***Supplementary material***

*S1: Phenotypic variation in the wild*

To assess to what extent phenotypic divergence of *R. arvalis* populations seen in the laboratory (at a combination of acid *versus* neutral pH and predator present *versus* predator absent treatments) parallels that in the wild, *R. arvalis* tadpoles were sampled in the six study ponds (see Material and Methods in main text) during two time periods (May and June 2009). At each pond, 7-40 tadpoles were collected using dip net (32 cm diameter, 0.8 mm mesh size) sweeps, and stored in 4% formalin. Formalin fixed tadpoles were later photographed on a piece of millimeter paper to obtain a digital lateral and dorsal-view image.

For each tadpole, the developmental stage was recorded (Gosner, 1960) and five morphological traits (body depth, body length, tail depth, tail length and tail muscle depth) measured as in Experiment 1. Measurements were done (to the nearest 0.001 mm) with the software Pro-Plus 4.5.0.29 for Windows (Media Cybernetics, Silver Spring, MD, USA). As tadpole mass was not available for the wild collected tadpoles, we used PC1 axis (explained 92% of variation) from a principal component analysis on the five morphological variables as an estimate of body size. Only undamaged tadpoles were included in the analyses.

We present data on larval body size (PC1), developmental stage, relative tail depth and relative tail muscle depth. (Tail depth and tail muscle depth were chosen as they had the strongest loadings in PC analyses). Phenotypic traits were analyzed with analyses of (co)variance (AN(C)OVA), where population and time (May and June) were used as fixed factors. In the analyses of tail depth and tail muscle depth and developmental stage, PC1 was used as a covariate to correct for size variation. Subsequently, linear orthogonal polynomials (Quinn & Keough 2002) were used to test for a linear relationship between a given phenotypic trait and pond pH. Non-significant three way interactions were removed from the analyses. All statistical analyses were conducted in SAS 9.3 (SAS Insitute, Inc.). Note: we here present results for the same six populations on which laboratory experiments were conducted, but analyses on a total of nine populations from the wild show qualitatively similar patterns (not shown).

**References:**

Gosner K L. 1960 A simplified table for staging anuran embryos and larvae with notes on identification. *Copeia* **1960,** 183-190.

Quinn GP, Keough MJ. 2002 *Experimental design and data analysis for biologists*. Cambridge University Press, Cambridge, U.K.

**Table S1.** Analysis of (co)variance on development stage, tadpole size (PC1), tail depth and tail muscle depth of tadpoles collected in the field from six *R. arvalis* populations occurring along a pH gradient. Sampling was conducted during two time periods (May and June). Significant values ($P < 0.05$) are shown in **bold.**

| | **Development stage** | | | **Larval size (PC1)** | | | **Tail depth** | | | **Tail muscle depth** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Effect* | *ndf, ddf* | *F* | *P* | *ndf, ddf* | *F* | *P* | *ndf, ddf* | *F* | *P* | *ndf, ddf* | *F* | *P* |
| Population | 5, 191 | 15.89 | **<0.001** | 5,122 | 6.89 | **<0.001** | 5, 101 | 8.64 | **<0.001** | 5, 146 | 16.99 | **<0.001** |
| Time | 1,191 | 314.94 | **<0.001** | 1, 122 | 17.49 | **<0.001** | 1, 101 | 78.79 | **<0.001** | 1, 146 | 142.56 | **<0.001** |
| Larval size (PC1) | - | - | - | - | - | - | 1, 101 | 73.99 | **<0.001** | 1, 146 | 8.82 | **0.004** |
| Dev. stage | - | - | - | 1,122 | 0.49 | 0.486 | - | - | - | - | - | - |
| *Linear Contrasts* | | | | | | | | | | | | |
| Pond pH | 1, 191 | 35.60 | **<0.001** | 1,122 | 10.63 | **0.001** | 1, 101 | 4.31 | **0.040** | 1, 146 | 14.77 | **<0.001** |

