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A shift in erythrocyte histone H1 complement following selection in quail (*Coturnix japonica*)

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ABSTRACT: This work was aimed at comparing distribution of isoforms for polymorphic histone H1 variants H1.b and H1.z and variably abundant histone H1.d subtype between quail (*Coturnix japonica*) population selected for a high egg yolk cholesterol content and the control birds. The isoforms of histone H1.b (H1.b1, H1.b2) and histone H1.z (H1.z1, H1.z2) differed in their apparent molecular weights judging from their differential migration rates in one- and two-dimensional SDS-polyacrylamide gels. Stained histone H1.d bands and spots in one-dimensional acetic acid-urea and two-dimensional SDS-polyacrylamide gel patterns, respectively, exhibited differential intensities among quail individuals. Histone H1.d phenotypes with high (d^h), intermediate (d^hd^l), and low (d^l) amount of protein, displaying a small within-phenotype variability of the protein band intensities (coefficients of band variation below a threshold value of 0.25) were shown to be inherited in a Mendelian fashion with two alleles at a locus contributing to the band intensity. The frequencies of histone H1 alleles at loci *H1.b* (χ^2 = 13.32, d.f. = 1, $P < 0.001$), *H1.z* (χ^2 = 21.84, d.f. = 1, $P < 0.001$), and *H1.d* (χ^2 = 8.98, d.f. = 1, $P < 0.01$) were found to be statistically significant among the control and selected population. In general, a moderate degree of genetic divergence (F_{ST} equal to 0.07 and 0.1 at loci *H1.b* and *H1.z*, respectively) was observed among the control and selected quail populations. Selection may directly or indirectly affect the complement of H1 histones because of their presumably differential interactions with DNA and/or DNA-associated proteins resulting in alterations in the chromatin function.

Keywords: allelic variants; erythrocyte; histone H1 variants; phenotypic variants; quail; selection

INTRODUCTION

Histone H1 is thought to function as a modifier protein interacting dynamically with chromatin components (Bustin et al. 2005; Catez et al. 2006; Raghuram et al. 2009; Lu et al. 2013). By acting as an architectural protein, it forms and maintains higher-order chromatin structure (Hansen 2002). In concert with a variety of non-histone proteins (McBryant et al. 2010), the histone H1 regulates transcription, replication, and DNA repair following damage that underlie many cellular processes including cell division, differentiation, and aging (Izzo et al. 2008). A functional diversification of histone H1 is linked with its heterogeneous composition reflected by the existence of several non-allelic subtypes in mammals (Parseghian and Hamkalo 2001; Happel and Doenecke 2009), birds (Shannon and Wells 1987; Palyga 1991), and plants (Berdnikov et al. 1993, Kosterin et al. 1994). Some histone H1 non-allelic subtypes play specialized functions (Kowalski and Palyga 2012a). For example, human histone H1.1 was shown to be essential for a proper activity of chromatin-associated protein, barrier to autointegration factor (BAF), involved in the integration of retroviral preintegration complexes (Montes de Oca et al. 2005). Likewise, a specific cooperation of mouse histone H1b with the Msx1 homeoprotein leading to the inhibition of expression of the skeletal muscle differentiation regulatory factor MyoD was observed (Lee et al. 2004). The individual histone

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H1 subtypes may also act as specific regulators involved in repression genes controlling cell proliferation and cell cycle progression (Sancho et al. 2008). A specific amino acid composition of histone H1 C-terminal domain is essential for a molecular recognition and function (Lu et al. 2009). The C-terminal domain may adopt diverse conformations in the nucleosome-bound state with a high potential for formation of α-helix structures (Caterino et al. 2011). This indicates that C-terminal tail is a typical disordered domain, which in common with a disordered nature of N-terminal domain allows to classify H1 histones as highly disordered proteins (Peng et al. 2012). Many histone H1 non-allelic subtypes may consist of allelic isoforms, thus the presence of allelic polymorphism is a distinctive feature of this histone class (Kowalski and Palyga 2012a). Since histone H1 allelic variants were shown to differ in the C-terminal domains (Palyga et al. 2000; Berdnikov et al. 2003; Sarg et al. 2005; Gornicka-Michalska et al. 2006; Bogdanova et al. 2007; Villar-Garea and Imhof 2008; Kowalski et al. 2011a), they could be able to interact with chromatin through the allele-specific effects as manifested by their fluctuated frequency in a given population (Palyga 1998a; Palyga et al. 2000; Kowalski and Palyga 2014).

