Supplementary information for:

The interplay between movement, dispersal and morphology in *Tetrahymena* ciliates

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(1) *Tetrahymena thermophila* genotypes used and culture conditions

In this study, we used a set of 44 genetically distinct genotypes (synonyms: clonal lines, strains) of the ciliate protist *Tetrahymena thermophila* that have a different history in terms of geographical location at date of isolation (Pennekamp et al. 2014, see Table S1). We previously showed they differ in several life history traits such as growth rate, maximum cell density and survival under starvation conditions (Fjerdingstad et al. 2007, Pennekamp 2014). Cells only reproduced clonally because all cells from one genotype share the same mating type, preventing sexual reproduction through conjugation (Collins 2012); this ensures stability of the genotype over the duration of our study.

After defrosting cells from stock cultures kept in suspended animation in liquid nitrogen (Altermatt et al. 2015), the 44 genotypes were maintained under standard culture conditions before and during the experiment: axenic liquid culture in a nutrient medium (broth consisting of 2% Proteose peptone and 0.2% yeast extract [Becton Dickinson], diluted in ultrapure water), kept at constant 27°C temperature in a light controlled incubator with a 14:10 hours light/dark cycle. Culture stocks were renewed every 10 days by inoculating a 2 mL sample of fresh medium with 100 µL of culture and maintained in 2 mL multi-well plates (CELLSTAR® multiwell plates, Ref. 662102 from Greiner BioOne, Belgium). All manipulations of axenic cultures were conducted under sterile conditions in a laminar flow hood (Ultrasafe 218 S, Faster, Italy).

(2) Experimental design with two patch dispersal systems

We quantified dispersal and movement behaviour of *T. thermophila* cells using the same standardized two patch system developed in our previous work (Fjerdingstad et al. 2007, Schtickzelle et al. 2009, Chaine et al. 2010, Pennekamp et al. 2014) (Fig. S1).

In addition to the strict control of all our *T. thermophila* culture conditions, two standardization steps were performed prior to the experiment. First, a pre-culture of each genotype was started from the stock by transferring 100 µL of culture into 2 mL of fresh nutritive medium (2% Proteose peptone and 0.2% yeast extract [Becton Dickinson], diluted in ultrapure water) on a 24 well plate (CELLSTAR® multiwall plate, Ref. 662102 from Greiner BioOne, Belgium) and allowed to grow exponentially for 4 days to synchronize populations to the logarithmic phase of population growth (Collins 2012). Second, at the end of this synchronization phase, cell density was estimated for each genotype, and new cultures, to be used for the experiment, were launched at an equal starting density of 10000 cells/mL in culture flasks (CELLSTAR® Cell Culture Flask 50 mL, Ref. 690175 from Greiner BioOne, Belgium). These cultures grew for three days allowing them to reach sufficiently high cell densities for the experiment.

Each dispersal system consisted of two standard 1.5 mL microtubes connected by a silicon pipe (length: 17 mm; external diameter: 6 mm; Ref. 228-0709 from VWR, Belgium). The dispersal system was filled prior to the experiments with 3 mL of the standard nutritive medium through one tube to ensure fluid transition between the tubes and a connecting pipe free of air bubbles. The system was then closed by placing a clamp in the middle of the connecting pipe. To start the experiment, cells were inoculated into the "start" tube of the system at a density of density of 300000 cells / mL (i.e. 450000 cells for a 1.5 mL volume) and the tube content was homogenized to encourage the cells to move freely throughout the start tube. After 30 minutes of acclimation to the new medium, the clamp closing the connecting pipe was removed and cells could freely disperse between the two tubes for 6 h. After these 6 h, the pipe was clamped

again, and five independent samples taken from the “start” and “target” tubes after culture homogenization. Each sample was loaded into the chamber of a counting slide (Precision cell 301890, Vacutest Kima, Italy).

