Electronic supplementary material for:

Obligately silent males sire more offspring than singers in a rapidly evolving cricket population

Justa L. Heinen-Kay*, Ellen M. Urquhart, and Marlene Zuk

Department of Ecology, Evolution & Behavior
University of Minnesota, Saint Paul, MN 55108 USA

*justa.heinenkay@gmail.com

Supplementary methods:

Construction of the pure-breeding flatwing and normal-wing colony lines began in 2013, using crickets from the general Kauai lab colony. First, 5 mating pairs for each wing morph founded the first homozygous generation. Then 8-25 F1 females were each mated with a different male from the general Kauai colony and housed separately until the offspring were confirmed to have the expected wing phenotype. All F1 males were sacrificed to avoid potential for inbreeding. For each wing morph, we housed 125 F2 homozygous females with 125 males with appropriate wing morph from the general Kauai lab colony, spread amongst 5 rearing boxes. Next, 25 males were added to each of the 5 female boxes for each wing morph. Boxes were periodically mixed within wing morph, and a subset of males from each wing morph colony were replaced with males from the general Kauai colony every two weeks. For the next and subsequent generations, the wing morph pure-breeding colonies were reared in the same way as our other laboratory colonies, except that males from the general Kauai colony are added periodically to the appropriate colony. Crickets were housed in 15L plastic containers with ad libitum access to Teklad rabbit chow, moist cotton, and egg carton for shelter within Caron incubators set to 26°C, 75% humidity, and a 12:12 photo-reversed LD cycle.

Supplementary sample size information:

Table S1. Number of cricket pairings for each treatment combination at various stages of the experiment. There are many reasons, biological and non-biological, why a mounting may not proceed to the next experimental stage of successful oviposition. These include premature termination of copulation, failed spermatophore transfer, and female removal of the spermatophore, in addition to other factors like cricket escape from the experimental arena.

Wing morph	Acoustic treatment	Age treatment	Attempted matings	Female mounted the male	Successful spermatophore transfer & retention; 1 week oviposition period	Produced offspring
FW	Song	Young	59	41	29	24
		Old	60	50	29	25
	No song	Young	54	39	31	27
		Old	65	50	30	27
NW	Song	Young	48	39	30	26
		Old	41	36	31	30
	No song	Young	50	35	31	29
		Old	51	40	30	27

Supplementary results:

(A) Latency to mount

Table S2. We used a forward model selection approach that constructed all possible models to understand the most important contributors to cube-root transformed female latency to mount. We included the following covariates: female pronotum width (FPW), male pronotum width (MPW), female age (FemAge); and main effects: male wing morph (WM), male age category (Age), and male acoustic treatment (Song). We also included all interactions among main effects, and employed a heredity restriction. Below are the top five models as determined by AICc.

Model	AICc	ΔAICc
WM + Age + Song + WM*Age + WM*Age + Age*Song + WM*Age*Song	696.06	0
MPW + WM + Age + Song + WM*Age + WM*Age + Age*Song + WM*Age*Song	697.43	1.37
FemAge + WM + Age + Song +Wing*Age + WM*Song + Age*Song + Wing*Age*Song	697.52	1.46
FPW + WM + Age + Song +Wing*Age + WM*Song + Age*Song + Wing*Age*Song	698.01	1.95
Age	700.26	4.20

(B) Reproductive success

Table S3. We employed a forward model selection approach that constructed all possible models to understand which factors best predict male reproductive success. We included the following covariates: female pronotum width (FemPW), male pronotum width (MalePW), female age (FemAge); and main effects: male wing morph (WingMorph), male age category (Age), and male acoustic treatment (Song). We also included all possible interactions among main effects, and employed a heredity restriction. Below are the five best models as determined by AICc.

Model	AICc	ΔAICc
FemPW + MalePW + FemAge + WingMorph	2591.83	0
FemPW + MalePW + FemAge + WingMorph + Song	2593.91	2.08
FemPW + MalePW + FemAge + WingMorph + MaleAge	2593.96	2.13
FemPW + MalePW + FemAge	2594.69	2.86
FemPW + FemAge + WingMorph	2595.29	3.46

(C) Mounting success

Table S4. Results of a generalized linear mixed model with binomial error distribution, which included male and female IDs as random effects because crickets were allowed multiple mating opportunities.

Factor	Z	P
Wing morph	0.400	0.689
Age Treatment	-0.146	0.884
Song Treatment	0.769	0.442
Wing*Age	-0.626	0.531
Wing*Song	0.346	0.730
Age*Song	-0.994	0.320
Wing*Age*Song	0.620	0.535

(D) Mating failure

Table S5. Results of nominal logistic regression examining factors that influence mating failure, where a mated female failed to produce offspring. P-values <0.05 are bolded.

Factor	χ^2_1	P
Female pronotum width	1.868	0.172
Male pronotum width	6.565	**0.010**
Female age	1.111	0.292
Wing morph	1.912	0.166
Age treatment	0.311	0.577
Song treatment	0.035	0.851
Wing*Age	0.031	0.861
Wing*Song	0.707	0.400
Age*Song	0.507	0.477
Wing*Age* Song	1.390	0.238