

Eggshell patterning in the Red-backed Shrike *Lanius collurio*: relation to egg size and potential function

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Abstract. Despite numerous studies on avian eggs, the function of eggshell patterning remains largely unknown. Using a museum collection of Red-backed Shrike clutches, we estimated the repeatability of characteristics describing the intensity and contrast of egg pigmentation, as well as egg size and shape. Digitalized photographs of eggs were used for all measurements. Repeatability was highest for overall pigmentation intensity and overall pigmentation contrast ($R = 0.81$ and $R = 0.82$ respectively). Lower values were recorded for egg breadth ($R = 0.66$) and shape ($R = 0.57$). These findings suggest that eggshell patterning, as well as the variables describing egg size and shape, may respond to natural selection. Among the possible functions of this trait, condition signalling within species and mimicry are the most probable. To obtain more evidence of this, however, further studies in wild populations are required.

Key words: digital image analysis, egg size, egg pigmentation, egg shape, *Lanius collurio*, measurements, museum collection, repeatability

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INTRODUCTION

The egg quality plays important role in avian reproduction. However, to date the majority of studies have focused on quality expressed as egg size and/or egg contents chemical composition (Blount et al. 2000). For example, egg size positively affects the reproductive output of birds, particularly, through influencing chick survival during the first days of their life (review in Williams 1994). It has been also well documented, that egg size traits are heritable and thus respond to natural selection (Hendricks 1991). However, much fewer data provide explanation for function of eggshell colour and patterning. So far, several hypotheses have been proposed (see Kilner 2006 for a review). In the past, the most common were anti-predation and anti-parasitic mechanisms which resulted in crypsis and mimetism of avian eggs respectively. Other functions like filtering solar radiation or strengthening the eggshell, has also been postulated (Moreno & Osorno 2003, Gosler et al. 2005). More recently, eggshell pattern was considered as sexually selected trait signaling female and

offspring condition to mates (Moreno & Osorno 2003, Siefferman et al. 2006). This assumption has been shown in the case studies on pied flycatcher *Ficedula hypoleuca* (Moreno et al. 2004, Moreno et al. 2005, Soler et al. 2005).

Although egg size, shape and eggshell pattern are species specific and even serve as a clue for species identification, eggs produced by females of the same species differ slightly from each other. Understanding basis of this variation is crucial in further investigations on the role of eggshell patterning (Gosler et al. 2000). One of the methods used for analysing variation of any phenotypic traits in animals is repeatability measure. Repeatability represents the proportion of phenotypic variance due to genetic plus environmental variance (Falconer 1981). Thus, repeatability sets an upper limit for heritability. Straightforward studies on inheritance of phenotypic traits demand tracking consecutive generations of a given species what is not always possible in the field conditions (Boag & Noordwijk 1987, Bańbura & Zieliński 1990). In such situations repeatability may be used as an approximation of heritability.

In this article, we made an attempt to assess constancy of eggshell patterning in relation to egg size and shape in the Red-backed Shrike. We are aware of the fact that data on within clutch repeatability are not sufficient to test any hypothesis explaining egg spot pattern function (e.g. Moreno & Osorno 2003, Gosler et al. 2005). On the other hand, to our knowledge, the article is the second one (after Gosler et al. 2000) that deals with the problem of sources of variation in egg patterning. We believe that presented results are good point of departure for further studies on possible functions of this egg trait.

MATERIAL AND METHODS

Egg collection

Data from 1904–1925 were obtained from the oological collection of Upper Silesian Museum in Bytom. Most of the clutches were collected by E. Drescher (Potrzebowska-Dutka 1969). For analysis, only clutches collected in the Opole Silesia and Upper Silesia regions were used. For describing clutch size parameters, information from the museum labels was used. In total data from 73 clutches were used. For more details on the study collection with special attention to the Red-backed Shrike data set see Tryjanowski (2002).

