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Supplementary Materials for

Kinematic flexibility allows bumblebees to increase energetic efficiency when carrying heavy loads

Stacey A. Combes*, Susan F. Gagliardi, Callin M. Switzer, Michael E. Dillon

*Corresponding author. Email: sacombes@ucdavis.edu

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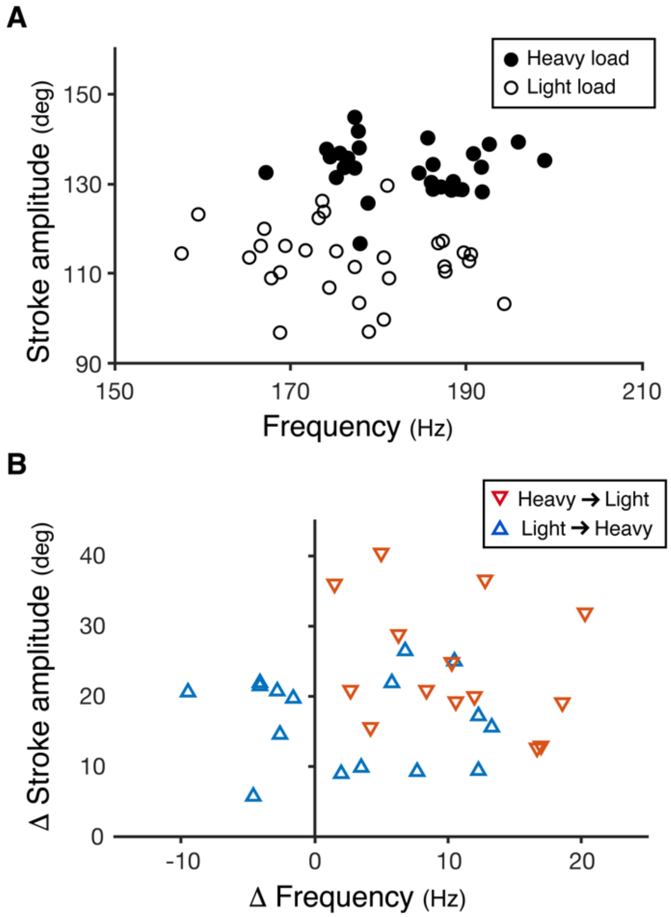

Fig. S1. There was no relationship between stroke amplitude and wingbeat frequency or between the change in amplitude and change in frequency between trials. (**A**) Measured stroke amplitude was unrelated to flapping frequency for bees in the heavy (filled circles) and light (open circles) loading treatments (heavy, $R^2 = 0.0047$; light, $R^2 = 0.0307$). (**B**) Individual changes in amplitude between trials (heavy – light loading) were unrelated to changes in frequency ($R^2 = 0.0066$). Bees tested in the heavy to light treatment order are shown in red and those in the light to heavy order are shown in blue.

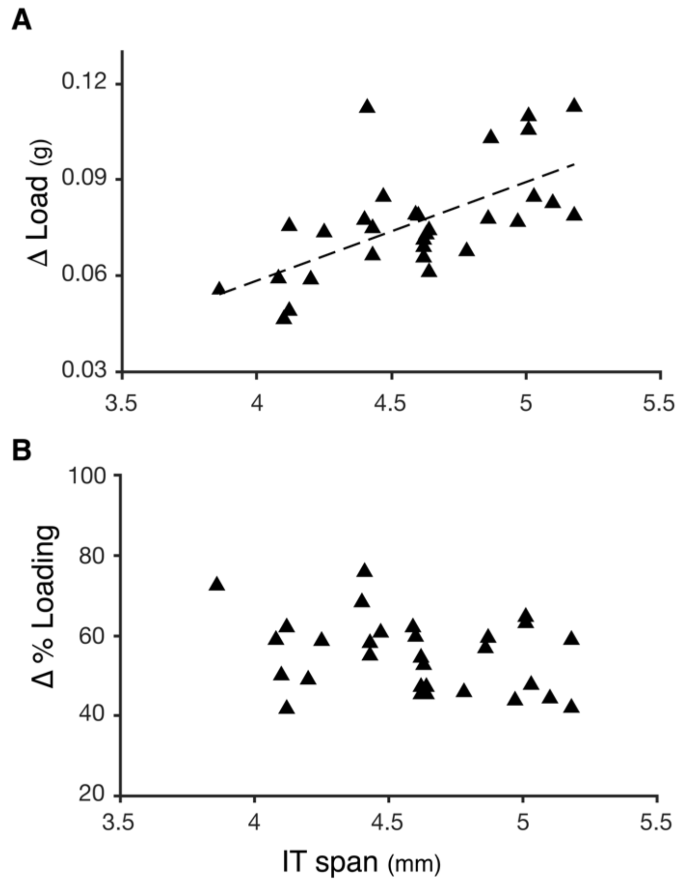

Fig. S2. Change in loading between trials scaled with bee size, but change in % loading was independent of size. (**A**) Change in absolute loading for individual bees (total mass during heavy loading – mass during light loading) increased with bee size, as measured by intertegular (IT) span (R^2 = 0.4016). (**B**) Change in % loading (change in load normalized by body mass; Fig. 2) was not associated with bee size (R^2 = 0.0876). Note that IT span (a common measure of bee size) was used as the independent variable because body mass was used in calculation of the independent variable (Δ % loading).

