AGRICULTURAL BIOLOGY,
2018, V. 53, № 6, pp. 1162-1168
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 636.52/.58:636.082.2

doi: 10.15389/agrobiology.2018.6.1162eng
doi: 10.15389/agrobiology.2018.6.1162rus

SELECTION OF PREPARENTAL LINES OF PLYMOUTH ROCK CHICKEN USING MARKER GENES *K* AND *k*

D.N. EFIMOV[1], Zh.V. EMANUYLOVA[1], E.V. ZHURAVLEVA[2], A.V. EGOROVA[3], V.I. FISININ[3]

[1]*Smena Breeding and Genetic Center*, pos. Bereznyaki, Moscow Province, 141357 Russia, e-mail zhanna.emanujlov@mail.ru (✉ corresponding author), dmi40172575@yandex.ru;
[2]*Ministry of Science and Education of the Russian Federation*, 11, ul. Tverskaya, Moscow, 125009 Russia, e-mail zhuravla@yandex.ru;
[3]*Federal Scientific Center All-Russian Research and Technological Poultry Institute RAS,* 10, ul. Ptitsegradskaya, Sergiev Posad, Moscow Province, 141311 Russia, e-mail vnitip@vnitip.ru, egorova@vnitip.ru
ORCID:
Efimov D.N. orcid.org/0000-0002-4152-2476
Emanuylova Zh.V. orcid.org/0000-0002-8855-2947
Zhuravleva E.V. orcid.org/0000-0002-3253-0730
Egorova A.V. orcid.org/0000-0001-9981-5768
Fisinin V.I. orcid.org/0000-0003-0081-6336
The authors declare no conflict of interests
Acknowledgements:
Supported financially by FANO (the Ministry of Science and Higher Education) of Russia (subprogram of Federal Program for the Development of Agriculture 2017-2025). Additional State Task № 007-00507-18-02 of 10.23.2018
Received July 16, 2018

Abstract

Chicken lines with marker genes of sex-linked economically important traits are of a particular breeding interest. Here we report on the first results on the creation of an autosex maternal parental form of dual purpose Plymouth Rock breed by sequential selection of chickens from experimental Russian breeding lines. The paternal and maternal pre-parental Plymouth Rock lines were selected for productivity indices and for growth rate of wing feathers. Genotypes with slow (line X4) and fast (line X3) feathering rates were maintained via phenotypic evaluation at 1 day of age and culling individuals with fast and slow feathering rate, respectively; heterozygous males and their progeny were also culled. Chicks with slow feathering rate feature poor development of tectrix and remex, the tectrix being longer or equal to the remex; chicks with fast feathering rate feature the well-developed tectrix which is shorter than the remex. The quantitative PCR technique (real-time polymerase chain reaction) was also used to identify individuals with homo- and heterozygous sex-linked *K* and *k* alleles; the analyses were performed using feather pulp samples. Live bodyweight at 5 weeks of age increases significantly ($p \le 0.001$) in the selected individuals of generation F_5 compared to F_1, i.e in X3, the index is 13.6 % higher in males and 15.4 % higher in females; and in X4, it is 15.2 and 14.2 % higher ($p \le 0.001$), respectively. Breast muscle score is improved by 7.3 and 5.0 % in males and by 6.2 and 5.0 % ($p \le 0.001$) in females of X3 and X4 lines, respectively; leg muscle scores is 7.5 and 5.3 % higher in males and 10.5 and 2.5 % higher ($p \le 0.001$) in females. The progress in the reproductive performance also occurs: egg production during 52 weeks of age was improved by 4.1 eggs per hen (3.39 %) in X3 line and by 4.7 eggs (3.7 %, $p \le 0.001$) in X4 line; percentage of eggs suitable for incubation was 0.6 and 1.0 % higher, and hatch was 0.5 and 2.2 % higher in X3 and X4 lines, respectively. These results in better chick output per hen (by 3.9 and 7.0 % in X3 and X4 lines). Thus the selection improved the most of the productivity indices in every successive generation; the resulting paternal (X3) and maternal (X4) lines differed from the initial purebred chicken in the productive performance and carried marker genes of slow (X4) and fast (X3) feathering rate. Their crossing brings to a maternal form which is autosex for *K* and *k* genes with sexing accuracy 99.6 %.

