How Maggots Move: Allometry and Kinematics of Crawling in Larval Diptera

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We describe the kinematics and allometry of limbless locomotion in the larvae of four species of Diptera: *Drosophila melanogaster*, *Musca domestica*, *Protophormia terraenovae* and *Sarcophaga bullata*. In the small species, *D. melanogaster* and *M. domestica*, crawling involves a series of telescoping peristaltic movements; in the two larger species, it consists of a combination of telescoping and caterpillar-like movements. Speed, contractions per second and the distance traveled per contraction are correlated with mass. We contrast these relationships to the kinematics of caterpillar and limbed insect locomotion. Crawling by larval flies is notable for its low speed compared to limbed insects and for the fact that both contraction frequency and the distance traveled per contraction increase with speed.

Allometry   Kinematics   Locomotion   Crawling   Diptera

INTRODUCTION

Comparative studies of locomotion have attempted to relate morphological and ecological variables to differences in locomotor performance (Alexander, 1982; Peters, 1983; Full, 1994). Work on insect locomotion has focused on swimming, flying and limbed terrestrial locomotion [see the reviews in Full (1989, 1994), Casey (1989), Ellington (1984), Herreid (1981) and Nachtigall (1985)], even though many insects spend their larval periods without limbs or with rudimentary limb buds or prolegs and move by crawling and/or burrowing (Chapman, 1971; Birket-Smith, 1984). Limbless organisms with hydrostatic skeletons show diverse mechanisms of locomotion (Heffernan and Wainwright, 1974; Trueman, 1975). However, locomotion by insect larvae remains poorly described: for example, Diptera larvae are thought to crawl using telescoping peristaltic motion (Duncan and Pickwell, 1939), but detailed and comparative studies are lacking. The analysis of limbless locomotion in insects could prove invaluable in assessing the ecological causes of variation in locomotor performance because of the diverse habits of larval insects. Similarly, insects could provide a model for understanding the functional significance of variation in morphology, because larval insects display substantial variation in size and shape. One major goal of this study is to assess the extent to which larval Diptera differ in shape and to determine if differences in morphology are related to differences in the kinematics of crawling.

Casey (1991) describes the energetics and kinematics of locomotion in a caterpillar and Berrigan and Lighton (1993) estimated the cost of transport in a larval fly, *Protophormia terraenovae*. These studies found that crawling is energetically costly, about 4 times the predicted cost for a limbed insect of the same mass in caterpillars and about 10 times the predicted cost in larval flies. Many insect larvae spend all or part of the larval period burrowing in a host (e.g. Diptera) or essentially immobile (as in many Hymenoptera). In these larvae, the cost of crawling *per se* may not be particularly important. However, a number of holometabolous insects go through a wandering phase just prior to pupation. During this phase the larvae cease feeding and search for a suitable pupation site. The wandering phase can last several days or more and represents a substantial fraction of the insect life cycle (Denlinger and Zdarek, 1994). Fly larvae (Sarcophagidae and Calliphoridae) may pupate 10 m from the host (Greenberg, 1990) and anecdotal evidence indicates larvae will sometimes crawl much farther (Dethier, 1976). Larvae may also be particularly vulnerable to predators and parasites during this period, because they are not concealed in the larval medium or surrounded by many other individuals.

In this study we describe the kinematics and allometry of limbless locomotion in four species of Diptera: *Drosophila melanogaster*, *Musca domestica*, *Protophormia terraenovae* and *Sarcophaga bullata*. The main goals of this project were to characterize the locomotor behavior of fly larvae that span a wide range of body sizes, assess the impact of variation in contraction frequency and distance traveled per contraction on speed and document the impact of variation in larval shape on the

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kinematics of locomotion. If the inter- and intraspecific scaling relationships between size, morphological and kinematic parameters of larval Diptera are similar, then useful estimates of the energetic costs of crawling and performance advantages of changes in morphology could be based on allometric relationships obtained from studies of particular species (e.g. Casey, 1991; Berrigan and Lighton, 1993).

MATERIALS AND METHODS

Experimental organisms

The *D. melanogaster* used in this study are from long-term lab populations. Some of the flies were obtained from Dr Linda Partridge at the University of Edinburgh (U.K.). They were originally collected from a fruit stall near Brighton in the U.K. and have been held in large population cages at 25°C for the last 50 100 generations. Additional larvae were from a stock collected by Dr L. Harshman in central California and reared at 25 C for about 20 generations. The *M. domestica* and *S. bullata* were purchased from Carolina Biological Supply Co. (Burlington, NC, U.S.A.) and the *P. terraenovae* were supplied by B. Greenberg (University of Illinois, Urbana, IL, U.S.A.) and J. Werren (University of Rochester, Rochester, NY, U.S.A.). Some of the descriptions of crawling by *P. terraenovae* analyzed here were described by Berrigan and Lighton (1993), who provide details of culture conditions for this species.

Observations

We collected third instar larvae that had entered the wandering phase of each of the four species and recorded videotapes of episodes of crawling (Sony Hi-8 format or Super-VHS in the case of the *P. terraenovae*). The recordings were made at room temperature, 23–25°C. After videotaping a larva, we weighed it on a Cahn electronic balance to the nearest 0.001 mg or, for the *S. bullata*, on a Mettler top-loading balance to the nearest 0.01 mg. The recordings of *D. melanogaster*, *M. domestica* and *S. bullata* were made of larvae crawling on agar (40 g/l) plates. The recordings of *P. terraenovae* were made on individuals crawling in glass tubes. We also recorded a few individuals crawling on glass, cardboard, wood and porcelain. The kinematics of crawling were not obviously sensitive to substrate texture: the larvae in this study (see below) showed some backwards motion of the head regardless of substrate, as in the results of Berrigan and Lighton (1993).

