Colour polymorphism
in female *Coenagrion puella*: differences in egg shape
(Odonata: Coenagrionidae)

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ABSTRACT

The maintenance of female colour polymorphism in coenagrionids is still an open issue. Here we ask if the three different female morphs of *Coenagrion puella* represent different reproductive traits in terms of clutch and egg size. Therefore clutch size and egg morphometry of the three female colour morphs were examined. We found that female colour morphs did not differ in clutch or egg size. However, we also found that the female morphs differ in egg shape, with the intermediate morph having more elongated eggs compared to the hetero- and andromorphic females. Our results are discussed in terms of potential different preferences in oviposition substrate.

INTRODUCTION

Colour polymorphism is a widespread feature, described for many animals of different taxa (e.g. Galeotti et al. 2003; Munday et al. 2003; Hatadani et al. 2004; Wente & Philips 2005). One of the best-known examples is the peppered moth, which has become a textbook example with the occurrence of at least two colour morphs (Cook 2003). Literature suggests that sexual selection (Robertson 1985; Hinnekint 1987; Kingston et al. 2003), or natural selection in the form of predation (Hort 2004; Wente & Philips 2005), directly selects on colour polymorphism. But, for several species, the actual factors maintaining colour polymorphism remain unknown (Galeotti et al. 2003). Often colour polymorphism is restricted to one sex and most of the work on sex-specific colour polymorphisms has so far been done on males (e.g. Sundberg 1995; Sinervo & Lively 1996; Tsubaki et al. 1997; Gamble et al. 2003). However, female colour polymorphism has also received broad attention (e.g. Cordero 1990; Fincke 1994; Van Gossum et al. 2001; Svensson et al. 2001; Roulin et al. 2003).

It has been shown that colour morphs not only differ in colour, but may also exhibit different correlated reproductive strategies (Gray & McKinnon 2007). Male morphs of the zygopteran *Mnais pruniosa costalis* Selys, differing in their wing patterning, display different mating strategies by either being territorial or non-territorial (Tsubaki et al. 1997). For female lizards, Svensson et al. (2001) revealed that one colour morph acts as a K-strategist while the other as an r-strategist. Roulin et al. (2003) found a trade-off between offspring quality and frequency of breeding in female colour morphs of the tawny owl *Strix aluco*. Those differences in reproductive strategies, or in other traits like physiology (Rolff & Siva-Jothy 2004), life history (Roulin et al., 2004) and behaviour (Van Gossum et al. 2001, 2004), may be the actual factors under selection influencing occurrence and maintenance of colour morphs (Gray & McKinnon 2007).

In coenagrionids colour polymorphism is limited to one sex and is shown to be genetically determined in females (Johnson 1964, 1966; Cordero 1990; Andrés & Cordero 1999; Sánchez-Guillén et al. 2005). Thereby the coloration, sometimes also the black pattern, of females either resembles that of the males, or differs from the male. In Zygoptera the selective factors maintaining colour polymorphism remain unsolved (Andrés et al. 2000). Common hypotheses discussed are the male mimic hypothesis (Johnson 1966; Robertson 1985; Hinnekint 1987), and the learning hypothesis (Fincke 1994; 2004), with a momentary trend to focus on frequency-dependent selection (cf. Cordero 1989; Andrés et al. 2000; Sherratt 2001; Fincke 2004; Svensson et al. 2005; Van Gossum et al. 2005a, b). However, a recent study revealed that female colour morphs in Zygoptera also differ in other traits besides colour, as offspring larval development time correlated with maternal colour morph (Abbott & Svensson 2005). Male and female offspring of one colour morph do not differ in development time though. This suggests that selection may also act on other phenotypic traits, besides natural and sexual selection on colour itself (Abbott & Svensson 2005; Svensson & Abbott 2005).

