Online supplementary material

Hybridization as a threat in climate relict *Nuphar pumila* (Nymphaeaceae)

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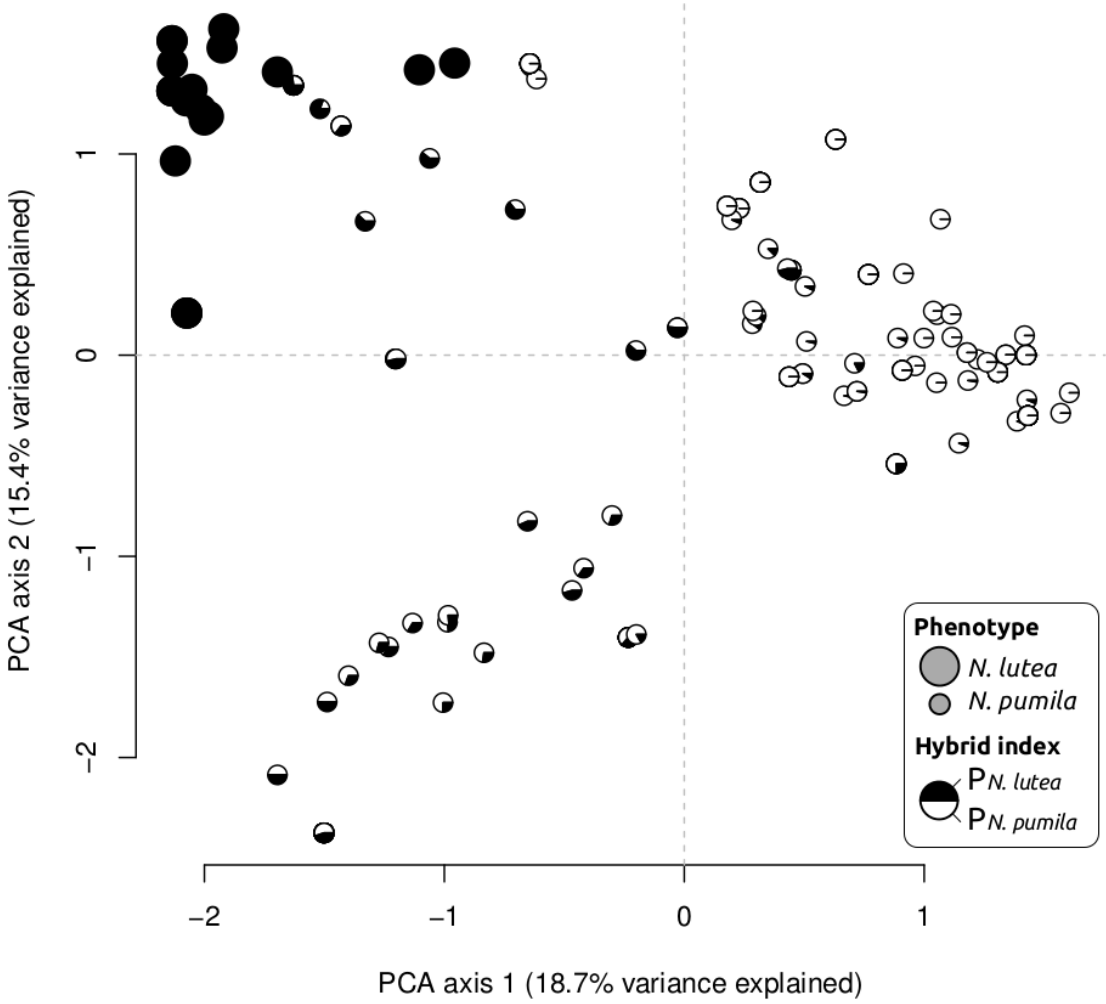
Online resource 1. Primers and PCR amplification conditions. The primer name and sequences, fluorophore, mutliplex pool, annealing temperature, PCR temperature ramps, template DNA dilution (applied for limiting the effects of PCR inhibitors), allele size range, number of alleles and publication are detailed for each marker used in the study.

