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TECHNICAL NOTE

"Bug-eggs" for Common Swifts and other small birds: minimally-invasive and stress-free blood sampling during incubation

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Abstract The use of haematophagous bugs (Triatominae) for minimally-invasive blood sampling has increasingly gained interest. We developed a small bug-containing dummy egg (size: 25 × 19 [mm]) for stress-free blood sampling during incubation in Common Swifts (*Apus apus*) and potentially even smaller bird species. Our design expands on an application of a method previously used successfully on larger birds. In our study, 40 % of blood-sampling attempts were successful. Success was highest in the early breeding season, higher at noon than later in the day and unaffected by nest infestation with ectoparasitic louse-flies (*Crataerina pallida*). We recommend this method for blood-sampling birds without trapping during the sensitive period of incubation and encourage its application in small bird species.

Keywords *Dipetalogaster maxima* · *Apus apus* · Blood-sucking bug · Reproduction · Incubating birds · Triatominae

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Zusammenfassung

„Wanzen-Eier" für Mauersegler und andere kleine Vögel – minimal-invasive und stressfreie Blutentnahme während der Inkubationszeit

Der Einsatz blutsaugender Wanzen (Triatominae) zur minimal-invasiven Blutprobengewinnung hat zunehmend an Interesse in der Forschung gewonnen. Zur stressfreien Blutentnahme während der Inkubationszeit entwickelten wir für Mauersegler (*Apus apus*) – und potentiell noch kleinere Vogelarten – ein kleines künstliches Ei (25 x 19 [mm]), in das eine Wanze gesetzt wird. Unser „Wanzen-Ei"-Design erweitert die Anwendung einer Methode, die bei größeren Vogelarten bereits erfolgreich war. In unserer Studie lag die Erfolgsquote der Blutentnahme bei 40 %. Sie war vor allem zu Beginn der Brutsaison hoch, war in den Mittagsstunden höher als in den Stunden danach und war unabhängig vom Befall des Nestes mit Lausfliegen (*Crataerina pallida*). Wir empfehlen diese Methode zur stressfreien Blutentnahme bei Vögeln ohne deren Fang während der empfindlichen Zeit der Inkubation - auch bei kleineren Vogelarten.

Introduction

Blood sampling represents a basic prerequisite for many research purposes. However, standard techniques involve trapping and handling the animal and drawing blood with conventional needles and may thus cause stress for the animal under investigation (Fair et al. 2010). This effect is unacceptable in sensitive or endangered species or during challenging periods like reproduction, e.g. when trapping

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may increase the risk of nest abandonment in birds. Furthermore, stress influences the results when measuring energy expenditure (Butler et al. 2004) or monitoring baseline stress hormone titres (Romero and Reed 2005). Von Helversen et al. (1986) described a gentle method of minimally-invasive blood-sampling using haematophagous bugs (Triatominae, Heteroptera). These bugs are able to obtain blood even from small vessels, usually unnoticed by the host, and no haematoma or wound remains (von Helversen et al. 1986).

In the last decade, this method has gained interest, reflected in numerous publications on the validation of different parameters in blood samples taken via this method: various blood parameters for clinical chemistry and haematology (Bauch et al. 2010; Markvardsen et al. 2012), doubly-labelled water experiments (Voigt et al. 2003), hormones (Voigt et al. 2004; Arnold et al. 2008; Riechert et al. 2012), telomeres (Bauch et al. 2013) and virus-neutralising antibody titres (Voigt et al. 2006; Vos et al. 2010). Becker et al. (2006) refined the procedure for its application in incubating Common Terns (*Sterna hirundo*; natural egg size 42 × 31 (mm); Becker and Ludwigs 2004) using bug-containing dummy eggs ("bug-eggs"). These hollow artificial eggs had a gauze-covered window, and a wire fixed the egg in the nest. A modified version, consisting of hollow eggs with a small opening around the circumference plus additional small holes and a screw-in thread in the middle of the egg holding the halves together, further increased blood-sampling success rate (Arnold et al. 2008; Bauch et al. 2010). Whereas successful blood collection was reported for Common Terns (Becker et al. 2006; Arnold et al. 2008; Bauch et al. 2010) and Montagu's Harriers (*Circus pygargus*; Janowski, personal communication), it failed in smaller species like Great Tits (*Parus major*; Bähnisch 2011). Due to small egg size, the author placed the bug into a small bag and added it to the clutch. However, this method was described as unsuccessful as the bugs did not suck blood or the birds removed the foreign material.

