

Genetic structure of natterjack toad (*Epidalea calamita*) populations in Flanders, Belgium, and its implications for conservation

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Abstract. Unique evolutionary potential could be lost when a population goes extinct or when individuals are translocated to other existing populations. Therefore, in order to identify priorities and to predict the efficiency and consequences of conservation actions, information is needed on the genetic structure of natural populations. In the urbanized and diverse landscapes of Flanders, Belgium, natterjack toad (*Epidalea calamita*) populations have been declining over the last decades. Therefore, this species is subjected to a wide range of different types of conservation measures (e.g. habitat management, corridor development, translocations). However, more information is needed on its genetic population structure. In this study, we sampled egg clutches from six populations and studied their genetic structure with six microsatellite markers. In total, 184 samples from 99 different egg strings were genotyped. Observed heterozygosity was generally high, even for the small and isolated populations (overall mean $H_O = 0.43$). The weak clustering by the Bayesian analyses (STRUCTURE, Adegnet and BAPS) does not allow us to make strong conclusions on the population structure. However, the significant Φ_{ST} values between the populations underline the importance of genetic information when conservation priorities are discussed. Unique evolutionary potential could be lost when one or more natterjack toad populations would go extinct, and translocation of individuals to other existing populations should be considered with caution.

Keywords: conservation, *Epidalea calamita*, genetic differentiation, microsatellites, population genetics.

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Supplementary material

Table S1. Sample locations, sample year, number of egg string and total sample size of natterjack toad populations (*Epidalea calamita*) in Flanders, Belgium, for analyses of genetic structure.

Population (subpopulation)	Sample year	<i>N</i> egg strings	<i>N</i> samples
Tielrode	2008	6	21
Liereman (subpopulation 1)	2008	18	21
Liereman (subpopulation 2)	2008	3	9
Kalmthout (subpopulation 1)	2008	2	6
Kalmthout (subpopulation 2)	2008	10	13
Kalmthout (subpopulation 3)	2008	2	8
Linkeroever (subpopulation 1)	2008	13	19
Linkeroever (subpopulation 2)	2008	7	13
Linkeroever (subpopulation 3)	2008	17	23
Den Diel	2010	10	10
Kikbeekbron	2010	11	41
Total number		99	184

