

# Microsatellite analysis of *Maculinea alcon* (Lepidoptera, Lycaenidae) eggs and legs using a new method for DNA extraction from tiny amounts of tissue

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museum

naturalis



## Introduction

In The Netherlands, two forms of *Maculinea alcon* (Lycaenidae) were described as separate subspecies: *alcon arenaria* occurring in the dunes near Wassenaar (Lempke, 1942) until ca. 1970 and *alcon ericae* occurring in heathlands throughout The Netherlands (Lempke, 1956). Lempke based his descriptions on characters deviating from the type such as colour (darker), size (smaller), host plant (*Gentiana cruciata* instead of *G. pneumonanthe*) and eyespots (lower number and size). In the rest of the distribution area, several other forms were named: *alcon alcon* (Austria), *alcon arirang* (Korea), *alcon kondakovi* (China), *alcon monticola* (Georgia) and *alcon rebeli* (Austria). Whether or not these forms are sufficiently different from each other is questionable as a darker colour, smaller size and deviating amount of eyespots have been described for multiple Lepidoptera as an effect of temperature (Elfferich, 1966; True, 2003; Davis et al. 2005). Also, at least one population of *M. alcon alcon* uses two different species of Gentianaceae as host plant (Sielezniew and Stankiewicz, 2004).

## Goal

Microsatellites were generated from freshly collected eggs (Fig. 1) and museum specimen legs (Fig. 2) to shed more light on the genetic difference of geographically separated populations of *M. alcon* using different host plants and occurring in different environments.

## Methods

DNA was extracted in the special Ancient DNA facility at Leiden (Fig. 3) from freshly collected eggs and specimens preserved in Dutch museums (butterflies and herbaria (eggs accidentally collected on host plants - Fig. 4). Standard protocols often failed to amplify DNA from these specimens due to low amounts of tissue and degradation over time. A new protocol was therefore developed for DNA extraction from single eggs and legs using DNARELEASE (NIPPON Genetics).

Microsatellites Malc169 and Macu11 developed by Zeisset et al. (2005) were amplified using *Phire* Hot Start Polymerase (Finnzymes). After amplification, PCR fragments were tailed, processed using the QiaGen MinElute PCR Purification Kit and cloned with the TOPO TA Cloning Kit (Invitrogen) to detect possible effects of denaturation and assess allelic diversity. Sequencing was performed by Macrogen on an ABI 3730xl (Applied Biosystems).



Fig. 1. *M. alcon* depositing eggs on *Gentiana pneumonanthe* (left: floral buds; right: opening flowers). Photographs by Gerard Oostermeijer

