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Polymorphism in *dopamine receptor D4* gene is associated with pigeon racing performance

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Description: Personality traits are considered to have a major influence on sport achievements.^{1,2} Polymorphisms within *dopamine receptor D4* (*DRD4*) gene are known to be associated with those traits.³ Here, we report SNPs in the pigeon *DRD4* gene and their association with racing performance during short races.

Sequencing and genotyping: Sequencing of PCR products (Table S1) covering the complete exon 3 and parts of introns 2 and 3 from the *DRD4* gene revealed four SNPs: the intronic g.129954C>T (ss1751581452), two missense mutations g.129562A>C (p.Arg139Ser; ss1751581453) and g.129456C>T (p.Leu175Phe; ss1751581455) and the synonymous g.129484T>C (ss1751581454). The last one was not further considered. The study involved 123 racing pigeons derived from two unrelated pigeon flocks. SNP g.129562A>C and SNP g.129456C>T were in complete linkage disequilibrium, and only the latter was considered in association analyses. The frequencies of SNPs and of combined genotypes of the *DRD4* gene are presented in Table S2.

Association analysis: Ace points acquired by pigeons in races were calculated as described before⁴ on the basis of 1380 race records from eight short (<400 km) and six long (>500 km) races. Associations between ace points and SNPs were analysed with a mixed model using the lme4 package for R software. The model included effects of genotype, breeder, gender, weather at the start, weather at the end, race category, individual (to account for repeated observations) and a random polygenic component. The additive relationship matrix was based on a three-generation pedigree using the kinship2 R package. A detailed description is provided in Appendix S1.

Comments: This study reports an association analysis of pigeon racing performance with SNP g.129954C>T, SNP g.129456C>T and the combined genotype of both loci (Table S3). We found the g.129456C>T SNP to be associated with pigeon racing performance for short races ($P = 0.0327$). Heterozygotes acquired significantly more ace points than did CC individuals. Moreover, the combined genotype of those loci also influenced pigeon racing performance in short races category ($P = 0.0018$). The CTCT genotype was linked to the highest ace points mean and the CCCC genotype to the lowest mean. Possible dependence of pigeon racing ability on genetic variation has been reported before⁵ and, also, association of the *LDHA* polymorphism with performance of pigeons in races at short distances has been indicated recently.⁴ In sum, our data suggest that *dopamine receptor D4* variants could play an important role in pigeon performance during short races.

References

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Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Statistical analysis.

Table S1 Primer sequences and restriction enzymes used for the PCR-RFLP genotyping of the selected single nucleotide polymorphisms in pigeon *DRD4* gene.

Table S2 Genotypic frequencies of selected SNPs in pigeon *DRD4* gene.

Table S3 Effect of *DRD4* genotypes on Ace Points mean values acquired by pigeons on races.

Appendix S1. Statistical Analysis

Associations between AP and SNPs were analysed with following mixed model using lme4 package for R software:

$$y = \mu + g + s + h + k + ps + pp + k + i + a + e$$

where:

y - analysed trait;

μ - overall mean;

g - effect of genotype (AA, AB, BB);

s - gender effect (males, females);

h - breeder effect (A, B);

ps - weather at the start effect (sunny, changeable);

pp - weather at the end effect (sunny, changeable, rainy, windy, cloudy);

k - race category effect (short, long);

i - effect of individual accounting for repeated observations (1-123);

a - a random polygenic component account for all known pedigree relationships (3 generations);

e - random error.

Pedigree data was handled using Pedigree Viewer 6.5b. The additive relationship matrix was based on a three-generation pedigree using the kinship2 R package.

Table 1 The *P*-values of fixed factors included in the statistical model excepting genotype effect

Factor	All races	Short races	Long races
Gender	0.0005	0.0070	0.0044
Breeder	0.2887	0.6830	0.0438
Weather at the start	0.0090	0.3027	0.0190
Weather at the end	0.0000	0.0002	0.0962
Race category	0.7899	NA	NA

Table S1 Primer sequences and restriction enzymes used for the PCR-RFLP genotyping of the selected single nucleotide polymorphisms in pigeon *DRD4* gene.

