pigeons (Dybus et al., 2006). In contrast, Ramadan et al. (2013) in an Egyptian pigeon population showed the presence of six polymorphic sites, including a microsatellite region (TTTAT)3-5 located in the 6th intron of this gene. Further research based on statistical analysis incorporating the BLUP model to estimate breeding value (EBV) for total distance during an athletic career (referred to by the authors as survival; *survivability*), showed a highly significant association of individual microsatellite sequence variants with estimated breeding value (EBV) at distances above 2000 km (Ramadan et al., 2018). In a study conducted by Proskura et al. (2014), analysed the effect of a polymorphism in the *LDHA* gene on the flight performance of racing pigeons in Poland. It included 123 pigeons (60 females and 63 males) from two *lofts*. All birds involved in the experiment were trained according to the widowhood method and flew during the season, earning ACE points. The birds in this study were genotyped using an easy and inexpensive PCR-RFLP method by which three polymorphic sites in the *LDHA* gene g.2582481G>A, g.2583935G>A and g.2584057C>T were determined. Statistical analysis including the association between the effect of the genotypes obtained and the ACE scores achieved in short (less than 400 km) and long (more than 500 km) distance flights showed that the polymorphic variation of g.2582481G>A potentially influenced flight performance at all flight distances, but gender was also a significant factor here, as female pigeons had statistically significantly higher flight performance, relative to males.

Polymorphism in the gene *DRD4*

Based on the results of studies in other animal and human species, the gene encoding dopamine receptor 4*(DRD4*) was analysed. In humans, mutations in this gene have been linked to various behavioural phenotypes, including autonomic nervous system dysfunction, attention deficit/hyperactivity disorder and the novelty-seeking personality trait. In pigeons, three exons and flanking intron fragments were analysed using Sanger sequencing. Four *single nucleotide polymorphism (SNP*) variations have been identified in this gene. Using the PCR-RFLP technique, it was shown that g.129954C>T; g.129456C>T have a significant effect on the flight ability of birds expressed in ACE scores. It was also shown that the change g.129456C>T and the T allele in the double combination g.129954T; g.129456T significantly influenced pigeon performance over short distances (less than 400 km) (Proskura et al., 2017). The highest ACE scores were achieved by pigeons with the *CTCT* genotype, the lowest by *CCCC*. In addition, the work Shao et al, 2019, showed that the region containing the *DRD4* gene is under selection pressure in sporting pigeons.

Polymorphism in the gene *F-Ker*

The study also analysed the *F-KER* gene, which encodes the keratin protein that builds birds' feathers. To identify polymorphic sites, four regions of this gene were sequenced in two sport pigeons and two controls. Two polymorphic sites have been demonstrated: in the 5'UTR region and the site encoding the keratin protein (Dybus and Haase, 2011). Subsequently, a polymorphic substitution g.701T>G in the 83rd codon of the amino acid chain changing cysteine (C) to glycine (G) in the protein sequence was selected as significant, which showed a significant effect on the long-distance flight performance of pigeons expressed in the average ACE score. In a study with a sample size of 123 birds, birds with the TT genotype achieved statistically significantly more ACE scores on long distances (P = 0.0243), while individuals with the GT genotype performed better on short distances, but without statistical significance (Proskura et al., 2017).

Polymorphisms in genes *LPR8* and *GSR*

Work is currently underway to evaluate potentially important markers for pigeonpea flight in the *LRP8* gene, which encodes the LDL8 receptor-related protein (also known as apolipoprotein E receptor 2 or ApoER2), a member of the low-density lipoprotein receptor family. LRP8 and its ligand Relin are essential for the induction of *long term potentiation* (LTP) and are crucial for learning, memory and cognitive function. In pigeons, a sense-change mutation changing glutamine (Q) to histidine (H) was identified in a conserved domain named the class A low-density lipoprotein receptor domain. This mutation has the potential to alter the helix-forming ability and affinity of lipids for further interaction and normal function of this protein. Therefore, a mutation in the *LRP8* gene may alter certain pathways in a part of the brain - the hippocampus - to improve spatial memory ability in homing pigeons. The frequency of the identified mutation (H allele) in racing pigeons is more than 81%, compared to only 24% in other pigeons. The favourable variant for racing pigeons is variant H. However, work is still underway to assess the impact of the identified mutation on the flight performance of racing pigeons.