(3) Reconstructing movement trajectories from videos

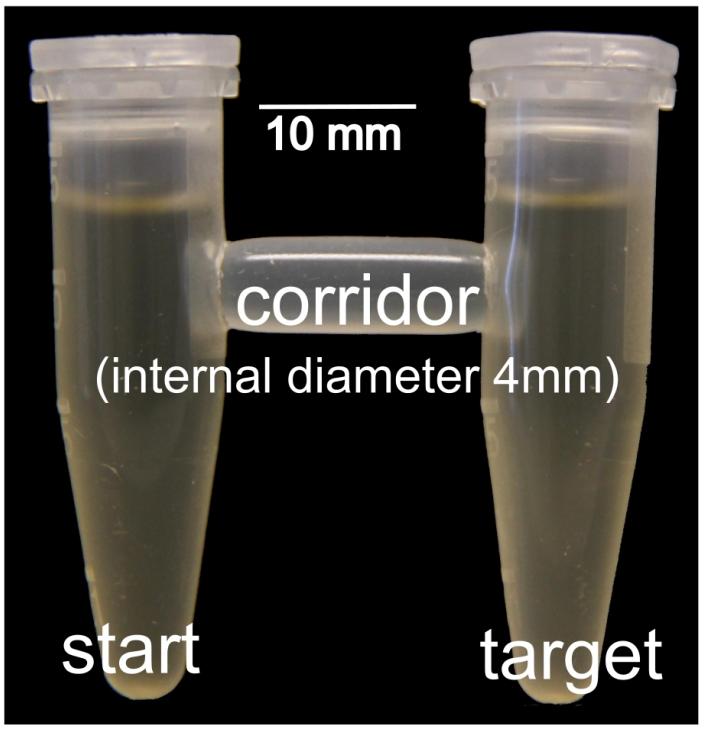
We developed for this study a workflow to extract movement trajectories from digital videos in a standardized and automated fashion, which was later transformed into the R package BEMOVI (Pennekamp et al. 2015). We customized the ParticleTracker plug-in for ImageJ software, originally aimed at tracking intracellular movements of cell structure (http://mosaic.mpi-cbg.de/ParticleTracker/). The ParticleTracker links each position of a given cell, as recorded on every frame of the video, into a unique trajectory of time-stamped X and Y coordinates, which are output as text files. The 25 frames per second acquisition speed for our videos guarantees correct assembly of positions into individual trajectories even when many cells are tracked simultaneously. ParticleTracker's feature point tracking algorithm is described in detail in Sbalzarini & Koumoutsakos (2005) and has several powerful features: 1) tracking of many individuals simultaneously is feasible and due to low computational requirements longer video sequences may be analysed; 2) the plug-in deals with unrestricted viewing fields, i.e. cells may leave and enter the video because the viewing field is not physically bounded; 3) in case the algorithm is unable to retain the identity of a given cell because of collisions between tracked cells or with artefacts on the video such as dust, it acts conservatively by terminating the current trajectories and assigning new trajectory identities when cells are again separated.

Home-made SAS scripts (www.sas.com) were used to read the raw trajectory data extracted by ParticleTracker (Fig. S2). 49% of the recorded trajectories (258,592 out of 525,328) were discarded because they lasted less than 1 s or had a total net displacement (i.e. the bee line distance between the start and the end position of the trajectory) less than 50 µm (corresponding to one body length of an average *Tetrahymena* cell); such trajectories correspond to non-moving cells. For analysis with continuous time movement models (Fleming et al. 2014, Gurarie et al. 2017), which is highly computationally demanding, we then subsampled 23

trajectories for each genotype x replicate x tube combination, resulting in 6072 trajectories sampled from a total of 266,736 trajectories of moving cells.

