AERIAL PERFORMANCE OF *DROSOPHILA MELANOGASTER* FROM POPULATIONS SELECTED FOR UPWIND FLIGHT ABILITY

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Summary

A computerized system for three-dimensional tracking of large numbers of individual free-flying insects was used to assess the performance of *Drosophila melanogaster* from populations that had undergone 160 generations of selection for upwind flight ability. Compared with control lines, the selected lines showed significant increases in mean flight velocity, decreases in angular trajectory and a significant change in the interaction between velocity and angular trajectory. Maximal flight velocity was apparent as a sharply defined upper boundary of the distribution of horizontal and vertical velocity as a function of angular trajectory; this upper bound ($0.85\,\mathrm{m\,s^{-1}}$) differed little between the selected and control lines, although individuals from the selected lines attained maximal performance levels much more frequently. Maximum induced power output was calculated directly from the product of maximum vertical velocity and body weight. This measure ($28\,\mathrm{W\,kg^{-1}}$ muscle) was closely predicted by a scaling relationship derived from the load-lifting limits of larger insects and vertebrates, as well as tethered *D. melanogaster* stimulated *via* their optomotor reflex to produce maximal lift. These results indicate that selection for flight performance can readily alter the relative effort and/or the frequency of phenotypes capable of attaining population-wise maximal performance levels, but shows little ability to increase population-wise maximal performance.

Key words: flight, *Drosophila melanogaster*, phenotype selection, performance, fruit fly, motion analysis.

Introduction

A recent study reported a large increase in 'mean apparent flying speed' of *Drosophila melanogaster* (from 0.02 to $1.7\,\mathrm{m\,s^{-1}}$) as a result of 100 generations of directional selection for upwind flight ability in a compartmented wind tunnel (Weber, 1996). This result is different from what physiologists would have predicted. Comparative studies using a wide variety of flying animals have shown conformity to a single scaling relationship for maximum load-lifting capacity and induced power output in relation to flight muscle mass (Marden, 1987, 1990, 1994; Ellington, 1991). Although power outputs substantially greater than predicted by this scaling relationship have been reported for euglossine bees and hummingbirds (Dudley, 1995; Chai and Dudley, 1995), this difference has subsequently been attributed to the application of a more detailed aerodynamic model to similar levels of performance (Chai *et al.* 1997). Wing anatomy and muscle power limits appear to have evolved in close coordination, as wing stroke amplitude reaches its geometrical limit (180°) at maximal muscle power output in both hummingbirds and *Drosophila melanogaster* (Chai and Dudley, 1995; Chai *et al.* 1997; Lehman and Dickinson, 1997). Together, these studies suggest that flying animals share a common upper limit of muscle mass-specific burst performance, which may not be improvable by either natural or artificial selection.

Species that experience intense natural selection for flight ability do show improved burst performance, but not on a muscle mass-specific basis. Palatable butterflies that use flight to evade avian predators invest a greater portion of their total body mass in flight muscle than do unpalatable butterflies (Marden and Chai, 1991). Similarly, male hummingbirds and dragonflies that engage in aerial competition for mating territories, as well as robberflies that use short-burst flights to capture flying prey, allocate an unusually high proportion of their body mass to flight muscle (Hartman, 1961; Marden, 1989; Morgan *et al.* 1985). All of these taxa show enhanced body mass-specific burst performance, but do not deviate from the general trend for muscle mass-specific performance (Marden, 1987). Thus, the common response to natural selection for burst flight performance has been an alteration of overall body design, such that flight muscle with approximately equal mass-specific performance occupies an increasing share of total body mass. However, Weber's (1996) *Drosophila melanogaster* do not appear to have followed this evolutionary path: selected lines at generations 50 and 85

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showed no loss in relative fitness compared with control lines, i.e. they showed no indication of allocating less body mass to reproductive tissues.

Could it be possible that *D. melanogaster*, contrary to expectations based on prior physiological research, could so markedly improve their flight performance without altering their body design and fecundity? Here we test that hypothesis by employing a computerized three-dimensional tracking system to measure directly the free-flight performance of Weber's control and selected populations of *D. melanogaster*.

Materials and methods

Weber's selection regime has been described in detail (Weber, 1996), but features relevant to the present study are reviewed here. Selection for flight performance was accomplished by releasing batches of up to 15 000 flies into the downwind end of a compartmented wind tunnel. A light at the upwind end of the otherwise darkened tunnel attracted the flies to move upwind through a linear series of 40 compartments. At each compartment, a light-proof valve allowed some air to escape, thus forming a gradient of air speeds that opposed the flies with increasing intensity as they approached the light. The downwind faces of the compartment walls had a slippery coating so that flies could only advance by flying. Performance in the wind tunnel was expressed as the airflow velocity at the most upwind intercompartmental passages reached by flies during a constant experimental duration. However, since turbulence and shearing affect local wind velocities at each passage, and flies were observed to maneuver along the edges of the airstream at upwind passages, the true flight speeds were unknown.

The selection experiment was conducted on a large scale. The original base population was founded from 350 wild-caught isofemale lines and was later split into two control lines (here designated C1 and C2). Over 9 million flies were processed in the first 100 generations of selection on the two replicate selection lines (here designated S1 and S2), which were founded from the control lines. The mean selection pressure was 4.5 %. The effective population size was estimated to vary between 500 and 1000 per generation.