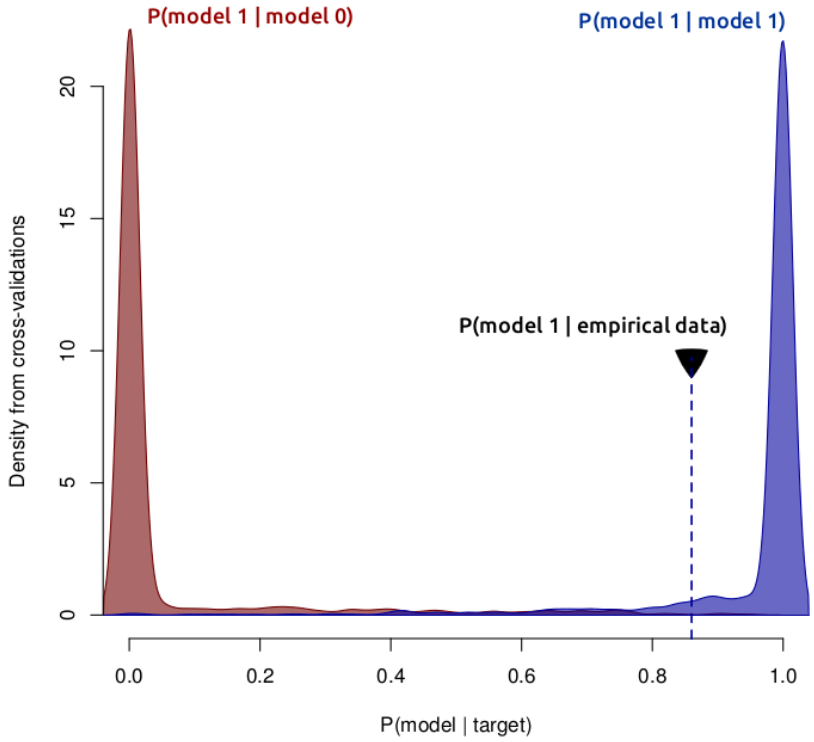
Marker name	Primer forward (5'-3')	Primer reverse (5'-3')	Fluorophore	Multiplex	T. Anneal	T. ramp	DNA dil.	Size range	N.alleles	Reference
NLGA2	CTTTAGGAGGGTCTTTAGCC	CCAATCTCTAGTAGGAGGAGC	ATTO	II	52°C	5°C/s	dil 1/20	93-123	12	Ouborg *et al.* (2000)
NLGA3	GTTGTAACGTAAATGCCGTCC	CTTGCCGATGAAACCCAT	ATTO	I	55°C	5°C/s	dil 1/20	99-183	14	Ouborg *et al.* (2000)
NLGA5	CCCGCCATATCTGATGAC	AAGTGGAGGGGACGAAAG	HEX	I	52°C	5°C/s	dil 1/20	70-100	5	Ouborg *et al.* (2000)
NLGA7	ATTTATTCCCAGCACTTTGG	CTTGACATGATTTCTCTGAACC	HEX	II	52°C	2°C/s	dil 1/5	58-104	12	Ouborg *et al.* (2000)
NLCA1	CTCAGAAACGAGGCTCTATG	TTTGGTTGGAAGACAAGAAG	FAM	II	52°C	5°C/s	dil 1/20	182-242	15	Ouborg *et al.* (2000)
NLTG/GA1	AAGCAGCAGCAAAATTTGTA	TGTGCAAGTTACCTGTTTCC	FAM	II	52°C	5°C/s	dil 1/20	117-135	8	Ouborg *et al.* (2000)
Nsub033	ACACACACACACTCTCTCTCTC	ACTTGCAAAGATCCTCTCAGAT	ATTO	I	57°C	5°C/s	dil 1/10	222-241	6	Yokogawa *et al.* (2008)
Nsub176	AGAGAGAGAGAGACACACACAC	GGCAACAGGTCTATTAATCTCA	FAM	I	57°C	2°C/s	dil 1/5	91-146	12	Yokogawa *et al.* (2008)

Online resource 2. Morphological and ecological differences between *Nuphar pumila* (specialist) and *N. lutea* (generalist).