Figures

Figure S1: A two patch dispersal system made of two 1.5 mL microtubes connected by a silicon pipe, filled with nutritive medium, used to quantify dispersal rate from cells inoculated in the start patch and allowed to move freely during 6 h.



$$\text{dispersal rate} = \frac{\text{abundance}_{\text{target}}}{\text{abundance}_{\text{start}} + \text{abundance}_{\text{target}}}$$

Figure S2: An illustration of the raw trajectory data extracted from videos

Different colours show different individual trajectories. The linearity differed among trajectories with some being very linear and others more tortuous (see arrows). Some very short (in time or space) trajectories correspond to non-moving cells.

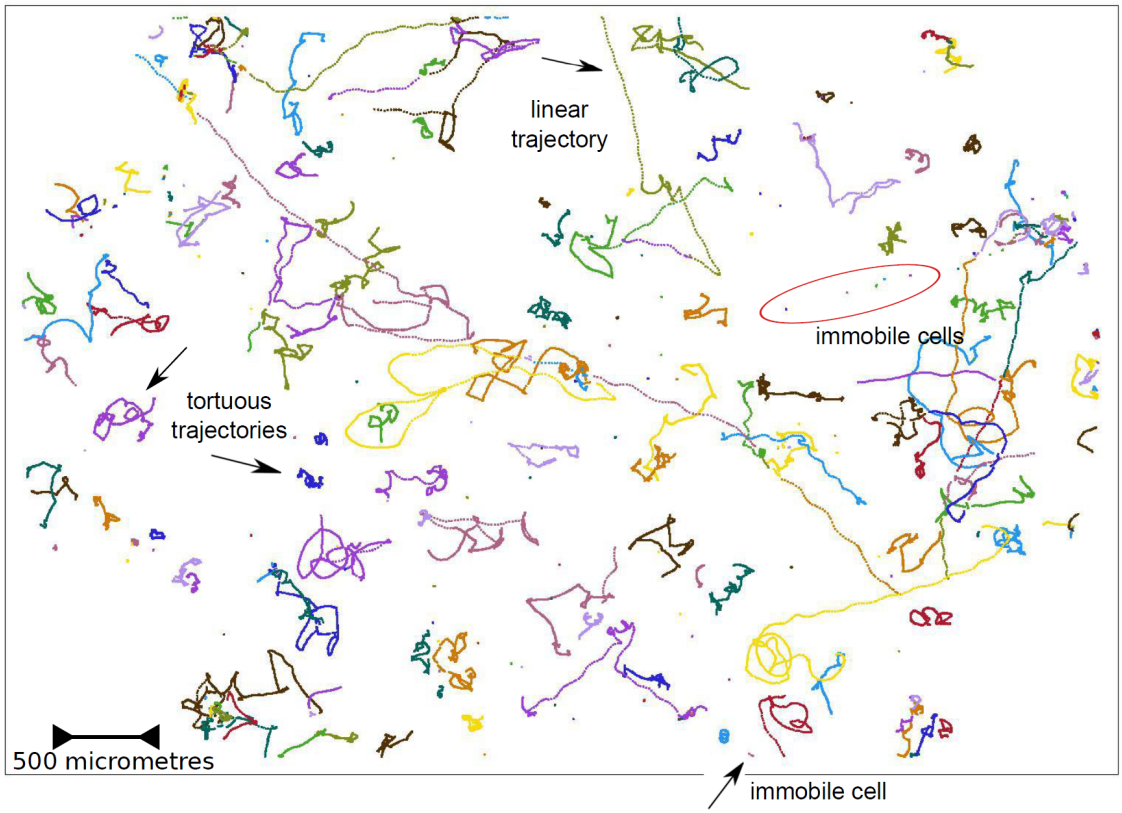


Figure S3: A positive correlation between movement speed and linearity was found across genotypes. Faster genotype moved more linear. The strength of the relationship did not differ with dispersal status.

