Plötner et al._Supplementary Material 4 (Figures S1–S5)

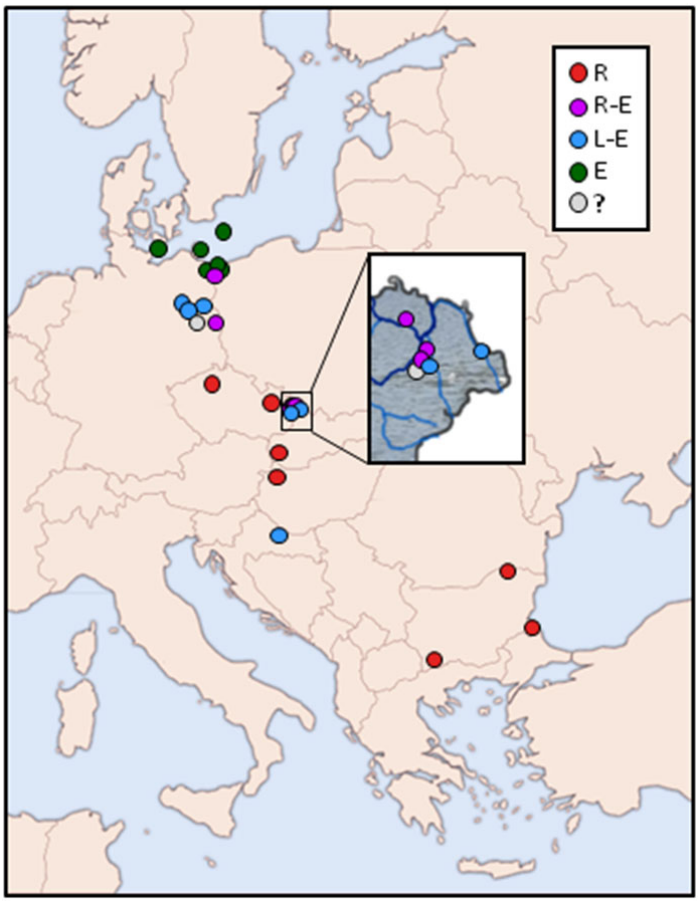

Fig S1 Map of the sampling area.
R: all-*ridibundus* populations,
R-E: *ridibundus-esculentus* populations,
L-E: *lessonae-esculentus* populations,
E: all-hybrid (*esculentus*) populations,
?: population system unclear.