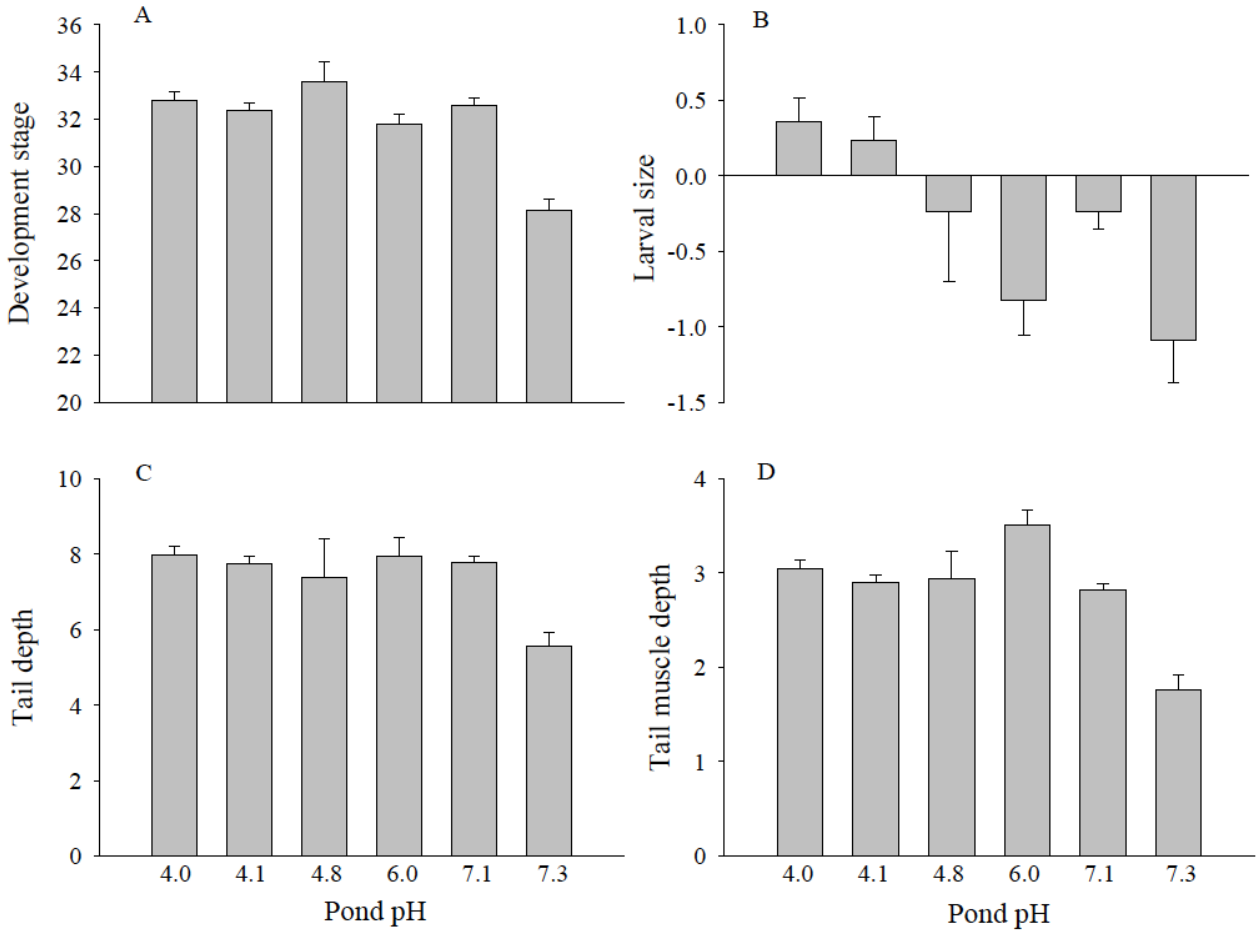


**Fig. S1.** LS mean + SE of A) developmental stage (Gosner stage), B) larval size (PC1), C) relative tail depth (mm) and D) relative tail muscle depth (mm) of tadpoles collected from the wild in six *R. arvalis* populations plotted against pond pH. Pond predator densities increase with pond acidity.

*S2: Parentage assignment*

256 out of the 407 experimental tadpoles survived the exposure to predators. Microsatellite markers were used to determine the source population of the surviving tadpoles by assigning tadpole genotypes to putative parents. As five pairs were crossed within each population, this resulted in a total of 30 families with 30 female and 30 male putative parents. Six polymorphic microsatellite loci (WRA1-22, WRA1-28, WRA1-160, WRA6-8, P. Arens, unpublished, Genbank accession AJ1419881-84; RLaCa41 (Garner and Tomio 2001) and Gala 19 (Arioli 2007)) were used to identify the parents.

