HERPETOZOA 24 (1/2): 3 - 11
Wien, 30. Juli 2011

Changes in blood-serum proteins, erythrocyte count, and size of *Pelophylax bedriagae* (CAMERANO, 1882) during metamorphosis

(Anura: Ranidae)

Veränderungen an den Serumproteinen des Blutes, sowie an der Erythrozytenzahl und -größe bei *Pelophylax bedriagae* (CAMERANO, 1882) während der Metamorphose

(Anura: Ranidae)

HÜSEYIN ARIKAN & KERIM ÇİÇEK

KURZFASSUNG

Die vorliegende Untersuchung an Tieren aus Menemen (İzmir, Türkei) weist eine Zunahme der Albumin- und Globulinfraktionen am Gesamteiweiß des Blutserums von *Pelophylax bedriagae* (CAMERANO, 1882) im Verlauf der Metamorphose nach. Nach Abschluß der Metamorphose veränderten sich die Anteile der Proteinfraktionen nicht mehr. Zusätzlich war feststellbar, daß die in der Larvalperiode deutlich elliptischen Erythrozyten während der Metamorphose durch Breitenzunahme rundlicher wurden und daß die Erythrozytendichte mit zunehmender Erythrozytengröße abnahm.

ABSTRACT

In the present study, an increase was found in both albumin and globulin fractions relative to the total amount of blood-serum proteins during metamorphosis of *Pelophylax bedriagae* (CAMERANO, 1882), from Menemen (İzmir, Turkey). The concentration of the protein fractions remained stable after the completion of metamorphosis. In addition, it was observed that the following processes happened during metamorphosis: the erythrocytes, which had been more oval in the larval period, became less ellipsoid upon a gradual increase in their widths, and the erythrocyte concentration decreased with increasing erythrocyte size.

KEY WORDS

Amphibia: Anura: Ranidae; *Pelophylax bedriagae*, metamorphosis, blood-serum proteins, erythrocyte count and size, physiology, electrophoresis, Turkey

INTRODUCTION

There are many studies on the changes in soluble proteins (HERNER & FRIEDEN 1960; DENIS 1961; BROWN & CASTON 1962; INABA & FRIEDEN 1967; CHARLEMAGNE 1967a, 1967b; CHEN 1968, 1970; RAKOTOARIVONY & GASSER 1973) and the ontogenetic changes in erythropoiesis and erythrocyte morphology (HOLLYFIELD 1966; BENBASSAT 1970; BROYLES et al. 1981; WAKAHARA & YAMAGUCHI 2001; DAVIS 2008) during the metamorphosis of various urodelan and anuran species.

Despite the availability of numerous studies on both the electrophoretic analysis of blood-serum proteins (e.g., ÖZETI & ATATÜR 1979; ARIKAN 1990, 1991; ARIKAN et al. 2001; TOSUNOĞLU & ARIKAN 2007) and the morphology, count and size of blood cells (e.g., ARIKAN 1989; ATATÜR et al. 1998, 1999; ARIKAN et al. 2001, 2003, 2010) in adult amphibians, we still have inadequate knowledge about the changes both in the blood-serum proteins and in erythrocyte count and size during larval development of urodelan and anuran species (HOLLYFIELD 1966; BENBASSAT 1970; DAVIS 2008; GRENAT et al. 2009). This study aims to acquire information about the changes in blood-serum proteins and in erythrocyte counts and sizes of *Pelophylax bedriagae* (CAMERANO, 1882) during metamorphosis.

MATERIALS AND METHODS

A total of 15 larvae of GOSNER (1960) stages 38 [*n* = 5], 41 [*n* = 5], and 43 [*n* = 5], and 10 adult individuals of *P. bedriagae* [5 males, 5 females] were captured from temporary ponds in June 2010 in Menemen, İzmir (Turkey) [38.583035° N, 26.999625° E, 4 m a.s.l.] then brought to the laboratory. Blood samples were taken from etherized tadpoles and frogs by means of cardiac ventricular puncture with heparinized hematocrit capillaries.

The electrophoretic separation of blood-serum proteins was performed according to ÖZETI & ATATÜR (1979), who slightly modified and applied the polyacrylamide gel electrophoresis method by DAVIS (1964). The blood obtained was centrifuged for 5 min at 600 g and preserved at -20°C. Electrophoretic separations were run using a Canalco Model 1200 electrophoresis apparatus (Canalco Inc., Rockville, Md., USA) at room temperature (20-25°C). Gels containing separated proteins were stained with 0.5% Amido Black (Naphtol Blue Black 10-B), and excess stain was passively discharged in 7% acetic acid baths. The densitometric curves of the separations were obtained at 500 nm by means of a Gelman ACD-15 39430 densitometer (Gelman Instrument Co., Ann Arbor, Mi., USA), and they were photographed. The qualitative evaluation of the gels was made directly from the electropherograms.

