

1 **Most published selection gradients are underestimated: why this is and how to fix it**

2 Niels J. Dingemanse, Yimen G. Araya-Ajoy, David F. Westneat

3 **--Supplementary Material--**

4 **Glossary**

5 Supplementary Text S1.....Page 1
6 Supplementary Text S2.....Page 13
7 Supplementary Text S3.....Page 13
8 Supplementary Text S4.....Page 16
9 Supplementary Text S5.....Page 18
10 Supplementary Text S6.....Page 20
11 Supplementary Text S7.....Page 23
12 Supplementary Text S8.....Separate Document

14 **Supplementary Text S1**

15 *Literature Review*

16 To assess the extent of awareness of trait repeatability as an important source of bias in
17 estimates of selection, and the type of estimates that might often be biased, we surveyed the
18 literature for papers presenting estimates of selection. We decided to take a sample of all such
19 estimates by focusing on papers published within the journal *Evolution* in the years 2010-2019
20 inclusive. Our strategy was first to identify all papers published in that time period that cited
21 Lande & Arnold (1983). These were then pruned by skimming abstracts to assess if selection
22 gradients of empirical data were likely to be presented. We then retrieved each paper and read
23 the methods and results to confirm that gradients were indeed estimated. If so, we assessed the
24 following variables: the trait(s) that were analysed, labelled as the authors chose and scored as
25 to type (morphological, life history, physiological, performance, or behavioural) because
26 repeatability varies considerably among these classes of traits. We used information in the
27 methods to assess whether traits were mean-centred prior to analysis and whether repeated
28 measures of the trait were taken (and if so, how many repeats). We further scored whether the
29 authors used individual values, the mean, or some other technique such as principal components
30 analysis that combines individual data points (and mean-centres as well), and whether trait
31 correlations were presented in analyses of correlational selection. We also noted if the author(s)
32 mentioned measurement error and repeatability in the paper, and if so, if they specified the trait
33 repeatability. Finally, we noted what type of selection was estimated (directional, quadratic, or
34 correlational) and any unusual elements of the analysis beyond the standard regression approach
35 described by Lande & Arnold (1983), such as use of path analysis, linear mixed models, aster
36 analysis, or bivariate mixed-effects models.

37 Our survey resulted in 68 papers producing 311 trait estimates (we did not count
38 replicate populations or years). Most estimates were on morphological traits (195; 63%), but
39 38 (12%) were of behavioural traits, 26 (8%) of physiological, 31 (10%) were of life history
40 characters, and 21 (7%) of performance traits. All traits were used to estimate directional
41 selection; quadratic selection was also measured for 178 (57%), and correlational selection was
42 assessed among 107 (34%). Table 1 (Main Text) provides summary statistics over all papers
43 and Table S1 provides information extracted per paper.

44 **Table S1.** Studies publishing estimates of linear and nonlinear selection in *Evolution* from 2010 -2019, with species, trait studied, category of trait
 45 (MO=morphological, BEH = Behavioral, LH = Life history, PHY = Physiological, PER = Performance), fitness measure (L = lifetime, typically
 46 survival; E = one measure of an episode of fitness; E2 = at least two measures of episodic fitness), number of measures taken, whether the mean was
 47 used if more than 1 measure (or if ≥ 2 traits were combined with PCA), whether repeatability was mentioned and its magnitude if known, type of
 48 selection measured (D = directional, Q = quadratic, C = correlational), whether multivariate models were used, if traits were mean-centred before
 49 analysis (? = either authors did not say or simply stated they “standardized” without defining; residuals and PCA were counted as mean-centred) and
 50 if among-trait correlations were provided in cases of non-linear selection. Entries left blank if non-applicable.

Authors	Year	Citation	Species	Trait	Category	Fitness	Measures	Used mean	Mentioned repeatability	Type of selection measured	Used multivariate	Traits mean-centred	Trait correlations estimated
Reynolds et al.	2010	64(2):358-369	<i>Silene virginica</i>	Petal length	MO	L	2+	Yes	No	D,Q,C	No	?	No
				Petal width	MO	L	2+	Yes	No	D,Q,C	No	?	No
				Flower height	MO	L	2+	Yes	No	D,Q,C	No	?	No
				Corolla length	MO	L	2+	Yes	No	D,Q,C	No	?	No
				Corolla width	MO	L	2+	Yes	No	D,Q,C	No	?	No
				Stigma exertion	MO	L	2+	Yes	No	D,Q,C	No	?	No
van de Pol et al.	2010	64(3):836-851	<i>Haematopus ostralegus</i>	Bill shape	MO	E	1		Yes	D	No	No	
Cox & Calsbeek	2010	64(3):798-809	<i>Anolis sagrei</i>	Body size	MO	L	1		No	D,Q	No	?	No
Siepeliski & Benkman	2010	64(4):1120-1128	<i>Pinus flexilis</i>	PC1	MO	E	2	Yes	Yes	D	No	PCA	
Freeman-Gallant et al	2010	64(4):1007-1017	<i>Geothlypis trichas</i>	UV brightness	MO	E	4	Yes	No	D	No	Yes	
				Mask area	MO	E	4	Yes	0.9	D	No	Yes	
				Bib area	MO	E	1		0.9	D	No	Yes	
				Carotenoid chroma	MO	E	4	Yes	No	D	No	Yes	
				Yellow brightness	MO	E	4	Yes	No	D	No	Yes	
Weese et al.	2010	64(6):1802-1815	<i>Poecilia reticulata</i>	Body size	MO	L	1		No	D	No	Yes	
				Black	MO	L	1		No	D	No	Yes	
				Green	MO	L	1		No	D	No	Yes	
				Carotenoid	MO	L	1		No	D	No	Yes	
				Structural	MO	L	1		No	D	No	Yes	
				Total Color	MO	L	1		No	D	No	Yes	
Perez & Munch	2010	64(8):2450-2457	<i>Fish sp.</i>	Body size	MO	L	?		No	D,Q,C	No	Yes	No
						E					No	Yes	No
				Bower base	BEH	E	1		No	D,Q,C	No	Yes	No
				Bower platform	BEH	E	1		No	D,Q,C	No	Yes	No
Baythavong & Stanton	2010	64(10):2904-2920	<i>Erodium cicutarium</i>	Emergence timing MP	LH	L	6		No	D	No	?	No

				Emergence timing L	LH	L	6	No	D	No	?	No
				Emergence timing Q	LH	L	6	No	Q	No	?	No
				Cotyledon width MP	MO	L	6	No	D	No	?	No
				Cotyledon width L	MO	L	6	No	D	No	?	No
				Cotyledon width Q	MO	L	6	No	Q	No	?	No
				Early leaf number MP	MO	L	6	No	D	No	?	No
				Early leaf number L	MO	L	6	No	D	No	?	No
				Early leaf number Q	MO	L	6	No	Q	No	?	No
				Leaf turnover MP	MO	L	6	No	D	No	?	No
				Leaf turnover L	MO	L	6	No	D	No	?	No
				Leaf turnover Q	MO	L	6	No	Q	No	?	No
				FFdateMP	MO	L	6	No	D	No	?	No
				Ffdate L	MO	L	6	No	D	No	?	No
				Ffdate Q	MO	L	6	No	Q	No	?	No
				Leaves MP	MO	L	6	No	D	No	?	No
				Leaves L	MO	L	6	No	D	No	?	No
				Leaves Q	MO	L	6	No	Q	No	?	No
Sullivan-Beckers & Cocroft	2010	64(11):3158-3171	<i>Enchenopa binotata</i>	Survival	LH	E	1	No	D,Q	No	?	No
				Weight	MO	E	1	No	D,Q	No	?	No
				Signals per bout	BEH	E	1	0.27	D,Q	No	?	No
				Period	BEH	E	1	0.1	D,Q	No	?	No
				Whine length	BEH	E	1	0.06	D,Q	No	?	No
				Pulse rate	BEH	E	1	0.22	D,Q	No	?	No
				Frequency	BEH	E	1	0.32	D,Q	No	?	No
Rundle & Chenoweth	2011	65(3):893-899	<i>Drosophila serrata</i>	CHC1	PHY	E	1	No	D,Q,C	No	Yes	No
				CHC2	PHY	E	1	No	D,Q,C	No	Yes	No
				CHC3	PHY	E	1	No	D,Q,C	No	Yes	No
				CHC4	PHY	E	1	No	D,Q,C	No	Yes	No
				CHC5	PHY	E	1	No	D,Q,C	No	Yes	No
				CHC6	PHY	E	1	No	D,Q,C	No	Yes	No
				CHC7	PHY	E	1	No	D,Q,C	No	Yes	No
				CHC8	PHY	E	1	No	D,Q,C	No	Yes	No
				CHC9	PHY	E	1	No	D,Q,C	No	Yes	No
Sahli & Conner	2011	65(5):1457-1473	<i>Raphanus raphanistrum</i>	Flower number	MO	E2	1	No	D,Q	No	Yes	No
				Flower size	MO	E2	1	No	D,Q	No	PCA	No
				Anther exertion	MO	E2	1	No	D,Q	No	Yes	No
				Stamen dimorphism	MO	E2	1	No	D,Q	No	Yes	No
Postma et al.	2011	65(8):2145-2156	<i>Poecilia reticulata</i>	Black patch size	MO	E	1	No	D,Q,C	No	?	Yes
				Fuzzy black size	MO	E	1	No	D,Q,C	No	?	Yes
				Orange size	MO	E	1	No	D,Q,C	No	?	Yes

				Iridescent size	MO	E	1		No	D,Q,C	No	?	Yes
				Tail size	MO	E	1		No	D,Q,C	No	?	Yes
				Body size	MO	E	1		No	D,Q,C	No	?	Yes
Martin & Pfennig	2011	65(10):2946-2958	<i>Spea multiplicata</i>	Denticle rows	MO	E	1		No	D,Q,C	No	Yes(residuals)	No
				Orbitohyoideus muscle width	MO	E	1		No	D,Q,C	No	Yes(residuals)	No
				Interhyoidius muscle width	MO	E	1		No	D,Q,C	No	Yes(residuals)	No
				Gut length	MO	E	1		No	D,Q,C	No	Yes(residuals)	No
				Mouthpart shape	MO	E	1		No	D,Q,C	No	Yes(residuals)	No
				Denticle rows	MO	E	1		No	D,Q,C	No	Yes(residuals)	No
Leinonen et al.	2011	65(10):2916-2926	<i>Gasterosteus aculeatus</i>	Lateral plate number	MO	L	1		No	D	No	No	Yes
				Spine triangle size	MO	L	1		No	D	No	No	Yes
				Head depth	MO	L	1		No	D	No	No	Yes
				Head length	MO	L	1		No	D	No	No	Yes
				Body depth	MO	L	1		No	D	No	No	Yes
				Pelvic girdle length	MO	L	1		No	D	No	No	Yes
				Caudal peduncle length	MO	L	1		No	D	No	No	Yes
Formica et al.	2011	65(10):2771-2781	<i>Bolitotherus cornutus</i>	Body Size (PC1)	MO	L	5	PCA	No	D	No	PCA	
				Social Body Size	MO	L	10+	Yes	No	D	No	PCA	
				Body Size (PC1)	MO	E	5	PCA	No	D	No	PCA	
				Social Body Size	MO	E	10+	Yes	No	D	No	PCA	
Crean et al.	2011	65(11):3079-3089	<i>Styela plicata</i>	Larval size	MO	L	3	Yes	No	D,Q,C	No	Yes	No
				Hatch time	LH	L	1		No	D,Q,C	No	Yes	No
				Settle time	BEH	L	1		No	D,Q,C	No	Yes	No
				Larval size	MO	E	3	Yes	No	D,Q,C	No	Yes	No
				Hatch time	LH	E	1		No	D,Q,C	No	Yes	No
				Settle time	BEH	E	1		No	D,Q,C	No	Yes	No
Lorenzi & Thompson	2011	65(12):3527-3542	<i>Polistes biglumis</i>	Head width	MO	L	1		No	D,Q	No	?	Yes
				Building effort	PER	L	1		No	D,Q	No	?	Yes
				Brood investment	PER	L	1		No	D,Q	No	?	Yes
				Protective effort	BEH	L	1		No	D,Q	No	?	Yes
Egan et al.	2011	65(12):3543-3557	<i>Belonocnema treatae</i>	Gall size	MO	L	1		No	D,Q	No	Yes	
Kulbaba et al.	2012	66(5):1344-1359	<i>Polemonium brandegeei</i>	Herkegomy	MO	E	1		No	D,Q,C	No	?	No
				Corolla tube length	MO	E	1		No	D,Q,C	No	?	No
				Corolla tube width	MO	E	1		No	D,Q,C	No	?	No
				Sex organ height	MO	E	1		No	D,Q,C	No	?	No
				Flower mass	MO	E	1		No	D,Q,C	No	?	No