Among odonates, species differ in reproductive traits such as clutch and egg size (Schenk & Söndgerath 2005). Egg size is an important trait since it affects larval size and developmental performance (Schenk & Söndgerath 2005; and references therein). Consequently, we might assume that differences in larval development in offspring of female colour morphs may be related to differences in clutch size, egg size and/or egg shape among female colour morphs. However, nothing is yet known of whether female colour morphs differ in these reproductive traits. Here we ask whether female morphs in *Coenagrion puella* (Linnaeus) differ in clutch size, egg size and egg shape. The measurement of these traits will help enable us to unravel differences between the morphs in reproductive traits, and potentially indicate traits that are under selection to maintain colour polymorphism.

MATERIALS AND METHODS

Coenagrion puella is a common species in central Europe (Dijkstra 2006). As with many other coenagrionid species, females are described to be polymorphic in colour while males are always blue. The female morphs are termed as andromorphic (blue),

heteromorphic (green) and intermediate (green thorax, blue abdomen) (Sternberg & Buchwald 1999; for terminology cf. Fincke et al. 2005; Joop et al. 2006). In coenagrionids heteromorphs often show higher numbers within a population than any of the other female morphs (for a review, see Fincke et al. 2005). *C. puella* oviposits in tandem and females place their eggs endophytically in floating plants and reeds by means of an abdominal ovipositor. Eggs hatch before winter and larvae hibernate mainly in the F-4 stadium (Sternberg & Buchwald 1999). First adults emerge from early May onwards, and the species is on the wing until September (Dijkstra 2006).

All animals were caught at a pond near Braunschweig, Germany, with a population size of ca 50,000 emerging individuals (cf. Rolff 1999). To obtain the eggs, females were caught out of copula and brought into the laboratory. Age is known to not affect clutch size in another coenagrionids species (Svensson et al. 2005). However, we only sampled mature females with undamaged wings and as this is a good indicator of young age (Corbet 1999: 638), thereby controlled for possible age effects. For oviposition each female was put in a separate plastic box (20 x 8 cm, 5 cm in height) that contained wet filter paper over a wooden stick as oviposition substrate. Boxes were covered with gauze. After 48 h females were freed, after measuring head width with a stereomicroscope as a common measurement for size in odonates (Benke 1970). Eggs from each clutch were counted on a light table and about 30 eggs from each female were preserved in 80% EtOH and stored until further measurements. Before measuring length, width and area, eggs were re-hydrated for 48 h at 4°C in distilled water with 4% formalin to avoid mould. All egg size and area measurements were taken from standardized images using an image analyzer (Optimas 6.0). Subsequently, all eggs were dried on a pre-weighed foil at 60°C over night. After cooling to room temperature the eggs were weighed on the foil. For each female, we calculated the average egg length, width, area and dry weight. Egg shape was calculated as the ratio of egg length to egg width, using the individual's average egg length and width. Data for clutch size of the different female colour morphs are from 2003 and 2004, while data for egg size are from 2004 only. For clutch size (2003/2004) our sample size was eight blue females (3/5), 40 intermediate females (24/17) and 131 green ones (54/74). For egg size and shape we measured 30 eggs each of three blue, 12 intermediate, and 45 green females.

Statistics

In all analyses female head width was used as an independent covariate to correct results for potential maternal effects that might be caused by female size differences (Corbet 1999: 37). Furthermore, in all analyses error type III was used to correct for unbalanced sample size.

Clutch size was analysed using an ANCOVA with colour class (andromorphic, heteromorphic or intermediate) and year (2003 or 2004) as independent variables and egg number as the dependent variable. Egg size was analysed in a MANCOVA with egg length, - width, -area and egg dry weight as dependent variables and colour class as an independent variable. Egg shape was used as a dependent variable in an ANCOVA. The closer the ratio of egg length : egg width is to 1, the more spherical (less spindle-shaped) the egg is. All analyses were done using SPSS 11.

