# Supplementary Material

## Supplementary Methods 1: Species identification

We ensured that only eggs and adults of *Radix balthica* were included in this study in two ways. First, different *Radix* species can be distinguished by comparing sequence variation in the mitochondrial cytochrome oxidase subunit I (COI) gene (Pfenninger *et al.*, 2006; Pfenninger *et al.*, 2011; Lawton *et al.*, 2015). We therefore sequenced a 600 bp segment of the COI gene of 86 adult snails collected from the Uerikon population at peak breeding season in the year before sampling of egg clutches took place (sampling day 24.04.2013). We extracted DNA from head tissue using the NucleoSpin Plant II Kit from Macherey-Nagel, followed by two nested PCRs. The total reaction volumes of 30 µl contained 15 µl GoTaq Colorless Master Mix (Promega), 12.4 µl water, 0.3 µl each of forward primer (name: LCO1490, sequence: 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and reverse primer (name: HCO2198, sequence: 5'-TAA ACT TCA GGG TGA CCA AAA AAT-3'), and 2 µl extracted DNA. Both primers had concentrations of 0.125 µM. The second PCR was run with 2 µl PCR product that was diluted 1:200 with water. Both reactions were exposed to the following temperature regime: an initialization for 2 min at 95°C, followed by 35 cycles of denaturation at 90°C for 30 s, annealing at 48°C for 1 min, and elongation at 72°C for 1 min, followed by a final elongation at 72°C for 10 min. PCR products were visualized on an agarose gel and subsequently purified using the Wizard SV Gel and PCR Clean-Up System (Promega). Gene products were sequenced in one direction by Microsynth and were then aligned with BioEdit version 7.2.2, jointly with 164 sequences kindly provided for comparison by Markus Pfenninger (Pfenninger *et al.*, 2006). While a few of these sequences pertain to outgroup taxa, most belong to *Radix* snails collected throughout Europe and represent

much of the molecular variation present within the genus. A phylogenetic tree was then constructed using maximum-likelihood methods implemented in MEGA 5.2 (Tamura *et al.*, 2011) using a T92+G+I substitution model. For the purpose of this study, all individuals that grouped with sequences belonging to the Molecularly defined Operational Taxonomic Unit 2 (MOTU2, for details see (Pfenninger *et al.*, 2006; Pfenninger *et al.*, 2011)), were considered to be *R. balthica*. Based on this comparison, 85 out of the 86 snails collected in 2013 (98.8%) were identified as *R. balthica*, confirming that adult snails collected in spring from the Uerikon population are very likely to belong to this species. All sequences have been uploaded to GenBank (Accession No. KX832428-KX832513).

Second, we tested the microsatellite markers used in this study on ten snails each that had been identified as *R. ampla*, *R. auricularia* and *R. lagotis* based on previous COI sequencing. In addition, we tested them on five individuals of *Lymnaea stagnalis*, which belong to the same family as *Radix* snails (Lymnaeidae), and on five individuals of *Physa acuta*, a more distantly related species of freshwater snails. *L. stagnalis* individuals were taken from our laboratory stock population, and *P. acuta* individuals from a nearby pond upon inspection under the binocular. All five species showed characteristic and consistent patterns of locus-specific amplification failure, with no PCR products being visible at two loci in *R. ampla*, at three loci in *R. lagotis*, at four loci in *L. stagnalis*, at five loci in *P. acuta* and at seven loci in *R. auricularia*. In addition, all species had fixed alleles at some of the loci that did amplify. With this information we screened all embryo and adult genotypes for individuals that were not *R. balthica* and aimed to exclude them from all analyses, yet only found *R. balthica*, again corroborating the purity of the Uerikon population.