Out of eight histone H1 subtypes (H1.a, H1.a′, H1.b, H1.b′, H1.c, H1.c′, H1.d, and H1.z) identified among avian erythrocyte histone H1 (Palyga 1991), five, including H1.a in duck (*Anas platyrhynchos*) (Kowalski et al.1998), chicken (*Gallus gallus*) (Gornicka-Michalska et al. 2006) and quail (*Coturnix japonica*) (Palyga 1998a), H1.a′ in grey partridge (*Perdix perdix*) (Kowalski et al. 2008), H1.b in quail (Palyga 1998a), duck (Palyga et al. 2000) and guinea fowl (*Numidea meleagris*) (Kowalski et al. 2011a), H1.c in pheasant (*Phasianus colchicus*) (Kowalski et al. 2010) and guinea fowl (Kowalski et al. 2011a), and H1.z in duck (Palyga et al. 1993) and quail (Palyga 1998a), have been recognized to be polymorphic due to the presence of two or three electromorphs differently migrating in the polyacrylamide gels as a result of variation in their net charges and/or apparent molecular weights.

Both frequent and rare allelic isoforms for the avian polymorphic histone H1 subtypes were already observed in both conservative (Kowalski et al. 1998; Palyga et al. 2000; Kowalski and Palyga 2014) and breeding populations (Palyga et al. 1993; Palyga 1998b; Kowalski and Palyga 2014). Since allele distribution of histone H1 subtypes H1.a, H1.b, and H1.z in quail variety Pharaoh was found to fluctuate in the course of divergent selection for amount of weight loss after transient starvation (Palyga 1998b), in this work we sought to examine likely differences in histone H1 heterogeneity between the control quail population and population selected for a high yolk cholesterol content in the egg. Besides allelic isoforms for subtypes H1.b and H1.z that differentially migrated in two-dimensional polyacrylamide gels due to their disparate molecular weights, in this work we have also detected a variable abundance of protein spots for histone H1.d. In quail populations, the histone H1.d spots with high (phenotype d^h), intermediate (phenotype d^hd^l), and low amount of protein (phenotype d^l) in two-dimensional gel patterns have been revealed. Altered allele frequencies for all tested histone H1 subtypes have been detected during screening in the control quail population and the line selected for a high yolk cholesterol content. While the levels of both *H1.b* alleles were virtually the same, and those at loci *H1.z* and *H1.d* were roughly similar in the unselected control quail population, the frequencies of the alleles $H1b^2$, $H1z^2$, and $H1d^1$ increased by about a half in the selected quail line. We believe that a diversification of the allele levels among tested quail populations may suggest that selection for a quantitative trait (cholesterol content in the egg yolk) is associated with alterations in the distribution of linker histone H1 isoforms that may exert modulatory effects on structure and function of chromatin.

MATERIAL AND METHODS

Animals. Two quail (*Coturnix japonica*) lines, 37 individuals from unselected control population (mean cholesterol content in the egg yolk – 1763.06 mg/100 g yolk) and 41 individuals from population selected for high yolk cholesterol content (mean cholesterol content in the egg yolk – 1931.26 mg/100 g yolk) (Baumgartner et al. 2007) bred at the University of Life Sciences in Lublin, Poland, were used. These birds were selected by Dr. J. Baumgartner (Slovak Agricultural Research Centre, Nitra, Slovak Republic) and then relocated to the University of Life Sciences in Lublin. The founding outbred, egg type, quail stock (07)

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population housed at the Poultry Breeding Station Ivanka pri Dunaji was used for a divergent selection for high and low cholesterol content in the egg yolk. The selection procedure and phenotypic correlations in the selected quail lines were reported in detail by Baumgartner et al. (2007). Briefly, one male was mated to two females. The criteria for selection included cholesterol content in fresh egg yolk as well as family and individual records. Male individuals were chosen based on the performance of their sisters. The selection was performed by 18 generations (Baumgartner et al. 2007). In this work we used only the quail population selected for high cholesterol content in the egg yolk.

Blood samples collected into SSC solution (0.15M NaCl and 0.015M sodium citrate) containing 1mM phenylmethylsulfonyl fluoride (PMSF) were used to isolate erythrocytes by triple consecutive suspending and washing of the blood in SSC solution and centrifugation.

Linker histone extraction from erythrocyte nuclei. Linker histones were extracted twice with dilute perchloric acid using first 1M and then 0.5M solution from saponin-lysed erythrocyte nuclei (3% saponin in 0.1M sodium phosphate buffer, pH 7.0) according to Neelin et al. (1995). After precipitation with 20% trichloroacetic acid, the protein was first washed with acetone acidified with HCl (500 : 2, v/v) followed by pure acetone and air-dried. One-milligram aliquots of protein preparations were dissolved in a sample buffer (8M urea, 0.9M acetic acid, and 10% 2-mercaptoethanol) for electrophoretic separation.

