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Males do not adjust feeding rate to egg colour in a Norwegian population of pied flycatchers *Ficedula hypoleuca*

Master thesis 2007

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Abstract

According to the differential allocation hypothesis, an individual will invest more in an offspring when mated to a high than a low quality mate. The blue/green eggshell colouration of pied flycatcher *(Ficedula hypoleuca)*, has been suggested to be a sexually selected trait working as a post-mating signal of female phenotypic quality that influences male willingness to invest in nestlings. We tested the egg signalling hypothesis by moving clutches between nests to see if the male adjusted his investment according to the old or the new colour of the eggs. Egg colour was measured by reflctanc spectometry and male feeding effort was quantified by videofilming and by recording nestling body mass. Our results showed no adjusted effort in male feeding rate of nestlings in relation to egg colour.

Introduction

According to the differential allocation hypothesis, an individual will invest more in an offspring when mated to a high than a low quality mate (Burley, 1986). The hypothesis thus predicts an effect of mate attractiveness on the level of reproductive investment of individuals (Johnsen et al., 2005; Sheldon, 2000), thereby influencing the trade-off between current and future reproductive effort (Rutstein et al., 2005; Sheldon, 2000).

The blue/green colour of the eggs of the pied flycatcher *(Ficedula hypoleuca)* has formerly been explained as (1) a by-product of metabolism reflecting female stress during the laying period (Moreno et al. 2003), (2) pigmented eggshells being beneficial to embryos directly by having antibacterial properties (Soler et al., 2005) or (3) that egg colouration facilitates discrimination of eggs from those laid by brood parasite among others (Moreno et al., 2003). Lately, it has been suggested to be a sexually selected trait working as a post-mating signal of female phenotypic quality that influences male willingness to invest in nestlings (Moreno et al., 2006a; Moreno et al., 2005; Moreno et al., 2006b; Moreno and Osorno, 2003; Moreno et al., 2004). The blue-green colouration on the eggshell derives from the pigment biliverdin (Kaur et al., 2003; Moreno et al., 2006a; Moreno and Osorno, 2003; Moreno et al., 2004; Siefferman et al., 2005), which together with its reduction product, bilirubin, acts as a potent antioxidant (Stocker et al., 1987). Spending this pigment on egg colouration may be costly for the female and reprisent a “handicap” during the laying period (Kaur et al., 2003; Moreno et al., 2006a; Moreno et al., 2005; Moreno et al., 2006b; Moreno and Osorno, 2003; Moreno et al., 2004).

A descriptive study of a Spanish population of pied flycatchers by Moreno et al. in (2005) has given support to this theory, showing that the males had a higher feeding rate of offspring deriving from more colourful eggs. Recently, the same scientists presented experimental evidence showing that the biliverdin content in the eggshell does reflect the female condition at the time of laying (Moreno et al., 2006b). However, alternative explanations for the above results may be that the quality of the territory or the female quality is the main factor influencing feeding rate. Males with better territories may have a higher quality, and females may compete for the best males. A high quality

female might therefore produce colourful eggs because she is in a good territory providing the extra energy needed to do so.

In the present study, we wanted to test the egg signaling theory experimentally and differentiate between four hypotheses:

a) The male asjusts his effort according to the colour of the eggs.
b) There is an idirect relationship between male effort and egg colouration, because both factors are related to a third factror, namely territory quality. In high quality territories, females produce dark blue/green eggs and males can afford to have a high feeding rate.
c) There is an indirect relationship between male effort and egg colouration through female mate choice. High quality females, producing blue/green eggs, get the best males, which feed at the highest rate.
d) There is no relationship between egg colour and territory quality, nor between male and female quality.

We tested these hypotheses by moving clutches between nests to see if the male will adjust his investment according to the old or the new colour of the eggs. If the male adjusts his feeding rate according to the color of the eggs, he is expected to bring more food to nestlings deriving from darker blue/green eggs. If the feeding rate is related to territory quality then we can expect a low feeding rate in nests were the initial egg colour was light and has become darker. If the feeding rate is related to female quality, we can expect the same result as we would according to territory quality. Finally, if there are no relationship between egg colour and territory quality, nor between male and female quality, we would expect an average feeding rate in nests with both initial light and darker egg colour. This will be a critical test of the effect of egg colouration because territory qualities will not be manipulated nor male or female quality (tab. 1). As measures for male investment, we used his share of nestlings provisioning, and the body mass of the nestlings.

Table 1. Predicted feeding effort of male pied flycatcher after manipulating egg colour.

Initial egg colour	**Egg colour after switching of clutches**	**Expected male effort from hypothesis** a)	b)	c)	d)
Light	**Dark**	High	Low	Low	Average
Dark	**Light**	Low	High	High	Average

Material and methods

The studied site

The data for this study was collected at the study site Dæli (59°50`N,10°33`E), west of Oslo, Norway, from April to July 2005. The 1.6 km^2 study plot contains of approximately 400 nest boxes in a lush broad-leaved forest. These boxes are mainly used by great tit (*Parus major)*, blue tit *(Cyanistes caeruleus)* and pied flycatcher, but also by coal tit *(Parus ater)* and nuthatches *(Sitta europaea)***.**

Clutch switching, egg measurements and objective colorimetries

The length and width of each egg was measured with a calliper by the same person (TS), and volume was calculated according to Hoyt`s formula ($V = 0.51 \times (length \times width)^2$). Egg reflectance was quantified with a USB 2000 spectrometer (Ocean Optics Eerbek, The Netherlands) connected by a bifurcated fiberoptic probe to a Xenon (PX-2) pulsed light source. A plastic cylinder was mounted on the probe to ensure a fixed measurement area (diameter: 4 mm) and to exclude ambient light. Three reflectance measurements were taken from three different spots of each egg. Egg reflectance was calculated relative to a WS-2 white standard (Avantes, Eerbek, The Netherlands).

To analyse egg reflectance, we computed three objective colour parameters describing the variation in spectral shape (Andersson and Prager, 2006), based on averaged and smoothed reflectance spectra: brightness as the average reflectance (R_{av}) in the 320-700 nm interval, chroma (spectral purity/saturation) as the difference between maximum and minimum reflectance divided by brightness ((R_{max}- R_{min})/R_{av} and UV chroma (UV reflectance ratio) as $R_{320-400}/R_{320-700}$ (Andersson et al., 1998; Andersson and Prager, 2006; Delhey et al., 2003; Johnsen et al., 2005; Johnsen et al., 2001). We used the average of all eggs in a clutch in the analyses. We also calculated principal component coefficients (PC 1-3) to describe spectral variation (Chuthill et al., 1999; Hunt et al., 1998). However, since the first two principal components were highly correlated with the parameters based on spectral shape (brightness vs. PC1: r = 0.98, p = 0.0001; chroma vs.

PC2: r = - 0.95, p = 0.0001; UV chroma vs. PC2: r = 0.91, p = 0.0001), we only present the tests based on the latter. Brightness, UV chroma, chroma were analyzed by Analysis of variance (ANOVA). Egg colour was also assessed by a human observer (KV), using a colour-scale made by seven different colours in the range of pale white (score 1) to darker greenish-blue (score 2). Classification based solely on human perceived colours, will not include UV-A (320-400nm) (Bennett and Cuthill, 1993), and may a give misleading results (Hunt et al., 1998). We used human-based methods combined with the objective measurement of the full spectral range including UV by spectrometry.

Clutches were measured early in the incubation period, with a mean of 2.7 days (SD = 1.2) after incubation started. During the measurements, the eggs were replaced with other similar eggs to ensure continued female incubation. A total of 304 eggs from 48 clutches were measured, during four different days over a period of 11 days (25 May, 28 May, 1 June and 5 June). Upon completed measurements, we returned the clutches to different nests. The pair wise exchange of clutches was based on the following criteria:

- The egg colour should be as different between pairs of clutches as assessed by human observer.
- The clutches should have a similar number of eggs (+/- 1 egg).

All the pairs accepted the clutch exchanges.

Nests studied

During the 2005 nesting season, pied flycatchers used 67 of the nest boxes. 57 of them were used for this experiment. 48 of the boxes were used in clutch exchanges and 46 were video filmed. During the laying period each egg was marked with a number in the order they were laid. At about the expected hatching time, the nest boxes were checked every second day to determent the hatching date. Hatching date was defined as the day when the first egg hatched. In cases where haching date was not observed directly, the smallest and the largest individuals of the brood were weighed, and hatching date was estimated from a growth curve. Each brood was weighed for a second time at the age of 11-13 days.

We caused damage to four eggs during the period of egg laying and four eggs were damage during colour measurement. These eggs were excluded when calculating nesting success, hatching success and fledging success. In four boxes, all the nestlings died after hatching because of adult mortality and was not used in the analyses. Data on nestling mass on days 11-13 was missing from four boxes, and in one box, only the female was feeding during filming and male feeding rate could not be recorded.

Video filming

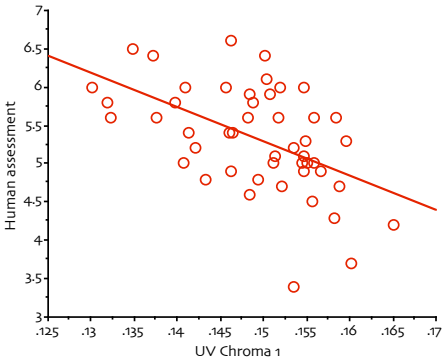
46 nest boxes were filmed for 1–1.5 h. when the nestlings were 10-12 days old. The camera was placed on a tripod about 1-2 m. from the nest box entrance and all filming was done in favourable weather. The purpose of filming was to register two parameters in relation to the feeding rate:

1) The total amount of feeding per hour (male and female).
2) The relative amount of male feeding per hour in percent of total feeding.