Our objective was to modify "bug-eggs" in a way that they can be applied to a smaller bird species, the Common Swift *Apus apus*; adult body mass, 2009–2012 (our study colony): 29.8–52.5 g, mean ± SD = 42.0 ± 4.1 (g), $n = 107$. We developed a new egg design and investigated possible influences of sampling time, ambient temperature, nest temperature and parasite infestation on blood-sampling success.

Methods

Field studies were carried out in a Common Swift colony situated inside a concrete highway bridge (51°02′33″N, 07°49′40″E) spanning the Bigge Reservoir near Olpe, Germany (Wellbrock et al. 2012) during the breeding season of 2010 [incubation period: May 28 (first clutch)–July 9 (last chick hatched)]. In Common Swifts, both partners incubate. Target birds were either fitted with passive transponders (Trovan™ ID-100; Euro I.D., Weilerswist, Germany) and could be identified remotely by nest antennae or they were colour-marked (Tipp-Ex™; Société BIC, Clichy, France) on the back of the head to distinguish breeding partners.

The Common Swift is host to an obligate ectoparasitic louse-fly (*Crataerina pallida,* Hippoboscidae; Glutz von Blotzheim 1980). Parasite load in individual nests was counted to investigate its effect on blood-sucking of the bug due to an eventually restless bird.

Ambient temperature was recorded inside the bridge chambers (accuracy ± 0.5 °C, measuring interval 10 min, EL-USB-2, Humidity & Temperature USB data logger; Lascar Electronics, Salisbury, UK). Additionally, we recorded temperature in the nest at 10-min intervals using temperature loggers (accuracy ± 0.5 and ± 1.0 °C, respectively; iButtons™: DS1922L, DS1921G; Maxim Integrated™; San Jose, CA, USA) to assess incubation intensity and the influence of temperature on the blood-sucking bugs. In statistical analyses, mean ambient or nest temperature over sampling time was introduced.

"Bug-eggs" were produced from artificial white eggs (solid plastic eggs, 25 × 19 (mm); JOKO-Systemtechnik, Syke-Ristedt, Germany) of similar size to Common Swift eggs (25.5 × 16.4 (mm); Glutz von Blotzheim 1980). They were cut into halves and hollowed. To hold the halves together, 3 mini-magnets (disc-shaped: 2 mm diameter, 1 mm height, adhesive force 130 g; magnets4you, Lohr/Main, Germany) were integrated in the cut surface on both sides so that a small gap (about 1 mm) remains when put together (Fig. 1). This circular opening and small holes (about 3 mm diameter) all along the gap gives the bug the opportunity to pierce its proboscis through the "bug-egg" to reach the bird. Incubating birds can follow their natural behaviour and roll their eggs in the nest, and bugs are able to reach the bird no matter in which position the egg lies. The application of mini-magnets instead of a screw-in thread leaves the entire space inside the egg for the bug.

We used laboratory-bred second instar larvae (L2) of *Dipetalogaster maxima* (Triatominae), a very aggressive blood-sucking bug (Stadler et al. 2011). Until application, L2 were kept in an incubator (FB 50-R; Jaeger Bruttechnik, Wächtersbach, Germany) at 27 °C and 60–70 % relative humidity.

We performed 27 trials on 21 birds (from 15 nests). To bleed an incubating bird, a starved bug was placed into the "bug-egg" and added to the clutch, or alternatively one egg was replaced for the time of the experiment (max. 1.5 h).