Measurements

The following procedure was developed for taking egg's measurements. Photographs of eggs were taken with a 35 mm camera in a darkroom. There was fixed distance between the lens, flashes and eggs. After films were processed, colour prints were scanned with the resolution of 600 dpi. On each photograph the reference colour scale was present (for radiometric calibration) and control points (for geometric calibration). Scanned images were ortorectified using control points and resampled to the resolution of 100 pixels/cm. Then, the radiometric calibration was performed using the greyscale present on photographs. Individual objects (eggs) on images were automatically extracted using custom functions written in MatLab language with the use of Image Processing Toolbox (MATLAB® The MathWorks). Egg length and breadth were calculated as the major and the minor axis length of the ellipse encompassing on the extracted object. The shape index was calculated by dividing egg length by egg breadth. The measured angle of the major axis was used to rotate eggs and its horizontal

placement. After the rotation, B-spline function was fitted by least squares to the egg envelope (spap2 function from the Matlab Spline Toolbox). Values of the B-spline function in the domain assigned by taking percentages (5%, 10%, 20%, 30%, 50%, 70%, 80%, 90%, 95%) of the major axis length were used for parametrisation of the egg curvature. Egg volume was estimated by rotation of its envelope around its symmetry axis:

$$V = \pi \int_a^b y^2 dx,$$

where: V — estimated egg volume, a, b — integration limits (respectively, minimum and maximum coordinates of the major axis), y — values of the B-spline function fitted to the egg envelope. Integration of the spline function was performed numerically and approximated by summation.

The MatLab functions developed by us for undertaking the computations described above can be downloaded from the web site <http://zbiep.amu.edu.pl/lechu/jaja>.

The functional relationship between egg volume and its measurements (length and breadth) can be described by assuming that egg volume is linearly proportional to the volume of the cylinder encompassing the egg. Based on this simple assumption, Hoyt (1979) proposed a formula:

$$V = a * L * B^2,$$

where: V — egg volume [cm^3], L — egg length [mm], B — egg breadth.

The coefficient a from equation 2 can be estimated for each egg by division of its volume (calculated with equation 1) by the product $L * B^2$. The mean value of coefficient a for our sample was 0.5322 (95% bootstrap confidence intervals: 0.5321–0.5323).

To calibrate photographic egg measurements, 106 eggs were measured with a calliper. We assumed that calliper measurements were more precise as they were taken directly from objects. Both egg length and breadth measured with a calliper were smaller by 0.24 mm than the corresponding dimensions achieved by automatic object extraction and measurement from images. Taking the calliper measurements as a reference, this bias was on average -1.1% in case of egg length and -1.4% in case of egg breadth.

The local egg lightness (reverse pigment concentration), was estimated in 5 sectors assigned by division of the egg major axis on 5 equal-length segments. Standard deviation of pixel lightness

within each sector was used as a measure of contrast. Only pixels lying at least 2 mm apart from the egg edge were used in calculations of egg colour intensity.

Statistical analysis

Principal components analysis was used to extract and interpret egg features. Three separate analyses were undertaken: 1) for egg curvature measurements, 2) for egg pigmentation intensity, and, 3) for egg pigmentation contrast.

To avoid pseudoreplications, correlations between eggs pigmentation and size parameters were computed on mean clutch values.

RESULTS

Interpretation of principal components

Egg size and shape. The three most important components explain more than 96% of the whole variance in egg size and shape measurements. All loadings of the first component are positive, and this axis can be interpreted as an index of overall egg size (Table 1). A strong correlation with the egg breadth supports this interpretation ($R^2 = 0.97$, $df = 458$, $p < 0.0001$). The second component loadings are negative for the blunt end positive for the pointed end. Thus, this component is an index of a difference between dimensions of both egg ends and can be interpreted as a measure of egg shape asymmetry (Fig. 1). Loadings of the third component are negative for the middle part

Table 1. Loadings of the principal components for egg envelope measurements. The values denoted by Y_i are measurements taken at the i -th percentages of the major axis length, where $i = 5\%$, 10% , 20% , 30% , 50% , 70% , 80% , 90% , 95% .

