

Predation shapes sperm performance surfaces in guppies

Supplementary material

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Population sampling

Male guppies were collected from nine rivers spanning the Caroni, Oropouche and Northern drainages in Trinidad's Northern Range Mountains in May-June 2011 [see table S1 and 1 for details]. Trinidadian rivers are characterized by the presence of natural and artificial waterfalls that impede fish migration from downstream to upstream [2]. As a result, in upstream sites, the main guppy predator is *Rivulus haarti*, a small killifish, which feeds mainly on guppy juveniles, while in downstream sites *Crenicichla alta* and *Hoplias malabaricus* exert strong predation pressure on adults [3].

Overall, we sampled 540 males from nine rivers (see table S1); in each river, 30 males came from a low predation site (upstream populations) and 30 were taken from a high predation site (downstream populations). We used a combination of hand seine netting and dip nets (depending on the river conditions) to capture fish. Collections were approved by the Director of Fisheries, Fisheries Division, Ministry of Food Production (Trinidad and Tobago) and all animal procedures were approved by UWA's Animal Ethics Committee (permit number RA/3/100/513).

Table S1. List of the rivers and GPS coordinates of the locations where fish were collected from in Trinidad's Northern Range Mountains.

River Name	High predation site		Low predation site	
	GPS coordinates (DM)		GPS coordinates (DM)	
	North	West	North	West
Aripo	10° 39.029'	61° 13.401'	10° 41.134'	61° 13.942'
Caura	10° 41.354'	61° 21.524'	10° 42.683'	61° 20.982'
Guanapo	10° 37.248'	61° 15.047'	10° 42.706'	missing
	10° 38.389'	61° 14.904'		
	10° 38.530'	61° 14.783'		
Lopinot	10° 38.329'	61° 19.058'	10° 41.595'	61° 19.330'
Quare	10° 39.147'	61° 11.382'	10° 40.554'	61° 11.788'
St Joseph	10° 39.245'	61° 24.749'	10° 42.806'	61° 23.889'
Tacarigua/Tunapuna	10° 38.520'	61° 22.468'	10° 40.140'	61° 23.410'
Turure	missing	missing	10° 40.783'	61° 10.024'
Yarra	10° 47.372'	61° 21.216'	10° 44.405'	61° 19.279'

Sperm assays

Ejaculates were manually obtained via abdominal massage, using standard procedures [1], into a sperm extender medium (207 mM NaCl, 5.4 mM KCl, 1.3 mM CaCl₂, 0.49 mM MgCl₂, 0.41 mM MgSO₄, 10mM Tris, pH 7.5) to ensure that sperm

remained quiescent until activated for sperm performance assays. The stripped ejaculates were divided into two sub-samples for analysing sperm velocity and sperm morphology. To estimate sperm velocity, sperm were activated with 150 mM KCl [4] and immediately placed in a single well of a 12-well multitest slide (MP Biomedicals, Aurora, OH, USA).

The sample, viewed under x100 magnification (negative phase x10 objective), was recorded using a Canon EOS 600 digital camera fitted to a Leica DM750 microscope. Obtained video-footage of motile sperm were subsequently analysed using computer assisted sperm analysis (CASA) via a CEROS Sperm Tracker (Hamilton Thorne Research, Beverly, MA, USA) to quantify sperm velocity. Sperm velocity was assessed for 480 males, meaning that usable samples were not obtained from 60 males (i.e. ejaculates were either unable to be extracted, there were technical difficulties in obtaining usable sperm velocity data, or number of motile sperm cells was <10, our minimum value for sample inclusion). Sperm velocity was measured for 70.6 ± 1.3 (mean \pm SE) sperm per male (see supplementary table 1 for details). To estimate sperm morphology, sperm were preserved in 10% buffered formalin and 20 individual sperm cells from each male were measured. Due to poor preservation of samples from some males, sperm morphology could only be assessed in 264 males (134 males from low predation sites and 130 males from high predations sites). We photographed sperm cells under a 400x magnification microscope

(Leica DM750) and then used ImageJ [5] to measure sperm head, midpiece and flagellum length.