We then digitized the position both of the head and the rear of several larvae during 5–10 s episodes of crawling in an approximately straight line. Measurements were made on a single episode of crawling by each larva. The digitizing was performed on a NextStep/NextDimension computer using software written by J. Brasher. We used a frame-grabber to obtain 30 frames/s of video images and digitized the position of the larvae in every third frame, resulting in 0.1 s intervals between data points. We were able to resolve individual pixels on the computer screen and the actual resolution of this system ranged from 0.0033 cm/pixel for the videotapes of *D. melanogaster* to 0.0125 cm/pixel for the videotapes of *S. bullata*. We measured larval width at the fourth segment from the posterior end of the larvae at a single time-point during a recording. This measurement was made on individuals at the midpoint of a contraction cycle. Total length of the larvae was calculated as the average length over a complete contraction cycle. For *P. terraenovae*, some of the width data was obtained by extrapolation based on length and mass using multiple regression analysis. This was necessary because videotapes of some individuals were unavailable. Velocity was calculated as the slope of the line relating tail position and time. The regression equations used to estimate this slope always had values of $r^2 > 0.9$ and usually had values of $r^2 > 0.95$.

Statistical analysis

All the statistical analyses described here were performed using the program JMP (SAS Institute Inc., Cary, NC, U.S.A.) on an Apple Macintosh microcomputer. The data were natural log (ln) transformed prior to analysis to meet the assumptions of analysis of variance (Sokal and Rohlf, 1981). We chose to use ln transformations rather than $\log_{10}$ transformation, because $\log_{10}$ transformations failed to result in normal distributions for some of the data. We report means and standard errors (SEs) where appropriate. The slopes and intercepts of regression lines for the individual species were estimated using analysis of covariance (ANCOVA). We chose to use ANCOVA rather than ratios of kinematic or dimensional data to mass because the analysis of ratios can be misleading when variables do not scale isometrically [reviewed in Packard and Boardman (1987)]. ANCOVA estimates the slope and intercept of regression lines for each fixed effect in the model. If the interaction term between the covariate and the fixed effect is significant then there are significant differences between two or more of the estimated slopes. In the ANCOVAs presented here, mass or stride characteristics were treated as covariates and species was treated as a fixed effect. To test for relationships between dimensional and kinematic properties of crawling larvae independent of body size, we tested for correlations between the residual values of length and width estimated from regressions using all the data and the residual values of speed, stride length and stride frequency estimated from regressions using all the data. We made similar calculations (not shown) using the residuals from the ANCOVAs described above.

RESULTS

Qualitative aspects of larval crawling

Larval flies moved by performing a series of peristaltic contractions. The head moves rapidly forward and then hooks downward while the rear of the larvae moves forward at a more constant rate. Figure 1(a) illustrates a
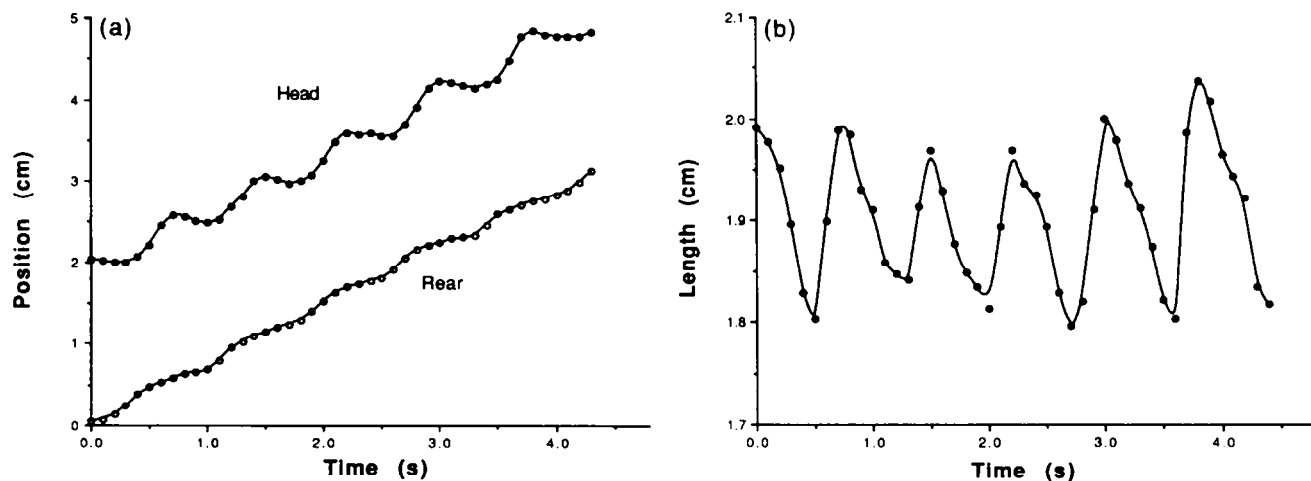
FIGURE 1. Typical result of our digitizing procedure. Part (a) shows the movements of the head and rear of a larva crawling in a straight line. Part (b) illustrates the change in body length of the same larva during locomotion. These data were collected by making Super VHS or Hi-8 video recordings of crawling larvae and then measuring the position of the head and rear every 0.1 s. Stride length is defined as the distance traveled during one complete lengthening and shortening of the larva and stride frequency is the number of these contractions/s. These data were obtained from an *S. bullata* larva weighing 219 mg.

typical example of this pattern of locomotion for a flesh fly, *S. bullata*. Berrigan and Lighton (1993) show a similar illustration for a blowfly, *P. terraenovae* and graphs (not shown) of head and tail positions for *D. melanogaster* and *M. domestica* are also comparable. When the head of the larvae hooks downward they are attempting to establish a *point d'appui* or anchor point. However, in all four of these species the head often slips backward, even on rougher surfaces. This motion results in regular lengthening and shortening of the body [Fig. 1(b)]. Digitized recordings of the very smallest larvae did not always show the regular changes in body length seen in Fig. 1, due in part to the limits of resolution imposed by pixel size in our recordings. Following Berrigan and Lighton (1993) and many past workers, we define stride length as the distance traveled during a complete cycle of lengthening and shortening of the body, and stride frequency as the frequency of these contractions.