Results

1. How does human colour assessment reflect reflectance values?

a)



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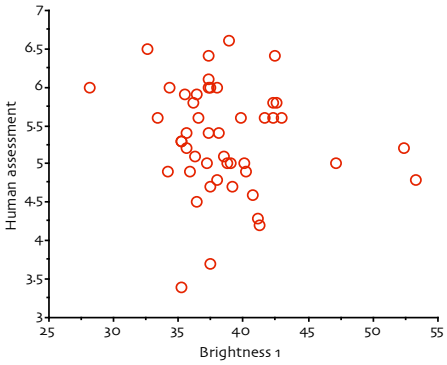

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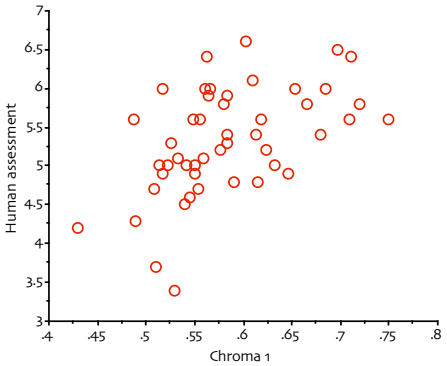


Fig. 1.1. Human egg colour assessment in relation to mean UV chroma (a), mean brightness (b) and mean chroma (c).

When comparing human colour vision with reflectance spectrometry, we found a correlation between human colour assessment, two aspects of reflectance recorded by spectrometry, viz. UV chroma (fig. 1.1.a; r = - 0.52, n = 48, p = 0.0001) and chroma (fig. 1.1.c; r = 0.52, n = 48, p = 0.0002). There was no significant correlation with brightness (fig. 1.1.b; r = - 0.15, n = 48, p = 0.30).

2. Does egg colour reflectance measurements differ in clutches before and after clutch exchange?

2.1. Human assessment

We found no correlation in human assessment of egg colour when comparing the clutches before and after the clutch exchange (fig. 2.1; r = - 0.12, n = 48, p = 0.41).

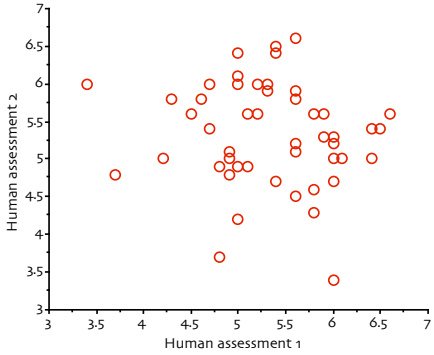


Fig 2.1. Human egg colour assessment before clutch exchange in relation to human assessment after clutch exchange.

2.2. Reflectance values

We found no correlation among any of the reflectance components when compairing the clutches before and after clutch exchange, viz. UV chroma (fig. 2.2.a; r = 0.002, n = 48, p = 0.99), brightness (fig.2.2.b; r = 0.15, n = 48, p = 0.32) and chroma (fig. 2.2.c; r = - 0.08, n = 48, p = 0.61).

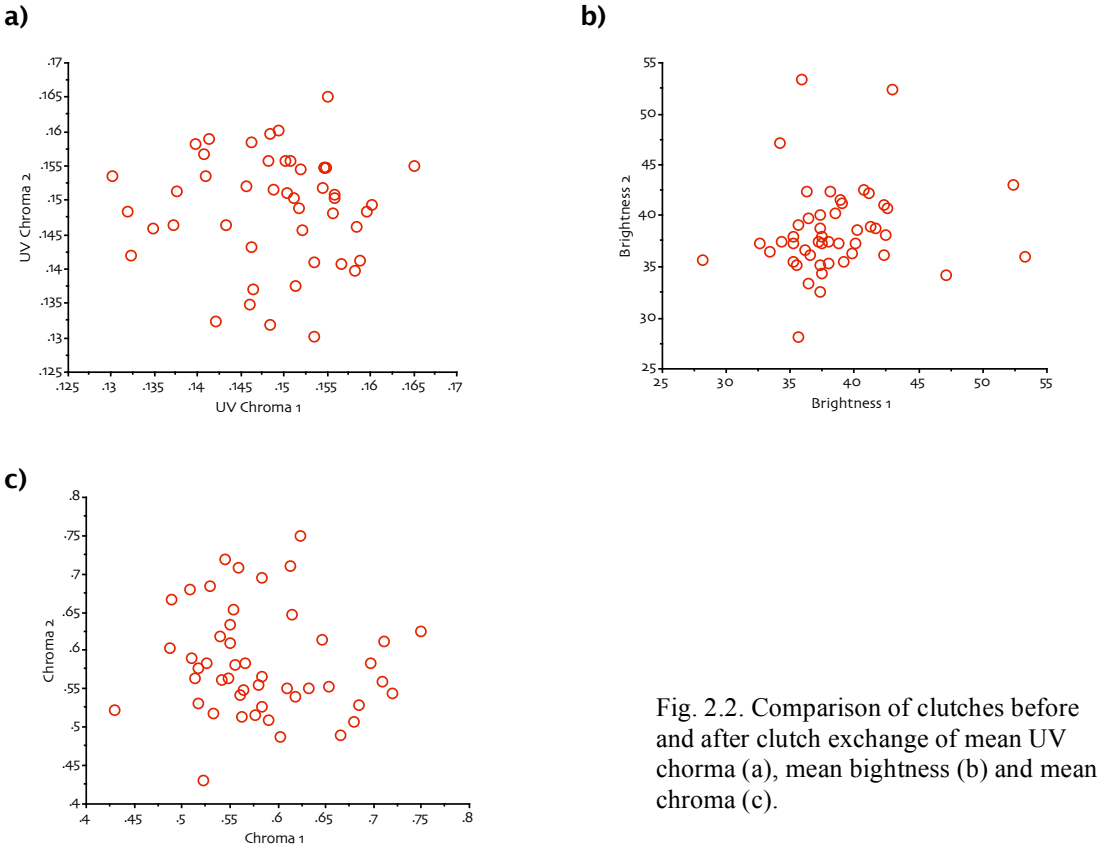


Fig. 2.2. Comparison of clutches before and after clutch exchange of mean UV chorma (a), mean bightness (b) and mean chroma (c).

3. Does egg colour reflect female quality?

We tested the correlation of the egg colour of clutches, using both human colour assessment and reflectance values with reproductive variables that could affect colour.

3.1. Human assessment and laying date, egg volume and clutch size

We found a positive correlation between human colour assessment and the laying date before clutch exchange (fig. 3.1.a; r = 0.32, n = 48, p = 0.03) illustrating that the mean colour as assessed by human vision increased during the nesting season.
Human assessment colour was not significant related to egg volume before clutch exchange (fig. 3.1.b; r = 0.09, n = 48, p = 0.53) and we found no significant correlation between human colour assessment and clutch size before (fig. 3.1.c; r = 0.12, n = 48, p = 0.41).

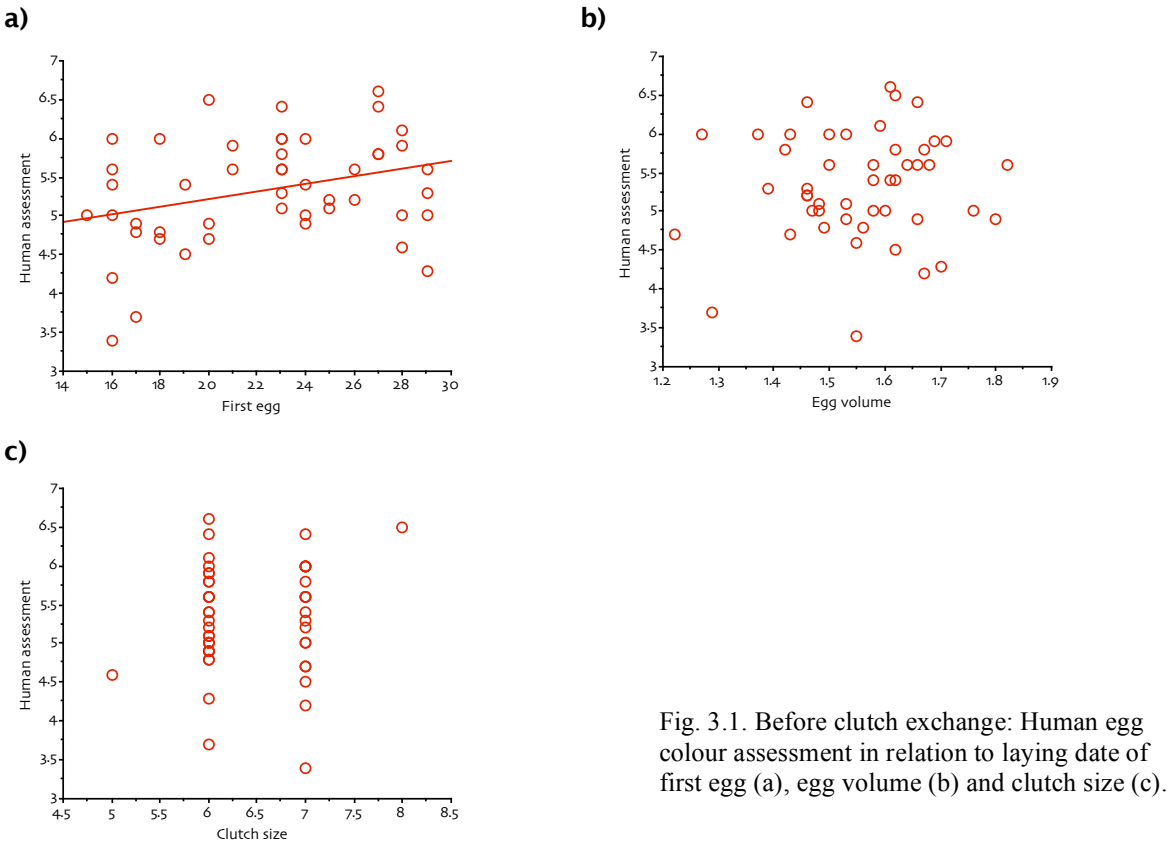


Fig. 3.1. Before clutch exchange: Human egg colour assessment in relation to laying date of first egg (a), egg volume (b) and clutch size (c).