Godsen et al.	2012	66(7):2106-2116	<i>Drosophila melanogaster</i>	Nectar volume	PHY	E	1		No	D,Q,C	No	?	No
				Sugar content	PHY	E	2	Yes	No	D,Q,C	No	?	No
				CHC1	PHY	E	1		No	D	No	Yes	No
				CHC2	PHY	E	1		No	D	No	Yes	No
				CHC3	PHY	E	1		No	D	No	Yes	No
				CHC4	PHY	E	1		No	D	No	Yes	No
				CHC5	PHY	E	1		No	D	No	Yes	No
Fitzpatrick et al.	2012	66(8):2451-2460	<i>Mytilus galloprovincialis</i>	CHC6	PHY	E	1		No	D	No	Yes	No
				CHC7	PHY	E	1		No	D	No	Yes	No
				Sperm density	PER	E	2	Yes		D,Q,C	No	?	No
				Sperm head volume	MO	E	30	Yes		D,Q,C	No	?	No
				Sperm flagellum length	MO	E	30	Yes		D,Q,C	No	?	No
				Sperm motility PC1	PER	E	2	Yes	Yes	D,Q,C	No	PCA	No
				Sperm motility PC2	PER	E	2	Yes	Yes	D,Q,C	No	PCA	No
Guerreiro et al	2012	66(11):3615-3623	<i>Mus musculus</i>	Sperm age	PER	E	1		No	D,Q,C	No	?	No
				Egg age	PER	E	1		No	D,Q,C	No	?	No
				IL-6	PHY	L	1		No	D,Q,C	No	Yes	No
				IL-10	PHY	L	1		No	D,Q,C	No	Yes	No
Benkman et al	2013	67(1):157-169	<i>Pinus contorta latifolia</i>	Cone width/length	MO	E	3	Yes	Yes	D,Q	No	No	Yes
				Cone mass	MO	E	3	Yes	Yes	D,Q	No	No	Yes
				Distal scale	MO	E	6	Yes	Yes	D,Q	No	No	Yes
				Scale length	MO	E	6	Yes	Yes	D,Q	No	No	Yes
				Full seeds	PER	E	3	Yes	Yes	D,Q	No	No	Yes
				Empty seeds	PER	E	3	Yes	Yes	D,Q	No	No	Yes
				Seed mass	MO	E	5	Yes	Yes	D,Q	No	No	Yes
Marshall & Monro	2013	67(2):328-337	<i>Watersipora subtorquata</i>	Offspring size	MO	E	1		No	D,Q	No	?	No
				Zooid size	MO	E	1+	Yes	No	D,Q	No	?	No
				Senescence size	MO	E	1		No	D,Q	No	?	No
Wacker et al.	2013	67(7):1937-1949	<i>Gobiusculus flavescens</i>	Length	MO	E	3	Yes	Yes	D	No	Yes	
				Torso area	MO	E	3	Yes	Yes	D	No	Yes	
				Blue spots	MO	E	3	Yes	Yes	D	No	Yes	
				Tail length	MO	E	3	Yes	Yes	D	No	Yes	
Fritzsche & Arnqvist	2013	67(7):1926-1936	<i>Callosobruchus maculatus</i>	Male body size	MO	E	2	Yes	No	D	No	Yes	
				Male body size	MO	E	2	Yes	No	D	No	Yes	
				Male body size	MO	E	2	Yes	No	D	No	Yes	
				Male body size	MO	E	2	Yes	No	D	No	Yes	
				Female body size	MO	E	2	Yes	No	D	No	Yes	
				Female body size	MO	E	2	Yes	No	D	No	Yes	
				Female body size	MO	E	2	Yes	No	D	No	Yes	
				Female body size	MO	E	2	Yes	No	D	No	Yes	

Morrissey & Sakrejda	2013	67(7):2094-2100	<i>Homo sapiens</i>	Birth mass	MO	E	1		No	D,Q,C	No	?	No	
				Gestation length	LH	E	1		No	D,Q,C	No	?	No	
Kulbaba&Worley	2013	67(8):2194-2206	<i>Polemonium brandegeei</i>	Nectar conc	PER	E	2	Yes	No	D,Q	No	Yes	No	
				Tube length	MO	E	2	Yes	No	D,Q	No	Yes	No	
				Tube diameter	MO	E	2	Yes	No	D,Q	No	Yes	No	
				Herkogamy	MO	E	2	Yes	No	D,Q	No	Yes	No	
				Bird visits	PER	E	2	Yes	No	D,Q	No	Yes	No	
				Pollen in anthers	PER	E	2	Yes	No	D,Q	No	Yes	No	
				Pollen on stigma	PER	E	2	Yes	No	D,Q	No	Yes	No	
				Seeds set	PER	E	2	Yes	No	D,Q	No	Yes	No	
Mezquida & Benkman	2014	68(6):1710-1721	<i>Pinus uncinata</i>	Seeds sired	PER	E	2	Yes	No	D,Q	No	Yes	No	
				Cone mass	MO	E	4	Yes	No	D	No	Yes		
				Full seeds	PER	E	4	Yes	No	D	No	Yes		
				Empty seeds	PER	E	4	Yes	No	D	No	Yes		
Sletvold & Agren	2014	68(7):1907-1918	<i>Dactylorhiza lapponica</i>	Seed mass	MO	E	4	Yes	No	D	No	Yes		
				Height	MO	E	1		No	D	No	?		
				Num Flowers	MO	E	1		No	D	No	?		
				Corolla size	MO	E	1		No	D	No	?		
				Spur length	MO	E	1		No	D	No	?		
Gillespie et al	2014	68(12):3421-3432	<i>Narnia femorata</i>	Flowering day	LH	E	1		No	D	No	?		
				Male body size (PC)	MO	E	7	PCA	No	D	No	PCA		
				Female body size (PC)	MO	E	7	PCA	No	D	No	PCA		
Ercit & Gwynne	2015	69(2):419-430	<i>Oecanthus nigricornis</i>	Tegmen width	MO	L	1		No	D,Q,C	No	?	No	
				Leg size (PC1)	MO	L	3	PCA	No	D,Q,C	No	PCA	No	
				Pronotum length	MO	L	1		No	D,Q,C	No	?	No	
				Head width	MO	L	1		No	D,Q,C	No	?	No	
				Tegmen width	MO	E	1		No	D,Q,C	No	?	No	
				Leg size (PC1)	MO	E	3	PCA	No	D,Q,C	No	PCA	No	
				Pronotum length	MO	E	1		No	D,Q,C	No	?	No	
Campobello et al.	2015	69(4):916-925	<i>Falco naumanni</i>	Head width	MO	E	1		No	D,Q,C	No	?	No	
				Individual attendance	BEH	E	1		No	D	No	Yes		
				<i>Corvus Monedula</i>	Individual attendance	BEH	E	1		No	D	No	Yes	
				<i>Falco naumanni</i>	Conspecific attendance	BEH	E	>2	Yes	No	D	No	Yes	
				<i>Corvus Monedula</i>	Conspecific attendance	BEH	E	>2	Yes	No	D	No	Yes	
				<i>Falco naumanni</i>	Heterospecific attendance	BEH	E	>2	Yes	No	D	No	Yes	
				<i>Corvus Monedula</i>	Heterospecific attendance	BEH	E	>2	Yes	No	D	No	Yes	
Weis et al.	2015	69(6):1361-1374	<i>Brassica rapa</i>	Emergence time	LH	L	1		No	D	No	Yes		

Eck et al.	2015	69(9):2525-2532	<i>Manduca sexta</i>	Group emergence	LH	L	>2	Yes	No	D	No	Yes	
				Age to 2nd instar	LH	L	1		No	D,Q,C	No	?	No
				Mass at 2nd instar	MO	L	1		No	D,Q,C	No	?	No
				Mass at eclosion	MO	L	1		No	D,Q,C	No	?	No
Chevin et al.	2015	69(9): 2319-2332	<i>Parus major</i>	First egg date	BEH	E2	1		No	D,Q	No	?	No
Reid et al.	2015	69(11): 2846-2861	<i>Melospiza melodia</i>	Inbreeding Coefficient	BEH	L	1+	Yes	No	D	No	Yes	
Akcay et al.	2015	69(12):3186-3193	<i>Melospiza melodia</i>	Aggression	BEH	L	3.2	Yes	0.48	D,Q,C	No	?	No
Austen & Weis	2016	70(1):111-125	<i>Brassica rapa</i>	Soft song	BEH	L	3.2	Yes	0.3	D,Q,C	No	?	No
				Age at flowering	LH	L	1		No	D	No	Yes	
				Root mass	MO	L	1		No	D	No	Yes	
				Leaves at flowering	MO	L	1		No	D	No	Yes	
Marie-Orleach et al.	2016	70(2):314-328	<i>Macrostomum lignano</i>	Height	MO	L	1		No	D	No	Yes	
				Body size	MO	E	1		0.57 – 0.97	D	No	Yes	
				Testes size	MO	E	1		0.57 – 0.97	D	No	Yes	
				Ovary size	MO	E	1		0.57 – 0.97	D	No	Yes	
				Seminal vesicle size	MO	E	1		0.57 – 0.97	D	No	Yes	
				Stylet centroid size	MO	E	1		0.57 – 0.97	D	No	Yes	
				Stylet RWS1	MO	E	1		0.57 – 0.97	D	No	Yes	
				Stylet RWS2	MO	E	1		0.57 – 0.97	D	No	Yes	
Stylet RWS3	MO	E	1		0.57 – 0.97	D	No	Yes					
Martin	2016	70(6):1265-1282	<i>Cyprinidon sp.</i>	DF1	MO	L	1		No	D,Q	No	Yes	No
				DF2	MO	L	1		No	D,Q	No	Yes	No
Outomuro et al.	2016	70(7):1582-1595	<i>Lestes sponsa</i>	Wing size	MO	L	1		No	D,Q,C	No	Yes	No
				Wing shape PC1	MO	L	1		No	D,Q,C	No	PCA	No
				Wing shape PC2	MO	L	1		No	D,Q,C	No	PCA	No
				Wing size	MO	E	1		No	D,Q,C	No	Yes	No
				Wing shape PC1	MO	E	1		No	D,Q,C	No	PCA	No
				Wing shape PC2	MO	E	1		No	D,Q,C	No	PCA	No
Kvalnes et al.	2016	70(7):1486-1500	<i>Alces alces</i>	Birth date	LH	E2	1		No	D,Q,C	No	Yes	Yes
				Birth mass	MO	E2	1		No	D,Q,C	No	Yes	Yes
Wise & Rausher	2016	70(10): 2411-2420	<i>Solanum carolinense</i>	Damage1	MO	E	1		No	D,Q,C	No	Yes	No
				Damage2	MO	E	1		No	D,Q,C	No	Yes	No
				Damage3	MO	E	1		No	D,Q,C	No	Yes	No
				D4	MO	E	1		No	D,Q,C	No	Yes	No
				D5	MO	E	1		No	D,Q,C	No	Yes	No