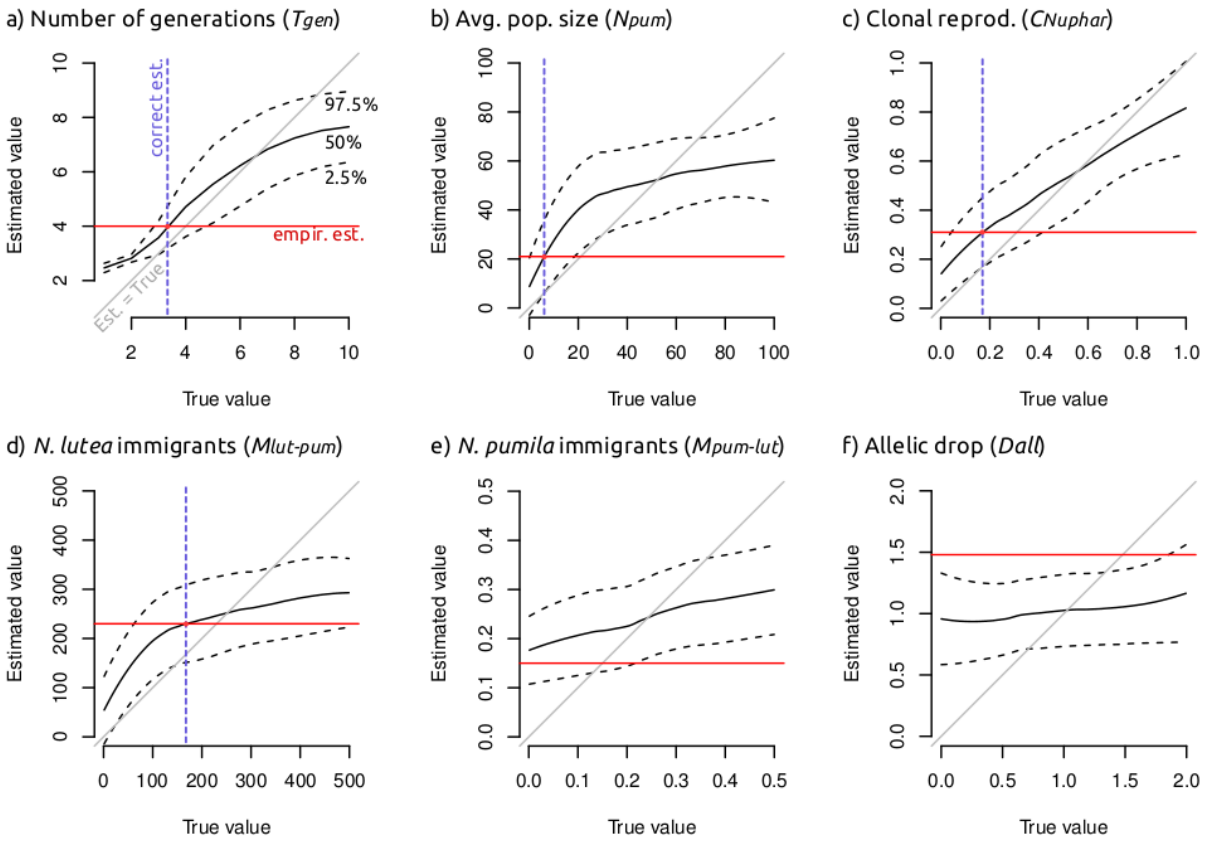
Trait/attribute	***N. pumila***	***N. lutea***
Morphology		
Stigmatic disc diameter	6-8.8 mm	10-20 mm
Stigmatic disc form	deeply lobed	entire
Stigmatic rays	6-13	12-25
Flower diameter	2-3 cm	4-5 cm
Perigon length	1-2 cm	2-3 cm
Fruit length	1-3 cm	2-4 cm
Fruit form	slightly curved, grooved	straight, not grooved
Floating leaves	10 x 12 cm	30 x 40 cm
Petiole length	50-150 (350) cm	50-250 (500) cm
Petiole form (under the blade)	compressed	trigonous
Rhizome diameter	1-2 cm	3-8 cm
Ecology		
Flowering period	VI-VIII	VII-IX
Waterbodies/habitat	stagnant	stagnant to slowly flowing
Tolerance to water movement	no	yes
Tolerance to wave action	no	yes
Tolerance to salinity	no	yes
Water trophic level	dystrophic to mesotrophic	moderately eutrophic
Water temperature	cool	wide amplitude
Water pH	slightly acidic	wide amplitude
Substrates	mainly over mud or peat	wide amplitude (also sand and gravel)
Maximal depth of water	3.5 m	6 m
Maximum length of peduncle	2 m	4 m
Maximum altitude in Europe	ca. 1700 m a.s.l.	ca. 1000 m a.s.l.
Distribution southwards	to the Alps	to Northern Africa