3.2. Reflectance values and laying date, egg volume and clutch size

3.2.1. Laying date

We found no correlation between laying date of first egg and any of the reflectance components recorded before clutch exchange, viz. UV chroma (fig. 3.2.1.1.a; r = - 0.01, n = 48, p = 0.95), brightness (fig. 3.2.1.1.b; r = 0.12, n = 48, p = 0.43) and chroma (fig. 3.2.1.1.c; r = - 0.06, n = 48, p = 0.69).

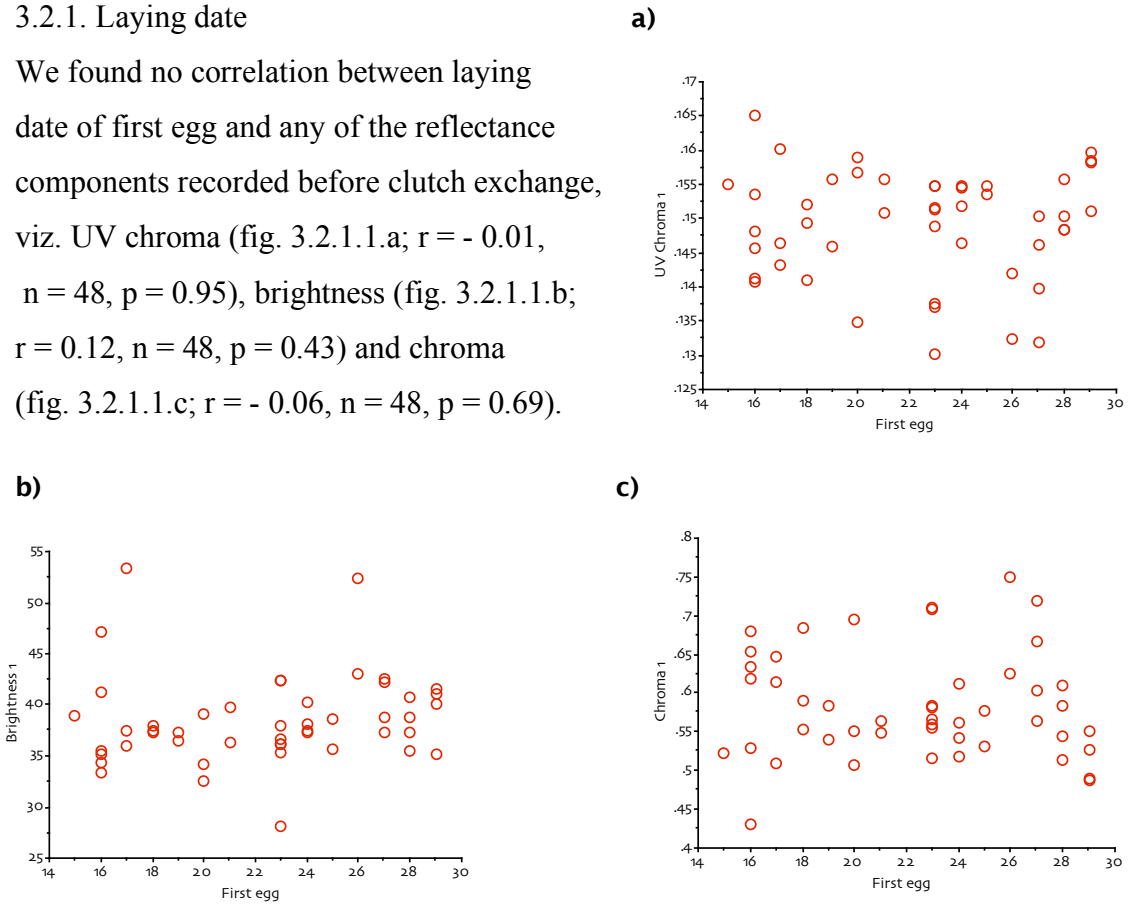


Fig. 3.2.1.1. Before clutch exchange: mean UV chroma (a), mean brightness (b) and mean chroma (c) in relation to laying date.

We found no correlation between laying date of first egg and any of the reflectance components recorded by spectrophotometer after clutch exchange, viz. UV chroma (fig. 3.2.1.2.a; r = - 0.18, n = 48, p = 0.23), brightness (fig. 3.2.1.2.b; r = 0.12, n = 48, p = 0.41) and chroma (fig. 3.2.1.2.c; r = 0.74, n = 48, p = 0.62).

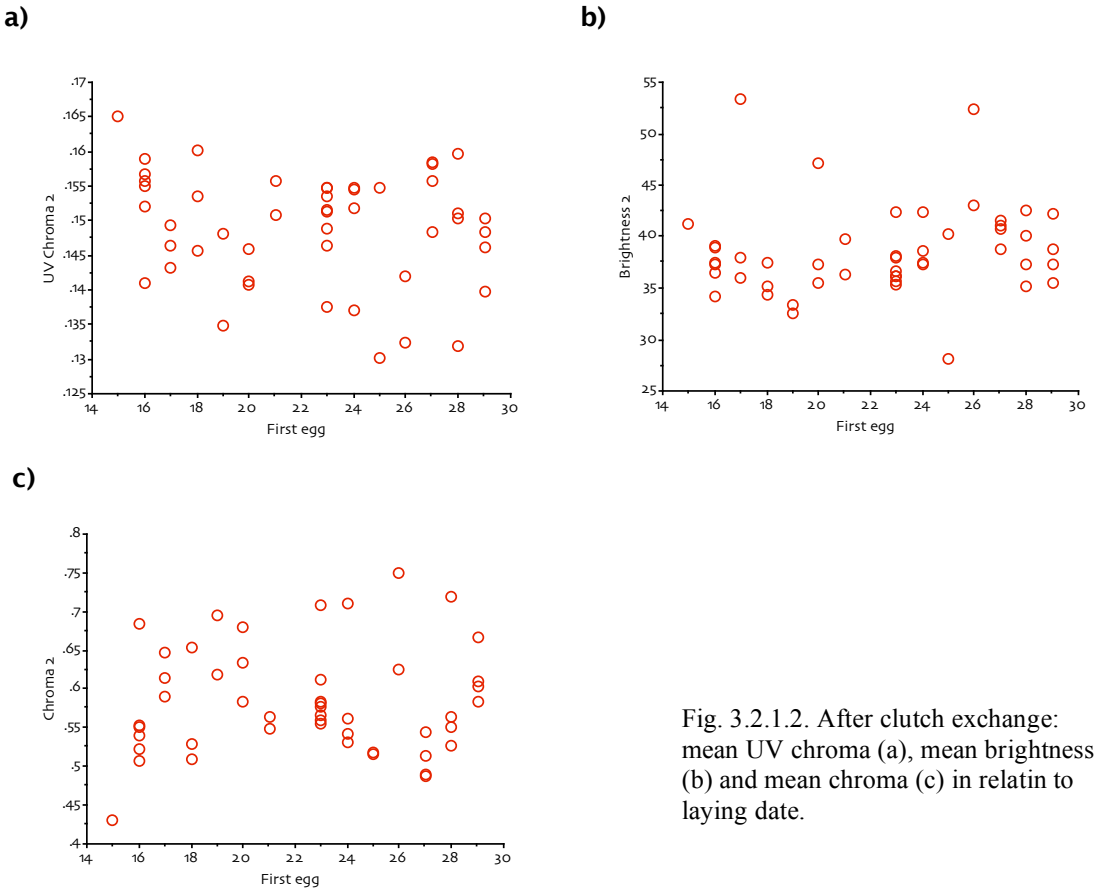
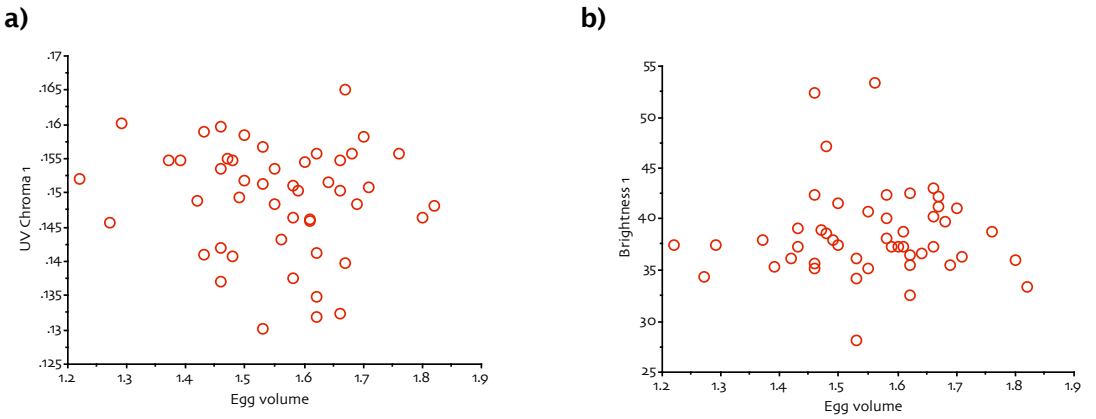


Fig. 3.2.1.2. After clutch exchange: mean UV chroma (a), mean brightness (b) and mean chroma (c) in relatin to laying date.