The erythrocytes counts were done utilizing a Neubauer hemocytometer. As diluting solution for erythrocytes, the standard Hayem's solution was used. The blood smear preparations prepared using Wright's stain were utilized to measure the morphology and size of erythrocytes. The erythrocytes were measured using a MOB-1-15x ocular micrometer. On each blood smear preparation, the length (L) and width (W) of 40 random erythrocytes and their nuclear length (NL) and nuclear width (NW) were measured. The erythrocyte cytoplasm (C) and nuclear (N) sizes were calculated according to the formulae $C = (L \cdot W \cdot \pi)/4$ and $N = (NL \cdot NW \cdot \pi)/4$. The cellular and nuclear shapes were compared using the L/W and NL/NW ratios, while the comparison of the nucleuo-cytoplasatic ratio was made on the basis of the N/C ratio. The photographs of the erythrocytes were taken with an Olympus CX31-Altra 20 Soft Imaging system. One-way ANOVA was used to compare the larvae and adults, whereas Pearson's correlation was used to determine the relationship between erythrocyte count and size. Alpha was set at 0.05.

RESULTS

Electrophoretic analysis: The gel photographs of the electrophoretic analyses of the blood-serum proteins of the larvae at stages 38, 41 and 43 and of an adult male of *P. bedriagae* are presented in Figure 1; Figures 2, 3, 4 and 5 show the gel photographs of the electrophoretic analysis of the blood-serum proteins from each of the larval stages studied and from an adult male, along with their densito-metric curves.

In the electrophoretic analysis of *P. bedriagae*, the blood-serum proteins could be divided into a total of 12 fractions or fraction groups in the larvae at stage 38 – 2 in the albumin region and 10 (1-10) in the globulins region (Figure 2); into a total of 12 fractions or fraction groups in the larvae at stage 41 – 1 in the albumin region and 11 (1-11) in the globulins region (Figure 3); into a total of 13 fractions or fraction groups in the larvae at stage 43 – 1 in the albumin region and 12 (1-12) in the globulins region (Figure 4); and into a total of 13 fractions or fraction groups in the adult individuals – 1 in the albumin region and 12 (1-12) in the globulins region (Figure 5). According to the results of the electrophoretic analysis, a gradual increase was observed in the concentrations of both albumin and globulin fractions in the total number of blood-serum protein fractions during metamorphosis (Figure 1).

Erythrocyte count and size: The characteristic erythrocyte and nuclear

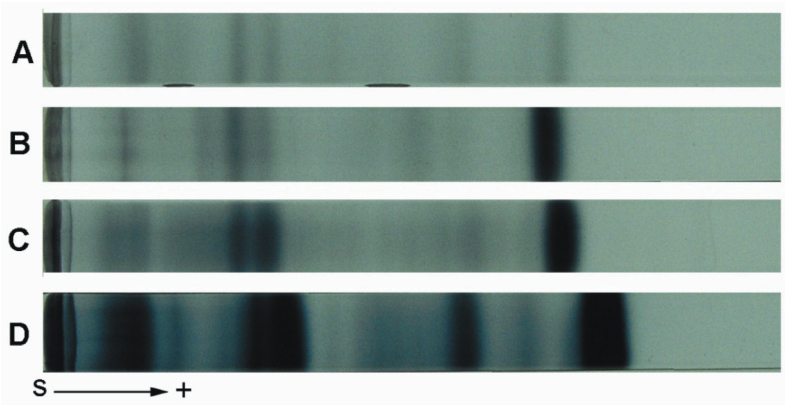

Fig. 1: Photograph of the gel showing the electrophoretic separation of the blood-serum protein sample of adult and larval *Pelophylax bedriagae* (Camerano, 1882).
A - larvae of stage 38, B - larvae of stage 41, C - larvae of stage 43 (according to Gosner 1960), D - adult male, S - start, junction between the stacking and separation gels.

Abb. 1: Photo des Gels mit Darstellung der elektrophoretischen Auftrennung der Blutserum-Proteine larvaler und adulter *Pelophylax bedriagae* (Camerano, 1882).
A - Larven im Stadium 38, B - Larven im Stadium 41, C - Larven im Stadium 43 (nach Gosner 1960), D - adultes Männchen, S - Start, Grenze von Sammelgel und Trenngel.