Keywords: Plymouth Rock breed, breeding, chicken lines, feathering type, genotype, marker genes, feathering genes, *K* and *k* alleles, sexing, productivity

The challenge of planning and identification of chicken sex in commercial egg and meat poultry farming remains acute [1]. Study of sex genes allows practitioners to implement breeding programs [2, 3]. Routine sex determination methods in poultry are sexing by cloacae and fecal steroids, laparoscopy, and karyotyping. However, such procedures are not reliable, expensive, take long time;

and some of them are painful and even dangerous for poultry.

It is known that sex genes in poultry are located in sex chromosomes [4-6], one Z and one W chromosome in females, and two Z chromosomes in males [7, 8]. Recently, DNA technologies for sex identification have been developed [9, 10]. Genes marking autosexed 1 day old chicks are successfully used in selection of egg-laying and meet-type chickens [11, 12]. DNA markers also allow identification of chromosome regions controlling main properties and critical traits, genetic polymorphisms [13-15]. Study of such loci and their functional activity [16] attributes to perspectives of more effective targeted breeding for economically significant traits [14, 15, 17-19].

Presence of autosexing markers significantly facilitates separation of 1 day old chickens of both sexes. Selection by coat color (color sex) and by growth of tectrix and remex (feather sex) is possible [17, 20-22]. Feathering is under the same genetic control as sexual differentiation. Slow feathering is determined by *K* allele (dominant), fast feathering by *k* allele (recessive). Crossing of fast feathering heterozygous male chickens with slow feathering female chickens give offspring with slow feathering males and fast feathering females. When autosexing method is used, accuracy of separation of 1 day old resultant broiler hybrids and chickens of the female parental line into males and females is significantly increased with double reduction of spent time. Besides, sex determination accuracy is not decreased with growth of chickens. Evidently, creation of specialized chicken lines which are the carriers of marker genes of sex-linked qualitative traits is of special interest.

Here we report our first results on the creation of an autosex maternal parental form of Plymouth Rock breed based on the experimental Russian breeding lines accounting for homo- and heterozygous state of sex-linked *K* and *k* alleles. This form would be further used to create the cross of meat-type chicken with autosex maternal parental form.

Purpose of the research was to assess and to select poultry of paternal and maternal lines by the growth rate of wing feathers and by productive capacity to create cross of meat-type chicken with autosex maternal parental form.

Techniques. Birds of Plymouth Rock breed (experimental paternal line of maternal parental form X3 and experimental maternal line of maternal parental form X4) were used in the study under farming conditions (Selection and Genetic Center Smena, Moscow Region, 2014-2018). Birds were kept in floor pens. Feeding and keeping conditions were as per recommendations (Methodological guidelines for feeding of agricultural poultry. Sergiev Posad, 2015) and technological reglament (Methodological guidelines for technical designing of poultry farms RD-APK 1.10.0504-13. Moscow, 2013).

For selection, 10-15 nestles (13 females and one male in each) were formed in each line, and at least 364 descendants were assessed in each gneration (F_1-F_5). Selection group of X3 line consisted of *kk* enhancers homozygous for fast feathering gene and neutral individuals accounting for valuable economical traits (live body weight, chest and leg weights, feed conversion, egg-laying performance). In X4 line, *KK* individuals homozygous for slow feathering gene, were selected for egg-laying, yield of hatching eggs, their weight, hatchability, live body weight of poultry, chest and leg muscle scores, and feed conversion).

Productive capacity in nestles was accounted individually. Live body weight, egg-laying capacity, egg weight, puberty, and chest and leg muscle scores were determined by commonly accepted methodologies [23]. Individual caps and standard wing band set were used to control pedigree origin at incubation.

Feathering type was visually established in 1 day old chickens separated by sex using Japanese method (by the presence and form of sex bump), at slow growth rate of wind the tectrix feathers are longer than or the same as remex ones, at fast growth rate the tectrix are shorter than remex and are well developed.