Samples of the control and selected lines, after 160 generations of selection (i.e. an additional 60 generations beyond that reported in Weber's 1996 paper) were cultured in J. H. Marden's laboratory, without selection, for approximately 25 generations. Separate samples of selected flies showed little or no decline in performance in Weber's wind tunnel after cessation of selection for 10 and 30 generations (Weber, 1996); therefore, we assume that the flies tested for performance in the present study retained the flight phenotype that enabled enhanced upwind performance in Weber's device.

Our aim in the present experiment was to obtain a direct measure of free-flight performance, which Weber (1996) assessed only in an indirect fashion. To accomplish this, we devised a method for tracking individual free-flying *Drosophila melanogaster* in three-dimensional space. Spatial coordinate data were then used to compare mean and maximal flight velocities, accelerations and angular trajectories between control and selected lines.

Flies were shaken from their home vial (approximately 40 flies per vial) into a food-free vial, then immediately allowed to ascend through a tube that led out of the top of this vial. Most flies then voluntarily initiated flight into still air within a temperature-controlled cabinet (26 °C) and were tracked following takeoff (Fig. 1) using a computerized motion-analysis system. The tracking system consisted of two spatially calibrated CCD cameras with dedicated processors and software (MacReflex; Qualisys Inc., Glastonbury, CT, USA; http://www.qualisys.com). Each camera had a 30 000×30 000 pixel sensor, of which approximately 19 500×27 000 pixels were usable (i.e. not blocked by the lens mount). Rather than storing information for every pixel, these processors store only the pixel locations of high-contrast objects, which in conventional use are infrared-reflective markers that stand out against a dark background. Our system was configured to reverse the polarity of the detection system and thus to detect dark objects (unmarked individual flies) against a uniformly bright background (translucent white Plexiglas covered with a red filter and illuminated from behind by a bank of well-ventilated fluorescent lights). Pixel coordinates of the centroid of dark objects (flies) were recorded 60 times per second and transformed in near real time (using the manufacturer's proprietary algorithm) into three-dimensional coordinates with a spatial resolution of less than 1 mm (Fig. 1). This arrangement allowed us rapidly and accurately to determine free-flight performance of large numbers of flies that had never been anesthetized or handled.

Cameras were spatially calibrated by recording the positions of opaque markers placed at the corners of a rigid Plexiglas frame (14 cm×13 cm×12 cm). The manufacturer's calibration routine was then followed. The calibration was checked periodically by computing the gravitational acceleration of a small clay sphere (approximately 2 mm diameter) dropped through the calibrated airspace. For sixteen such tests conducted over a period of 5 months (during which camera positions were occasionally altered slightly and new calibrations were performed), the mean gravitational acceleration was -9.83 ± 0.03 m s^{-2} (mean ± S.D.).

The calibrated airspace was not physically bounded, and the flies were free to fly anywhere within the 0.5 m×0.5 m×0.5 m cabinet. In order to elicit the widest possible range of flight behaviors, a black light used as a phototactic stimulus was positioned vertically (90 °), diagonally (45 °) or horizontally (0 °) at a point approximately 0.5 m from where flies were released. Flies were discarded after each trial and no individuals were resampled; 444 flights were successfully tracked.

Tracking data for each fly consisted of a single set of 3–79 (mean ± S.D., 14±10) coordinates following takeoff (Fig. 1), i.e. a segment spanning a duration that averaged 0.23 s, which is ample time for insects with a wingbeat frequency of
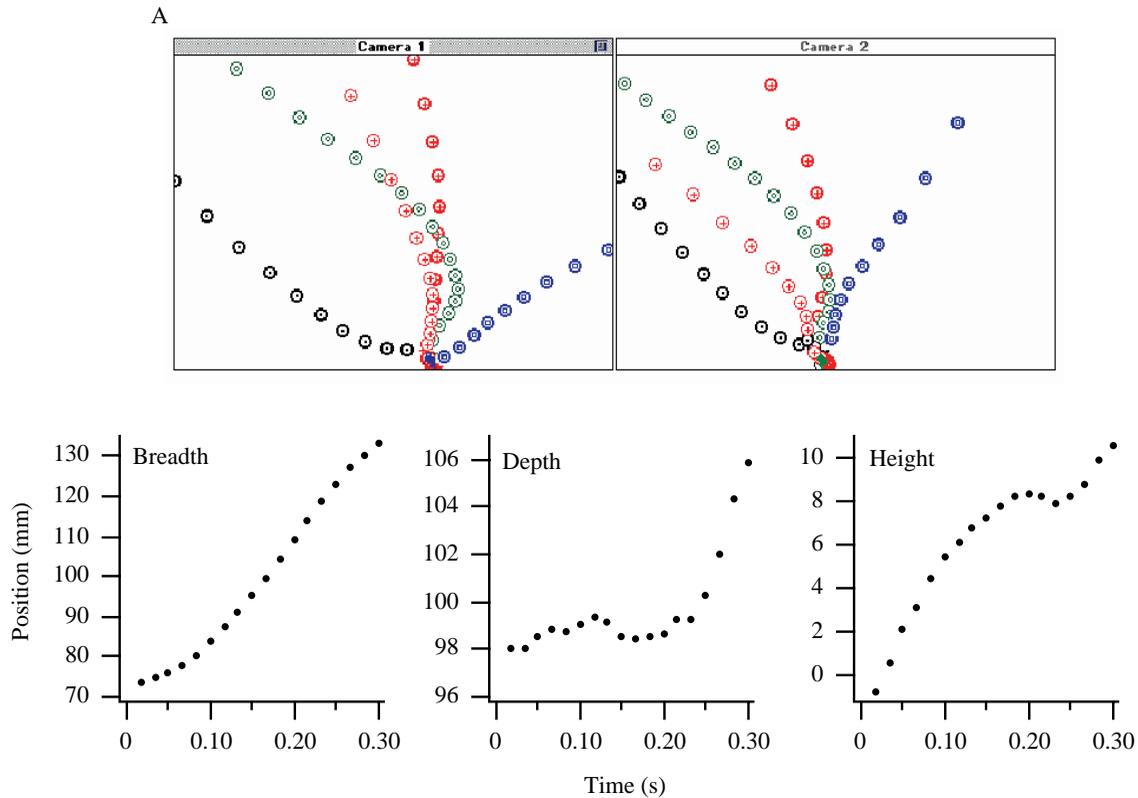
Fig. 1. (Top) Two-dimensional camera coordinates of five flies that were tracked at 60 samples s^{-1} as they flew upwards from a release point at the bottom center. Units are pixels on a 19 500×27 000 array for each camera. (Bottom) Three-dimensional coordinates of a representative flight. Note the fine resolution of motion, which in the depth dimension of this flight shows orderly tracking from 0 to 0.25 s within a total distance of less than 2 mm. A rotating three-dimensional plot of additional representative tracking data is available at http://cac.psu.edu/ jhm10/project3.html.

