SUPPLEMENTARY MATERIAL

for

Sex reversal and performance in fitness-related traits during early life in agile frogs

Front. Ecol. Evol. 9:745752.

doi: 10.3389/fevo.2021.745752

by

Veronika Bókony[1,2*], Nikolett Ujhegyi[1], Zsanett Mikó[1], Réka Erös[3], Attila Hettyey[1], Nóra Vili[4], Zoltán Gál[5], Orsolya Ivett Hoffmann[5], Edina Nemesházi[1,4]

[1] Lendület Evolutionary Ecology Research Group, Plant Protection Institute, Centre for Agricultural Research, Eötvös Loránd Research Network, Herman Ottó út 15, 1022 Budapest, Hungary

[2] Institute of Biology, Department of Systematic Zoology and Ecology, Eötvös Loránd University, Pázmány Péter sétány 1/c, 1117 Budapest, Hungary

[3] Hungarian Department of Biology and Ecology, Babeș-Bolyai University, Clinicilor 5-7, 400006 Cluj-Napoca, Romania

[4] Conservation Genetics Research Group, Department of Ecology, University of Veterinary Medicine Budapest, István u. 2, 1078 Budapest, Hungary

[5] Animal Biotechnology Department, Institute of Genetics and Biotechnology, Hungarian University of Agriculture and Life Science, Szent-Györgyi Albert u. 4, H-2100, Gödöllő, Hungary

* Correspondence:
Veronika Bókony
bokony.veronika@atk.hu

Table S1. Number of genetic females (XX females and sex-reversed XX males), genetic males (XY males), and female-to-male sex-reversed individuals (XX males) in each family in the two experiments. Three families with 100% genetic females are highlighted with bold text.

Experiment	Population	Family	All genetic females	All genetic males	Sex-reversed
1	Kerek-tó	K1	11	7	
1	Kerek-tó	K2	11	9	1
1	Kerek-tó	K3	7	13	
1	Kerek-tó	K4	15	2	
1	Kerek-tó	K5	9	9	
1	Kerek-tó	K6	8	11	
1	Kerek-tó	K7	9	10	
1	Kerek-tó	K8	12	8	1
1	Kerek-tó	S8	14	6	1
1	Pilisvörösvár	P1	8	12	2
1	Pilisvörösvár	P2	12	8	
1	Pilisvörösvár	P3	14	6	
1	Pilisvörösvár	P4	10	9	
1	Pilisvörösvár	P5	10	10	
1	Pilisvörösvár	P6	8	9	
1	Pilisvörösvár	P7	8	11	
1	Pilisvörösvár	P8	15	5	
1	Szárazfarkas	S1	8	11	
1	Szárazfarkas	S2	11	9	
1	Szárazfarkas	S3	14	6	
1	Szárazfarkas	S4	12	8	
1	Szárazfarkas	S5	10	10	
1	Szárazfarkas	S6	12	8	
1	Szárazfarkas	S7	13	7	
2	Szárazfarkas	Z1	5	7	
2	Szárazfarkas	Z2	5	13	
2	**Szárazfarkas**	**Z3**	**10**	**0**	**3**
2	**Szárazfarkas**	**Z4**	**12**	**0**	**9**
2	Szárazfarkas	Z5	8	10	
2	Szárazfarkas	Z6	6	6	
2	Szárazfarkas	Z7	7	5	3
2	**Szárazfarkas**	**Z8**	**18**	**0**	
2	Szárazfarkas	Z9	8	8	
2	Szárazfarkas	Z10	4	8	1

Table S2. Summary of our statistical analyses.

Question	Outcome variable	Experiment	Method[§]	Predictors (fixed effects)	Random effects	Further settings
Does sex-reversal rate depend on treatment?	incidence of sex reversal (yes/no)	1 & 2	GLMM	treatment	family nested in experiment	subset: genetic females, excluding carbamazepine treatment groups
	incidence of sex reversal (yes/no)	1	Fisher's exact test	carbamazepine treatment	–	excluding terbuthylazine treatment groups
Does survival depend on genetic sex?	survival time (days)	1 & 2	CoxME	genetic sex (XX/XY)	family nested in experiment	individuals that survived used as censored data
Do the fitness-related traits depend on sex[*]?	time to metamorphosis (days)	1 & 2	CoxME	sex, treatment	family nested in experiment	
	body mass at metamorphosis (mg)	1 & 2	LMM	sex, treatment	family nested in experiment	different variances by sex
	body mass at metamorphosis (mg)	1 & 2	LMM	sex × time to metamorphosis, treatment	family nested in experiment	different variances by sex & family
	body mass at dissection (mg)	1 & 2	LMM	sex, age, treatment	family nested in experiment	different variances by sex
	incidence of individuals observed in the open (yes/no)	1	Fisher's exact test	sex	–	
	incidence of individuals feeding (yes/no)	1	GLMM	sex, date, time of day, treatment, observer identity, temperature, shelf height	individual nested in family	
	incidence of individuals feeding (yes/no)	1	GLMM	sex × date, time of day, treatment, observer identity, temperature, shelf height	individual nested in family	