Electrophoresis. Dissolved aliquots of histone H1 were electrophoresed in long (240 × 240 cm) two-dimensional polyacrylamide gels according to Kowalski and Palyga (2012b). The Coomassie Blue R-250 stained stripes containing histone H1 bands resolved in a one-dimensional acetic acid-urea polyacrylamide slab gel (15% acrylamide, 0.5% N,N'-methylenebisacrylamide, 8M urea, 0.9M acetic acid) were cut out from the gel and incubated (2 × 15 min) in an adaptation buffer (2.1% SDS, 2% 2-mercaptoethanol, 10% glycerol, 0.124M Tris-HCl, pH 6.8). The adapted gel stripes were then separated in a second dimension in the SDS-polyacrylamide gel (13.5% acrylamide, 0.36% N',N'-methylenebisacrylamide, 0.1% SDS).

Processing of the gel images and the statistical analysis. The electrophoretic patterns of resolved H1 histone subtypes were recorded by Doc-Print II gel documentation system (Vilber Lourmat) and processed by using ImageJ software (Version 1.42q, 2009) (www. rsbweb.nih.gov/ij). The volumes of histone H1.d bands in the preparations from individuals of different phenotypes were evaluated both using densitometric profiles of the protein resolved in one-dimensional acetic acid-urea polyacrylamide gel and scans of protein spot separated in the two-dimensional polyacrylamide gel, and expressed either as a percentage of H1.d peak volume in relation to a total volume of all histone H1 bands or grey intensities (in pixels) of H1.d spots in a given preparation. The coefficient of variation (CV), which is a standard deviation divided by a mean value, below a threshold of 0.25 was regarded as a low relative variability of the protein band in the tested populations.

Chi-square (χ^2) test of goodness-of-fit was applied for testing a possible deviation of the selected quail population from Hardy-Weinberg equilibrium. Genetic divergence among populations was determined by means of Wright's *F*-statistics. The probability $P < 0.05$ was considered to be statistically significant.

RESULTS

Quail (*Coturnix japonica*) histone H1 isolated with a dilute perchloric acid solution from saponine-lysed erythrocyte nuclei was resolved in a two-dimensional polyacrylamide gel electrophoresis into subtypes H1.a, H1.b, H1.b', H1.c, H1.c', H1.d, and H1.z (Figures 1 and 2). Gel patterns of histones H1.b, H1.d, and H1.z differed among preparations from quail individuals whereas the remaining histone bands and spots occupied the same in-gel locations and showed similar intensities in all individuals screened (Figures 1 and 2). Here, we have identified two-allelic polymorphism of histone subtypes H1.b and H1.z (Palyga 1998a, b) and a phenotypic diversity in the staining intensity of histone H1.d bands reflecting altered protein levels. Furthermore, the existence of two histone H1 alleles at loci *H1.b* and *H1.*z as well as three phenotypes of the subtype H1.d is consistent with their co-dominant inheritance patterns in progeny of various quail crosses (Table 1). In contrast to unselected (control) quail population, the line selected for a high cholesterol content in the egg yolk exhibited altered allele and phe-

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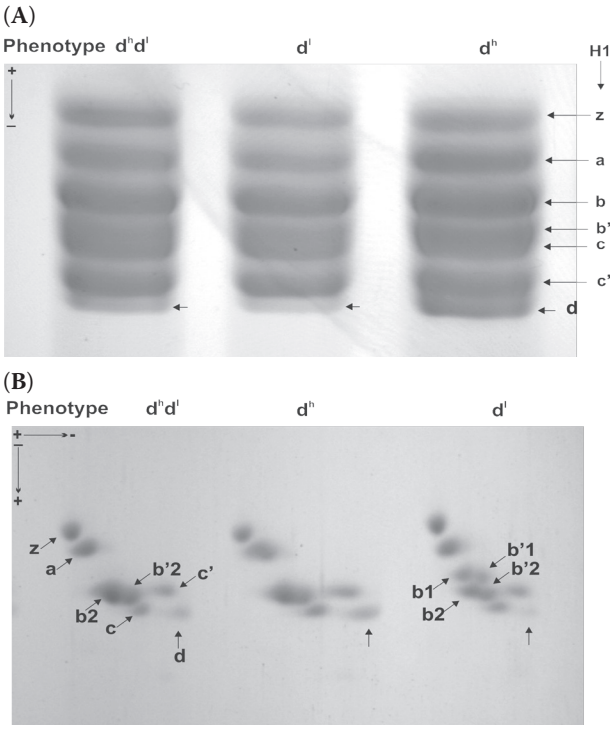

