Tethered-flight performance of thermally-acclimated pest fruit flies (Diptera: Tephritidae) suggests that heat waves may promote the spread of *Bactrocera* species

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Supplementary methods

FLY HUSBANDRY

Ceratitis capitata

Cultures of *C. capitata* were established from pupae collected from field infested fruits (oranges, mandarins and apples) from the area of Volos (Greece) and reared at the Laboratory of Entomology and Agricultural Zoology at the University of Thessaly under constant conditions (temperature: 25±2°C and RH: 55±5% 14L: 10D). The fruits were placed on sterile sand in plastic containers and sifted at regular intervals (at least once a week) to retrieve the pupae. Pupae (ca. 300) were then placed in wooden cages 30×30×30cm (custom made) and remained at the same conditions until adult emergence. Upon emergence, adults had *ad libitum* access to standard adult diet (sugar: yeast hydrolysate and water in ratio 4:1:5) and water. Females were allowed to oviposit into artificial oviposition substrates (domes) that consisted of a red, plastic, hollow hemisphere 5 cm in diameter (Euro-matik kfc, Budapest, Hungary) with 40 to 50 evenly distributed holes (diameter 0.7 mm). Each dome was fitted into a 5 cm diameter hole cut on the lid of a 5.5 cm diameter plastic Petri dish. Water was placed into the petri dish to maintain humidity levels in the dome. A plastic container with 0.5 ml of orange juice was placed in the base of the Petri dish to stimulate oviposition. Eggs were collected from the inner surface of the dome, and larvae were reared on a standard artificial diet. Rearing of the experimental generations was conducted following the same methodology but after emergence, *C. capitata* adults were sexed and placed in Plexiglas cages 20×20×20 (Plastil, Thessaloniki, Greece) in groups of 10 (one per temperature group/sex) with *ad libitum* access to food (hydrolyzed yeast and sugar) and water. The same 14L: 10D light cycle was used in the incubators for acclimation treatment. Experimental flies were from the third laboratory generation.

Bactrocera dorsalis

Cultures of *B. dorsalis* were established from wild pupae provided by Citrus Research International (Nelspruit, South Africa) from which the females were mated with laboratory adapted males (less than 15 generations). Pupae (ca. 400) were placed in insect cages (32.5 × 32.5 × 32.5 cm, BugDorm43-030, MegaView Science, Taichung, Taiwan) with unrestricted access to food (hydrolysed yeast and sugar in separate dishes) and water (water-soaked cotton wool). The cultures were maintained at ~ 25° C in a climate room (University of Pretoria, Pretoria, South Africa) with a 14:10 light:dark photocycle. To create optimal mating conditions, the first and last hour of the light phase simulated dawn and dusk with 8 W fluorescent tubes (T4, Eurolux, Sandton, South Africa) that were placed obliquely to the fly culture and turned on before, and turned off after, the main room lights. The remaining room lights, comprising a combination of 20 W (G5, Eurolux, Sandton, South Africa) and 58 W (58W/840, Osram, Germany) fluorescent tubes were also turned on for the remainder of the light period. Each new generation was obtained by allowing females of 20-40 days of age to lay eggs on a 125 mL plastic container (Plastilon, South Africa) covered with a layer of laboratory film (Parafilm M, Bemis, USA) pierced several times

with a pin. Tissue paper soaked with 3 mL of guava juice concentrate (Hall's, Tiger Consumer Brands Limited, Bryanston, South Africa) and a slice of guava fruit were placed in the plastic container to encourage females to oviposit through the film. Eggs were then washed out of the artificial substrate with water and placed on 125 mL of a carrot-based larval rearing medium (Citrus Research International, Nelspruit, South Africa) in a plastic container at an approximate density of 2.5 eggs/mL of medium. The container of larval rearing medium was then placed in a 2 L plastic box with a layer of sand and a ventilated lid. After 15 days, during the pupal phase, the sand was sifted and pupae placed in a Petri-dish (ø 65 mm) and transferred into an insect cage (32.5 × 32.5 × 32.5 cm) with unrestricted access to food and water for emerging adults. Experimental flies were produced in the same manner, except that before one week of age (prior sexual maturity), females and males were separated and transferred to a 2 L transparent plastic cage (ca. 50 individuals per cage) with unrestricted access to food and water. The same 14L: 10D light cycle was used in the incubators for acclimation treatment. Experimental flies were produced 6 to 12 generations after crossing laboratory cultures with the wild flies provided by Citrus Research International.