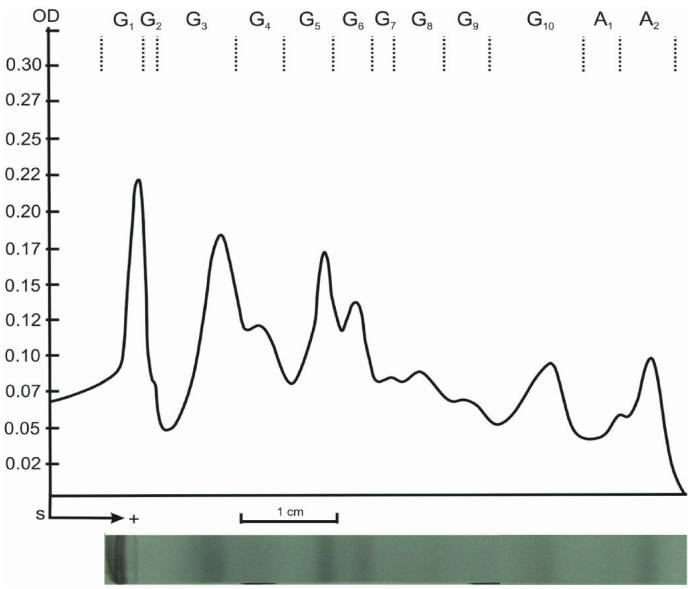

Fig. 2: Gel photograph showing the electrophoretic separation of the blood-serum protein sample of *Pelophylax bedriagae* (Camerano, 1882) larval stage 38 (Gosner 1960), together with its densitometric curve.

Abb. 2: Photo des Gels mit Darstellung der elektrophoretischen Auftrennung der Blutserum-Proteine von Larven von *Pelophylax bedriagae* (Camerano, 1882) des Stadiums 38 (Gosner 1960) und densitometrische Kurve.

Table 1: Descriptive statistics (*n*, Arithmetic Mean, Minimum, Maximum, SE - Standard Error of the Mean, SD - Standard Deviation) of the measurements of the erythrocytes and their nuclei from cardiac blood of larvae and adults of *Pelophylax bedriagae* (CAMERANO, 1882). L - Erythrocyte length, W - Erythrocyte width, ES - Erythrocyte size, NL - Nucleus length, NW - Nucleus width, NS - Nucleus size, NS/ES - Nucleocytoplasmic ratio. Developmental stage according to GOSNER (1960).

Tab. 1: Beschreibende Statistiken (*n*, arithmetisches Mittel, Minimum, Maximum, SE - Standardfehler des Mittelwertes, SD - Standardabweichung) der Maße der Erythrozyten und ihrer Kerne aus dem kardialen Blut von Larven und Adulten von *Pelophylax bedriagae* (CAMERANO, 1882). L - Erythrozytenlänge, W - Erythrozytenbreite, ES - Erythrozytengröße, NL - Kernlänge, NW - Kernbreite, NS - Kerngröße, NS/ES - Kern-Zytoplasma-Verhältnis. Entwicklungsstadium nach GOSNER (1960).

Stage Stadium	Parameter Kenngröße	L (µm)	W (µm)	L/W	ES (µm²)	NL (µm)	NW (µm)	NL/NW	NS (µm²)	NS/ES
38	Sample size / Umfang	*n* = 40	*n* = 40	*n* = 40	*n* = 40	*n* = 40	*n* = 40	*n* = 40	*n* = 40	*n* = 40
	Mean / Mittel	20.14	11.10	1.82	175.63	8.51	5.83	1.46	38.94	0.22
	SE	0.151	0.072	0.013	2.138	0.054	0.026	0.010	0.333	0.003
	Min	18.00	10.25	1.59	151.11	7.75	5.50	1.40	33.46	0.19
	Max	22.00	12.00	2.00	198.31	9.25	6.00	1.60	42.39	0.26
	SD	0.956	0.456	0.083	13.521	0.344	0.162	0.062	2.109	0.018
41	Sample size / Umfang	*n* = 40	*n* = 40	*n* = 40	*n* = 40	*n* = 40	*n* = 40	*n* = 40	*n* = 40	*n* = 40
	Mean / Mittel	18.59	11.03	1.69	160.92	8.31	5.98	1.39	38.99	0.24
	SE	0.119	0.062	0.012	1.509	0.069	0.025	0.013	0.353	0.003
	Min	17.00	10.50	1.55	140.12	7.00	5.80	1.20	32.97	0.20
	Max	21.00	11.75	1.89	179.18	9.00	6.20	1.60	42.93	0.28
	SD	0.752	0.391	0.079	9.544	0.438	0.158	0.085	2.235	0.016
43	Sample size / Umfang	*n* = 40	*n* = 40	*n* = 40	*n* = 40	*n* = 40	*n* = 40	*n* = 40	*n* = 40	*n* = 40
	Mean / Mittel	18.45	11.29	1.64	163.38	8.66	5.95	1.46	40.46	0.25
	SE	0.172	0.087	0.022	1.752	0.105	0.016	0.018	0.492	0.004
	Min	16.00	10.25	1.43	144.83	7.50	5.80	1.30	33.85	0.20
	Max	20.00	12.50	1.95	184.48	9.50	6.00	1.70	44.75	0.30
	SD	1.091	0.551	0.140	11.081	0.662	0.101	0.116	3.113	0.024
Adult	Sample size / Umfang	*n* = 40	*n* = 40	*n* = 40	*n* = 40	*n* = 40	*n* = 40	*n* = 40	*n* = 40	*n* = 40
	Mean / Mittel	20.98	13.39	1.57	220.63	8.61	5.44	1.58	36.77	0.17
	SE	0.200	0.064	0.016	2.419	0.064	0.017	0.013	0.288	0.002
	Min	19.00	11.75	1.36	182.17	7.50	5.20	1.40	32.38	0.14
	Max	24.00	14.00	1.77	253.65	9.50	5.50	1.70	41.02	0.20
	SD	1.266	0.404	0.102	15.300	0.404	0.106	0.083	1.819	0.013