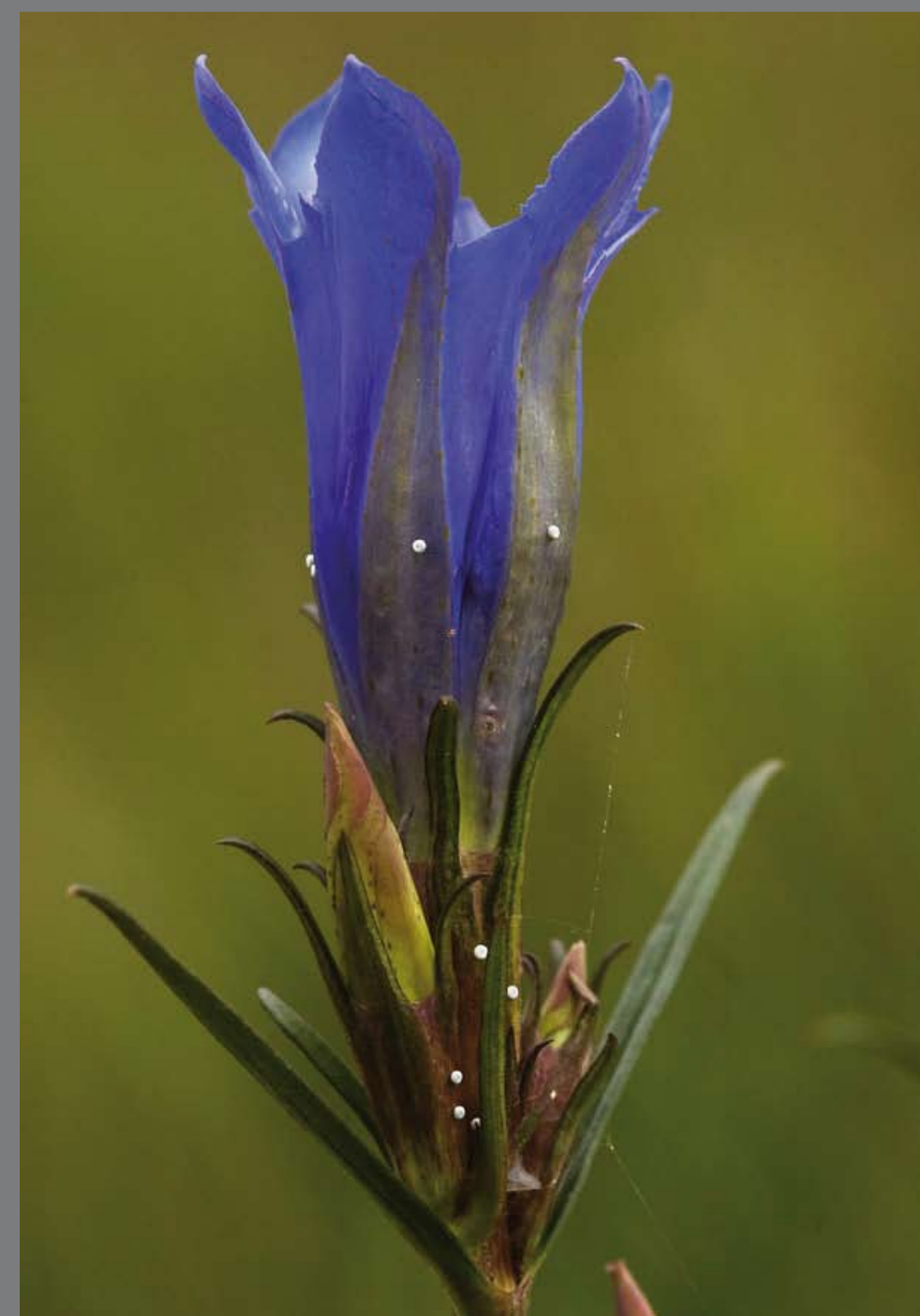


Fig. 4. Eggs of *M. alcon* analyzed on herbarium specimen of *Gentiana cruciata*. Photographs by Ben Kieft



Table 1. Allelic diversity of *M. alcon* over the past 70 years (- not analyzed)

Locality sampled	Year	Nr. of alleles		Alleged subspecies
		Malc169	Macu11	
Meijendel (The Netherlands)	1937	3	4	<i>Maculinea alcon arenaria</i>
Donderden (The Netherlands)	1939	-	1	<i>Maculinea alcon ericae</i>
Panticosa (Spain)	1961	3	-	<i>Maculinea alcon monticola</i>
Nunspeet (The Netherlands)	1966	-	4	<i>Maculinea alcon ericae</i>
Staverden (The Netherlands)	1967	2	-	<i>Maculinea alcon ericae</i>
Col de la Rama (France)	1978	3	-	<i>Maculinea alcon rebeli</i>
Erzurum (Turkey)	1989	3	-	<i>Maculinea alcon monticola</i>
Sarikamis (Turkey)	1990	3	2	<i>Maculinea alcon monticola</i>
Aosta Ponder (Italy)	1998	4	-	<i>Maculinea alcon rebeli</i>
Namos (Slovenia)	1999	5	2	<i>Maculinea alcon rebeli</i>
Gyttegard (Denmark)	2005	4	-	<i>Maculinea alcon alcon</i>
Buuserzand (The Netherlands)	2008	3	-	<i>Maculinea alcon ericae</i>
Dwingeloo (The Netherlands)	2008	3	2	<i>Maculinea alcon ericae</i>
Kootwijk (The Netherlands)	2008	1	-	<i>Maculinea alcon ericae</i>

## Results

Allelic diversity of *M. alcon* could be monitored for a period of 70 years (Table 1). The forms of *M. alcon* analyzed could not be distinguished using the microsatellites generated (Fig. 5). Als et al. (2004) and Berezki et al. (2005) also found an absence of genetic differentiation in Alcon Blues using *CO1*, *COII*, *EF1* sequences and allozymes, respectively.