Bactrocera zonata

Cultures of *B. zonata* were established from pupae retrieved from fruits (*Terminalia catappa*) in La Réunion. Laboratory rearing was conducted in insect cages (37.5 × 37.5 × 37.5 cm, BugDorm 4S4545, MegaView Science, Taichung, Taiwan) with unrestricted access to food (hydrolysed yeast and sugar) and water (water-soaked sponge). The cultures were maintained at ~ 25° C in a climate room (CIRAD, Saint-Pierre, La Réunion, France) with natural light supplemented with artificial white light from 6 a.m. to 5 p.m. Each new generation was obtained by allowing females of 10-40 days of age to lay eggs on an artificial oviposition device (perforated tennis ball) with a small piece of fruit inside to encourage females to oviposit. Eggs were washed out with 0.2 % Nipagin/sodium benzoate solution and were transferred in a carrot-based larval-rearing medium [1] in a plastic container. The container of larval rearing medium was then placed in a 3L plastic box with a layer of sand and a ventilated lid. After 15 days, during the pupal phase, the sand was sifted, and the retrieved pupae (ca. 1000 individuals per cage) were placed into a new insect cage with unrestricted access to food and water for emerging adults. Experimental flies come from generations 176 to 189 of the laboratory cultures. They were produced in the same manner, except that before one week of age (prior sexual maturity), females and males were separated and transferred in a 2 L transparent plastic cage (ca. 50 individuals per cage) with unrestricted access to food and water. The same 11L: 13D light cycle was used in the incubators for acclimation treatment.

TETHERED FLIGHT PROCEDURE

For *C. capitata*, the flight mill assays were conducted at the University of Thessaly in a room with an average temperature of 25±2°C and average relative humidity of 55±5%RH. To record flight performance, 8 flight mills were constructed in the laboratory according to Attisano, Murphy, Vickers and Moore [2]. The flight mills were connected to an 8-channels flight mill data acquisition system and a free proprietary software (WinDaq/Lite, DATAQ Instruments, Ohio, USA) was used to record the data. We followed Attisano et al. (2015) and used Python (version 3.7.9), to extract the data. In preparation for the flight mill assays, the mass of 1.5 mL empty microcentrifuge tubes (ScientAct, Greece) was recorded using an analytical balance (accurate to 0.0001g, Precisa 40 SM-200A, SWISSQUALITY, Switzerland). Then the acclimated flies were individually placed in the microcentrifuge tube from the cages using an aspirator and the mass of the fly plus the tube was measured to determine the body mass of the fly. Flies were then briefly chilled (ca. 2 min) by placing the microcentrifuge tubes on fine ice shavings. Once chilled, the fly was placed on a paper towel, and a small drop of hot melted glue collected on the tip of a #1 entomological pin was immediately applied to the center of the thorax in a perpendicular and upright position. After receiving the pin, each fly placed on a Styro-foam board by

sticking the pointed end of the pin into it. Flies were allowed to recover until the last fly (8 flies per session) was pinned. Starting with the first fly to be pinned, flies were attached to the flight apparatus by inserting the pin into the opening of the hypodermic tube of the arm of the flight mill and secured with a small piece of Patafix white glue pad (UHU, Greece) to ensure that the pin would not slip out of the tube. A small foil flag of the same weight of the fly was placed with a piece of Patafix on a pin inserted in the opposite arm of the flight mill to balance the mass and maximize disruption of the infrared beam in the sensor. One to two flies from each temperature group were flown per session, and two sessions a day were run. At the end of each session, flies were returned to their microcentrifuge tubes and placed in a -80°C freezer.

For *B. dorsalis*, the flight mill assays were conducted in a climate room at the University of Pretoria, with an average temperature of 25.4 ± 0.4 °C and average relative humidity of 28.3 ± 7.9 % RH. To record flight performance, we used computerized flight mills connected to a 15-channels flight mill data acquisition (DAQ) system that was controlled from a laptop. The DAQ was built by the Vehicle Dynamics Group at the University of Pretoria using the design developed by the USDA-ARS Arid Land Agricultural Research Centre in Mariscopa, Arizona [3]. In preparation for the flight mill assays, the mass of 1.5 mL empty microcentrifuge tubes (Plastpro Scientific, South Africa) was recorded using an analytical balance (accurate to 0.0001g, NewCLassic MF model #MS204S, Mettler-Toledo, Greifensee, Switzerland). The flies were then individually captured from the 2 L plastic cages using the microcentrifuge tube and the mass of the fly plus the tube was measured to determine the body mass of the fly. Flies were then briefly chilled (ca. 2 min) by placing the microcentrifuge tubes on fine ice shavings. Once chilled, the fly was placed on a paper towel, and a small drop of hot melted glue collected on the tip of a #1 entomological pin was immediately applied to the center of the thorax in a perpendicular and upright position. After receiving the pin, each fly was placed on a Styro-foam board by sticking the pointed end of the pin into it. Flies were allowed to recover until the last fly (9 flies per session) was pinned. Starting with the first fly to be pinned, flies were attached to the flight apparatus by inserting the pin into the opening of the hypodermic tube of the arm of the flight mill and secured with a small piece of pressure-sensitive non-permanent adhesive (Bostik, South Africa) to ensure that the pin would not slip out of the tube. A small piece of adhesive of the same size was placed on a pin inserted in the opposite arm of the flight mill to balance the mass. Three flies of the same sex from each temperature group were flown per session, and two sessions a day were run. At the end of each session, flies were returned to their microcentrifuge tubes and placed in a -80°C freezer.