DNA was extracted (from tail tip in the tadpoles and from the skin fold among toes in the adults) using a high salt extraction procedure (Aljanabi and Martinez 1997). Multiplexed polymerase chain reaction (PCR) amplifications were performed in a total volume of 5µl containing some 50ng DNA template, 2.5µg PCR mix (QIAGEN Multiplex PCR Kit), 1µl 10xprimer mix, 1.5µl RNAse-free water in a TProfessional Basic (Biometra) thermal cycler. PCR profiles consisted of 15min denaturation at 95°C followed by 30-38 cycles of 30s denaturation at 94°C, 90s annealing at 50-59°C and 60s extension at 72°C with a final step of 30min at 72°C. PCR products were visualized using an ABI 3739xI DNA analyzer (Applied Biosystems) and alleles were scored using GeneMapper software v3.7 (Applied Biosystems). Parentage assignment was performed using the PROBMAX (version 1.3) program (Danzmann 1997). 244 out of the 256 survived tadpoles could be assigned to the parents with a probability of >90%. Assignment probability of the remaining 12 tadpoles was < 70% and they were treated as dead in the analyses.

**References:**


Aljanabi SM, Martinez I. 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Res*. **25,** 4692-4693.

Arioli M. 2007 *Reproductive patterns and population genetics in pure hybridogenetic water frog populations of Rana eculenta.* Ph.D. thesis. Univ. of Zurich, Ecology department. Available at www.dissertationen.unizh.ch.

Danzmann RG. 1997 PROBMAX: A computer program for assigning unknown parentage in pedigree analysis from known genotypic pools of parents and progeny. *J. Hered*. **88,** 333.

Garner TWJ, Tomio G. 2001 Microsatellites for use in studies of the Italian agile frog, *Rana latastei* (Boulenger). *Cons. Genet*. **2,** 77-80.

**Table S2.** Generalized linear mixed model of tadpole survival in six *R. arvalis* populations when exposed to free-hunting predators at two pH treatments (acid, neutral) following rearing in four treatment combinations (acid/predator present, neutral/predator present, acid/predator absent or neutral/predator absent). Linear contrasts were conducted against pond pH. Significant effects ($p<0.05$) are shown in **bold**.

| *Random effects* | *Variance ± SE* | | *Z* | *P* |
|---|---|---|---|---|
| Tank (predator treatment × pH treatment) | 29.20 ± 14.30 | | 2.04 | **0.021** |
| | | | | |
| *Fixed effects* | *ndf* | *ddf* | *F* | *P* |
| Predator treatment | 1 | 64 | 1.7 | 0.199 |
| pH treatment | 1 | 64 | 7.4 | **0.009** |
| Population | 5 | 317 | 3.4 | **0.006** |
| Predator treatment × pH treatment | 1 | 64 | 0.1 | 0.715 |
| Predator treatment × Population | 5 | 317 | 1.1 | 0.378 |
| pH treatment × Population | 5 | 317 | 0.8 | 0.539 |
| Predator treatment × pH treatment × Population | 5 | 317 | 2.3 | **0.048** |
| Predator size | 1 | 317 | 9.9 | **0.002** |
| | | | | |
| *Linear contrasts pond pH* | | | | |
| pond pH | 1 | 317 | 9.4 | **0.002** |
| Predator treatment × pond pH | 1 | 317 | 3.3 | 0.072 |
| pH treatment × pond pH | 1 | 317 | 0.7 | 0.400 |
| predator treatment × pH treatment × pond pH | 1 | 317 | 4.3 | **0.039** |

*S3. Multiple regression on distance matrices*

To test to what extent relative fitness differences (i.e. survival under predation) correlate with phenotypic divergence among populations, population pairwise differences (i.e. phenotypic distances) in larval trait means (activity, larval mass, tail depth or tail muscle depth from Experiment 1, see above) were calculated within the treatments and analyzed with a multiple regression on distance matrices (MRM ; Legendre et al. 1998; Lichstein 2007). The analyses were conducted within each of the four treatment combinations (Acid/Predator absent (ANP), Acid/Predator present (ANP), Neutral/Predator absent (NNP) and Neutral/Predator present (NPP). A full model was first run with activity, larval mass and tail depth (or tail muscle depth) as predictors, followed by stepwise regression removing the non-significant effects (starting from the least significant).

