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Coordination of legs during straight walking and turning in *Drosophila melanogaster*

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Summary. Leg coordination of *Drosophila melanogaster* was studied using frame-by-frame film analysis.

1. For fastest walking alternating tripod coordination is observed which slightly deviates towards tetrapody as a function of step period. During acceleration or deceleration legs may transiently recover in diagonal pairs.
2. Mean step length increases with step frequency.
3. Mean recovery stroke duration increases with step period and plateaus beyond a period of about 110 ms. Middle legs recover significantly faster than others.
4. Ipsilateral footprints are transversally separated.
5. Walking is usually initiated in tripod coordination (frequently in combination with a turn), otherwise in an accelerating sequence which rapidly shifts towards tripod pattern. Flies can stop abruptly or decelerate over about one metachronal wave.
6. Short interruptions in walking are observed. Legs interrupted during swing phase stay lifted and finish recovery thereafter.
7. Slight changes in walking direction are obtained by altering step lengths only. Tight turns are composed of two or three phases with backward, zero and forward translatory components. In fast turning tripod coordination is maintained. Otherwise body sides can decouple widely. In all turns numbers of contralateral metachronal waves were equal.

Results are compared to those for other walking insects and their relevance in screens for locomotor mutants is discussed.

Key words: *Drosophila* behavior – Insect locomotion – Leg coordination – Turning behavior

Introduction

For hexapod walking coordination stick insect, grasshopper and cockroach are the most extensively studied organisms and a vast body of behavioral data has been obtained for these species (Wendler 1964; Graham 1972, 1978; Hughes 1952; Delcomyn 1971). Common to these insects and others studied by Wilson (1966) is a change in leg coordination for slow walking speeds. While for fast walking alternating tripod coordination is observed, for low walking speeds successive ipsilateral metachronal waves do not overlap anymore and at any time not more than two legs are lifted off ground (Graham 1972). Especially for the first instar stick insect Graham (1972) described two different gaits in an overlapping range of walking speeds within the same animal. Behavioral investigations of unrestrained and tethered walking animals together with electrophysiological experiments, elaborate surgery and a multiplicity of other experimental approaches reviewed recently (Bässler 1983; Graham 1985) have led to a detailed description of sensory subsystems of the single leg, of coordinating influences between different legs and to the construction of a variety of models concerning leg coordination (Wendler 1968, 1978; Graham 1972, 1977; Cruse 1979a, 1980a, b).

While in *Drosophila* electrophysiology and surgery are cumbersome due to the small size of the animal, a powerful spectrum of methods has been developed in classical and molecular genetics over nearly a century (review: Rubin 1988) and has successfully been employed in a variety of neurobiological fields (review: Hall and Greenspan 1979; Hall 1982). Surprisingly, the study of the flies' leg coordination has been left out so far, though mutants disturbed in this property are described (e.g. *uncoordinated* (Lindsley and Grell 1968; Schalet 1972); *wobbly* (Grigliatti et al. 1973; Schmidt-Nielson and Hall 1977)). Walking is an important mode of locomotion to the fly. Search for food in the nearfield is accomplished by walking. During courtship males track females in the walking mode. *Drosophila* is a persevering runner: In 'Buridan's paradigm' (Götz 1980), Bülthoff et al. (1982) showed that, restrained from flight, individuals can walk persistently for hours until complete exhaustion.

Taking advantage of *Drosophila* genetics it should be possible to contribute to the functional analysis of the insect locomotor system. Mutants with well-defined partial defects in walking might help to separate motor-subsystems which are not separable by surgical means. At all levels mutants have contributed to the understanding of behavioral subsystems. Considering the visual system, for example, mutant flies *optomotor-blind* are impaired in a specific optomotor response to large field stimuli (Heisenberg and Wolf 1984; Bausenwein et al. 1986). *Sevenless* flies lack color vision due to the absence of R7 receptor cells in all ommatidia but are not affected in motion-dependent tasks (Harris et al. 1976; Heisenberg and Buchner 1977). Beyond the dissection of behavior, genetics addresses the question of how motor programs are determined genetically.

With the present work on the walking coordination of *Drosophila* we provide quantitative behavioral data on which a search for locomotor mutants can be based. To our knowledge, the present study is the first one dealing with leg coordination in dipterans.

Materials and methods

Flies. The flies used throughout this study were intact 1 to 2 day old females of the wildtype strain Berlin. The average body length was 2.4 to 2.6 mm measured from the onset of antennae to the abdominal tip. To prevent flight, wings were shortened by 2/3rds under cold anesthesia (+4 °C) in a stream of dry air. At least 4 h of recovery were given prior to filming.

Study of straight walk. One fly at a time was walking freely in the horizontal plane on smooth transparent graph paper illuminated from below. It was filmed from above with a 16 mm high speed motion picture camera (Locam 51-0002) at 200 frames/s on Eastman 7224 4-X negative film (500 ASA). An electronic time mark system provided independent control for proper film speed.

In order to elicit straight walks flies were placed in a visual surround known as 'Buridan's paradigm' (Götz 1980; Bülthoff et al. 1982). There flight-restricted flies keep running to and fro between two identical inaccessible black objects in an otherwise uniformly white arena. In our setup an elevated circular platform (∅ 85 mm) with the film window of 20 × 26 mm in the center was surrounded by a water-filled moat. Temperature was constant at (28 ± 1) °C.