Some aspects of crawling behavior were not quantified by our digitizing procedure. First, in *D. melanogaster* and *M. domestica*, the telescopic nature of larval locomotion

was particularly apparent. As the head moves forward, the cuticle appears to slide over the body sac containing the hemolyph and internal organs of the larvae. In contrast, this process was more difficult to observe in *P. terraenovae* and *S. bullata* because these larvae are more nearly opaque, regardless of the kind of illumination used to obtain video images of the larvae. Second, *M. domestica* larvae often displayed an undulatory motion while crawling. This results in about a 5% of body length lateral displacement of the body. We rarely observed undulations in the other species. Third, larvae of all four species sometimes moved their heads from side to side in synchrony with waves of contraction, although *P. terraenovae* almost always displayed this behavior whereas the other species only occasionally exhibited it. Finally, *D. melanogaster* and *M. domestica* larvae showed little or no vertical displacement of the body during crawling, whereas *P. terraenovae* and *S. bullata* had a distinct caterpillar like wave of vertical displacement. This displacement begins at the rear of the larvae, with about a 5–10% rise in the segment of the body that is contracting

TABLE 1. Mean values and ranges of the morphological and kinematic parameters described in this paper

Variable	D. melanogaster	M. domestica	P. terraenovae	S. bullata
Sample size	20	20	25	20
Mass (mg)	1.23	13.91	51.86	142.4
	0.46–2.18	3.87 22.2	11.7–87.9	50.0 219.0
Speed (cm/s)	0.10	0.32	0.38	0.58
	0.07 0.17	0.085–0.43	0.11 0.70	0.16 1.0
Stride frequency (contractions/s)	1.13	1.53	1.98	1.35
	0.71 1.7	0.58–2.43	1.39–2.78	0.64 1.74
Stride length (cm/contraction)	0.094	0.20	0.18	0.42
	0.06–0.18	0.12–0.23	0.022 0.29	0.21 0.59
Length (cm)	0.37	0.86	1.23	1.59
	0.29 0.46	0.52–1.1	0.82 1.74	1.16 1.90
Width (cm)	0.082	0.17	0.31	0.45
	0.06 0.10	0.12–0.21	0.18 0.42	0.32 0.57

Note that we do not present SEMs because the larvae were selected to span a range of sizes.

and moves forward along the body, gradually diminishing in height.

Allometry of kinematic and dimensional parameters

Table 1 summarizes the morphological and kinematic characteristics of the larvae used in this study. Overall, the larvae measured here ranged in mass from 0.46 to 219 mg wet wt. They traveled at speeds ranging from 0.07 to 1.0 cm/s with stride frequencies from 0.58 to 1.74 contractions/s and stride lengths of 0.022–0.59 cm/contraction. Ignoring interspecific differences, speed, stride length and stride frequency increase linearly with mass on ln–ln plots [Fig. 2(a–d)]. However, species differed with respect to the slopes or intercepts of regression lines relating mass and kinematic or dimensional parameters as well as the slopes and intercepts of regression lines relating stride characteristics and speed.

We estimated regression statistics using ANCOVA (Tables 2 and 3) for the relationship between ln mass and ln speed, stride length, stride frequency, length and width. Speed increases with mass [Fig. 2(a)] in the three largest species with slopes ranging from 0.51 to 0.89 and it tends to increase (slope = 0.30, SE = 0.16, $P = 0.058$) in *D. melanogaster*. The intercepts of the lines relating speed
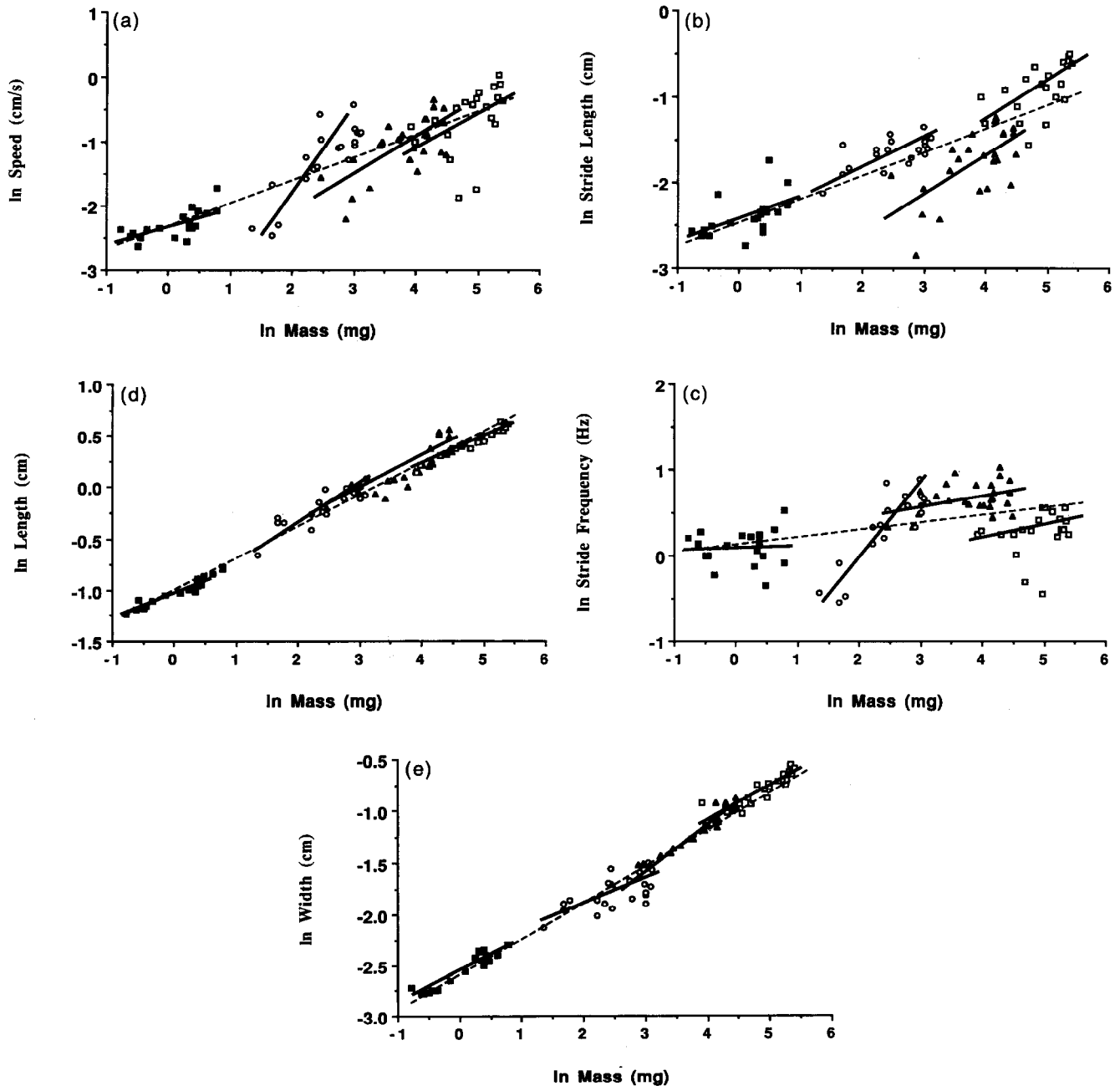