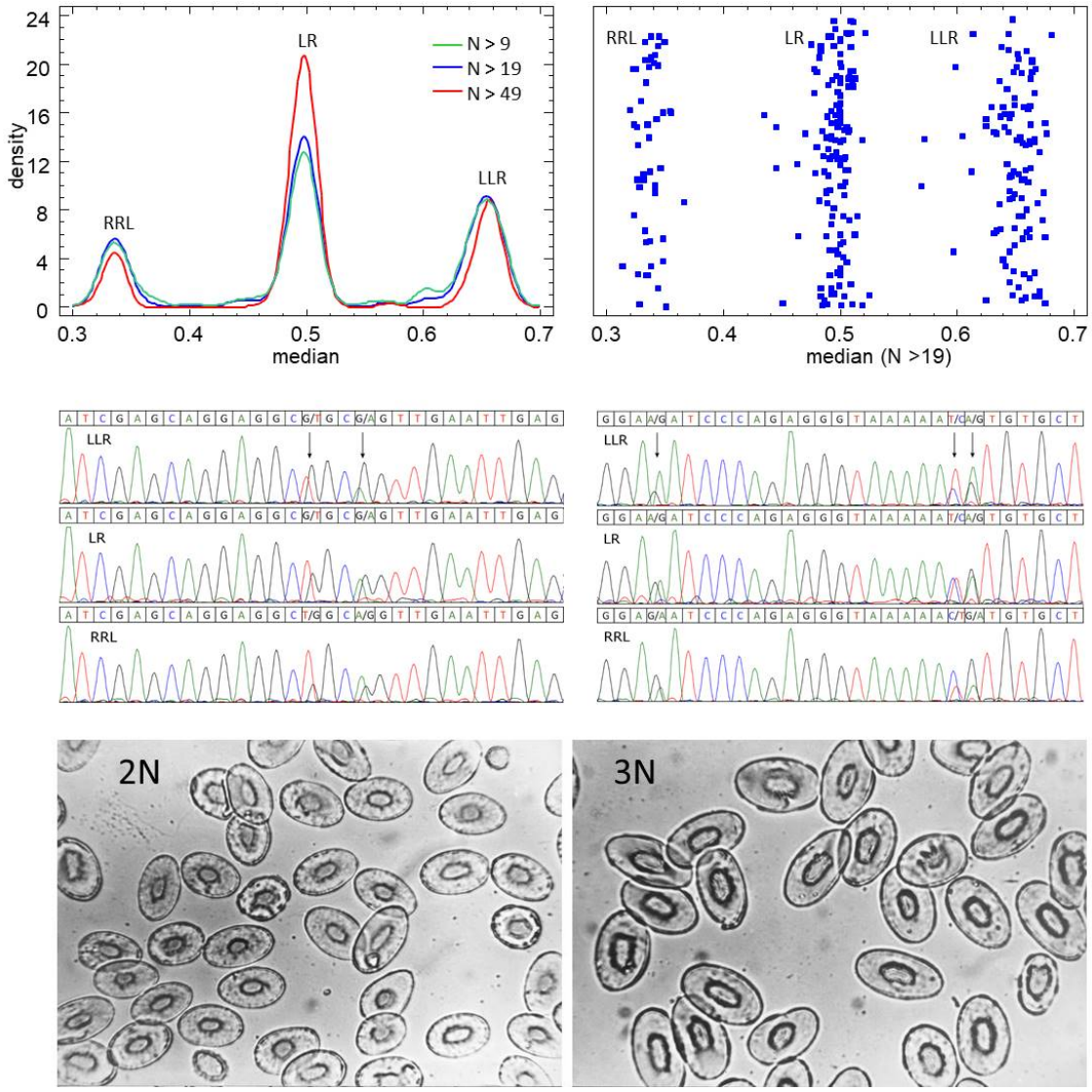

Fig. S2 Methods used in this study to determine the ploidy and genotype of *Pelophylax esculentus*. Top: Read-based genotyping. The number of L-specific reads per SNP was divided by the total coverage (L+R). Because of gene dosage effects, triploid hybrids with two L genomes and one R genome (LLR) should have a higher proportion of L-specific reads, resulting in values of 0.67, whereas diploid hybrids (LR) and triploid RRL hybrids are expected to have values of 0.5 and 0.33, respectively. The data shown in the density plot (left) and scatter plot (right) are largely consistent with these expectations, even when individuals with fewer SNPs (9 < N < 20) were included in the analysis. Center: Sanger sequence-based genotyping, as described by Tecker et al. (2017) and Krage et al. (2022); this method also uses gene dosage effects. As seen in the electropherograms of *uqcrfs1* sequences, LLR genotypes display significantly higher L-specific peaks, while RRL genotypes show higher R-specific signals at the heterozygous (species-specific) positions. In LR hybrids, the heights of L- and R-specific peaks are nearly equal. Bottom: Ploidy determination by measuring erythrocytes (e.g., Uzzell and Berger 1975; Günther 1977). The photographs show blood smears of diploid (left) and triploid (right) hybrids (640x magnification, from Günther 1977). Erythrocytes of triploid hybrids (LLR, RRL) are, on average, one-third larger than those of diploids (LR) due to an additional set of chromosomes.