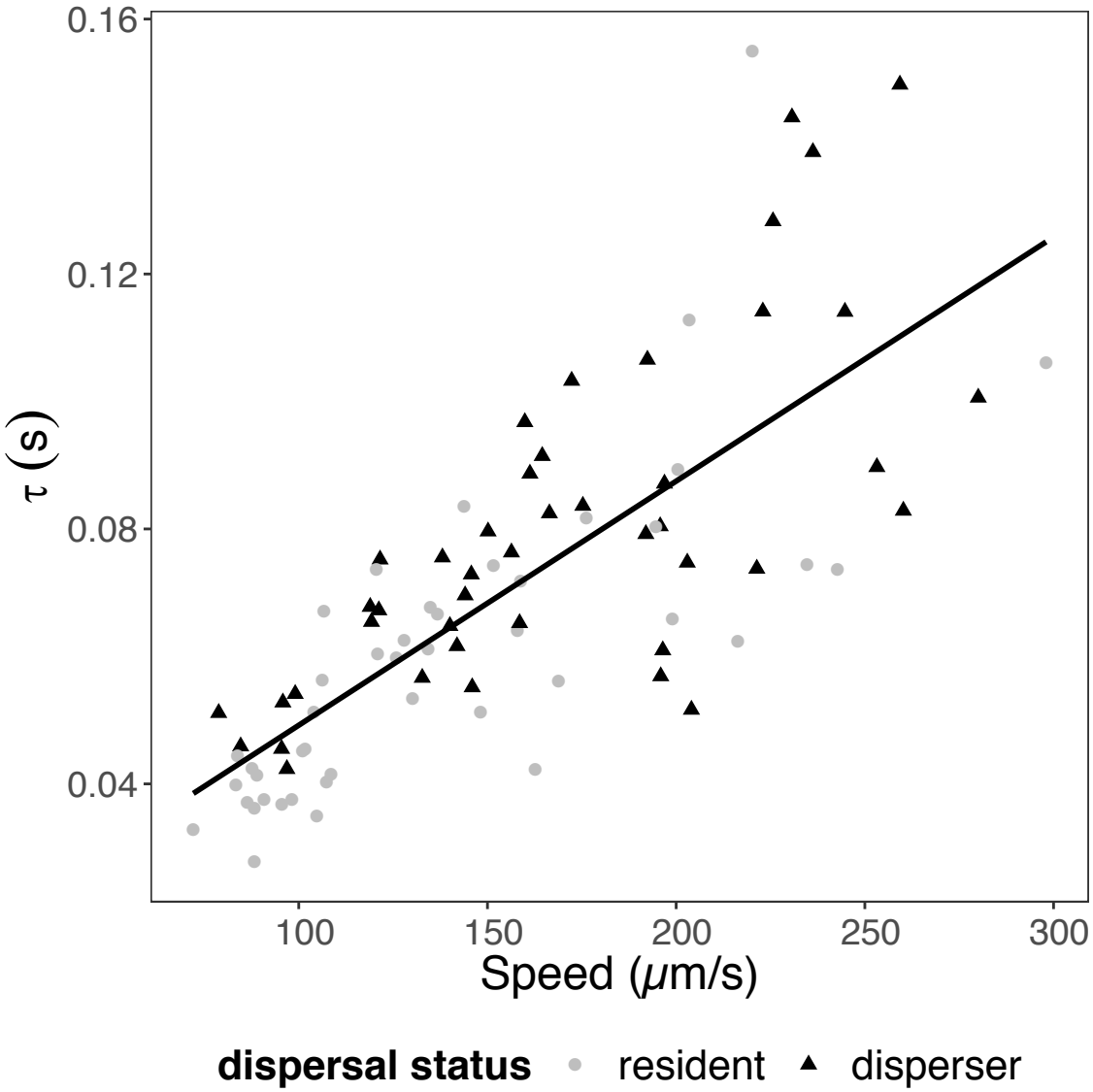