approximately 200 Hz to attain steady-state aerodynamic conditions. Horizontal, vertical and resolved velocities (hereafter referred to as the path velocity) were determined from frame-to-frame changes in location. The framewise angular trajectory (0° is horizontal; 90° is vertical) was calculated as the inverse cosine of the ratio of horizontal velocity to path velocity.

Statistical analyses of means were based on the single frame for each flight (i.e. $N=1$ data point per individual fly; $N=444$ flies) that showed the highest path velocity. Maxima were examined both visually (using graphs of all framewise data; $N=6122$) and statistically (Gaines and Denny, 1993) by using the maximal framewise velocity observed within each vial of flies ($N=36$ vials), corrected for sampling effort. Except where otherwise indicated, framewise segments showing negative vertical velocities ($N=368$) were omitted in order to eliminate the effects of gravitational augmentation of velocity and acceleration.

At the start of the experiments, we separated newly emerged flies by sex during cold-induced immobilization at 4 °C. Because the mean path velocity for 157 known-sex flies did not differ according to sex or sex × line ($P=0.22$), we discontinued this procedure in order to streamline the experiment.

Table 1. *Mean path velocity of selected and control lines of Drosophila melanogaster*

Line	N	Mean velocity (m s^{-1})	S.E.M.
Selected 1	113	0.696	0.013
Selected 2	88	0.644	0.019
Control 1	99	0.464	0.015
Control 2	144	0.516	0.012

ANOVA; $F=54$, $P<0.0001$.

Results

Flies from the two selected lines showed a significantly higher mean flight velocity in our tracking arena [1.25- to 1.50-fold improvement; analysis of variance (ANOVA); $F=55$, $P<0.0001$; Table 1] than that of flies from the two control lines. Age (mean ± S.D., 6.6±2.6 days post eclosion, range 3–15 days) had no independent effect on velocity ($P=0.13$).

Flies from selected and control lines also differed in the angular trajectory of their flights. Trajectories of selected flies were much more horizontal than were trajectories of control flies (Fig. 2; ANOVA; $F=30.1$, $P<0.00001$).

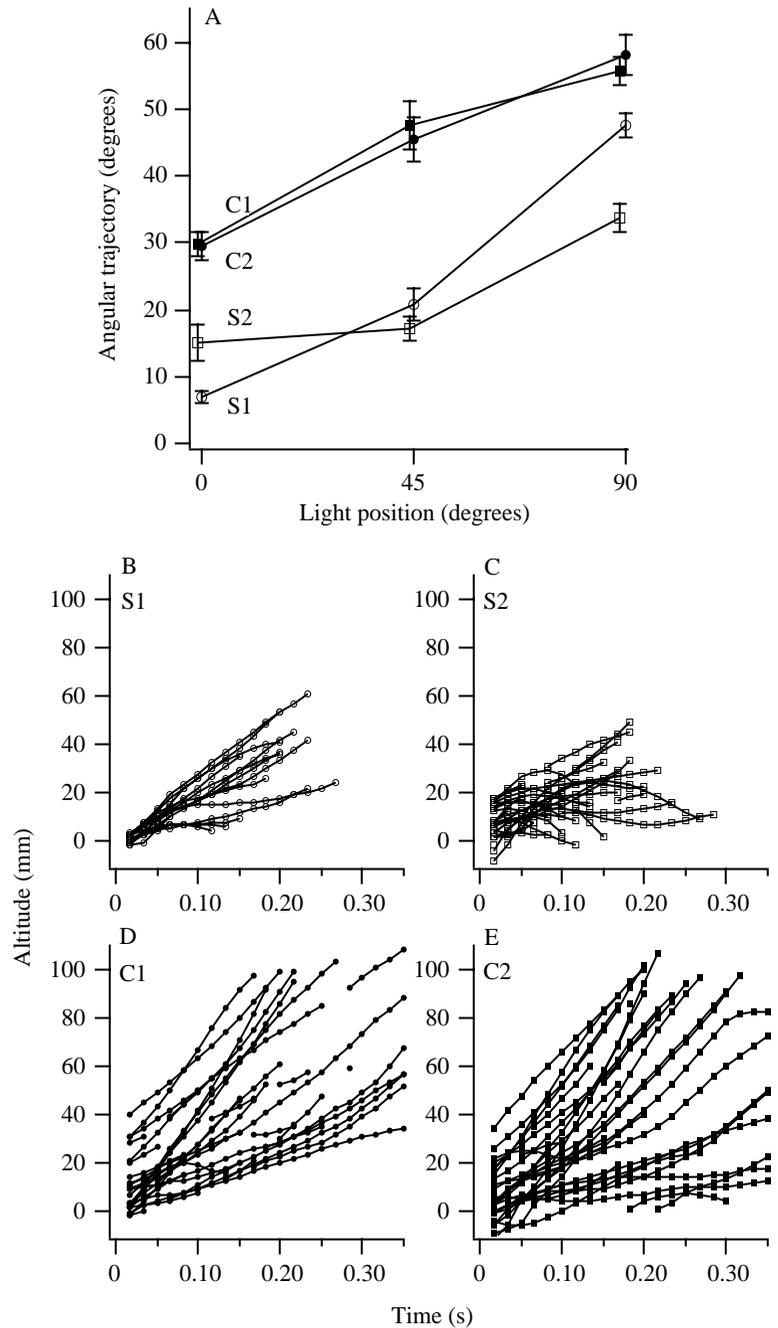A statistical comparison of maximal flight velocities