Differences between high and low-predation populations in male traits

We compared male traits (fish area, orange, iridescent, black, sperm velocity and the sperm length measures) between high and low predation sites using a series of linear mixed-effects models fit by REML, using the lmer function in lme4 R package [6]. For each model, the trait of interest was entered as the dependent variable and predation regime (hereafter “Predation”) was entered as a fixed effect with two levels (high/low). To account for variation in the effect of predation both within and among rivers, our analyses incorporate the random effects of river (treated as random intercepts) and Predation (treated as random slopes). Table S2 shows the number of individuals measured for each trait in each river. In table S3 we show results of the models.

The R syntax of the models was:
`lmer(trait~Predation+(Predation+1|River))`.

Table S2. Number of individuals measured for each trait in each sampled population.

	Site	Number of complete measures	Sperm performance*	Fish morphology and colouration**	Sperm morphology***
High predation	Aripo	18	27	30	20
	Caura	19	29	30	20
	Guapo	8	29	30	9
	Lopinot	27	29	30	28
	Quare	8	30	30	8
	St Joseph	13	27	29	15
	Tacarigua/Tunapuna	10	28	30	10
	Turure	11	29	30	12
	Yarra	8	26	30	8
	High predation, total	122	254	269	130
Low predation	Aripo	20	28	30	20
	Caura	6	13	30	12
	Guapo	8	25	30	9
	Lopinot	25	29	30	25
	Quare	11	30	30	11
	St Joseph	15	24	30	17
	Tacarigua/Tunapuna	16	29	30	16
	Turure	3	27	30	5
	Yarra	14	21	30	19
	Low predation, total	118	226	270	134

* Sperm velocity, VAP.

** Body area, orange area, iridescent area and black area.

*** Sperm head, midpiece and flagellum length.

Table S3. Differences between guppy populations from high and low-predation sites in body size (lateral fish area, caudal fin included. Hereafter body area), ornamentation (size of coloured spots) and ejaculate characteristics (sperm velocity and sperm morphology). Linear mixed models fit by REML (lmer). Predation regime was entered as fixed factor (two-level factor: high/low predation). River and predation regime within river were entered as random factors.

Trait	Low Predation mean \pm S.D. (N)	High Predation mean \pm S.D. (N)	Effect size (d)	F. value	Den DF	p
Body area (mm ²)	78.72 \pm 12.89 (270)	65.94 \pm 12.04 (269)	1.02	9.27	8.00	0.02
Orange area (mm ²)	6.7 \pm 3.93 (270)	4.36 \pm 2.4 (269)	0.72	16.17	8.00	<0.01
Iridescent area (mm ²)	10.31 \pm 3.26 (270)	8.37 \pm 2.86 (269)	0.63	3.44	8.00	0.10
Black area (mm ²)	1.34 \pm 0.97 (270)	1.01 \pm 0.66 (269)	0.40	9.52	7.99	0.02
Sperm velocity (VAP, μ m/s)	93.75 \pm 22.02 (226)	95.31 \pm 21.91 (254)	0.07	0.41	8.13	0.54
Sperm head (μ m)	4.32 \pm 0.16 (134)	4.3 \pm 0.13 (130)	0.13	0.09	7.46	0.77
Midpiece (μ m)	3.03 \pm 0.3 (134)	2.98 \pm 0.23 (130)	0.17	1.64	7.61	0.24
Flagellum length (μ m)	48.04 \pm 1.15 (134)	47.17 \pm 0.95 (130)	0.82	15.61	6.89	<0.01

Performance analysis method step by step

In their seminal papers [7-9], Arnold and coauthors developed a two-step methods consisting of 1) determining the effect of morphology on performance and 2) determining the effect of performance of fitness. Here we focused and developed the first part of Arnold’s approach, which we called “performance analysis”. Performance analysis comprises the same steps as standard selection analysis [7, 10], with the difference that the dependent variable is not a fitness estimate but a general performance estimate (e.g. swimming speed, strength of bite, intensity of display). The detailed steps of the procedure include the following:

1. The first step consists of standardizing the trait’s measurement to a mean of zero and standard

deviation of one. Standardised trait scores are calculated by subtracting the population mean value from the observed value and dividing this difference by the standard deviation. Similar to estimates of relative fitness, performance estimates are also standardized to a mean of one by dividing the observed value by the population [sample] mean.

