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Determinants of Eggshell Strength in Endangered Raptors

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ABSTRACT We compared eggshell strength in a group of falcon taxa including the peregrine falcon (*Falco peregrinus peregrinus*), the red shaheen falcon (*F. peregrinus babylonicus*), the saker falcon (*F. cherrug*), the gyr falcon (*F. rusticolus*) and some interspecific and intraspecific hybrids. Our results showed that smaller falcons ($<1{,}000$ g) of the peregrine group have eggshells that are significantly softer ($\bar{x} = 13.3$ N) and thinner ($\bar{x} = 0.26$ mm) ($n = 107$ eggs) than larger falcons ($>1{,}000$ g) of the gyr-saker group ($\bar{x} = 20.8$ N and 0.39 mm, respectively, $n = 81$ eggs). We found a significant positive correlation between egg hardness and eggshell thickness. Linear mixed models showed that clutches from heavier females consisted of larger and harder eggs with thicker shells and thicker egg membranes. Eggs produced by older females and eggs laid later in the laying sequence were relatively smaller and softer and had relatively thin egg membranes and eggshells. Individual females, irrespective of their age, contributed significantly to the observed variation in egg strength. Egg size and hardness of hybrid eggs were similar to that of the pure species suggesting that hybridization does not affect eggshell hardness or thickness. Our study provides quantitative evidence of several factors, other than levels of contamination, which may affect eggshell thickness and hardness in falcons. *J. Exp. Zool. 311A:303–311, 2009.* © 2009 Wiley-Liss, Inc.

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Reduction of egg viability is an important cause of reproductive failure and has been suggested to contribute to decreases in bird populations (Drent and Woldendorp, ’89; Graveland and Drent, ’97). Population collapse and reproductive failure in raptors have occurred in many parts of the northern hemisphere from about 1950 onwards (Ratcliffe, ’80; Crick and Ratcliffe, ’95; Newton, 2004). The relationship between the use of DDT and its effect on bird populations was first detected in peregrine falcons by Ratcliffe in England (Ratcliffe, ’58, ’67). Subsequently, high levels of pesticides have been related to a reduction in eggshell thickness in falcons and other bird species, although not in all (Cade et al., ’71; Peakall and Lincer, ’96; Falk et al., 2006), and eggshells have been suggested to be useful tools to monitor the health of bird populations over long periods. However, long-term thinning of eggshells may not be only related to pollution, and other factors may also influence variation in eggshell thickness. For example, a significant decrease in

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eggshell thickness has been noted in birds (e.g., thrushes, *Turdus* spp.) even before the introduction of organochlorine pesticides (Green, '98; Scharlemann, 2003).

Previous results from poultry and wild birds have indicated that eggshell breaking strength and/or eggshell thickness are influenced by the egg size and color (Kennedy and Vevers, '73), the location of the egg where these parameters are measured (Gosler et al., 2005), egg developmental stage (Vanderstoep and Richards, '70; Bunck et al., '85; Bennett, '95; Castilla et al., 2007), shell microstructure (Massaro and Davis, 2005), female age, body mass and health status, the time the eggs spend in the uterus, the length of incubation period, egg laying sequence and clutch number (Ar et al., '79; Massaro et al., 2002; Massaro and Davis, 2004; Castilla et al., 2009), the type of diet (Connor and Arnold, '72) and genetics (Francesch et al., '97).

Ecological factors may also determine egg hardness. For example, bird species nesting in hard soils or cavities likely benefit from stronger eggs as this may provide them with a protection from natural breakage (Mallory and Weatherhead, '90; Brooker and Brooker, '91; Boersma et al., 2004) and in some cases, intraspecific egg destruction has led to the evolution of unusually strong eggs (Picman et al., '96; Picman and Honza, 2002). Although eggshell hardness thus appears to be important to birds, very little research has been devoted to this topic in wild species. Because many factors may influence on eggshell strength, it is important to identify and quantify these in order to understand the patterns of the observed variation in many wild bird populations.