Fig. 2. (A) Trajectory (mean angle of inclination of flights) in relation to the placement of a phototactic stimulus (a black light). Filled symbols represent control lines (C1, C2); open symbols represent selected lines (S1, S2). Values are means ± S.E.M. (N=10–88). (B–E) Raw tracking data for four vials of flies tested under identical conditions (diagonally sited phototactic stimulus) on a single day.

between the selected and control lines was performed as follows. A replicate series of maxima based on a sufficiently large sample (Gaines and Denny, 1993) was obtained from our data by using the maximal framewise path velocity observed from within each of the 36 vials of flies. These maxima varied asymptotically according to the number of velocity estimates obtained from each vial (Fig. 3A). Residuals from an exponential curve fitted to these data were normally distributed, homoscedastic and varied significantly among lines (ANOVA; $P<0.02$; $r^2=0.26$; Fig. 3B). Flies from the C1 line had maximal velocity residuals significantly lower than those of the S1 line (Tukey–Kramer test; $P<0.05$), whereas

values for the C2 line did not differ significantly from values for the S2 line.

A graphical comparison of all framewise velocities is helpful for interpreting this result. For all four lines, the distribution of framewise vertical and horizontal velocity as a function of angular trajectory showed a common and sharply defined upper boundary (Fig. 4). This upper bound conforms remarkably well to a trigonometrically derived curve for flies that have a maximum path velocity of $0.85\,\mathrm{m\,s^{-1}}$. Two important points emerge from this result. First, the strict adherence of maximal horizontal and vertical velocities to the trigonometrically derived curve demonstrates that our maximal values are not