				D6	MO	E	1		No	D,Q,C	No	Yes	No
				D7	MO	E	1		No	D,Q,C	No	Yes	No
				D8	MO	E	1		No	D,Q,C	No	Yes	No
				D9	MO	E	1		No	D,Q,C	No	Yes	No
				D10	MO	E	1		No	D,Q,C	No	Yes	No
				D11	MO	E	1		No	D,Q,C	No	Yes	No
				D12	MO	E	1		No	D,Q,C	No	Yes	No
Lange et al.	2016	70(10):2404-2410	<i>Watersipora subtorquata</i>	Early modules	LH	E	1		No	D	No	Yes	
				Intermediate modules	LH	E	1		No	D	No	Yes	
				Late modules	LH	E	1		No	D	No	Yes	
				Growing edge	LH	E	1		No	D	No	Yes	
				Onset senescence	LH	E	1		No	D	No	Yes	
Reed et al.	2016	70(10):2211-2225	<i>Parus major</i>	Lay date	LH	E2	variable	Yes	No	D	Yes	NA	
				Clutch size	LH	E2	variable	Yes	No	D	Yes	NA	
Tofts et al.	2016	70(12):2899-2908	<i>Brachyistius frenatus</i>	FA	MO	L	2		Yes	D,Q	No	Yes	No
Langeloh et al.	2017	71(2):227-237	<i>Lymnaea stagnalis</i>	Growth rate	LH	E2	2	Yes	Yes	D,Q	No	Yes	No
				PO-like activity	PHY	E2	2	Yes	<0.43	D,Q	No	Yes	No
				Antibacterial activity	PHY	E2	2	Yes	<0.43	D,Q	No	Yes	No
Thomson et al.	2017	71(3):716-732	<i>Cyanistes caeruleus</i>	Clutch size(F)	LH	E2	1+	BLUPS	No	D	No	Yes	
				Male attendance	BEH	E2	1+	Yes	No	D	No	No	
				Nestling body mass	MO	E2	6	BLUPS	No	D	No	Yes	
Agren et al	2017	71(3):550-564	<i>Arabidopsis thaliana</i>	Flowering time	LH	E	20	Yes	No	D,Q	No	?	No
Kooyers et al	2017	71(5):1205-1221	<i>Mimulus alsinoides</i>	Peduncle length	MO	E	1		No	D	No	No	
				Node of flowering	MO	E	1		No	D	No	No	
				Plant height	MO	E	1		No	D	No	No	
				Number flowers	MO	E	1		No	D	No	No	
Tanner et al.	2017	71(7): 1742-1754	<i>Hyla chrysoscelis,</i>	Call duration	BEH	E	3	Yes	No	D,Q,C	No	Yes	No
				Call rate	BEH	E	3	Yes	No	D,Q,C	No	Yes	No
				Pulse rate	BEH	E	3	Yes	No	D,Q,C	No	Yes	No
				Dominant freq	BEH	E	3	Yes	No	D,Q,C	No	Yes	No
				Relative amplitude	BEH	E	3	Yes	No	D,Q,C	No	Yes	No
Fisher et al.	2017	71(7):1841-1854	<i>Tamiasciurus hudsonicus</i>	Growth rate	PER	L	1		No	D	No	Yes	
				Birth date	LH	L	1		No	D	No	Yes	

Kalvnes et al.	2017	71(8):2062-2079	<i>Passer domesticus</i>	Tarsus	MO	L	1		No	D	No	Yes	Yes
				Wing	MO	L	1		No	D	No	Yes	Yes
				Bill length	MO	L	1		No	D	No	Yes	Yes
				Bill depth	MO	L	1		No	D	No	Yes	Yes
				Condition	MO	L	1		No	D	No	Yes	Yes
O'Brien et al.	2017	71(11):2584-2598	<i>Sagra femorata</i>	Hind leg length	MO	E	1		No	D,Q,C	No	?	Yes
				Elytra length	MO	E	1		No	D,Q,C	No	?	Yes
				Residual leg length to elytra	MO	E	1		No	D,Q,C	No	Yes	NA
McCollough et al	2018	72(4):893-905	<i>Onthophagus taurus</i>	Testes mass	MO	E	1		No	D,Q,C	No	Yes	No
				Horn length	MO	E	1		No	D,Q,C	No	Yes	No
				Soma mass	MO	E	1		No	D,Q,C	No	Yes	No
Hunter et al.	2018	72(4):851-855	<i>Ovis aries</i>	Mass	MO	E	1		Yes	D	No	No	
Ferris & Willis	2018	72(6):1225-1241	<i>M.lacinatus x M. guttatus hybrids</i>	Flowering time	LH	L	1		No	D,Q	No	Yes	No
				Leaf area	MO	L	1		No	D,Q	No	Yes	No
				Leaf lobes	MO	L	1		No	D,Q	No	Yes	No
Hamala et al	2018	72(7):1373-1386	<i>Arabidopsis lyrata</i>	Flowering time	PER	L	1		No	D,Q	No	Yes	No
				Shoot length	MO	L	1		No	D,Q	No	Yes	No
				Inflorescence Num	MO	L	1		No	D,Q	No	Yes	No
				Fruit maturation	LH	L	1		No	D,Q	No	Yes	No
				Flowering cessation	LH	L	1		No	D,Q	No	Yes	No
Exposito-Alonzo et al.	2018	72(8):1570-1586	<i>Arabidopsis thaliana</i>	Max Recruitment	PER	L	1		No	D,Q	No	Yes	Yes
				Flowering time	LH	L	1		No	D,Q	No	Yes	Yes
Taylor et al	2018	72(10):2090-2099	<i>Urosaurus ornatus</i>	Snout-vent length	MO	L	1		No	D,Q	No	Yes	No
				Hind-limb length	MO	L	1		No	D,Q	No	Yes	No
				Mass	MO	L	1		No	D,Q	No	Yes	No
				MaxSprint	PER	L	2		No	D,Q	No	Yes	No
Hamann et al.	2018	72(12):2682-2696	<i>Brassica rapa</i>	Flowering time	LH	L	2	Yes	No	D,Q	No	?	No
				Stem diameter	MO	L	2	Yes	No	D,Q	No	?	No
Wang & Althoff	2019	73(2):303-316	<i>Drosophila melanogaster</i>	ADH activity	PHY	L	2	Yes	No	D,Q	No	Yes	No
				ADH_P010	PHY	L	2	Yes	No	D,Q	No	Yes	No
				ADH_P016	PHY	L	2	Yes	No	D,Q	No	Yes	No
				ADH_P1016	PHY	L	2	Yes	No	D,Q	No	Yes	No
Ramakers et al.	2019	73(2):175-187	<i>Parus major</i>	First-egg date Intercept first egg date	LH	L	2	Yes	No	D	Yes	Yes	Yes

				Slope in FED with temperature	LH	L	2	Yes	No	D	Yes	Yes	Yes
LeGrice et al..	2019	73(4):762-776	<i>Lasiorrhynchus barbicornis</i>	Body length	MO	E	1		No	D,Q	No	?	No
Keith & Mitchell-Olds	2019	73(5):947-960	<i>Boechera stricta</i>	ConGS-R	MO	E	1		No	D	No	Yes	
				BC-ratio-R	MO	E	1		No	D	No	Yes	
				ConGS-C	MO	E	1		No	D	No	Yes	
				BC-ratio-C	MO	E	1		No	D	No	Yes	
				ConGS-F	MO	E	1		No	D	No	Yes	
				BC-ratio-F	MO	E	1		No	D	No	Yes	
Tonnabel et al.	2019	73(5): 897-912	<i>Murcurialis annua</i>	Height	MO	E	1		No	D,Q,C	No	Yes	Yes
				Diameter	MO	E	1		No	D,Q,C	No	Yes	Yes
				Mean branch length	MO	E	2	Yes	No	D,Q,C	No	Yes	Yes
				Ped inflorescences	MO	E	1		No	D,Q,C	No	Yes	Yes
				Peduncle length	MO	E	5	Yes	No	D,Q,C	No	Yes	Yes
				Seeds	MO	E	1		No	D,Q,C	No	Yes	Yes
				Vegetative weight	MO	E	1		No	D,Q,C	No	Yes	Yes
				Germination rate	MO	E	1		No	D,Q,C	No	Yes	Yes
Watts et al	2019	73(9):1927-1940	<i>Schizocosa crassipes</i>	Mass	MO	E	1		No	D	No	Yes	Yes
				Thorax width	MO	E	3	Yes	No	D	No	Yes	Yes
				Brush area	MO	E	1		Yes	D	No	Yes	Yes
				Tibial darkness	MO	E	3	Yes	No	D	No	Yes	Yes
				Latency to Signal	BEH	E	1		No	D	No	Yes	Yes
				Latency to Bounce	BEH	E	1		No	D	No	Yes	Yes
				Bounce Rate	BEH	E	1		No	D	No	Yes	Yes
				Bounce Interval	BEH	E	>1	Yes	No	D	No	Yes	Yes
				Number Wave Bouts	BEH	E	1		No	D	No	Yes	Yes
				Total Wave Duration	BEH	E	1		No	D	No	Yes	Yes
				Mean Wave Duration	BEH	E	>1	Yes	No	D	No	Yes	Yes
				Prop. Variance	BEH	E	1		No	D	No	Yes	Yes
				Cummulative Variance	BEH	E	1		No	D	No	Yes	Yes
				Courtship Effort (PC1)	BEH	E	12	PCA	No	D	No	Yes	Yes
				Size-ornament (PC2)	MO	E	12	PCA	No	D	No	Yes	Yes
				Leg-Wave duration (PC3)	BEH	E	12	PCA	No	D	No	Yes	Yes

52 **Supplementary Text S2**

53 *Calculating bias in directional selection gradients that ignore effects of trait repeatability*

54 We derive here Eqn. 5 (Main Text). We start with reprinting the mathematical relationship
 55 between b_1^* , the unstandardized linear selection gradient that ignores effects of within-
 56 individual variance, the trait's repeatability (R_t), and the true unstandardized linear selection
 57 gradient (b_1) (Eqn. 4, Main Text):

$$58 \quad b_1^* = b_1 R_t \quad (\text{S2.1})$$

59 Standardized linear selection gradients (β) measure the change in relative fitness per standard
 60 deviation unit trait (Lande and Arnold 1983), calculable by dividing the unstandardized linear
 61 selection gradient by mean fitness (\bar{W}) and by multiplying it by the square-root of the variance
 62 in trait value at the focal level of analysis, thus:

$$63 \quad \beta_1^* = b_1^* \frac{\sqrt{V_{p_t}}}{\bar{W}} \quad (\text{S2.2})$$

64 The true standardized linear selection gradient (β_1) represents the multiplication of b_1 with $\frac{\sqrt{V_{i_t}}}{\bar{W}}$,
 65 where V_{i_t} represents the true individual variance in trait values:

$$66 \quad \beta_1 = b_1 \frac{\sqrt{V_{i_t}}}{\bar{W}} \quad (\text{S2.3})$$

67 Eqn. S2.2 may therefore be rephrased as:

$$68 \quad \beta_1^* = \frac{\beta_1}{\sqrt{V_{i_t}}} R_t \sqrt{V_{p_t}} \quad (\text{S2.4})$$

69 Expressing R_t in its underlying variance components gives:

$$70 \quad \beta_1^* = \frac{\beta_1}{\sqrt{V_{i_t}}} \frac{V_{i_t}}{V_{i_t} + V_{e_t}} \sqrt{V_{i_t} + V_{e_t}} = \beta_1 \frac{\sqrt{V_{i_t}}}{\sqrt{V_{i_t} + V_{e_t}}} \frac{\sqrt{V_{i_t}}}{\sqrt{V_{i_t} + V_{e_t}}} \frac{\sqrt{V_{i_t} + V_{e_t}}}{\sqrt{V_{i_t}}} = \beta_1 \frac{\sqrt{V_{i_t}}}{\sqrt{V_{i_t} + V_{e_t}}} \quad (\text{S2.5})$$

71 Re-expressing variance components in terms of repeatability, leads to Eqn. 5 (Main Text):

$$72 \quad \beta_1 = \beta_1^* / \sqrt{R_t} \quad (\text{S2.6})$$

73 **Supplementary Text S3**

74 *Calculating bias in quadratic selection gradients ignoring effects of trait repeatability*