In this study we focused on different falcon taxa. Falcons are not known to suffer from nest parasitism or high nest predation (Cramp and Simmons, '80), and thus variation in egg hardness is likely not affected by these characteristics. Falcons are endangered species and many populations are declining worldwide. In order to restore populations through conservation programs recovery centers where falcons are bred under captive conditions have been established (e.g., Rahbek, '93). Because of the captive conditions of the birds, a general weakness of working on captive-bred birds can be turned into an advantage because the birds are likely unaffected by naturally occurring pollutants. In addition, the eggs are produced under near-optimal conditions (e.g., females are provided with a high-quality diet) as breeders are interested in obtaining large and healthy clutches with good hatching success for commercial purposes. Another advantage of working on these captive birds is that a large sample of eggs can be obtained and measured, and important information related to the female age, clutch size, egg laying sequence, etc. can be obtained.

In this study we explored several factors that could affect eggshell strength variation among falcon taxa, including egg characteristics (egg size, length, width, mass; membrane thickness, eggshell thickness, egg design and color), female characteristics (individual identity, age, body mass), egg laying sequence and zone. We also tested the prediction that eggshells from bigger eggs are stronger than those from smaller eggs, and falcons with a higher body mass lay stronger eggs. We also examined the relationship between egg hardness and eggshell thickness among taxa, and explored possible effects of hybridization on eggshell strength.

MATERIALS AND METHODS

Study animals and eggs

The falcon species examined in this study are protected, rare or endangered and included on the CITES list (Cramp and Simmons, '80). They include the peregrine falcon, *Falco peregrinus peregrinus*, the red shaheen falcon, *F. peregrinus babylonicus*), the intraspecific hybrid *F. peregrinus peregrinus*∗*F. peregrinus babylonicus*, the saker falcon, *F. cherrug*, the gyr falcon, *F. rustcolus* and their hybrid (*F. cherrug*∗*F. rustcolus*).

We examined eggs obtained from two different falconries located in two different areas in Catalonia (NE Spain), separated by ca. 200 km. One zone was near the coast at 97 m above sea level (mean annual temperature = 14°C, mean annual precipitation = 650 mm, mean relative humidity = 80%). The other zone was in the Pyrenees mountains at 800 m asl (mean annual temperature = 12°C, mean annual precipitation = 650 mm, mean relative humidity = 65%). We examined the clutches of 58 different females with an age between 3 and 14 years and a body mass between 650 and 1,680 g. The age of the females was provided by the breeders. Body mass was measured at the end of summer after reproduction was finished. The birds were captured in their cages and weighed with an electronic KRUPS 840 balance (Hamburg, Germany) (to 1 g).

Most females laid four (45%, 26 of 58) or eight eggs (47%, 27 of 58 females) and only five females

(9%) laid between 9 and 11 eggs between March and July 2007. Egg pulling (i.e., removing eggs as they are laid) was conducted in both zones, so females did not produce true clutches, as they would do in the wild. However, data on egg laying sequence were obtained by writing down a number on the eggshell as the female was laying them.

Egg collection and measurements

Egg hardness in some birds is significantly influenced by developmental stage (Castilla et al., 2007). Consequently, we only used nondeveloped eggs for all taxa. These included infertile eggs ($n = 133$) and eggs aborted during the first week of incubation ($n = 55$). Fertilization was checked using an ovoscope (OB-1-60-1) (Cherkassy, Ukarine). Egg mass was measured only for fresh eggs that were recently laid. Egg length and width were measured after egg incubation and failure. We used an electronic Sartorious AG, balance (Goettingen, Germany) (to 0.01 g) and digital calipers Mitutoyo (Tokyo, Japan) (to 0.01 mm) to obtain egg measurements. Egg design (uniform or spotted) and egg color (pale, dark) was recorded upon visual inspection of the eggs.

To investigate the force needed to break the eggs, we used an isometric Kistler force transducer (type 9203, Kistler Inc., Winterthor, Switzerland) attached to a portable charge amplifier with peak-hold function (type 5995A). A screw with a flat surface (surface area of $3\,\text{mm}^2$) was mounted on the force transducer and pushed onto the egg until the eggshell broke (see Castilla et al., 2007). We measured egg strength around the equator of the egg. When possible the puncture was done at pale spots on the egg only to reduce variation in hardness owing to differences in pigmentation (see Gosler et al., 2005).