For *B. zonata,* the flight mill assays were conducted in a climate room at the CIRAD Réunion, with an average temperature of 26.4 ± 1.8°C and average relative humidity of 54.7 ± 9.5 % RH. We used an 8-channels flight mill connected to a data acquisition system controlled by a Rasberry Pi 3 B+ (Rasberry Pi, United Kingdom) to record flight performance. The flight mill was built by the CIRAD Réunion using the design developed by Attisano, Murphy, Vickers and Moore [2]. Flies were individually weighed before each flight session. The flies were individually captured from the 2 L plastic cages, and a #1 entomological pin was glued to the center of the thorax in a perpendicular and upright position with a small drop of the hot melted glue. After receiving the pin, each fly was placed on a Styro-foam board by sticking the pointed end of the pin into it until the last fly was pinned. Flies were attached to the flight apparatus by inserting the pin into the opening of the hypodermic tube of the arm of the flight mill. Eight flies were flown per session, chosen randomly according to sex and temperature group. Each session started 2 minutes after the last fly was attached to the flight mill and recorded for precisely 2 hours. At the end of each session, flies were put in microcentrifuge tubes and placed in a -80°C freezer. A group of *Bactrocera dorsalis* from La Reunion Island were tested in the same conditions.

WING MORPHOMETRY

Both wings of the flies tested on the flight-mill were removed from the thorax using a razor blade and mounted face up on a microscope slide using clear double-sided tape, and a second microscope slide was added on top to cover the wings. Photographs of both wings were taken at ×20 magnification using a stereo-microscope (brands differed between species) with a digital camera mounted on it. To scale the images, a photograph of a 0.5 mm ruler was also taken at the beginning of each session. For both wings (one if damaged), open-source ImageJ software (Wayne Rasband, National Institute of Health, USA) was used to take measurements of wing length, wing width and 7 landmarks used to calculate wing area. The landmarks for wing measurements differed between *Bactrocera* and *Ceratitis* species (see Fig. S1 and S2). These measurements were used to calculate the aspect ratio (wing length^2 / wing area) and the wing loading (body mass / wing area).

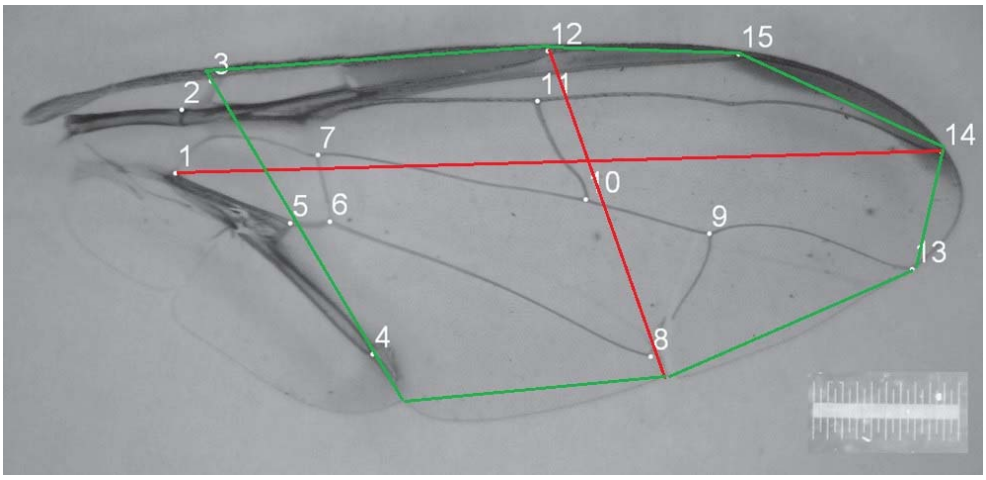

Figure S1. Landmarks used in *Bactrocera* species for measuring wing length (1 to 14), wing width (12 to the edge of the wing pass landmark 8) and wing area (green lines). From Schutze *et al.*[4].