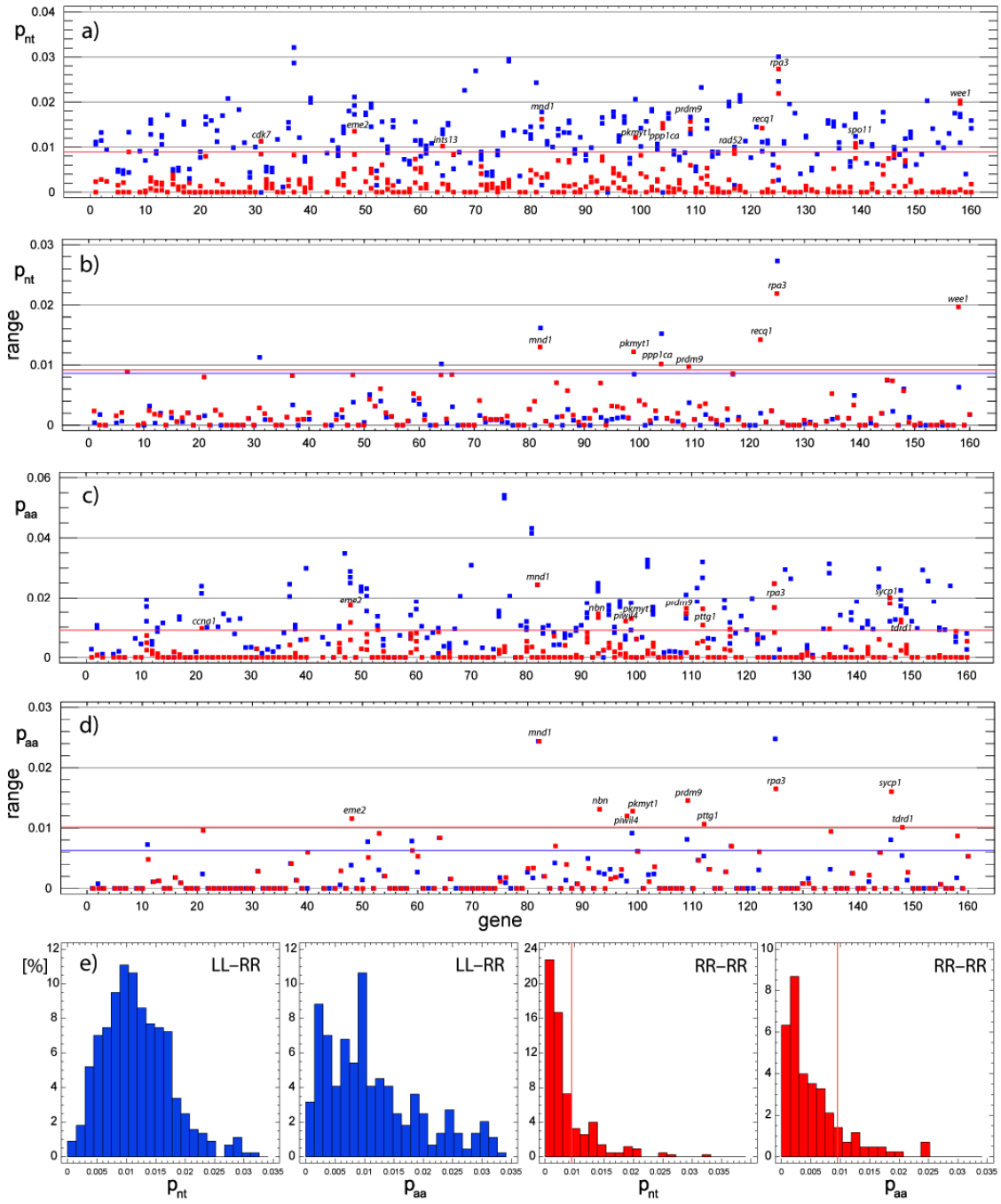

Fig. S3 Scatterplots of pairwise uncorrected p_{nt} distances (a) and p_{aa} distances (c), their corresponding ranges of variation (b, d), and frequency distributions (e), calculated for 160 gametogenic genes. Blue: Interspecific comparisons between *P. lessonae* (LL) and *P. ridibundus* (RR); red: intraspecific comparisons across P. ridibundus individuals (RR1/RR2, RR1/RR3, RR2/RR3). Values falling outside the 95% percentile (indicated by red and blue lines) correspond to genes exhibiting unusually low interspecific or unusual high intraspecific distance values. Gene names are listed in Table S2, Supplementary Material 1.

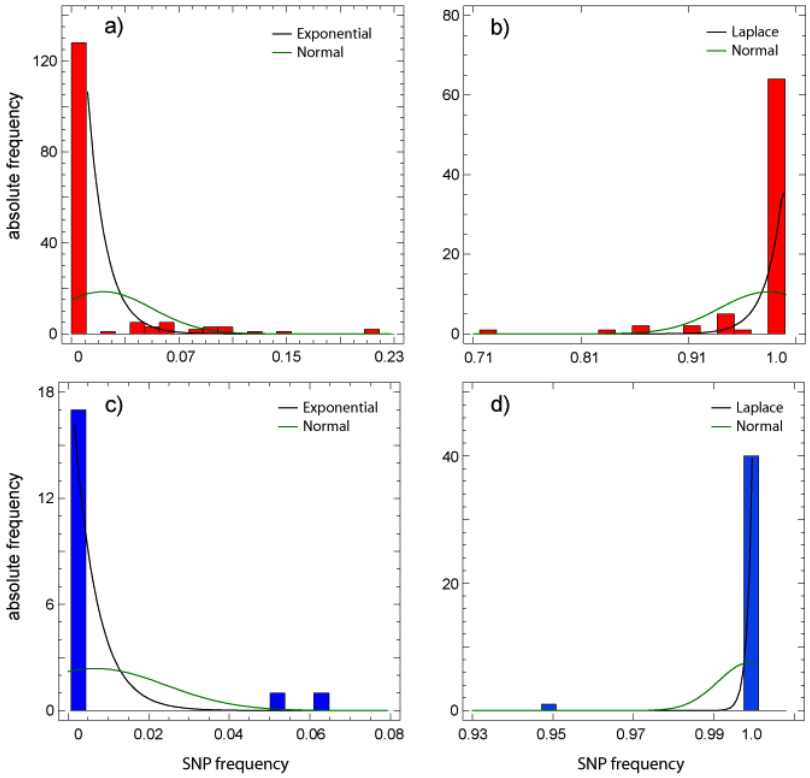

Fig. S4 Zero-inflated (a, c) and one-inflated (b, d) distributions of the frequencies of putative species-specific SNPs for *P. ridibundus* (red) and *P. lessonae* (blue). Frequencies differing from 0 or 1 may indicate introgression of *lessonae*-specific alleles into the *ridibundus* gene pool, or vice versa.