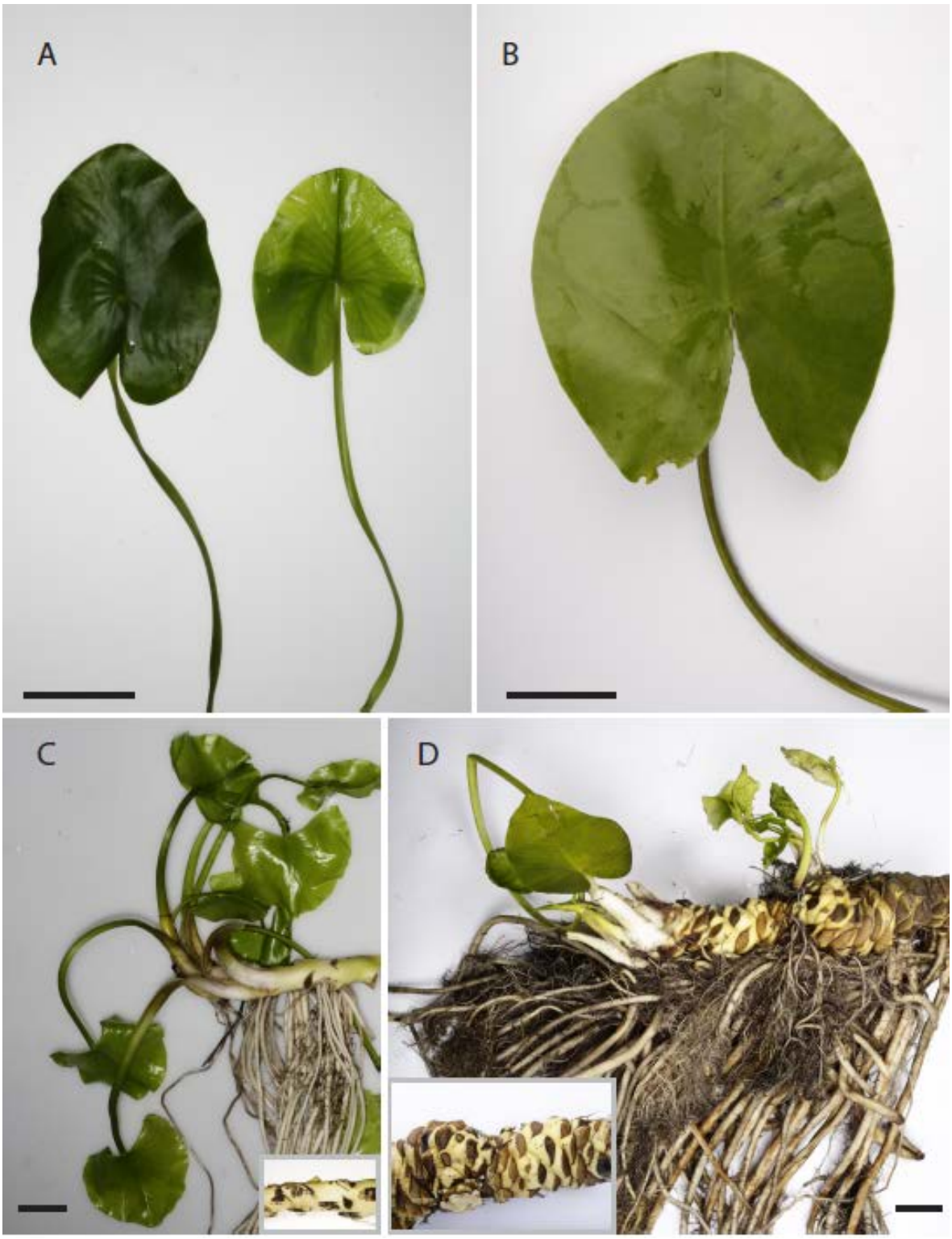
Online resource 3. Principal coordinates analysis of individual genotypes. Our sampling includes 194 *N. pumila* specimens (small pie charts) collected in 13 natural populations, completed with 20 *N. lutea* specimens (large pie charts) from natural populations (KES – 15 specimens, STI – 2 specimens) and botanical gardens (LAU – 3 specimens). Distances among specimens are computed according to their genotype, as characterized by 8 SSR loci. Allele sizes are not accounted in distance calculations. In parallel, we display the admixture levels of specimens, estimated with the "hybrid index" (Buerkle 2005), using pie-charts. Every specimen is assigned either to *N. lutea* (black) or *N. pumila* (white) genetic pool using a probabilistic framework; pure breed specimens receive a probability of 0 (*N. lutea*) or 1 (*N. pumila*) while first generation hybrids and further admixed genotypes get intermediate probabilities. Note that several hybrid specimens appear as differentiated from both *N. pumila* and *N. lutea* genotypes (i.e. appearing with negative coordinates on the PCA1 and PCA2 eigenaxes). Those hybrids actually carry alleles with SSR sizes suggesting a *N. lutea* origin, that were however not directly observed in pure *N. lutea* specimens. This pattern arises due to the limited amount of specimens sampled for *N. lutea*. It should be noted that *Hindex* accommodates such data limitations by excluding the alleles being absent from both reference pools (i.e. representing here 28 alleles / 84 in total).