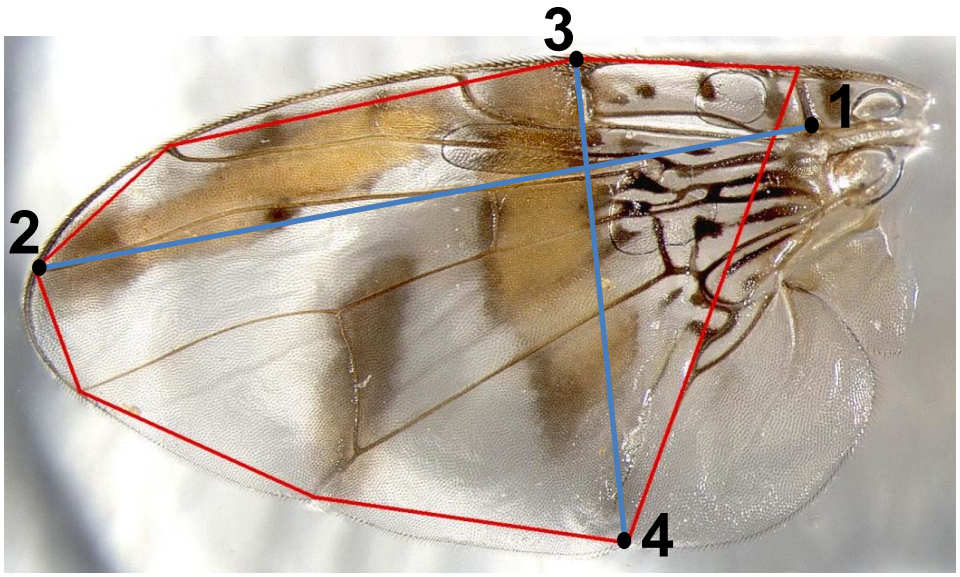

Figure S2. Landmarks used for measuring the wing length (1 to 2), wing width (3 to 4) and wing area (red lines) of *Ceratitis capitata*. From Esterhuizen *et al.*[5].

STATISTICAL ANALYSES

We first checked if flight performance was similar between individuals of the same species (*B. dorsalis*) tested on different flight mill apparatus in different laboratories (see Table S1 and Fig. S3).

Intraspecific comparisons of flight performance

Using the cor function (R built in package), we checked in each species for correlation between body mass, aspect ratio, wing loading, wing area, distance, average speed and maximum speed (Fig. S4). Data for body mass and flight performance traits were analyzed using generalized mixed effects models with a Gaussian distribution (residuals of the models were normally distributed), except for the number of flight events where a Poisson distribution was used because this variable has count data. The models were built using the glmmTMB function from the package of the same name [6]. Acclimation temperature, sex and their interaction were entered as fixed effects, body mass as a covariate (not included in the model for body mass) and flight mill channel as a random effect. No random effect was added to the model for the distance flown as the random variance was null and a generalized linear model was used. Model reduction was performed by removing the interaction term if it was not significant, and models (full and reduced) were compared using the anova function to determine the best one based on the lowest AIC. If a significant main effect or interaction was detected, *post hoc* pairwise comparison tests of the estimated marginal means were performed using the emmeans function from the package of the same name [7]. The pairwise comparisons from the emmeans function returns an estimate that is the difference between the estimated marginal means of the two compared groups and indicates the direction of the difference. To control for age variation in *B. zonata*, the same models for body mass and all flight performance traits were used, and age was entered as a covariate (without any other covariate). The results are displayed in Table S2.

Wing and body morphometry

For wing measurements, species were compared to one another for all traits (aspect ratio, wing loading and wing area) using generalized linear models. The glm function from the car package was used to build the models and species, sex, acclimation temperature and their interactions were added as fixed effect, and body mass as a covariate (except for wing loading). Residuals of the models were inspected and because the normal distribution assumption was not met a logarithmic transformation of the response variables was necessary. The minimal adequate model was determined using the step function. If a significant main effect or interaction was detected, *post hoc* pairwise comparison tests of the estimated marginal means were performed using the emmeans function.

Table S1. Flight performance and body mass comparison between *B. dorsalis* females and males originating from populations in South Africa and Reunion Island. Only the parameters retained in the minimal adequate model are presented.

	$\chi 2$	df	p
Distance			
Body mass	20.65	1	**< 0.001**
Average speed			
Body mass	25.97	1	**< 0.001**
Maximum speed			
Body mass	21.64	1	**< 0.001**
Flight events			
Population	2.51	1	0.113
Sex	2.02	1	0.155
Body mass	7.6	1	**0.006**
Flight duration			
Population	6.34	1	**0.012**
Sex	3.19	1	0.074
Body mass			
Population	204.92	1	**< 0.001**
Sex	15.33	1	**< 0.001**

Generalized linear models were used to compare the flight performance of two *B. dorsalis* populations originating from laboratories in South Africa and Reunion Island. Flies were acclimated at 25°C only and tested at the same temperature. Flies from South Africa were larger which resulted in longer distance being flown and higher average speed (Fig. S3), but this was only driven by body mass (Table S1). For flight performance traits, there was only a significant effect of population on the flight duration, which was lower in flies from Reunion Island than from South Africa (estimate = -1347, p = 0.014).