After egg breakage, we confirmed the developmental stage of each egg previously assigned using the ovoscope. After the measurement of egg strength, eggshells were cleaned and immersed in a plastic box with water for 10 min. The time allowed the membrane to become soft so that it could be separated from the eggshell. We put shells and membranes on a dry absorbent paper for 15 additional minutes and proceeded with eggshell and membrane thickness measurements.

Measurements of eggshell thickness were conducted in three equidistant locations (at 1/3 intervals) around the equator of the egg. The average was calculated to obtain an overall indicator of eggshell thickness. Membrane thickness was recorded for one location only. Both thickness measurements were performed using a micrometer (Mitutoyo) to the nearest 0.001 mm.

Statistical procedures

As all egg dimensions were highly correlated ($P < 0.01$ or higher in all cases), we based comparisons among taxa, zones and other variables on a multivariate summary of the data. We first performed a principal component analysis to reduce the dimensionality of the data set. This resulted in a new set of uncorrelated variables that can be analyzed separately. Results of the principal component analysis were represented graphically using biplots, where the cosine of the angle between two vectors provides an estimate of the correlation between the respective variables. Principal components were used as dependent variables in a mixed linear model containing taxa, zone, egg color and egg design as fixed factors. Laying sequence, female age and body mass were added as continuous covariates. Female identity was added as random effect to estimate among-female variation, and to take the dependency of the data for eggs from a single female into account. Tests of fixed effects were based on F tests with degrees of freedom approximated by Kenward and Rogers method. The random female effect was tested using a likelihood ratio test. The data were analyzed using linear mixed models (GLMMs). GLMMs were fitted with the GLIMMIX procedure in SAS.

The relationships between body mass and egg hardness, and between eggshell thickness and egg hardness were examined using Pearson correlations of individual means, using SPSS V. 15.

RESULTS

We found a large variation among species in all egg measurements (Tables 1 and 2) and all traits were highly correlated ($P < 0.01$ or higher in all cases) (Fig. 1). Zone, however, did not influence on the observed egg variation among species (Table 3). We found a significant correlation between eggshell thickness and egg hardness ($r = 0.80$, $P > 0.01$, $N = 187$) (Fig. 2).

The principal component analysis of the egg characteristics revealed that the first three components explained nearly 90% of all variation. Biplots of all combinations of these showed that the first principal component, explaining 61% of all variation, can be regarded as a measure of

TABLE 1. Measurements of falcon eggs from different taxa

	Egg length (mm)					Egg width (mm)					Egg mass (g)				
Taxa	Mean	SD	Max	Min	*N*	Mean	SD	Max	Min	*N*	Mean	SD	Max	Min	*N*
P	49.2	2.38	54.0	44.3	65	39.2	1.48	41.6	36.9	65	42.4	3.18	47.6	37.2	60
R	48.5	1.88	51.6	43.3	39	37.0	1.76	39	30.6	39	37.2	3.31	42.0	29.9	31
PR	47.8	0.68	48.6	47.4	3	37.1	0.96	37.9	36.05	3	34.6	2.07	36.7	32.5	3
S	54.1	1.70	58.6	51.6	33	41.5	1.57	43.6	37.4	33	52.0	3.68	57.6	43.1	27
G	55.3	1.98	58.7	53.0	9	44.3	1.10	45.8	43	9	61.0	5.14	69.0	56.1	9
SG	53.3	2.70	58.7	47.9	39	42.0	2.01	49.6	36.7	39	52.7	6.06	64.9	36.7	36

Indicated are the means, standard deviations (SD), the maximum and minimum values, and the sample size (*N*). P, peregrine falcon (*Falco peregrinus peregrinus*); R, red shaheen falcon (*F. peregrinus babylonicus*); PR, intraspecific hybrid peregrine*red shaheen (*F. peregrinus peregrinus * F. peregrinus babylonicus*); S, saker falcon (*F. cherrug*); G, gyr falcon (*F. rustcolus*); SG, interspecific hybrid saker*gyr (*F. cherrug * F. rustcolus*).