3.2.2. Egg volume

We found no correlation between egg volume and any of the aspects of reflectance viz. UV chroma (fig. 3.2.2.a; r = - 0.12, n = 48, p = 0.46), brightness (fig. 3.2.2.b; r = 0.01, n = 48, p = 1.00) or chroma (fig. 3.2.2.c; r = 0.06, n = 48, p = 0.67).



c)

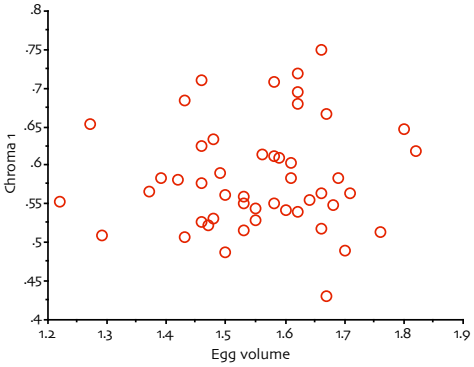
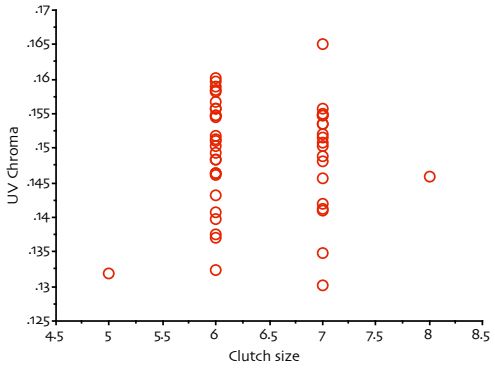


Fig. 3.2.2. Mean UV chroma (a), mean brightness (b) and mean chroma (c) in relation to egg volume.

3.2.3. Clutch size

Larger clutches showed a negative correlation with brightness (fig. 3.2.3.b; r = - 0.34, n = 48, p = 0.02). However, we found no correlation between UV chroma and clutch size. (fig. 3.2.3.a; r = 0.004, n = 48, p = 0.98) and chroma (fig. 3.2.3.c; r = - 0.04, n = 48, p = 0.81).

a)



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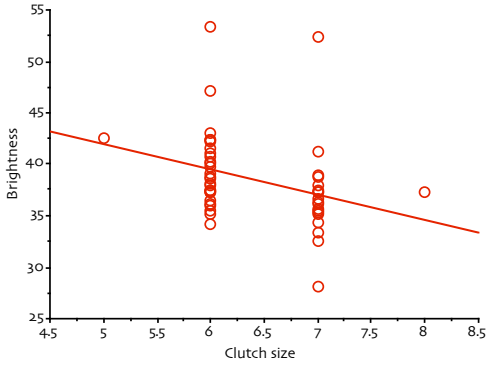

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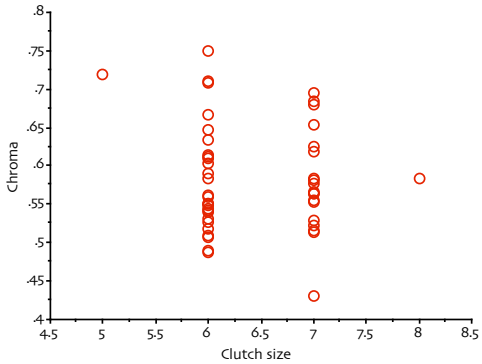


Fig 3.2.3 Mean UV chroma (a), mean brightness (b) and mean chroma (c) in relation to clutch size.

4. Do males invest in relation to egg colour?

4.1. Pair wise comparison

We used a pair wise design exchanging clutches between two and two nests, one with lighter eggs and one with darker eggs. Human assessment of light eggs had a mean value of 5.00 (SD = 0.67, n = 24), and for dark eggs a mean value of 5.72 (SD = 0.52, n = 24). There was a clear difference in egg colour according to human assessment (paired t-test; t = 4.90, df = 23, p = 0.0001). However, we found no difference in male proportion of feeding in percent of total feeding of the paired males (paired t-test; t = 0.61, df = 17, p = 0.55). Male proportion of feeding in percent of total feeding with initially light eggs was 49.8 % (SD = 13.5, n = 18, range 22-71), and for males with initially dark eggs it was 47.5 % (SD = 10.5, n = 18, range 29-63).

4.2. Human assessment

We found a negative correlation between male feeding rate per hour and egg colour as assessed by human vision before clutch exchange (fig. 4.2.a; r = - 0.41, n = 41, p = 0.01). However, we did not find a significant correlation between male proportion of feeding and human assessment before clutch exchange (fig. 4.2.b; r = - 0.18, n = 41, p = 0.25), nor did we find any significant correlation between male feeding rate per hour and human egg colour assessment after clutch exchange (fig. 4.2.c; r = - 0.19, n = 41, p = 0.23) or male proportion of feeding (fig. 4.2.d; r = - 0.14, n = 41, p = 0.40).

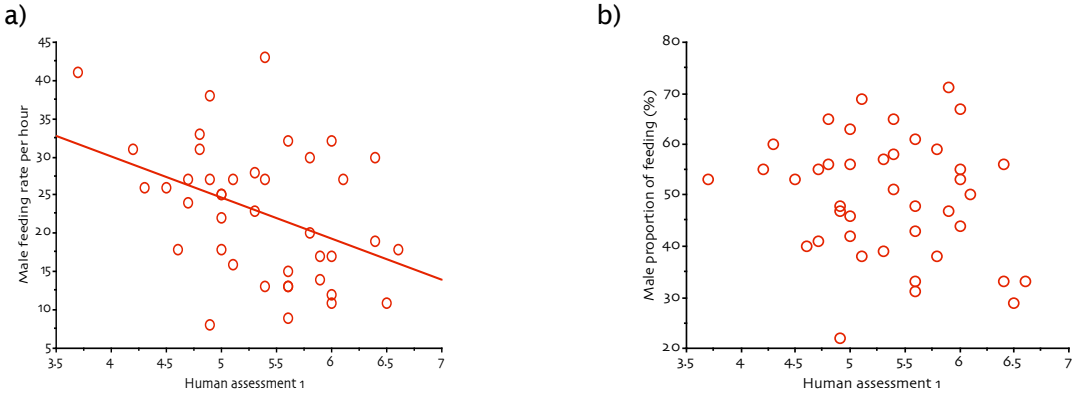


Fig. 4.2. Before clutch exchange: male feeding rate per hour (day 10-12) (a) and male proportion of feeding in % of total feeding (day 10-12) (b), in relation to human colour assessment.

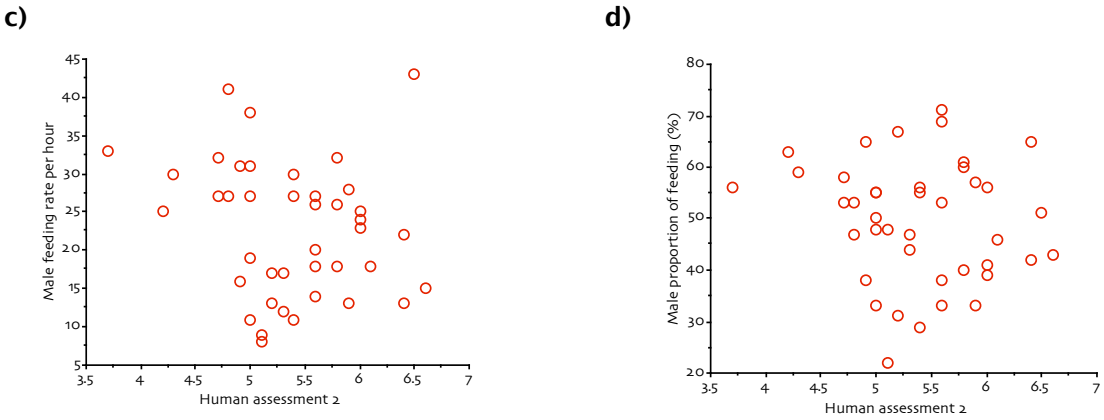


Fig. 4.2. After clutch exchange: male feeding rate per hour (day 10-12) (c) and male proportion of feeding in % of total feeding (day 10-12) (d), in relation to human egg colour assessment.

4.3. Reflectance values

4.3.1. Male feeding rate per hour in relation to reflectance before and after clutch exchange

We found no correlation between male feeding per hour or any of the reflectance components recorded before clutch exchange, viz. UV chroma (fig. 4.3.1.1.a; r = 0.21, n = 41, p = 0.18), brightness (fig. 4.3.1.1.b; r = 0.003, n = 41, p = 0.99) and chroma (fig. 4.3.1.1.c; r = - 0.11, n =41, p = 0.49).