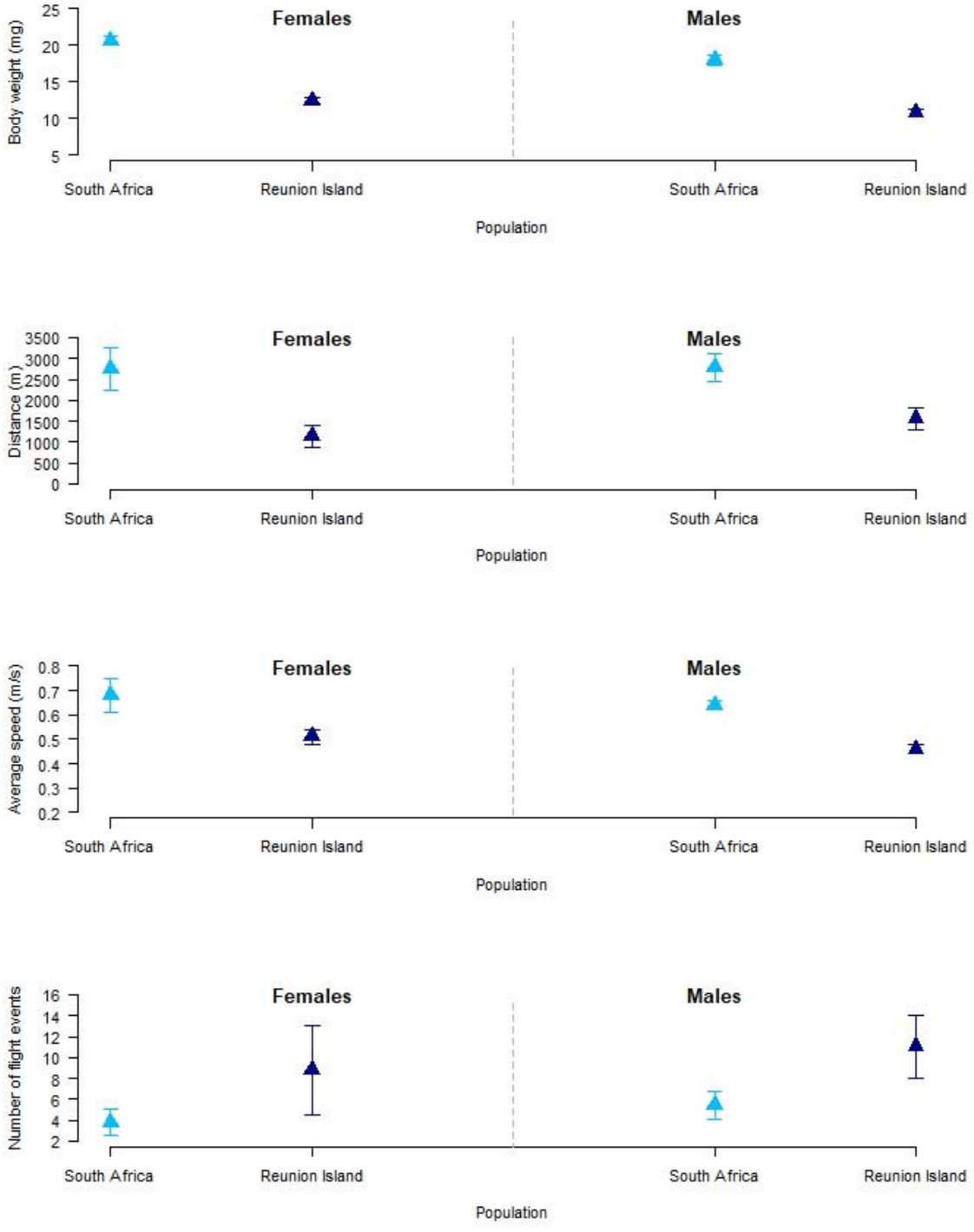

Figure S3. Average body mass and flight performance traits of two populations of *Bactrocera dorsalis* tested for 2 hours on flight mills at 25°C. Each triangle represents the means from 15 to 20 individuals, and the error bars represent the standard error of the mean.

Table S2. Effects of temperature acclimation, sex and age (covariate) on the body mass and flight performance traits of *B. zonata* acclimated for 48 hours at either 20, 25 or 30°C and tested for flight performance for 2 hours at 25°C. Generalized mixed effects models with a Gaussian distribution were used for all traits except flight events where a Poisson distribution was used.

	χ2	**df**	**p**
Distance			
Acclimation	1.82	1	0.402
Sex	5	1	0.822
Age	2.14	1	0.143
Sex x Acclimation	**12.28**	**2**	**0.002**
Average Speed			
Acclimation	5.43	2	0.066
Sex	2.07	1	0.149
Age	**8.13**	**1**	**0.004**
Sex x Acclimation	1.03	2	0.596
Maximum Speed			
Acclimation	5.68	2	0.058
Sex	0.36	1	0.545
Age	**7.87**	**1**	**0.005**
Sex x Acclimation	4.09	2	0.129
Flight Events			
Acclimation	**85.94**	**2**	**< 0.001**
Sex	1.53	1	0.216
Age	0.74	1	0.388
Sex x Acclimation	**14.12**	**2**	**< 0.001**
Flight Duration			
Acclimation	3.67	2	0.159
Sex	0.46	1	0.499
Age	0.92	1	0.336
Sex x Acclimation	**6.21**	**2**	**0.044**
Body mass			
Acclimation	**39.82**	**2**	**< 0.001**
Sex	**14.77**	**1**	**< 0.001**
Age	0.52	1	0.468
Sex x Acclimation	**17**	**2**	**< 0.001**

We found that only speed was affected by the age of the flies, with average speed (coefficient = -0.004, p = 0.004) and maximum speed (coefficient = -0.006, p = 0.005) slightly decreasing as age increases. Nevertheless, this did not affect how temperature acclimation and sex affects flight performance (similar outputs to the table presented in the main text).