FIGURE 2. Relationship between mass and kinematics of crawling larvae: (a) mass vs speed; (b) mass vs stride length; (c) mass vs stride frequency; (d) mass vs length; (e) mass vs width. All data are ln transformed. The solid lines are the estimated regression lines for each species and the dotted lines are the overall regression lines for all the data points: ●, *D. melanogaster*; ○, *M. domestica*; ▲, *P. terraenovae*; □, *S. bullata*.

TABLE 2. ANCOVA on the relationship between ln mass and ln transformed kinematic and dimensional data for four species of Diptera

Dependent variable	df	SS	F-ratio	P ≤
ln speed				
Model	7	38.30	49.57	0.0001
Error	77	8.50		
Total	84	46.80		
Effect test				
Species	3	1.56	4.70	0.004
ln mass	1	6.50	58.87	0.0001
Species × ln mass	3	0.91	2.75	0.048
ln stride length				
Model	4	24.78	104.87	0.0001
Error	80	4.73		
Total	84	29.51		
Effect tests				
Species	3	4.39	24.75	0.0001
ln mass	1	2.49	42.13	0.0001
ln stride frequency				
Model	7	6.72	20.99	0.0001
Error	77	3.52		
Total	84	10.23		
Effect tests				
Species	3	1.78	13.00	0.0001
ln mass	1	1.20	26.27	0.0001
Species × ln mass	3	1.43	10.45	0.0001
ln length				
Model	4	26.32	1178.6	0.0001
Error	80	0.45		
Total	84	26.77		
Effect tests				
Species	3	0.14	8.58	0.0001
ln mass	1	1.95	341.17	0.0001
ln width				
Model	7	36.43	775.74	0.0001
Error	77	0.52		
Total	84	36.95		
Effect tests				
Species	3	0.08	3.86	0.0125
ln mass	1	1.99	296.9	0.0001
Species × ln mass	3	0.13	6.46	0.0006

Species is treated as the fixed effect and mass as the covariate. A significant interaction term indicates heterogeneity of slopes. Where the interaction terms are absent the interaction was not significant (P > 0.05). Table 3 gives the regression equations estimated using these analyses.

and mass also differed significantly; *D. melanogaster* has a significantly greater intercept for the line relating speed and mass than the other three species. This indicates that *Drosophila* crawl relatively rapidly for their size.

Stride length increases with mass [Fig. 2(b)] in all four species. The ANCOVA indicates that these species share a common slope (0.34), with significantly different intercepts. *M. domestica* had the greatest intercept for the line relating stride length to mass, while *D. melanogaster* had the smallest. Therefore, *M. domestica* had the longest relative stride length and *D. melanogaster* the shortest. Stride frequency increased with mass [Fig. 2(c)] overall, but this relationship is significant only in *M. domestica*.

There were small but significant differences in the shape of larvae from these four species. Length and width are strongly correlated with mass [Fig. 2(d, e)], with the interaction term in the ANCOVA testing for homogen-

eity of slopes (Table 2) significant for width but not for length. This indicates that there were significant differences in the slopes of the lines relating mass and width among these species but not among the lines relating mass and length. Width increased with mass relatively little in *M. domestica* (slope = 0.20) compared to the other three species (slopes = 0.32–0.39). This indicates that *M. domestica* is relatively long and slender, whereas the other species are relatively short and stout.

Relationship between speed and kinematics

These flies increased speed by increasing both stride length and stride frequency [Fig. 3(a, b)]. Table 4 shows ANCOVAs for the relationship between ln speed and ln stride frequency and ln stride length. Notice that in both analyses the interaction between the fixed effect (species) and the covariate (stride frequency or stride length) is significant. This indicates that the slope of the lines relating speed and stride frequency and stride length differ significantly between species. Speed increased with stride frequency [Fig. 3(a)] significantly (Table 5) in all species except *D. melanogaster*, where the slope of the line relating speed and stride frequency is positive (0.35) but not significant (P > 0.2). Speed increased with stride length [Fig. 3(b)] in all four species, most rapidly in *M. domestica* and least rapidly in *D. melanogaster* (Table 5). Notice that for a given speed, *S. bullata* had relatively high stride frequencies and relatively low stride

TABLE 3. Regression equations obtained from ANCOVA for the relationship between mass and the kinematics of locomotion in four species of Diptera