We ran tail depth and tail muscle depth in separate models as they were highly correlated within both neutral treatments (Pearson $r > 0.7$). For tail depth and tail muscle depth, we here used absolute (rather than size-corrected) relative differences as absolute differences are expected to be more directly related to fitness differences. Pairwise differences in absolute tail morphology were mostly relatively weakly correlated with pairwise differences in body mass (Pearson $r$: -0.03 to 0.64). However, in the NPP treatment, both tail depth and tail muscle depth differences were highly correlated with larval mass differences (Pearson $r > 0.75$) and hence were not included in the same model with larval mass differences within this treatment. Instead alternative models were conducted (activity + mass *versus* activity + tail depth or tail muscle depth). In analyses using larval activity, population means estimated in the *presence* of predators (see Experiment 1 in main manuscript) within a given pH treatment were used. All trait distances were standardized (to a mean of 0 and standard deviation of 1) prior to analyses. The MRM analyses were conducted using permutation tests with 10 000 permutations in the package 'Ecodist' in R (version 3.0.2).

**References**

Legendre P, Lapointe F-J, Casgrain P. 1994 Modeling brain evolution from behavior: a permutational regression approach. *Evolution* **48,** 1487-1499

Lichstein JW. 2007 Multiple regression on distance matrices: a multivariate spatial analysis tool. *Plant Ecol.* **188,** 117 –131. doi 10.1007/s11258-006-9126-3

**Table S3**. Multiple regression analyses on pairwise distance matrices (MRM) of tadpole survival (when exposed to free-hunting predators) and phenotypic trait divergence. A) full models and B) reduced final models ($R^2$ for models, as well as slopes and significance of individual variables are shown) are shown. For NPP, two alternative types of models were conducted due to high trait correlations (see above). Significant effects ($p<0.05$) are shown in bold.

*A) Full models*

| | *Treatments* ANP $R^2 = 0.043$, $P = 0.373$ | | APP $R^2 = 0.164$, $P =$ **0.025** | | NNP $R^2 = 0.127$, $P =$ **0.027** | | NPP $R^2 = 0.053$, $P = 0.189$ | |
|---|---|---|---|---|---|---|---|---|
| *Traits* | *b* | *P* | *b* | *P* | *b* | *P* | *b* | *P* |
| Intercept | 0.1275 | 0.120 | 0.0847 | 0.989 | 0.1020 | 0.583 | 0.1651 | 0.639 |
| Activity | -0.0138 | 0.234 | -0.0080 | 0.701 | -0.0125 | 0.112 | -0.016 | 0.396 |
| Mass | 0.0078 | 0.550 | 0.0494 | **0.017** | -0.0167 | 0.066 | 0.0279 | 0.174 |
| Tail depth | -0.0128 | 0.145 | 0.0419 | **0.017** | 0.0349 | **0.010** | - | - |
| | | | | | | | $R^2 = 0.020$, P = 0.541 | |
| | | | | | | | *b* | *P* |
| Intercept | | | | | | | 0.2043 | 0.223 |
| Activity | | | | | | | -0.0218 | 0.244 |
| Tail depth | | | | | | | -0.0007 | 0.972 |

*B) Reduced models*

| | *Treatments* ANP $R^2 = 0.014$, $P = 0.184$ | | APP $R^2 = 0.151$, $P =$ **0.010** | | NNP $R^2 = 0.090$, $P =$ **0.013** | | NPP $R^2 = 0.010$, $P = 0.249$ | |
|---|---|---|---|---|---|---|---|---|
| *Traits* | *b* | *P* | *b* | *P* | *b* | *P* | *b* | *P* |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Intercept | 0.1184 | 0.071 | 0.0764 | 0.998 | 0.0722 | 0.987 | 0.1206 | 0.118 |
| Mass | | | 0.0492 | **0.019** | | | -0.010 | 0.259 |
| Tail depth | -0.0111 | 0.184 | 0.0414 | **0.016** | 0.0318 | **0.013** | | |

| | $R^2 = 0$, $P = 0.972$ | |
|---|---|---|
| | *b* | *P* |
| Intercept | 0.1776 | 0.611 |
| Tail depth | 0.0007 | 0.972 |