Fig. 3. (A) Maximum framewise path velocity observed from each vial of flies ($N=36$ vials) as a function of the number of framewise path velocities measured per vial. Symbols are as in Fig. 2. The curve is an iterative fit of an exponential equation (Igor; $V_{max}=0.81-0.82e^{(-0.41\sqrt{n})}$, where V_{max} is maximum velocity and n is sample size). (B) Means (± 1 S.D.) of residual maximum velocities from the fitted curve (A) for selected and control populations.
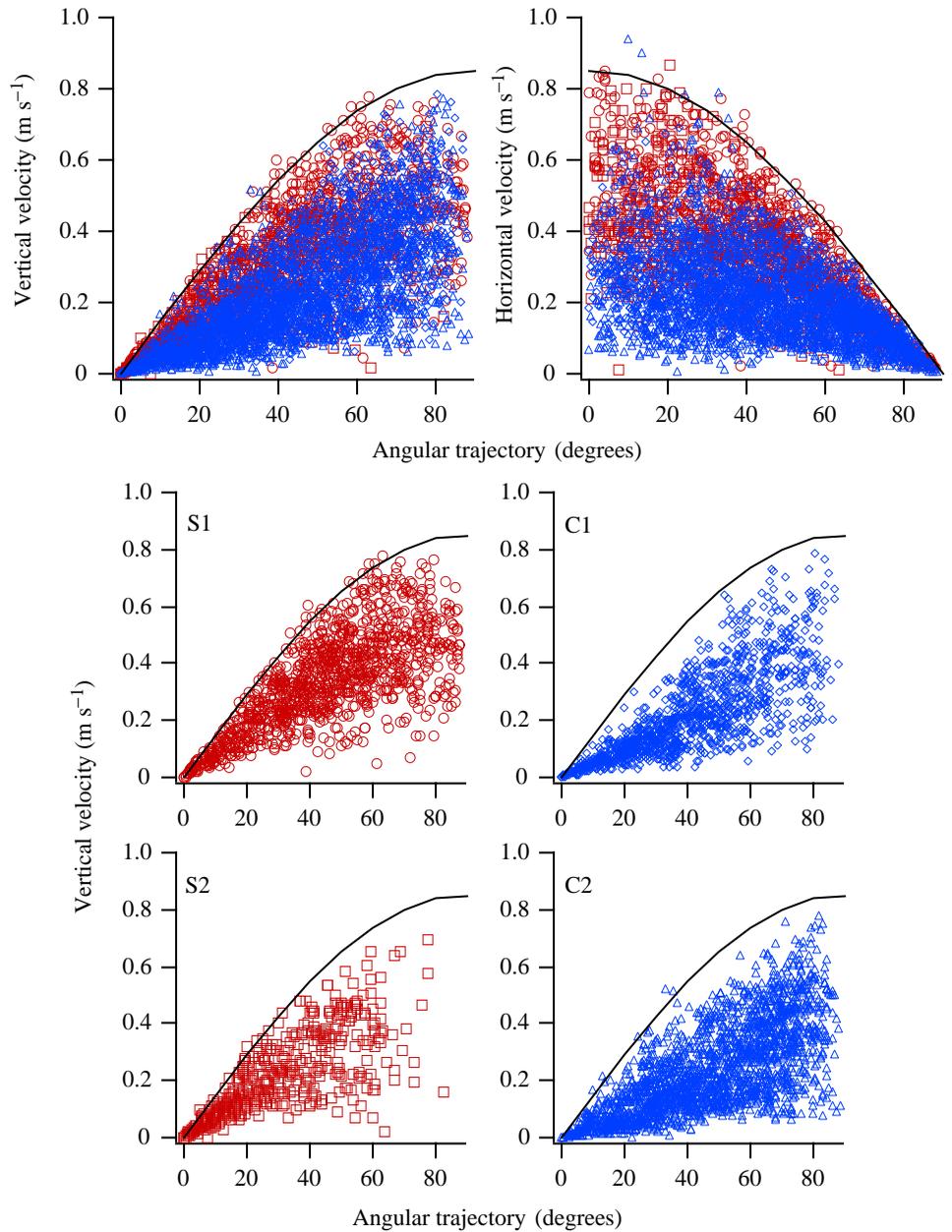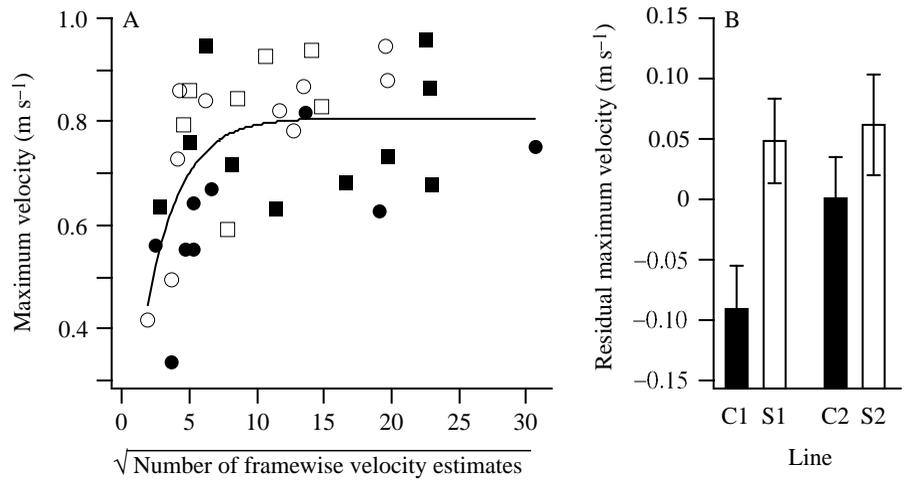

Fig. 4. Vertical and horizontal velocity as a function of the inclination of the flight path ($0°$ is horizontal motion; $90°$ is vertical motion). Curves show the predicted upper bound for a maximum path velocity of $0.85\,\mathrm{m\,s^{-1}}$ (i.e. a right-angled triangle whose sides are vertical and horizontal velocity, and whose hypotenuse is a constant $0.85\,\mathrm{m\,s^{-1}}$). All of the framewise data ($N=6122$) where vertical velocity was positive (i.e. upward motion not augmented by gravity) are shown in the upper two graphs. The four lower graphs show the same data for flies from each line (C1, C2, S1 and S2).

simply the largest measurement errors, which would be random and would not adhere to any consistent pattern. Second, flies from the two control lines occasionally flew at this upper limit of performance (or very close to it in the case of C1 flies), whereas flies from the two selected lines frequently displayed this level of performance. What cannot be determined from these data is whether the selected flies had an increased tendency to voluntarily use high levels of exertion or whether more individuals in the selected lines were physiologically capable of attaining path velocities of $0.85\,\mathrm{m\,s^{-1}}$. In either case, what Fig. 4 clearly shows is that the selection regime greatly amplified the *frequency* of maximal levels of performance, without markedly affecting that maximum *per se*.

Our tracking system did not distinguish between flies that had just taken off from the release point (the vast majority of flights) and flies that occasionally re-entered the tracking airspace from other locations in the chamber. We made no effort to filter the data for re-entry flights, which include both downward and upward flight segments; however, it is clear that flies moving downwards through the tracking arena were capable of path velocities greater than $0.85\,\mathrm{m\,s^{-1}}$. Of the 368 framewise segments showing downward motion, 10 had path velocities greater than $1.0\,\mathrm{m\,s^{-1}}$ (maximum $1.20\,\mathrm{m\,s^{-1}}$; Fig. 5), whereas only one ($1.105\,\mathrm{ms^{-1}}$) of the 6122 flight segments showing upward motion had a path velocity greater than $1.0\,\mathrm{m\,s^{-1}}$ (Fig. 5).

The highest accelerations ($>10\,\mathrm{m\,s^{-2}}$) were observed during the first $0.1\,\mathrm{s}$ of tracking, at path velocities less than $0.8\,\mathrm{m\,s^{-1}}$

(Fig. 6). Framewise acceleration of each individual at its highest observed path velocity did not vary as a function of line ($P=0.07$), path velocity ($P=0.71$) or the interaction between line and path velocity ($P=0.60$; $r^2=0.02$). These data suggest that our tracking distances were sufficient to allow the flies to reach steady-state performance and that the selected and control lines did not differ in the rate at which they reached steady-state performance.