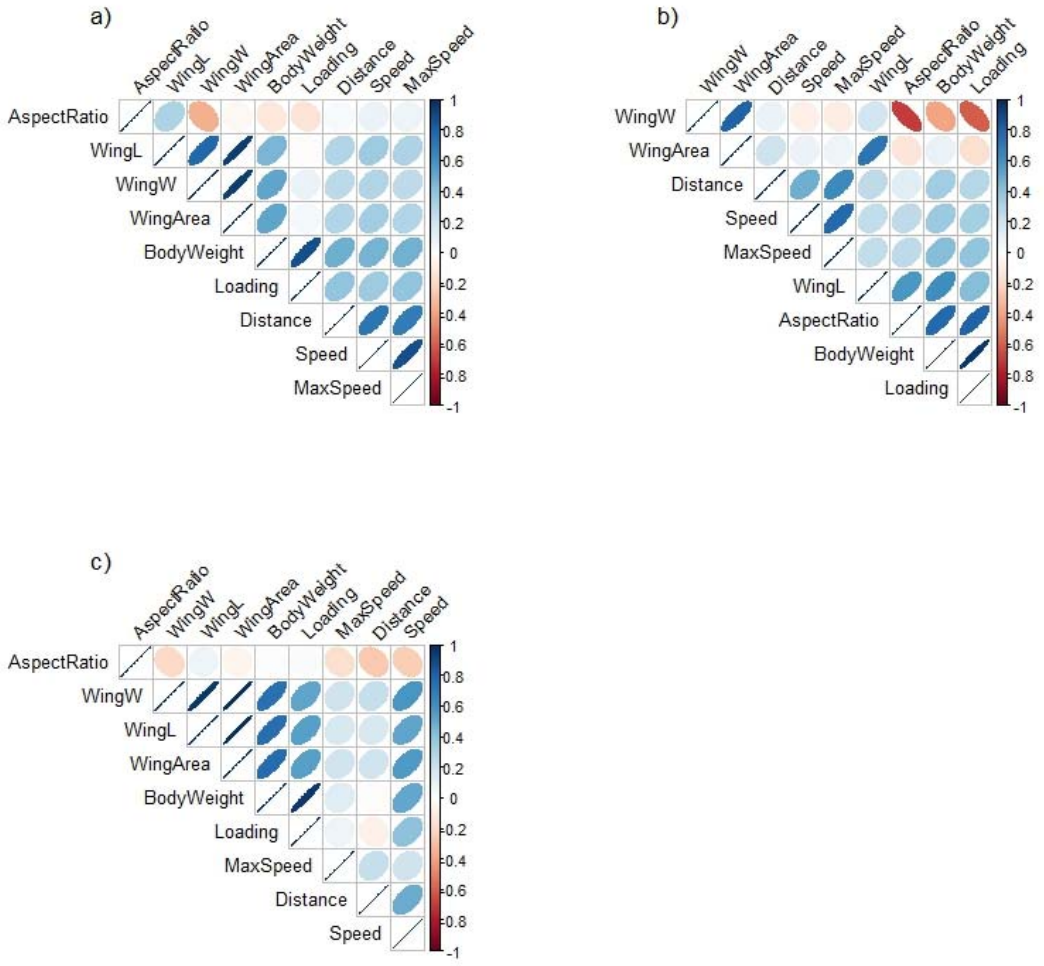

Figure S4. Correlation matrix between flight performance traits and wing and body characteristics in *C. capitata* (a), *B. dorsalis* (b) and *B. zonata* (c). The color gradient indicates the magnitude of the correlation (dark red strong negative and dark blue strong positive) and the direction whether the correlation is positive (top pointing to the right) or negative (top pointing to the left).