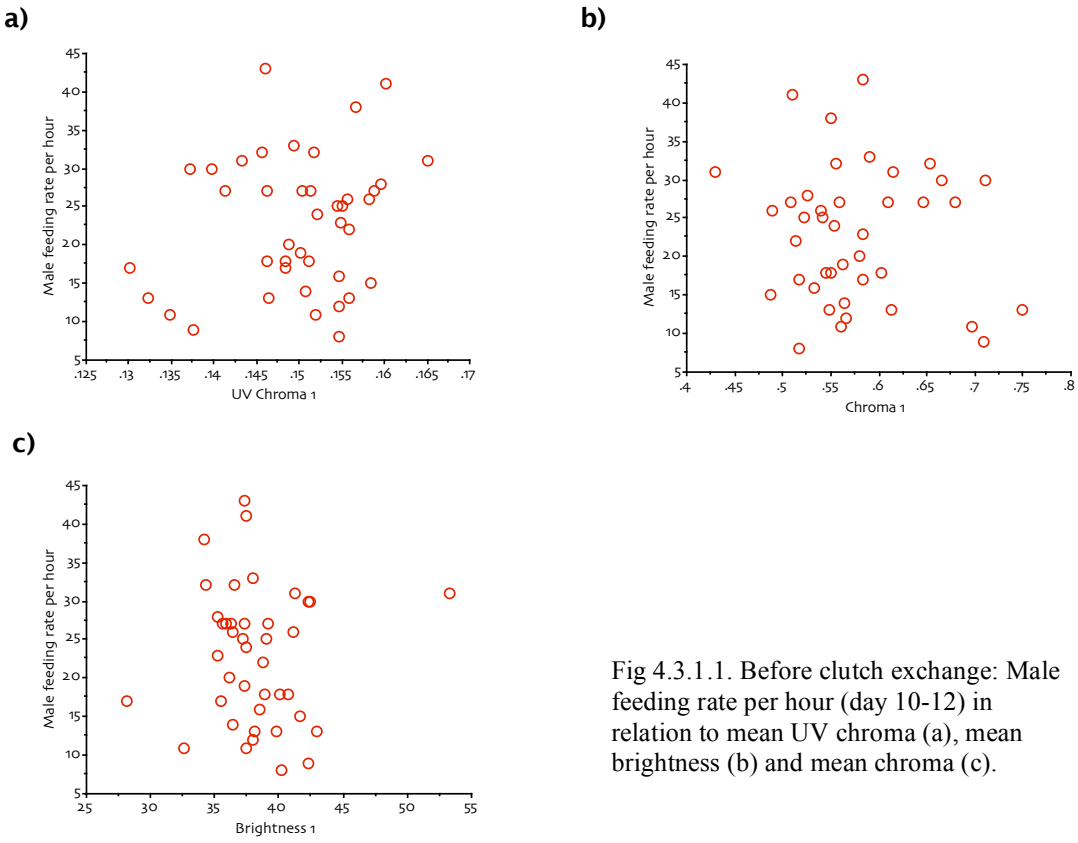


Fig 4.3.1.1. Before clutch exchange: Male feeding rate per hour (day 10-12) in relation to mean UV chroma (a), mean brightness (b) and mean chroma (c).

We found no correlation between male feeding per hour and any aspects of reflectance recorded after clutch exhange, viz. UV chroma (fig. 4.3.1.2.a; r = - 0.17, n = 41, p = 0.28), brightness (fig. 4.3.1.2.b; r = - 0.04, n = 41, p = 0.8) and chroma (fig 4.3.1.2.c; r = 0.15, n = 41, p = 0.35).

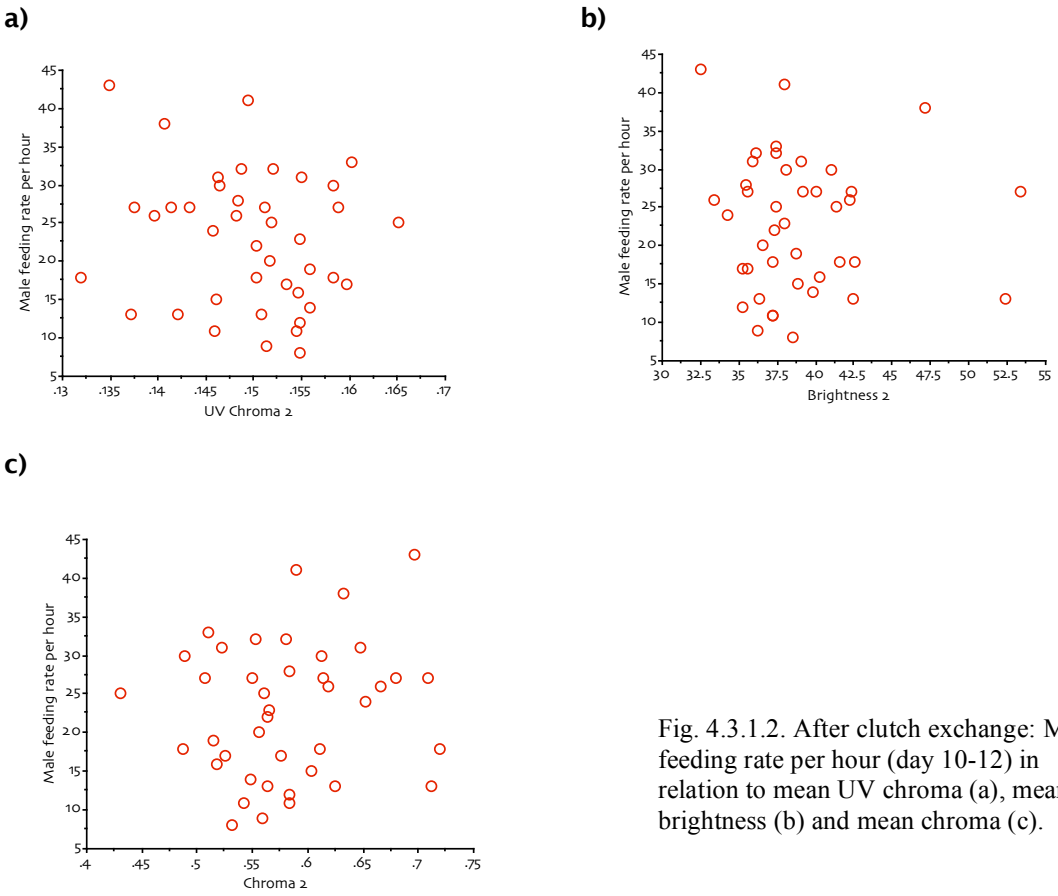


Fig. 4.3.1.2. After clutch exchange: Male feeding rate per hour (day 10-12) in relation to mean UV chroma (a), mean brightness (b) and mean chroma (c).

4.3.2. Male proportion of feeding in relation to reflectance before and after clutch exchange

We found no correlation between male proportion of feeding and aspects of reflectance recorded by spectrophotometer, viz. UV chroma (fig. 4.3.2.a; r = 0.00, n = 41, p = 1.0), brightness (fig. 4.3.2.b; r = - 0.02, n = 41, p = 0.92) and chroma (fig. 4.3.2.c; r = - 0.12, n = 41, p = 0.47).

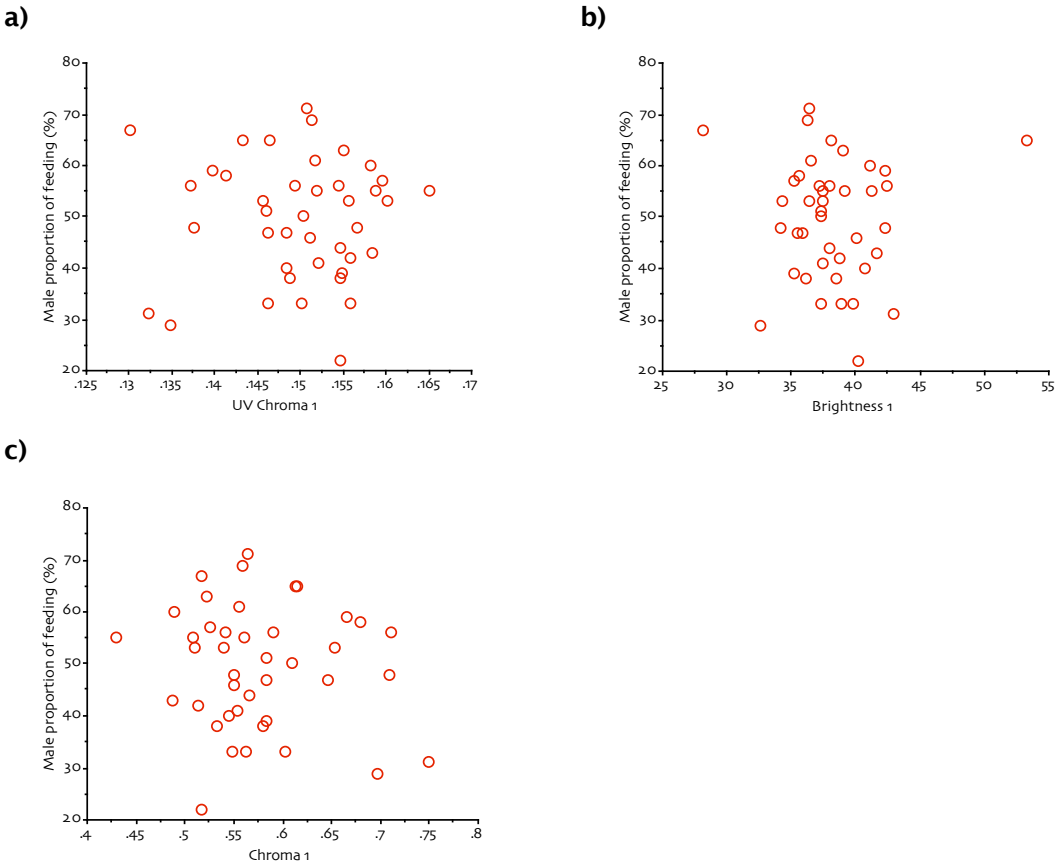
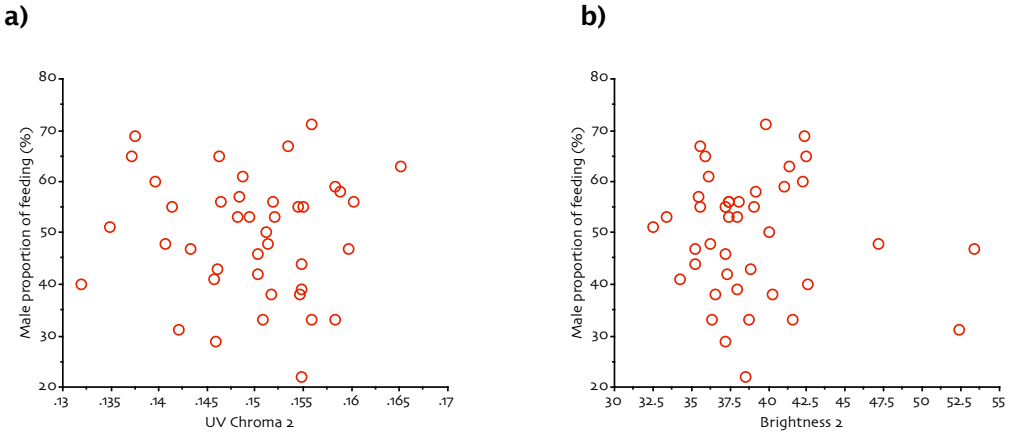


Fig. 4.3.2. Before clutch exhange: Male proportion of feeding in % of total feeding (day 10-12) in relation to mean UV chroma (a), mean brightness (b) and mean chroma (c).