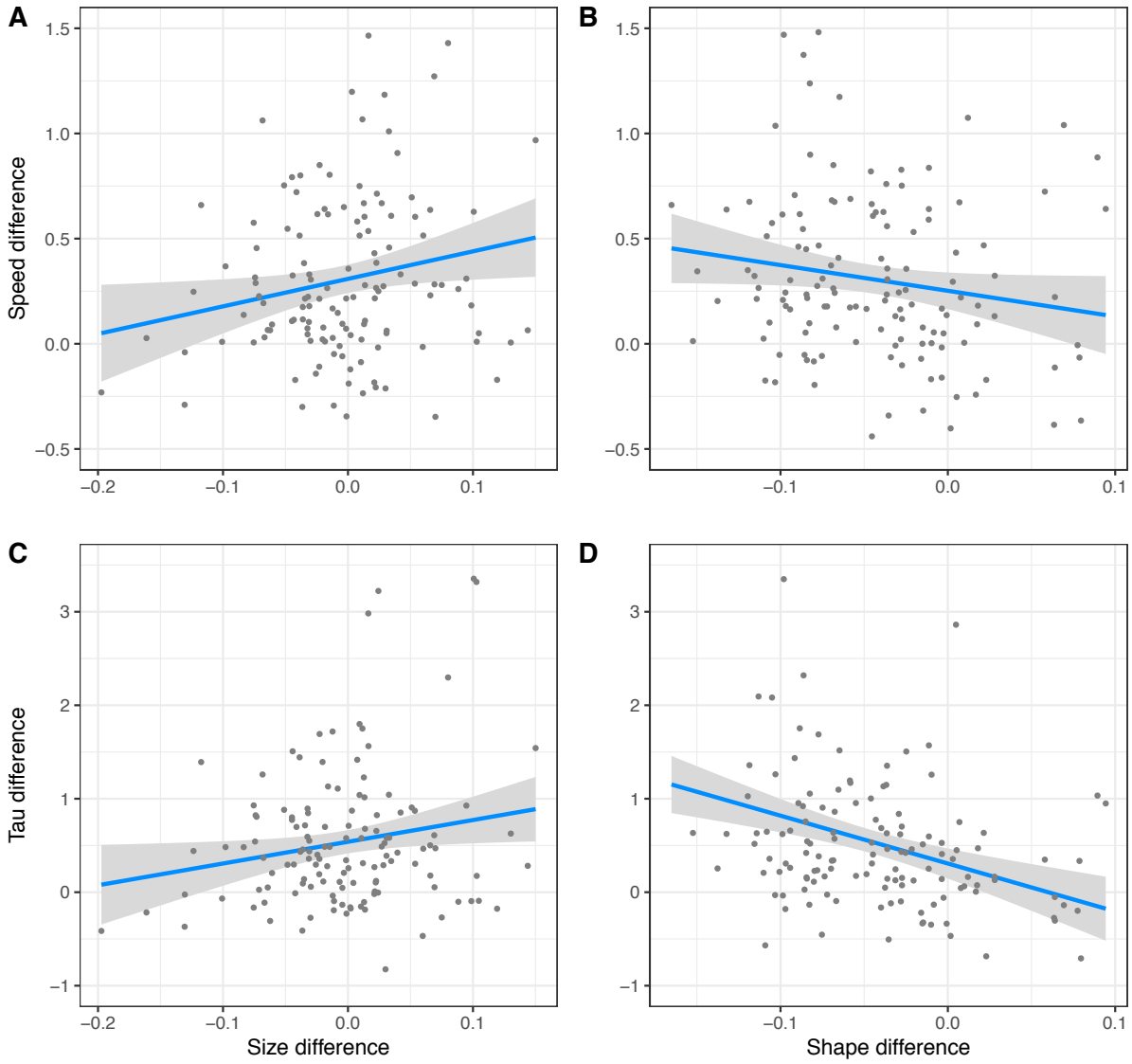


