MATERIALS AND METHODS

3D High-speed videography

Starved flies were released into an enclosed flight chamber (diameter 0.3 m). Three orthogonally aligned high speed cameras (Kodak MotionCorder, 5000 frames per second, shutter speed 50 μs, resolution 146 x 88 pixels) were aimed on a small region in front of an odor-baited visual target (3 cm high and 1 cm wide cylindrical vessel containing a drop of vinegar). Arrays of near infra-red (λ = 880 nm) LEDs covered with diffusive paper provided the back lighting required for image capture in a spectral range well below the flies' sensitivity (*S1*) and hence did not compromise the visually-mediated components of the animals' flight behavior. Hungry flies will orient toward dark vertical objects in the presence of attractive odors (*S2*). As they approach, they either land on or saccade away from the target as it looms within their visual field (*S3, S4*). Six complete object-avoidance saccades were captured and analyzed.

Measurement of wing and body kinematics

A custom built interactive graphic user interface (developed using Matlab, The MathWorks, Inc.) was used to extract the 3-dimensional body and wing positions from the 3 images (12 positional degrees of freedom). The positions of the head and tip of the abdomen (blue and green dots, respectively) were adjusted until the displayed objects were aligned in all 3 views. Similarly, displayed silhouettes of the right (red) and left (yellow) wing could be moved until the best fit was achieved. In this way over 3700 frames and roughly 30,000 individual measurements were obtained from the 6 flight sequences analyzed (also see Movie S1).

Measurement of aerodynamic wing forces

To measure the aerodynamic forces, the wing motion in body-centered coordinates was played through a newly developed dynamically-scaled flapping robot. The device was nearly identical to one described previously (*S5*), except that wing motion was controlled by feedback-driven servo-motors and not stepper motors. Inertial forces due to the wing mass of the robot were below the noise limit of our sensor and thus did not contaminate the measurements of aerodynamic force.

Dynamic scaling and body mass

The stroke frequency of the robotic fly was precisely adjusted to match the calculated Reynolds number in the real fly (*S5*). The magnitude of aerodynamic forces on an actual fly, F_{fly}, is related to those measured on the robotic model, F_{robot}, according to the relationship:

$$F_{fly} = F_{robot} \frac{\rho_{air} \cdot n_{fly}^2 \cdot R_{fly}^2 \cdot S_{fly} \cdot \hat{r}_2^2(S)_{fly}}{\rho_{oil} \cdot n_{robot}^2 \cdot R_{robot}^2 \cdot S_{robot} \cdot \hat{r}_2^2(S)_{robot}}, \quad (1)$$

where ρ is fluid density, n is stroke frequency, R is wing length, S is wing area, and $\hat{r}_2^2(S)$ is the normalized second moment of wing area (see *S6*, p. 25 for definition). By substituting $R^2 \cdot n^2 \cdot S = \nu^2 \cdot Re^2$, where ν is kinematic viscosity and Re is Reynolds number, this relationship simplifies further to:

$$F_{fly} = F_{robot} \frac{\rho_{air} \cdot \nu_{fly}^2 \cdot \hat{r}_2^2(S)_{fly}}{\rho_{oil} \cdot \nu_{robot}^2 \cdot \hat{r}_2^2(S)_{robot}}. \tag{2}$$

It is of interest to note that for truly isometric objects this latter expression depends only on the density and viscosity of the fluid. The values of $\hat{r}_2^2(S)_{robot}$ and $\hat{r}_2^2(S)_{fly}$ (0.40 and 0.35, respectively), differed due to a small notch at the base of the robotic wing required to accommodate the force sensor. Based on a measured relationship between wing length and body mass (N=53), we estimated the mass of the fly in the sequence shown in Figs. 1D, and 2 of the manuscript to lie between 1.16 and 1.40 mg (95% confidence limits), corresponding to a weight range of 11.4 to 13.7 μN.

Estimates of body inertia and frictional damping

Body inertia, *I,* was estimated from body morphology using a standard formula for a cylinder rotating around its central transverse axis:

$$I = \tfrac{1}{12} M \cdot L^2, \tag{3}$$

where L is body length (2.5 mm), D is diameter (0.7 mm), and M is body mass (1 mg). The frictional damping coefficient, *C,* was calculated using an integration of Stokes' law (S*7*) as:

$$C = \frac{\pi \cdot \mu \cdot L^3}{3 \cdot \ln(\frac{1}{2} \cdot L / D)}, \tag{4}$$

where μ is the dynamic viscosity of air at 20° C (1.84×10^{-5} kg m^{-1} s^{-1}). The alternate estimates of *I* and *C* based on body kinematics and measured torque represent the average of 6 multilinear regressions performed independently on each sequence. The average root mean square error was $6.5 \times 10^{-10} \pm 2.5 \times 10^{-10}$ (mean ± SD, N=6). Our estimates of *I* are supported by previous results based on torsional oscillations of a tethered fruit fly, which yielded a value of 9×10^{-13} Nms^2 (*S8*). In contrast, our upper estimate of *C* is roughly 44 times smaller than the upper estimate of 2.3×10^{-11} Nms given in the same study. We believe this overestimate to be due to the assumption, now known to be invalid, that frictional forces alone are sufficient to decelerate the animal at the end of a turn.

Assumptions and controls

A successful experimental synthesis of free flight kinematics of real flies with force measurements on a dynamically-scaled robot relies on several critical assumptions. First, we approximated the wings of the tiny fly as flat plates. Although this simplification is largely justified by the 3D images, the forces produced by the real fly may be influenced in part by aeroelastic effects operating on the flexible wings. Second, the forces produced by a translating and rotating fly were measured on a stationary robot. Although adequate for hovering, the motion of the fly through the air must influence to some degree the flow and forces on the flapping wings. Third, because we could not directly measure torque about the wing base, our estimates of aerodynamic moments required an assumption of a fixed center of pressure throughout the stroke. The results of the analysis, however, suggest that these assumptions are sufficiently satisfied to provide an accurate measure of free flight dynamics. The forces measured during hovering were quantitatively consistent with the balance required for stationary flight (Fig 3B). Further, the time course of measured yaw torque during the saccades matched well the torque required to rotate a fly through the observed motion according to the dynamics of a mass-damped system (Fig. 3D). Collectively, these observations support the accuracy of the methodology.

References

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Movie S1. A visualization of a fly's wing and body motion, with superimposed arrows indicating the measured aerodynamic forces.