Molecule typing of homo- and heterozygotes by *K* and *k* alleles was done in CJSC Sintol (Moscow) with the use of qualitative real-time PCR (qPCR), relevant primers and amplifying modes [24]. Feather pulp samples were collected to extract DNA [24]. Multiplex qPCR was conducted using an ANK-32M device (Institute of Analytic Instrument Engineering RAS, Russia) with sequencing of amplification products using a genetic analyzer Nanophore 05 (Institute of Analytic Instrument Engineering RAS, Russia) according to the manufacturer's protocols [24].

Software Statistica 10.0 (StatSoft, Inc., USA) and Microsoft Excel were used for statistical processing. Results were presented as means (*M*) and standard errors of means (±SEM). Statistical significance of the compared indicators was determined by *t*-Student criteria. Statistically significant values were at $p \le 0.05$. Values m_r (standard error of mean correlation coefficient of the analyzed traits by line) and t_r (significance of correlation significance) were calculated based on the following formulae:

$$m_r = \frac{1-r^2}{\sqrt{n}},\ t_r = \frac{r}{m_r} = \frac{r\sqrt{n}}{1-r^2},$$

where *n* is the number of birds in a sample; the values were considered statistically significant at $p \le 0.05$.

Results. Genotype determining slow feathering was maintained by rigorous selection for phenotype and by exclusion of fast feathering 1 day old chickens. Homozygosity of males in line X4 for slow feathering was verified by assessment of its manifestation in males, selected to form nestles, by estimation of the offspring until reproduction of initial lines. Both heterozygous males and their offspring were excluded. Genotypes with fast feathering was similarly maintained; homozygosity of males in line X3 was controlled by assessment of males destined for nestles by quality of offspring until reproduction of initial lines, without further use of heterozygous males and their offspring.

1. Dynamics of proportion (%) of low and fast feathering 1 day old Plymouth Rock chickens in experimental maternal parental form during selection (F_1-F_5) (Selection and Genetic Center Smena, Moscow Region, 2014-2018)

Generation	Feathering at age of 1 day	Line X3		Line X4	
		males	females	males	females
F_1	Slow	88.9	99.1	92.4	81.9
	Fast	11.1	0.9	7.6	18.1
F_2	Slow	77.2	82.7	99.3	84.9
	Fast	22.8	17.3	0.7	15.1
F_3	Slow	66.6	59.2	100	99.5
	Fast	33.4	40.8	0	0.5
F_4	Slow	31.3	26.9	100	99.9
	Fast	68.7	73.1	0	0.1
F_5	Slow	0	0	100	100
	Fast	100	100	0	0

Note. X3 — paternal line of maternal parental form, X4 — maternal line of maternal parental form.

For consolidation of the lines X3 and X4 population by feathering for a number of years, males in nestles were assessed by feathering rate of 1 day old offspring chickens (in line X3 the total number was from 2319 in F_1 to 10880 in F_5; in line X4 the number was from 2466 to 14916, respectively) (Table 1). These results are coherent with other known reports [21, 25-27]. Inside lines X3 and X4, homogeneity by wing feathering rate was reached in different times, but finally, in 2018 feathering rate in all poultry of X3 line was fast, and of X4 slow (see Table 1). The autosex poultry of maternal parental form (for *K* and *k*) with sexing accuracy of 99.6 % was produced from their crossing.

Assessment of productive performance of poultry from the experimental lines had shown (Table 2) that live body weight of 5 week old chickens of maternal parental form of X3 line was higher than in maternal parental form of X4 line: in males by 6.21 and 4.79 %, in females by 7.09 and 8.28 % ($p \leq 0.001$) in F_1 and F_5, respectively. Body weight of F_5 chickens aged 5 weeks was higher than in F_1, in line X3 by 13.6 and 15.4 % ($p \leq 0.001$) (for males and females), in line X4 by 15.2 and 14.2 % ($p \leq 0.001$). Chest muscle score in F_5 chickens had increased in line X3 for males by 7.3 %, in line X4 by 5.0 %, for females by 6.2 and 5.0 % ($p \leq 0.001$). The same trend was noted in leg muscle score: increase in males by 7.5 and 5.3 %, and in females by 10.5 and 2.5 % ($p \leq 0.001$) in lines X3 and X4, respectively. The total number of chickens estimated for body weight, leg and chest muscle scores in lines X3 and X4 were 2210 and 2370 for F_1, and 10700 and 14810 for F_5 (see Table 2).