2. Standardized trait values and performance estimates are entered as predictors and dependent variables, respectively, in a multiple regression analyses to obtain linear performance gradients (β_p coefficients). A second regression is then performed with the same predictors along with the quadratic [squared] and correlational [cross-product] terms. Coefficients of these variables represent the non-linear performance gradients (γ_p

coefficients). Note that we used the subscript “p” (performance) for the gradients to differentiate these values from those generated through classic selection analyses.

3. Canonical analysis involves rotating γ_p (through a matrix diagonalization) to obtain a new set of multivariate variables similar to a PCA and representing the directions of the fitted surface where the non-linear relationships are stronger [11]. In the new matrix, λ_p eigenvalues describe the curvature of the surface.
4. The new transformed variables (the value for each individual in each vector) can be used in a full multiple regression [12] to obtain linear gradients (θ_p) and p-values for both θ_p and λ_p .
5. Alternatively, significance of new gradients can be tested with a permutational approach [e.g. 13].
6. To visualize the relationships between original traits or vectors and performance, non-parametric 2D and/or 3D spline functions [14] can be drawn.

Testing the effect of predation on performance gradients

To test if performance gradients (β_p and γ_p) are affected by predation regime we developed an approach based on permutations and Monte Carlo simulations similar to that proposed by Bisgaard and Ankenman [12] and used by different authors [15-17]. This method consists of generating a distribution of performance gradients with 10000

simulated populations of 120 individuals (the average number of individuals in low and high predation populations) shuffled from the full dataset. The gradients are calculated with the regression method described above. Observed gradients obtained with a full multiple regression within each population type are then compared to the null distribution and shown in table S5 (see also figure S1). Observed values that fall outside the upper or lower 95 percentiles in the simulated distribution are considered significantly different from the respective simulated values. Importantly, our approach does not shuffle performance against trait values, but instead generates new populations of individuals where the association between traits and performance is maintained but the distribution of the phenotypes in the simulated populations is random and independent of predation regime. We thus did not remove association between traits and performance at the individual level, rather we removed the differences at the population level due to the grouping factor (predation). Each randomized population was therefore different in the level of predation regime, which could potentially vary from “complete high predation” (when all individuals were drawn from populations of high predation) to “complete low predation” (when all individuals were drawn from populations of low predation). With a similar procedure we tested if the observed differences in performance gradients between low and high predation populations are bigger than expected by chance. To this end we first calculated the observed absolute

difference between the coefficients in low and high predation populations. We then compared the observed differences with a distribution of 10000 differences obtained from regressions performed in simulated populations as above. Observed

differences greater than the 95% upper percentile of the simulated distribution were considered significant (shown in table S6 and figure S2). Monte Carlo simulations were performed with PopTools [18] and R.

Table S4. Performance gradients (β_p and γ_p coefficients) representing the relationships between sperm swimming speed (VAP) and other male traits, including body size (body area, mm²), ornamentation (orange, black and iridescent colouration, mm²), and sperm morphology (head, midpiece and flagellum lengths, μ m) traits for (a) low and (b) high predation populations. In parenthesis P values obtained by multiple regression models. Quadratic performance gradients have been doubled [19]. Low and high predation populations were treated separately. Significant values are indicated in bold.

