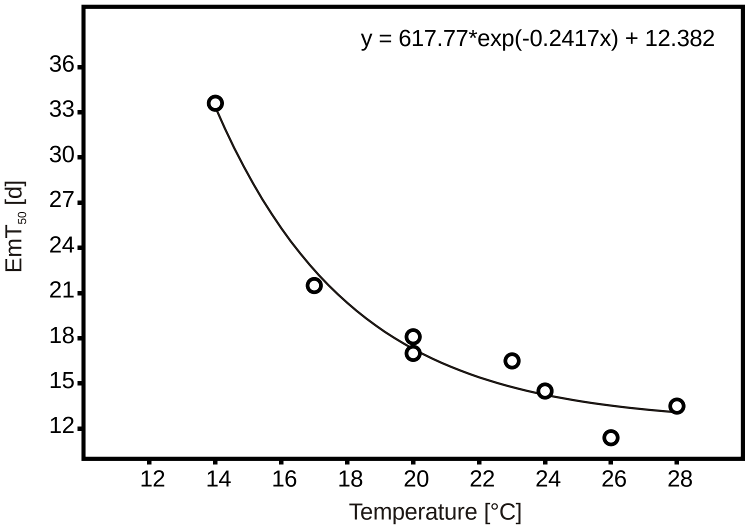
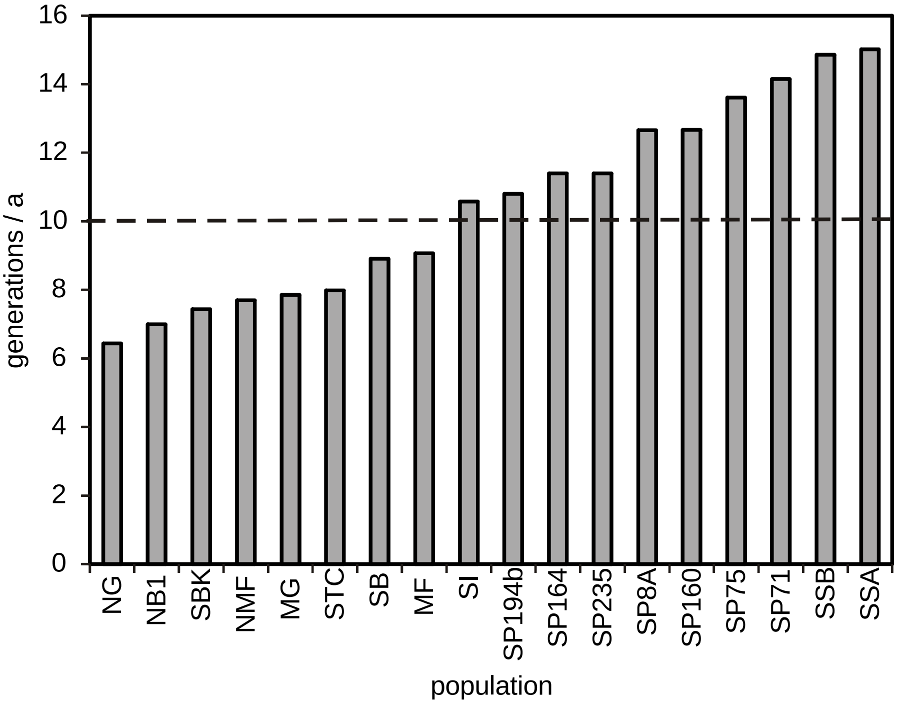
Electronic supplementary material S1

Supplementary Figure S1.1



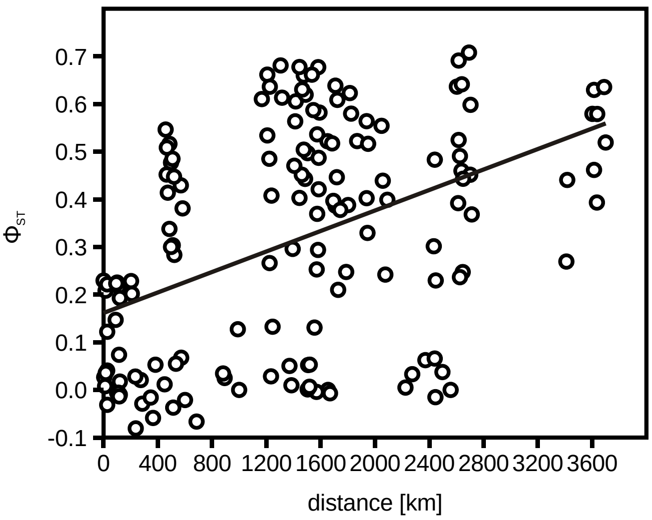
Experimentally assessed mean EmT_{50} of *C. riparius* at different test temperatures. The exponential decay curve sets the model of the EmT_{50}-temperature dependency.