75 To derive the bias in quadratic selection gradients caused by ignoring within-individual
 76 variation, we provide the mathematical relationship between the unstandardized quadratic
 77 selection gradient that ignores the effects of within-individual error (b_{11}^*), the true
 78 unstandardized quadratic selection gradient (b_{11}), and the repeatability of the squared value of
 79 the focal trait t (R_{t^2}), i.e., we apply Eqn. 4 to an unstandardized quadratic selection gradient:

$$80 \quad b_{11}^* = b_{11} R_{t^2} \quad (\text{S3.1})$$

81 where R_{t^2} represents the repeatability of the square of trait t . Estimating standardized selection
 82 gradients requires expressing trait values in standard deviation units (Lande and Arnold 1983).
 83 As we have seen above, the standardized trait value (z) equals the raw trait value (t) divided by
 84 the square root of the variance in trait values ($\sqrt{V_t}$), where $z = t / \sqrt{V_t}$. Z-transforming the raw
 85 trait value (t) thus results in $z^2 = t^2 / \sqrt{V_{t^2}}$. As we show above (Supplementary Text S2), the
 86 bias in the standardized linear gradient represents the square root of the bias in unstandardized
 87 gradients. Applied to quadratic selection gradients:

$$88 \quad \gamma_{11} = \gamma_{11}^* / \sqrt{R_{t^2}} \quad (\text{S3.2})$$

89 As above, the repeatability of the squared term of trait t is attributable to within- and among-
 90 individual variance components:

$$91 \quad R_{t^2} = \frac{V_{i_{t^2}}}{V_{i_{t^2}} + V_{e_{t^2}}} \quad (\text{S3.3})$$

92 where $V_{i_{t^2}} + V_{e_{t^2}}$ equals the total phenotypic variance in squared trait values ($V_{p_{t^2}}$). The
 93 phenotypic variance in squared trait values ($V_{p_{t^2}}$) represents the variance of a product (i.e.,
 94 $V_{p_{t^2}} = V_{p_{x,y}}$; where $x = y = t$); this variance can be broken down into the following
 95 components (e.g., Mood et al. 1973; Cacoullos 1989):

$$96 \quad V_{p_{x,y}} = C_{p_{x^2,y^2}} + \mu_x^2 \mu_y^2 - \left(C_{p_{x,y}} + \mu_x \mu_y \right)^2 \quad (S3.4)$$

97 Variance of products thus vary as a function of mean trait values (μ_x, μ_y), means of squared
 98 trait values (μ_{x^2}, μ_{y^2}), as well as trait covariances ($C_{p_{x^2,y^2}}, C_{p_{x,y}}$). Assuming multivariate
 99 normality, we can apply the following transformation:

$$100 \quad C_{p_{x^2,y^2}} = \left(C_{p_{x,y}}^2 + 2\mu_x \mu_y \sqrt{V_{p_x} V_{p_y}} \right) \quad (S3.5)$$

101 Substituting Eqn. S3.5 into S3.4 gives:

$$102 \quad V_{p_{x,y}} = \left(C_{p_{x,y}}^2 + 2\mu_x \mu_y \sqrt{V_{p_x} V_{p_y}} \right) + \mu_{x^2} \mu_{y^2} - \left(C_{p_{x,y}} + \mu_x \mu_y \right)^2 \quad (S3.6)$$

103 Re-expressing S3.6 in terms of trait values rather than squared trait values gives:

$$104 \quad V_{p_{x,y}} = \left(C_{p_{x,y}}^2 + 2\mu_x \mu_y \sqrt{V_{p_x} V_{p_y}} \right) + (\mu_x^2 + V_{p_x})(\mu_y^2 + V_{p_y}) - \left(C_{p_{x,y}} + \mu_x \mu_y \right)^2 \quad (S3.7)$$

105 Because $x = y = t$, $C_{p_{x,y}} = V_{p_x} = V_{p_y} = V_{p_t}$, we can substitute V_{p_t} for $C_{p_{x,y}}$:

$$106 \quad V_{p_{t^2}} = 2(V_{p_t}^2 + 2\mu_t \mu_t \sqrt{V_{p_t} V_{p_t}}) + (\mu_t^2 + V_{p_t})(\mu_t^2 + V_{p_t}) - (V_{p_t} + \mu_t \mu_t)^2 \quad (S3.8)$$

107 This then simplifies to:

$$108 \quad V_{p_{t^2}} = 2V_{p_t}^2 + 4V_{p_t} \mu_t^2 \quad (S3.9)$$

109 Applying Eqn. S3.9 to the among-individual ($V_{i_{t^2}}$) instead of total phenotypic variance in
 110 squared trait values ($V_{p_{t^2}}$) gives:

$$111 \quad V_{i_{t^2}} = 2V_{i_t}^2 + 4V_{i_t} \mu_t^2 \quad (S3.10)$$

112 We can now express the factor causing bias in quadratic selection gradients ($\sqrt{R_{t^2}}$; Eqn. S3.2)
 113 in terms of variances in trait values rather than squared trait values:

$$114 \quad \sqrt{R_{t^2}} = \sqrt{\frac{2V_{i_t}^2 + 4V_{i_t} \mu_t^2}{2V_{p_t}^2 + 4V_{p_t} \mu_t^2}} = \sqrt{\frac{V_{i_t}^2 + 2V_{i_t} \mu_t^2}{V_{p_t}^2 + 2V_{p_t} \mu_t^2}} \quad (S3.11)$$

115 This formula shows that bias is much more complex for quadratic versus linear gradients. Bias
 116 in standardized linear selection gradients varies solely as a function of the relative magnitudes
 117 of among- (V_{i_t}) and within-individual (V_{e_t}) variances and equals the trait's square-root
 118 repeatability ($\sqrt{R_t}$; Eqn. S2.6). By contrast, bias in quadratic selection gradients varies as a
 119 function of the among- (V_{i_t}) and within-individual (V_{e_t}) variances, and the square of the mean
 120 trait value (μ_t^2). Importantly, Eqn. S3.11 implies that bias in quadratic selection gradients is
 121 lowest when the trait mean (μ_t) equals zero. We show this by comparing bias ($\sqrt{R_{t^2}}$) when the
 122 trait mean is zero versus infinite. When $\mu_t = 0$, Eqn. S3.11 simplifies into:

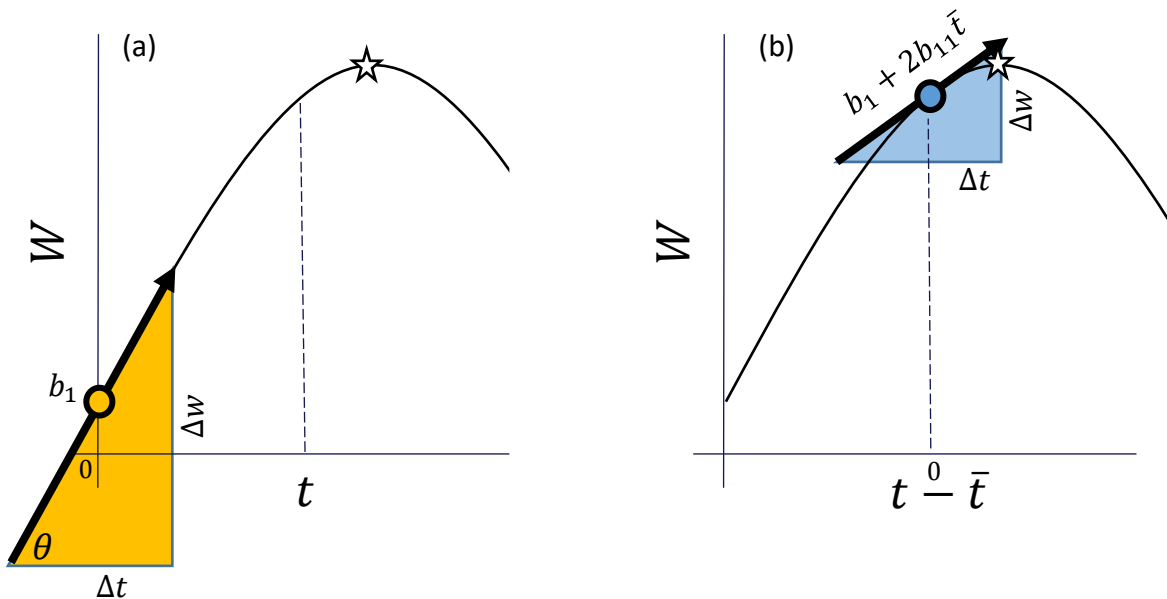
$$123 \quad \sqrt{R_{t^2}} = \sqrt{\frac{V_{i_t}^2}{V_{p_t}^2}} = \sqrt{R_t^2} = R_t \quad (S3.12)$$

124 By contrast, when $|\mu_t|$ approaches infinity, $\sqrt{R_{t^2}}$ (Eqn. S3.11) becomes:

$$125 \quad \lim_{|\mu_t| \rightarrow \infty} \sqrt{R_{t^2}} \approx \sqrt{\frac{V_{i_t}}{V_{p_t}}} = \sqrt{R_t} \quad (S3.13)$$

126 The magnitude of attenuation bias in quadratic selection gradient analyses ($\sqrt{R_{t^2}}$; Eqn. S3.11)
 127 thus varies between R_t (when $\mu_t = 0$; Eqn. S3.12) and $\sqrt{R_t}$ (when $|\mu_t| \rightarrow \infty$; Eqn. S3.13).
 128 Because R_t is always smaller than $\sqrt{R_t}$, attenuation bias therefore increases with decreasing
 129 value of $|\mu_t|$, and is, in fact, smallest when $\mu_t = 0$. This insight is important as our literature
 130 review (Text S1) implies that many studies (Table 1) mean-centre traits prior to analysis as part
 131 of trait standardization (i.e., $z = (t - \mu_t)/\sqrt{V_{p_t}}$). This transformation effectively replaces μ_t
 132 for $\mu_z = 0$ in Eqn. S3.11 and thereby (accidentally) minimizes attenuation bias.

133 Mean-centring traits minimizes attenuation bias but also affects the interpretation of the
 134 linear component of a quadratic selection gradient analyses. In a quadratic regression model,
 135 the linear effect (b_1) of the trait (t) on absolute fitness (W) represents the slope of the tangent
 136 line where the trait has the value zero as illustrated by the orange dot in Figure S3a, where the
 137 arrow represents b_1 for $t = 0$. A biologically meaningful zero-point represents the population-
 138 mean trait value (\bar{t} ; dotted line in Fig. S3), because the linear effect of the trait on fitness then
 139 provides information on whether the fitness peak/valley (white star in Fig. S3) is above or below
 140 the population mean trait value (\bar{t}). Said differently, it provides information on the expected
 141 strength of directional selection on the trait. The arrow in Fig. S3b represents the tangent line
 142 at this population-mean value (blue dot), and is calculated by adding $2b_{11}\bar{t}$ to b_1 (as defined in
 143 Eqn. 14). Applying this transformation to the scenario presented in Fig S3, would result in $\beta_1 >$
 144 0 at the population-mean trait value (Fig. S3b), implying that the population mean is below the
 145 optimal trait value, as illustrated.



146 **Figure S3.** Illustration of a parabolic relationship between trait (t) on absolute fitness (W), where the
 147 dotted line represents the population-mean trait value, the star represents the optimal trait value; **(a)** the
 148 orange dot represents the tangent line where the trait value has the value zero. **(b)** the blue dot represents
 149 the tangent line at the population-mean trait value.