## Supplementary Methods 2: Microsatellite genotyping

From thawed egg clutches assigned for genotyping, a random sample of both developed and undeveloped embryos was placed in individual tubes of a 96-well plate. Adult snails were thawed and a small piece of head tissue was taken from each snail, dried on a paper towel and placed in individual tubes of 8-tube strips. Forceps and scalpels were washed with bleach and wiped on a paper towel after handling each sample. DNA was extracted using the HotSHOT method (Montero-Pau *et al.*, 2008). Each embryo and adult snail was genotyped for ten microsatellite markers (GenBank Accession No. KX830983-KX830992) that had been newly developed for this population and assembled into two multiplex Polymerase Chain Reactions (PCRs) by Ecogenics (Zurich, Switzerland), each amplifying five loci. Details on these loci can be found in Table 4. Both multiplex reactions had a total volume of 12 µl, consisting of 6 µl HotStarTaq Master Mix (Qiagen, Hilden, Germany), 2.08 µl water, 1.92 µl of a mixture of the primers for five loci, and 2 µl extracted DNA. In reactions using adult snail DNA, 0.6 µl of the 2.08 µl water was replaced by bovine serum albumin (10 µg per µl) to overcome PCR inhibition caused by snail mucus. In multiplex reaction 1, the 1.92 µl mixture of primers consisted of 0.48 µl of primers Rb_1 (F + R) and Rb_2, 0.36 µl of primers Rb_3 and Rb_5, and 0.24 µl of primer Rb_4 (all with concentrations of 10 µM). In multiplex reaction 2, the 1.92 µl mixture of primers consisted of 0.36 µl of primers Rb_6 (F + R), Rb_7, Rb_8 and Rb_10, and 0.48 µl of primer Rb_9 (all with concentrations of 10 µM). All reactions had the following protocol: an initialization for 15 min at 95°C, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 1.5 min, and elongation at 72°C for 1 min, followed by a final elongation at 72°C for 30 min. DNA fragments were analyzed for length polymorphisms on a 3730xl DNA Analyzer (Applied Biosystems). With each 96-well plate we ran one extraction control (no tissue added) and one PCR control (no extracted DNA added). They were all negative.

Microsatellite peaks were called using STRand (Toonen and Hughes, 2001) and binned using a custom-built R routine, inspired by the R package MsatAllele (Alberto, 2009), that ensured identical binning rules for all samples. To further improve scoring consistency, all scored microsatellite peaks were double-checked by the same person (AB). Adult snails whose genotype could not be determined with certainty at eight or more loci (25/317 snails, i.e. 7.9%) were extracted and genotyped again. Embryos with ambiguous genotypes at more than two loci (25/1059 embryos, i.e. 2.4%) were replaced by other embryos from the same clutch, as DNA cannot be extracted twice from the same embryo. This provided us with 298/316 (94.3%) adults and 1033/1034 (99.9%) embryos with complete genotypes at ≥8 loci, or 304/316 (96.2%) adults and 1032/1034 (99.8%) embryos with complete genotypes at ≥7 loci (without locus Rb_3). Note that one adult was excluded from downstream analyses because it belonged to another *Radix* species (see Supplementary Methods 1).

We compared the replicate genotypes of the 25 adults that were re-genotyped due to genotyping difficulties, plus of 29 unproblematic snails as a control, leaving us with 54 replicated samples (17.1% of adult genotypes), which is more than the recommended 5-10% of samples (Bonin *et al.*, 2004; Pompanon *et al.*, 2005). All replicate genotypes were obtained from newly extracted DNA in order to quantify the overall rate of genotyping errors resulting from all potential sources of error, including errors during DNA extraction. This comparison revealed 51/110 (46.4%) locus-specific mismatches between replicate genotypes in difficult snails, but only 10/236 (4.2%) in control snails. This difference is highly significant (two-sample test for equality of proportions with continuity correction: $\chi_1^2$=88.8, $P$<0.0001) and suggests a high genotype quality for samples amenable to genotyping (92.1% of adults and 97.6% of embryos). Furthermore, most mismatches in both difficult (66.7%) and control snails (80.0%) were caused by a partial or complete

amplification failure of one of the two alleles in heterozygotes (i.e. a suspected null allele) (Dabrowski *et al.*, 2015), and could thus be resolved.