We found no correlation between propotion of male feeding and any aspects of reflectance recorded after clutch exchange, viz. UV chroma (fig. 4.3.2.a; r = - 0.03, n = 41, p = 0.84), brightness (fig. 4.3.2.b; r = - 0.10, n = 41, p = 0.55) and chroma (fig. 4.3.2.c; r = 0.15, n = 41, p = 0.36).



c)

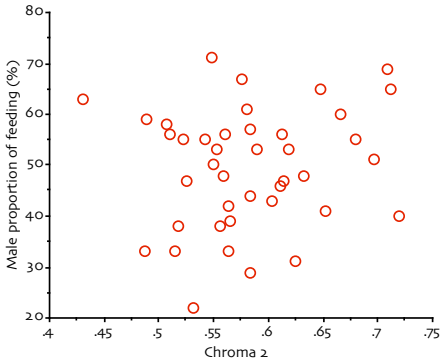


Fig. 4.3.2. After clutch exchange: Male proportion of feeding in % of total feeding (day 10-12) in relation to mean UV chroma (d) mean brightness (e) and mean chroma (f).

4.4. Male feeding effort in relation to egg volume, brood size and laying date.

4.4.1. Egg volume

We found no correlation between male feeding rate per hour and egg volume before clutch exchange (fig. 4.4.1a; r = - 0.20, n = 41, p = 0.21), male proportion of feeding and egg volume before clutch exchange (fig. 4.4.1.b; r = - 0.42, n = 41, p = 0.79), male feeding rate per hour and egg volume after clutch exchange (fig. 4.4.1.c; r = - 0.81, n = 41, p = 0.61) or male proportion of feeding and egg volume after clutch exchange (fig. 4.4.d; r = 0.05, n = 41, p = 0.74).

a)

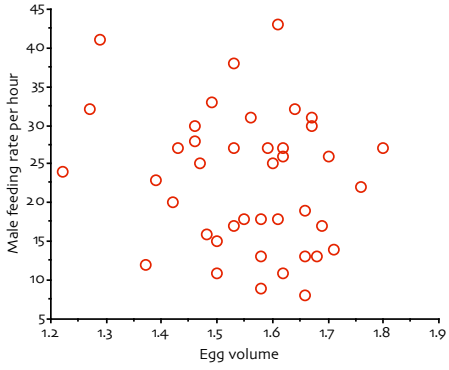


Fig. 4.4.1.a. Before clutch exchange: Male feeding rate per hour (day 10-12) in relation to egg volume.

b)

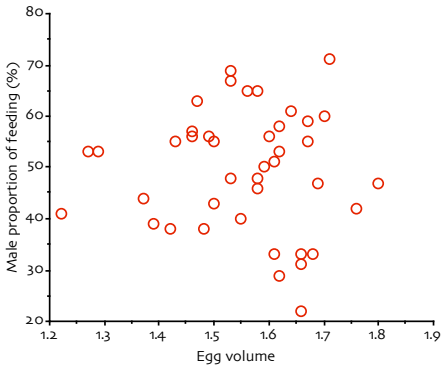


Fig. 4.4.1.b. Before clutch exchange: Male proportion of feeding in % of total feeding (day 10-12) in relation to egg volume.

c)

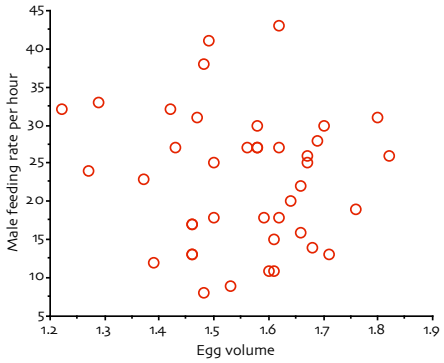


Fig. 4.4.1.c. After clutch exchange: Male feeding rate per hour (day 10-12) in relation to egg volume.

d)

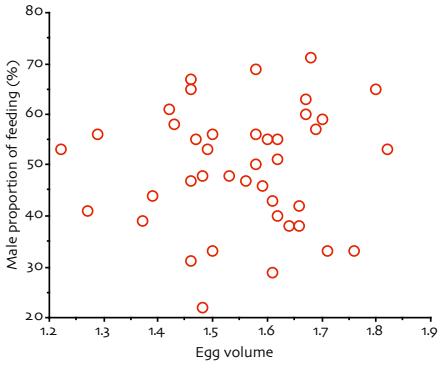


Fig. 4.4.1.d. After clutch exchange: Male proportion of feeding in % of total feedig (day 10-12) in relation to egg volume.

4.4.2. Laying date

We found a negative correlation between male feeding rate per hour and laying date (fig. 4.4.2.a; r = 0.42, n = 41, p = 0.007). However we found no correlation between male proportion of feeding in percent of total feeding and laying date (fig. 4.4.2.b; r = - 0.26, n = 41, p = 0.12).

a)

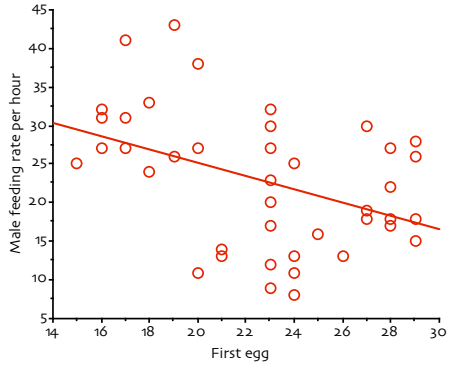


Fig. 4.4.2.a. Male feeding rate per hour (day 10-12) in relation to laying date.

b)

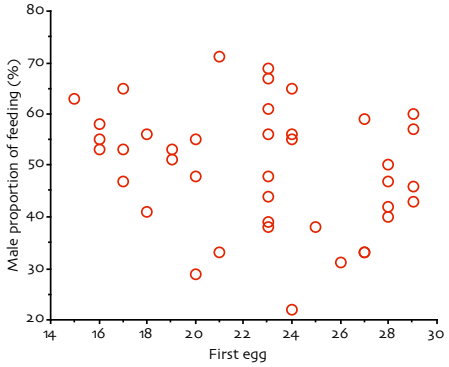


Fig. 4.4.2.b. Male proportion of feeding (day 10-12) in % of total feeding in relation to laying date.

4.4.3. Brood size

Male feeding rate per hour was positively correlated with higer brood size (fig. 4.4.3.a; r = 0.42, n = 41, p = 0.01). However, we found no correlation between male proportion of feeding and brood size (fig. 4.4.3.b; r = - 0.13, n = 41, p = 0.43).

a)

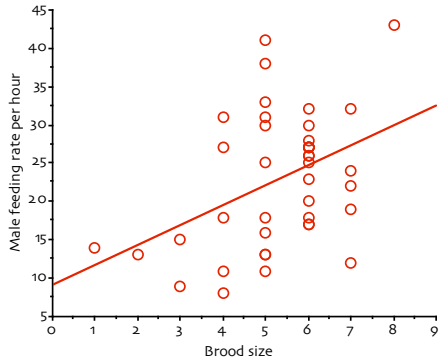


Fig. 4.4.3.a. Male feeding rate per hour (day 10-12) in relation to brood size (day 10-12).

b)

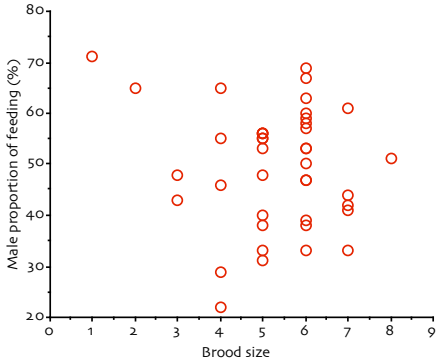


Fig. 4.4.3.b. Male proportion of feeding in % of total feeding (day 10-12) in relation to brood size (day 10-12).

