Rapid and simple method for isoelectric focusing of egg white reveals maternity in passerine clutches

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⮜ INTRODUCTION ⮞

Individual eggs/clutches (Figure 1) can be assigned to individual females by the "protein fingerprinting", which is based on isoelectric focusing (IEF) of albumen from birds' eggs (Anderson *et* Åhlund, 2001). Albumen is a strictly maternal origin secreted by specific cells in the magnum section of the oviduct. Protein composition of albumen is strictly inherited genetically so it can be used for genetic differentiation among females.

Isoelectric focusing may be defined as electrophoresis in a pH gradient formed by small amphoteric molecules called ampholytes (Anonymous, 1982). In contrast to zone electrophoresis, IEF is an end point method. This means, that the pattern – once the proteins have reached their isoelectric points (pI) – is stable without time limit. Because of the focusing effect sharps protein zones a high resolution is obtained.

Ultrathin polyacrylamide (PAA) gels were used for our protein separation. These gels are totally synthetic, they can be made to closely controlled specification and pore size can be varied (Anonymous, 1982). The gels are chemically inert and mechanically strong. The ultrathin gels can be more efficiently cooled, so loaded samples need short running time and higher resolution. Staining times are rapid, as the diffusion pathway is very short (Westermeier, 2005).

Figure 1. A Blackcap nest with a full clutch of eggs.

⮜ MATERIAL AND METHODS ⮞

The present research is based on the separation of egg white diluted in water (1:21) in horizontal PAA gels (T5C3 gel with SERVALYT pH 4-5 and glycerol, thickness 0,25 mm, 20 cm distance between electrodes). To protect the PAA gels against damage and torn, they were prepared between two glass plates (24 x 24 x 0,3 and 24 x 25 x 0,3 cm). The first plate (24 x 24 cm) was treated by 3-methacryloxypropyltrimethoxysilane to bind chemically PAA gel to the glass plate surface with covalent linkages.

Both glass plates were divided after the gel polymerization and gel-linked glass plate was set on the precooled IEF apparatus (Figure 2). The gels were pre-run (30 minutes, 3000 V, 150 mA, 20 W, 4°C). This step avoids artifacts, increases protein stability and decreases formation of straighter bands. Then 5 µl of each sample was loaded directly onto the gel surface through the cut outs in the plastic sample applicator mask (22 samples per one gel) and they were separated for 5 hours (3000 V, 150 mA, 20 W, 4°C). If the sample was unable to interpret undiluted egg white was rerun. The gels with separated samples were silver-stained (Smith *et al.*, 1995) and photographed (Figure 3). The total number of bands and presence or absence of each band was scored. The eggs with the same egg white band pattern were assigned to the same female.

Figure 2. Technical equipment for isoelectric focusing is comprised of (from left to right) Electrophoresis Constant Power Supply EPSC 3000/150, electrophoresis unit Flat Bed Apparatus FBE-3000, cooling unit Julabo F12. Instead of cooling unit is possible to use cold water from draw-off tap. The next necessary equipment for preparing of gel is fume-hood and a rotating shaker for silver-staining of the gel.

⮜ RESULTS AND DISCUSSION ⮞

The IEF method was optimized to be faster and cheaper. Our own gels were prepared instead of precast gels. An electrophoresis time (5 instead of 10 hours) and a much faster silver-staining method (approximately 1 instead of more than 12 hours) were used than were used by Anderson *et* Åhlund (2001). The method is applicable only to freshly laid eggs; samples from partly incubated eggs were unable to score. The presence of yolk or embryo's blood in egg white did not cause any problems in electrophoresis or scoring. When individual female identification through yolk protein electrophoresis (Cariello *et al.,* 2002) and egg white isoelectric focusing are compared, the IEF egg white method does not destruct the eggs and only slightly reduces egg hatchability.

⮜ ADVANTAGES OF PROTEIN FINGERPRINTING ⮞

- Determination of maternity, not confounded by paternal identity.
- Reproducible female band pattern.
- Albumen sampling which is nondestructive and represents little extra effort to the standard field protocol of nesting studies.

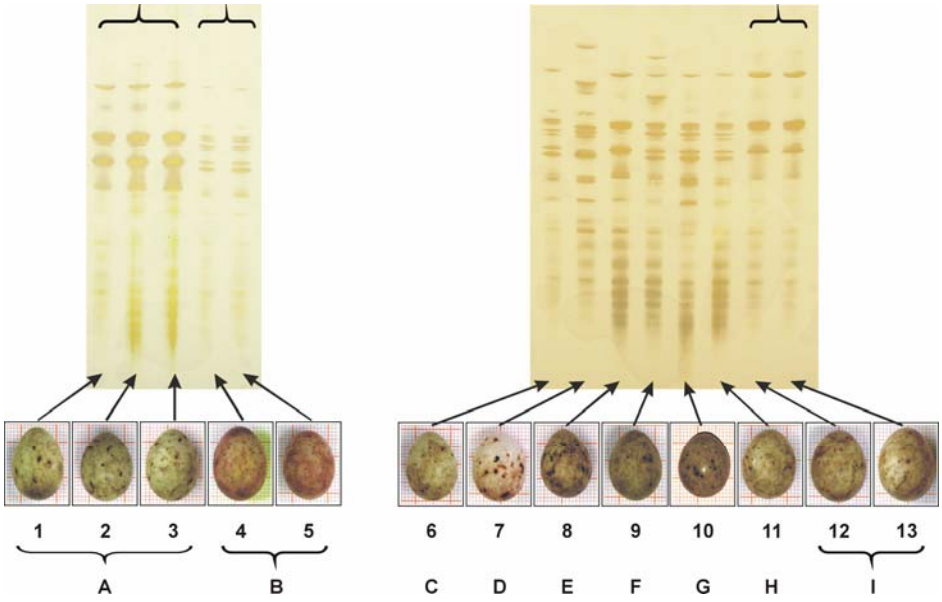

Figure 3. An example of Blackcap female discrimination by means of IEF. Thirteen nests (1-13) from two study plots could be assigned to nine individual females (A-I). Albumen was sampled from one egg per nest.

⮜ ADVANTAGES OF MODIFIED PROTEIN FINGERPRINTING ⮞

- Inexpensive gel and staining system.
- Short running time of electrophoresis.
- Fast staining.
- Clear background.
- High resolution of proteins.

⮜ PRACTICAL APPLICATION ⮞

The Blackcap (*Sylvia atricapilla*) is an open-nesting songbird characterised by stable or increasing population and, simultaneously, by low nest success. The low nest success is likely balanced by frequent renesting. Yet, the number of nesting attempts per season per female is difficult to measure in field, as it requires catching and tracking of individually marked females. We tried to overcome this problem by assigning clutches to individual females by means of IEF. To date, about 120 samples of Blackcap eggs from one breeding season have been analyzed. Preliminary results suggests applicability of the method to this species (Figure 3).

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