RESULTS

Clutch size (Fig. 1) did not differ among the female morphs (ANCOVA: df = 2, F = 0.183, p = 0.833). Neither was a difference found between years (ANCOVA: df = 1, F = 1.267, p = 0.262), and head width did not influence our results (ANCOVA: df = 1, F = 0.752, p = 0.387). We also did not find differences among the female morphs in egg width, egg length, egg area, and egg weight (Pillai's trace: F = 0.518, Hypothesized df = 8, Error df = 102, p = 0.840) (Fig. 2), nor was there any influence of head width (Pillai's trace: F = 0.397, Hypothesized df = 4, Error df = 50, p = 0.810). Even though we found no significant differences for clutch- and egg-size measurements among female morphs, it is obvious that egg length, -area and -weight follow the same pattern for all morphs (Fig. 2). In contrast, the morphs show a different pattern for egg width that should lead to a less spindle-shaped three-dimensional shape in eggs of blue females, while intermediate females should have the most oval eggs. Results revealed differences in egg shape among female colour morphs (colour class: df = 2, F = 4.535, p = 0.015, head width: df = 1, F = 0.264, p = 0.609) (Fig. 3). In a pairwise comparison (LSD adjustment for multiple comparisons) eggs of blue females differed significantly from those of intermediate females (p = 0.008), as did eggs from green females (p = 0.03). The difference in shape between eggs from blue and green females was not significant, though they did show a strong tendency p = 0.089).
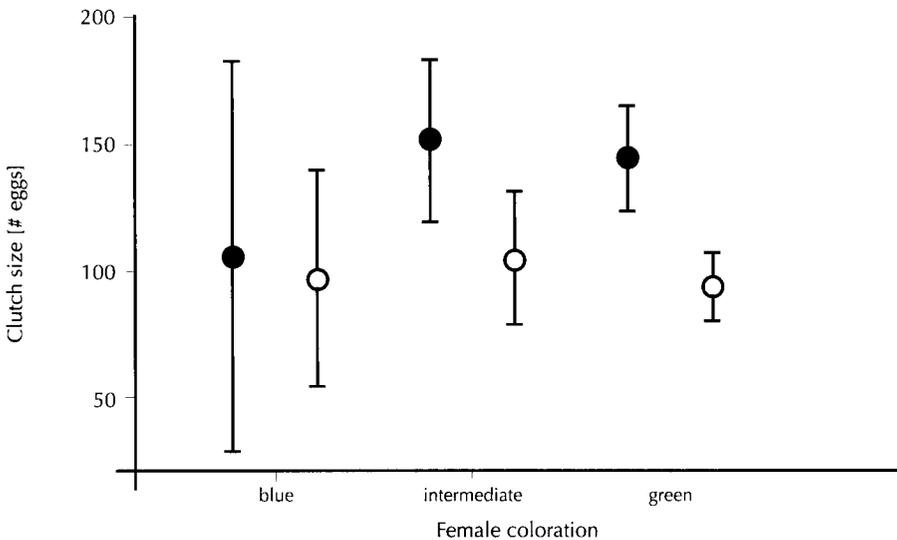