Measurement	PC 1	PC 2	PC 3
Y_5	0.16	-0.34	0.40
Y_{10}	0.24	-0.45	0.28
Y_{20}	0.32	-0.43	0.01
Y_{30}	0.38	-0.32	-0.19
Y_{50}	0.43	-0.03	-0.46
Y_{70}	0.42	0.29	-0.30
Y_{80}	0.39	0.36	0.02
Y_{90}	0.32	0.33	0.41
Y_{95}	0.25	0.27	0.51
Cumulative % of variance	73.40	92.90	96.60

of an egg (around the minor axis or maximum breadth) and positive for both ends. This component portrays the divergence of dimensions near ends comparing to dimensions at the middle and was interpreted as a measure of both ends point-ness (Fig. 1). The fourth component, explaining only 1.5% of the total variance, was rather not interpretable and, likewise the next components, was excluded from the analysis.

Egg pigmentation. The first three principal components explain 99.9% of the total variance in egg pigmentation intensity. Loadings of the first component are positive for lightness in all 5 sectors (Table 2). This component can be interpreted as a reverse measure of the overall egg pigment

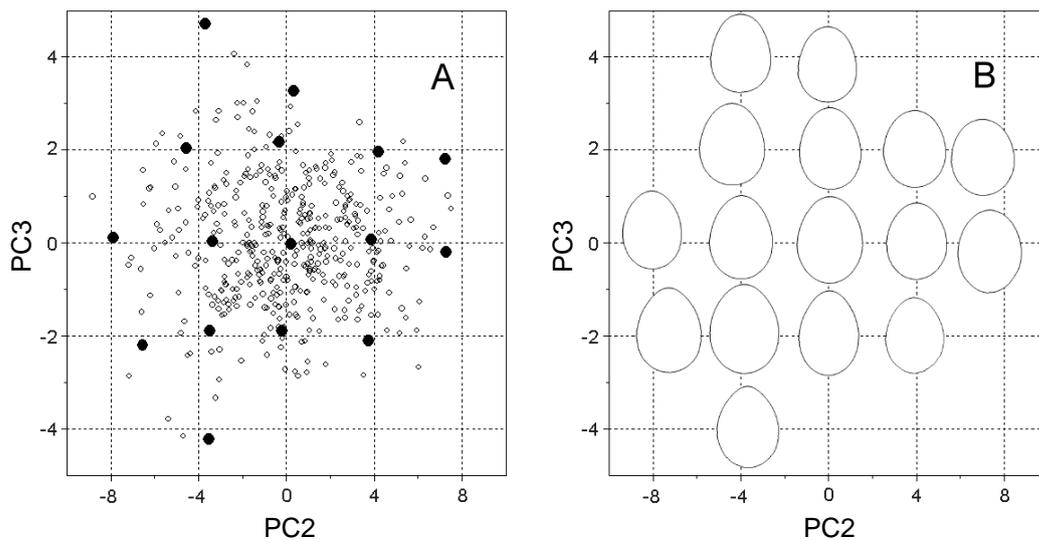


Fig. 1. A: the 2-nd (PC2) and the 3-rd (PC3) principal components of the egg measurements describing its shape. The solid black points are the closest eggs to the vertices of a grid presented on the plot. B: the egg silhouettes corresponding to the black points. For detailed explanation of component interpretation see text and Table 1.

Table 2. Loadings of the principal components of mean lightness (reverse pigmentation intensity, M) measured in five, equally spaced along the major axis, sectors (1-pointed end, 5-blunt end).

Measurement	PC 1	PC 2	PC 3
M_1	0.50	-0.65	0.53
M_2	0.49	-0.27	-0.55
M_3	0.46	0.18	-0.48
M_4	0.40	0.48	0.25
M_5	0.38	0.49	0.34
Cumulative % of variance	96.20	99.50	99.90

content — the higher values indicate lower pigment content (Fig. 2A). Loadings of the second component are negative for the pointed end and positive for the blunt end. This component is a measure of difference between pigmentation of both ends. High values of this component are associated with eggs which are dark on the pointed end (Fig. 2B). Loadings of the third component are negative for the second and third sectors and positive for others. This component describes the placement of mass pigmentation on the egg. High values of this component are typical for eggs with dark ring near the pointed end. Low values of this component represent eggs with the pigmentation concentrated around the blunt end (Fig. 2C).

