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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:
Primer 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*^{TM} 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 $MgCl_2$, 200 µM dNTP and 0.3 units Taq-polymerase (*Eur*$_{\chi}$) in a total volume of 15 µl. The following cycles were applied: 94ºC/5 min, followed by 33 cycles at 94ºC/30 sec, 60 ºC/40 sec, 72 ºC/90 sec, and final synthesis at 72 ºC/5 min. Amplified DNA samples were digested with *Apo*I, *Bsu*RI, *Hinf*I, *Msp*I, *Mva*I, *Pvu*II, *Rsa*I, *Vsp*I and *Tai*I 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 on an ABI Prism Sequencer (Perkin-Elmer) and Chromas software. Distribution frequencies of genotypes and haplotypes were compared using χ^2 test (Fisher's Exact Test).

Results: In case of *Hinf*I and *Tai*I enzymes RFLP were observed (Figure). Molecular basis of *LDHA/Hinf*I and *Tai*I 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/Hinf*I polymorphism frequencies of genotypes in the homing and non-homing group of pigeons were similar (χ^2=1.89). (Table 1). Statistical significant differences between homing and non-homing pigeons were observed in genotypes frequencies for *LDHA/Tai*I polymorphism (χ^2=20.54) and also haplotypes frequencies (χ^2=29.41) (Table 2).
The higher frequency of *LDHA/Tai*I^A detected in the group of homing pigeons and its effect on homing performance should be verified in further, more advanced studies.

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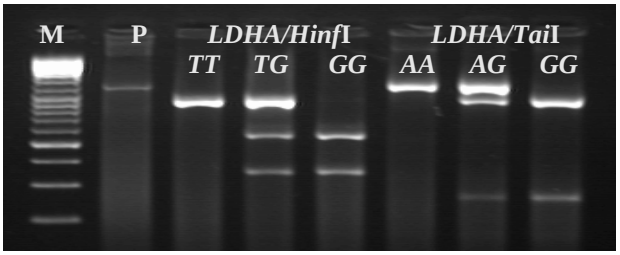

Figure: Representative results of *LDHA/Hinf*I and *LDHA/Tai*I analysis
(P: PCR product, M: DNA Ladder Plus MBI Fermentas)

Table 1
Frequencies of *LDHA/Hinf*I and *LDHA/Tai*I genotypes

Polymorphism	Group of pigeons	n	Genotype frequency		
*LDHA/Hinf*I	non-homing	77	0.247 TT (n=19)	0.376 TG (n=29)	0.376 GG (n=29)
	homing	68	0.368 TT (n=25)	0.294 TG (n=20)	0.338GG (n=23)
*LDHA/Tai*I	non-homing	77	-	0.065 AG** (n=5)	0.935 GG (n=72)
	homing	68	0.044 AA (n=3)	0.324 AG** (n=22)	0.632 GG (n=43)

** - $P \leq 0.01$, * - $P \leq 0.05$.

Table 2
Frequencies of *LDHA/Hinf*I-*Tai*I haplotypes

Group	n	Haplotype frequencies (*Hinf*I-*Tai*I)					
		TT/GG	TG/AG	TG/GG	GG/AA	GG/AG	GG/GG
H	68	0.368 (n=25)	0.176** (n=12)	0.118* (n=8)	0.044 (n=3)	0.147 (n=10)	0.147* (n=10)
N-H	77	0.247 (n=19)	0.013** (n=1)	0.364* (n=28)	- -	0.052 (n=4)	0.324* (n=25)

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

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