4.4.4. Feeding per nestling per hour and brood size and laying date

We found a negative correlation between the amount of feeding per nestling per hour and brood size (fig. 4.4.4.a; r = - 0.47, n = 41, p = 0.0019) and laying date of the first egg (fig. 4.4.4.b; r = - 0.33, n = 41, p = 0.033).

a)

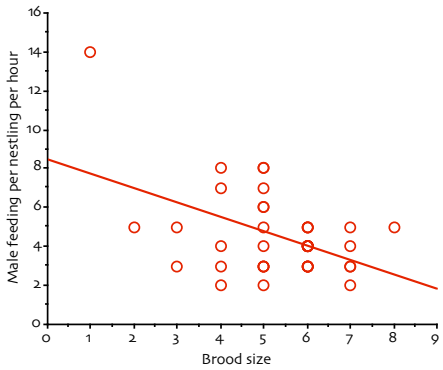


Fig. 4.4.4.a. Amount of feeding per nestling per hour in relation to brood size.

b)

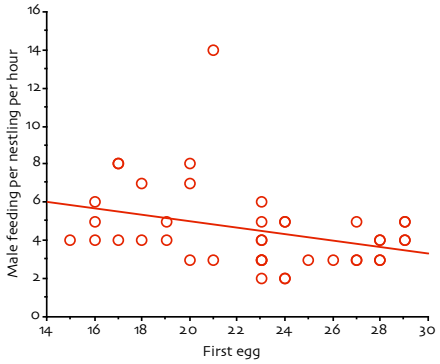


Fig. 4.4.4.b. Amount of feeding per nestling per hour in relation to laying date of first egg.

5. Was nestling body mass related to egg colour, egg volume or laying date?

5.1. Human assessment

We found no correlation between mean nestlings body mass and human assessment of egg colour before clutch exchange (fig. 5.1.a; r = 0.05, n = 41, p = 0.77). However, we found a positive correlation between nestling weight and human assessment after clutch exchange (fig. 5.1.b; r = 0.35, n = 41, p = 0.03), which shows that heavier nestlings were found in nests with darker eggs.

a)

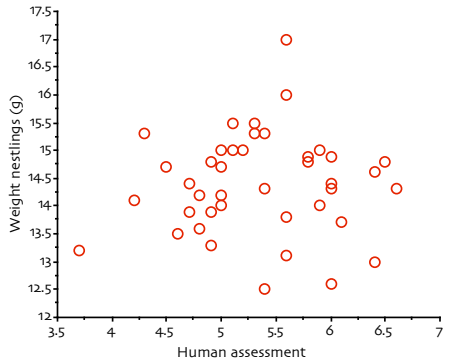


Fig. 5.1.a. Before clutch exchange: Mean nestling body mass (day 12-13) in relation to human egg colour assessment.

b)

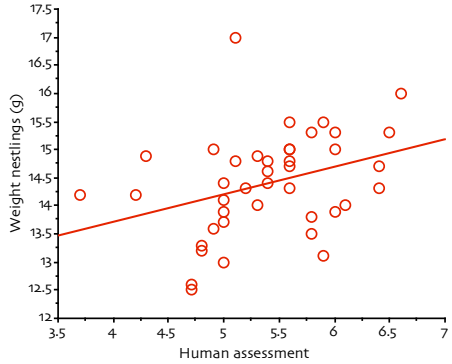


Fig. 5.1.b. After clutch exchange: Mean nestling body mass (day 12-13) in relation to human egg colour assessment.

5.2. Reflectance values

5.2.1. Nestling body mass and reflectance values of eggs before clutch exchange

None of the reflectance components showed any significance in correlation with mean body mass of nestlings, viz. UV chroma (fig. 5.2.1.a; r = 0.01, n = 41, p = 0.94), brightness (fig. 5.2.1.b; r = 0.14, n = 41, p = 0.39) or chroma (fig. 5.2.1.c; r = - 0.05, n = 41, p = 0.77).

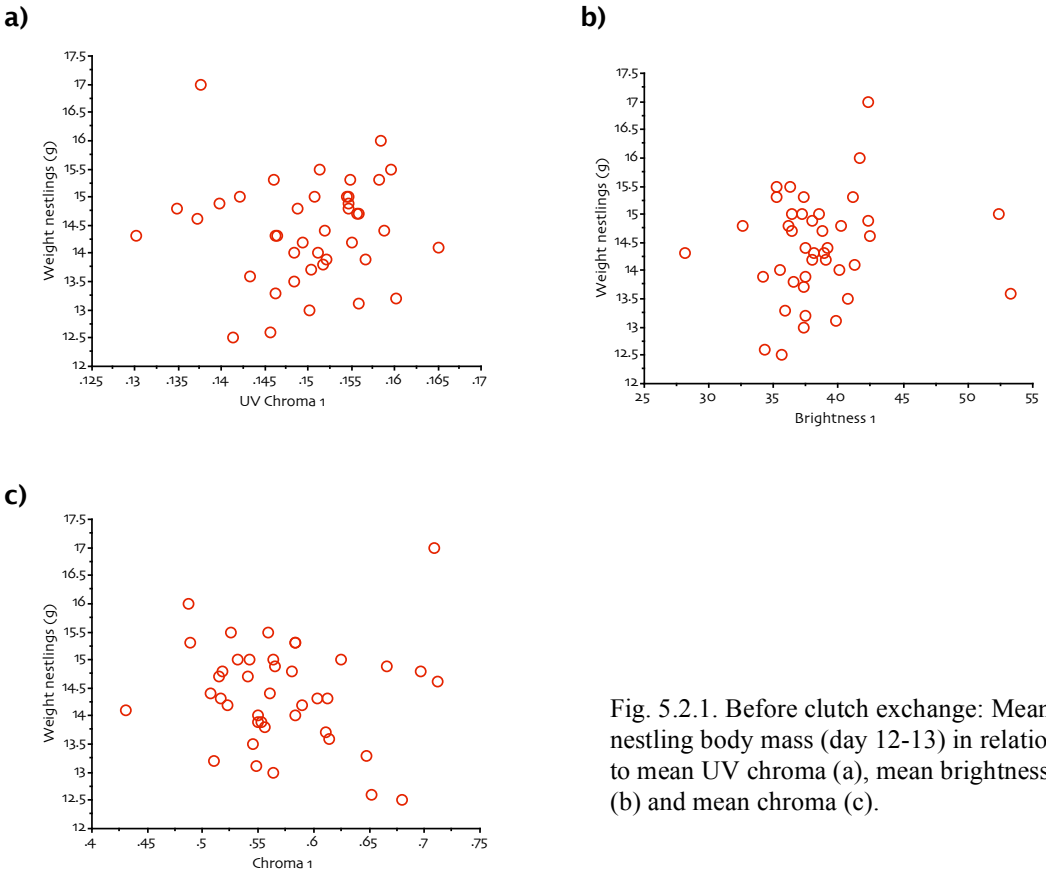


Fig. 5.2.1. Before clutch exchange: Mean nestling body mass (day 12-13) in relation to mean UV chroma (a), mean brightness (b) and mean chroma (c).

5.2.2. Nestling body mass and reflectance spectrophotometer values after clutch exchange.

We found no correlation between mean body mass of nestlings and any aspects of reflectance recorded, viz. UV chroma (fig. 5.2.2.a; r = - 0.14, n = 41, p = 0.38), brightness (fig. 5.2.2.b; r = - 0.16, n = 41, p = 0.32) and chroma (fig. 5.2.2.c; r = 0.14, n = 41, p = 0.38).

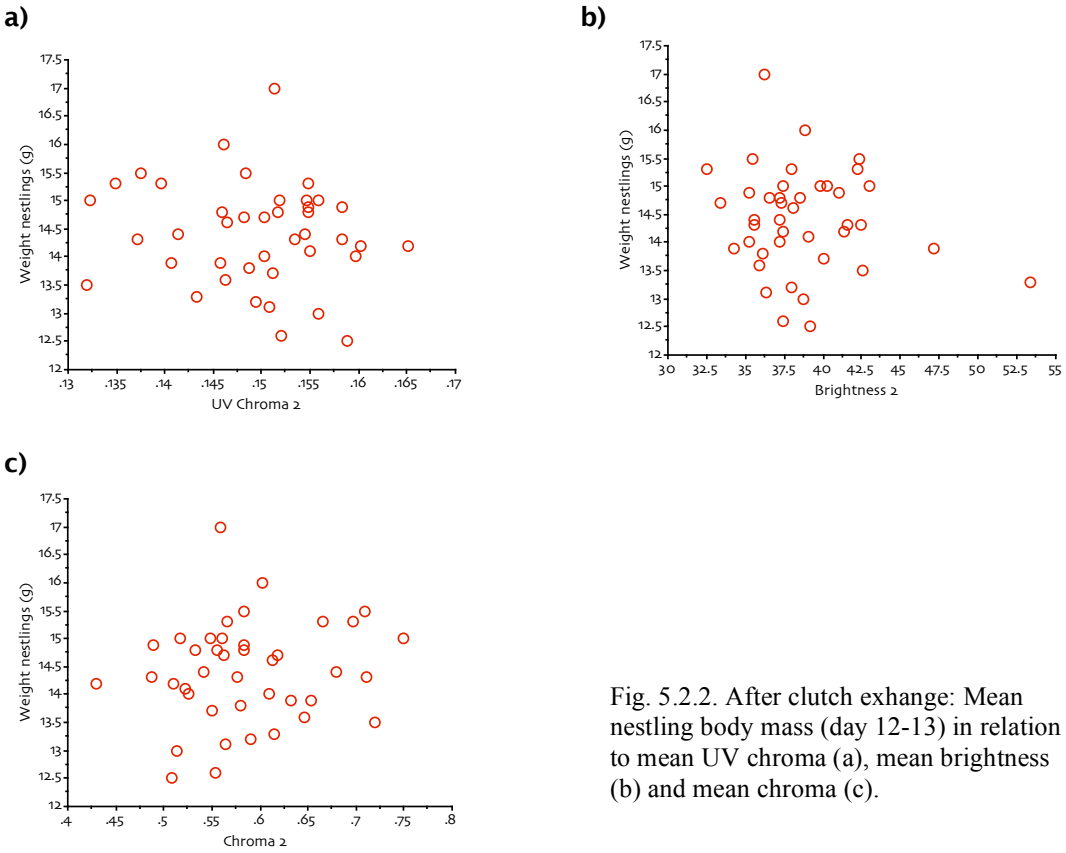


Fig. 5.2.2. After clutch exhange: Mean nestling body mass (day 12-13) in relation to mean UV chroma (a), mean brightness (b) and mean chroma (c).

