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Kazimierz Walasz

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Title: **Sexual dimorphism of egg size in the European blackbird *Turdus merula***

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26 **Abstract**

27 Recently, a number of studies have shown that female birds are able to control the sex of their  
28 progeny at the stage of the gamete. There is also some evidence that females adjust their  
29 investment in offspring depending on the sex of the embryo during egg formation.  
30 Differential maternal investment to the eggs depending on their sex is usually interpreted as  
31 an adaptive strategy, by which females can increase competitive abilities of the smaller sex, or  
32 preferentially invest towards the sex with the potentially higher fitness returns. Here, we  
33 studied variation in egg size in relation to embryo sex and laying order in the European  
34 blackbird *Turdus merula*. We found male and female eggs to differ in size, with larger eggs  
35 containing male embryos, as well as a significant interaction between embryo sex and laying  
36 order. This interaction resulted from the fact that egg size increased with the laying sequence  
37 among eggs bearing females but did not change with laying order among eggs bearing males.  
38 There was no relationship between offspring sex and the laying sequence within a clutch. We  
39 suggest that sexual dimorphism in egg size recorded in the European blackbird may reflect  
40 favoritism of the sex which may give higher fitness returns.

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42 **Key words:** egg volume, hatching asynchrony, laying sequence, maternal effects, sex  
43 allocation

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51 **Introduction**

52 Sex allocation theory predicts that if a social or ecological circumstance differentially affects  
53 the reproductive value of male and female offspring, parents should skew their investment  
54 towards the sex with higher fitness returns. Differential sex allocation can be potentially  
55 achieved in two ways, by the production of different numbers of sons and daughters, or by  
56 unequal allocation of resources to the members of each sex (Trivers and Willard 1973,  
57 Charnov 1982). In birds, females are the heterogametic sex, and thus have the opportunity to  
58 vary their investment in sons and daughters both during egg production and after hatching by  
59 differential provisioning of the offspring. In most bird species, the male is usually the sex  
60 with higher variance in reproductive success (Griffith et al. 2002). Thus, according to sex  
61 allocation theory mothers may be expected to allocate more resources to male offspring  
62 (Charnov 1982). The first evidence showing disparities in size between eggs bearing male and  
63 female embryos comes from studies on the White-crowned sparrow *Zonotrichia leucophrys*  
64 *orianta*, in which authors detected that male offspring hatch from larger eggs (Mead et al.  
65 1987). The same pattern of egg sexual dimorphism where males (the larger sex) come from  
66 larger eggs has also been found in other bird species (e.g. Cordero et al. 2000, Cunningham  
67 and Russell 2001, Leitner et al. 2006). If male offspring benefit more from increased maternal  
68 investment early in life compared to female offspring in terms of lifetime reproductive  
69 success, the observed pattern of egg provisioning may be considered as an adaptive favoritism  
70 of the sex with potentially higher fitness returns (e.g. Mead et al. 1987, Cordero et al. 2000).  
71 Alternatively, it is suggested that females may differentiate investment to eggs to boost the  
72 competitive ability of the smaller sex. Two studies on sexually size dimorphic birds, the  
73 American kestrel *Falco sparverius* and the Brown songlark *Cinclorhamphus cruralis*, show  
74 that eggs containing the smaller, less competitive sex are larger (Anderson et al. 1997,  
75 Magrath et al. 2003). Females may also affect performance of sons and daughters by placing

76 male and female eggs early or late in the laying sequence (e.g. Cichoń et al. 2003, Lezalova et  
77 al. 2005). Such differential allocation to sons and daughters according to the laying order  
78 might be an important strategy of compensating negative effects of asynchronous hatching  
79 (e.g. Rutkowska and Cichoń 2005).

80         Here, we examined whether eggs containing male and female embryos differ in size  
81 and whether sexes are non-randomly ordered in the laying sequence in the European blackbird  
82 *Turdus merula*. Sexual dimorphism in egg size might be expected in this species as sexes  
83 differ in size (adult males are ca. 5% larger than adult females; Cramp 1988, Wysocki 2002,  
84 Walasz, unpublished data), and fitness returns resulting from additional maternal investment  
85 to sons and daughters may differ, due to the high frequency of extra-pair paternity (in the  
86 studied population up to 18% of offspring originate from extra-pair copulation, Rutkowska et  
87 al, unpublished data) and possibility of facultative male polygyny (Wysocki 2004). As the  
88 European blackbird shows substantial hatching asynchrony (Magrath 1989, Walasz,  
89 unpublished data), strategies of allocation of offspring sex to early or late eggs in the laying  
90 order might be expected. Thus, we predicted that males would be placed in larger and earlier  
91 eggs.