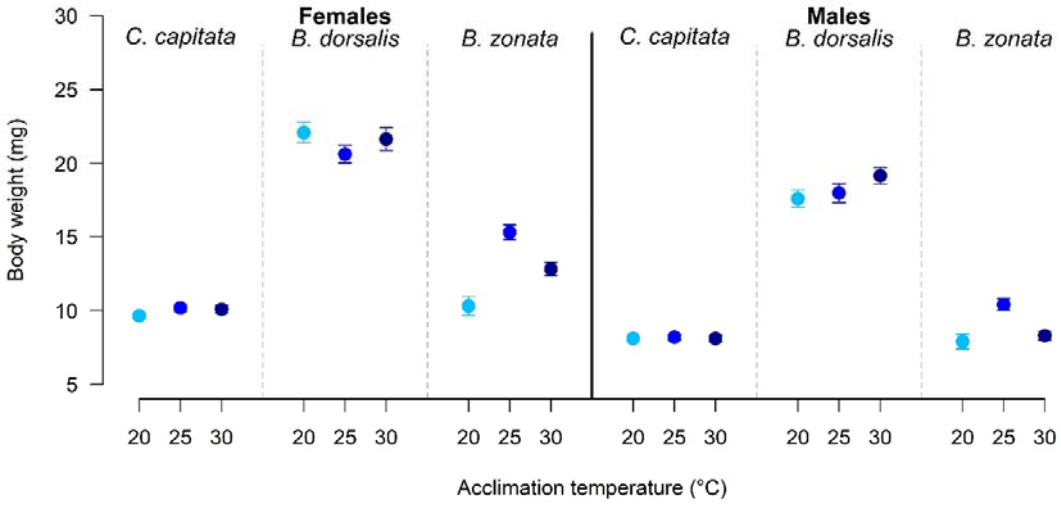

Figure S5. Average body mass of females and males from three species of fruit flies acclimated at either 20, 25 or 30°C. Each circle represents 14 to 24 individuals and error bars represent the standard error of the mean.

In *C. capitata* and *B. dorsalis*, body mass was affected by sex only with males being lighter than females (*C. capitata*: coefficient = -1.53, $p < 0.001$; *B. dorsalis*: coefficient = -4.35, $p < 0.001$) (Table 1, Fig. S5). However, in *B. zonata* there was also an interaction between acclimation temperature and sex (Table 1). This is because females acclimated at 20°C were lighter than those acclimated at 25 or 30°C (respectively, estimate = -4.92, $p < 0.001$; estimate = -3.40, $p < 0.001$), and males acclimated at 20°C lighter than males acclimated at 25°C (estimate = -2.48, $p = 0.002$), while males from the 25°C were heavier than those acclimated at 30°C (estimate = 3.35, $p < 0.001$). As in the other species, male *B. zonata* were lighter than females overall (coefficient = -2.47, $p < 0.001$).

When aspect ratio, wing loading or wing area were added to the models in replacement of body mass, we found that wing loading affected the distance flown and the maximum speed in *C. capitata*, and average speed was affected by wing area in *B. zonata* (Table S3). Distance (coefficient = 5479.14, $p = 0.017$) and maximum speed (coefficient = 0.54, $p = 0.009$) increased as wing loading increased in *C. capitata*. The average flying speed in *B. zonata* increased with greater wing area (coefficient = 0.11, $p = 0.002$).

Table S3. Effect of wing aspect ratio, wing loading and wing area on flight performance traits in *C. capitata*, *B. dorsalis* and *B. zonata*. Aspect ratio, wing loading and wing area were added separately to the models as covariates.

		$\chi 2$	df	p
Distance				
C. capitata	Aspect ratio	0.61	1	0.434
	Wing loading	**6.06**	**1**	**0.013**
	Wing area	2.85	1	0.091
B. dorsalis	Aspect ratio	0.13	1	0.717
	Wing loading	0.08	1	0.775
	Wing area	0.23	1	0.631
B. zonata	Aspect ratio	0.96	1	0.325
	Wing loading	1.67	1	0.195
	Wing area	3.67	1	0.055
Speed				
C. capitata	Aspect ratio	0.23	1	0.627
	Wing loading	3.66	1	0.055
	Wing area	2.49	1	0.114
B. dorsalis	Aspect ratio	0.01	1	0.935
	Wing loading	0.02	1	0.871
	Wing area	0.01	1	0.912
B. zonata	Aspect ratio	3.11	1	0.077
	Wing loading	3.22	1	0.073
	Wing area	**10.05**	**1**	**0.001**
Maximum speed				
C. capitata	Aspect ratio	0.23	1	0.631
	Wing loading	**7.35**	**1**	**0.006**
	Wing area	3.02	1	0.082
B. dorsalis	Aspect ratio	0.04	1	0.841
	Wing loading	0.16	1	0.691
	Wing area	0.28	1	0.596
B. zonata	Aspect ratio	1.94	1	0.163
	Wing loading	0.01	1	0.928
	Wing area	0.48	1	0.485

When accounting for size differences between species in the interspecific models, body mass was a significant predictor for all flight performance traits except for the number of flight events (Table S5). Differences between species for average and maximum speed disappeared when taking body mass into account (Table S5).

Table S4. Across species effects of acclimation temperature (20, 25 and 30°C) and sex on the flight performance at 25°C of three tephritid species.