Flies from control lines were much more likely to fly with a highest recorded path velocity of less than $0.45\,\mathrm{m\,s^{-1}}$ (Fig. 7). When control flies did fly with maximal individual velocities higher than $0.45\,\mathrm{m\,s^{-1}}$, they used increasingly vertical trajectories. In contrast, selected flies nearly always flew with a highest recorded path velocity greater than $0.45\,\mathrm{m\,s^{-1}}$ and their flight trajectories became increasingly horizontal as their velocities increased. This covariance between line, effort and trajectory is demonstrated statistically by an ANOVA model using 'light orientation', 'line' and the 'line × path velocity' interaction as independent variables to explain the angular trajectory of flights (Table 2; $r^2=0.46$). 'Line' did not have a significant effect independently of the 'line × path velocity'
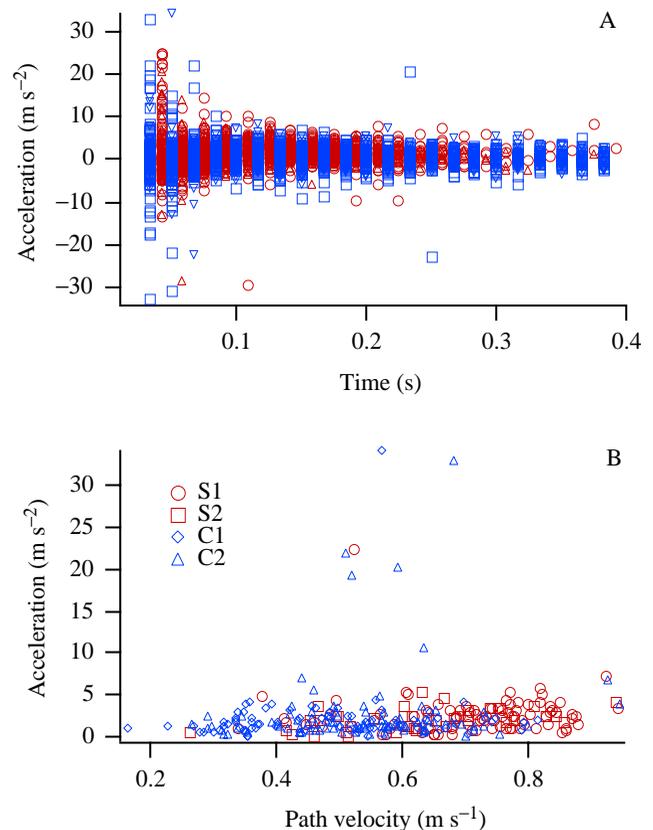
Fig. 6. Acceleration as a function of time in flight for all framewise data (A) and as a function of path velocity for each individual fly at its highest recorded path velocity (B). Both plots include only those data where vertical velocity was positive. In A, data for selected lines S1 and S2 (red symbols) have been shifted to the right by half of the sampling period ($0.0082\,\mathrm{s}$) in order to improve visual resolution. Control lines C1 and C2 are shown with blue symbols.
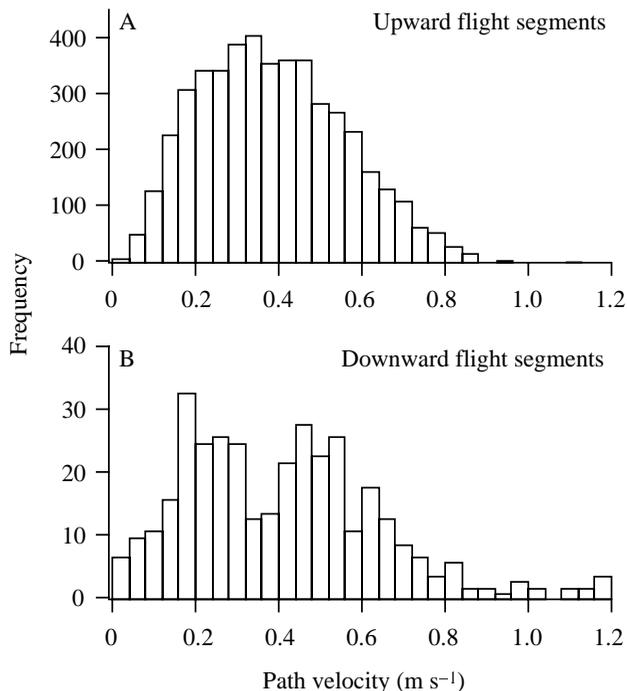

Fig. 5. Frequency distributions for all framewise path velocities from (A) upward (i.e. positive vertical velocity) and (B) downward flight segments.

Table 2. *ANOVA results for the effect of light orientation (vertical, diagonal or horizontal), line and line × path velocity on the angular trajectory of flights*

Source	d.f.	Sum of squares	F ratio	P
Light orientation	2	43164	63.5	0.0000
Line	3	1461	1.4	0.23
Line × path velocity	3	3205	3.1	0.025

interaction. When 'line' is removed from the model, fitted estimates for the interaction term are negative for the selected lines and positive for the control lines. A simple way to illustrate this interaction is to count the number of flights (Fig. 7) that had both a horizontal velocity greater than $0.45\,\mathrm{m\,s^{-1}}$ and a vertical velocity of less than $0.2\,\mathrm{m\,s^{-1}}$ (i.e. a relatively fast, horizontally oriented flight). Of those flights, 17 and 13 were by flies from the two selected lines, whereas five and nine were by flies from the two control lines. For the reverse condition (relatively fast upward flights; vertical velocity greater than $0.45\,\mathrm{m\,s^{-1}}$ and horizontal velocity less than $0.2\,\mathrm{m\,s^{-1}}$), only seven and one flights were by the two selected lines, whereas 11 and 22 were by flies from the control lines.

