

# No evidence for effects of infection with the amphibian chytrid fungus on populations of yellow-bellied toads

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## Genetic data

### Laboratory work and data analysis

Total DNA was isolated from the swabs using the DNeasy Blood and Tissue Kit (Qiagen) and following the manufacturer's protocol. Extracted DNA was stored at -20°C until further processing. For population genetic analysis, ten autosomal microsatellite loci were analyzed using primers published by Stuckas & Tiedemann (2006) and Hauswaldt et al. (2007) for *Bombina orientalis*. The same ten markers (9H, F22, 12F, B14, B13, 5F, 1A, 10F, F2, 8A) were chosen as in Weihmann et al. (2009). Except for F2 the same primers and two more from Nürnberger et al. (2003) were also used by Cornetti (2013).

PCR amplification was performed in a 10 µl reaction volume containing 10-100 ng of total genomic DNA, 0.4 µM each of forward and reverse primer, 0.2 mM of each dNTP, 2.5 mM MgCl<sub>2</sub>, 0.02 U/µl of DNA polymerase (Phusion High Fidelity DNA polymerase F-530, Thermo Scientific) and 1 x reaction buffer (F-518 Phusion HF reaction buffer, Thermo Scientific). After an initial denaturation step (94 °C, 3 min), 35 cycles were performed at 94 °C for 30 s, the locus specific annealing temperature for 30 s, and 72 °C for 30 s. A final elongation step at 72 °C for 10 min followed. Optimal annealing temperatures as determined in a gradient PCR were 56.2 °C for 9H, F22 and 12F, 62.6 °C for B14, 53.0 for B13, 58.9 for 5F, 60.9 for 1A, 60.3 for F2, and 63.5 for 10F and 8A. The amplified products were genotyped on an ABI Prism 310 Genetic Analyzer (Applied Biosystems). Differential labelling of the primers allowed to analyze three loci plus a size standard (350 TAMRA Size Standard; Applied Biosystems, GeneScan) in each run. Data were collected with the 310 Data Collection Software vers. 3.1.0., and allele lengths were determined with the help of GeneScan Analyzer 3.7 and ABI Prism Genotyper 2.5.

All loci have been tested for two-locus linkage disequilibrium (LD). For five locus pairs indication of a possible LD was found in two or three out of 14 populations tested. Because the few significant results were not consistent over populations or loci, we assumed that genotypes at one locus were

independent of those at the other loci (see also Weihmann et al. 2009, Cornetti 2013). All loci were further tested for the presence of null alleles, allelic drop-out and scoring errors using MicroChecker 2.2.3 (Van Oosterhout et al. 2004).

Population genetic analysis of microsatellite data was performed using various software packages. To examine within-population genetic diversity, the mean number of alleles per locus and allelic richness (a measure of the number of alleles standardized for sample size, in our study five; El Mousadik & Petit 1996) were calculated with the program Fstat 2.9.3.2 (Goudet 2002). Mean observed and expected heterozygosities ( $H_o$  and  $H_e$ ; Nei 1987) and the inbreeding coefficient (FIS) were obtained with Arlequin 3.5.1.2 (Excoffier & Lischer 2010).

**Table S1. Characteristics of the *Bombina variegata* populations in Northern Hesse, which were considered in the second Generalized Linear Model due to available genetic data.**

Predominant land use is stated within the location's name; otherwise, the locations were situated in the restored floodplain of the Fulda.

Location	<i>Bd</i> Prevalence (%)	Forest cover (%) in terrestrial habitats (300 m radius)	Elevation (m above sea level)	Distance (m) to permanent waters	Expected heterozygosity
Former military training area 'Treysa'	38.46	46.57	242	129	0.46
Military training area 'Homberg'	28.57	13.48	260	833	0.37
'Ellenberg'	30.30	0	196	139	0.41
'Melsungen'	5.71	33.4	220.2	244	0.4
'Bad Soden Allendorf Nord'	27.27	39.39	159	315	0.5
'Trimberg'	0.00	31.64	247	159	0.51
'Breitau'	40.00	37.20	279	351	0.44
Gravel pit 'Obersuhl'	9.38	0	211	114	0.51
'Obere Aue Heringen'	4.76	0	216	0	0.56
'Hünfeld Rückers'	6.25	47.94	270	24	0.45
Disposal site 'Schrimpf'	10.81	34.05	427	0	0.4
'Blankenheim'	66.67	46.49	202	192	0.52
'Bebra gravel pit'	28.89	16.71	189	0	0.5
'Baumbach marl pit / Hergershausen stone pit'	41.18	5.00	212	502	0.59

## LITERATURE

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