92

### 93 **Methods**

94 The study was carried out in an urban blackbird population inhabiting the Dr Jordan Park in  
95 Kraków (southern Poland). The European blackbird is a medium-sized, sexually dichromatic,  
96 strongly territorial, socially monogamous, and open cup nesting passerine bird (Cramp 1988).  
97 The park is located close to the city center and covers 21.3 ha; density of the breeding  
98 blackbirds averaged 1.9 pairs per 1 ha (ca. 40 pairs every year). Most males occupy their  
99 territories from March and April and some of them stay on their territories throughout the  
100 whole year. Females start laying eggs at the end of March and continue into July. In the study

101 population, females usually lay 3-5 eggs (mean  $\pm$  SD:  $4.3 \pm 0.7$ , N = 99 clutches). As a result  
102 of high nest predation, about 95% of females repeat breeding attempts. Most nests are  
103 destroyed during egg laying (very often after laying first or second egg) and incubation. All  
104 territories were monitored daily and nests were found by following nest-building females and  
105 searching bushes and trees throughout the park. Almost all breeding birds in the park were  
106 individually marked with an aluminum band and a combination of colour bands. In years  
107 2000-2003 and 2005-2006 laying date of the first egg, clutch size, hatching date and number  
108 of nestlings were recorded in most of nests. Additionally, data on laying and hatching order  
109 were recorded in a sample of nests. In most cases, data on egg size and sex were obtained  
110 from clutches deserted during incubation (N = 45 eggs from 25 clutches). In this case,  
111 samples of tissue from embryos were collected. In some nests nestlings hatched successfully  
112 (N = 33 hatchlings from 27 clutches), and blood was sampled for further sex determination  
113 within 1-2 days after hatching of the first nestling. In the case of 2 clutches, genetic material  
114 was collected from both unhatched eggs (N = 2) and alive nestlings (N = 2). Laying order was  
115 determined by inspection of nests every day during egg laying. On the day of laying, eggs  
116 were numbered with a permanent marker, measured with an electronic calliper (length and  
117 width to the nearest 0.1 mm) and weighed with an electronic balance (to the nearest 0.01 g).  
118 Starting from the day before expected hatching, nests were visited daily to assign each  
119 marked egg to its corresponding hatchling. Nestlings were then marked by nail clipping. In  
120 some nests, we were not able to assign hatchlings to the eggs from which they hatched and so  
121 they were excluded from the analyses. To calculate egg volume, we used the formula,  $V =$   
122  $0.51 \times LW^2$ , where L = egg length and W = egg width, which can be applied to most bird  
123 eggs (Hoyt 1979). Egg volume calculated according to this formula is highly correlated with  
124 the egg mass ( $r = 0.85$ ,  $P < 0.0001$ , N = 87). Overall, data on laying order and sex were  
125 collected for 82 eggs originating from 54 different females (clutches). 93% of these clutches

126 were incomplete because of egg infertility (N = 4), unknown hatching order of nestlings (N =  
127 4), partial predation (N = 17) and discovery of the nest after egg-laying initiation (N = 25).

128 Blood and tissue samples were kept in 0.5 ml of 96% ethanol and stored at room  
129 temperature. DNA was extracted using Nucleo Spin Blood kit (Macherey-Nagel) following  
130 the manufacturer's protocol. Molecular sexing was performed by PCR amplification of two  
131 homologous genes (CHD1-W and CHD1-Z) following the protocol of Griffiths et al. (1998).  
132 PCR products were separated by electrophoresis on 3% agarose gels, which were stained with  
133 ethidium bromide and visualized under UV trans-illumination. The sex was assessed  
134 according to the presence of one band for a male or two bands for a female. This method was  
135 positively validated by blindly testing blood samples originating from 10 adult birds of known  
136 sex.

137 To examine variation in egg volume in relation to embryo sex and laying sequence, we  
138 used the linear mixed model (PROC MIXED in SAS). In this model, the sex of the embryo  
139 was introduced as a categorical variable and laying order as a covariate. To control for  
140 possible non-linear effects, the laying order was introduced to the model as a linear and  
141 quadratic term and their interactions with sex were also tested. In addition, year as a  
142 categorical variable and laying date and clutch size as covariates were introduced to the initial  
143 model. To distinguish between within and among clutch effects, clutch sex ratio was entered  
144 to the initial model and although non-significant it remained in the final model. This clutch  
145 sex ratio was derived only of eggs with known sex and laying order. The level of significance  
146 was set at  $P < 0.05$  and non-significant terms were systematically removed from the initial  
147 model to increase the power of tests. Female identity was entered in each model as a random  
148 factor. To analyze the relationship between offspring sex and laying order, we used the  
149 generalized linear mixed model using GLIMMIX macro in SAS with logit link function and  
150 binomial error variance. To check possible non-linear effects of the position of the egg in the