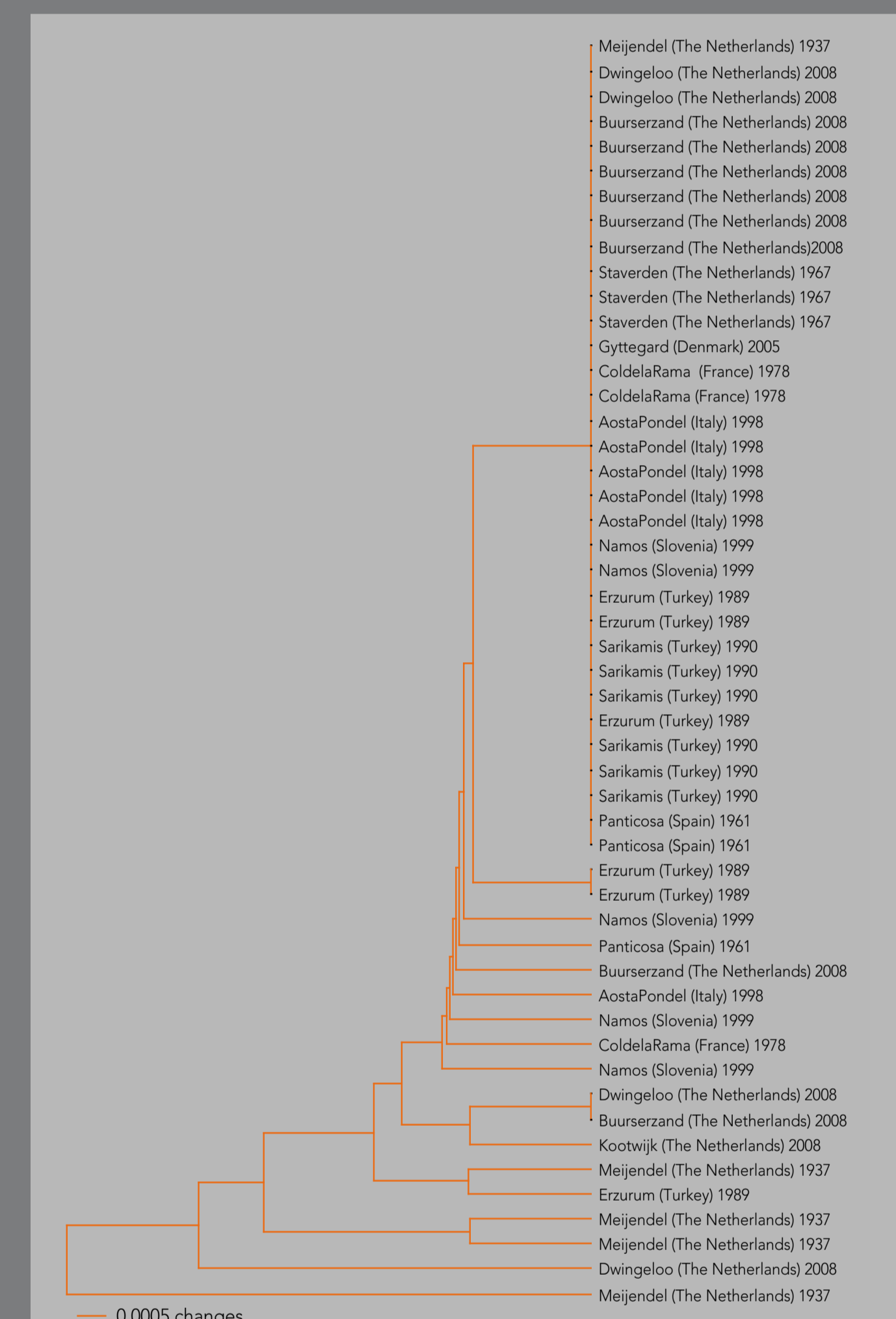


Fig. 5. UPGMA phenogram of Malc169 data generated in this study

## Conclusion

The fact that no distinct genetic differences were found, in combination with the very small morphological differences and the absence of any correlation in sequence variation with either host plant species, altitude or alleged subspecies indicates that *M. alcon* is still a single species in which geographical isolation - at least in the West Palearctic - has not yet resulted in genetic differentiation at all loci investigated so far.