Discussion

Drosophila melanogaster from Weber's (1996) selected lines showed higher velocities and more horizontal trajectories in unconstrained free flight than did control lines. These
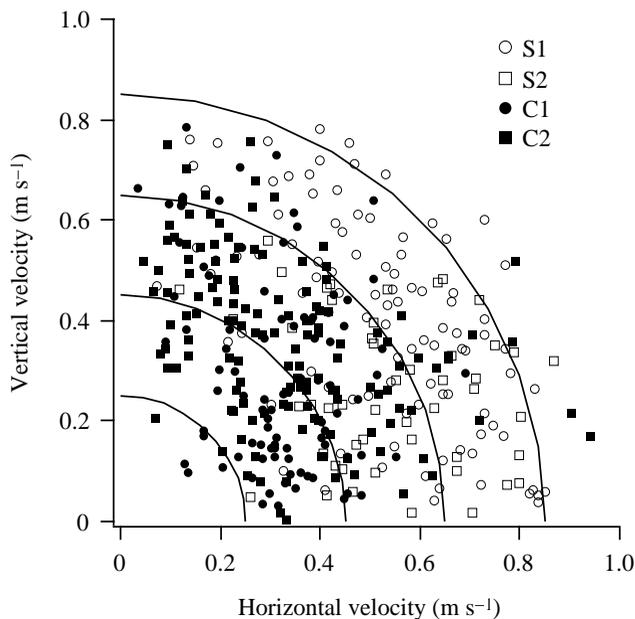

Fig. 7. Horizontal and vertical velocities for each individual fly at its highest observed path velocity. Isovelocity arcs are superimposed for path velocities of 0.25, 0.45, 0.65 and $0.85\,\mathrm{m\,s^{-1}}$. Control lines C1 and C2 are shown with open symbols; selected lines S1 and S2 are shown with filled symbols.

tendencies covaried: the trajectories of selected flies became flatter as their velocity increased. This relationship between speed and trajectory is the reverse of the behavior shown by control flies, whose flights became more vertical as their velocity increased. The flight phenotype of selected flies is well suited to the characteristics of Weber's selection device, wherein flies were required to progress upwind through openings in the center of a linear series of horizontally arranged compartments. Flies that tended to use horizontally directed bursts of high effort were apparently favored in that environment.

Because the motion detection and control systems in *Drosophila melanogaster* are known in more detail than for perhaps any other organism, we can speculate on the possible physiological source of differences in preferred flight paths and velocities. The wing kinematics controlling lift and thrust in fruitflies are surprisingly inflexible (Vogel, 1966; David, 1978; Götz and Wandel, 1984); wing pitch and stroke plane are fixed, with the lift/thrust ratio controllable only by variation of the body axis angle. Total flight force is varied solely by changes in wingbeat amplitude and frequency. Thus, it is likely that Weber's (1996) selected flies utilize a more horizontal body axis angle, together with greater stroke amplitude and frequency, without varying other wing kinematic parameters. Selection for more horizontal flight paths in Weber's selected lines may have arisen from variation in the response of motion detectors in the upper frontal part of the visual field, which has been shown to differ widely among individual wild-type flies (Buchner *et al.* 1978). Antennal feedback from air motion is also known to affect the preferred directionality of vertical course control (Götz and Biesinger, 1983), although its variability among individual wild-type flies is unknown.

The significantly increased mean velocity and altered trajectory of Weber's (1996) selected flies measured in the present study demonstrates that the original populations contained genetic variation for flight-related traits, that the selection regime effectively sorted flies according to performance, and that there was a particular selective advantage for horizontally directed bursts of high-speed flight. Yet, the population-wise upper bound of performance was either unaffected or improved only slightly (Fig. 4). Do these results allow us to conclude that maximum performance in *Drosophila* cannot readily be improved by selection?

To evaluate this question, it is important first to consider the possibility that flies in our tracking experiment might not have exhibited maximal performance. Voluntary free flight does not necessarily evoke maximal effort (in fact, we suspect that the vast majority of flights represent submaximal effort, and that only a very large sample size permits delimitation of population-wise maximal performance). However, the sharply defined upper bound of velocity *versus* angular trajectory (Fig. 4) must represent either an invariant maximal performance or invariant ceiling on submaximal effort. If the latter were true, why would selected flies, which have evolved a higher mean velocity (Table 1), limit their performance to the same sharply bounded submaximal level? Furthermore,

why would flies using submaximal effort to move horizontally at a velocity of 0.85 m s⁻¹ show such a predictable decline in horizontal velocity as their trajectories became increasingly vertical (i.e. conforming precisely to a trigonometric prediction)? Acceleration as a function of path velocity did not differ between control and selected lines (Fig. 6), and thus there is no evidence to suggest that selected lines would have shown higher flight velocities had we been able to track them over greater distances. Definitive rejection of the 'unrevealed maximum' hypothesis is not possible, but it appears that invariant population-wise maximal performance is a far more likely explanation for these data.