150 An important question is whether estimates of optimal trait values in stabilising
 151 selection scenarios are also affected by failure to acknowledge biasing effects of within-
 152 individual error. The optimal trait value represents the trait value at the vertex of the parabola,
 153 calculable as $\frac{-\beta_1}{2\gamma_{11}}$ (Bronshtein et al. 2015). The trait value at the parabolic peak calculated while
 154 ignoring biasing effects of within-individual variance ($\frac{-\beta_1^*}{2\gamma_{11}^*}$) is mathematically related to the true

155 trait value at the parabolic peak ($\frac{-\beta_1}{2\gamma_{11}}$) because $\gamma_{11} = \gamma_{11}^*/\sqrt{R_{t^2}}$ (Eqn. S3.2) and $\beta_1 = \beta_1^*/\sqrt{R_t}$
 156 (Eqn. S2.6), thus:

$$157 \quad \frac{-\beta_1}{2\gamma_{11}} = \frac{-\beta_1^* \sqrt{R_{t^2}}}{2\gamma_{11}^* \sqrt{R_t}} \quad (\text{S3.14})$$

158 where $\frac{\sqrt{R_{t^2}}}{\sqrt{R_t}}$ represents the bias in the trait value at the parabolic peak. Substituting $\sqrt{R_{t^2}}$ for
 159 Eqn. S3.11 gives:

$$160 \quad \frac{\sqrt{R_{t^2}}}{\sqrt{R_t}} = \frac{\sqrt{\frac{V_{i_t}^2 + 2V_{i_t}\mu_t^2}{V_{p_t}^2 + 2V_{p_t}\mu_t^2}}}{\sqrt{R_t}} \quad (\text{S3.15})$$

161 Eqn. S3.15 simplifies for traits that were mean-centred (using Eqn. S3.12):

$$162 \quad \frac{\sqrt{R_{t^2}}}{\sqrt{R_t}} = \frac{R_t}{\sqrt{R_t}} = \sqrt{R_t} \quad (\text{S3.16})$$

163 Eqn. S3.15 simplifies differently when the mean trait value approaches infinity (using Eqn.
 164 S3.13):

$$165 \quad \lim_{|\mu_t| \rightarrow \infty} \frac{\sqrt{R_{t^2}}}{\sqrt{R_t}} \approx \frac{\sqrt{R_t}}{\sqrt{R_t}} = 1 \quad (\text{S3.17})$$

166 In other words, for variance-standardized traits that are also mean-centred, failure to control for
 167 within-individual error causes an overestimation of the optimal trait value by a factor equal $\sqrt{R_t}$
 168 (Eqn. S3.16). Importantly, Eqn. S3.17 implies that bias in the placement of the parabolic peak
 169 is greatest when the trait mean (μ_t) equals zero. Moreover, Eqn. S3.17 shows that bias in the
 170 placement of the parabolic peak ($\frac{\sqrt{R_{t^2}}}{\sqrt{R_t}}$) disappears entirely when the mean trait value
 171 approximates infinity (and thus will not happen in practice). Mean-centring traits prior to
 172 analysis therefore decreases attenuation bias in standardized quadratic selection gradients (Eqn.
 173 S3.12) but simultaneously leads to an overestimation of the placement of the parabolic peak
 174 (Eqn. S3.16).

175 **Supplementary Text S4**

176 *Calculating bias in correlational selection gradients ignoring effects of trait repeatability*

177 To derive the bias in correlational selection gradients, we print the mathematical relationship
 178 between the unstandardized correlational selection gradient ignoring effects of trait
 179 repeatability (b_{12}^*), the true unstandardized correlational selection gradient (b_{12}), and the
 180 repeatability ($R_{t_1 t_2}$) of the interaction between two traits, trait 1 (t_1) and trait 2 (t_2), i.e., we
 181 apply Eqn. 4 to an unstandardized correlational selection gradient:

$$182 \quad b_{12}^* = b_{12} R_{t_1 t_2} \quad (\text{S4.1})$$

183 Estimating standardized selection gradients requires expressing trait values in standard
 184 deviation units (Lande and Arnold 1983). As we demonstrated above, the standardized trait
 185 value (z) equals the raw trait value (t) divided by the square root of the variance in trait values
 186 ($\sqrt{V_t}$), where $z = t/\sqrt{V_t}$. Z-transforming t_1 and t_2 leads to $z_1 z_2 = t_1 t_2 / \sqrt{V_{t_1 t_2}}$. We showed
 187 above (Supplementary Text S2) that the bias in the standardized linear gradient is equal to the
 188 square root of the bias in unstandardized gradients. Applying the same logic to correlational
 189 selection gradients, we obtain:

$$190 \quad \gamma_{12} = \gamma_{12}^* / \sqrt{R_{t_1 t_2}} \quad (\text{S4.2})$$

191 As above, the repeatability of the multiplication of the traits is attributable to within- and
 192 among-individual variance components:

$$193 \quad R_{t_1 t_2} = \frac{V_{i_{t_1 t_2}}}{V_{i_{t_1 t_2}} + V_{e_{t_1 t_2}}} \quad (\text{S4.3})$$

194 We noted in Supplementary Text S3, the variance in the multiplication of the two traits ($V_{t_1 t_2}$)
 195 is equal to the variance of a product; this variance can be broken down into the following
 196 components (Eqn. S3.7):

$$197 \quad V_{t_1 t_2} = (C_{t_1 t_2}^2 + 2\mu_{t_1} \mu_{t_2} \sqrt{V_{t_1} V_{t_2}}) + (\mu_{t_1}^2 + V_{t_1})(\mu_{t_2}^2 + V_{t_2}) - (C_{t_1 t_2} + \mu_{t_1} \mu_{t_2})^2 \quad (\text{S4.4})$$

198 The among-individual variance in the multiplication of the two traits ($V_{i_{t_1 t_2}}$) thus equals:

$$199 \quad V_{i_{t_1 t_2}} = C_{i_{t_1 t_2}}^2 + 4\mu_{t_1} \mu_{t_2} \sqrt{V_{i_{t_1}} V_{i_{t_2}}} + (\mu_{t_1}^2 + V_{i_{t_1}})(\mu_{t_2}^2 + V_{i_{t_2}}) - (C_{i_{t_1 t_2}} + \mu_{t_1} \mu_{t_2})^2 \quad (\text{S4.5})$$

200 The total phenotypic variance in the multiplication of the two traits ($V_{p_{t_1 t_2}} = V_{i_{t_1 t_2}} + V_{e_{t_1 t_2}}$)
 201 instead equals:

$$202 \quad V_{p_{t_1 t_2}} = C_{p_{t_1 t_2}}^2 + 4\mu_{t_1} \mu_{t_2} \sqrt{V_{p_{t_1}} V_{p_{t_2}}} + (\mu_{t_1}^2 + V_{p_{t_1}})(\mu_{t_2}^2 + V_{p_{t_2}}) - (C_{p_{t_1 t_2}} + \mu_{t_1} \mu_{t_2})^2 \quad (\text{S4.6})$$

203 where $C_{p_{t_1 t_2}} = C_{i_{t_1 t_2}} + C_{e_{t_1 t_2}}$, $V_{p_{t_1}} = V_{i_{t_1}} + V_{e_{t_1}}$, $V_{p_{t_2}} = V_{i_{t_2}} + V_{e_{t_2}}$. We express the bias in
 204 correlational selection gradients ($\sqrt{R_{t_1 t_2}}$; S4.2) in terms of variances in trait values rather than
 205 trait products by substituting, obtaining:

$$206 \quad \sqrt{R_{t_1 t_2}} = \frac{\sqrt{C_{i_{t_1 t_2}}^2 + 4\mu_{t_1} \mu_{t_2} \sqrt{V_{i_{t_1}} V_{i_{t_2}}} + (\mu_{t_1}^2 + V_{i_{t_1}})(\mu_{t_2}^2 + V_{i_{t_2}}) - (C_{i_{t_1 t_2}} + \mu_{t_1} \mu_{t_2})^2}}{\sqrt{C_{p_{t_1 t_2}}^2 + 4\mu_{t_1} \mu_{t_2} \sqrt{V_{p_{t_1}} V_{p_{t_2}}} + (\mu_{t_1}^2 + V_{p_{t_1}})(\mu_{t_2}^2 + V_{p_{t_2}}) - (C_{p_{t_1 t_2}} + \mu_{t_1} \mu_{t_2})^2}} \quad (\text{S4.7})$$

207 This formula shows that bias is much more complex for correlational versus quadratic gradients
 208 because bias in the former additionally varies as a function of covariances between the traits
 209 within and among individuals. When traits are mean-centred prior to analysis (i.e., $\mu_{t_1} = \mu_{t_2} =$
 210 0, Eqn. S4.7 can simplify into:

$$211 \quad \sqrt{R_{t_1 t_2}} = \frac{\sqrt{V_{i_{t_1}} V_{i_{t_2}} + C_{i_{t_1 t_2}}^2}}{\sqrt{V_{p_{t_1}} V_{p_{t_2}} + C_{p_{t_1 t_2}}^2}} \quad (\text{S4.8})$$

212 Expressing covariances in correlations gives $r_{i_{t_1 t_2}} = \frac{C_{i_{t_1 t_2}}}{\sqrt{V_{i_{t_1}} V_{i_{t_2}}}}$. Hence, $C_{i_{t_1 t_2}}^2 = r_{i_{t_1 t_2}}^2 V_{i_{t_1}} V_{i_{t_2}}$.

213 Similarly, $r_{p_{t_1 t_2}} = \frac{C_{p_{t_1 t_2}}}{\sqrt{V_{p_{t_1}} V_{p_{t_2}}}}$. Eqn. S4.8 can thus be rephrased into:

$$214 \quad \sqrt{R_{t_1 t_2}} = \frac{\sqrt{V_{i_{t_1}} V_{i_{t_2}} + C_{i_{t_1 t_2}}^2}}{\sqrt{V_{p_{t_1}} V_{p_{t_2}} + C_{p_{t_1 t_2}}^2}} = \frac{\sqrt{V_{i_{t_1}} V_{i_{t_2}} + r_{i_{t_1 t_2}}^2 V_{i_{t_1}} V_{i_{t_2}}}}{\sqrt{V_{p_{t_1}} V_{p_{t_2}} + r_{p_{t_1 t_2}}^2 V_{p_{t_1}} V_{p_{t_2}}}} = \frac{\sqrt{V_{i_{t_1}} V_{i_{t_2}} (r_{i_{t_1 t_2}}^2 + 1)}}{\sqrt{V_{p_{t_1}} V_{p_{t_2}} (r_{p_{t_1 t_2}}^2 + 1)}} =$$

$$215 \quad \sqrt{R_{t_1} R_{t_2}} \sqrt{\frac{r_{i_{t_1 t_2}}^2 + 1}{r_{p_{t_1 t_2}}^2 + 1}} \quad (\text{S4.9})$$

216 This shows that $\sqrt{R_{t_1 t_2}}$ equals the geometric mean repeatability of the two traits ($\sqrt{R_{t_1} R_{t_2}}$)
 217 when the correlations between the two traits do not differ between the levels (i.e., $r_{i_{t_1 t_2}} =$
 218 $r_{e_{t_1 t_2}} = r_{p_{t_1 t_2}}$) and traits are mean-centred prior to analyses. Therefore, when $|r_{i_{t_1 t_2}}| > |r_{e_{t_1 t_2}}|$

219 it follows that $\sqrt{R_{t_1 t_2}} > \sqrt{R_{t_1} R_{t_2}}$; by contrast, when $|r_{i_{t_1 t_2}}| < |r_{e_{t_1 t_2}}|$, it follows that $\sqrt{R_{t_1 t_2}} <$
 220 $\sqrt{R_{t_1} R_{t_2}}$.