shapes both in the larval period and in adults are oval in *P. bedriagae*, as in vertebrates other than mammalians. On the preparations prepared using Wright's stain, the cytoplasm was light yellowish-pink, whereas the chromophilic nucleus was dark purplish-blue. The erythrocytes were more ellipsoid in the larval period (Figure 6a), whereas, they turned into a less ellipsoid form in adults (Figure 6b).

In 1 mm^3 of blood, the mean erythrocyte counts were 305,500 in the larvae at stage 38 [n = 3], 310,000 in the larvae at stage 41 [n = 3], 315,000 in the larvae at stage 43 [n = 4] and 300,000 in adults [n = 3 males, 2 females], respectively.

The erythrocyte and nuclear lengths, widths, sizes, L/W and NL/NW ratios and the nucleocytoplasmic ratios are presented in Table 1. As can also be seen in Table 1, the mean erythrocyte length was calculated as 20.14 µm at stage 38, 18.59 µm at stage 41, 18.45 µm at stage 43 and 20.98 µm in adults ($F_{3,156}$ = 56.29; P = 0.000); the mean erythrocyte width as 11.10 µm at stage 38, 11.03 µm at stage 41, 11.29 µm at stage 43 and 13.39 µm in adults ($F_{3,156}$ = 220.04; P = 0.000); the mean erythrocyte size as 175.63 μm^2 at stage 38, 160.92 μm^2 at stage 41, 163.38 μm^2 at stage 43 and 220.63 μm^2 in adults ($F_{3,156}$ = 177.61; P = 0.000); and L/W ratio as 1.82 at stage 38, 1.69 at stage 41, 1.64 at stage 43 and 1.57 in adults ($F_{3,156}$ = 39.30; P = 0.000). It was found that the erythrocyte width gradually increased with metamorphosis and that the more ellipsoid erythrocyte form in the larval period turned into a less ellipsoid form in adults, as also seen in the L/W ratios (Table 1). Some variation in nucleus size was observed in the larval period. There were differences between the larvae at stages 38, 41 and 43 and the adults in terms of NL ($F_{3,156}$ = 3.88; P = 0.010), NW ($F_{3,156}$ = 136.79; P = 0.000) and NS ($F_{3,156}$ = 15.74; P = 0.000) values, as well as NL/NW ($F_{3,156}$ = 29.74; P = 0.000) and NS/ES ($F_{3,156}$ = 171.17; P = 0.000) ratios.

DISCUSSION

Electrophoretic analysis: There are many studies on the changes in soluble proteins during larval development (metamorphosis) in amphibians. HERNER & FRIEDEN (1960) stated that an increase occurred in the concentration of serum protein fractions in anurans upon metamorphosis and that when metamorphosis was completed, the variation ceased. CHEN (1968) reported that the embryo proteins of *Bombina variegata* (LINNAEUS, 1758), *Lithobates pipiens* (SCHREBER, 1782) and *Ichthyosaura alpestris* (LAURENTI, 1768) increased at the HARRISON (1969) stages 28 to 36. Similar results were shown by BROWN & CASTON (1962) in *L. pipiens* and by DENIS (1961) in *Pleurodeles waltl* MICHAHELLES, 1830. BROWN & CASTON (1962) revealed that in *L. pipiens,* the cytoplasmic ribosomes, in which protein synthesis takes place, also increased in number, with an increase in concentration of proteins. According to CHEN (1968), the new protein fractions occurring during metamorphosis supposedly are involved in the synthesis of new proteins. Significant increases were observed by CHARLEMAGNE (1967a, 1967b) in the concentrations of serum proteins from the larval to the adult condition with completed metamorphosis in *Ambystoma mexicanum* (SHAW & NODDER, 1798) and *I. alpestris* that were electrophoretically examined. RAKOTOARIVONY & GASSER (1973) found an increase in the concentration of soluble proteins in the course of metamorphosis of *P. waltl*. INABA & FRIEDEN (1967) observed a great change in the plasma oxydase activity of the anuran species *Lithobates grylio* (STEJNEGER, 1901), *L. catesbeianus* (SHAW, 1802), and *L. pipiens* during metamorphosis. As a result of the analysis of the blood-serum proteins of *P. bedriagae* by means of polyacrylamide gel electrophoresis, a gradual increase was observed in the number of protein fractions and, especially in the concentrations of albumin and globulin fractions. This finding is compatible with the results found in different urodele and anuran species by various researchers.