Comparison of our data with results from other experiments provides further support for the hypothesis the fastest flights observed in our experiments were the result of maximal effort. The highest *D. melanogaster* velocity recorded by Ennos (1989) was 0.82 m s⁻¹; Vogel (1966) reported velocities of 1.2 m s⁻¹ for a larger species, *D. virilis*; and David (1978) reported velocities up to 0.9 m s⁻¹ for the even larger *D. hydei*. The best advance ratio, which may limit velocity for animals and aircraft powered by wings and propellers, is thought to be approximately 0.33 for *D. melanogaster* (Vogel, 1994). For our *D. melanogaster* with wing lengths averaging 2.3 mm, using a maximal combination of wing stroke amplitude and frequency (177 °; 220 Hz; Lehman and Dickinson, 1997), an advance ratio of 0.33 should limit maximum velocity to 1.03 m s⁻¹ (see equation 12.2 in Vogel, 1994).

Flight velocities can also be used as a direct measure of induced power output, which can then be compared with the predicted value from other flying animals lifting maximal loads, as well as a recent estimate that is specific to *D. melanogaster*. The product of vertical velocity and body weight provides a direct measure of climbing power (i.e. force×distance/time), which should be equal and opposite to the mean momentum of the downward airflow created by wing flapping. A vertical velocity of 0.85 m s⁻¹ represents a climbing power of 28 W kg⁻¹ muscle (using our measure of 1.0 mg flies and Lehman and Dickinson's (1997) measure of a flight muscle ratio of 0.3). This measure is reasonably close to Lehman and Dickinson's estimate (32 W kg⁻¹) for induced power output of *D. melanogaster* that were stimulated *via* their optomotor response to exert maximal effort during tethered flight and also to the value predicted by a scaling equation for induced power output for maximally loaded insects, birds and bats (Marden 1987, 1990; Fig. 8; least-squares regression prediction 31 W kg⁻¹ muscle). These comparisons show that the useful (induced power) component of total power output estimated from our velocity measures, as opposed to the non-useful profile, inertial and parasite power components that constitute the majority of total power output for insects the size of *Drosophila* (Curtsinger and Laurie-Ahlberg, 1981; Zanker and Götz, 1990; Dickinson and Lighton, 1995; Lehman and Dickinson, 1997), and which our data do not reveal, is consistent with other results from *D. melanogaster* and an apparently universal scaling relationship for flying animals exerting maximal effort.
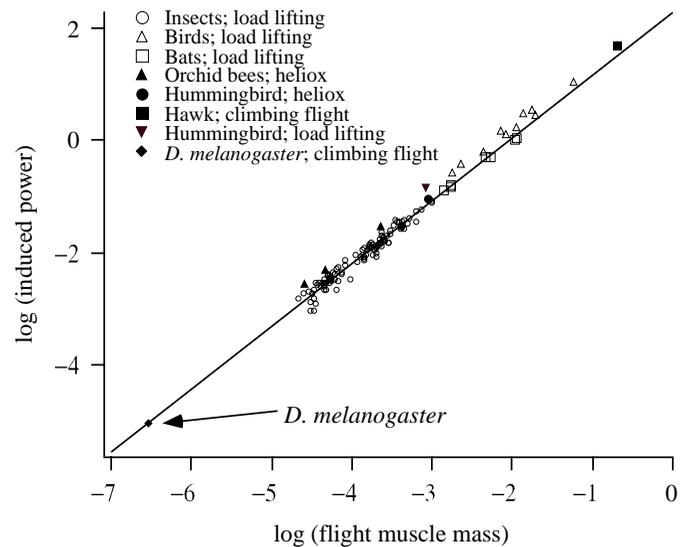

Fig. 8. Induced power output of *Drosophila melanogaster* flying vertically at 0.85 m s⁻¹ in comparison with an independently derived scaling relationship from 121 individual conventional wingbeat flyers measured for load-lifting capacity (open symbols; Marden, 1987, 1990; $\log P_{ind}=1.1227\log M_m+2.2918$, where P_{ind} is induced power in W and M_m is flight muscle mass in kg). Data collected by other investigators using other methods to elicit maximal effort are shown as filled symbols (hawk, Pennycuick *et al.* 1989; orchid bee, Dudley, 1995; hummingbird, Chai and Dudley, 1995; Chai *et al.* 1997).

On the basis of these considerations, we can conclude with reasonable certainty that the upper limit of the flight velocities that we observed represents flies using maximal effort, that selected and control lines of *D. melanogaster* differed in the frequency with which they voluntarily utilized or were physiologically capable of attaining population-wise maximal performance, but that the upper limit of performance remained very nearly constant. It is remarkable that 160 generations of intensive selection by Weber for wind tunnel performance failed to improve markedly the maximum velocity and induced power output of the selected flies over controls. This same selection regime resulted in major changes in other traits that contribute to performance in a horizontal wind tunnel. Our data reveal substantial shifts in angular trajectory and in the interaction between relative effort and trajectory, and the original report (Weber, 1996) showed striking improvements in the rate of upwind progression. Although Weber's wind tunnel performance variable is an 'apparent mean flying speed' and cannot be interpreted literally as a velocity, it is an accurate parameter of large genetic and phenotypic changes. Thus, while other aspects of the organism can be modified greatly by selection, it appears that the population-wise maximal performance of wild-type flies cannot be readily improved. We conclude that natural selection on flies, and perhaps flying animals in general, already maintains population-wise maximal flight performance at or very near its physical limit.

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