Species and variable	Intercept a	Slope b	r²
D. melanogaster			
Speed	−2.33(0.076)	0.30*(0.16)	0.82
Stride length	−2.43(0.054)	0.34(0.05)	0.84
Stride frequency	0.098(0.049)	0.029*(0.10)	0.66
Length	−1.03(0.02)	0.30(0.016)	0.98
Width	−2.56(0.02)	0.35(0.04)	0.99
M. domestica			
Speed	−3.49(0.36)	0.89(0.14)	0.82
Stride length	−2.49(0.14)	0.34(0.05)	0.84
Stride frequency	−1.35(0.23)	0.68(0.09)	0.66
Length	−0.91(0.04)	0.30(0.016)	0.98
Width	−2.29(0.11)	0.20(0.03)	0.99
P. terraenovae			
Speed	−3.22(0.466)	0.56(0.12)	0.82
Stride length	−3.06(0.21)	0.34(0.05)	0.84
Stride frequency	0.18*(0.30)	0.13(0.078)	0.66
Length	−0.95(0.06)	0.30(0.016)	0.98
Width	−2.70(0.11)	0.39(0.03)	0.99
S. bullata			
Speed	−3.14(0.83)	0.51(0.17)	0.82
Stride length	−2.56(0.26)	0.34(0.05)	0.84
Stride frequency	−0.40*(0.53)	0.14*(0.11)	0.66
Length	−0.99(0.08)	0.30(0.016)	0.98
Width	−2.36(0.20)	0.32(0.04)	0.99

The equations refer to ln transformed values and the numbers in parentheses are SEs. Sample sizes are n = 20 except n = 25 for *P. terraenovae*. Mass is in mg, speed is in cm/s, stride length in cm/contraction, stride frequency in cm/s and length and width in cm.
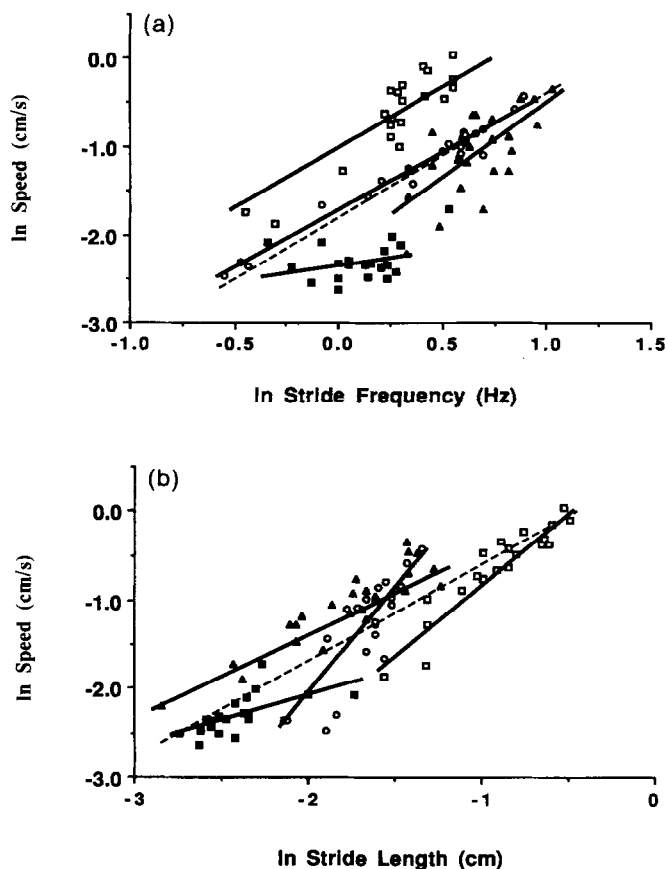*Not significantly different from 0, P > 0.05.

FIGURE 3. Relationship between stride characteristics and speed in crawling larvae: (a) relationship between stride frequency and speed; (b) relationship between stride length and speed. All data are ln transformed. The solid lines are the estimated regression lines for each species and the dotted lines are the overall regression lines for all the data points: ●, *D. melanogaster*; ○, *M. domestica*; ▲, *P. terraenovae*; □, *S. bullata*.

lengths, whereas *D. melanogaster* had low stride lengths and frequencies and *P. terraenovae* had relatively low stride frequencies and high stride lengths. These species differed in how they altered stride length and frequency to determine speed.

Not surprisingly, stride length and stride frequency were also correlated with each other, regressions of ln stride frequency on ln stride length gave significant positive relationships for *M. domestica* and *S. bullata*, a positive, but not quite significant relationship for *P. terraenovae* and a significant negative relationship between stride frequency and stride length for *D. melanogaster*. In other words, these species differ in how stride frequency changes with increasing stride length. The regression equations for the four species are: for *D. melanogaster*, ln stride frequency = − 0.46·ln stride length − 0.99, SE slope = 0.17 and intercept = 0.41, $r^2 = 0.28$, $P = 0.016$; for *M. domestica*, ln stride frequency = 1.66·ln stride length + 3.07, SE slope = 0.38 and intercept = 0.62, $r^2 = 0.52$, $P = 0.0004$; for *P. terraenovae*, ln stride frequency = 0.16·ln stride length + 0.95, SE slope = 0.09 and intercept = 0.16, $r^2 = 0.13$, $P = 0.086$; and for *S. bullata*, ln stride

TABLE 4. ANCOVA for the relationship between ln speed and ln transformed stride characteristics for four species of Diptera

Dependent variable	df	SS	F-ratio	$P \leqslant$
ln speed				
Model	7	42.3	103.36	0.0001
Error	77	4.5		
Total	84	46.8		
Effect tests				
Species	3	9.00	51.32	0.0001
ln mass	1	7.22	123.56	0.0001
Species × ln stride frequency	3	1.08	6.17	0.0008
ln speed				
Model	7	42.80	117.63	0.0001
Error	77	4.00		
Total	84	46.80		
Effect tests				
Species	3	1.57	10.07	0.0001
ln mass	1	9.72	187.09	0.0001
Species × ln stride length	3	1.92	12.33	0.0001