151 laying sequence on offspring sex we introduced to the model laying order as a linear and  
152 quadratic term. Female identity was introduced to the model as a random factor. Because we  
153 did not find any non-linear effects, laying order squared was removed from the final model.  
154 All analyses were performed in SAS version 9.1 and denominator degrees of freedom were  
155 approximated by the Satterthwaite method.

156

## 157 **Results**

158 We found that within a clutch, eggs containing male and female embryos to differ in size  
159 (Table 1). Male eggs were significantly larger (least sq mean  $\pm$  SE; egg volume:  $6988.7 \pm$   
160  $112.9 \text{ mm}^3$ ) in comparison to female eggs (least sq mean  $\pm$  SE; egg volume:  $6842.6 \pm 110.9$   
161  $\text{mm}^3$ ). There was also a significant interaction between embryo sex and laying order (Table 1,  
162 Figure 1). Separate analysis performed for each sex showed that egg volume significantly  
163 increased with the laying sequence among eggs bearing female embryos ( $F_{1, 25.4} = 8.46$ ,  $P =$   
164  $0.007$ ; Figure 1). The effect of laying order was not significant among eggs bearing male  
165 embryos ( $F_{1, 23.4} = 1.44$ ,  $P = 0.24$ ; Figure 1). We also found a non-linear effect of position of  
166 the egg in the laying sequence on egg size (Table 1). This results from the fact that initial eggs  
167 were larger than middle eggs and final eggs were the largest within a clutch. There were no  
168 between clutch effects on egg volume (Table 1), thus the observed within-clutch effects were  
169 independent from differences in sex ratio. The sex ratio in the studied sample of eggs was not  
170 significantly different from parity (49% males; Binomial test,  $P = 0.90$ ). Offspring sex did not  
171 vary with the position of the egg in the laying sequence analyzed both as a linear (laying  
172 order:  $F_{1, 80} = 1.62$ ,  $P = 0.21$ ) and quadratic term (laying order:  $F_{1, 79} = 0.26$ ,  $P = 0.61$ ; laying  
173 order laying order:  $F_{1, 79} = 0.08$ ,  $P = 0.78$ ).

174

## 175 **Discussion**

176 In this study, we report within-clutch differences in eggs size between sexes but do not find  
177 evidence that offspring sex is differently allocated in early and late laid eggs in the European  
178 blackbird. Sexual egg size dimorphism has been reported in at least six passerine species  
179 (Mead et al. 1987, Cordero et al. 2000, 2001, Arnold and Griffiths 2003, Magrath et al. 2003,  
180 Leitner et al. 2006). There are also similar findings from non-passerine species (Anderson et  
181 al. 1997, Petrie et al. 2001; see also Cunningham and Russell 2001, Blanco et al. 2003, Müller  
182 et al. 2005). However, the observed patterns of egg sexual size dimorphism are inconsistent  
183 and multiple hypotheses have been suggested to explain the possible adaptive value of  
184 disparities in egg size between male and female eggs. Among passerines, female offspring of  
185 the Spotless starling *Sturnus unicolor* and the Brown songlark hatched from larger eggs  
186 (Cordero et al. 2001, Magrath et al. 2003). This pattern of egg provisioning was interpreted as  
187 an adaptive strategy of support for the more vulnerable smaller sex (Cordero et al. 2001,  
188 Magrath et al. 2003). In contrast, in the White-crowned sparrow, the House sparrow *Passer*  
189 *domesticus* and the captive canaries *Serinus canaria*, males were found in larger eggs, despite  
190 them being larger than females (Mead et al. 1987, Cordero et al. 2000, Arnold and Griffiths  
191 2003, Leitner et al. 2006). Mead et al. (1987) and Cordero et al. (2000) explained their results  
192 in light of the sex allocation theory, arguing that better provisioning of eggs bearing male  
193 embryos may be a adaptive strategy of increased investment to the sex with the potential to  
194 give higher fitness returns. In fact, males of those species exhibit higher variation in  
195 reproductive success compared to females resulting from extra-pair copulation, very common  
196 in these species. Therefore, the increased maternal investment towards male offspring might  
197 be expected. Support for this hypothesis comes from studies on mammals, which indicate that  
198 the breeding success of a male is related to maternal investment at early stages of life,  
199 however, only mothers in good condition can produce more successful males (e.g. Clutton-  
200 Brock et al. 1984, 1986, Sheldon and West 2004). Thus, extra investment in eggs containing