2. Improvement of productive performance of Plymouth Rock experimental lines of maternal parental form during selection (F_1-F_5) (Selection and Genetic Centre Smena, Moscow Region, years 2014-2018)

Trait	Sex	Line X3		Line X4	
		F_1	F_5	F_1	F_5
Body weight of 5 week old chicks, kg	Males	1.54±0.004	1.75±0.004	1.45±0.005	1.67±0.005
	Females	1.36±0.003	1.57±0.004	1.27±0.003	1.45±0.004
Chest muscle score, points	Males	4.10	4.40	4.00	4.2
	Females	4.05	4.30	4.00	4.2
Leg muscle score, points	Males	2.00	2.15	1.90	2.00
	Females	1.90	2.10	2.00	2.05
Chicken survivability, %		96.8	97.0	97.0	97.1
Egg-laying performance per initial laying bird, units:					
for 30 weeks		17.10±0.038	19.70±0.375	19.80±0.377	22.00±0.321
for 52 weeks		121.00±1.750	125.10±1.235	126.40±1.730	131.10±1.234
Egg weight of 30-week old females, g		56.90±0.171	57.20±0.176	56.50±1.174	56.90±0.177
Puberty, days		183.40±0.415	184.00±0.396	183.10±0.418	183.70±0.397
Yield of hatching eggs, %		91.5	92.1	91.8	92.8
Egg fertility, %		89.8	91.7	91.6	93.0
Hatching rate, %		74.5	75.0	76.3	78.5
Chickens per layer		82.5	86.4	88.5	95.5
Female survivability, %		96.8	97.0	97.0	97.1

Note. X3 — paternal line of maternal parental form, X4 — maternal line of maternal parental form.

Reproductive properties have been improved during 5 years of selection in lines X3 and X4. The egg-laying capacity during 52 weeks of life had increased by 4.1 (3.39 %) and 4.7 eggs (3.7 %) ($p \leq 0.001$); yield of hatching eggs by 0.6 and 1.0 %, hatching rate by 0.5 and 2.2 %, which had increased hatching rate per one layer by 3.9 and 7.0 % (the sample size of $n = 1040$). Annual selection effect in lines X3 and X4 for body weight on day 35 comprised 2.7 % (42 g) and 3.0 % (44 g) in males, 3.1 % (42 g) and 2.8 % (36 g) in females; for chest muscle score 1.46 and 1.0 % (males), 1.23 and 1.00 % (females); for egg-laying performance 0.68 and 0.74 % (lines X3 and X4). Thus, the majority of chicken economic traits became better from generation to generation upon intensive selection. Birds of these lines, being carriers of relevant marker genes of slow (line X4) and fast (line X3) feathering rates, differed from the initial genetic material by productive properties and could be used for production of cross lines with improved productive properties. Crossing of these lines had resulted in maternal parental autosexed form for genes of slow (*K*) and fast (*k*) feathering rates with 99.6 % sexing accuracy.

Correlation coefficients between the body weight of 35 day old chicken and chest muscle score were high, positive and slightly differed both between males and females, and between the lines or generations (Table 3). Positive and valid correlation was preserved between the live body weight and leg muscle score, but its value was slightly lower. High positive and valid relationship was noted between the live body weight in chickens aged 35 days and width of chest, length of thigh s. These results are in line with regularities described for a relationship of the body weight with other indicators at early age [28].

3. Correlations between body weight and other meat yield indicators in 35 day aged Plymouth Rock chickens in the experimental lines of maternal parental form during selection (F_1-F_5) (Selection and Genetic Centre Smena, Moscow Region, years 2014-2018)