Egg pigmentation contrast. The first three components explain 97.3% of the total variance in pigmentation contrast. All loadings of the first component are positive and this component was interpreted as a measure of an overall egg spot contrast (Table 3). High values of this component are associated with eggs uniformly colored, without distinct spots, especially on the blunt end (Fig. 3A). Loadings of the second component are negative for sectors near the pointed end and the middle sector and positive for the rest. It can be interpreted as a measure of difference in contrast of pigmentation on the both ends of an egg. Eggs with high values of this component are uniformly pigmented with distinct spots on the blunt end. Low values of this component are characteristic for eggs with bright, uniformly colored blunt end and highly contrasting spots on the pointed end (Fig. 3B). Loadings of the third component are positive for the middle sectors and negative for others. Eggs with high values of this component have a contrasting ring of spots in the middle, without spots at the ends. Low values of this component are typical for eggs uniformly coloured in the middle part with spots at both ends (Fig. 3C).

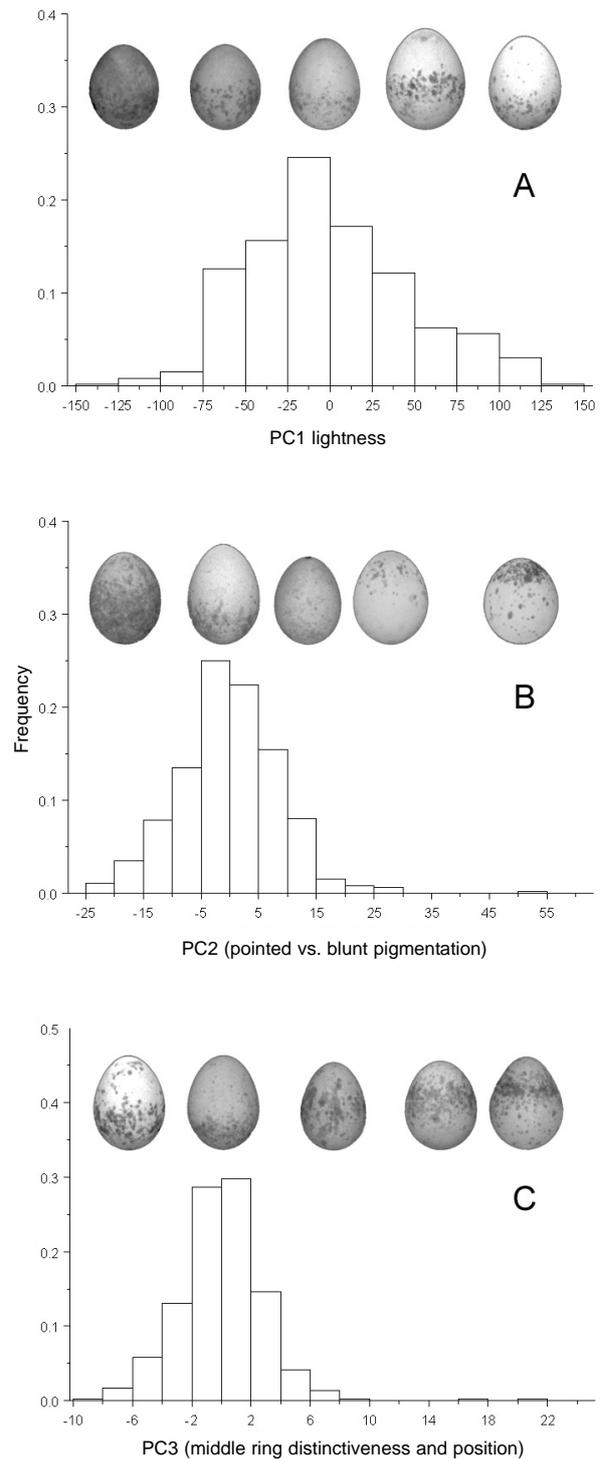


Fig. 2. Variability of principal components describing egg pigmentation intensity. Frequency distributions are shown and greyscale images of eggs corresponding to the values depicted on the axes. A: the gradient of egg overall lightness (= overall reverse pigmentation intensity). B: gradient of egg pigmentation pattern describing the difference between pigmentation of both ends. C: gradient of egg pigmentation pattern describing the placement and distinctiveness of mass pigmentation on the egg. For detailed explanation of component interpretation see text and Table 2.

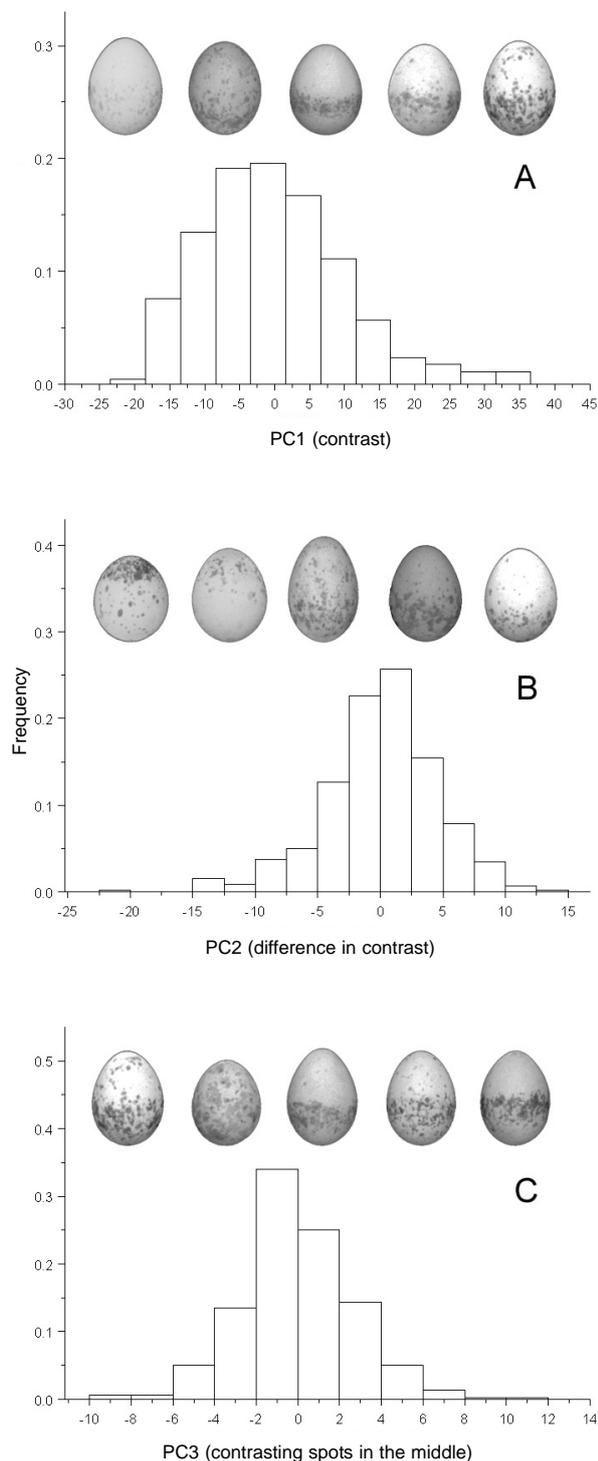


Fig. 3. Variability of principal components describing egg pigmentation contrast (= distinctiveness and placement of spots). Frequency distributions are shown and greyscale images of eggs corresponding to the values depicted on the axes. A: the gradient of overall egg spot contrast. B: gradient of a difference in contrast of pigmentation between the both ends of an egg. C: gradient of spot patterning showing placement of contrasting ring on the egg. For detailed explanation of component interpretation see text and Table 3.