	$\chi 2$	df	p
Body mass			
Species	**440.59**	**2**	**< 0.001**
Acclimation	0.66	2	0.718
Sex	**5.05**	**1**	**0.024**
Species x Acclimation	**55.51**	**4**	**< 0.001**
Species x Sex	**9.04**	**2**	**0.011**
Acclimation x Sex	0.38	2	0.825
Species x Acclimation x Sex	**24.23**	**4**	**< 0.001**
Distance			
Species	3.78	2	0.151
Acclimation	1.62	2	0.444
Sex	0.125	1	0.723
Species x Acclimation	**13.51**	**4**	**0.009**
Species x Sex	0.26	2	0.875
Acclimation x Sex	1.08	2	0.581
Species x Acclimation x Sex	**10.28**	**4**	**0.036**
Average speed			
Species	**37.78**	**2**	**< 0.001**
Acclimation	0.02	2	0.989
Sex	0.15	1	0.695
Species x Acclimation	6.29	4	0.178
Species x Sex	4.44	2	0.108
Acclimation x Sex	0.13	2	0.936
Species x Acclimation x Sex	4.22	4	0.376
Maximum speed			
Species	**44.52**	**2**	**< 0.001**
Acclimation	0.07	2	0.967
Sex	0.52	1	0.468
Species x Acclimation	3.93	4	0.414
Species x Sex	3.72	2	0.155
Acclimation x Sex	0.18	2	0.914
Species x Acclimation x Sex	4.04	4	0.401
Flight events			
Species	2.26	2	0.322
Acclimation	3.97	2	0.137
Sex	0.07	2	0.788
Species x Acclimation	**12.89**	**4**	**0.012**
Species x Sex	0.66	2	0.719
Acclimation x Sex	2.27	2	0.321
Species x Acclimation x Sex	5.36	4	0.251
Flight duration			
Species	0.49	2	0.783

Acclimation	2.96	2	0.226
Sex	0.01	1	0.941
Species x Acclimation	8.26	4	0.082
Species x Sex	0.28	2	0.868
Acclimation x Sex	1.44	2	0.486
Species x Acclimation x Sex	7.68	4	0.104

Table S5. Across species effects of acclimation temperature (20, 25 and 30°C) and sex on the flight performance at 25°C of three species of fruit flies. Body mass was added to the models as a covariate.

	χ2	**df**	**p**
Distance			
Species	**7.86**	**2**	**0.019**
Acclimation	1.39	2	0.497
Sex	0.08	1	0.771
Species x Acclimation	**24.58**	**4**	**< 0.001**
Species x Sex	1.36	2	0.505
Acclimation x Sex	1.16	2	0.561
Species x Acclimation x Sex	**17.23**	**4**	**0.002**
Body mass	**25.07**	**1**	**< 0.001**
Average speed			
Species	0.35	2	0.838
Acclimation	0.04	2	0.979
Sex	0.06	1	0.801
Species x Acclimation	0.67	4	0.955
Species x Sex	2.56	2	0.277
Acclimation x Sex	0.04	2	0.982
Species x Acclimation x Sex	1.32	4	0.858
Body mass	**22.31**	**1**	**< 0.001**
Maximum speed			
Species	0.17	2	0.917
Acclimation	0.09	2	0.954
Sex	0.01	1	0.939
Species x Acclimation	2.06	4	0.723
Species x Sex	2.96	2	0.227
Acclimation x Sex	0.28	2	0.867
Species x Acclimation x Sex	1.54	4	0.818
Body mass	**26.82**	**1**	**< 0.001**
Flight events			
Species	0.28	2	0.866
Acclimation	3.77	2	0.151
Sex	0.26	1	0.609
Species x Acclimation	**13.84**	**4**	**0.008**
Species x Sex	1.07	2	0.585
Acclimation x Sex	2.29	2	0.318
Species x Acclimation x Sex	6.93	4	0.139

Body mass	3.59	1	0.058
Flight duration			
Species	3.36	2	0.186
Acclimation	2.86	2	0.238
Sex	0.07	1	0.789
Species x Acclimation	**10.82**	**4**	**0.028**
Species x Sex	1.07	2	0.586
Acclimation x Sex	1.32	2	0.516
Species x Acclimation x Sex	**9.605**	**4**	**0.047**
Body mass	**9.29**	**1**	**0.002**

Table S6. Across species effects of acclimation temperature (20, 25 and 30°C) and sex on the morphometry of three tephritid species.

	$\chi 2$	**df**	**p**
Aspect ratio			
Species	**2451.66**	**2**	**< 0.001**
Sex	**102.12**	**1**	**< 0.001**
Acclimation	3.11	2	0.211
Species x Sex	**106.33**	**2**	**< 0.001**
Species x Acclimation	8.24	4	0.083
Sex x Acclimation	**7.1**	**2**	**0.028**
Wing loading			
Species	**91.707**	**2**	**< 0.001**
Acclimation	5.38	2	0.067
Sex	**6.59**	**1**	**0.011**
Species x Acclimation	**45.57**	**4**	**< 0.001**
Species x Sex	1.44	2	0.487
Acclimation x Sex	2.58	2	0.275
Species x Acclimation x Sex	**13.67**	**4**	**0.008**
Wing area			
Species	**30.38**	**2**	**< 0.001**
Acclimation	1.79	2	0.407
Sex	2.21	1	0.145
Body mass	**76.98**	**1**	**< 0.001**
Species x Acclimation	**11.59**	**4**	**0.021**
Species x Sex	0.41	2	0.813
Acclimation x Sex	0.68	2	0.711
Species x Acclimation x Sex	**24.34**	**4**	**< 0.001**

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