Table S1. Morphological, kinematic, and metabolic variables for all bees tested, grouped by treatment order (light to heavy, L-H; heavy to light, H-L). Variables shown include area of one forewing (S_{wing}), span length of one wing (L_{wing}), raw body mass (m_{body}), total mass with light loading (m_{light}) and heavy loading (m_{heavy}), stroke amplitude (Φ), frequency measured from audio data (n_{audio}; used in analyses), and frequency from video data (n_{video}).

		Morphology				Light loading trials						Heavy loading trials					
Bee ID	Load order	IT span (mm)	S_{wing} (mm^2)	L_{wing} (mm)	m_{body} (g)	m_{light} (g)	% Load	CO_2 out (mL/hr)	Φ (deg)	n_{audio} (Hz)	n_{video} (Hz)	m_{heavy} (g)	% Load	CO_2 out (mL/hr)	Φ (deg)	n_{audio} (Hz)	n_{video} (Hz)
1	L-H	4.6	32.3	11.2	0.1577	0.1854	17.5	10.39	111.5	177.3	181.2	0.2597	64.6	12.54	128.7	189.5	184.9
5	L-H	5.0	35.3	11.7	0.1755	0.1991	13.4	8.98	116.2	166.6	159.8	0.2759	57.2	12.20	125.7	178.8	173.1
12	L-H	4.4	29.9	10.8	0.1363	0.1731	27.0	10.34	110.5	187.6	182.4	0.2481	82.0	11.43	130.3	186.0	184.9
20	L-H	5.2	36.9	12.0	0.1886	0.2428	28.7	13.43	122.4	173.2	173.1	0.3218	70.6	14.38	131.4	175.2	169.8
26	L-H	4.6	30.8	10.9	0.1384	0.1943	40.4	10.01	129.6	181.0	175.3	0.2672	93.0	10.37	135.5	176.4	172.0
28	L-H	4.8	31.9	11.3	0.1472	0.1960	33.1	12.24	111.6	187.5	192.9	0.2636	79.1	14.33	132.4	184.6	180.0
35	L-H	4.6	30.5	11.1	0.1565	0.1794	14.6	9.08	103.5	177.8	172.0	0.2506	60.1	11.42	128.6	188.3	183.7
36	L-H	4.4	29.9	10.6	0.1147	0.1635	42.5	5.51	110.3	168.8	159.8	0.2301	100.6	7.81	136.8	175.6	168.8
37	L-H	4.1	24.1	9.6	0.0931	0.1256	34.9	5.91	112.8	190.3	177.6	0.1723	85.0	6.53	134.3	186.2	180.0
41	L-H	4.3	27.8	10.4	0.1258	0.1893	50.5	8.99	116.8	186.8	187.5	0.2630	109.1	10.01	138.8	192.6	188.8
42	L-H	4.9	31.4	11.0	0.1371	0.2074	51.2	11.53	117.3	187.3	184.9	0.2853	108.1	12.28	138.0	177.8	170.9
44	L-H	4.2	27.7	10.3	0.1200	0.1572	31.0	6.41	115.0	175.2	180.0	0.2160	80.0	10.19	130.5	188.5	182.4
45	L-H	5.0	37.3	11.7	0.1776	0.2272	27.9	11.96	106.9	174.4	173.1	0.3120	75.7	13.64	116.7	177.9	175.3
49	L-H	4.1	28	10.7	0.1182	0.1691	43.0	10.27	114.7	189.7	184.9	0.2184	84.8	11.13	129.3	187.1	181.2
50	L-H	4.6	31	11.1	0.1392	0.2142	53.9	11.15	113.6	180.6	181.2	0.2800	101.1	11.52	135.7	176.5	178.8
59	L-H	5.1	39.1	11.8	0.1868	0.2391	28.0	12.83	123.2	159.5	160.7	0.3220	72.4	15.01	132.5	167.2	161.7
3	H-L	4.9	34.6	11.4	0.1732	0.2064	19.2	9.21	120.0	167.0	168.8	0.3093	78.6	11.78	144.8	177.3	176.5
9	H-L	4.4	24.4	9.9	0.1135	0.1408	24.0	7.58	103.3	194.3	181.2	0.2183	92.3	9.47	139.3	195.9	194.2
10	H-L	4.6	29.6	10.6	0.1269	0.1441	13.6	7.16	96.9	168.8	154.3	0.2133	68.0	11.03	128.8	189.1	188.8
11	H-L	4.6	29.4	10.7	0.1350	0.1589	17.7	6.81	116.2	169.4	167.7	0.2201	63.0	10.24	128.8	186.2	183.7
16	H-L	5.0	34.5	12.0	0.1694	0.1991	17.5	9.53	114.5	157.6	157.9	0.3089	82.3	14.26	133.6	176.1	175.3
24	H-L	5.0	31.8	11.4	0.1670	0.2147	28.6	12.33	109.0	167.8	166.7	0.3202	91.7	15.72	137.7	174.1	167.7
30	H-L	4.6	29.7	11.1	0.1317	0.1819	38.1	9.36	126.2	173.6	172.0	0.2607	97.9	10.94	141.7	177.7	178.8
31	H-L	4.5	33.8	11.6	0.1395	0.1877	34.5	8.79	113.6	165.3	157.0	0.2725	95.3	11.86	133.5	177.3	176.5
32	H-L	4.1	25	9.7	0.1008	0.1139	13.0	5.51	97.1	178.9	170.9	0.1733	71.9	7.46	133.7	191.7	184.9
33	H-L	4.1	27.5	10.3	0.1213	0.1458	20.2	7.22	109.0	181.2	182.4	0.2213	82.4	13.08	128.2	191.8	187.5
39	H-L	4.4	31.9	11.2	0.1480	0.1966	32.8	12.91	99.8	180.6	170.9	0.3089	108.7	15.10	140.2	185.6	184.9
53	H-L	5.2	36.6	12.1	0.1909	0.2368	24.0	13.21	115.2	171.7	170.9	0.3495	83.1	16.48	136.0	174.5	169.8
54	H-L	4.6	29.2	10.6	0.1274	0.1534	20.4	8.39	123.8	173.8	173.1	0.2327	82.6	11.00	136.7	190.8	190.1
56	H-L	3.9	20.1	8.7	0.0767	0.1016	32.4	7.27	114.3	190.5	195.7	0.1572	104.9	8.45	135.2	198.9	204.5
Mean all		4.6	30.7	10.9	0.1411	0.1815	29.1	9.48	113.2	176.8	173.9	0.2584	84.2	11.72	133.4	183.2	180.0
S.D. all		0.4	4.3	0.8	0.0288	0.0361	11.6	2.39	8.0	9.6	10.6	0.0488	14.8	2.46	5.5	7.7	9.0

