Electronic supplementary material for

Sexual selection and ageing: interplay between pre- and post-copulatory traits senescence in the guppy

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Table S1. Correlation matrix of pre- and post-copulatory traits measured in young (a) and old (b) males. Asterisks indicate significant correlations.

(a)

	Brightness	Orange area	Iridescent area	Sperm velocity fresh	Sperm number fresh	Sperm velocity stored	Sperm number stored
Brightness	1						
Orange area	-0.01	1					
Iridescent area	-0.12	-0.36 *	1				
Sperm velocity fresh	0	-0.03	0.13	1			
Sperm number fresh	-0.15	0.13	-0.23	-0.26 *	1		
Sperm velocity stored	0.03	-0.03	0.04	0.37 *	-0.09	1	
Sperm number stored	-0.34 *	0.16	-0.13	-0.11	0.53 *	-0.05	1

(b)

	Brightness	Orange area	Iridescent area	Sperm velocity fresh	Sperm number fresh	Sperm velocity stored	Sperm number stored
Brightness	1						
Orange area	-0.06	1					
Iridescent area	-0.29	0.01	1				
Sperm velocity fresh	0.14	0.15	-0.02	1			
Sperm number fresh	-0.12	0.02	0.12	0.02	1		
Sperm velocity stored	0.26	-0.04	-0.14	0.28	0.19	1	
Sperm number stored	-0.1	-0.13	-0.2	0.24	0.35 *	0.47 *	1

Table S2. Senescence of pre- and post-copulatory traits. Senescence of traits between young and old males (reported as old – young trait values), traits that were significantly different are reported in bold. Colours are reported relative to body area. P-values were obtained using separated paired t-tests for each trait and are reported after correction for multiple comparisons (false discovery rate, FDR). Sperm number is reported $x10^6$.

Trait	Mean difference %	Mean difference (± SE)	CI	Young (mean ± SE)	Old (mean ± SE)	N	*t*	adjusted *p*	direction
Body size (SL)	0.90	0.139 ± 0.06	0.02; 0.25	16.15 ± 0.09	16.34 ± 0.10	48	2.364	**0.037**	**increase**
Orange area	-19.46	-1.866 ± 0.40	-2.67; -1.06	8.44 ± 0.37	3.03 ± 0.42	45	4.679	**<0.001**	**decrease**
Orange brightness	-21.65	-0.987 ± 0.23	-1.46; -0.51	4.10 ± 0.11	3.03 ± 0.21	48	4.203	**<0.001**	**decrease**
Iridescent area	-27.92	-4.591 ± 0.75	-6.11; -3.07	13.75 ± 0.39	9.71 ± 0.58	45	6.102	**<0.001**	**decrease**
Sperm number *	-9.97	-0.900 ± 0.44	-1.79; -0.01	7.17 ± 0.26	6.47 ± 0.45	47	2.037	0.067	NS
Sperm velocity *	-2.56	-3.969 ± 2.10	-8.19; 0.25	129.26 ± 1.12	124.97 ± 0.45	47	1.891	0.081	NS
Sperm number (fresh)	-3.17	-0.281 ± 0.47	-1.23; 0.67	4.89 ± 0.21	4.70 ± 0.49	42	0.597	0.616	NS
Sperm number (stored)	-12.67	-1.792 ± 0.64	-3.09; -0.50	9.53 ± 0.38	8.29 ± 0.59	43	2.793	**0.016**	**decrease**
Sperm velocity (fresh)	-5.96	-8.975 ± 2.51	-14.03; -3.90	136.21 ± 1.31	128.34 ± 1.90	43	3.576	**0.002**	**decrease**
Sperm velocity (stored)	0.72	-0.169 ± 2.66	-5.53; 5.19	122.32 ± 1.39	120.41 ± 1.97	44	0.064	0.950	NS

* average between fresh and stored sperm

Table S3. Cohen's d effect sizes (difference/SD_{diff}) with their standard errors. Positive and negative effect size values indicate an increased and a decreased phenotypic value in old males, respectively. Standard errors were calculated using a bootstrap procedure (10,000 iterations), using PopTools in Excel [1]. *P* values were derived from the proportion of bootstrapped values that were greater or smaller than 0, according to whether the corresponding Cohen's d value was positive or negative. Effect sizes' standard errors correspond to the standard deviation of the bootstrapped distribution. Both pre- and post-copulatory traits had significantly negative Cohens' d values, but mean effect size was significantly larger (in absolute value) for pre-copulatory traits.