Figure 1. Polymorphic variation of quail histone H1.d

Total histone H1 preparation was first resolved in an acetic acid-urea gel (first dimension) (**A**) and then in SDS gel (second dimension) (**B**). A whole array of quail erythrocyte histone H1 subtypes resolved in one-dimensional acetic acid-urea gel was designated on the right by letters z, a, b, b′, c, c′, and d. Histone H1.d bands (one-dimensional gel) and spots (two-dimensional gel) of high (variant H1.d^h), intermediate (variant H1.d^hd^l), and low intensities (variant H1.d^l), corresponding to the phenotypes d^h, d^hd^l, and d^l, respectively, are indicated with short arrows. In addition, single spots of electromorphs H1.b2 and H1.b′2, and double spots of electromorphs H1.b1b2 and H1.b′1b′2 are also indicated in the two-dimensional gel pattern

notype distribution for all three variable histone H1 subtypes.

Phenotypic variability of histone H1.d. Histone H1.d was found to be differentially expressed among individuals of tested quail populations (Figure 1A). High, intermediate, and low intensities of histone H1.d bands were regarded to indicate a variation of the H1.d subtype represented by three distinct phenotypes d^h, d^hd^l, and d^l, respectively, in the population. Differential staining intensities of histone H1.d bands (Figure 1A and 1B) can reflect alterations in protein levels as evaluated by measuring peak heights in densitometric tracings of one-dimensional acetic acid-urea polyacrylamide gels and by scanning the two-dimensional polyacrylamide gels (Table 2). The intensity of protein bands from individuals d^h was by about 2.3-fold higher than of those from quail d^hd^l, and this in turn was by about 2.4-fold higher than in birds d^l (Figure 1A, Table 2). Similar ratios (about 2.2-fold differences) were found by comparing the grey levels of d^h and d^hd^l spots, as well as d^hd^l and d^l spots (Figure 1B, Table 2). As the CV values expressing a ratio of standard deviation (SD) to the mean (M) were below the threshold value of 25% (Table 2), low relative variations both within the protein bands and protein spots for each histone H1.d phenotype were confirmed.

A distribution of H1.d histone phenotypes in unselected and selected quail populations is briefly summarized in Table 3. Although the phenotypic proportions of H1.d in quail populations conformed to the Hardy-Weinberg principle ($\chi^2 = 0.523$, $P = 0.469$ and $\chi^2 = 0.03$, $P = 0.862$ in control and selected line, respectively) and similar frequencies of heterozygous individuals containing phenotype d^hd^l (0.432 in the unselected line vs 0.365 in the selected line) were found, considerable alterations in the distribution of phenotypes d^h ($\chi^2 = 4.714$, $P = 0.029$) and d^l ($\chi^2 = 6.833$, $P = 0.008$) were detected between the unselected (control) birds and those subjected to selection. Similarly, a statistically significant difference between populations was indicated based on a test of phenotype homogeneity ($\chi^2 = 6.69$, $P = 0.035$). Although roughly similar frequencies of phenotypes d^h (0.216) and d^l (0.351) were detected in the unselected group of birds, approximately four times fewer individuals d^h (frequency 0.048) and almost two times more individuals d^l (frequency 0.583) were found in the population affected by the selection.

Polymorphic variability of histone H1.z. Slow migrating isoform H1.z1 and faster moving isoform H1.z2 in one-dimensional SDS-polyacrylamide gel patterns (Figure 2A) might differ in apparent molecular weights. Although no differences in the histone H1.z band mobilities were detected in the patterns of total histone H1 in one-dimensional acetic acid-urea polyacrylamide gel (Figure 2B, upper panel (UP)), two protein spots corresponding to isoforms H1.z1 and H1.z2 were discernible in two-dimensional polyacrylamide gel patterns from heterozygous birds (Figure 2B, the middle of lower panel (LP)). Single bands/spots containing the isoforms H1.z1 or H1.z2 represented homozygous phenotypes z1 and z2, respectively, whereas double band/spots contain-

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Table 1. Distribution of genotypes and phenotypes for histone H1 subtypes in progeny from various quail matings