Traits		β_p coefficients	γ_p coefficients						
			Body Size	Ornamentation			Sperm morphology		
			Body area	Orange	Black	Iridescent	Head	Midpiece	Flagellum
(a) Low Predation (n=118)	Body area	0.018 (0.567)	0.136 (0.103)						
	Orange area	-0.003 (0.913)	-0.076 (0.145)	-0.022 (0.706)					
	Black area	0.009 (0.696)	-0.001 (0.977)	0.065 (0.040)	0.012 (0.776)				
	Iridescent area	0.046 (0.127)	-0.063 (0.395)	0.049 (0.302)	0.01 (0.863)	0.016 (0.849)			
	Sperm head	0.001 (0.950)	0.011 (0.846)	-0.055 (0.276)	0.035 (0.359)	0.112 (0.035)	0.106 (0.097)		
	Midpiece	0.026 (0.265)	-0.03 (0.471)	-0.019 (0.554)	0.009 (0.777)	0.045 (0.413)	0.046 (0.335)	-0.01 (0.792)	
	Flagellum length	0.027 (0.253)	0.053 (0.308)	0.023 (0.480)	-0.037 (0.333)	-0.039 (0.419)	0.071 (0.094)	0.069 (0.071)	0.018 (0.732)
(b) High Predation (n=122)	Body area	0.017 (0.545)	0.036 (0.574)						
	Orange area	0.024 (0.269)	-0.031 (0.397)	0.110 (0.008)					
	Black area	-0.001 (0.946)	0.026 (0.518)	-0.129 (0.002)	0.060 (0.070)				
	Iridescent area	-0.002 (0.942)	-0.049 (0.295)	-0.001 (0.968)	-0.037 (0.360)	0.092 (0.103)			
	Sperm head	0.036 (0.086)	0.047 (0.234)	-0.03 (0.328)	-0.054 (0.094)	0.019 (0.574)	0.040 (0.334)		
	Midpiece	0.007 (0.718)	0.029 (0.472)	0.002 (0.918)	0.003 (0.922)	-0.047 (0.208)	0.036 (0.165)	-0.002 (0.939)	
	Flagellum length	0.041 (0.064)	-0.039 (0.402)	-0.044 (0.145)	-0.003 (0.933)	0.066 (0.150)	0.065 (0.024)	-0.027 (0.388)	0.040 (0.322)

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Table S5. Observed performance gradients obtained with a full regression for the two predation regimes separately and compared to 95% C.I. obtained with 10000 simulated full regressions. Values with * are significant regression values. Values with # are values that fall outside the 95% C.I. intervals of the simulated distribution. Simulated performance gradients (mean and 95% C.I.) are also reported.

	Trait	Observed value		Simulated (10000 regressions)	
		High predation	Low predation	Mean	95%C.I.
Linear β_p	Size	0.021	0.004	0.011	-0.046 0.068
	Orange	0.009	0.001	0.000	-0.046 0.045
	Black	-0.042	0.014	0.007	-0.050 0.064
	Iridescent	0.001	0.055	0.019	-0.035 0.072
	Sperm head	-0.013	0.053	0.028	-0.022 0.080
	Midpiece	0.004	0.003	-0.001	-0.044 0.042
	Flagellum length	0.011	-0.013	-0.005	-0.054 0.045
Quadratic γ_p	Size	0.037	0.137	0.065	-0.034 0.170
	Orange	0.110*	-0.022	0.044	-0.036 0.122
	Black	0.060#	0.012	-0.010	-0.094 0.059
	Iridescent	0.092	0.015	0.040	-0.070 0.145
	Sperm head	0.041	0.106	0.036	-0.050 0.113
	Midpiece	-0.003	-0.010	0.014	-0.044 0.071
	Flagellum length	0.041	0.017	0.039	-0.026 0.114
Quadratic-Correlational γ_p	Size*Orange	-0.031	-0.076	-0.021	-0.089 0.045
	Size*Black	0.026	-0.001	0.015	-0.059 0.091
	Size*Iridescent	-0.049	-0.063	-0.053	-0.132 0.026
	Size*Sperm head	0.047	0.011	0.019	-0.053 0.095
	Size*Midpiece	0.029	-0.030	0.014	-0.059 0.084
	Size*Flagellum	-0.039	0.053	0.009	-0.053 0.068
	Orange*Black	-0.128*#	0.066*	0.004	-0.070 0.069
	Orange*Iridescent	-0.001	0.050	-0.010	-0.072 0.051
	Orange*Sperm head	-0.030	-0.055	-0.015	-0.073 0.045
	Orange*Midpiece	0.002	-0.019	0.005	-0.037 0.054
	Orange*Flagellum	-0.044	0.023	-0.012	-0.059 0.036
	Black*Iridescent	-0.037	0.010	-0.027	-0.111 0.045
	Black*Sperm head	-0.054	0.036	-0.019	-0.080 0.042
	Black*Midpiece	0.003	0.009	0.008	-0.045 0.065
	Black*Flagellum	-0.003	-0.037	-0.005	-0.066 0.057
	Iridescent*Sperm head	0.019	0.112*#	0.030	-0.040 0.096
	Iridescent*Midpiece	-0.047	0.045	-0.018	-0.085 0.054
	Iridescent*Flagellum	0.066	-0.039	0.009	-0.059 0.076
	Sperm head*Midpiece	0.036	0.046	0.033	-0.019 0.093
	Sperm head*Flagellum	0.065*	0.071	0.052	-0.008 0.103
Midpiece*Flagellum	-0.027	0.069#	0.015	-0.036 0.064	