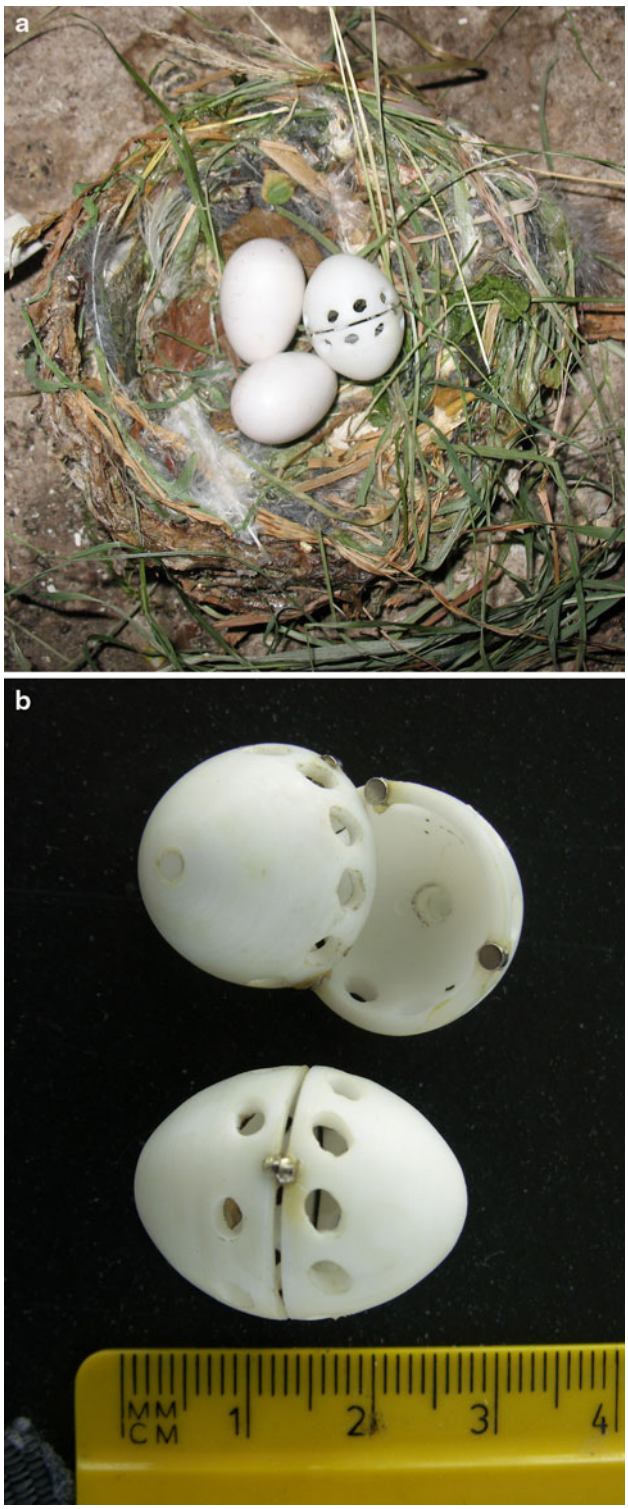

Fig. 1 **a** "Bug-egg" in the nest with two Common Swift (*Apus apus*) eggs. The different shape of the dummy egg had no influence on the incubating birds. **b** "Bug-eggs" are held together by mini-magnets

After about 1 h or if the target bird left the nest, we checked if bleeding via the bug was successful. In this case, we immediately punctured the bug's abdomen and carefully withdrew the blood with a syringe (Myjector^{TM}, U-40 Insulin, 29G; Terumo Europe, Leuven, Belgium), thereby avoiding haemolysis of red blood cells. This syringe with permanently attached needle limits dead space and is thus advantageous for small blood quantities. Blood was transferred into Eppendorf tubes and processed for further analysis.

In an exploratory approach to investigate possible relationships between successful bleeding via bug and environmental or time parameters, we performed Spearman Rank correlations using SPSS^{TM} 18.0. All tests were two-tailed, and the level of significance was set at $p \leq 0.05$.