Histone H1 locus	Type of mating female × male	Number of progeny	Genotype distribution in progeny observed/expected		
			b1b1	*b1b2*	*b2b2*
H1.b	*b1b1* × *b1b2*	1	1/0.5	0/0.5	
	b1b1 × *b2b2*	1		1/1	
	b1b2 × *b1b1*	6	4/3	2/3	
	b1b2 × *b1b2*	2	1/0.5	1/1	0/0.5
	b1b2 × *b2b2*	7		3/3.5	4/3.5
	b2b2 × *b1b1*	1		1/1	
	b2b2 × *b1b2*	15		5/7.5	10/7.5
	b2b2 × *b2b2*	7			7/7
			d^hd^h	d^hd^l	d^ld^l
H1.d	$d^h \times d^h$	2	2/2		
	$d^hd^l \times d^hd^l$	13	4/3.25	6/6.5	3/3.25
	$d^hd^l \times d^l$	4		2/2	2/2
	$d^l \times d^h$	3		3/3	
	$d^l \times d^hd^l$	12		8/6	4/6
	$d^l \times d^l$	7			7/7
			z1z1	*z1z2*	*z2z2*
H1.z	*z1z1* × *z1z1*	2	2/2		
	z1z1 × *z2z2*	2		2/2	
	z1z2 × *z2z2*	9		5/4.5	4/4.5
	z2z2 × *z1z1*	4		4/4	
	z2z2 × *z1z2*	4		2/2	2/2
	z2z2 × *z2z2*	19			19/19

ing both isoform H1.z1 and H1.z2 constituted a heterozygous phenotype z1z2 (Figure 2A and 2B).

Both control (χ^2 = 1.43, P = 0.23) and selected (χ^2 = 0.87, P = 0.349) quail populations were in Hardy-Weinberg equilibrium, supported by the Wright's F-statistics values oscillating around zero (F_{IS} = –0.04 and F_{IT} = 0.064). The frequencies of genotypes *z1z1* and *z1z2* were several-fold lower in the selected line than in the control population while the frequency of the genotype *z2z2* was almost four times higher in the selected population as compared to the control quail population (Table 4). While the proportion of allele $H1.z^1$ was lower in the selected line, the frequency of allele $H1.z^2$ increased 1.7-fold in the selected population so that a test for the homogeneity of allele frequency indicated statistically significant difference (χ^2 = 21.84, P < 0.001) between these quail populations.

Polymorphic variability of histone H1.b. The presence of polymorphism for histone H1.b was confirmed by the resolution of total histone H1 preparations in one-dimensional SDS-polyacrylamide gel (Figure 2A) in which a single band of isoform H1.b1 (phenotype b1), an apparent lack of the protein band (phenotype b2) or the presence of both isoforms, b1 and b2, (phenotype b1b2) were revealed among tested quail individuals. The apparent lack of the isoform H1.b2 resulted from its co-migration with neighbouring histone subtype H1.c (Figure 2A). While histone H1.b was found to migrate as a single protein band in all histone H1 preparations screened in one-dimensional acetic acid-urea polyacrylamide gel (Figure 2B, upper panel (UP)), two spots of histone H1.b isoforms were detected in the two-dimensional polyacrylamide gel patterns from heterozygous animals (Figure 2B, the middle of lower panel (LP)). In addition to allelic variants forming homozygous phenotypes b1 (genotype *b1b1*) and b2 (genotype *b2b2*), a heterozygous phenotype (genotype *b1b2*) containing both protein spots was also identified (Figure 2B, LP). A shift in the

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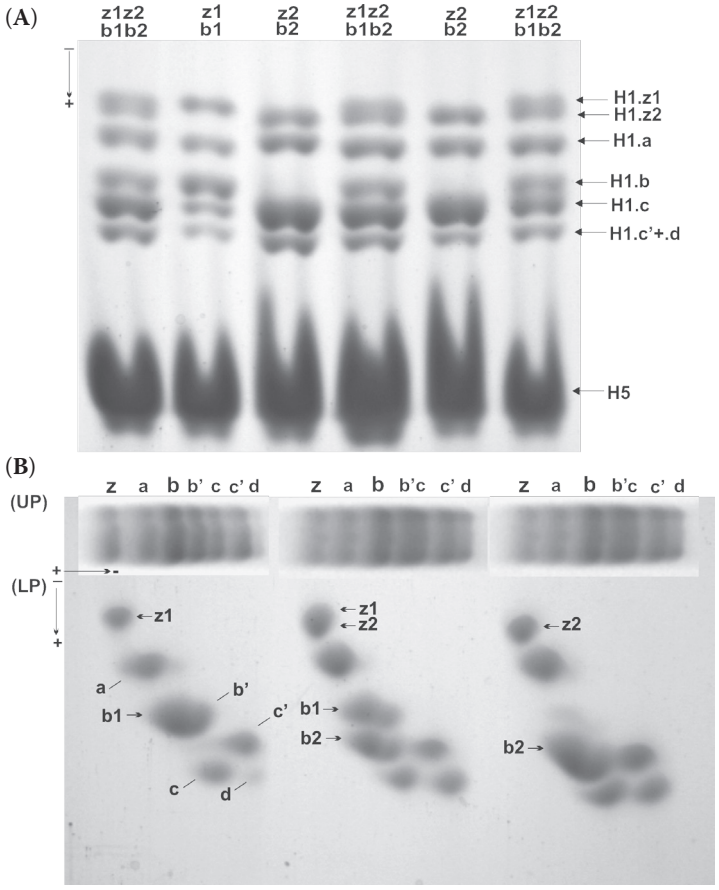