5.3. Egg volume

We found no correlation between mean body mass nestlings and mean egg volume before clutch exchange (fig. 5.3.a; r = 0.02, n = 41, p = 0.9) or after clutch exchange (fig. 5.3.b; r = - 0.2, n = 41, p = 0.21).

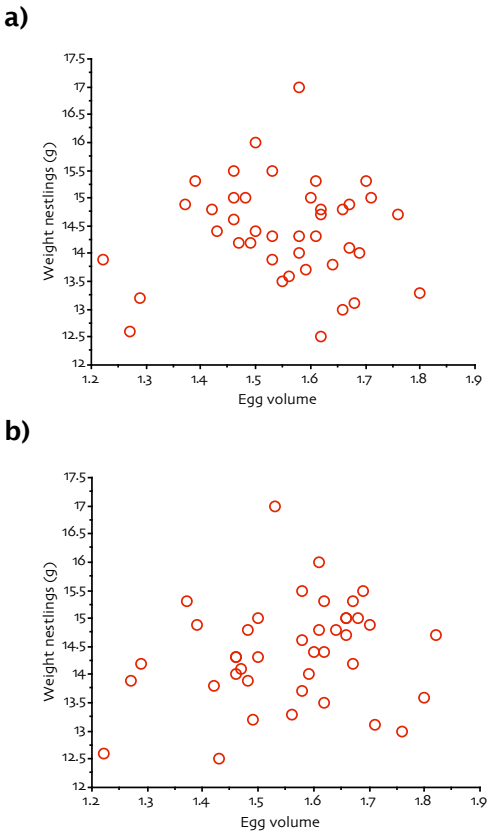


Fig. 5.3. Mean nestling body mass at day 12-13 in relation to egg volume before clutch exchange (a) and after clutch exchange (b).

5.4. Laying date

We found a positive correlation between mean nestlings body mass and laying date, broods deriving from egg laid late in the season showed a higher mean weight (fig. 5.4.a; r = 0.38, n = 41, p = 0.02). Mean body mass of nestlings was not significantly correlated with brood size (fig. 5.4.b; r = - 0.25, n = 41, p = 0.11).

a)

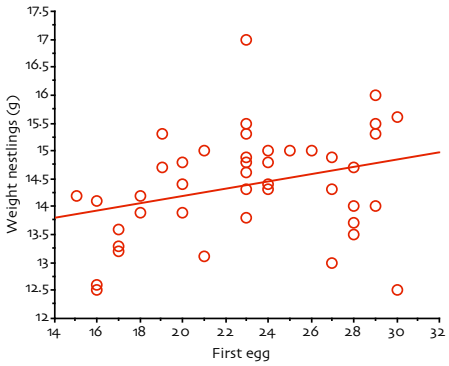


Fig. 5.4.a. Mean nestling body mass at day 12-13 in relation to laying date.

b)

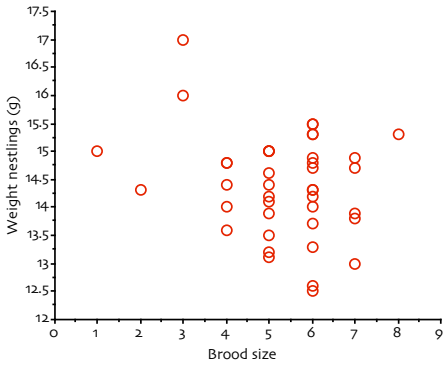


Fig. 5.4.b. Mean nestling body mass at day 12-13 in relation to brood size.

Discussion

We succeeded in our attempt to present the birds with a new clutch of eggs with colours that were independent of those of their on initial clutch, which enabled us to separate possible relationships between male provisioning effort, female quality and colour of clutches. This is shown by the non-significant results when comparing the two clutches as measured by spectrophotometer and assessed by a human. Interestingly, we found a significant correlation between human colour assessment and UV chroma and chroma. However, considering human inability to see in UV wavelengths, the relationship with UV chroma is obviously a side-effect of the strongly negative correlation between this variabble and chroma (unpublished data). Human colour assessment therefore predomiantly reflects variation in saturation of the colour at medium to long wavelengths, caused by a variation in the amount of the biliverdin pigment.

Previous studies of a population of pied flycatchers breeding in Central Spain (Morales et al., 2006; Moreno et al., 2005; Moreno et al., 2006b) supporting the sexually selected egg colour hypothesis, demonstrated that eggshell colour, and biliverdin content, reflect female condition at laying. In our study, spectrophotometer measurements showed that the last positioned egg in clutches of six eggs, and the two last eggs in clutches of seven eggs, had a significant lower chroma than the rest (unpublished data). This supports previous findings that eggs become lighter in the course of the laying sequence (Moreno et al., 2005). However, in testing egg colour of clutches to reproductive variables that we can expect to affect female quality and thus colour, like laying date, clutch size, egg volume and mean nestling weight, we failed in finding strong correlations with egg colour. Human colour assessment showed that females laid more colourful eggs early in the breading season, but this result was not supported by any of the spectrophotometer variables. Brightness was significantly correlated with clutch size, but to none of the other reflectance components, nor human colour assessment. In several species, there have been observed larger eggs in later position in the laying sequence, assumed to function as a strategy to facilitate the survival of the nestlings hatched from eggs laid later in the sequence (Slagsvold et al., 1984). In our study we found no correlation between laying date and egg volume. This result represents an interesting contrast to Siefferman et al. (2005) in which both egg coloration and egg size increased

with position in the laying sequence in eastern bluebirds (*Sialia sialis*), and eggs laid later in the sequence had more intense egg colouration.

Hypothesis A predicted an adjusted male effort according to the colour of the eggs after clutch exchange. In theory, we would expect males to invest more heavily in nests where egg colour had become darker, whereas from hypothesis B and C we would expect the opposite effect (tab. 1). During video filming we registered two parameters in relation to male feeding rate; 1) the total amount of feeding visits per hour by male and female, and 2) the amount of male feeding visits per hour in percent of total feeding visits. Interestingly, our results showed that these two parameters, which were significantly correlated ($r = 0.43$, $n = 41$, $p = 0.005$), gave different results. Our results showed no adjusted effort in male feeding rate of nestlings deriving from more colourful eggs and therefore no support for hypotheses A. We found a negative correlation between male feeding rate per hour and egg colour as assessed by human vision before clutch exchange. However, this was not supported by spectrometry. For nest with larger broods we found a higher feeding rate per hour, than for nests with smaller broods, showing a possible adjustment by the male according to the number of nestlings. However, when comparing feeding rate per nestling per hour with brood size, we found a negative correlation. Further, male feeding rate per hour was negative correlated with laying date, showing a decrease in feeding rate per hour with time of breeding. In contrast, male proportion of feeding visits in percent of total feeding visits did not significantly correlate with any of the other parameters tested (colour measurements, laying date, brood size or egg volume).

In further testing of hypothesis A, we compared nestling body mass to variables that may influence male effort, like egg colour, egg volume and laying date. Nestling body mass was positively correlated with human assessment of egg colour after clutch exchange. However, mean nestling body mass was not significantly correlated with any of the objective colour variables, which gives no support to the hypothesis. We did find a positive correlation between body mass of nestlings and laying date, which does not corroborate with the previous results showing a negative correlation between laying and egg colouration (human assessment), male feeding per hour and male feeding per nestling per hour. An alternative explanation may be variation in female feeding effort.

According to hypothesis B and C, we would expect no adjustment in feeding rate according to egg colour, but an indirect relationship between egg colour and male feeding effort through a third factor, territory or female mate choice. A good territory may provide high food access and could also be a factor when selecting a mate (Kern et al., 2000). Hypothesis B predicts that females from high quality territories produce darker blue/green eggs and that males can afford to have a high feeding rate. High quality females may get the best males due to age, experience and extended prospecting for a mate (Dale and Slagsvold, 1996) or males may be responding to female morphological or other behavioural traits that are not associated with egg colour, like other aspects of female condition not measured. In contrast to Moreno et al (2006) hypothesis that biliverdin pigmentation in eggshell reflects female condition, our results showed no strong indications of egg colour reflecting female quality for our population of pied flycatchers. Apparently, there was no higher male feeding rate for nests where eggs initially were darker providing no support for hypothesis B or C (table 1). Our results showed that feeding rate was more or less independent of egg colour and egg colour exchange, and thereby gives support only for hypothesis D. Our results differ from previous studies on the same subject, and this may be explained by a number of factors. This study is based on a population of pied flycatchers from a different area, which may give unmeasured differences. Provision rate was based on male visits observed during a period of only 1-1.5 h. of filming, but similar to previous studies, and may not be representative. However, we measured nestling body mass as an additional parameter to the feeding rate. We assumed that males inspect the eggs during the laying and incubation stage and hence can be affected by the clutch exchange made during this period. However, the lack of any correlation between male feeding effort and egg colour before and after clutch exchange, suggests that males do not pay attention to egg colour during this period. As proposed by Moreno et al. (2006), males may also adjust their effort to quality of nestlings and adjust their level of investment to other parameters than egg colour signalling the quality of the clutch and brood.

In conclusion, our study showed no relationship between male feeding effort and egg colour, or egg colour exchange. This does not reveal a support for any of the hypotheses A, B, or C.

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