Online resource 4. Model selection. A model selection procedure was used to test for the presence of introgression among *N. lutea* and *N. pumila* (i.e. model 1) versus a null hypothesis (model 0) assuming no gene flow among species. The procedure started by estimating the posterior probability of each model [P(model | empirical data)], using a pairwise model comparison where 500'000 simulations were performed for each model (using priors and parameters defined as in the main manuscript) and compared to the empirical data. The 1'000 best simulations, collected from the compared models, were then used to estimate posterior probabilities [i.e. P(model | empirical data), using neural-nets implemented in the "abc" R CRAN package, with 50 iterations]. Cross-validations were then used to evaluate the robustness of this selection procedure. Briefly, 1'000 simulations were randomly picked from each of the compared models and used as pseudo-observations to feed the model selection procedure. This allowed to check i) whether the compared models could be discriminated from each other and ii) check how often posterior probabilities were designating either the correct [i.e. in blue on density plots, P(model X | model X), true positives] or the wrong model [i.e. in red on density plots, P(model X | model Y), false positives]. These counts then allowed computing p-values, indicating the risk of picking the wrong model, at a given posterior probability value. The p-value was estimated as Pval(model X) = 1 – $D_{post.prob.emp}$[P(model X | model X)] / ($D_{post.prob.emp}$[P(model X | model X)] + $D_{post.prob.emp}$[P(model X | model Y)]), where $D_{post.prob.emp}$ is the density of cross-validations picking a given model, at the posterior probability obtained with empirical observations.



Online resource 5. Cross-validation of ABC estimates. We used 1,000 simulations as pseudo-observations to assess the robustness and accuracy of our ABC estimations. The results are displayed as scatterplots, where the estimated parameters (i.e. Estimated value) are compared to those that were actually used to set the simulations (i.e. True value). The median and 95% confidence intervals of the obtained estimates as displayed as solid and dashed lines, respectively. These curves outline the level of technical uncertainty and systematic bias (i.e. deviations from the 1:1 gray line) associated to the ABC procedure itself. **a) - d)** Note that most of our estimations actually overestimate the actual parameter values (i.e. the median lines are shifted left compared to the 1:1 line), at least when considering the parameter space close to the empirical estimates (i.e. "empir. est.", displayed as a red line). It is therefore likely that our empirical estimations suffer from this same systematic bias. Hence, bias-corrected values (i.e. "correct est." - displayed as the blue-dashed line), obtained by intersecting of our empirical estimates with the median line, were considered for further discussion. **e) – f)** The cross-validations indicate that our ABC procedure yields essentially random estimations for these two parameters.



Online resource 6. Morphological differences between *N. pumila* and *N. lutea.* Leaves and rhizomes of *N. pumila* (A, C) and *N. lutea* (B, D). Bar: 5 cm. Plants obtained from the Botanic Garden of the University of Fribourg (*N. pumila*) and Botanic Garden of Lausanne (*N. lutea*). Photographs by Hans-Rüdiger Siegel (Natural History Museum Fribourg, Switzerland).