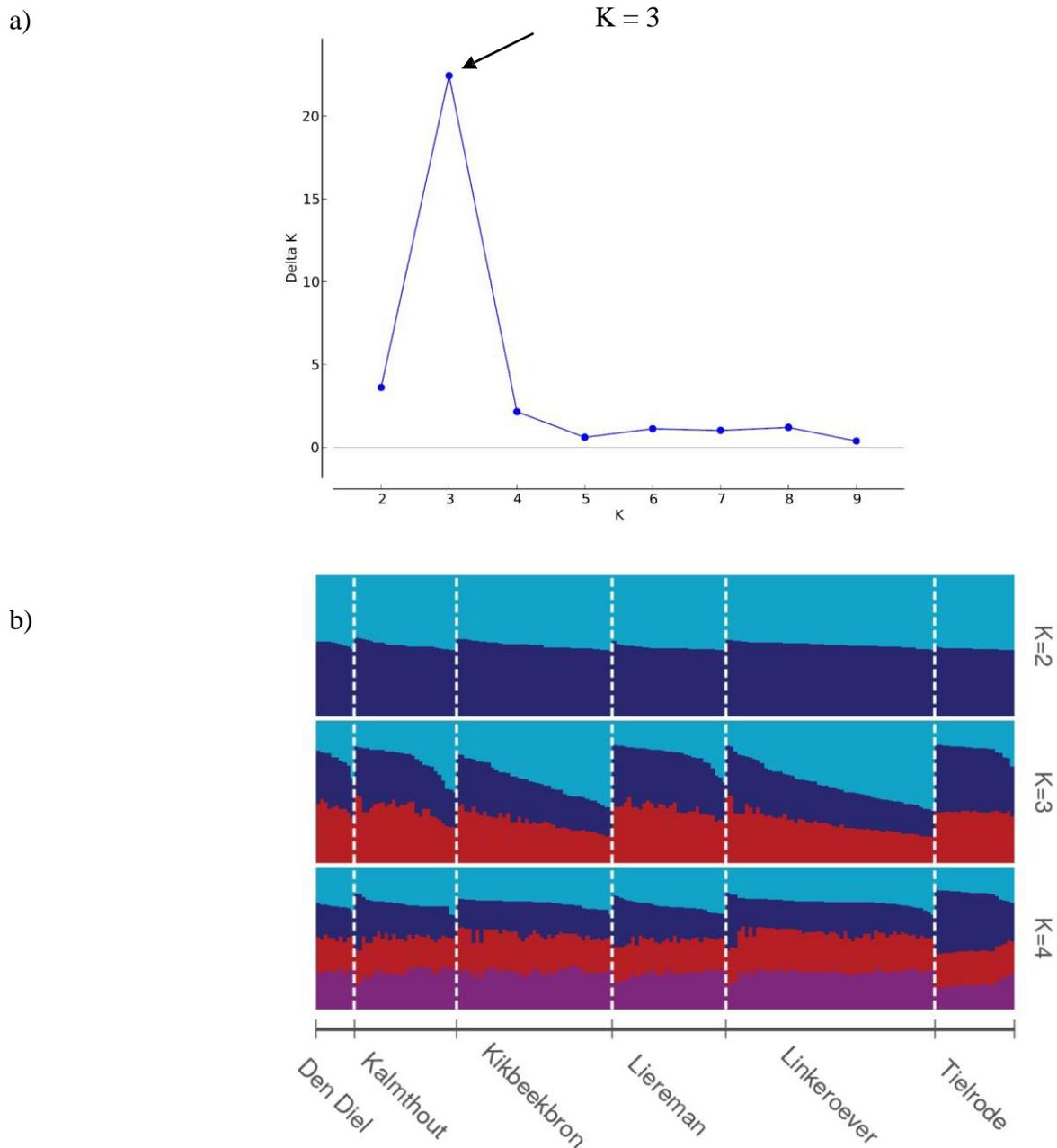


Figure S1. The result of STRUCTURE for the natterjack toad study populations in Flanders based on six polymorphic microsatellites (a) for Delta K, the second order rate of change in the likelihood, at each K and (b) the population structure as estimated with a Bayesian approach for K = 2, K = 3 and K = 4. Each bar represents an individual toad and individuals are clustered per population (labels above each cluster). For graphics, POPHELPER Structure Web App (version 1.0.10) is used.

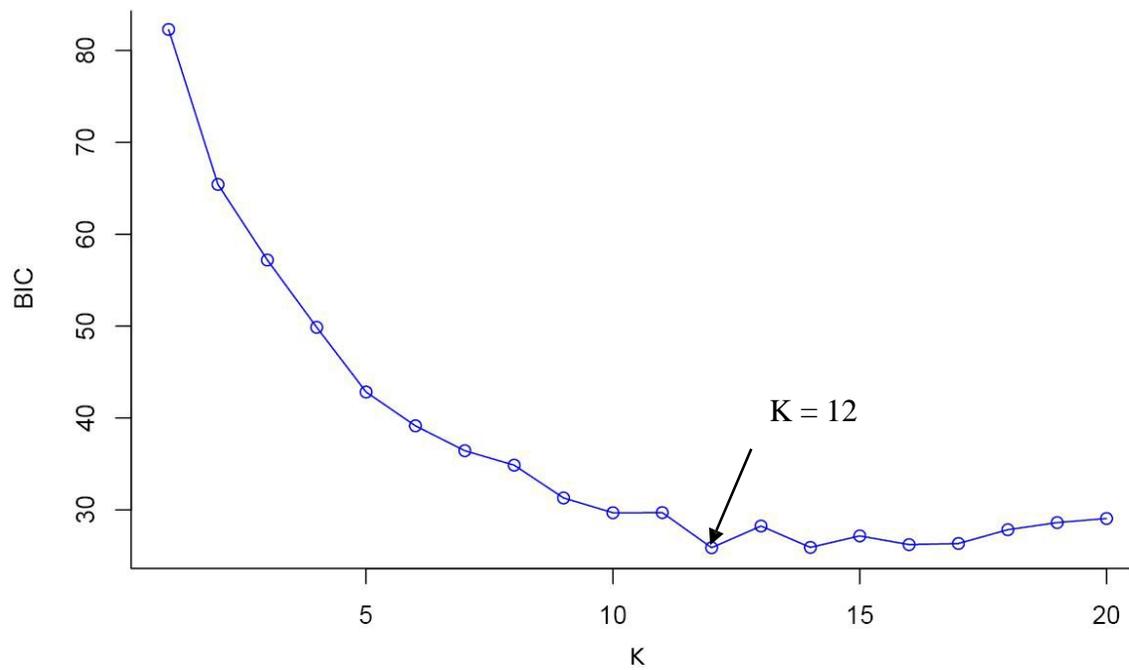


Figure S2. The result of Adegenet for the natterjack toad study populations in Flanders based on six polymorphic microsatellites: changes in mean Bayesian Information Criterion (BIC) values in successive K-means clustering.