Figure S4: Relationships between movement and morphology differences between residents and dispersers. Genotypes with relatively larger dispersers showed relatively faster and more linear movements. Relatively more elongated dispersers showed relatively slower and less linear movements.



Tables

Table S1: List of *Tetrahymena thermophila* strains used in this study, as well as the distributor, isolator and geographic location from which the strain was obtained.

Our name	Distributor and reference	Isolator and isolation date	Geographic isolation
7	ATCC 30306	D.L. Nanney, 1953	Woods Hole, MA
20	TSC SD01236	E. Orias	Laboratory created
4A	CCAP 1630/4A	Nanney & McCoy, 1976	Unknown
B	ATCC 30384 (B-18687)	Simon & Nanney, 1968	Laboratory created
C	TSC SD01216	Yuhua Shang, 2002	Laboratory created
E	ATCC 205043	Simon & Nanney, 1986	McCurdy Pond, ME
F	TSC SD00086	P. J. Bruns	Laboratory created
G	TSC SD00112	Unknown	Unknown
H	TSC SD00270	Unknown	Unknown
I	TSC SD01206	Unknown	Unknown
J	TSC SD00626	Unknown, 1976	Laboratory created
K	TSC SD01590	Unknown	Laboratory created
L	TSC SD01223	Unknown, 1979	Laboratory created
M	CCAP 1630/1M	Phelps, 1948/9	Unknown, AZ
N	CCAP 1630/1N	Phelps, 1948/9	Unknown, AZ
O	TSC SD01422 (NP1)	L. Rasmussen, 1968	Laboratory created
P	CCAP 1630/1P	Phelps, 1948/9	Unknown, AZ
Q	CCAP 1630/1Q	Phelps, 1948/9	Unknown, AZ
R	TSC SD00703 (SB210)	E. Orias	Laboratory created
S	TSC SD01532	Unknown	Laboratory created
T	TSC SD01538	Unknown	Laboratory created
U	CCAP 1630/1U (WH14)	Elliott, 1952	Woods hole, MA
D1	TSC SD01546	Doerder, 8/2002	CRWP, PA
D2	TSC SD01547	Doerder, 8/2002	CRWP, PA
D3	TSC SD01548	Doerder, 06/2003	SG29, PA
D4	TSC SD01549	Doerder, 06/2003	SG29, PA
D5	TSC SD01550	Doerder, 06/2003	SG29, PA
D6	TSC SD01551	Doerder, 06/2003	SG29, PA
D7	Doerder AK III	Doerder	Unknown
D8	TSC SD01553	Doerder, 07/2008	FS136NW, PA
D9	TSC SD01552	Doerder, 07/2008	Beaver Meadows, PA
D10	TSC SD01557	Doerder, 07/2009	Lake Warren, NH
D11	TSC SD01558	Doerder, 07/2009	IslandPond#1, NH
D12	TSC SD01556	Doerder, 08/2008	SG69-1, PA
D13	TSC SD01555	Doerder, 08/2008	SG69-4, PA
D14	TSC SD01554	Doerder, 08/2008	SG69-6, PA
D15	TSC SD01560	Doerder, 07/2009	Gregg Lake, NH
D16	TSC SD01559	Doerder, 07/2009	Gregg Lake, NH
D17	TSC SD01561	Doerder, 07/2009	Willard Pond, NH
D18	TSC SD01562	Doerder, 07/2009	Willard Pond, NH
D19	TSC SD01564	Doerder, 07/2009	Childs Bog, NH
D20	TSC SD01563	Doerder, 07/2009	Russell Reservoir, NH
D21	TSC SD01565	Doerder, 07/2009	Perkins Pond, NH
D22	TSC SD01566	Doerder, 07/2009	South Pond, VT

Model selection among and within genotypes:

Table S2: Model selection to identify whether dispersal status and morphological properties (predictors) explain variation in movement speed (response). The most parsimonious model is shown in bold. K = number of parameters, AICc = Akaike information criterion value, delta = difference with the lowest AIC value, weight = AIC weight.