Figure 1: Mean clutch size of the different female morphs in *Coenagrion puella* — ● data from 2003 and ○ 2004; s.e. is given.
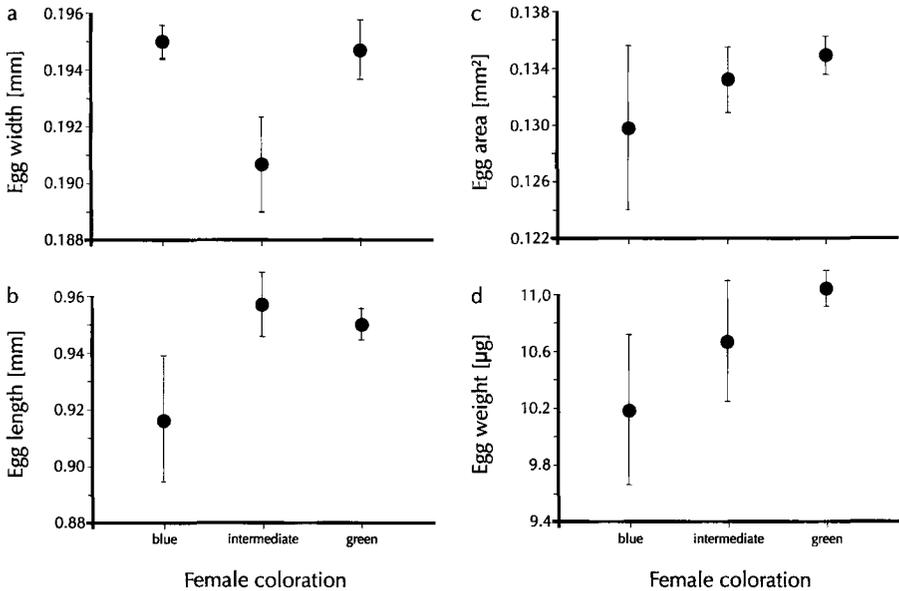
Figure 2: Mean egg data for the different female morphs in *Coenagrion puella* — (a) egg width; (b) egg length; (c) egg area; and (d) egg dry weight; s.e. is given.

DISCUSSION

Besides hypotheses about direct selection on female colour morphs (e.g. Van Gossum et al. 2005a), it is discussed whether selection on traits correlated to female colour morphs may explain maintenance of and variation in abundance of different female morph types. Recent studies reveal differences in behavioural (Van Gossum et al. 2001) and larval traits (Abbott & Svensson 2005) linked to specific colour morphs. Our results revealed no differences in clutch or egg size between female colour morphs in *Coenagrion puella* but egg shape differed significantly.

Consistent with our findings, assuming that oviposition duration is positively cor-related with clutch size, no differences in oviposition duration was detected in sev-eral coenagrionid species in the field (cf. Fincke 2004, and references therein). However, this correlation between oviposition duration and clutch size remains to be shown in the field as well as in the lab.

Since Abbott & Svensson (2005) found differences in offspring development time for female colour morphs in *Ischnura elegans* (Vander Linden), we expected egg size to differ between the colour morphs. This is because larval development time is as-sumed to be dependent on vitellin content in the egg, and this again may be linked to egg size (Nocolaro & Bradley 1980; Takesue et al. 1983; Bradley & Estridge 1997; for assumed links in odonates, see Hottenbacher & Koch 2006). However we did not detect any such differences in *C. puella*, neither in egg length, width or height, nor

in weight. It was not possible to raise eggs of blue female *C. puella* in the lab as all eggs became mouldy and died although we had no problems raising eggs of green or intermediate females. Therefore we cannot give any further data on larval development time according to maternal morph in this species. Furthermore, if there had been a difference between the female morphs in egg size, we also would have expected to find a difference in clutch size, due to a size-number trade off (Sahlén & Suhling 2002). But again, no such difference was detected in *C. puella*.