Figure 2. Polymorphic variation of quail erythrocyte histone H1.b and histone H1.z

(**A**) In one-dimensional polyacrylamide gel containing SDS, the band representing histone H1.b can migrate either above the histone H1.c band (isoform H1.b1) or comigrate with the histone H1.c (isoform H1.b2). The histone H1.z isoforms are well separated in one-dimensional SDS gel – the slow moving band is designated H1.z1 while the faster band represents isoform H1.z2

(**B**) Two-dimensional polyacrylamide gel patterns of histone H1.b and histone H1.z. Single histone H1.b and histone H1.z bands, resolved in the first dimension in an acetic acid-urea gel (upper panel – UP), can migrate either as slow or faster moving single protein spots designated as isoforms H1.b1 or H1.b2, and H1.z1 or H1.z2, respectively, or as double spots b1b2 or z1z2 in the two-dimensional gel (lower panel – LP)

localization of the histone isoforms H1.b1 and H1.b2 in the polyacrylamide gels containing SDS (Figure 2A and 2B, LP) suggested that they differed in their apparent molecular weights.

While the selected quail line conformed to the Hardy-Weinberg principle (χ^2 = 0.14, P = 0.708), our mated at random control quail population deviated from the Hardy-Weinberg equilibrium (χ^2 = 11.97, P < 0.001) at the locus *H1.b*. A lack of fit to the Hardy-Weinberg equilibrium in the unselected control quail population resulted from the excess of heterozygotes b1b2; both types of homozygotes (b1b1 and b2b2) exhibited the same frequency (0.108) (Table 5). The excess of heterozygotes at locus *H1.b* was supported by the negative values of Wright's *F*-statistics (F_{IS} = –2.84 and F_{IT} = –0.193).

A two-fold decrease in the frequency of genotype *b1b2* and over five-fold increase in that of genotype

Table 2. Quantification of histone H1.d phenotypes quantitative variability of histone H1.d bands was measured as a protein peak area in the densitometric tracing of acetic acid-urea polyacrylamide gel profiles (one-dimensional gel) and as a protein spot area in two-dimensional gel images. Histone H1.d band intensities were evaluated for 10, 36, and 30 histone H1 preparations with phenotype d^h, d^l, and d^hd^l, respectively, and expressed as a percentage of H1.d peak area in the respective phenotype in relation to the total peak area of all histone H1 subtypes in a given preparation (one-dimensional gel), and as integrated density, i.e. the sum of the values of pixels in a given protein spot. The coefficient of variation (CV) was calculated as a ratio of standard deviation (SD) and the mean (M)

Histone H1.d phenotype	Number of individuals	One-dimensional gel			Two-dimensional gel		
		M	SD	CV[a]	M	SD	CV[a]
d^h	10	7.23	0.54	0.08	6.77	0.69	0.1
d^l	36	1.31	0.23	0.17	1.35	0.2	0.14
d^hd^l	30	3.2	0.4	0.1	3.03	0.43	0.14

[a]CV values below 0.25 indicate a low relative protein spot variability

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Table 3. Frequency of histone H1.d phenotypes among quail populations

Quail line	Phenotype	Number of individuals observed/expected	Frequency of phenotype observed/expected
Unselected[a]	d^h	8/6.905	0.216/0.186[b]
	d^hd^l	16/18.157	0.432/0.490[c]
	d^l	13/11.937	0.351/0.322[d]
Selected[a]	d^h	2/2.188	0.048/0.053[b]
	d^hd^l	15/14.566	0.365/0.355[c]
	d^l	24/24.245	0.583/0.585[d]