Table 3. Loadings of the principal components of pigmentation contrast (distinctness of spots, SD) measured in five, equally spaced along the major axis, sectors (1-pointed end, 5-blunt end).

Measurement	PC 1	PC 2	PC 3
SD ₁	0.13	-0.61	-0.56
SD ₂	0.14	-0.55	-0.14
SD ₃	0.40	-0.43	0.71
SD ₄	0.64	0.19	0.09
SD ₅	0.63	0.33	-0.39
Cumulative % of variance	75.80	92.00	97.20

Relations between egg size and pigmentations

There were no significant correlations between the index of overall egg size and any of six parameters describing pigmentation intensity and contrast ($p > 0.05$ in all cases or even $p > 0.2$ if Bonferroni test for multiple comparisons was used).

Within clutch repeatability of egg traits

Among traditional characteristics describing egg sizes the highest repeatability values were recorded for egg breadth (B) and the lowest for egg length (L) and shape index (S, Table 4). When comparing principal components derived from egg envelope measurements, the highest repeatability value was noted for egg size and the lowest for end pointedness (Table 4). In the case of pigmentation intensity, the most consistent was the first principal component describing overall pigmentation intensity, while the placement of mass pigmentation was the least repeatable. Among variables connected with pigmentation contrast, the highest repeatability was obtained for overall pigmentation contrast and the lowest for distinctiveness of the middle ring. When comparing all egg traits together the most repeatable trait were overall egg pigmentation ($R = 0.82$) and the least consistent was placement of mass pigmentation ($R = 0.22$).

DISCUSSION

Our results show that egg patterning is more consistent than egg size and shape in Red-backed Shrike. This was demonstrated by within clutch repeatability values for overall pigmentation intensity and pigmentation contrast compared to egg volume and breadth (Table 4). According to idea of repeatability measure, this results show

Table 4. Repeatability (R) of egg features.

Feature	R	95% confidence limits	
Egg dimensions from calliper measuring			
Egg volume	0.64	0.55	0.73
Egg length	0.57	0.47	0.66
Egg breadth	0.66	0.57	0.74
Shape index	0.57	0.47	0.67
Principal components for egg envelope measurements			
Egg size	0.64	0.55	0.72
Shape symmetry	0.49	0.38	0.59
End pointedness	0.32	0.21	0.43
Principal components of mean pigmentation intensity			
Overall lightness (reverse pigmentation intensity)	0.82	0.77	0.87
Difference in pigmentation between egg ends	0.61	0.51	0.70
Placement of mass pigmentation	0.22	0.12	0.33
Principal components of pigmentation contrast			
Overall pigmentation contrast	0.81	0.76	0.86
Difference between pigmentation contrast of both ends	0.31	0.20	0.42
Distinctiveness of the middle ring	0.24	0.14	0.36

that variation in eggshell patterning is high heritable trait. Moreover, at least some aspects of the egg patterning are more repeatable than egg shape and size. In other words, traits connected with egg size and shape seem to be more affected by environmental conditions than eggshell patterning. Likewise other passerines, Red-backed Shrikes' females rely on current environmental resources during egg formation (income breeder). The mean clutch size in a studied population was ca. 5 eggs (Tryjanowski 2002). Knowing that Red-backed Shrike lays one egg daily, we may assume that conditions during egg formation could vary in terms of e.g. food availability, male provisioning rate or weather. The question remains why ambient conditions affect more egg size and shape than eggshell patterning? It is probable that egg dimensions, especially length, are more sensitive to the short-term environment resources fluctuations because it demands more energy to keep egg size constant. Moreover, the bulk of evidences show that fitness of females depends most of all on egg dimensions, not on their coloration (e.g. Pinowska et al. 2004, see Williams 1994 for a review). According to Fisherian models, traits related to individual's fitness tend to lose their additive genetic variability and thus become less heritable. This would explain why general patterning patterns in Red-backed Shrike's eggs are more consistent than their dimensions.