Supplementary Figure S1.2



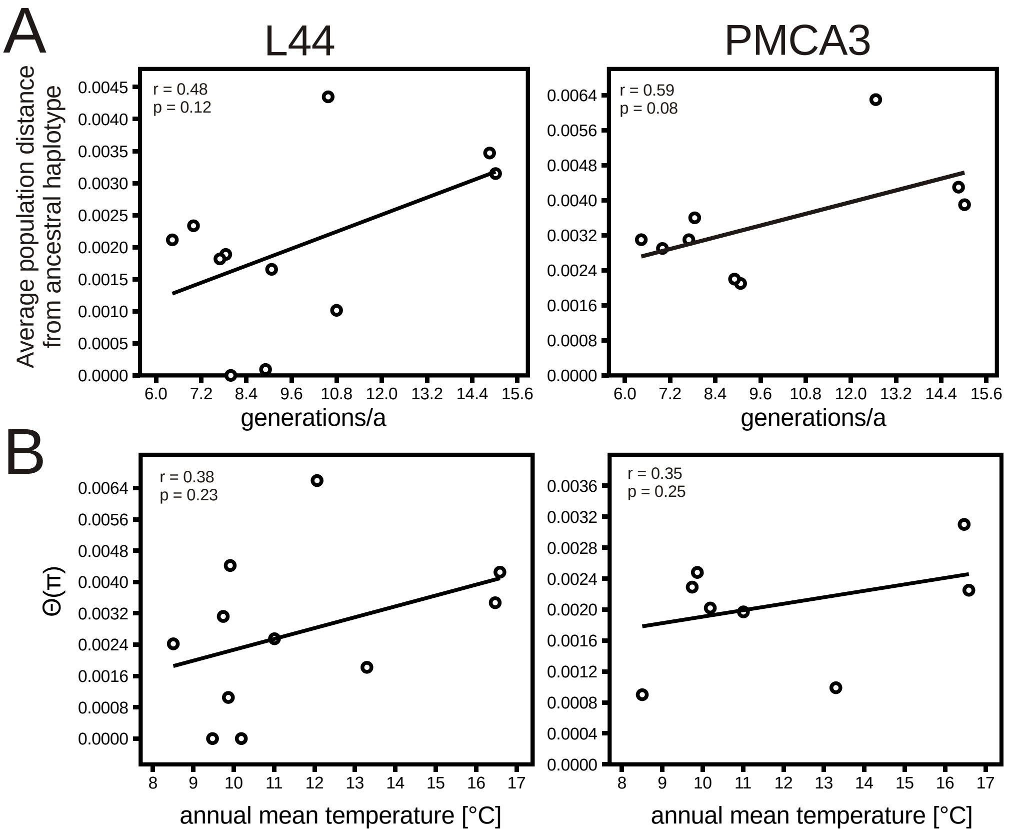
Number of potential generations per year modelled on the basis of monthly mean temperatures and a model of the EmT_{50}-temperature dependency for each sampled *C. riparius* population. Threshold between evolutionary slower and faster populations set at ten generations per year.

Supplementary Figure S1.3



Pairwise Φ_{ST} of the mitochondrial locus COI for *C. riparius* with respect to geographical distance shows highly significant isolation-by-distance pattern (Mantel's test p=0.0001).

Supplementary Figure S1.4



(A Plot of the average population distance from the ancestral haplotype in *C. riparius* and the number of potential generations per year. Average distances are based on a statistical parsimony network and the (uncorrected) p-distance from the ancestral haplotype and numbers of generations per year were modelled according to the experimentally assessed EmT$_{50}$-temperature dependency.

(B) Correlation between the population mutation parameter $\theta(\pi)$ and the annual mean temperature. Climate data was assessed from WorldClim and $\theta(\pi)$ was calculated on the nuclear sequence data.