221 One key question is whether the shape of the selection surface is also affected by failure
 222 to acknowledge biasing effects of within-individual variation. Surface shape is a function of the
 223 ratio of the product of the quadratic selection gradients of two focal traits over the square of
 224 their correlational selection gradient (i.e., $\frac{\gamma_{11}\gamma_{22}}{\gamma_{12}^2}$), which describes a saddle-shaped fitness
 225 surface when below one (assuming γ_{11} and γ_{22} are both negative) but a fitness peak when above
 226 one (Phillips and Arnold 1989). For mean-centred traits, the shape of the selection surface
 227 calculated while ignoring biasing effects of within-individual variance $\left(\frac{\gamma_{11}^* \gamma_{22}^*}{\gamma_{12}^{*2}}\right)$ is
 228 mathematically related to true surface $\left(\frac{\gamma_{11}\gamma_{22}}{\gamma_{12}^2}\right)$ in the following way:

$$229 \frac{\gamma_{11}\gamma_{22}}{\gamma_{12}^2} = \frac{\gamma_{11}^* \gamma_{22}^*}{\sqrt{R_{t_1}^2} \sqrt{R_{t_2}^2}} \frac{R_{t_1 t_2}}{\gamma_{12}^{*2}} = \frac{\gamma_{11}^* \gamma_{22}^*}{\gamma_{12}^{*2}} \frac{R_{t_1 t_2}}{\sqrt{R_{t_1}^2 R_{t_2}^2}} \quad (\text{S4.10})$$

230 The shape of the selection surface is not affected when $\frac{R_{t_1 t_2}}{\sqrt{R_{t_1}^2 R_{t_2}^2}} = 1$. For mean-centred traits,

$$231 \sqrt{R_{t_2}} = R_t \text{ (Eqn. S3.12)}, \quad \sqrt{R_{t_1 t_2}} = \sqrt{R_{t_1} R_{t_2}} \sqrt{\frac{r_{i_{t_1 t_2}}^2 + 1}{r_{p_{t_1 t_2}}^2 + 1}} \text{ (Eqn. S4.9)},$$

232 therefore bias in the selection surface is described as:

$$233 \frac{R_{t_1 t_2}}{\sqrt{R_{t_1}^2 R_{t_2}^2}} = \frac{R_{t_1} R_{t_2} \left(\frac{r_{i_{t_1 t_2}}^2 + 1}{r_{p_{t_1 t_2}}^2 + 1}\right)}{R_{t_1} R_{t_2}} = \frac{r_{i_{t_1 t_2}}^2 + 1}{r_{p_{t_1 t_2}}^2 + 1} \quad (\text{S4.11})$$

234 This demonstrates that the bias in the fitness surface is *not* a function of (geometric mean)
 235 repeatability of the traits for mean-centred traits. Eqn. S4.11 shows instead that the fitness
 236 surface is unbiased when the correlations between the two traits do not differ between the levels
 237 (i.e., $r_{i_{t_1 t_2}} = r_{e_{t_1 t_2}} = r_{p_{t_1 t_2}}$). However, when correlations among individuals are tighter than
 238 those within individuals (i.e., $|r_{i_{t_1 t_2}}| > |r_{e_{t_1 t_2}}|$), failure to acknowledge within-individual
 239 variance can cause bias in the shape of the selection surface because it makes the fitness surface
 240 appear more saddle-shaped. Along the same lines, when correlations within individuals are
 241 tighter than those among individuals (i.e., $|r_{i_{t_1 t_2}}| < |r_{e_{t_1 t_2}}|$), failure to acknowledge within-
 242 individual variance can cause bias in the shape of the selection surface because it makes the
 243 fitness surface appear more peaked. Such simple rules, notably, do not apply when correlational
 244 selection gradient analyses were based on traits that were not mean-centred prior to analyses
 245 because bias in the correlational selection gradient ($\sqrt{R_{t_1 t_2}}$) is then much more complex (Eqn.
 246 S4.7) thus also any effects on fitness surfaces.

247 **Supplementary Text S5**

248 *Bias in selection gradients used on mean trait values*

249 Researchers commonly calculate individual-mean trait values prior to conducting phenotypic
 250 selection analyses (Table 1). In what follows, we logically assume that researchers working
 251 with individual-mean trait values would apply trait standardization *after* rather than *before*
 252 calculating individual-mean trait values. We note that none of the papers using individual-
 253 means in phenotypic selection analyses in our review (Text S1) clarified when standardization

254 was applied. However, this would be the only sensible decision when one views the variance
 255 in individual-mean trait values as the best proxy of the among-individual variance.

256 In previous sections, we derived the equations for bias in linear (Text S2), quadratic
 257 (Text S3), and correlational (Text S4) selection gradients resulting from failure to acknowledge
 258 residual within-individual variance. Based on few additional assumptions, we can readily
 259 modify these equations to be applied to analyses using individual-mean trait values.
 260 Specifically, following Snijders & Bosker (1999), we assumed that the total phenotypic
 261 variance among individual-mean trait values ($V_{p_{\bar{t}}}$) can be approximated as:

$$262 \quad V_{p_{\bar{t}}} = V_{i_t} + \frac{V_{e_t}}{n} \quad (S5.1)$$

263 where V_{i_t} and V_{e_t} represent the among-individual and residual within-individual variance in trait
 264 values, respectively, and n represents the number of replicate samples collected per individual
 265 (assuming equal replication among all individuals). We further assumed that the phenotypic
 266 covariance between mean traits ($C_{p_{\bar{t}_1\bar{t}_2}}$) can then be approximated as (Snijders and Bosker
 267 1999):

$$268 \quad Cov_{p_{\bar{t}_1\bar{t}_2}} = Cov_{i_{t_1t_2}} + \frac{Cov_{e_{t_1t_2}}}{n} \quad (S5.2)$$

269 where $Cov_{i_{t_1t_2}}$ and $Cov_{e_{t_1t_2}}$ represent the among-individual and residual within-individual
 270 covariances between traits. The associated formula for the phenotypic correlation between
 271 individual-mean trait values ($r_{p_{\bar{t}_1\bar{t}_2}}$) is therefore (Dingemanse et al. 2012):

$$272 \quad r_{p_{\bar{t}_1\bar{t}_2}} = \frac{Cov_{i_{t_1t_2}} + \frac{Cov_{e_{t_1t_2}}}{n}}{\sqrt{\left(V_{i_{t_1}} + \frac{V_{e_{t_1}}}{n}\right)\left(V_{i_{t_2}} + \frac{V_{e_{t_2}}}{n}\right)}} \quad (S5.3)$$

273 In brief, any equation printed in Texts S2-S4 can therefore be modified to derive bias for
 274 phenotypic selection analyses that used individual-mean trait values, which may be achieved
 275 by replacing (i) V_{e_t} for $\frac{V_{e_t}}{n}$, (ii) V_{p_t} for $V_{p_{\bar{t}}}$, (iii) R_t for $\frac{V_{i_t}}{V_{i_t} + \frac{V_{e_t}}{n}}$, (iv) $C_{e_{t_1t_2}}$ for $\frac{C_{e_{t_1t_2}}}{n}$ and (v) r_{p_t} for
 276 $r_{p_{\bar{t}}}$. When applied to individual-mean trait values, main formulae for bias in standardized
 277 selection gradients owing to failure to acknowledge within-individual variation consequently
 278 become:

279 (i) For linear selection gradients: $\sqrt{\frac{V_{i_t}}{V_{i_t} + \frac{V_{e_t}}{n}}}$ instead of $\sqrt{R_t}$ (Eqn. S2.6).

280 (ii) For quadratic selection gradients (general formula): $\sqrt{\frac{V_{i_t}^2 + 2V_{i_t}\mu_t^2}{V_{p_{\bar{t}}}^2 + 2V_{p_{\bar{t}}}\mu_t^2}}$ instead of Eqn. S3.11.

281 (iii) For quadratic selection gradients (mean-centred traits): $\frac{V_{i_t}}{V_{i_t} + \frac{V_{e_t}}{n}}$ instead of Eqn. S3.12.

282 (iv) For correlational selection gradients (general formula):

$$283 \quad \frac{2C_{i_{t_1t_2}}^2 + 4\mu_{t_1}\mu_{t_2}\sqrt{V_{i_{t_1}}V_{i_{t_2}}} + (\mu_{t_1}^2 + V_{i_{t_1}})(\mu_{t_2}^2 + V_{i_{t_2}}) - (C_{i_{t_1t_2}} + \mu_{t_1}\mu_{t_2})^2}{\sqrt{2C_{p_{\bar{t}_1\bar{t}_2}}^2 + 4\mu_{t_1}\mu_{t_2}\sqrt{V_{p_{\bar{t}_1}}V_{p_{\bar{t}_2}}} + (\mu_{t_1}^2 + V_{p_{\bar{t}_1}})(\mu_{t_2}^2 + V_{p_{\bar{t}_2}}) - (C_{p_{\bar{t}_1\bar{t}_2}} + \mu_{t_1}\mu_{t_2})^2}}$$

instead of Eqn. S4.7.

284 (v) For correlational selection gradients (mean-centred traits): $\sqrt{\frac{V_{i_{t_1}}V_{i_{t_2}}}{V_{p_{\bar{t}_1}}V_{p_{\bar{t}_2}}}} \sqrt{\frac{r_{i_{t_1t_2}}^2 + 1}{r_{p_{\bar{t}_1\bar{t}_2}}^2 + 1}}$ instead of

285 Eqn. S4.9.

286 **Supplementary Text S6**

287 *Estimating quadratic selection gradients with multivariate mixed-effects models*

288 To estimate quadratic selection using a multivariate mixed-effects model, we expand the
 289 bivariate model used to estimate linear selection (Eqn. 9, 10). This introduces a general solution
 290 applicable to further extensions. Quadratic selection gradients calculated using analyses
 291 ignoring within-individual variance (γ_{11}^*) would normally be modelled by expanding Eqn. 1
 292 into (Stinchcombe et al. 2008):

293
$$\omega = \alpha + \beta_1^* z + \frac{1}{2} \gamma_{11}^* z^2 + \varepsilon \quad (\text{S6.1})$$

294 This model is applied when each individual's trait (e.g., tarsus) is measured only once, but
 295 would suffer the problem of attenuation. Unattenuated quadratic selection gradients (γ_{11}) may
 296 be acquired by expanding the bivariate into a trivariate mixed-effects model; again, this requires
 297 repeated measures. We propose here to estimate quadratic selection gradients by fitting the
 298 squared term of the trait (t_{hi}^2) as a third response. We note an apparent problem: our aim is
 299 estimating the effect of the square of individual-mean trait values (\bar{t}_i^2) on fitness rather than the
 300 effect of individual-means of squared trait values (\bar{t}_i^2 ; note the subtle difference in the coverage
 301 of the bar to distinguish the two values). The mixed-model would estimate effects of \bar{t}_i^2 not \bar{t}_i^2 ;
 302 fitting the squared value of each observation (t_{hi}^2) thus seems inappropriate. To assess if this is
 303 indeed a problem, we ran simulations with normally distributed data, for different levels of trait
 304 repeatability, and compared the two metrics. We found that mixed-model estimates of among-
 305 individual variance in mean-of-squares accurately approximate simulated variances of the
 306 square of individual-specific values (see Section "Mean-of-squares vs. Square-of-means"
 307 below). The proposed trivariate model has the following phenotypic equation and random
 308 effects structure:

309
$$\begin{bmatrix} t_{hi} \\ t_{hi}^2 \\ W_i \end{bmatrix} = \boldsymbol{\beta}_0 + \mathbf{I}_i + \mathbf{e}_{hi}$$

310
$$\begin{bmatrix} I_t \\ I_{t^2} \\ I_W \end{bmatrix} \sim MVN(0, \Omega_I) : \begin{bmatrix} V_{I_t} & C_{I_t, t^2} & C_{I_t, W} \\ C_{I_t, t^2} & V_{I_{t^2}} & C_{I_{t^2}, W} \\ C_{I_t, W} & C_{I_{t^2}, W} & V_{I_W} \end{bmatrix}$$

311
$$\begin{bmatrix} e_t \\ e_{t^2} \\ e_W \end{bmatrix} \sim MVN(0, \Omega_e) : \begin{bmatrix} V_{e_t} & C_{e_t, t^2} & C_{e_t, W} \\ C_{e_t, t^2} & V_{e_{t^2}} & C_{e_{t^2}, W} \\ C_{e_t, W} & C_{e_{t^2}, W} & V_{e_W} \end{bmatrix} \quad (\text{S6.2})$$