Some of characteristics connected with eggshell patterning obtained low within clutch repeatability values (placement of mass pigmentation, difference between pigmentation contrast of both ends, and distinctiveness of the middle ring;

Table 4). All characteristics listed above deal with distribution of spots, especially presence and location of so called "crown" or a ring of spots surrounding egg. It has been shown in gulls and terns that the degree of development of spots rings may depends of egg laying sequence (Preston 1957, Chamberlin 1977). It is probable that the same rule plays also in the case of Red-backed Shrike. The design of our study excludes possibility to point at any biological function connected with eggshell patterning. Indeed, if we assume that high within clutch repeatability represents high heritability, then high heritability of eggshell pigmentation could be evidence of any adaptive cause of this trait including crypsis, signalling to mates, eggshell strength etc. However, irrespective of the real function of eggshell patterning in Red-backed Shrike, one important conclusion could be derived from our results. Egg pigmentation is more heritable than egg dimensions, thus more prone to evolutionary response to selection for that trait. Considering massive disproportion between articles dealing with egg sizes and those about egg coloration, this should encourage researchers to focus more on the latter.

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REFERENCES

- Bañbura J., Ziełiński P. 1990. Within-clutch repeatability of egg dimensions in the Black-headed Gull *Larus ridibundus*. *J. Ornithol.* 131: 305–310.
- Blount J. D., Houston D. C., Møller A. P. 2000. Why egg yolk is yellow? *Trends Ecol. Evol.* 15: 47–49.
- Boag P. T., van Noordwijk A. J. 1987. Quantitative genetics. In: Buckley P. A., Cooke F. (eds). *Avian genetics*. London, pp. 45–78.
- Chamberlin M. L. 1977. Relationship between egg pigmentation and hatching sequence in the Herring Gull. *Auk* 94: 363–365.
- Falconer D. S. 1981. *Introduction to quantitative genetics*. Longmans, London, New York.
- Gosler A. A., Barnett P. R., Reynolds S. J. 2000. Inheritance and variation in eggshell patterning in the Great Tit *Parus major*. *Proc. Roy. Soc. B* 267: 2469–2473.
- Gosler A. A., Higham J. P., Reynolds S. J. 2005. Why are birds' eggs specked? *Ecol. Lett.* 8: 1105–1113.
- Hendricks P. 1991. Repeatability of size and shape of American Pipit eggs. *Can. J. Zool.* 69: 2624–2628.
- Hoyt D. F. 1979. Practical methods for estimating volume and fresh weight of birds eggs. *Auk* 96: 73–77.
- Kilner R. M. 2006. The evolution of egg colour and patterning. *Biol. Rev.* 81: 383–406.
- Moreno J., Morales J., Lobato E., Merino S., Tomás G., Martínez-de la Puente J. 2005. Evidence for the signaling function of egg color in Pied Flycatcher *Ficedula hypoleuca*. *Behav. Ecol.* 16: 931–937.
- Moreno J., Osorno J. L. 2003. Avian egg colour and sexual selection: does eggshell pigmentation reflect female condition and genetic quality? *Ecol. Lett.* 6: 803–806.
- Moreno J., Osorno J. L., Morales J., Merino S., Tomás G. 2004. Egg coloration and male parental effort in the Pied Flycatcher *Ficedula hypoleuca*. *J. Avian Biol.* 35: 300–304.
- Pinowska B., Barkowska M., Pinowski J., Bartha A., Hahm K.-H., Lebedeva N. 2004. The effect of egg size on growth and survival of the Tree Sparrow *Passer montanus* nestlings. *Acta Ornithol.* 39: 121–135.
- Potrzebowska-Dutka J. 1969. Die Sammlung der Vogelneuste im Museum Górnosławskie Bytom. *Roczniki Muzeum Górnosławskiego w Bytomiu, seria Przyroda* 4: 7–22.
- Preston F. W. 1957. Pigmentation of eggs: variation in the clutch sequence. *Auk* 74: 28–41.
- Siefferman L., Navara K. J., Hill G. E. 2006. Female coloration is correlated with egg coloration in Eastern Bluebirds *Sialia sialis*. *Behav. Ecol. Sociobiol.* 59: 651–656.
- Soler J. J., Moreno J., Avilés J. M., Møller A. P. 2005. Blue and green egg color intensity is associated to parental effort and mating system in passerines: support for the sexual selection hypothesis. *Evolution* 59: 636–644.
- Tryjanowski P. 2002. A long-term comparison of laying date and clutch size in the Red-backed Shrike *Lanius collurio* in Silesia, southern Poland. *Acta Zool. Hung.* 48: 101–106.
- Williams T. D. 1994. Intraspecific variation in egg size and egg composition in birds: effects on offspring fitness. *Biol. Rev.* 68: 35–59.