TABLE 2. Measurements of egg hardness (in newtons, N), membrane thickness (in millimetres, mm) and eggshell thickness (mm) of falcons of different taxa

		Egg hardness (N)				Membrane thickness (mm)				Eggshell thickness (mm)			
Taxa	*N*	Mean	SD	Max	Min	Mean	SD	Max	Min	Mean	SD	Max	Min
P	65	16.3	3.53	22.4	10.3	0.066	0.015	0.1	0.041	0.29	0.031	0.34	0.23
R	39	12.7	2.67	19.9	8.1	0.055	0.019	0.09	0.025	0.26	0.019	0.29	0.22
PR	3	11.7	2.31	13.9	9.3	0.064	0.002	0.065	0.1	0.25	0.006	0.25	0.24
G	33	19.9	3.64	27.1	14.6	0.074	0.013	0.094	0.057	0.33	0.028	0.37	0.29
S	9	21.7	3.81	27.8	17.4	0.076	0.007	0.088	0.066	0.33	0.023	0.37	0.30
SG	39	19.8	2.86	24.0	13.9	0.069	0.021	0.108	0.032	0.32	0.022	0.39	0.28

Indicated are the means and standard deviations (SD), the maximum and minimum values and the sample size (*N*). P, peregrine falcon (*Falco peregrinus peregrinus*); R, red shaheen falcon (*F. peregrinus babylonicus*); PR, intraspecific hybrid peregrine*red shaheen (*F. peregrinus peregrinus * F. peregrinus babylonicus*); S, saker falcon (*F. cherrug*); G, gyr falcon (*F. rustcolus*); SG, interspecific hybrid saker*gyr (*F. cherrug * F. rustcolus*).

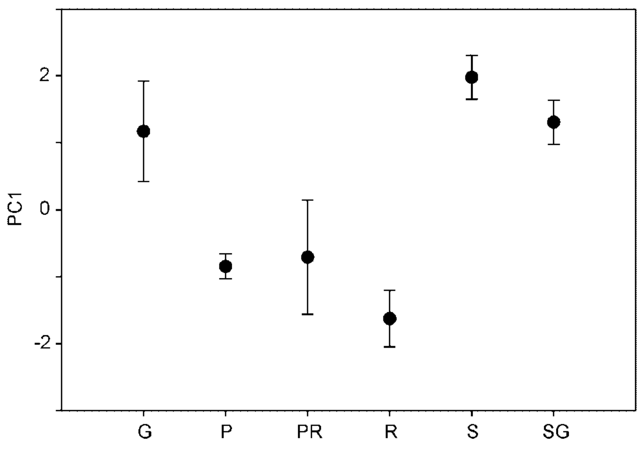

Fig. 1. Means (±SE) of the first principal component (least square means from mixed model) for the different falcon taxa. A high score of the first principal component corresponds to large eggs with thick shells and membranes. P, peregrine falcon (*Falco peregrinus peregrinus*); R, red shaheen falcon (*F. peregrinus babylonicus*); PR, intraspecific hybrid peregrine* red shaheen (*F. peregrinus peregrinus*F. peregrinus babylonicus*); S, saker falcon (*F. cherrug*); G, gyr falcon (*F. rustcolus*); SG, interspecific hybrid saker*gyr (*F. cherrug*F. rustcolus*).

overall egg size. A high score of the first principal component corresponds to large eggs with thick shells and thick membranes. The second component, explaining 16% of all variation, reflected a contrast between egg length and membrane thickness. High scores for this component correspond to eggs that were relatively long with a relatively thin membrane. The third component, explaining 12% of all variation, reflected a contrast between egg length and membrane thickness on the one hand and shell thickness and hardness on the other.