Line	Sex	F_1		F_5	
		$r \pm m_r$	t_r	$r \pm m_r$	t_r
		Chest muscle score			
X3	Males	0.689±0.007	98.37	0.692±0.012	57.67
	Females	0.611±0.008	76.43	0.616±0.011	56.07
X4	Males	0.669±0.007	100.53	0.685±0.010	68.50
	Females	0.701±0.007	126.64	0.707±0.009	78.59
		Leg muscle score			
X3	Males	0.329±0.012	27.65	0.337±0.010	33.70
	Females	0.344±0.011	30.60	0.349±0.010	34.96
X4	Males	0.509±0.009	56.99	0.495±0.013	38.08
	Females	0.471±0.009	52.37	0.483±0.007	69.03
		Chest width			
X3	Males	0.678±0.014	48.43	0.682±0.012	56.83
	Females	0.692±0.010	69.27	0.699±0.013	53.78
X4	Males	0.665±0.016	41.56	0.672±0.015	44.80
	Females	0.659±0.012	54.91	0.656±0.011	59.77
		Thigh length			
X3	Males	0.452±0.022	20.55	0.480±0.024	20.00
	Females	0.427±0.029	17.77	0.433±0.025	17.32
X4	Males	0.464±0.021	22.09	0.461±0.020	23.05
	Females	0.478±0.029	16.50	0.485±0.027	17.97

Note. X3 — paternal line of maternal parental form, X4 — maternal line of maternal parental form.

Thus, we have improved the majority of economic traits due to selection in paternal (X3) and maternal (X4) lines of maternal parental form in generations F_1-F_5, in these, only birds preserving marker alleles of slow (allele *K*, line X4) and fast (allele *k*, line X3) feathering rates were selected. Their crossing resulted in autosex (for alleles *K* and *k*) maternal parental form with sexing accuracy of 99.6 %.

REFERENCES

1. Chomaev A.M., Mityashova O.S., Tsygankov V.I. *Zootekhniya*, 2012, 8: 2-3 (in Russ.).
2. Bramwell P.K. Sexing chicks in the backyard flock. *The Poultry Site*. Available http://www.thepoultrysite.com/articles/95/sexing-chicks-in-the-backyard-flock/. Accessed December 02, 2018.
3. Cheng Y.H., Kuo T.E., Lee D.N., Weng C.F. Sex identification of the Black-faced Spoonbill (*Platalea minor*). *Zoological Studies*, 2006, 45: 104-113.
4. Clinton M. A rapid protocol for sexing chick embryos (*Gallus g. domesticus*). *Animal Genetics*, 1994, 25(5): 361-362 (doi: 10.1111/j.1365-2052.1994.tb00374.x).
5. Dawson D.A., Darby S., Hunter F.M., Krupa A.P., Jones I.L., Burke T. A critique of avian CHD-based molecular sexing protocols illustrated by a Z-chromosome polymorphism detected in auklets. *Molecular Ecology Notes*, 2001, 1(3): 201-204 (doi: 10.1046/j.1471-8278.2001.00060.x).
6. Ellegren H. Hens, cocks and avian sex identification. A quest for genes on Z or W? *EMBO Reports*, 2001, 2(3): 192-196 (doi: 10.1093/embo-reports/kve050).
7. Cerit H., Avanus K. Sex identification by CHDW and HDZ genes of avian sex chromosomes in *Nymphicus hollandicus. Turk. J. Vet. Anim. Sci.*, 2007, 31(6): 371-374.
8. Griffiths R.A., Korn R.M. A CHDI gene is Z chromosome linked in chicken *Gallus domesticus. Gene*, 1997, 197(1-2): 225-229 (doi: 10.1016/S0378-1119(97)00266-7).
9. Canon N.R., Canon N.R., Tell L.A., Needham M.L. Flow cytometric analysis of nuclear DNA for sex identification in three psittacine species. *Am. J. Vet. Res.*, 2000, 61(7): 847-850.
10. Cerit H., Avanus K. Sex identification in avian species using DNA typing methods. *World's Poultry Science Journal*, 2007, 63(1): 91-99 (doi: 10.1017/S0043933907001316).
11. Bondarenko V.Yu. *Materialy dokladov Konferentsii Rossiiskogo natsional'nogo otdeleniya WPSA* [Proc. Conf. of the Russian National Branch of WPSA]. Zelenograd, 2003: 46 (in Russ.).
12. Ustinova E.S., Gofman A.Yu. *Materialy IV Mezhdunarodnoi konferentsii «Ptitsevodstvo — mirovoi i promyshlennyi opyt»* [Proc. IV Int. Conf. "Poultry farming — global experience"]. Moscow,