## Supplementary Table 1

**Selfing rates with associated 95% confidence intervals in a natural population of the freshwater snail *R. balthica*, estimated using one direct and three indirect methods and separately assessed on six sampling days spanning three years.** Selfing rates were computed using nine highly polymorphic microsatellite loci that are largely free from null alleles, and also including an additional locus (locus Rb_3) with a high frequency of null alleles. Selfing rates were obtained directly from field-collected egg clutches (progeny-array method yielding "primary" selfing rates, $s$(PA); 15 egg clutches with a total of 259 ± 34 (mean ± SE) embryos for each of four sampling days) and indirectly from field-collected adult snails (population-level methods; 52.7 ± 8.1 adults for each of six sampling days). Population-level selfing rates were estimated in three ways: from the multilocus inbreeding coefficient $F_{IS}$ (Weir and Cockerham, 1984) using the relationship $s=2F_{IS}/(1+F_{IS})$ (Wright, 1969), from two-locus heterozygosity disequilibrium values ($g_2$) using RMES (David *et al.*, 2007), and by maximizing the log-likelihood of the multilocus heterozygosity structure of the sample (ML), also using RMES. Numbers in bold are significantly different from zero.

| Sampling day | $s$(PA) | CI95 | $s(F_{IS})$ | CI95 | $s(g_2)$ | CI95 | $s$(ML) | CI95 |
|---|---|---|---|---|---|---|---|---|
| Without locus Rb_3 | | | | | | | | |
| 24.04.2013 | - | - | **0.120** | [0.107, 0.134] | 0.032 | [0.000, 0.101] | 0.000 | [0.000, 0.064] |
| 06.03.2014 | 0.001 | [-0.001, 0.004] | **0.197** | [0.180, 0.214] | 0.049 | [0.000, 0.146] | 0.000 | [0.000, 0.092] |
| 22.03.2014 | 0.000 | [0.000, 0.000] | **0.131** | [0.114, 0.148] | 0.000 | [0.000, 0.073] | **0.069** | [0.008, 0.129] |
| 11.04.2014 | 0.000 | [0.000, 0.000] | **0.138** | [0.114, 0.162] | 0.000 | [0.000, 0.033] | 0.000 | [0.000, 0.081] |
| 23.05.2014 | 0.002 | [-0.001, 0.006] | **0.125** | [0.086, 0.164] | 0.095 | [0.000, 0.227] | 0.035 | [0.000, 0.113] |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 13.04.2015 | - | - | **0.074** | [0.050, 0.099] | 0.000 | [0.000, 0.054] | 0.000 | [0.000, 0.047] |
| With locus Rb_3 | | | | | | | | |
| 24.04.2013 | - | - | **0.226** | [0.202, 0.249] | 0.039 | [0.000, 0.109] | 0.000 | [0.000, 0.066] |
| 06.03.2014 | 0.003 | [-0.001, 0.006] | **0.290** | [0.262, 0.319] | 0.026 | [0.000, 0.117] | 0.000 | [0.000, 0.094] |
| 22.03.2014 | 0.000 | [0.000, 0.000] | **0.231** | [0.203, 0.258] | 0.020 | [0.000, 0.101] | **0.067** | [0.006, 0.129] |
| 11.04.2014 | 0.000 | [0.000, 0.001] | **0.227** | [0.197, 0.257] | 0.000 | [0.000, 0.048] | 0.014 | [0.000, 0.095] |
| 23.05.2014 | 0.002 | [-0.002, 0.006] | **0.216** | [0.169, 0.263] | 0.035 | [0.000, 0.142] | 0.000 | [0.000, 0.079] |
| 13.04.2015 | - | - | **0.215** | [0.175, 0.255] | 0.000 | [0.000, 0.060] | 0.000 | [0.000, 0.048] |

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