[a]phenotypic diversity among populations: $\chi^2 = 14.362$ ($P < 0.001$); difference in the distribution of phenotypes: [b]$\chi^2 = 4.714$ ($P = 0.029$), [c]$\chi^2 = 2.816$ ($P = 0.093$), [d]$\chi^2 = 6.833$ ($P = 0.008$)

Table 4. Frequency of genotypes and alleles among quail lines at locus *H1.z*

Quail line	Genotype	Number of individuals observed/expected	Frequency of genotype observed/expected	Allele	Frequency of allele
Unselected [a]	*z1z1*	9/10.789	0.243/0.29[b]	$H1.z^1$	0.54
	z2z2	6/7.829	0.162/0.211[c]	$H1.z^2$	0.46
	z1z2	22/18.381	0.594/0.469[d]		
Selected [a]	*z1z1*	3/1.966	0.073/0.048[b]	$H1.z^1$	0.219
	z2z2	26/25.008	0.634/0.610[c]	$H1.z^2$	0.781
	z1z2	12/14.025	0.292/0.342[d]		

[a]genotypic diversity among populations: $\chi^2 = 21.84$ ($P < 0.001$); difference in the distribution of phenotypes: [b]$\chi^2 = 0.02$ ($P = 0.887$), [c]$\chi^2 = 9.45$ ($P = 0.002$), [d]$\chi^2 = 0.04$ ($P = 0.841$)

Table 5. Frequency of genotypes and alleles among quail lines at locus *H1.b*

Quail line	Genotype	Number of individuals observed/expected	Frequency of genotype observed/expected	Allele	Frequency of allele
Unselected[a]	*b1b1*	4/9.25	0.108/0.25[b]	$H1.b^1$	0.5
	b2b2	4/9.25	0.108/0.25[c]	$H1.b^2$	0.5
	b1b2	29/18.5	0.783/0.5[d]		
Selected[a]	*b1b1*	2/2.5	0.049/0.061[b]	$H1.b^1$	0.247
	b2b2	23/23.247	0.561/0.567[c]	$H1.b^2$	0.753
	b1b2	16/15.251	0.390/0.372[d]		

[a]genotypic diversity among populations: $\chi^2 = 13.32$ ($P < 0.001$); differences in the distribution of phenotypes: [b]$\chi^2 = 0.08$ ($P = 0.841$), [c]$\chi^2 = 6.34$ ($P = 0.011$), [d]$\chi^2 = 8.86$ ($P = 0.002$)

b2b2 in the selected quail line (Table 5) might appear as a result of favouring the homozygous birds *b2b2*. The observed alterations in the frequency of *H1.b* alleles between control and selected quail lines (χ^2 for the exact test of allele homogeneity equals 13.32, $P < 0.001$) seem to suggest that they are coupled with processes affected by the selection. While the levels of both histone *H1.b* alleles in the control quail population were equal, the distribution of allele $H1.b^1$ dropped by half (frequency 0.247) and that of allele $H1.b^2$ increased (frequency 0.753) in the selected quail line.

DISCUSSION

Histone H1 is a family of non-allelic subtypes comprising from six or seven components in birds (Palyga 1991) and plants (Kosterin et al. 1994) to eleven members in mammals (Happel and Doenecke 2009). The specific functions of histone H1

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subtypes has been inferred from their selective distribution in chromatin (Trollope et al. 2010) and differential condensing capabilities (Clausell et al. 2009) which may determine formation of active and inactive chromatin regions. Acting as peculiar modifiers of chromatin organization, the histone H1 subtypes may control expression of individual genes that influence many important cellular processes, such as chromosome alignment and segregation (Takata et al. 2007), mitotic progression (Green et al. 2010), and DNA damage response (Kratzmeier et al. 1999; Hashimoto et al. 2007). Since histone H1 condensing function is mediated by a specific C-terminal domain amino acid composition (Hansen et al. 2006; Lu et al. 2009), the alterations in the C-terminal domain amino acid sequence (Kosterin et al. 1994; Palyga et al. 2000; Gornicka-Michalska et al. 2006; Kowalski et al. 2011a, b) seem to support functional diversity of these chromatin proteins. A specific role for the histone H1 allelic isoform might be assigned based on the cause and effect relationship between the appearance and/or disappearance of a given allele leading to the characteristic phenotypic effects (Kowalski and Palyga 2012a). The histone H1 allele frequencies were shown to correlate with growth dynamics (Kosterin et al. 1994; Bogdanova et al. 2007), accumulated temperature of vegetation period (Bogdanova et al. 2005), and geographic distribution of plants (Berdnikov et al. 1993; Dudnikov 2012), plumage colour in duck (Palyga et al. 2000), and the effects of environmental pollution in hare (*Lepus europaeus*).