Supplementary Material S1.5

Simulating the population genetic effects of shortened generation times and increased mutation rate

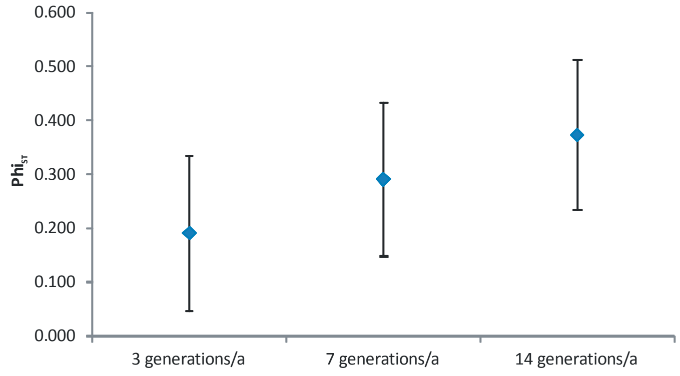


Fig.1: Dependence of Φ_{ST} on the number of generations per year. Coalescence simulations of 1000 population pairs over 100,000 years since divergence without gene-flow. Φ_{ST} was calculated from simulating a 600 bp DNA with *C. riparius* COI characteristics and the same per generation and site mutation rate.

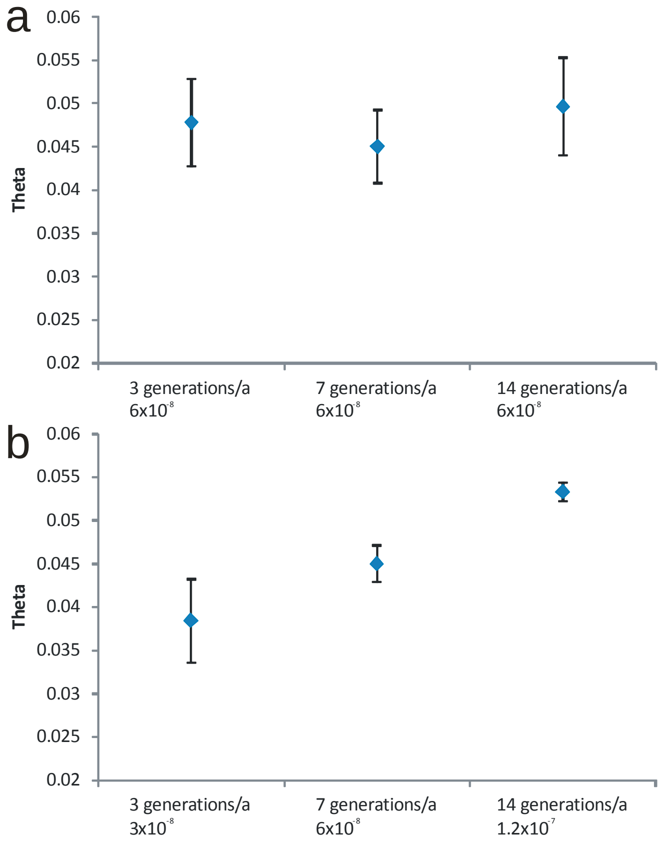


Fig.2. Dependence of theta from a temperature driven mutation rate. A) Simulations identical to the ones above. B) With a doubled mutation rate in each step in the range of $3x10^{-8}$ to $1.2x10^{-7}$.

In order to see whether the hypothesised effects of temperature on population genetic parameters via generation time and mutation rate may have a measurable effect on the chosen marker, we initially performed some exploratory simulations.

We used the coalescence simulation software SIMCOAL [1] to simulate genetic marker samples of the respective populations and evaluated the results with ARLEQUIN 3.5 [2]. The basic model simulated two populations of 1,000,000 individuals each that split 100,000 years ago. We simulated a DNA marker of 600 bp length with a transition bias of 4.3 and gamma shaped mutation rate distribution (shape parameter 0.05 and 4). These parameters reflect the empirically observed values of COI in *C. riparius* [3]. Each simulation was run 1000 times; 100 individuals were sampled to estimate the population genetic parameters of the respective population simulations.

Population differentiation

In a first set of simulations, we evaluated the effect of different generation times on population differentiation by comparing population pairs that split 100,000 years ago with three, seven and fourteen generations per year without subsequent gene-flow. As expected, the cumulative effect of more drift and mutation accumulation led to a larger differentiation in the population pairs with more generations per year (Figure 1).