2007: 274-276 (in Russ.).
13. Lessells S., Mateman C. Sexing birds using random amplified polymorphic DNA (RAPD) markers. *Molecular Ecology*, 2002, 7(2): 187-195 (doi: 10.1046/j.1365-294x.1998.00331.x).
14. Elferink M.G., Vallee A.A.A., Jungerius A.P., Crooijmans R.P.M.A., Groenen M.A.M. Partial duplication of the PRLR and SPEF2 genes at the late feathering locus in chicken. *BMC Genomics*, 2008, 9: 391 (doi: 10.1186/1471-2164-9-391).
15. Zhao J., Yao J., Li F., Yang Z., Sun Z., Qu L., Wang K., Su Y., Zhang A., Montgomery S.A., Geng T., Cui H. Identification of candidate genes for chicken early- and late- feathering. *Poultry Sci.*, 2016, 95(7): 1498-1503 (doi: 10.3382/ps.pew131).
16. Burt D.W. Chicken genome: current status and future opportunities. *Genome Res.*, 2005, 15(12): 1692-1698 (doi: 10.1101/gr.4141805).
17. Derks M.F.L., Herrero-Medrano J.M., Crooijmans R.P.M.A., Vereijken A., Long J.A., Megens H.-J., Groenen M.A.M. Early and late feathering in turkey and chicken: same gene but different mutations. *Genet. Sel. Evol.*, 2018; 50: 7 (doi: 10.1186/s12711-018-0380-3).
18. Simonsen M. The MHC the chicken genomic structure, gene products and resistance to oncogenic DNA and RNA viruses. *Veterinary Immunology and Immunopathology*, 1987, 17(1-4): 243-253 (doi: 10.1016/0165-2427(87)90144-9).
19. Bu G., Huang G., Fu H., Li J., Huang S., Wang Y. Characterization of the novel duplicated PRLR gene at the late-feathering K locus in Lohmann chickens. *J. Mol. Endocrinol.*, 2013, 51: 261-276 (doi: 10.1530/jme-13-0068).
20. Egorova A.V., Shakhnova L.V. *Ptitsa i ptitseproduky*, 2013, 3: 41-43 (in Russ.).
21. Emanuilova Zh.V. *Ptitsevodstvo*, 2008, 8: 25-26 (in Russ.).
22. Petrukovich T. *Zhivotnovodstvo Rossii*, 2017, 12: 11-12 (in Russ.).
23. Roiter Ya.S., Egorova A.V., Konopleva A.P., Tyapugin E.E., Shashina G.V., Degtyareva T.N., Karpenko L.S., Tyurikov V.M., Petrukhin O.N., Shcherbakova N.G. *Selektsionno-plemennaya rabota v ptitsevodstve* /Pod redaktsiei V.I. Fisinina, Ya.S. Roitera [Selection and breeding in poultry farming. V.I. Fisinin, Ya.S. Roiter (eds.)]. Sergiev Posad, 2016 (in Russ.).
24. Alekseev Ya.I., Borodin A.M., Nikulin A.V., Emanuilova Zh.V., Efimov D.N., Fisinin V.I. Molecular genotyping of chicken (*Gallus gallus* L.) feathering genes in connection with separation by sex. *Agricultural Biology*, 2017, 52(2): 367-373 (doi: 10.15389/agrobiology.2017.2.367eng).
25. Korshunova L., Royter Y., Egorova A. The usage of gene modifiers in selection of new forms of color- and feather-sex poultry. *Proc. XIV European Poultry Conference, 27 June 2014, Stavanger, Norway 23-27 June 2014*. Stavanger, 2014: 512.
26. Roiter Y., Egorova A., Sevastianova A., Korshunova L. The selection of autosex interlinear forms of poultry (chicken, geese, Guinea fowl). Proc. *XXV World's Poultry Congress*, *September 5-9, 2016*, *China*. Beijing, 2016: 257.
27. Lowe P.C., Merkley J.W. Association of genotypes for rate of feathering in broilers with production and carcass composition traits: effect of genotypes, sex, and diet on growth and feed conversion. *Poultry Sci.*, 1986, 65(10): 1853-1858 (doi: 10.3382/ps.0651853).
28. Dymkov A., Davydov D., Mal'tsev A., Spiridonov I., Chashchina G. *Ptitsevodstvo*, 2004, 10: 3-4 (in Russ.).