201 male embryos may be considered as an adaptive strategy by which females could increase  
202 reproductive success of their sons (e.g. Mead et al. 1987, Cordero et al. 2000). Such scenario  
203 may also contribute to explain sexual egg size dimorphism in the European blackbird, in  
204 which extra-pair paternity is common and generate a substantial variation in male  
205 reproductive success (Creighton 2000, Rutkowska et al, unpublished data). However, rigorous  
206 test of the adaptive role of sex-specific variation in egg size is missing. The observed pattern  
207 of sexual dimorphism in egg size may alternatively be explained without invoking adaptive  
208 explanations. For instance, if the same maternal hormones regulate oocyte growth and  
209 simultaneously affect sex-determining chromosome segregation, sexual egg size dimorphism  
210 may arise as a not-necessarily adaptive consequence of this hormonal mechanism (e.g. Young  
211 & Badyaev 2004).

212         In the current study we found a significant interaction between sex and laying order  
213 resulting from the fact that egg size increased with laying order among female but not male  
214 eggs. An increase in egg size is often explained in terms of compensatory mechanism of  
215 negative effect of hatching asynchrony (e.g. Clark and Wilson 1981). Such compensation  
216 could be expected in the blackbird, as eggs hatch asynchronously in this species (Magrath  
217 1989, Walasz, unpublished data). Interestingly, such compensation would occur only among  
218 eggs bearing daughters. Since eggs bearing daughters are smaller, they may pay larger cost of  
219 hatching asynchronously and the increase in egg size in late laid eggs may offset negative  
220 effects of hatching asynchrony. Such sex-specific effect has already been reported in  
221 asynchronously hatching zebra finches *Taeniopygia guttata*, in which egg mass and egg  
222 laying sequence were significant predictors of differential survival of male and female  
223 offspring (Rutkowska and Cichoń 2005).

224

225 **Zusammenfassung**

226 **Sexueller Dimorphismus der Eigröße in Amseln *Turdus merula***

227

228 In letzter Zeit haben mehrere Studien zeigen können, dass weibliche Vögel in der Lage sind,  
229 das Geschlecht ihrer Nachkommen während des Keimzellenstadiums zu kontrollieren. Ferner  
230 gibt es Hinweise darauf, dass Weibchen ihre Investitionen in ihre Nachkommen von dem  
231 Geschlecht des Embryos während der Eiformation abhängig machen können. Die  
232 unterschiedliche mütterliche Investition in Eier in Abhängigkeit des Geschlechts wird  
233 normalerweise als adaptive Strategie interpretiert, wobei Weibchen die  
234 Wettbewerbsfähigkeiten des kleineren Geschlechts verbessern können, oder vorzugsweise in  
235 das Geschlecht mit dem potenziell höheren Fitnessgewinn investieren. In der vorliegenden  
236 Arbeit haben wir Variation in Eigröße relativ zum Geschlecht der Embryos und  
237 Legereihenfolge in Amseln *Turdus merula* untersucht. Männliche und weibliche Eier waren  
238 unterschiedlich groß — größere Eier enthielten männliche Embryos. Wir fanden auch eine  
239 signifikante Interaktion zwischen dem Geschlecht von Embryos und der Legereihenfolge.  
240 Diese Interaktion entstand durch eine Zunahme von Eigröße mit Legereihenfolge innerhalb  
241 von Eiern die weibliche Embryos enthielten; die Eigröße von männlichen Embryonen  
242 veränderte sich dagegen nicht mit der Legereihenfolge. Wir fanden keinen Zusammenhang  
243 zwischen dem Geschlecht der Nachkommen und der Legereihenfolge innerhalb einzelner  
244 Gelege. Wir schlagen vor, dass der sexuelle Dimorphismus der Eigröße bei Amseln  
245 möglicherweise die Begünstigung des Geschlechts mit dem höheren  
246 Fitnessgewinnversprechens reflektiert.

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255

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323 Table 1.