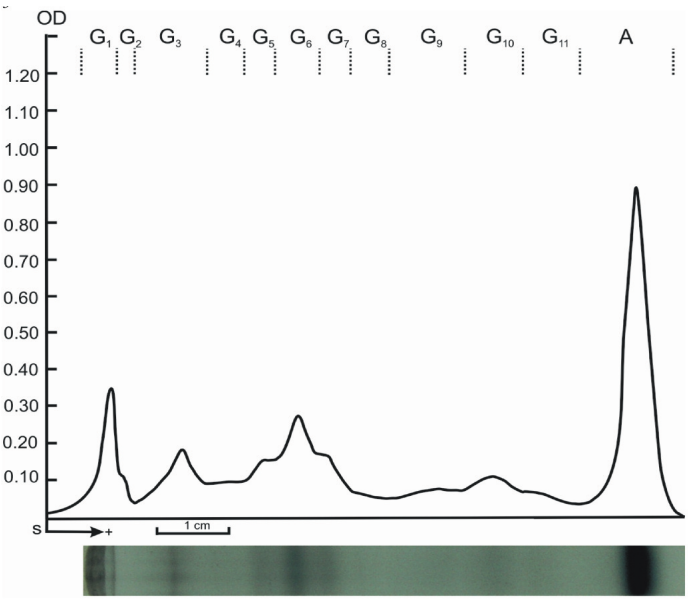

Fig. 3: Gel photograph showing the electrophoretic separation of the blood-serum protein sample of *Pelophylax bedriagae* (CAMERANO, 1882) larval stage 41 (GOSNER 1960), together with its densitometric curve.

Abb. 3: Photo des Gels mit Darstellung der elektrophoretischen Auftrennung der Blutserum-Proteine von Larven von *Pelophylax bedriagae* (CAMERANO, 1882) des Stadiums 41 (GOSNER 1960) und densitometrische Kurve.

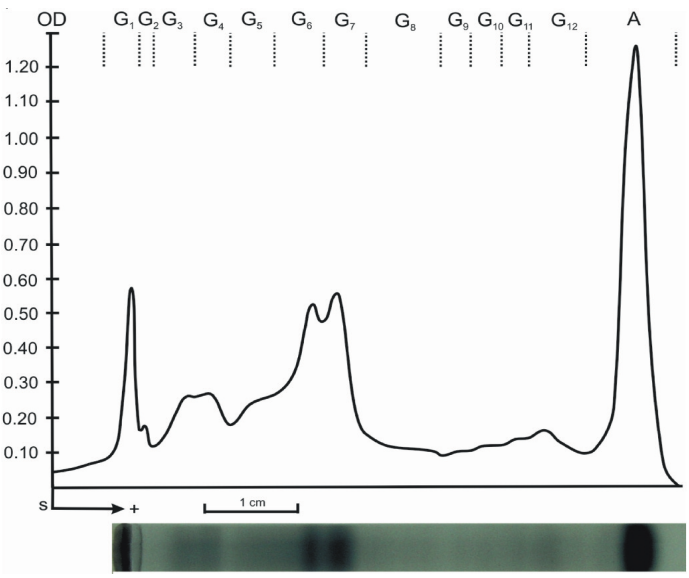

Fig. 4: Gel photograph showing the electrophoretic separation of the blood-serum protein sample of *Pelophylax bedriagae* (CAMERANO, 1882) larval stage 43 (GOSNER 1960), together with its densitometric curve.

Abb. 4: Photo des Gels mit Darstellung der elektrophoretischen Auftrennung der Blutserum-Proteine von Larven von *Pelophylax bedriagae* (CAMERANO, 1882) des Stadiums 43 (GOSNER 1960) und densitometrische Kurve.