Species is treated as the fixed effect and stride frequency or stride length as the covariate. A significant interaction term indicates heterogeneity of slopes. Where the interaction terms are absent the interaction was not significant ($P > 0.05$). Table 5 gives the regression equations estimated using these analyses.

frequency = 0.61·ln stride length + 0.83, SE slope = 0.15 and intercept = 0.14, $r^2 = 0.49$, $P = 0.0006$. These regression analyses ignore the confounding effects of size. However, note that partial correlations between ln stride length and ln stride frequency with the effects of size factored out, resulted in positive correlation coefficients for the three species with positive slopes in the regression analysis and a negative correlation coefficient for *D. melanogaster*. This indicates that the differing relationships between the kinematic variables are not a consequence of size alone.

Effect of shape on kinematics

We looked for relationships between shape and the kinematics of crawling by estimating the relationship

TABLE 5. Regression equations obtained from ANCOVA for the relationship between speed and the kinematics of locomotion in four species of Diptera

Independent variable and species	Intercept *a*	Slope *b*	r^2
Stride frequency			
D. melanogaster	−2.33(0.06)	0.35*(0.27)	0.90
M. domestica	−1.72(0.07)	1.30(0.13)	0.90
P. terraenovae	−2.18(0.19)	1.67(0.27)	0.90
S. bullata	−1.12(0.08)	1.78(0.21)	0.90
Stride length			
D. melanogaster	−0.96(0.54)	0.56(0.22)	0.91
M. domestica	2.85(0.46)	2.51(0.28)	0.91
P. terraenovae	0.75(0.21)	1.03(0.12)	0.91
S. bullata	0.81(0.17)	1.60(0.18)	0.91

The equations refer to ln transformed values and the numbers in parentheses are SEs. Sample sizes are $n = 20$ except $n = 25$ for *P. terraenovae*. Speed is in cm/s, stride length in cm/contraction and stride frequency in cm/s.

*Not significantly different from 0, $P > 0.05$.

TABLE 6. Regression statistics for the relationship between the residuals of ln transformed speed, stride length and stride frequency from regression equations on mass with the residuals of ln transformed length and width from regression equations on mass

Variable	Length		Width	
	r^2	P	r^2	P
Speed	0.03	0.11	0.01	0.36
Stride frequency	0.04	0.06	0.02	0.18
Stride length	0.00	0.79	0.00	0.71

Similar analysis using the residuals from the ANCOVAs or on a species × species × species basis always resulted in $P > 0.2$. The sample size for each analysis is 85.

between residual values of length and width and residual values of speed, stride length and stride frequency. Relative length and width were not significantly correlated with relative speed, stride length or stride frequency (Table 6). There was a trend ($P = 0.06$) for longer individuals to have higher stride frequencies. We also analyzed the entire data set using two multivariate approaches, principal components analysis and partial correlations. These analyses (not shown) lead to similar conclusions as the residual analysis. The relationships in Table 6 were obtained from regressions on all the data. When we performed similar analyses using the residuals from the regression equations generated for each species by ANCOVA, we also found no evidence for significant relationships between residual length and width and residual kinematic characteristics. In this analysis the P values for the relationship between length and stride frequency were all > 0.2.

Cost of locomotion

Berrigan and Lighton (1993) measured the cost of crawling in larval *P. terraenovae* using flow through respirometry with an IR gas analysis system to measure CO_2 production by individual crawling larvae. They found that these flies had a minimum cost of transport (MCOT) of about 2000 J/kg/m and used this in conjunction with descriptions of stride characteristics to estimate the cost/stride for larval *P. terraenovae*. We can use this estimate of the cost of transport to estimate the cost of crawling in the three additional species of flies described here by dividing the equation for metabolic rate as a function of speed (metabolic rate = intercept + MCOT·Speed) by average stride frequency. This calculation gives estimated costs/stride of 6.7, 7.7 and 12.4 J/kg/stride for *D. melanogaster*, *M. domestica* and *S. bullata*, respectively.

DISCUSSION

Larval flies spanning a wide range of sizes crawl using a combination of telescoping and caterpillar-like peristaltic motion. Overall, the flies increased speed by increasing both stride length and stride frequency. However, the data clearly demonstrate that these species differ in the relationship between mass and the kinematics

of crawling and in body shape. Past results on limbed invertebrates have found that in some species, increases in speed appear to be chiefly determined by increases in stride frequency (Casey, 1991; Joos, 1992; Full *et al.*, 1990), whereas in others, e.g. ants, speed is increased by increasing both stride length and stride frequency (Zollikofer, 1994). Direct contrasts of the kinematics of dipteran crawling to crawling by other insect larvae are currently difficult because of the lack of comparative data. The larvae studied here crawled at speeds ranging from about 0.07 to 1.0 cm/s at frequencies ranging from 0.6 to 2.8 Hz. Gypsy moth caterpillars (2.5 cm long) travel with average speeds of about 1 cm/s with 0.6 cm stride lengths and stride frequencies of 1.57 Hz at an average body temperature of 17.7°C (Joos, 1992). Joos (1992) found that speed increased with both stride length (slope ≈ 1.7) and stride frequency (slope ≈ 1.5). These estimates are fairly similar to our results for flies with the exception of *D. melanogaster*, where stride frequency was not correlated with speed.

A number of studies have been performed on the energetics and kinematics of limbless locomotion by invertebrates, but these studies have focused on earthworms, aquatic invertebrates and terrestrial gastropods (Trueman, 1975; Elder, 1980; Denny, 1980; DeMont, 1992). Leech and earthworm crawling is powered by successive or simultaneous contractions of circular muscles, followed by the establishment of an attachment point and then contraction of the longitudinal muscles in synchrony (Gray and Lissmann, 1938; Gray *et al.*, 1938). When the longitudinal muscles contract in a wave down the length of one side of the body more strongly then on the other side, the result is a wave of lateral movements down the body, as in leech swimming (Kristan *et al.*, 1982). It seems likely that the lateral movements we observed in *M. domestica* crawling are caused by a similar wave of contractions of longitudinal muscles along the length of the larva's body.