Final model	Rejected terms	Estimate	SE	d.f.	F	P
Sex		-720.03	281.67	1, 37.3	6.53	0.015
Laying order		-390.81	202.28	1, 36.7	2.47	0.124
Laying order laying order		73.95	30.57	1, 35.4	5.85	0.021
Sex laying order		187.51	78.52	1, 42.8	5.70	0.021
Clutch sex ratio		398.13	245.61	1, 74	2.63	0.109
	Laying date	6.90	3.73	1, 45.5	3.43	0.071
	Sex laying order laying order	-59.61	67.10	1, 38.3	0.79	0.380
	Year			5, 47.2	0.70	0.627
	Clutch size	41.69	168.11	1, 45.4	0.06	0.805

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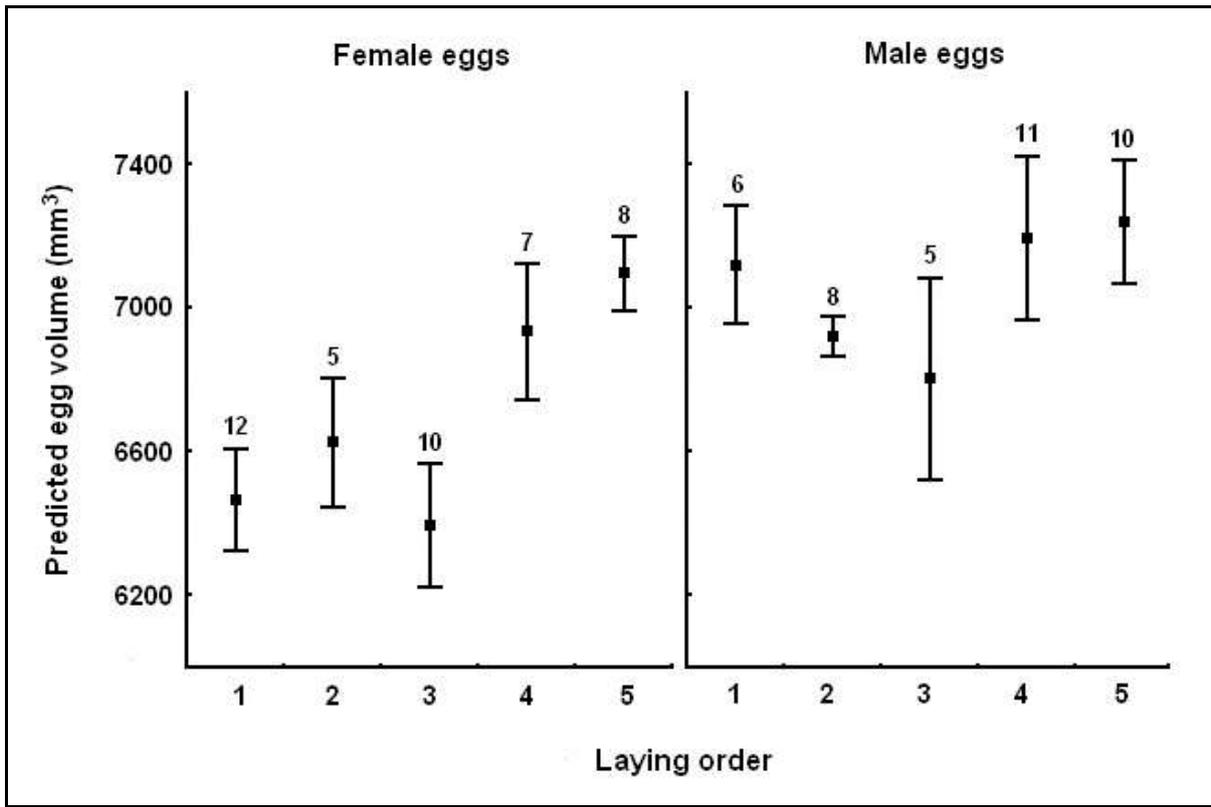
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332 Figure 1.



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347 Table 1. Results of the Linear Mixed Model (PROC MIXED in SAS) analyzing variation in  
348 egg volume in relation to embryo sex and year as categorical variables, laying order, clutch  
349 sex ratio, clutch size and laying date as covariates. Laying order was entered to the model  
350 both as a linear and quadratic term and all possible interaction between sex and laying order  
351 were tested. Non-significant terms were systematically removed from the initial model. In  
352 table F and P values, degrees of freedoms (d.f.) and estimates with SEs presents values for the  
353 final model, whereas in the case of rejected terms on the time when they were dropped from  
354 the model. Clutch sex ratio was entered to the initial model and remained in the final model to  
355 control for between clutch effects. Female identity was included as a random factor ( $Z = 3.75$ ,  
356  $P < 0.0001$ ; the final model).

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358 Figure 1. Predicted egg volume (least square means  $\pm$  SEs) in relation to embryo sex and  
359 laying order in the European blackbird. The respective sample sizes are indicated above bars.

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