In turn, the *GSR* gene encodes a protein that catalyses the reduction of glutathione disulphide (GSSG) to the sulfhydryl form of glutathione (GSH) by utilising the flavin adenine dinucleotide (FAD) prosthetic group and reduced nicotinamide adenine dinucleotide phosphate (NADPH) to reduce one mole equivalent of GSSG to two mole equivalents of GSH. Glutathione is a key molecule in the fight against oxidative stress and can prevent damage to important cellular components caused by reactive oxygen species such as free radicals,

peroxides, lipid peroxides and heavy metals. In pigeons, the GSR protein undergoes differential expression in the retina and thickening over the beak (cere) depending on a mutation located 410 bp downstream of the *GSR* gene, which has been linked to magnetoreceptivity, or so-called revertance. In homing pigeons, there is a predominance of the favourable T allele, but already in heterozygous CT individuals, problems with magnetorecognition are observed, and therefore the C allele is generally unfavourable in homing pigeon populations.

Polymorphisms in other candidate genes

A 2018 study by Dybus et al. analysed the relationship between SNP-type variations in genes encoding myostatin*(MSTN*), alphaglobin*(AGLOB*) and lactate dehydrogenase subunit H*(LDHB*) and flight performance in racing pigeons. A total of 123 individuals (63 males, 60 females) were used in the study. Four SNPs were analysed using the PCR-RFLP method. *AGLOB*: g.2899462C>T, *LDHB*: g.564756A>G, g.564102A>G and *MSTN*: g.11440232C>T. The results of short flights (less than 400 km) and long flights (more than 500 km), as well as all flights together, were used in the association studies: 2589 including 1463 short and 1126 long). Achievements were qualified using the ACE scale. However, there was no significant effect of the polymorphic changes studied on the flight performance of the pigeons. Recent studies have demonstrated the potential impact of the *CRY1* gene encoding a conserved flavinadenine dinucleotide binding protein that is a regulator of the circadian clock. Polymorphic changes in this gene have so far been linked to sleep and wakefulness disorders. In the study by Dybus et al. (2021) showed a potential association of the c.1086+287_1086+288delAGinsTT polymorphic change with average ACE scores (Dybus i in., 2021).

Application of high-throughput techniques in the search for changes in the pigeon genome resulting from selection pressures

The development and refinement of high-throughput next-generation sequencing techniques has made it possible to undertake studies aimed at understanding selection signatures, i.e. sites in the pigeon genome that are influenced by selection pressures in pigeon populations. By sequencing whole genomes and comparing them with the genomes of breeds selected for exterior traits, regions in the genes were selected: *CASK*, *LRP8*, *GSR*, *AVPR1A*, *SSBP3*, *LDLRAD1*, *TCEANC2*, *CDCP2*, *CPT2*, *LOC10208909*, *MRPL37*, *DMRTB1*, *GLIS1*, *MAGOH*, *MTUS2*, (Gazda et al., 2018; Shao et al., 2020), which should potentially be related to flight performance of racing pigeons. Therefore, among other things, an attempt was made to assess polymorphic changes in the *LRP8* and *GSR* genes . In turn, transcriptomic analysis of pigeon brain sections, including the hippocampus, visual and olfactory lobe using the RNASeq technique, enabled the identification of further genes: *MFSD2A*, *KIRREL3*, *KCNAB2*, *MAPL8IP2* potentially affecting the flight ability of sport pigeons (Shao et al., 2020). However, apart from the two indicated genes, no other gene has so far been analysed for use as a potential marker of pigeon flight ability.

Existing technological solutions

The current commercially available markers on the Polish as well as the European market for the *LDHA* (g.2582481G＞A) and *DRD4* (g.129954C>T; g.129456C>T) genes are off-patent, and information on their impact has so far only been described in scientific publications. Assessment of the above markers is extremely simple for the breeder, as the biological material used for DNA isolation can be peripheral blood, feathers or swabs taken from the beak. DNA is isolated using commercially available isolation kits, the gene fragment to be analysed is amplified in thermocyclers available in molecular genetics laboratories using primers described

previously in scientific publications. To determine genotype, amplicons are sequentially subjected to restriction cutting using enzymes that digest the DNA sequence only in specific regions, which determines after electrophoretic separation the stripe pattern characteristic of the polymorphism. Such an assessment can be performed in as little as a few days after the biological material is submitted. Therefore, in many laboratories in Poland and around the world, it is possible to carry out such genetic tests to identify individuals with favourable gene variants related to pigeon flight performance in order to consciously manage their breeding plans.

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SUMMARY

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Key words: racing pigeons, flight performance, racing