Next, we performed mixed linear model analyses with these three principal components as dependent variables. Results of tests are summarized in Table 3. PC1 differed between taxa, where two-by-two comparisons revealed that the species *F. cherrug*, *F. rusticolis* and their hybrid had the highest scores and thus the largest eggs with thickest shell and membrane and also the hardest shells. PC1 did not differ among these three groups (all $P > 0.3$). The lowest score for PC1 was observed for species *F.peregrinus babylonicus*,

TABLE 3. Overview of tests performed on the three first principal components using a mixed linear model (see text for details)

Model factors	PCA1 (61%)	PCA2 (16%)	PCA3 (12%)
Species	**$F_{5,40} = 38.1$, $P < 0.0001$**	$F_{5,46} = 0.79$, $P = 0.55$	$F_{5,46} = 0.50$, $P = 0.77$
Zone	$F_{1,38} = 2.97$, $P = 0.09$	$F_{1,44} = 1.11$, $P = 0.30$	$F_{1,45} = 0.01$, $P = 0.94$
Egg color	$F_{1,139} = 0.26$, $P = 0.61$	$F_{1,148} = 0.01$, $P = 0.91$	**$F_{1,135} = 6.11$, $P = 0.01$**
Egg design	$F_{2,143} = 0.55$, $P = 0.58$	$F_{2,150} = 1.21$, $P = 0.30$	**$F_{2,139} = 4.85$, $P = 0.01$**
Laying sequence	**$F_{1,121} = 7.20$, $P = 0.008$**	$F_{1,132} = 2.50$, $P = 0.12$	$F_{1,122} = 0.05$, $P = 0.82$
Female age	**$F_{1,52} = 7.28$, $P = 0.01$**	$F_{1,59} = 1.28$, $P = 0.27$	$F_{1,62} = 0.15$, $P = 0.70$
Female body mass	**$F_{1,46} = 5.80$, $P = 0.02$**	$F_{1,49} = 1.20$, $P = 0.29$	$F_{1,48} = 2.83$, $P = 0.10$
Female	**$\chi^2_1 = 33.9$, $P < 0.0001$**	**$\chi^2_1 = 27.8$**, $P < 0.0001$	**$\chi^2_1 = 44.2$, $P < 0.0001$**
(Random effect)	$\sigma^2_{\text{female}} = \mathbf{0.48}$	$\sigma^2_{\text{female}} = \mathbf{0.35}$	$\sigma^2_{\text{female}} = \mathbf{0.42}$
	$\sigma^2_{\text{residual}} = \mathbf{0.38}$	$\sigma^2_{\text{residual}} = \mathbf{0.44}$	$\sigma^2_{\text{residual}} = \mathbf{0.22}$

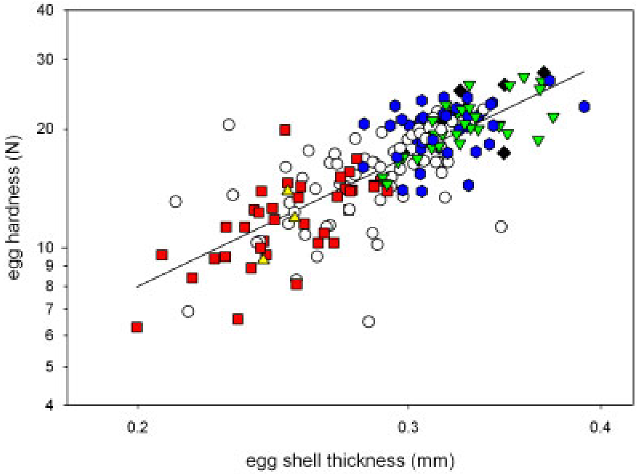

Fig. 2. Relationship between egg shell thickness and egg hardness (in Newtons) for the different falcon taxa: circle, peregrine falcon (*Falco peregrinus peregrinus*); square, red shaheen falcon (*F. peregrinus babylonicus*); triangle up, intraspecific hybrid peregrine*red shaheen (*F. peregrinus peregrinus*F. peregrinus babylonicus*); triangle down, saker falcon (*F. cherrug*); diamond, gyr falcon (*F. rustcolus*); hexagon, interspecific hybrid saker*gyr (*F. cherrug*F. rustcolus*). Regression equation: intercept = 2.21; slope = 1.87; $r^2 = 0.63$.