SNP ¹	Effect ²	Primer sequences ³	T _a ⁴	Length	RE ⁵
g.129954C>T	intronic	F-tttgggatcgctcgcttacc	60°C	191 bp	<i>Bst4CI</i>
ss1751581452		R- atgacaggggatgctacagc			
g.129562A>C	p.(Arg139Ser)	F- tttgggatcgctcgcttacc	60°C	679 bp	<i>BstC8I</i>
ss1751581453		R- gcaggacaacacagcgtctc			
g.129456C>T	p.(Leu175Phe)	F- gggccaacaggaagctctat	60°C	294 bp	<i>MnII</i>
ss1751581455		R- gcaggacaacacagcgtctc			

¹The descriptions of SNPs refer to their positions within the minus strand of Cliv_1.0 Primary Assembly (GenBank: NW_004973198.1). ²Predicted effect of the SNP on amino acid sequence of pigeon dopamine receptor D4 (Genbank: XP_005509894). ³Second primer pair was used for sequencing. ⁴Annealing temperature. ⁵Restriction enzyme.

Table S2 Genotypic frequencies of selected SNPs in pigeon *DRD4* gene.

Polymorphism	Genotype			Allele	
g.129954C>T	<i>CC</i>	<i>CT</i>	<i>TT</i>	<i>C</i>	<i>T</i>
	0.642	0.309	0.049	0.797	0.203
g.129562A>C	<i>AA</i>	<i>AC</i>	<i>CC</i>	<i>A</i>	<i>C</i>
	0.854	0.146	-	0.927	0.073
g.129456C>T	<i>CC</i>	<i>CT</i>	<i>TT</i>	<i>C</i>	<i>T</i>
	0.854	0.146	-	0.927	0.073
Combined genotypes of loci g.129954C>T and g.129456C>T					
	<i>CCCC</i>	<i>CCCC</i>	<i>CTCC</i>	<i>CTCT</i>	<i>TTCC</i>
	0.520	0.122	0.285	0.024	0.049

[†]Combined genotype of both loci: first 2 letters refer to the g.129954C>T genotype and next 2 letters refer to the g.129456C>T.

Table S3 Effect of *DRD4* genotypes on Ace Points mean values acquired by pigeons on races.

SNP	Genotype	All races			Short races			Long races		
		RR	AP	SE	RR	AP	SE	RR	AP	SE
g.129954C>T	<i>CC</i>	876	30.60	1.24	423	30.28	1.79	453	30.88	1.72
	<i>CT</i>	439	32.78	1.78	212	33.34	2.58	227	32.25	2.46
	<i>TT</i>	65	34.18	4.69	32	29.24	6.39	33	38.97	6.84
		<i>P</i> = 0.9991			<i>P</i> = 0.6521			<i>P</i> = 0.5772		
g.129456C>T	<i>CC</i>	1170	30.74	1.07	569	29.62	1.52	601	31.80	1.50
	<i>CT</i>	210	35.45	2.69	98	40.40	4.06	112	31.12	3.55
		<i>P</i> = 0.0843			<i>P</i> = 0.0327			<i>P</i> = 0.8605		
Combined ¹	<i>CCCC</i>	698	30.02 ^A	1.38	340	29.08 ^A	1.96	358	30.91	1.95
	<i>CCCT</i>	178	32.85	2.80	83	35.24 ^A	4.27	95	30.77	3.70
	<i>CTCC</i>	407	31.43 ^B	1.80	197	30.63 ^A	2.59	210	32.18	2.51
	<i>CTCT</i>	32	49.92 ^A	7.97	15	68.95 ^B	9.38	17	33.12	11.24
	<i>TTCC</i>	65	34.18	4.69	32	29.24 ^A	6.39	33	38.97	6.84
		<i>P</i> = 0.0357			<i>P</i> = 0.0018			<i>P</i> = 0.3626		

RR – race records, AP – ace points, SE – standard error.

Superscript letters indicate statistically significant differences – $P \leq 0.05$

¹Combined genotype of both loci: first 2 letters refer to the g.129954C>T genotype and next 2 letters refer to the g.129456C>T.

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