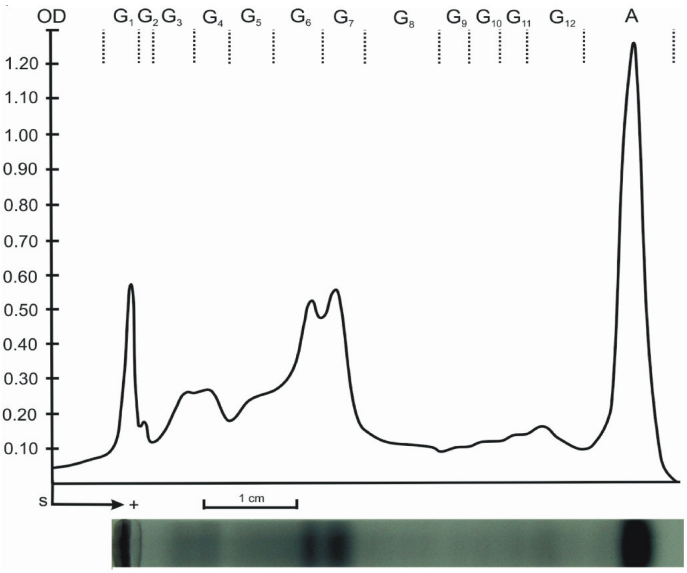

Fig. 5: Gel photograph showing the electrophoretic separation of the blood-serum protein sample of adult male *Pelophylax bedriagae* (CAMERANO, 1882), together with its densitometric curve.

Abb. 5: Photo des Gels mit Darstellung der elektrophoretischen Auftrennung der Blutserum-Proteine adulter Männchen von *Pelophylax bedriagae* (CAMERANO, 1882) und densitometrische Kurve.

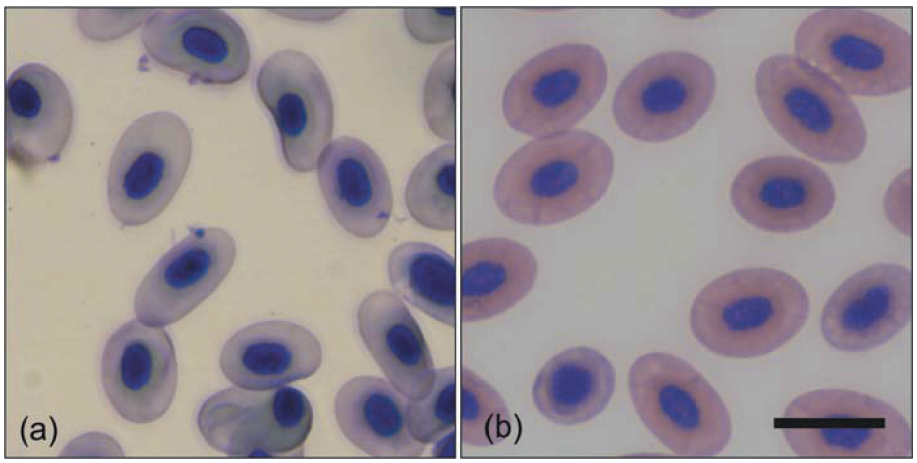

Fig. 6: Photomicrographs of erythrocytes of larval (a) and adult (b) *Pelophylax bedriagae* (CAMERANO, 1882). Horizontal bar represents 20 μm.

Abb. 6: Mikrophotographien der Erythrozyten von Larven (a) und erwachsenen Exemplaren (b) von *Pelophylax bedriagae* (CAMERANO, 1882). Die Balkenlänge entspricht 20 μm.

Erythrocyte count and size: The determination of erythrocyte sizes has been an essential point in standard hematological studies (ARIKAN et al. 2003, 2010), interspecies comparisons (ATATÜR et al. 1998, 1999; ARIKAN et al. 2001a, 2001b) and environmental, seasonal and altitude-related studies (RUIZ et al. 1989; PAGÉS et al. 1992; RUIZ et al. 2004). The knowledge of erythrocyte morphology of an animal provides important clues for genome size (KURAMOTO 1981; GRAGORY 2001), correlation between erythrocyte size and metabolic rate (SMITH 1925; VERNBERG 1955; MONNICKENDAM & BALLS 1973), and exposure to pollution (LLACUNA et al. 1996). In larval amphibians, two general types of erythrocytes, different from each other in terms of size and morphology are mentioned, namely larval and adult forms (HOLLYFIELD 1966; BENBASSAT 1970; BROYLES et al. 1981). BENBASSAT (1970) stated that erythrocytes of larval amphibians were large and extended, whereas, the erythrocytes of adults were smaller and more spherical. HOLLYFIED (1966) and HASEBE et al. (1999) reported that transitions from larval cells to adult cells started at the beginning of metamorphosis. DAVIS (2008) highlighted the positive correlation between the body size and erythrocyte width of *Ambystoma talpoideum* (HOLBROOK, 1838). Two different types of erythrocytes, i.e., larval and adult forms, were not observed in the blood of *P. bedriagae* during its larval development. Nevertheless, as DAVIS (2008) suggested, it is possible to speak of a correlation between body size and erythrocyte width in *P. bedriagae*. The erythrocyte width was observed to gradually increase from stage 38 to the adult form [mean value: 11.10 µm at stage 38; 13.29 µm in adults, Table 1]. Furthermore, as in adults, it is possible to speak of a partially inverse (negative) correlation between erythrocyte count and size (Pearson's correlation, $r = -0.85$, $P = 0.153$) during metamorphosis in that the erythrocyte concentration decreased with increasing erythrocyte size.