Results and discussion

Blood sampling was successful in 11 out of 27 trials (40 %). Similar to the result reported by Becker et al. (2006), bugs were most successful in the early breeding season (Table 1). We suggest that incubation intensity might explain this correlation by affecting the probability of an uninterrupted blood meal. Birds breeding early in the season are usually more experienced breeders with a higher breeding success (Verhulst and Nilsson 2008). Higher incubation intensity in these birds is supported by the fact that temperature measured in the nest was higher when bleeding via the bug was successful (Table 1).

Furthermore, blood-sampling success via the bug was higher at noon than later in the day (Table 1). From this finding, the hypothesis could be derived that restlessness of incubating birds could increase and thereby interrupt the bug meal, due to shorter incubation intervals later in the day and before nightfall, when swifts are leaving the nest more frequently to forage.

Neither number of days between clutch initiation and sampling day or days until hatching of chicks nor ambient temperature was related to sampling success (Table 1).

Two-thirds of the nests were infested with parasitic louse-flies. We expected ectoparasites to decrease the possibility of a successful bug meal by causing restlessness in the birds. Correlations were not significant, however, whether we took the number of louse-flies per nest or the infestation as a binary variable (Table 1). Temperature in the nest was not significantly correlated with ectoparasitic infestation.

The amount of blood withdrawn from a second instar larvae of *Dipetalogaster maxima* in our study was on average about 60–80 µl and maximally 130 µl, thus about twice the amount reported by Becker et al. (2006) for L2. Stadler et al. (2011) reported 0.2 g for L2. This makes already L2 suitable to yield enough blood for a variety of analyses in the field of physiology or genetics. According to the quantity of blood required, smaller L1 or the later

Table 1 Spearman rank correlations to investigate the relationships between successful blood sampling of Common Swifts (*Apus apus*) (no = 0/yes = 1) and the listed parameters

Parameter	Minimum	Maximum	Mean (±SD)	*r*	*p*
Sampling time of day (hh:mm; $n = 27$)	11:43	19:14	16:00 (±00:26)	−0.436	0.023
Sampling day (Julian date; $n = 27$)	167	182	171.4 (±0.8)	−0.436	0.023
Days betw. laying and sampling day ($n = 27$)	6	21	17.4 (±0.8)	0.196	0.327
Experiment duration (min; $n = 27$)	12	85	48 (±3)	−0.057	0.781
Number of louse-flies in indiv. nests ($n = 24$)	0	>10		0.076	0.725
Louse-flies (no = 0/yes = 1; $n = 24$)	0	1		0.194	0.363
Temperature inside the bridge (°C; $n = 27$)	14.5	26.8	19.1 (±0.6)	−0.136	0.500
Temperature in the nest (°C; $n = 20$)	21.5	32.8	28.0 (±0.7)	0.542	0.014

larvae stage L3, which suck less or more blood, respectively, can be used (Stadler et al. 2011). Note, however, animal welfare guidelines suggest blood samples equivalent to no more than 1 % body mass to be within safe limits (Fair et al. 2010).

The use of blood-sucking bugs failed in a study on Great Tits (Bähnisch 2011). One reason assigned was the removal of the little cotton bags containing the bugs, which were used instead of eggs due to the small egg size. Our new design for "bug-eggs", using mini-magnets instead of a screw-in thread in the middle of the egg, provides more space for the bug, even in small eggs. The bug has the possibility to move in every direction to easily reach the bird, particularly with regard to the increasing size of the abdomen, which assumes the shape of an inflated balloon when successfully sucking blood. The fact that dummy eggs were more round and slightly bigger than Common Swift eggs (Fig. 1a) had no impact on the incubation behaviour of the birds. Although Swifts are able to eject eggs from the nest cup, which occurs in connection with damaged or unfertilised eggs (personal observations; Glutz von Blotzheim 1980), we never observed this behaviour or any reaction to the bug during our experiments.

Conclusion

We present a successful method using haematophagous bugs in dummy eggs for blood sampling in birds as small as the Common Swift or we suggest even smaller. This minimally-invasive method allows stress-free and repeated blood sampling during the sensitive time of incubation without handling the target birds. Thus, nest abandonment or trap shyness are avoided.

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