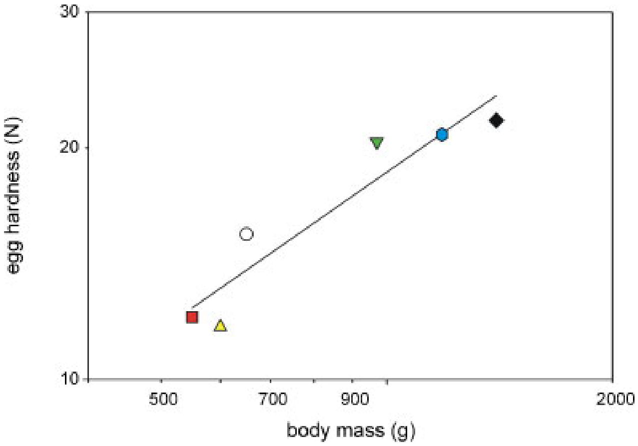

Fig. 3. Relationship between female body mass and the average eggshell strength (i.e., egg hardness, in Newtons) for the different falcon taxa: circle, peregrine falcon (*Falco peregrinus peregrinus*); square, red shaheen falcon (*F. peregrinus babylonicus*); triangle up, intraspecific hybrid peregrine* red shaheen (*F. peregrinus peregrinus*F. peregrinus babylonicus*); triangle down, saker falcon (*F. cherrug*); diamond, gyr falcon (*F. rustcolus*); hexagon, interspecific hybrid saker*gyr (*F. cherrug*F. rustcolus*). Regression equation: intercept = -0.77; slope = 0.68; $r^2 = 0.89$.

which differed from all others (all $P < 0.0001$). Intermediate levels were observed for *F.peregrinus peregrinus* and the intraspecific hybrid *F.peregrinus peregrinus*F.peregrinus babylonicus*, which did not differ from each other ($P = 0.33$), but had significantly lower scores than *F. cherrug*, *F. rusticolis* and their hybrid (all $P < 0.001$) (Figs. 1 and 2).

Each PC varied significantly between females (all random family effects highly significant) and thus individual female traits contributed strongly to the variation in egg characteristics. PC1 decreased with female age and laying sequence (Table 3). Thus, eggs produced by older females, which were laid later, were relatively small and soft and had a rather thin membrane and shell. PC1 increased significantly with female mass, indicating that clutches from heavier females contain larger and harder eggs with a thicker membrane and shell (Table 3, Fig. 3). Similar results were found when the data were analyzed using Pearson correlations ($r = 0.93$, $P = 0.007$, $N = 6$).

Because the effects that are important at the interspecific level do not necessarily have to be applied at the intraspecific level, we conducted a separate analysis to examine the relationships between body mass and egg hardness for the taxa for which sufficient sample size was available. We did not find a significant correlation between female body mass and average egg hardness in the big falcons (*F. cherrug*: $r = -0.262$, $P = 0.53$, $N = 8$, or the hybrid *F. cherrug∗F. rusticolus*: $r = 0.325$, $P = 0.24$, $N = 15$). The relationship was not significant either for the small *F. peregrinus babylonicus* ($r = 0.526$, $P = 0.12$, $N = 10$), but approached significance in the case of *F. peregrinus peregrinus* ($r = 0.475$, $P = 0.05$, $N = 17$; Fig. 4).

We found no effect of egg color or design on egg strength (Table 3). However, it should be noted that we did not quantify these traits with a spectrophotometer, but rather established broad categories based on visual inspection only. We also found that the color of eggs within clutches was more similar than the color of eggs from different clutches. Similar observations have been made for other bird species such as the kestrel *F. naummanni* (Negro et al. own observations), the red-legged partridge, *Alectoris rufa*; the gray partridge, *Perdix perdix*; the quail, *Coturnix japonica* (Jesús Nadal and Castilla, own observations); and for the eastern bluebirds, *Sialia sialis* (Siefferman et al., 2006).

Hybrid eggs showed similar size and hardness compared to that of the species of origin. Thus, hybridization appears not to have an effect on the eggshell characteristics measured here. The morphometric examination of adult falcons also showed the impossibility to discriminate accurately between gyr∗saker hybrids and their parent species (Eastham and Nicholls, 2005).

DISCUSSION

Variation in egg hardness has to our knowledge, not been reported for any falcon species, except for one report on egg hardness in two eggs of *F. naumanni* and nine eggs of *F. tinnunculus* (Ar et al., '79). Eggshell thickness has been examined in various populations of *F. peregrinus* subjected to different levels of environmental contamination (Falk et al., 2006, and references cited; Wegner et al., 2005).