Some aspects of snake locomotion also appear to display convergent mechanisms for crawling compared to those used by invertebrates. Snakes use four very different modes of crawling: concertina, lateral undulations, sidewinding and rectilinear (Gray, 1968; Gans, 1974; Jayne, 1986; Jayne and Davis, 1991; Secor *et al.*, 1992; Walton *et al.*, 1990). The first three modes are common and all involve sinuous lateral bending induced by asynchonous contractions of longitudinal muscles (Gans, 1974; Jayne, 1986). Rectilinear locomotion is similar in some ways to telescoping peristalsis. Snakes employing rectilinear movement anchor the ventral surface of the skin at successive points while the dorsal surface of the animal moves along smoothly (Lissmann, 1950). Another kind of limbless locomotion has been described in legless Amphisbaenians. These animals are organized in segmented rings which are involved in successive contractions during crawling or burrowing, reminiscent of larval locomotion in flies (Gans, 1974).

Despite the fact that these flies showed significant differences in the relationship between mass and length and width, we were unable to detect any effect of shape on the kinematics of crawling. However, the differences between species and between individuals in relative length and width were very small. Comparative studies of flies that differ more dramatically in shape might identify stronger effects of shape on the kinematics of crawling. Shape is likely to influence the relative efficiency of crawling versus burrowing, but little is known about the ecological habits of larvae, particularly in the wandering phase (Smith, 1986; Greenberg, 1990; Denlinger and Zdarek, 1994). This makes it more difficult to assess the importance of differences in shape with respect to burrowing vs crawling. We would like to know something about the average distance larvae travel during the feeding and wandering stages and something about the relative amount of time spent in different activities.

The allometry and kinematics of crawling are, not surprisingly, very different from those of walking or running. One notable aspect of crawling in insects is its low speed compared to limbed organisms (Heglund *et al.*, 1974; Joos, 1992; Full, 1994). In adult insects ranging in mass from about 7 to 200 mg, typical running speeds are from 7 to 10 cm/s at frequencies near 10 Hz. Thus, stride frequency and walking speed are an order of magnitude larger in limbed insects than than those found in larval flies and in caterpillars. The fact that crawling involves the progressive elongation of each body segment appears to result in rather different constraints than those found in limbed insects on the interaction between determinants of speed (Casey, 1991; Joos, 1992). The low speeds achievable by larval insects might contribute to the evolution of alternative methods for avoiding predators and parasites. Mediterranean fruit fly larvae, *Ceratitis capitata*, can jump 20 or more times their body length (Maitland, 1992); and eastern tent caterpillars, *Malacosoma americanum*, forage at night, possibly to avoid predators and parasites (Joos, 1992). Finally, many larval insects, particularly among the Lepidoptera, contain toxic substances to deter predators and parasites (Edmunds, 1974).

Sokolowski (1980, 1985) has described a genetic polymorphism that occurs in natural populations of flies and that influences the amount of crawling by larvae. Our estimate of the cost of crawling could be used in calculations designed to assess the costs and benefits of the gene controlling this behavior given information about the relative distances crawled by larvae with the alternative genotypes. These estimates (reported in the Results section) should be used with caution. Ideally we would like to make direct measurements of the cost of transport in species of interest. Unfortunately, this is likely to be impossible in many species: the study organisms must be large enough to produce sufficient CO_2 for measurement and must engage in the activity of interest for several minutes or more. The smaller the study organism, the longer the duration of activity required because respirometry on smaller individuals requires lower flow rates.

The major assumptions made in our procedure for estimating the cost of crawling is that the MCOT is the same in all four species described here. Berrigan and Lighton (1993) report no detectable effect of size on MCOT in *P. terraenovae*; on the other hand, MCOT is well known to scale with an allometric exponent of -0.25 in limbed insects (Full, 1989, 1994). There is not enough comparative data on limbless locomotion to assess how MCOT scales with body mass. Furthermore, the allometric relationships reported here between size and stride characteristics differ between species. This suggests that the relationship between size and the cost per stride could differ in these species. Biomechanical considerations suggest that the energy cost of crawling should be a function of frictional forces between the larvae and the surface it is crawling on, and the amount of energy used during a contraction cycle that is recovered as a consequence of elastic energy storage (T. Daniel, pers. commun.; Keller and Falkovitz, 1983). These variables could be measured for larvae varying in size and in conjunction with a biomechanical model used to estimate the scaling of MCOT with body size in larval flies. Measurements of this kind might complement additional studies of the energetics and kinematics of crawling in flies.

REFERENCES

Alexander R. McN. (1982) *Locomotion of Animals*. Blackie, Glasgow.

Berrigan D. and Lighton J. R. B. (1993) Bioenergetic and kinematic consequences of limblessness in larval Diptera. *J. exp. Biol.* **179**, 245–259.

Birket-Smith S. J. R. (1984) *Prolegs, Legs, and Wings of Insects*. Entomograph, Vol. 5. Scandinavian Science Press, Copenhagen.

Casey T. M. (1989) Oxygen consumption during flight. In *Insect Flight* (Eds Goldsworthy G. and Wheeler C.), pp. 257–272. CRC Press, Boca Raton, FL.

Casey T. M. (1991) Energetics of caterpillar locomotion: biomechanical constraints of a hydraulic skeleton. *Science* **252**, 112–114.

Chapman R. S. (1971) *The Insects, Structure and Function*. Elsevier, New York.

DeMont M. E. (1992) Locomotion of soft bodied animals. In *Advances in Comparative and Environmental Physiology* (Ed. Alexander R. McN.), Vol. 11, Chap. 6, pp. 167–190. Springer-Verlag, Berlin.