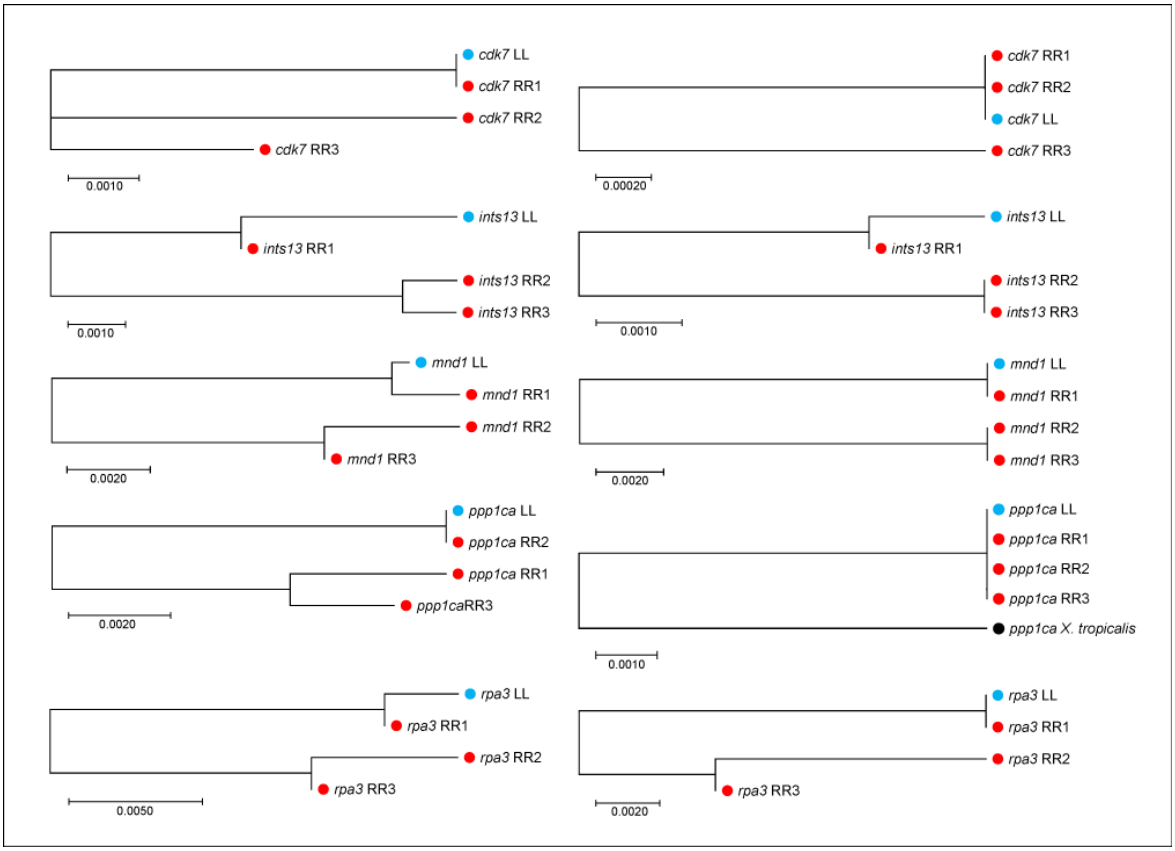

Fig. S5 Examples of putative gene introgressions. Coding sequences were selected from the testicular transcriptomes of *P. lessonae* (LL, blue circles) and *P. ridibundus* (RR, red circles). Neighbor-joining trees show clear differences between L- and R-specific alleles. In some cases, however, R alleles are more closely related to, or even identical with, L-specific orthologs rather than their conspecific R alleles, both in nucleotide (left) and/or amino acid sequences (right). In *pp1ca*, the amino acid sequences of the *Pelophylax* alleles were identical but clearly different from the *Xenopus* ortholog (black circle). These results indicate gene flow from the LL into the RR gene pool, especially considering the estimated divergence time between LL and RR is 8–17 mya (e.g. Uzzell 1978; Plötner et al. 2025) and because the *lessonae*-like alleles found in RR are distinct from R-specific alleles. Scale bar: uncorrected p distance. Gene names are listed in Table S2, Supplementary Material 1.

References

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