Two new PCR-RFLPs in the domestic pigeon (*Columba livia* var. *domestica*) lactate dehydrogenase A (*LDH-A*) gene (Brief report)

(Zwei neue PCR-RFLPs des Laktat-Dehydrogenase A (*LDH-A*) Gens bei der domestizierten Taube (*Columba livia* var. *domestica*))

**Background:** Traditional selection of racing pigeons has been focusing on spatial orientation, velocity, and endurance of flight. *LDHA* gene is involved in aerobic and anaerobic metabolism of the muscle tissue (VAN HALL et al., 1999). Mutations in the *LDHA* gene can potentially diversify the homing performance of racing pigeons. Previously, two polymorphic sites of *LDHA* gene have been identified (DYBUS and KMIEĆ, 2002, DYBUS et al., 2006).

**Procedures:**

**Prime sequences:**  
*LDHA45F* 5’-AACGACAAGAGCAACGTGAAG-3’  
*LDHA45R* 5’-CAAGAGCCCATTTCACCTACA-3’

DNA was isolated from blood samples of 145 domestic pigeons (68 homing of 14 lofts, 77 non-homing of 4 lofts) using *MasterPure™* kit (Epicentre Technologies). The PCR-RFLP method was used for detecting polymorphisms. Therefore, PCR primers were designed to produce 1112 base pair amplification product, encompassing a part of exons 4 and 5 with intervening intron, using Primer3 software. The PCR mixture contained 60 ng of genomic DNA, 0.1 µM of each primer, 1xPCR buffer, 1.5 mM MgCl₂, 200 µM dNTP and 0.3 units Taq-polymerase (*Eurx*) in a total volume of 15 µl. The following cycles were applied: 94 ºC/5 min, followed by 33 cycles at 94 ºC/30 sec, 60 ºC/40 sec, 72 ºC/90 sec, and final synthesis at 72 ºC/5 min. Amplified DNA samples were digested with *ApoI, BsuRI, HinfI, MspI, Mval, PvuII, Rsal, VspI* and *TaiI* restriction endonucleases. The digestion products were separated by horizontal electrophoresis through 2-4% agarose gels. Any observed variations of restriction patterns were characterized further and confirmed by sequence analysis using an ABI Prism Sequencer (Perkin-Elmer) and Chromas software. Distribution frequencies of genotypes and haplotypes were compared using χ² test (Fisher's Exact Test).

**Results:** In case of *HinfI* and *TaiI* enzymes RFLP were observed (Figure). Molecular basis of *LDHA/HinfI* and *TaiI* polymorphisms were T/G (at position 675) and A/G (at position 252) substitutions in intron 4 of *LDHA* gene, respectively. In case of *LDHA/HinfI* polymorphism frequencies of genotypes in the homing and non-homing group of pigeons were similar (χ²=1.89). (Table 1). Statistical significant differences between homing and non-homing pigeons were observed in genotypes frequencies for *LDHA/TaiI* polymorphism (χ²=20.54) and also haplotypes frequencies (χ²=29.41) (Table 2). The higher frequency of *LDHA/TaiI* detected in the group of homing pigeons and its effect on homing performance should be verified in further, more advanced studies.
Figure: Representative results of \textit{LDHA/Hinf}I and \textit{LDHA/Tai}I analysis.
(P: PCR product, M: DNA Ladder Plus MBI Fermentas)

Table 1

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Group of pigeons</th>
<th>n</th>
<th>Genotype frequency</th>
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</thead>
<tbody>
<tr>
<td>\textit{LDHA/Hinf}I</td>
<td>non-homing</td>
<td>77</td>
<td>0.247 TT ((n=19))</td>
</tr>
<tr>
<td></td>
<td>homing</td>
<td>68</td>
<td>0.368 TT ((n=25))</td>
</tr>
<tr>
<td>\textit{LDHA/Tai}I</td>
<td>non-homing</td>
<td>77</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>homing</td>
<td>68</td>
<td>0.044 AA ((n=3))</td>
</tr>
</tbody>
</table>

** - \(P \leq 0.01\), * - \(P \leq 0.05\).

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>TT/GG ((n=25))</th>
<th>TG/AG ((n=12))</th>
<th>TG/GG ((n=8))</th>
<th>GG/AA ((n=3))</th>
<th>GG/AG ((n=10))</th>
<th>GG/GG ((n=10))</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>68</td>
<td>0.368**</td>
<td>0.176**</td>
<td>0.118*</td>
<td>0.044</td>
<td>0.147</td>
<td>0.147**</td>
</tr>
<tr>
<td>N-H</td>
<td>77</td>
<td>0.247</td>
<td>0.013**</td>
<td>0.364*</td>
<td>-</td>
<td>0.052</td>
<td>0.324*</td>
</tr>
</tbody>
</table>

** - \(P \leq 0.01\), * - \(P \leq 0.05\); H – homing; N-H- non-homing

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References


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