|					
total distance moved (kpx)	2 (pre-startle)	LMM	sex, date, time of day, treatment	individual nested in family	different variances by sex
exploration (% of area used)	2 (pre-startle)	LMM	sex, date, time of day, treatment, total distance moved	individual nested in family	different variances by sex
time spent near wall (%)	2 (pre-startle)	LMM	sex, date, time of day, treatment	individual nested in family	different variances by sex
intensity of startle response	2 (post-startle)	CLMM	sex, stimulus type, date, time of day, treatment, total distance moved	individual nested in family	
intensity of startle response	2 (post-startle)	CLMM	sex × stimulus type, date, time of day, treatment, total distance moved	individual nested in family	
escape duration (sec)	2 (post-startle)	CoxME	sex, stimulus type, date, time of day, treatment, total distance moved	individual nested in family	subset: individuals that had >0 reaction intensity
escape duration (sec)	2 (post-startle)	CoxME	sex × stimulus type, date, time of day, treatment, total distance moved	individual nested in family	subset: individuals that had >0 reaction intensity
freezing duration (sec)	2 (post-startle)	CoxME	sex, stimulus type, date, time of day, treatment, total distance moved	individual nested in family	subset: individuals that had >0 reaction intensity
freezing duration (sec)	2 (post-startle)	CoxME	sex × stimulus type, date, time of day, treatment, total distance moved	individual nested in family	subset: individuals that had >0 reaction intensity

[*]In these analyses, sex was a 3-category factor: sex-reversed individual, concordant male, concordant female.

[§]Abbreviations: LMM = linear mixed model with Gaussian error distribution, GLMM = generalized linear mixed model with binomial error distribution, CoxME = Cox's proportional hazards model with mixed effects, CLMM = cumulative-link mixed model.

See the Data Availability Statement for a link to an annotated R script of the analyses.

Figure S1. Histological sections of the 6 ovotestes found in this study. Arrows point to oogonia.
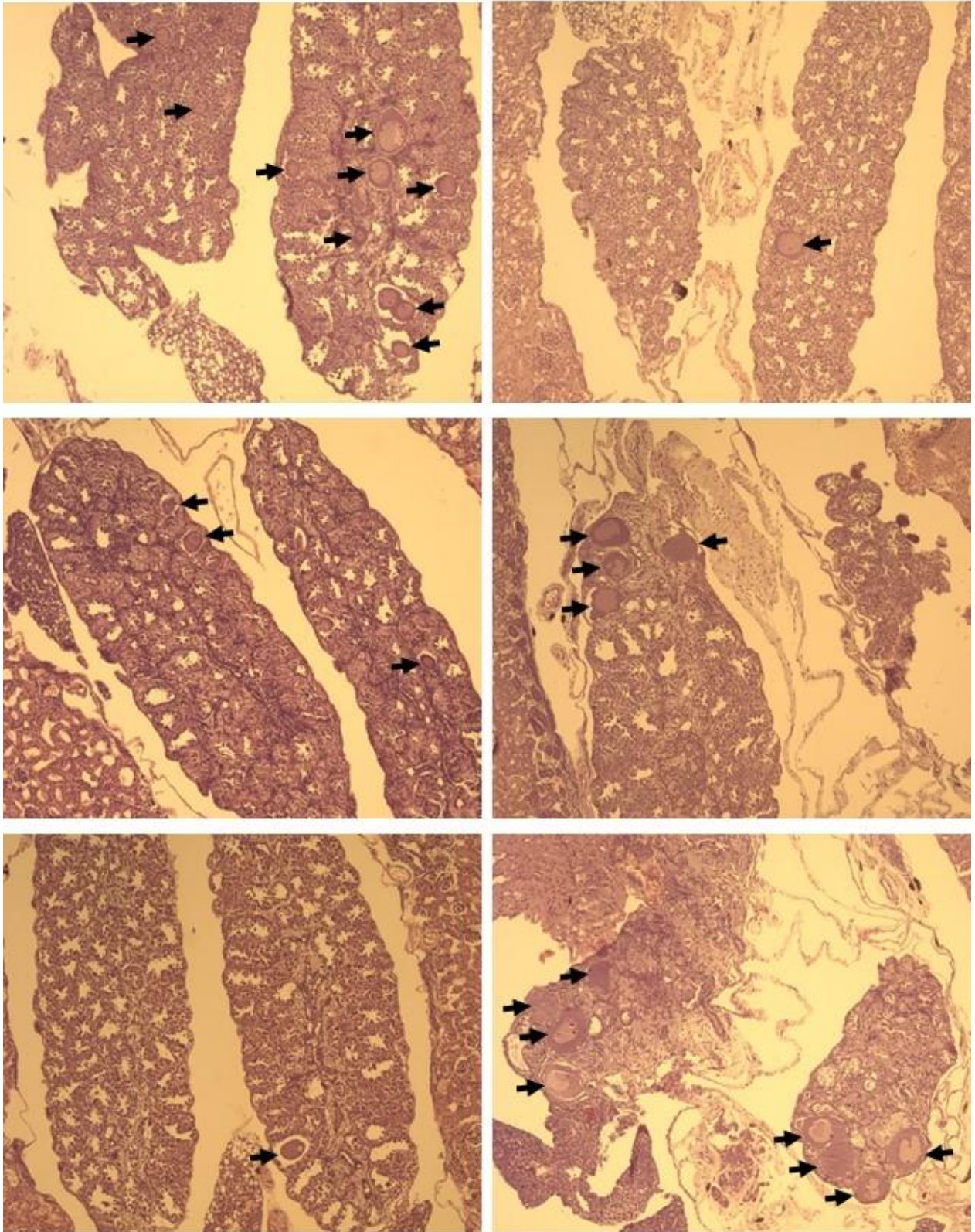
Figure S2. Relationship between mass at metamorphosis and duration of larval development (see Fig. 2A). Sex-reversed individuals are marked with their family IDs (see Table S1); circles represent individuals whose histological sections showed testicular oogonia (ovotestes). Note that families Z3 and Z4 contained only XX genotypes. Concordant males and females are shown as red circles and blue triangles, respectively.