However, female colour morphs differed in egg shape with intermediate females having more spindle-shaped eggs, while blue and green females have less spindle-shaped eggs. This might indicate the use of different oviposition substrates in the female morphs. *C. puella* oviposits in floating water plants, reed, and dead material (Martens 1994; Sternberg & Buchwald 1999). Females pierce the plant with their ovipositor and inject their eggs (Sternberg & Buchwald 1999). However, injecting eggs is likely to be easier with more spindle-shaped eggs than with less spherical-shaped ones, especially in harder substrates. Therefore intermediate females, having more spherical eggs, might prefer harder oviposition substrates while the other morphs might oviposit in slightly softer substrates. If the female morphs in *C. puella* preferred different oviposition substrates the species could inhabit a broader niche, i.e. different plants for oviposition. As intermediate females are the second most common morph in this population (in 2004: 52% green, 35% intermediate and 13 % blue females emerging; A. Gillen unpubl.), their egg shape might represent an alternative strategy to the egg shape of green and blue females. Sahlén (1995) found that Zygoptera differing in oviposition-plant preference also differ in the ultrastructure of the eggshell. Therefore it would be interesting to explore whether the female morphs of *C. puella* also differ in their eggshell composition.
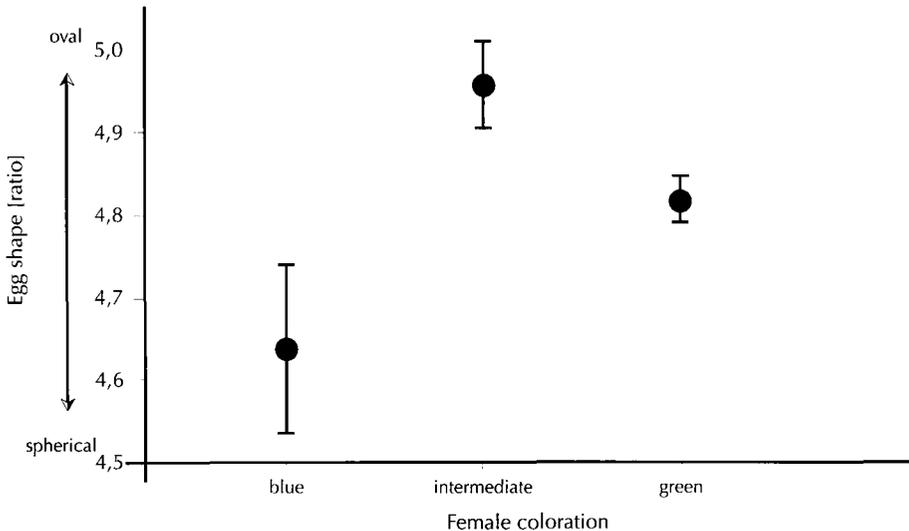

Figure 3: Egg shape of the different female colour morphs in *Coenagrion puella* — calculated as ratio of egg length to egg width; s.e. is given.

Among odonate species, other differences in egg shape, linked to oviposition, have been described previously: species ovipositing in plants have elongated, more fragile eggs that are easier to inject and are protected by the plant. On the other hand species ovipositing in open water have spherical eggs as these appear more robust against damage due to e.g. drift (Corbet 1999: 45 ff.). To our knowledge, it has not however been observed that females of *C. puella* oviposit in open water (or Coenagrionidae at all). For *Coenagriocnemis reuniensis* (Fraser) oviposition on rock surfaces has been described (Martens 2001). In this case spherical eggs might be advantageous over elongated ones as the current and heavy rain should not easily damage them, but little is known about egg shape in this species. Some Gomphidae however, even though they are ovipositing exophytically in running waters, have an intermediate egg shape between spindle-shape and spherical that might present the transition from endophytic oviposition to exophytic (Corbet 1999: 45).

As far as we are aware it has also not been shown that one female morph prefers one substrate or another in *C. puella*. Conagrionids are known to aggregate for oviposition (Martens 1994), but it is unknown whether the female colorations have any influence on the aggregation, and whether there are any substrate preferences. Therefore choice experiments on oviposition substrate for *C. puella* are needed along with observations in related species on oviposition substrate and egg shape.

So how can the existing colour-polymorph system in female *C. puella* be maintained via differences in egg shape? If males were aware of the potential differences in oviposition substrate preference they might choose one morph or another, depending on which water plants are present at the pond, as the water plant composition may change between seasons and/or due to natural succession (Begon et al. 1998). This might also provide a new aspect to explain the frequency dependent process regulated by male-female mate interaction found for the female morphs in *I. elgeans* by Svensson et al. (2005) as well as the population dynamics found in aging populations of *I. elegans* by Svensson & Abott (2005). Again, further behavioural examinations, in relation to environmental factors, or experiments providing different substrates to choose from, and with even more detailed egg measurements, e.g. of protein content or eggshell composition, might give us a clearer insight into this subject.

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