

Assignment of passerine clutches to individual females by albumen protein electrophoresis



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INTRODUCTION

Individual eggs/clutches can be assigned to individual females by the "protein fingerprinting", which is a method based on isoelectric focusing (IEF) of egg albumen (Anderson and Ahlund 2001). Albumen is a maternal origin secreted by the magnum section of the oviduct. Protein composition of albumen is inherited and can be used for genetic differentiation among females (eggs), not confounded by paternal identity.

AIMS

Our goal was to adapt this method to small passerines, which would make possible to assign discovered clutches to individual females, without need of catching, marking and tracking of individuals.

LABORATORY METHODS

This study is based on 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 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. The gels were pre-run (30 min, 3000 V, 150 mA, 20 W, 4°C) to avoid artefacts, 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 an undiluted egg white was rerun. The gels with separated samples were either silver-stained or stained in PageBlue™ Protein Staining Solution (Fermentas) for 30 - 60 minutes, destained in water (1 hour to overnight), air dried and photographed.

RESULTS

The IEF method was optimized to be faster and cheaper.

ADVANTAGES OF THE MODIFIED IEF METHOD

- Inexpensive gel and staining system (own gels were prepared instead of precast gels).
- Short running time of electrophoresis (5 instead of 10 hours).
- Fast staining (approximately 1hour instead of more than 12 hours).
- Clear background.
- High resolution.

REFERENCES

Anderson, M. and Ahlund, M. (2001). Protein fingerprinting: A new technique reveals extensive conspecific brood parasitism. *Ecology* 82:1433-1442.

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 the 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.

METHODS

We searched for nests with a constant effort from mid April until mid July. About 10 µl (< 1% of egg volume) of albumen was taken from fresh eggs (1-2 per clutch) by an insulin syringe and eggshell was then sealed with nail varnish. Hatchability was not significantly reduced below that of control eggs.

IEF revealed clear band patterns, which were reproducible within females (clutches) but variable among females.

INTERPRETATION OF RESULTS

High nest density, high nest losses and spatio-temporal distribution of nests suggested frequent renesting by a low number of females. Yet, IEF revealed contrasting pattern of renesting on the two study plots.

STUDY PLOT A

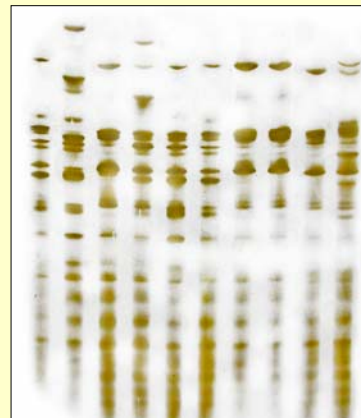
The 10 sampled nests (●) could be assigned to nine females. Successive nesting attempts located close to each other do not represent replacement clutches of the same females. This suggests a high turnover of breeding females on this plot during the breeding season.

STUDY PLOT B

The six sampled nests (●) could be assigned to two females. Each female had at least three nesting attempts, one of which was successful. The successive nests of the same female were spatially clustered.

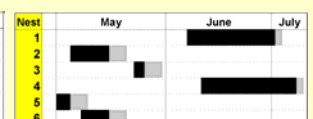
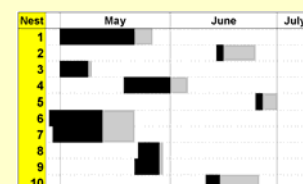
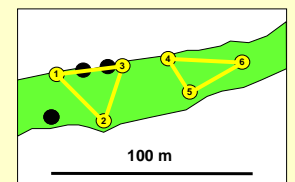
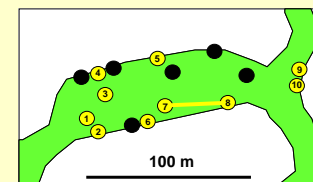
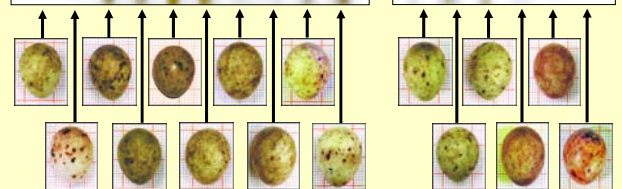
STUDY PLOT A

1 2 3 4 5 6 7 8 9 10



STUDY PLOT B

1 2 3 4 5 6



IMPLICATIONS FOR FIELD STUDIES

ADVANTAGES

- Sampling of albumen is non-destructive and represents little extra effort to the standard field protocol of nesting studies.
- Combination of cues (albumen band pattern, eggshell coloration, timing) permits clutch/female discrimination.

LIMITATIONS

- The IEF method is applicable only to freshly laid eggs. In spite of intensive nest searching, some nests are usually discovered already depredated or too late in the nesting cycle (●).