STRESZCZENIE

[Barwa skorupki jaj u gąsioraka: związek z rozmiarami jaja i potencjalne funkcje]

Celem niniejszych badań była ocena powtarzalności różnych parametrów jaj w obrębie

zniesienia. Badano zarówno cechy związane z rozmiarami i kształtem jaj, jak również z intensywnością oraz kontrastowością ubarwienia skorupki. Jako obiekt badań posłużyły jaja gąsioraka pochodzące ze zbiorów Górnosławskiego Muzeum w Bytomiu (w sumie 73 lęgi), zebrane w latach 1904–1925. Wszystkie jaja zostały sfotografowane w standardowych warunkach za pomocą aparatu małoobrazkowego, a wywołane zdjęcia zeskanowano. Uzyskane obrazy cyfrowe posłużyły do obliczenia wymiarów jaj, intensywności pigmentacji oraz kontrastowości pigmentacji. W przypadku wymiarów jaj, oprócz standardowych pomiarów (objętość, długość, szerokość, współczynnik kształtu), wykonano pomiary krzywizny skorupki oraz intensywności i kontrastowości zabarwienia w sektorach rozmieszczonych wzdłuż osi symetrii jaja. W celu ułatwienia interpretacji wyników, dane dotyczące krzywizny skorupki, intensywności oraz kontrastowości zabarwienia zredukowano za pomocą analizy składowych głównych. W każdym z trzech przypadków uzyskano interpretowalne składowe główne wyjaśniające co najmniej 96% zmienności (Tab. 1–3, Fig. 1–3). W celu sprawdzenia dokładności analizy zdjęć cyfrowych jako metody pomiaru wielkości jaj, 106 jaj zostało zmierzonych również za pomocą suwmiarki. Pomiary wykonane obiema metodami były ze sobą silnie skorelowane, chociaż wymiary uzyskane za pomocą suwmiarki były średnio o 0.24 mm mniejsze w stosunku do tych uzyskanych ze zdjęć.

W przypadku zmiennych opisujących wymiary jaj, najwyższą powtarzalność uzyskano dla szerokości ($R = 0.66$) oraz zmiennej opisującej ogólną wielkość jaja ($R = 0.64$, Tab. 4). Wyższe wskaźniki powtarzalności uzyskano dla ogólnej intensywności i kontrastowości pigmentacji (odpowiednio: $R = 0.82$, $R = 0.81$, Tab. 4). Powyższe wyniki sugerują, że cechy jaj związane z ich ubarwieniem są bardziej powtarzalne niż parametry opisujące rozmiary jaj. Wskazuje to, że czynniki środowiskowe działające na samice w czasie formowania się jaj w obrębie lęgu, wpływają bardziej na ich rozmiary niż ubarwienie skorupki. Na tej podstawie można założyć, że działanie doboru naturalnego na kolor i plamkowanie jaj może skutkować odpowiedzią ewolucyjną. Fakt ten nie wyjaśnia jednak funkcji plamkowania jaj u gąsioraka. Wyjaśnienie tego problemu wymaga dalszych badań na dziko żyjących populacjach ptaków.