312 Importantly, the standardized quadratic selection gradient (γ_{11}) to be calculated represents a
 313 partial regression coefficient (Lande and Arnold 1983). The linear (b_1) and quadratic (b_{11})
 314 slopes of the regression of the unstandardized trait on absolute fitness are partial regression
 315 coefficients; their calculation requires information embedded in the among-individual variance-
 316 covariance matrix (Ω_I):

317
$$b_1 = \frac{C_{I_t, W} V_{I_{t^2}} - C_{I_{t^2}, W} C_{I_t, t^2}}{V_{I_t} V_{I_{t^2}} - [C_{I_t, t^2}]^2}$$

318
$$b_{11} = \frac{C_{I_{t^2}, W} V_{I_t} - C_{I_t, W} C_{I_{t^2}, t^2}}{V_{I_t} V_{I_{t^2}} - [C_{I_t, t^2}]^2} \quad (\text{S6.3})$$

319 Mathematically, partial regression coefficients may directly be derived by inverting the
 320 among-individual covariance matrix (Ω_A). Briefly, matrix Ω_I (Eqn. S6.2) may be “split” into a
 321 matrix of predictors (Ω_A) and a matrix of covariances between predictors (traits) and response
 322 (fitness) (Ω_B), here taking the form of:

$$323 \quad \Omega_A : \begin{bmatrix} V_{i_t} & C_{i_t, t^2} \\ C_{i_t, t^2} & V_{i_{t^2}} \end{bmatrix}$$

$$324 \quad \Omega_B : \begin{bmatrix} C_{i_t, W} & C_{i_{t^2}, W} \end{bmatrix} \quad (\text{S6.4})$$

325 Partial regression coefficients (here, the unstandardized selection gradients) are then derived by
 326 multiplying $\mathbf{A}^{-1}\mathbf{B}$ (Bernstein 2005). In Supplementary Text S8 and on Github
 327 (<https://github.com/YimenAraya-Ajoy/SelectionBias>), we provide R-code to estimate and
 328 invert Ω_I and calculate partial regression coefficients. The standardized quadratic selection
 329 gradient (γ_{11}) then represents the multiplication of the unstandardized quadratic selection

330 gradient (b_{11} ; Eqn. S6.3) with $\frac{\sqrt{V_{i_{t^2}}}}{\beta_{0W}}$:

$$331 \quad \gamma_{11} = 2b_{11} \frac{\sqrt{V_{i_{t^2}}}}{\beta_{0W}} \quad (\text{S6.5})$$

332 A similar procedure can be applied to transform the unstandardized linear component
 333 in the quadratic selection model (b_1 ; Eqn. 6.3) into an interpretable standardized linear gradient
 334 (β_1). Adding a quadratic term, importantly, changes the meaning of this parameter, now
 335 representing the slope of the tangent where the trait has the value zero. A biologically
 336 meaningful zero-point represents the population-mean trait value, estimated as β_{0_t} in
 337 formulations like Eqn. S6.2 (illustrated in Fig. S3). Expressing β_1 relative to the population-
 338 mean trait value is insightful, for example, when $\gamma_{11} < 0$, the finding that $\beta_1 = 0$ implies
 339 stabilising selection with the optimal phenotype matching the population-mean trait value. $\beta_1 \neq$
 340 0 instead implies the adaptive peak is shifted away from the population-mean (see Fig. S3b),
 341 indicative of directional selection. The unstandardized linear gradient at the population-mean
 342 is the value of b_1 (as defined in Eqn. S6.3) plus $2b_{11}\beta_{0_t}$. An insightful standardized value of β_1
 343 is thus calculated by multiplying this sum with, $\frac{\sqrt{V_{i_t}}}{\beta_{0W}}$ (as in Eqn. 11, Main Text):

$$344 \quad \beta_1 = (b_1 + 2b_{11}\beta_{0_t}) \frac{\sqrt{V_{i_t}}}{\beta_{0W}} \quad (\text{S6.6})$$

345 The above assumes the trait was not mean-centred prior to analysis (see Discussion); the
 346 correction $+2b_{11}\beta_{0_t}$ would be unnecessary if it was.

347 *Estimating correlational selection gradients with multivariate mixed-effects models*

348 Expanding the model to estimate correlational selection gradients requires modifying Eqn. S6.2
 349 to instead fit two traits (t_1, t_2) and their product ($t_1 t_2$) as response variables. In many cases,
 350 researchers fit both the linear and quadratic of both traits in such models, in which case the
 351 multivariate mixed-model solution would fit six response variables. Unstandardized partial
 352 regression coefficients (here, b_1, b_2, b_{11}, b_{22} , and b_{12}) are again calculated by splitting Ω_I into
 353 Ω_A and Ω_B , and multiplying $\mathbf{A}^{-1}\mathbf{B}$. Standardized linear (β_1, β_2) and quadratic (γ_{11}, γ_{22})
 354 components are calculated as above (Eqn. S6.5, S6.6), while the standardized correlational
 355 selection gradient is calculated as:

$$356 \quad \gamma_{12} = b_{12} \frac{\sqrt{V_{i_{t_1 t_2}}}}{\beta_{0W}} \quad (\text{S6.7})$$

357 Where $V_{i_{t_1 t_2}}$ equals (Eqn. S4.5):

$$358 \quad V_{i_{t_1 t_2}} = 2C_{i_{t_1 t_2}}^2 + 4\beta_{0_{t_1}}\beta_{0_{t_2}}\sqrt{V_{i_{t_1}}V_{i_{t_2}}} + (\beta_{0_{t_1}}^2 + V_{i_{t_1}})(\beta_{0_{t_2}}^2 + V_{i_{t_2}}) - (C_{i_{t_1 t_2}} + \beta_{0_{t_1}}\beta_{0_{t_2}})^2 \quad (\text{S6.8})$$

359 For mathematical derivation see Text S4. A simulated example is described in Supplementary
 360 Texts S7; we provide R-code in Supplementary Text S8 and (future updates) on Github
 361 (<https://github.com/YimenAraya-Ajoy/SelectionBias>).

362 *Mean-of-squares vs. Square-of-means*

363 Above, we propose to estimate quadratic selection gradients by fitting the squared term of the
 364 focal trait (t_{hi}^2) as a response variable. Doing so assumes that the variance among-individuals
 365 in means of their squared trait values (\bar{t}_i^2) approximates the true among-individual variance in
 366 the square of individual-specific trait values (\bar{t}_i^2) of actual interest. Statistical simulations
 367 validated this assumption by demonstrating that the proposed multivariate mixed-effect model
 368 formulation produced unbiased estimates of the among-individual variance in squared values
 369 of individual-specific trait values.

370 We used the following simulation approach. We started by drawing individual-specific
 371 trait values (t_i) from a normal distribution with a mean (\bar{t}) equal to zero and $V_{i_t} = 3$. We then
 372 simulated 3 phenotypic observations for each of 800 individuals by adding an observation-
 373 specific error (drawn from a normal distribution with zero-mean and variance V_{e_t}). Next, we
 374 estimated the among-individual variance in the squared values in three different ways. (1) We
 375 squared the values of each observation, then calculated a mean value for each individual using
 376 all its squared values (\bar{t}_i^2), and finally, calculated the among-individual variance in this metric
 377 (among-individual variance in “mean-of-squared values”; Fig. S6). (2) We calculated the mean
 378 trait value over all observations per individual, squared this value (\bar{t}_i^2), and then calculated the
 379 among-individual variance in this metric (among-individual variance in “square-of-mean
 380 value”; Fig. S6). (3) We squared the values of each observation and fitted this variable (t_{hi}^2) as
 381 a response variable into a mixed-effects model with individual fitted as a random effects, and
 382 estimated the among-individual variance of this metric (“mixed-model estimate”; Fig. S7). We
 383 applied these simulations for two values of repeatability by setting $V_{e_t} = \frac{(V_{i_t} - R_t)}{R_t}$ using
 384 procedures detailed in Supplementary Texts S7 and S8. We repeated this procedure 100 times
 385 for both types of repeatability ($R_t = 0.3$ and $R_t = 0.7$).

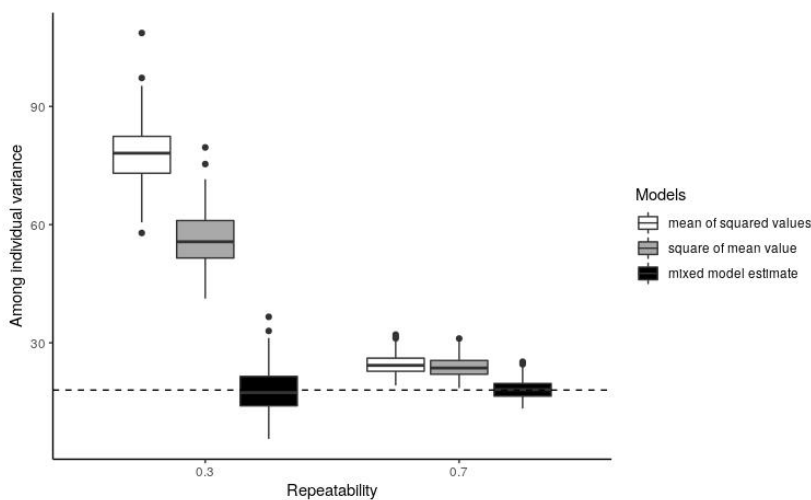


Fig. S6. The among-individual variance in squared values of a focal trait estimated in three different ways. The dashed line represents the true (simulated) among-individual variance in the square of individual-specific values.

392 Results show that the among-individual variance calculated using the mean of squares (white
393 bars), as well as the among-individual variance calculated using the square of means (light-grey
394 bars), overestimated the true among-individual variance in squared values of simulated
395 individual-specific trait values (dotted line) for both levels of repeatability (Fig. S6). By
396 contrast, the proposed mixed-effects model solution produced unbiased estimates of the true
397 among-individual variance in trait values.

398 **Supplementary Text S7**

399 *Linear selection analysis*

400 We used simulations to assess, first, whether classic approaches produced attenuated estimates
401 of linear selection gradients, and second, whether the proposed solutions (the application of
402 corrections (Table 1), multivariate mixed-models, or errors-in-variables models would address
403 this problem (for Results, see Table S7). We first studied a scenario where the absolute fitness
404 (W) of individual i was a function (b_1) of its true mean-centred trait value (t_i) (in its natural
405 scale; e.g., cm) plus an individual-specific stochastic environmental effect (e_i) with a variance
406 of 1 ($V_{e_w} = 1$).

$$407 \quad W_i = b_1 t_i + e_i \quad (S7.1)$$

408 The individual-specific values (t_i) were drawn from a normal distribution with a mean (\bar{t}) equal
409 to zero and among-individual variance (V_{i_t}) defined below. We then simulated 3 phenotypic
410 observations for each of 800 individuals by adding an observation-specific error drawn from a
411 normal distribution with zero-mean and residual variance ($V_{e_t} = \frac{V_{i_t}}{R_t} - V_{i_t}$) three separate times
412 to each t_i to produce the three measurements. As in the Main Text, the expected standardized
413 selection gradient β_1 equalled $b_1 \frac{\sqrt{V_{i_t}}}{\bar{W}}$, where $V_{i_t} = 3$, $b_1 = 0.346$, and $\bar{W} = 2$. Thus, $\beta_1 = 0.3$.