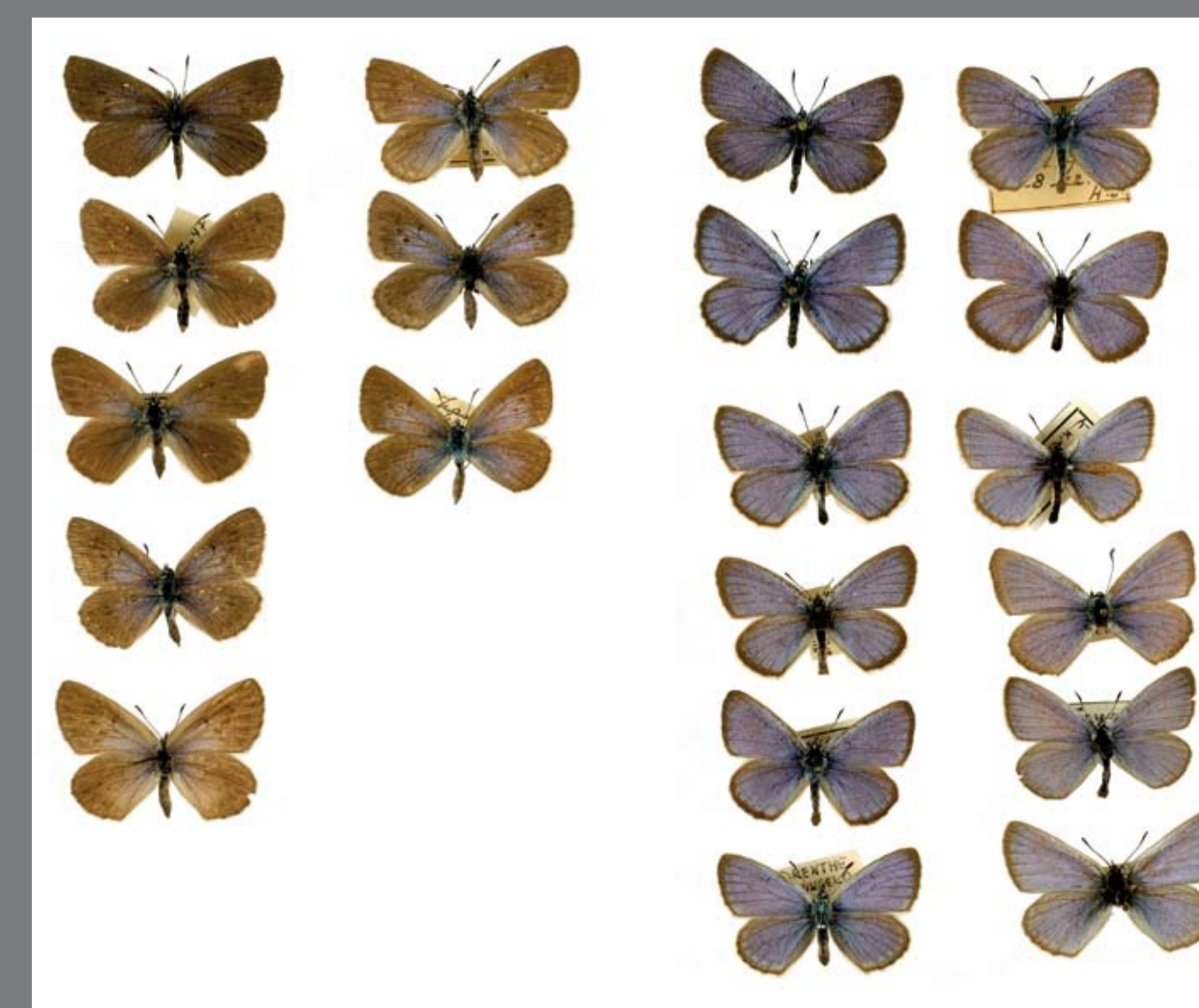


Fig. 2. Part of the *M. alcon* museum butterfly specimens analyzed. Photographs by Ben Kieft



Fig. 3. Ancient DNA facility at Leiden. Photograph by Barbara Gravendeel