	Cohen's d	**SE**	***p*** [(a)]
Body size	0.342	0.132	0.0060
Orange brightness	-0.640	0.210	0.0000
Orange area	-0.712	0.142	0.0000
Iridescent area	-0.936	0.194	0.0000
Sperm velocity	-0.272	0.151	0.0330
Sperm number	-0.339	0.173	0.0156
Mean pre-copulatory traits [(b)]	-0.762	0.106	0.0000
Mean post-copulatory traits [(c)]	-0.305	0.115	0.0026
Difference between the mean effect sizes of pre- and post-copulatory traits [(d)]	0.457	0.157	0.0024

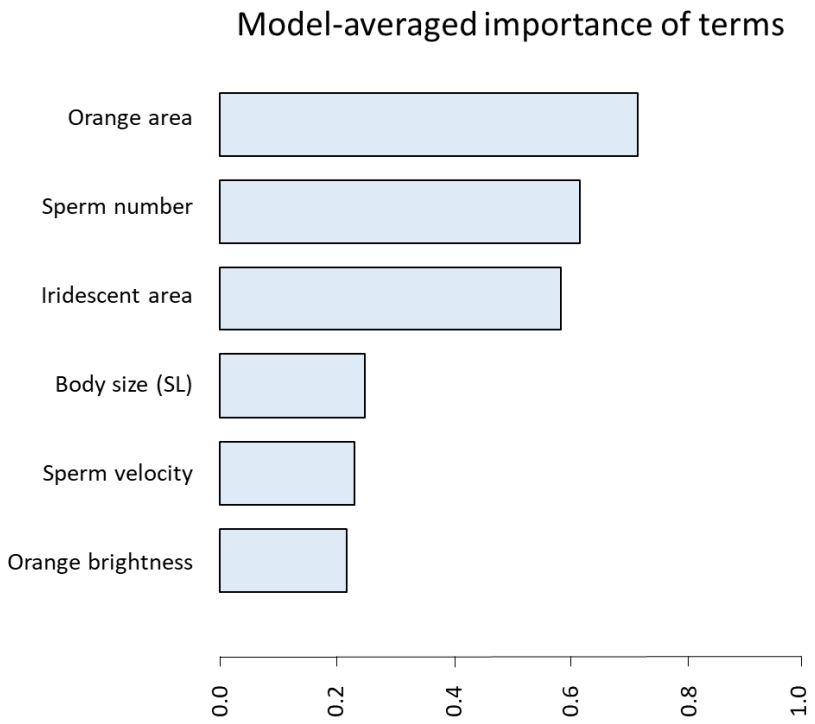
[(a)] represents the probability that Cohen's d is different from 0;

[(b)] average Cohen's d for brightness, orange and iridescent;

[(c)] average Cohen's d for sperm velocity and sperm number;

[(d)] difference between pre- and post-copulatory mean Cohen's d values.

Figure S1. A visual representation of the relative importance (see statistical analysis for details) of the various model terms that predict lifespan according to investment in pre- and post-copulatory traits across all models.



SUPPLEMENTARY METHODS

Measurements of orange brightness

The method we used circumvents the common problem of light reflectance on the body surface that may interfere with the assessment of the area, but most importantly with the spectral proprieties of the coloured spots. Briefly, each male was placed in a small tank (20x7x20 cm), lit by overhead circular fluorescence lamp (Circolux EL 24W, Osram), that limited excessive wandering of the fish. Once the male was acclimatised (usually within 1 minute), a net made of transparent acrylic was used to briefly confine the male close to the front glass of the tank, so that his left side could be photographed (using a Canon EOS 450D camera mounting a Canon EF-S 60-mm Macro USM lens). A ruler and a simulated Gretag Macbeth Pantone were included in each image to provide a reference for size and colour calibration. Spectral proprieties of orange spots were estimated using the software ColourWorker (http://colourworker.com/index.html) with orange (guppy-specific) spectra used as reference [2, 3]. For each male the largest orange spot was chosen, and the spectral data obtained used to calculate an index of brightness (the area under the reflectance spectrum), with low values indicating a dull orange spot [see 3].

References:

1. Hood G.M. 2011 *PopTools version 3.2.5*, Available on the internet. URL http://www.poptools.org.
2. Rahman M.M., Kelley J.L., Evans J.P. 2013 Condition-dependent expression of pre- and postcopulatory sexual traits in guppies. *Ecology and Evolution* **3**(7), 2197–2213.
3. Gasparini C., Kelley J.L., Evans J.P. 2014 Male sperm storage compromises sperm motility in guppies. *Biol Lett* **10**(11).