Model	K	AICc	delta	weight
speed ~ disp_status + shape + size + disp_status:shape + 1	**6**	**2846.68**	**0**	**0.69**
speed ~ disp_status + shape + size + disp_status:shape + disp_status:size + 1	7	2848.72	2.04	0.25
speed ~ disp_status + shape + disp_status:shape + 1	5	2851.37	4.68	0.07
speed ~ disp_status + shape + size + 1	5	2861.1	14.41	0
speed ~ disp_status + shape + size + disp_status:size + 1	6	2863.19	16.51	0
speed ~ disp_status + size + 1	4	2863.27	16.58	0
speed ~ disp_status + shape + 1	4	2865.25	18.56	0
speed ~ disp_status + size + disp_status:size + 1	5	2865.35	18.66	0
speed ~ disp_status + 1	3	2866.73	20.05	0
speed ~ size + 1	3	2881.54	34.86	0
speed ~ shape + size + 1	4	2883.29	36.6	0
speed ~ 1	2	2884.02	37.33	0
speed ~ shape + 1	3	2885.86	39.18	0

Table S3: Model selection to identify whether dispersal status and morphological properties (predictors) explain variation in tau, which describes movement linearity (response). The most parsimonious model is shown in bold. K = number of parameters, AICc = Akaike information criterion value, delta = difference with the lowest AIC value, weight = AIC weight.

Model	K	AICc	delta	weight
tau ~ disp_status + shape + disp_status:shape + 1	**5**	**-1129.43**	**0**	**0.58**
tau ~ disp_status + shape + size + disp_status:shape + 1	6	-1128.16	1.27	0.31
tau ~ disp_status + shape + size + disp_status:shape + disp_status:size + 1	7	-1126.06	3.37	0.11
tau ~ disp_status + shape + 1	4	-1114.82	14.6	0
tau ~ disp_status + shape + size + 1	5	-1113.47	15.96	0
tau ~ disp_status + shape + size + disp_status:size + 1	6	-1111.54	17.89	0
tau ~ disp_status + 1	3	-1106.99	22.44	0
tau ~ disp_status + size + 1	4	-1105.33	24.1	0
tau ~ disp_status + size + disp_status:size + 1	5	-1103.44	25.99	0
tau ~ 1	2	-1079.59	49.84	0
tau ~ shape + 1	3	-1079.1	50.32	0
tau ~ size + 1	3	-1077.73	51.7	0
tau ~ shape + size + 1	4	-1077.29	52.14	0

Model selection within genotypes:

Table S4: Model selection to identify whether differences in morphology (predictors) explain differences in speed (response). The most parsimonious model is shown in bold. K = number of parameters, AICc = Akaike information criterion value, delta = difference with the lowest AIC value, weight = AIC weight.

Model	K	AICc	delta	weight
speed_diff ~ shape_diff + size_diff + shape_diff:size_diff + 1	5	129.54	0	0.46
speed_diff ~ shape_diff + size_diff + 1	**4**	**130.32**	**0.78**	**0.31**
speed_diff ~ size_diff + 1	3	131.99	2.45	0.13
speed_diff ~ shape_diff + 1	3	133.43	3.89	0.07
speed_diff ~ 1	2	134.87	5.33	0.03

Table S5: Model selection to identify whether differences in morphology (predictors) explain differences in tau (response), which describes movement linearity. The most parsimonious model is shown in bold. K = number of parameters, AICc = Akaike information criterion value, delta = difference with the lowest AIC value, weight = AIC weight.

Model	K	AICc	delta	weight
tau_diff ~ shape_diff + size_diff + shape_diff:size_diff + 1	5	290.04	0	0.62
tau_diff ~ shape_diff + size_diff + 1	**4**	**291.51**	**1.47**	**0.3**
tau_diff ~ shape_diff + 1	3	294.22	4.18	0.08
tau_diff ~ size_diff + 1	3	307.8	17.76	0
tau_diff ~ 1	2	309.7	19.65	0

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