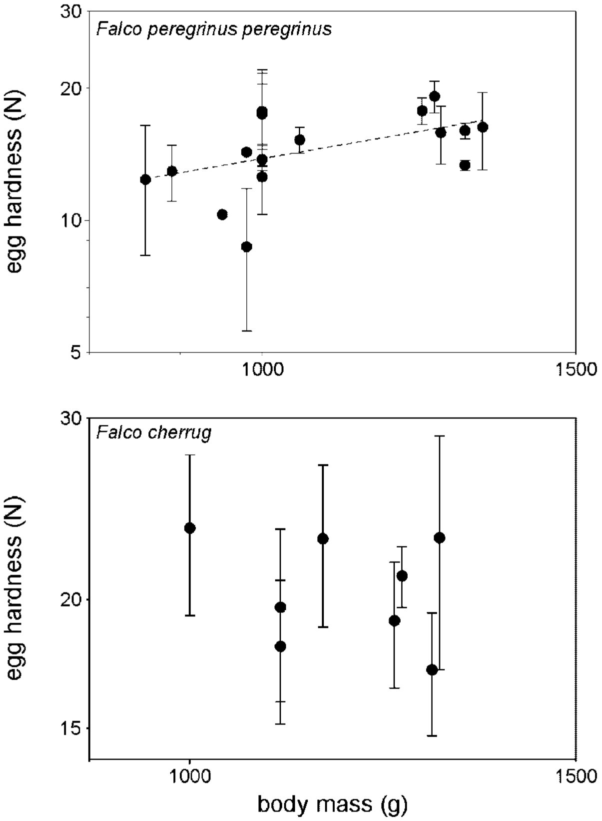

Fig. 4. Relationship between female body mass and the average eggshell strength (i.e., egg hardness, in Newtons) for falcons of different taxa. Symbols indicate the mean egg hardness for each female (±1 standard deviation). Relationships between body mass and eggshell strength are not significant (see results) but approach significance in the case of *Falco peregrinus peregrinus* (dashed line).

In our study, we examined the effect of different factors on egg hardness of nondeveloped eggs within a group of falcon species maintained under constant food quantity and quality, and not subjected to environmental contamination. We thus provide the first baseline data for egg strength and eggshell thickness in different falcon taxa.

We found that the smaller peregrine falcons produced smaller and softer eggs compared to the big falcons of the gyr-saker group. We cannot compare our values of egg hardness to those for other falcon populations owing the lack of data in the literature. However, data on eggshell thickness (which is highly correlated with egg hardness in our taxa) are available for other populations and subspecies of the peregrine falcon in Europe, Greenland and USA (range: 0.24–0.35 mm) (Falk and Møller, '90; Wegner et al., 2005, Falk et al., 2006). Our values of eggshell thickness in *F. peregrinus peregrinus* of 0.29 mm (Table 2) from 17 females are within the range reported in the literature. However, the values are at the low end of the range. Unfortunately, there are no data available on eggshell thickness for *F. peregrinus* in Spain, because no field studies have been conducted yet. Our ''low'' values could be attributed to food intake, but this is unlikely given the captive conditions of the animals. They could potentially also be related to the health or physiological characteristics of the females; however, this cannot be tested because we have no data on the physiological condition of the females. On the other hand, the observed differences may be owing to methodological bias in data obtained from different observers (e.g., presence/absence of membrane, location of the eggshell measured, equipment used).

Factors affecting on egg strength

Eggshell thickness

We found that eggshell thickness explained a high percentage of the variation in egg hardness for all falcon taxa (Fig. 2). Our results agree with those of Tyler ('69), who demonstrated that the main factor affecting strength in hen eggs was eggshell thickness. Although, such relationship has been suggested by other authors (Ar et al., '79, and references herein), the correlations between hardness and thickness are not always particularly good (Brooks, '60). In our study we minimized noise in our data set by measuring egg hardness and eggshell thickness only in nondeveloped eggs, at approximately the same location near the equator of the egg, and by removing the egg membrane in all species, which may explain the tight fit between both variables.