(Data are also available on-line at https://doi.org/10.5281/zenodo.2542885)

Table S2. Results of linear model investigating how Δ amplitude is affected by Δ % loading, treatment order, and bee size. Predictors dropped due to multicollinearity or because they did not significantly predict Δ amplitude include the following: treatment order, bee size (quantified by IT span), interaction of Δ % loading with bee size, interaction of Δ % loading with treatment order, and interaction of bee size with treatment order. Overall this model provides a significantly better fit than an intercept-only model – $F(1,28) = 15.47$, $p= 0.0005$.

Predictor	**Estimate (deg)**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-10.3904	7.902106	-1.31489	0.1992
Δ % loading	0.556958	0.141597	3.933409	0.0005

Table S3. Results from linear model investigating how Δ frequency is affected by Δ % loading, treatment order, and bee size. The same predictors were used as for Table S2, and all were dropped except for treatment order. Overall this model provides a significantly better fit than an intercept-only model – $F(1,28) = 9.70$, $p= 0.004$.

Predictor	**Estimate (Hz)**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	10.4619	1.7946	5.8297	2.90E-06
Order: light to heavy	-7.6536	2.4573	-3.1146	0.004

Table S4. Results from linear model investigating how Δ metabolic rate is affected by Δ % loading, Δ amplitude, Δ frequency, treatment order, and bee size. Predictors dropped due to multicollinearity or because they did not predict Δ metabolic rate include: Δ % loading, treatment order, and bee size (IT span). We made an *a priori* decision to keep both Δ amplitude and Δ frequency in the final model, even if they were not significant, because these kinematic values are mathematically related to total force production (Fig. 1). Overall this model provides a significantly better fit than an intercept-only model – $F(2,27) = 22.41$, $p = 1.834e-06$.

Predictor	**Estimate** (mL CO_2 * hr^{-1})	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.0604	0.2215	-0.2727	0.7871
Δ amplitude	0.0105	0.0098	1.0661	0.2958
Δ frequency	0.071	0.0109	6.5014	5.71e-07