Figure S1. Observed performance gradients obtained for low (blue triangles) and high (red dots) predation populations are compared to 95% C.I. of the distribution of performance gradients obtained with 10000 simulated regressions. In each of these regressions a simulated population ($n=120$) was obtained shuffling individuals from the original complete dataset ($n=240$).

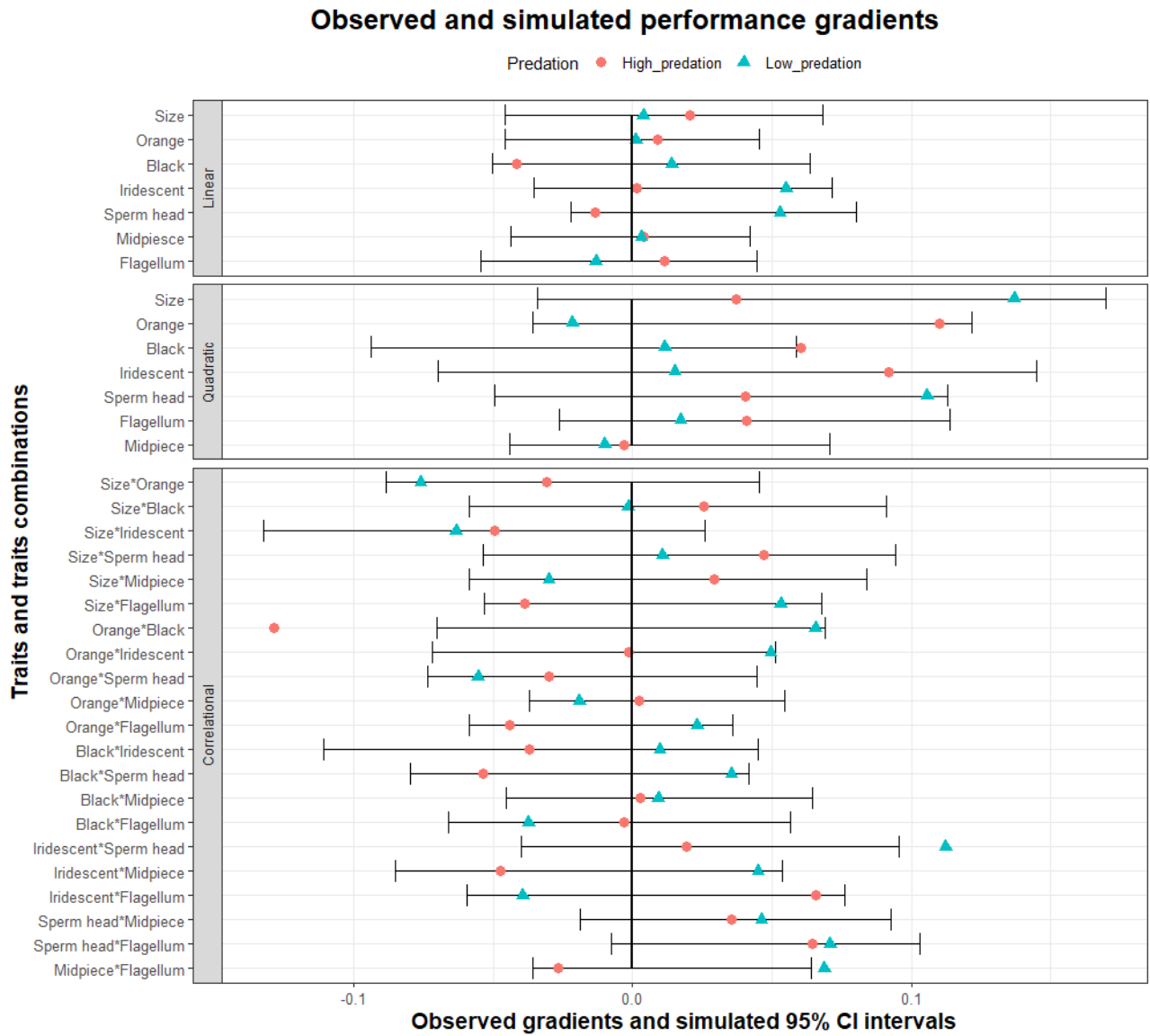
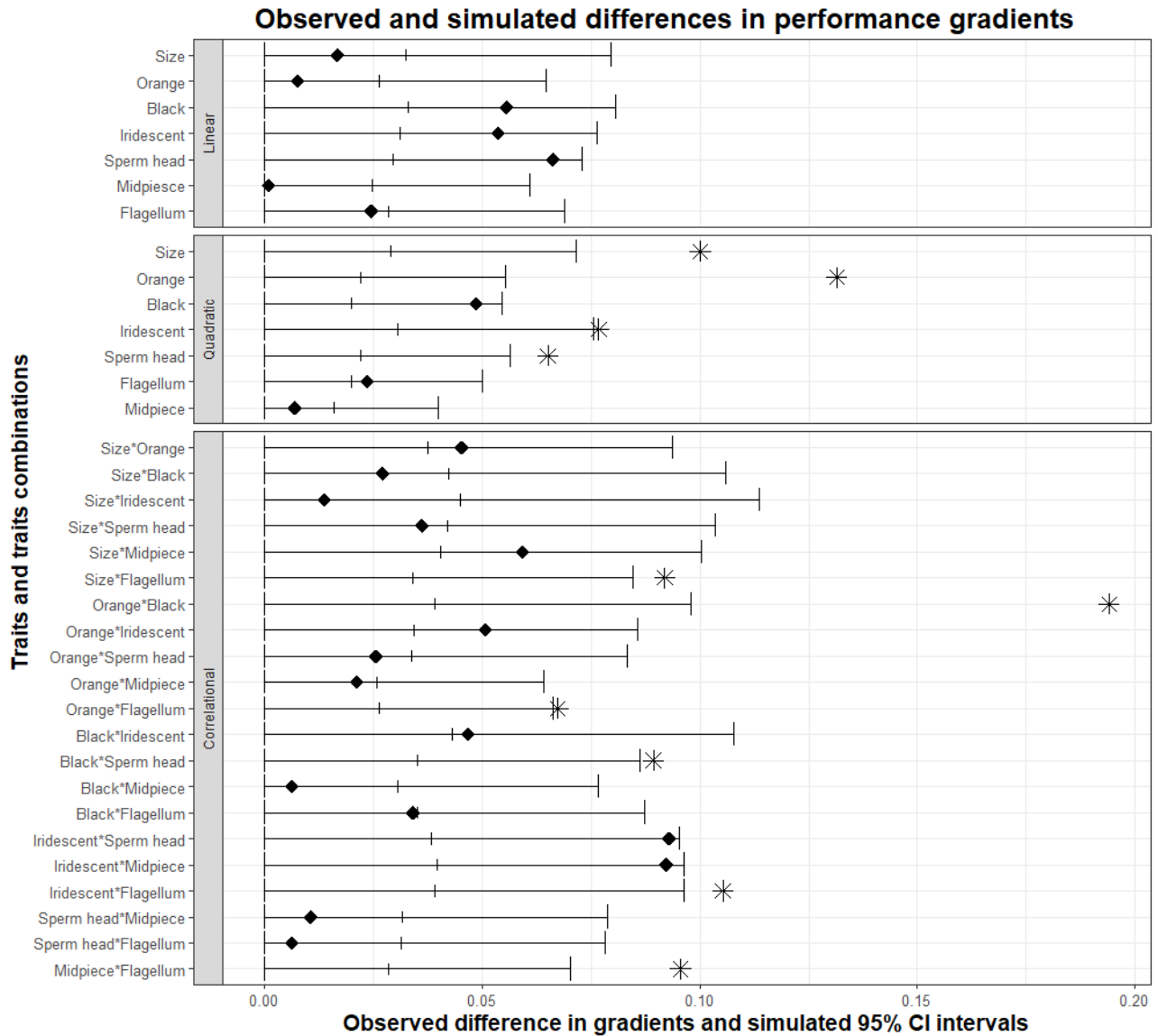