414 We ran simulations with (R_t) equal to 0.3 and 0.7 by varying $V_{e_t} = \frac{V_{i_t}}{R_t} - V_{i_t}$. We used $n=100$
415 replicate studies per level of repeatability. Following the generation of each full dataset (with n
416 = 2400 data points per simulation), we generated two sub-sets of data. The first subset contained
417 one randomly drawn trait value (of the three produced) per individual. The second subset
418 contained one mean value per individual calculated using all three observations. Fitness was
419 transformed into relative fitness and the trait transformed into standard deviation units for the
420 two sub-sets, where the standardization was applied after calculating trait means for the second
421 subset (for rationale, see Text S5); no transformations were applied to the full dataset.
422 Subsequently, we ran four analyses. First, we estimated the standardized linear selection
423 gradient using a linear regression, fitting the standardized trait as a predictor of relative fitness,
424 on the sub-set containing one random observation per individual. Second, we applied the latter
425 approach using the mean value per individual. Third, we ran a bivariate mixed-model with
426 random intercepts for individual identity on the full dataset, fitting the mean-centred trait and
427 absolute fitness as the two response variables, and estimated the standardized linear selection
428 gradient using Eqn. 11 (Main Text). We fitted the multivariate mixed-effects models in a
429 Bayesian framework using *MCMCglmm* (Hadfield 2010) in the R environment (R-Core-Team
430 2020). Finally, using RStan, we ran an errors-in-variables models. For all approaches, we
431 calculated estimation bias as the difference between the observed standardized selection
432 gradient minus the simulated standardized selection gradient divided by the simulated
433 standardized selection gradient. We provide R-code in Supplementary Text S8 and (future
434 updates) on Github (<https://github.com/YimenAraya-Ajoy/SelectionBias>).

435 Estimates of standardized linear selection gradients based on the first subset, containing
436 one randomly drawn trait value of the three produced per individual, were expected to be
437 attenuated by $\sqrt{R_t} = \sqrt{\frac{V_{i_t}}{V_{i_t} + V_{e_t}}}$ (Eqn. S2.6). Estimates of standardized linear selection gradients
438 based on the second subset, containing one mean value per individual calculated using all three
439 observations, were instead expected to be attenuated by $\sqrt{\frac{V_{i_t}}{V_{i_t} + \frac{V_{e_t}}{n}}}$ (see Text S5), where n
440 represents the number of observations per individual equal to three. As a follow-up analysis,
441 we fitted a univariate mixed-effects model with random intercepts for individual identity to
442 estimate V_{i_t} and V_{e_t} from the full dataset. We then corrected the standardized linear selection
443 gradient estimated for the first subset by dividing it by $\sqrt{R_t}$; the estimate for the second subset
444 was instead divided by $\sqrt{\frac{V_{i_t}}{V_{i_t} + \frac{V_{e_t}}{n}}}$. This procedure enabled us to assess whether corrections
445 applied to published data based on knowledge of trait repeatability could produce unbiased
446 estimates.

447 *Quadratic selection analysis*

448 We expanded our simulation to study how each approach (detailed above) performed when
449 applied to estimate standardized quadratic selection gradients. We did so by expanding Eqn.
450 S8.1 to include the effect of the quadratic component of the focal trait on absolute fitness (b_2):

$$451 \quad W_i = b_1 t_i + b_2 t_i^2 + e_i \quad (S7.2)$$

452 The expected value of β_1 equalled $b_1 \frac{\sqrt{V_{i_t}}}{\bar{W}}$. We set $V_{i_t} = 3$, $b_1 = 2.19$, and $\bar{W} = 2$. Thus, $\beta_1 =$
453 1.90. As in Eqn. S6.5, the expected value of γ_{11} equalled $2b_{11} \frac{\sqrt{V_{i_t^2}}}{\bar{W}}$. As $V_{i_t^2} = 2V_{i_t}^2 + 4V_{i_t}\mu_t^2$
454 (Eqn. S3.10), γ_{11} thus equalled $2b_{11} \frac{\sqrt{2V_{i_t}^2 + 4V_{i_t}\mu_t^2}}{\bar{W}}$. We set $b_2 = -0.14$ and $\mu_t = 0$, thus $\beta_2 = -0.3$. As
455 detailed above, we ran simulations with (R_t) equal to 0.3 and 0.7 by varying $V_{e_t} = \frac{V_{i_t}}{R_t} - V_{i_t}$. We
456 provide R-code in Supplementary Text S8 and (future updates) on Github
457 (<https://github.com/YimenAraya-Ajoy/SelectionBias>).

458 Estimates of standardized quadratic selection gradients based on the first subset,
459 containing one randomly drawn trait value of the three produced per individual, were expected
460 to be attenuated by $\sqrt{R_{t^2}} = R_t$ (Eqn. S3.12) because we pragmatically mean-centred the trait
461 prior to analysis. Estimates of standardized quadratic selection gradients based on the second
462 subset, containing one mean value per individual calculated using all three observations, were
463 instead expected to be attenuated by $\frac{V_{i_t}}{V_{i_t} + \frac{V_{e_t}}{n}}$ (instead of R_t ; see above). As a follow-up analysis,
464 we fitted a univariate mixed-effects model that had as a response variable t and random
465 intercepts for individual identity. We then corrected the standardized quadratic selection
466 gradient estimated for the first subset by dividing it by $R_t = \frac{V_{i_t}}{V_{i_t} + V_{e_t}}$; the estimate for the second

467 subset was instead divided by $\frac{V_{i_t}}{V_{i_t} + \frac{V_{e_t}}{n}}$. This procedure enabled us to assess whether corrections
 468 applied to published data based on knowledge of among- and within-individual variances and
 469 trait means could produce unbiased estimates.

470 *Correlational selection analysis*

471 Finally, we expanded our simulation to study how each approach (detailed above) performed
 472 when applied to estimate standardized correlational selection gradients. We did so by expanding
 473 Eqn. S8.2 to include the linear and quadratic effects of two focal traits (t_1, t_2), as well as their
 474 interaction, on absolute fitness:

$$475 \quad W_i = b_1 t_{1_i} + b_{11} t_{1_i}^2 + b_2 t_{2_i} + b_{22} t_{2_i}^2 + b_{12} t_{1_i} t_{2_i} + e_i \quad (S7.3)$$

476 For each trait (t_1, t_2), expected values for standardized linear (β_1, β_2) and quadratic (β_{11}, β_{22})
 477 selection gradients were set as detailed for the linear and quadratic selection examples above.

478 The expected value of the correlational selection gradient (γ_{12}) equalled $b_{12} \frac{\sqrt{V_{i_{t_1 t_2}}}}{\bar{W}}$ (Eqn. 18),
 479 where $V_{i_{t_1 t_2}} = C_{i_{t_1 t_2}}^2 + 2\mu_{t_1} \mu_{t_2} \sqrt{V_{i_{t_1}} V_{i_{t_2}}} + (\mu_{t_1}^2 + V_{i_{t_1}})(\mu_{t_2}^2 + V_{i_{t_2}}) - (C_{i_{t_1 t_2}} + \mu_{t_1} \mu_{t_2})^2$ (Eqn.
 480 S4.5). We provide R-code in Supplementary Text S8 and (future updates) on Github
 481 (<https://github.com/YimenAraya-Ajoy/SelectionBias>).

482 Estimates of standardized correlational selection gradients based on the first subset (one
 483 randomly drawn trait value of the three produced per individual) were expected to be attenuated
 484 by $\sqrt{R_{t_1 t_2}}$ (Eqn. S4.7). Estimates of standardized correlational selection gradients based on the
 485 second subset (containing one mean value per individual calculated using all three observations)

486 were instead expected to be attenuated by $\sqrt{\frac{V_{i_{t_1}} V_{i_{t_2}}}{V_{p_{\bar{t}_1}} V_{p_{\bar{t}_2}}} \frac{r_{i_{t_1 t_2}}^2 + 1}{r_{p_{\bar{t}_1 \bar{t}_2}}^2 + 1}}$ (Text S5). Our simulations

487 assumed a zero correlation between the traits such that the latter attenuation equated $\sqrt{\frac{V_{i_{t_1}} V_{i_{t_2}}}{V_{p_{\bar{t}_1}} V_{p_{\bar{t}_2}}}}$

488 while $\sqrt{R_{t_1 t_2}} = \sqrt{R_{t_1} R_{t_2}}$ (S4.9). As a follow-up analysis, we therefore two univariate mixed-
 489 effects model, with the traits were fitted as response variables, with random intercepts for
 490 individual identity on the full simulated dataset. We then corrected the standardized
 491 correlational selection gradient estimated for the first subset by dividing it by $\sqrt{R_{t_1} R_{t_2}}$; the

492 estimate for the second subset was instead divided by $\sqrt{\frac{V_{i_{t_1}} V_{i_{t_2}}}{V_{p_{\bar{t}_1}} V_{p_{\bar{t}_2}}}}$. This procedure enabled us to

493 assess whether corrections applied to published data based on knowledge of trait means and
 494 among- and within-individual correlations between traits could produce unbiased estimates.

495 **Table S7.** Estimates of accuracy and precision in linear (β_1), quadratic (γ_{11}), and correlational
496 (γ_{12}) selection gradients derived from regression models fitting one observed trait value or a
497 mean of three observed trait values, multivariate mixed-effects models, and errors-in-variables
498 models. We also show accuracy and precision after applying corrections to regressions. We
499 calculated bias (i.e., inaccuracy) as the difference between estimated minus true standardized
500 gradients, divided by the true gradient. This produced a mean percentage (upward/downward)
501 bias. The coefficient of variation (CV) among 100 datasets simulated for a given scenario was
502 used to measure imprecision. Estimates are provided for two levels of trait repeatability (R).

Model	R	%Bias β_1	CV β_1	%Bias γ_{11}	CV γ_{11}	%Bias γ_{12}	CV γ_{12}
1 obs	0.3	-45.04	0.07	-70.21	-0.64	-74.63	0.87
1 obs	0.7	-16.02	0.05	-33.13	-0.25	-31.05	0.23
Mean of 3 obs	0.3	-24.93	0.05	-42.58	-0.30	-49.35	0.36
Mean of 3 obs	0.7	-5.97	0.04	-15.03	-0.14	-14.87	0.15
1 obs corrected	0.3	0.85	0.06	1.32	-0.65	-1.92	0.86
1 obs corrected	0.7	0.45	0.04	-3.93	-0.24	1.16	0.24
Mean of 3 corrected	0.3	0.46	0.04	2.78	-0.31	-4.88	0.36
Mean of 3 corrected	0.7	0.56	0.04	-2.98	-0.14	-1.82	0.16
Multivariate mixed model	0.3	2.22	0.06	-1.10	-0.77	-7.02	1.61
Multivariate mixed model	0.7	0.66	0.04	-2.75	-0.20	-2.61	0.21
Errors-in-variables model	0.3	0.30	0.05	0.87	-0.13	-0.07	0.27
Errors-in-variables model	0.7	0.42	0.04	-0.25	-0.10	-1.58	0.15

503 References

- 504 Bernstein, D. S. 2005. Matrix Mathematics: Theory, Facts, and Formulas - 2nd Edition.
505 Princeton University Press.
- 506 Bronshtein, I. N., K. A. Semendyayev, G. Musiol, and H. Mühlig. 2015. Handbook of
507 Mathematics. Springer-Verlag, Berlin Heidelberg.
- 508 Cacoullos, T. 1989. Exercises in Probability. Springer Verlag, New York.
- 509 Dingemanse, N. J., N. A. Dochtermann, and S. Nakagawa. 2012. Defining behavioural
510 syndromes and the role of "syndrome deviation" to study its evolution. Behavioral
511 Ecology and Sociobiology 66:1543-1548.
- 512 Lande, R. and S. J. Arnold. 1983. The measurement of selection on correlated characters.
513 Evolution 37:1210-1226.
- 514 Mood, A. M., F. A. Graybill, and D. C. Boes. 1973. Introduction to the Theory of Statistics -
515 3rd Edition. McGraw Hill.
- 516 Phillips, P. C. and S. J. Arnold. 1989. Visualizing multivariate selection. Evolution 43:1209-
517 1222.
- 518 R-Core-Team. 2020. A language and environment for statistical computing. . R Foundation for
519 Statistical Computing, Vienna, Austria.
- 520 Snijders, T. A. B. and R. J. Bosker. 1999. Multilevel analysis - an introduction to basic and
521 advanced multilevel modelling. Sage, London.
- 522 Stinchcombe, J. R., A. F. Agrawal, P. A. Hohenlohe, S. J. Arnold, and M. W. Blows. 2008.
523 Estimating nonlinear selection gradients using quadratic regression coefficients: Double
524 or nothing ? Evolution 62:2435-2440.