REFERENCES

ARIKAN, H. (1989): Anadolu'daki *Rana ridibunda* (Anura, Ranidae) populasyonlarının kan hücrelerinin sayısı bakımından incelenmesi.- Turkish Journal of Zoology, Ankara; 13: 54-59.

ARIKAN, H. (1990) *Rana ridibunda* (Anura, Ranidae) populasyonları üzerinde morfolojik ve serolojik araştırmalar.- Turkish Journal of Zoology, Ankara; 14: 40-83.

ARIKAN, H. (1991): Göller Bölgesi *Rana ridibunda* PALLAS (Anura, Ranidae) populasyonlarının serolojik yönden incelenmesi.- Turkish Journal of Zoology, Ankara; 15: 85-90.

ARIKAN, H. & ÇEVIK, İ. E. & KAYA, U. & MERMER, A. (2001): Anadolu'daki dağ kurbağalarında eritrosit ölçümleri.- Anadolu University Journal of Science and Technology, Eskişehir; 2: 387-391. (in Turkish)

ARIKAN, H. & KESKIN, N. A. & ÇEVIK, İ. E. & ERIŞMIŞ, U. C. (2010): A study on the blood cells of Anatolian and Thracian populations of the Fire-Bellied Toad, *Bombina bombina* L. (Anura: Bombinatoridae).- Animal Biology, Leiden; 60: 61-68.

ARIKAN, H. & OLGUN, K. & ILGAZ, Ç. & BARAN, İ. & KUMLUTAŞ, Y. (2003): Erythrocyte size and number in *Neurergus strauchii* (Urodela: Salamandridae).-Russian Journal of Herpetology, Moskva; 10: 163-166.

ARIKAN, H. & OLGUN, K. & TOK, C. V. & ÇEVIK, İ. E. (2001): Morphological and serological investigations on the mountain frogs of mid-Taurus range between east longitudes 33° and 36°.- Turkish Journal of Zoology, Ankara; 25: 11-17.

ATATÜR, M. K. & ARIKAN, H. & ÇEVIK, İ. E. (1999): Erythrocyte sizes of some anurans from Turkey.- Turkish Journal Zoology, Ankara; 23: 111-114.

ATATÜR, M. K. & ARIKAN, H. & MERMER, A. (1998): Erythrocyte sizes of some urodeles from Turkey.- Turkish Journal Zoology, Ankara; 22: 89-91.

BENBASSAT, J. (1970): Erythroid cell development during natural amphibian metamorphosis.- Developmental Biology, San Diego; 21: 557-583.

BROWN, D. D. & CASTON, J. D. (1962): Biochemistry of amphibian development. Ribosome and protein synthesis in early development of *Rana pipiens*.- Developmental Biology, San Diego; 5: 412-434.

BROYLES, R. H. & JOHNSON, G. M. & MAPLES, P .B. & KINDELL, G. R. (1981): Two erythropoietic microenvironments and two larval red cell lines in bullfrog tadpoles.- Developmental Biology, San Diego; 81: 299-314.

CHARLEMAGNE, J. (1967a) : Étude par électrophorèse et immunoélectrophorèse des protéines sériques de l'axolot (*Ambystoma mexicanum* SHAW).- Bulletin de la Société Zoologique de France, Paris; 92: 739-748.

CHARLEMAGNE, J. (1967b) : Étude des protéines sériques de l'amphibien urodèle *Triturus alpestris* LAUR., chez l'adulte et au cours de la métamorphose.- Bulletin de la Société Zoologique de France, Paris; 92: 153-160.

CHEN, P. S. (1968): Patterns of soluble proteins and multiple forms of dehydrogenases in amphibian

development.- Journal of Experimental Zoology, Hoboken, New York; 168: 337-350.

Chen, P. S. (1970): Patterns and metamorphic changes of serum proteins in Amphibia.- Wilhelm Roux' Archiv für Entwicklungsmechanik der Organismen, Berlin, Heidelberg (Springer), München (Bergmann); 165: 132-149.

Davis, A. K. (2008): Ontogenetic changes in erythrocyte morphology in larval mole salamanders, *Ambystoma talpoideum*, measured with image analysis.- Comparative Clinical Pathology, London; 17: 23-28.

Davis, B. J. (1964): Disc electrophoresis - II. Method and application to human serum proteins.- Annals of the New York Academy of Sciences, New York; 121: 404-427.

Denis, H. (1961): Recherche sur la différenciation protéique au cours du développement des amphibiens.- Journal of Embryology and Experimental Morphology, London; 9: 442-445.