Denlinger D. L. and Zdarek J. (1994) Metamorphosis behavior of flies. *A. Rev. Ent.* **39**, 243–266.

Denny M. (1980) Locomotion: the cost of gastropod crawling. *Science* **208**, 1288–1290.

Dethier V. G. (1976) *The Hungry Fly: a Physiological Study of Behavior Associated with Feeding*. Harvard University Press, Cambridge, MA.

Duncan C. D. and Pickwell G. (1939) *The World of Insects*. McGraw-Hill, New York.

Edmunds M. (1974) *Defence in Animals*. Longman, London.

Elder H. Y. (1980) Peristaltic mechanisms. In *Aspects of Animal Movement* (Eds Elder H. Y. and Trueman E. R.), pp. 71–92. Cambridge University Press, Cambridge.

Ellington C. P. (1984) The aerodynamics of hovering insect flight. I. The quasi-steady analysis. *Phil. Trans. R. Soc. Lond. B* **305**, 1–15.

Full R. J. (1989) Mechanics and energetics of terrestrial locomotion: bipeds to polypeds. In *Energy Transformations in Cells and Organisms* (Eds Wieser W. and Gnaiger E.), pp. 170–174. George Thieme-Verlag, Stuttgart.

Full R. J. (1994) Invertebrate systems from a comparative viewpoint: locomotor system. In *Handbook of Comparative Physiology* (Ed. Dantzler W.). Oxford University Press, New York.

Full R. J., Zuccarello D. A. and Tullis A. (1990) Effect of variation in form on the cost of terrestrial locomotion. *J. exp. Biol.* **150**, 233–246.

Gans C. (1974) *Biomechanics: an Approach to Vertebrate Biology.* Lippincott, Philadelphia, PA.

Gray J. (1968) *Animal Locomotion.* Clowes, London.

Gray J. and Lissmann H. W. (1938) Studies in animal locomotion. VII. Locomotory refelexes in the earthworm. *J. exp. Biol.* **15**, 506–516.

Gray J., Lissmann H. W. and Pumphrey R. J. (1938) The mechanism of locomotion in the leech (*Hirudo medicinalis* Ray). *J. exp. Biol.* **15**, 408–430.

Greenberg B. (1990) Behavior of postfeeding larvae of some Calliphoridae and a Muscid (Diptera). *Ann. Ent. Soc. Am.* **83**, 1210–1214.

Heffernan J. M. and Wainwright S. A. (1974) Locomotion of the holothurian *Euapta lappa* and redefinition of peristalsis. *Biol. Bull.* **147**, 95–104.

Heglund N. C., Taylor C. R. and McMahon T. A. (1974) Scaling stride frequency and gait to animal size: mice to horses. *Science* **186**, 1112–1113.

Herreid C. F. (1981) Energetics of pedestrian arthropods. In *Locomotion and Energetics in Arthropods* (Eds Herreid C. F. and Fourtner C. R.), pp. 491–526. Plenum Press, New York.

Joos B. (1992) Adaptations for locomotion at low body temperatures in eastern tent caterpillars, *Malacosum americanum. Phys. Zool.* **65**, 1148–1161.

Jayne B. C. (1986) Kinematics of terrestrial snake locomotion. *Copeia* **1986(4)**, 915–927.

Jayne B. C. and Davis J. D. (1991) Kinematics and performance capacity for the concertina locomotion of a snake (*Coluber constrictor*). *J. exp. Biol.* **156**, 539–556.

Keller J. B. and Falkovitz M. S. (1983) Crawling of worms. *J. theor. Biol.* **104**, 417–442.

Kristan W. B., McGirr S. J. and Simpson G. V. (1982) Behavioural and mechanosensory neurone responses to skin stimulation in leeches. *J. exp. Biol.* **96**, 143–160.

Lissmann H. W. (1950) Rectilinear locomotion in a snake (*Boa occidentalis*). *J. exp. Biol.* **26**, 268–279.

Maitland D. P. (1992) Locomotion by jumping in the Mediterranean fruit fly larva *Ceratitis capitata. Nature* **359**, 159–161.

Nachtigall W. (1985) Swimming in aquatic insects. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology. Nervous System: Structure and Motor Function* (Eds Kerkut G. A. and Gilbert L. I.). Pergamon Press, New York.

Packard G. C. and Boardman T. J. (1987) The misuse of ratios to scale physiological data that vary allometrically with body size. In *New Directions in Ecological Physiology* (Eds Feder M. E., Bennet A. F., Burgren W. W. and Huey R. B.), pp. 216–235. Cambridge University Press, Cambridge.

Peters R. H. (1983) *The Ecological Implications of Body Size.* Cambridge University Press, Cambridge.

Secor S. M., Jayne B. C. and Bennet A. F. (1992) Locomotor performance and energetic cost of side winding by the snake *Crotalus cerastes. J. exp. Biol.* **163**, 1–14.

Smith K. G. V. (1986) *A Manual of Forensic Entomology.* Cornell University Press, Ithaca, NY.

Sokal R. R. and Rohlf F. J. (1981) *Biometry*, 2nd edn. Freeman, New York.

Sokolowski M. B. (1980) Foraging strategies of *Drosophila melanogaster*: a chromosomal analysis. *Behav. Genet.* **10**, 291–302.

Sokolowski M. B. (1985) Genetics and ecology of *Drosophila melanogaster* larval foraging and pupation behaviour. *J. Insect Physiol.* **31**, 857–864.

Trueman E. R. (1975) *Locomotion of Soft-bodied Animals.* Edward Arnold, London.

Walton M., Jayne B. C. and Bennet A. F. (1990) The energetic cost of limbless locomotion. *Science* **249**, 524–527.

Zollikofer C. P. E. (1994) Stepping patterns in ants: II. Influence of body morphology. *J. exp. Biol.* **192**, 107–118.