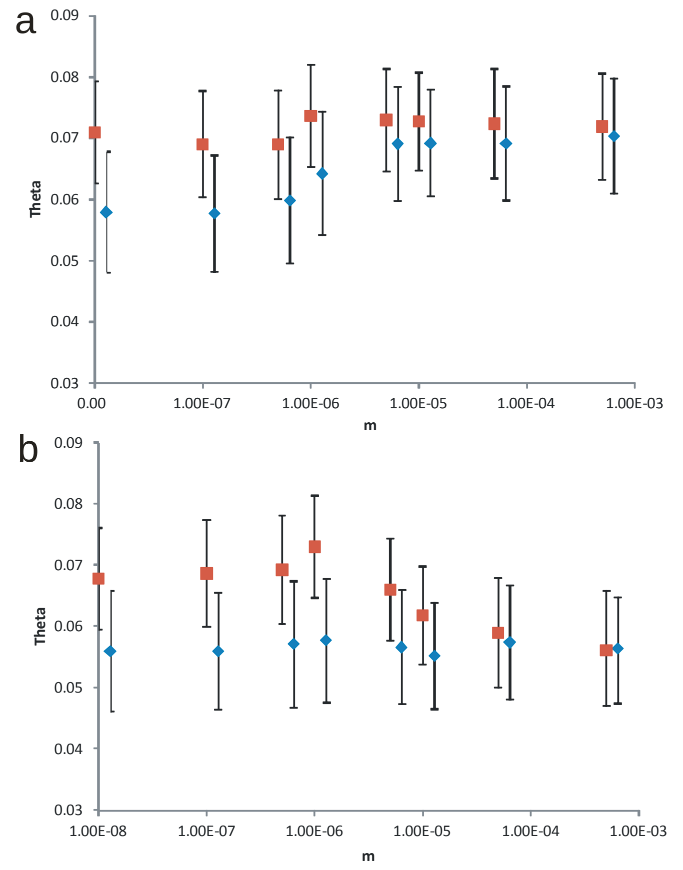


Fig.3. Influence of migration on theta. Red represents the larger (recipient) population, blue the smaller (donor), respectively. A) bidirectional gene-flow. B) unidirectional gene-flow.

Genetic diversity

More generations per year alone had no influence on estimates of mean theta (pi) (Figure 2a). We therefore increased with increasing generations per year (as expected under ESH) by doubling the mutation rate in each step in a realistic range of $3x10^{-8}$ to $1.2x10^{-7}$ mutations per site and generation [4]. This increased theta (Figure 2b).

Migration

Since migration tends to homogenise populations, we evaluated its effect on estimates of theta. To create population pairs with different thetas, we had to employ two different population sizes (2,000,000 and 1,000,000 individuals, respectively), because it is not possible to assign different mutation rates to populations in SIMCOAL. In a first set, we evaluated bi-directional migration with rates from 0 to 0.0005, corresponding to 0 to 1,000, respectively 500 migrants per generation. The thetas of the two populations remained distinguishable over a wide range of migration rates (up to 1 Nm) above which the values of the smaller population converged slowly to those of the larger population until they are not distinguishable any more at around 500 exchanged migrants per generation (Figure 3a). The mean differentiation between the simulated population pairs at 1 Nm (Φ_{ST} = 0.12 +/- 0.06) corresponds approximately to the y-axis cut of the isolation by distance regression line (Supplemental Figure 3).

Similar results were obtained with unidirectional gene-flow. Theta of the recipient population approaches slowly to the donor population’s value with a gene-flow of more than 1 migrant per generation until it reaches convergence at 500 individuals (Figure 3b).

The simulation results thus suggested that the predicted effects might be observed with the chosen marker and sampling strategy.

[1] Excoffier, L., Novembre, J. & Schneider, S. 2000 SIMCOAL: A general coalescent program for the simulation of molecular data in interconnected populations with arbitrary demography. *J. Hered.* **91**, 506-509.

[2] Excoffier, L., Laval, G. & Schneider, S. 2005 Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evol Bioinform* **1**, 47-50.

[3] Pfenninger, M. & Nowak, C. 2008 Reproductive Isolation and Ecological Niche Partition among Larvae of the Morphologically Cryptic Sister Species *Chironomus riparius* and *C. piger*. *PLoS One* **e2157**.

[4] Haag-Liautard, C., Dorris, M., Maside, X., Macaskill, S., Halligan, D.L., Charlesworth, B. & Keightley, P.D. 2007 Direct estimation of per nucleotide and genomic deleterious mutation rates in *Drosophila*. *Nature* **445**, 82-85.