Gosner, K. L. (1960): A simplified table for staging anuran embryos and larvae with notes on identification.- Herpetologica, Lawrence; 16: 183-190.

Gregory, T.R. (2001): The bigger the C-value, the larger the cell: genome size and red blood cell size in vertebrates.- Blood Cells Molecules and Diseases, San Diego; 27: 830-843.

Grenat, P. R. & Bionda, C. & Salas, N. E. & Martino, A. L. (2009): Variation in erythrocyte size between juveniles and adults of Odontophrynus americanus.- Amphibia-Reptilia, Leiden; 30: 141-145.

Harrison, R. (1969): Harrison stages and description of the normal development of the spotted salamanders, *Ambystoma punctatum* (Linn.); pp. 44-66. In: Harrison, R. (ed.): Organization and development of the embryo. New Haven, CT (Yale University Press).

Hasebe, T. & Oshima, H. & Kawamura, K. & Kikuyama, S. (1999): Rapid and selective removal of larval erythrocytes from systemic circulation during metamorphosis of the bullfrog, *Rana catesbeiana*.- Development Growth & Differentiation, Richmond; 41: 639-643.

Herner, A. E. & Frieden, E. (1960): Biochemistry of anuran metamorphosis. VII Changes in serum proteins during spontaneus and induced metamorphosis.- Journal of Biological Chemistry, Bethesda; 235: 2845-2851.

Hollyfield, J. G. (1966): Erythrocyte replacement at metamorphosis in the frog *Rana pipiens*.- Journal of Morphology, New York; 119: 1-6.

Inaba, T. & Frieden, E. (1967): Changes in ceruloplasmin during anuran metamorphosis.- Journal of Biological Chemistry, Bethesda; 242: 4789-4795.

Kuramoto, M. (1981): Relationships between number, size and shape of red blood cells in amphibians.- Comparative Biochemistry and Physiology, New York; (Part A: Molecular & Integrative Physiology) 69:771-775.

Llacuna, S. & Gorriz, A. & Riera, M. & Nadal, J. (1996): Effects of air pollution on hematological parameters in passerine birds.- Archives of Environmental Contamination and Toxicology, New York; 31: 148-152.

Monnickendam, M. A. & Balls, M. (1973): Relationship between cell sizes, respiration rates and survival of amphibian tissues in long-term organ cultures.- Comparative Biochemistry and Physiology, New York; 44: 871-880.

Özeti, N. & Atatür, M. K. (1979): A preliminary survey of the serum proteins of a population of *Mertensiella luschani finikensis* Başoğlu & Atatür from Finike in Southwestern Anatolia.- Istanbul Universitesi Fen Fakültesi Mecmuası, Istanbul; (B) 44: 23-29.

Pagés, T. & Peinado, V. I. & Viscor, G. (1992): Seasonal changes in hematology and blood chemistry of the freshwater turtle *Mauremys caspica leprosa*.- Comparative Biochemistry and Physiology, New York; (Part A: Molecular & Integrative Physiology) 103: 275-278.

Rakotoarivony, J. & Gasser, F. (1973): Analyse électrophorétique en gel de polyacrylamide des protéines solubles au cours du développement embryonnaire de l'amphibien urodèle *Pleurodeles waltlii* Michah.- Annales d'Embryologie et de Morphogenèse, Paris; 6: 229-242.

Ruiz, G. & Rosenmann, M. & Cortes, A. (2004): Thermal acclimation and seasonal variations of erythrocyte size in the Andean mouse *Phyllotis xanthopygus rupestris*.- Comparative Biochemistry and Physiology; New York; (Part A: Molecular & Integrative Physiology) 139: 405-409.

Ruiz, G. & Rosenmann, M. & Veloso, A. (1989): Altitudinal distribution and blood values in the toad, *Bufo spinulosus* Wiegmann.- Comparative Biochemistry and Physiology, New York; (Part A: Molecular & Integrative Physiology) 94: 643-646.

Smith, H. M. (1925): Cell size and metabolic activity in Amphibia.- Biological Bulletin, Woods Hole; 48: 347-378.

Tosunoğlu, M. & Arikan, H. (2007): A serological investigation of *Pelodytes caucasicus* and *Pelobates syriacus* (Amphibia, Anura) populations in Turkey.- Turkish Journal of Zoology, Ankara; 31: 395-398.

Vernberg, F. J. (1955): Hematological studies on salamanders in relation to their ecology.- Herpetologica, Lawrence; 11: 129-133.

DATE OF SUBMISSION: December 27, 2010 Corresponding editor: Heinz Grillitsch

AUTHORS: Hüseyin Arikan < huseyin.arikan@ege.edu.tr >, Kerim Çiçek (corresponding author) < kerim.cicek@ege.edu.tr >, Ege University, Faculty of Science, Department of Biology, Zoology Section, 35100 Bornova, Izmir, Turkey