In this work, we revealed that the pattern of quail (*Coturnix japonica*) histone H1.b and H1.z allelic variation resembled that in the quail variety Pharaoh (Palyga 1998a) with two alleles at the locus whose protein products differed in apparent molecular weights. In addition, here we have found that intensity of quail histone H1.d bands from different individuals can be assigned into 3 groups. We observed high (d^h), intermediate (d^hd^l), and low intensities (d^l) of histone H1.d bands. Low relative variability (CV < 0.25) within histone H1.d bands and spots from each phenotype (d^h, d^hd^l, and d^l), and a lack of deviation from the expected pattern of inheritance strongly confirms the presence of three phenotypes of histone H1.d.

Previously, it has been found (Palyga 1998a) that quail individuals (variety Pharaoh) with phenotype a^0 were either devoid of H1.a band or sometimes contained only a trace amount of the protein. Such a leaking histone H1.a expression (Palyga 1998a) and differential expression of H1.d (the present work) might result from numerous events influencing gene regulation at transcriptional and posttranscriptional levels. Single nucleotide polymorphisms, insertions, deletions, and rearrangements affecting local DNA sequence may change interactions of promoter/enhancer regions with transcription factors resulting in a variable expression of the phenotype (Feuk et al. 2006). Likewise, mRNA untranslated regions may influence translation by acting either as positive elements effective in increasing protein expression (Furger et al. 1997) or as negative elements substantially reducing the level of the cellular protein (Calvo et al. 2009).

Allelic variants of histone H1 were found to fluctuate in several avian breeding flocks (Kowalski et al. 1998, 2010, 2011a, b; Palyga et al. 2000; Gornicka-Michalska et al. 2006). Changes in the distribution of histone H1 alleles can be associated with a selection process (Palyga 1998b). A divergent selection of quail (variety Pharaoh) conducted for the amount of weight loss after transient starvation was accompanied by the changes in the allele frequencies of three polymorphic histone H1 subtypes H1.a, H1.b, and H1.z (Palyga 1998b). The selection caused a decrease in the frequency of allele $H1.b^1$ at locus *H1.b* and increase in the frequency of alleles $H1.z^2$ at locus *H1.*z in the line selected for a low body mass reduction with a concomitant fall of the levels of allele $H1.a^0$ at the locus *H1.a* in the lines selected divergently for the high and low reduction of body mass. It is worth noting that when the selection was stopped, the frequencies of alleles $H1.b^1$ and $H1.z^2$ returned to the levels at the onset of screening the selection, while the frequency of allele $H1.a^0$ in the line selected for the high reduction in body mass returned to that in the control line and was maintained at a relatively stable level in the line selected for the low body mass reduction (Palyga 1998b). Likewise, in the current study we have observed changes in the distribution of phenotypes and alleles for three histone H1 loci between the control outbred quail population and that selected for the high yolk cholesterol content. These populations considerably differed in the cholesterol level by about 168.2 mg cholesterol/100 g yolk (Baumgartner et al. 2007). While no violation of the Hardy-Weinberg

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principle was observed at all tested histone H1 loci in the selected population and at loci *H1.d* and *H1.z* in the control population, we found a departure from the Hardy-Weinberg law at the locus *H1.b* in the unselected control population caused by a heterozygote excess as reflected by negative values of Wright's *F*-statistics F_{IS} and F_{IT}. The heterozygote excess at the locus *H1.b* in the control quail population might be the result of an accidental and uncontrolled event in the outbred stock leading to a loss of homozygotes at the time of sampling.

In general, our results indicate that the differences observed between the control and selected quail populations might be triggered by the selection acting against the histone H1 phenotype (d^h) and alleles (*H1.b*^{1} and *H1.*z^1) and favouring otherwise infrequent alleles (*H1.b*^{2} and allele *H1.*z^2) and rare phenotype (H1.d^l). A selection-dependent shift in the distribution of histone H1 alleles and phenotypes in quail seems to suggest that there is a relationship between histone H1 variants and the selection process. It is likely that these alleles might have exerted either positive or negative effects on the expression of physiological traits subjected to the selection and this association is reflected by a decrease or increase in their frequencies in the selected population. Thus, our results seem to suggest that the function of a histone H1 subtype might be additionally influenced by its allele-specific protein-DNA and/or protein–protein interactions.

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