Table S6. Observed absolute difference in performance gradients obtained with a full regression for the two predation regimes separately and compared to the upper 95% percentile of a distribution of absolute differences obtained after 10000 iterations where two full regressions on two different simulated populations were performed. Values with # are values bigger than the upper 95% percentile of the simulated distribution. Simulated differences (mean and upper 95% percentile) are also reported.

		Trait	Observed difference	Simulated (10000 iterations)	
				Mean	Upper 95% percentile
Linear β_p		Size	0.017	0.033	0.080
		Orange	0.008	0.026	0.065
		Black	0.056	0.033	0.081
		Iridescent	0.054	0.031	0.076
		Sperm head	0.066	0.030	0.073
		Midpiece	0.001	0.025	0.061
		Flagellum length	0.025	0.029	0.069
Quadratic γ_p		Size	0.100#	0.029	0.072
		Orange	0.131#	0.022	0.055
		Black	0.049	0.020	0.055
		Iridescent	0.077#	0.030	0.076
		Sperm head	0.065#	0.022	0.056
		Midpiece	0.007	0.016	0.040
		Flagellum length	0.024	0.020	0.050
Quadratic-Correlational γ_p		Size*Orange	0.045	0.038	0.094
		Size*Black	0.027	0.042	0.106
		Size*Iridescent	0.014	0.045	0.114
		Size*Sperm head	0.036	0.042	0.103
		Size*Midpiece	0.059	0.040	0.100
		Size*Flagellum	0.092#	0.034	0.085
		Orange*Black	0.194#	0.039	0.098
		Orange*Iridescent	0.051	0.034	0.086
		Orange*Sperm head	0.026	0.034	0.083
		Orange*Midpiece	0.021	0.026	0.064
		Orange*Flagellum	0.067#	0.026	0.066
		Black*Iridescent	0.047	0.043	0.108
		Black*Sperm head	0.089#	0.035	0.086
		Black*Midpiece	0.006	0.031	0.077
		Black*Flagellum	0.034	0.035	0.087
		Iridescent*Sperm head	0.093	0.038	0.095
		Iridescent*Midpiece	0.092	0.040	0.096
		Iridescent*Flagellum	0.105#	0.039	0.096
		Sperm head*Midpiece	0.011	0.032	0.079
		Sperm head*Flagellum	0.006	0.031	0.078
	Midpiece*Flagellum	0.096#	0.029	0.070	

Figure S2. Observed absolute difference (diamonds and asterisks) in performance gradients between low and high predation populations are compared to 95% upper percentile of the distribution of differences in performance gradients obtained with 10000 simulations. In each of these simulations the difference between the same gradients was obtained from two regressions performed in two simulated populations ($n=120$) made by shuffled individuals from the original complete dataset ($n=240$). Asterisks represents absolute differences greater than the 95% upper percentile of the simulated distribution.



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