Laying sequence

We found that eggs produced later in the laying sequence were relatively smaller and softer and had rather thin membranes and shells. Our results are congruent with the results of Burnham et al. ('84) who observed a decreasing mean shell thickness in later clutches of captive peregrines. Falk and Møller ('90) also suggested that in large clutches of the peregrine falcon the last laid eggs should be expected to have thinner shells because of decreased levels of calcium available in the female body. In other bird species, eggshell thickness and egg size or egg volume also decrease with laying order irrespective of the sex of the embryo (Reynolds, 2001; Lezalova et al., 2005; Lislevand et al., 2005). In contrast, egg size and eggshell thickness appear to increase with female age in penguins (Massaro et al., 2002; Massaro and Davis, 2004).

Differences in egg quality may arise from differential allocation of resources across the laying sequence by the parent and may suggest a parental strategy to alleviate the detrimental effects of within-brood hierarchies on the last-hatching chick (Muck and Nager, 2006). It has been suggested that females may adaptively allocate resources to eggs of different laying order to affect breeding conditions (D'Alba and Torres, 2007). In addition, variation in egg quality in the sequence appears to be owing to a combination of environmental conditions, reflected in food resources, individual quality, and allocation trade-offs during the laying period (Ardia et al., 2006). However, in populations without environmental food limitation, such as the one studied here, we should not expect differences in calcium allocation to eggs laid in different order but rather differences owing to female condition or ageing, or to embryo sex allocation (e.g., Blanco et al., 2003). Unfortunately, we do not know the sex of the embryos because the eggs used in this study were mainly infertile or aborted at early developmental stages.

Female mass, age and identity

In our study, we found that clutches from heavier females contained larger and harder eggs with thicker shells and membranes. Ar et al. ('79) hypothesized that for an effective incubation (i.e. without breaking the eggs in the nests), big birds have to produce bigger and thicker eggs than small birds. They found a positive relationship when comparing 47 species of nonrelated birds from 26 families and 11 orders, from passerines of ca. 1 g to ostrich (*Struthio camelus*) of 1,500 g. The results of our study using a group of closely related species (e.g., only falcons) support the female mass hypothesis. However, the relationships between body mass and egg hardness at the intraspecific level showed similar trends in some taxa but not in all, suggesting that the effects that are observed at the interspecific level do not necessarily apply at the intraspecific level. Although we did not find significant correlations, similar trends were observed and larger sample sizes may prove to uphold the observed interspecific pattern, at least in some falcon taxa.

Eggs produced by older females, were relatively small and soft and had rather thin membranes and shells. A decrease in egg dimension with increase in female age has been already shown in different populations of *F. peregrinus* (Ratcliffe, '80; Burnham et al., '84; Wegner, 2005). Our results also show that individual females contribute significantly to the observed variation in egg strength. Some individuals produced larger-thicker-harder eggs than others, suggesting the importance of female physical condition and health on egg characteristics. In some species, oviductal problems accounted for poor laying performance, including soft-shelled eggs at the onset of lay (Johnston and Gous, 2007) and body condition is often suggested to be the most plausible proximate determinant of breeding performance in birds (Drent and Daan '80). Indeed, females in good condition and health typically lay significantly larger eggs compared to those in poor condition (Hanssen et al., 2002; Lifjeld et al., 2005; Ardia et al., 2006).

Geographic area

In our study, egg hardness variation among taxa was not significantly different between flacons from different zones. Interestingly, however, previous research has shown that trends in daily temperature change before and during laying can influence egg mass and volume (Lessells et al., 2002; Barkowska et al., 2003). Heat stress (42 vs 26°C) of both short and long duration (6–15 days) can cause significant hyperthermia and the reduction in egg production and egg weight (Rozenboim et al., 2007). In addition, laying gaps have been shown to correlate with cold weather and may have an effect on egg production (Low, 2008). Larger samples of eggs from birds living in different areas are needed to better understand the possible effect of climatic conditions on eggshell strength.

In summary, our data provide quantitative support that different factors other than pollution may significantly affect eggshell strength in falcons. However, further studies should investigate the effects of female health and condition on eggshell characteristics.

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