Study of turning behavior. In an initial setup flies were filmed from above with a stationary Super-8-Camera (Beaulieu 4008 ZMII) at its maximum speed of 70 frames/s. Instead of cutting wings flies were covered with a Petri dish of 2 mm rim height. When the fly was about to leave the film window it was brought back by shifting the graph paper appropriately. Due to the open field situation flies predominantly showed random search marked by frequent changes in walking direction. Therefore recordings have been used for a qualitative survey of the flies' repertoire of turning maneuvers.

Comparison of straight episodes obtained in the latter setup with those obtained as described under 'straight walk' showed no detectable effect of either cutting wings or moving the walking surface on coordination or any other parameters described in this work.

The terms and conventions established by Wilson (1966) and Graham (1972) have been followed:

Propulsion stroke: Movement of a leg relative to the body while being in contact to the ground (stance phase).

Recovery stroke: Movement of a lifted leg relative to ground and body (swing phase).

Step length: Defined here as the distance between two successive footprints of the same leg; no normalization in relation to the direction of propagation.

Period: Time taken to complete one leg cycle consisting of swing and stance phase.

Metachronal wave: The successive recovery of legs on one body side in the order of hind, middle, front.

Legs are numbered from front to rear, prefixes L and R marking body sides. Leg numbers without prefix refer to ipsilateral legs and expressions or figures are valid for either body side.

Lag: Time interval between the beginning of swing phase in leg x and the immediately following swing phase onset of another leg y, written ${}_xL_y$.

Metachronal lag: ${}_3L_1$ ipsilateral (see insert in Fig. 2) includes ${}_{L3}L_{L1}$ and ${}_{R3}L_{R1}$.

Phase: Lag between two legs divided by the period of the first. The phase of R1 on L1 e.g. is written R1:L1.

Results

In a natural surround rich in visual and olfactory stimuli freely walking *Drosophila melanogaster* in general do not move straight for long distances. While such a situation provides an excellent opportunity to study the fly's behavioral repertoire, it is demanding on time and film material if one intends to record leg coordination of straight walking. For the evaluation of these data therefore flies were filmed in 'Buridan's paradigm' (Götz 1980) taking advantage of the recurrent inversion of fixation and antifixation behavior in an arena with two opposing landmarks.

The maximum walking speed for female *Drosophila* flies is 4.2 cm/s and continuous straight walks have been observed down to about 1.5 cm/s. Lower average walking speeds are generated by stop and go behavior and changes in the walking direction are frequent, then. In continuous straight walks the duration of a single step ranges from 60 ms to 150 ms corresponding to a rate of 16.5 to 6.7 steps/s.

Coordination during straight walking

The alternating tripod gait is the standard coordination for fast walking flies. Fore- and hindlegs of one body side recover together with the contralateral middle leg. Legs belonging to opposite tripod subsets are recovering in exact antiphase. A typical fast run (3.8 cm/s) is shown in Fig. 1a. Instead of describing the coincidence of certain swing phases a more universal description can be given for leg coordination if the parameter metachronal lag is evaluated (Graham 1972). An adequate description for tripod coordination is the statement, that lag ${}_3L_1$ on both sides equals period and any contralateral legs are in antiphase.

Examining slower walks (Fig. 1b, 2.2 cm/s), the pattern is slightly shifted: While the metachronal wave of recovery strokes from rear to front is still conserved in that the lag ${}_3L_2$ equals ${}_2L_1$, the onset of swing phase of leg 3 belonging to the subsequent ipsilateral metachronal wave is somewhat delayed: Ipsilateral hind- and forelegs no longer start recovery synchronously. In a plot of the metachronal lag ${}_3L_1$ versus period of the hindleg P_3 (Fig. 2) a weak frequency-dependence of the deviation from tripod gait is shown. The drawn curve

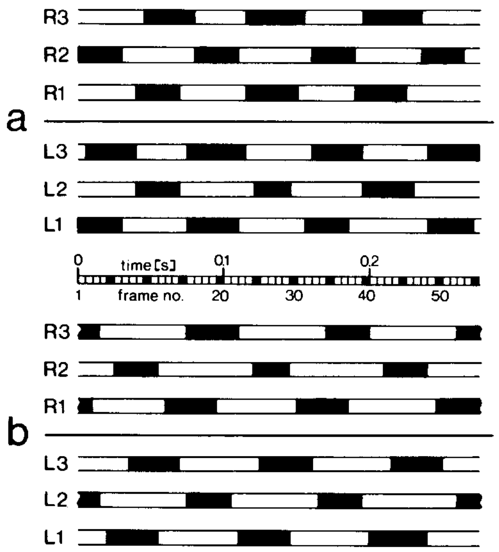

Fig. 1 a, b. Two examples of leg coordination during straight walking obtained from the same individual. Legs are numbered from front to rear, prefixes R and L marking body sides. Black bars indicate swing phases. In fast walking **a** (3.8 cm/s; 12.7 steps/s) perfect tripod coordination is observed, while for slower walks **b** (2.2 cm/s; 10 steps/s) recovery strokes of hindlegs are delayed relative to those of ipsilateral frontlegs

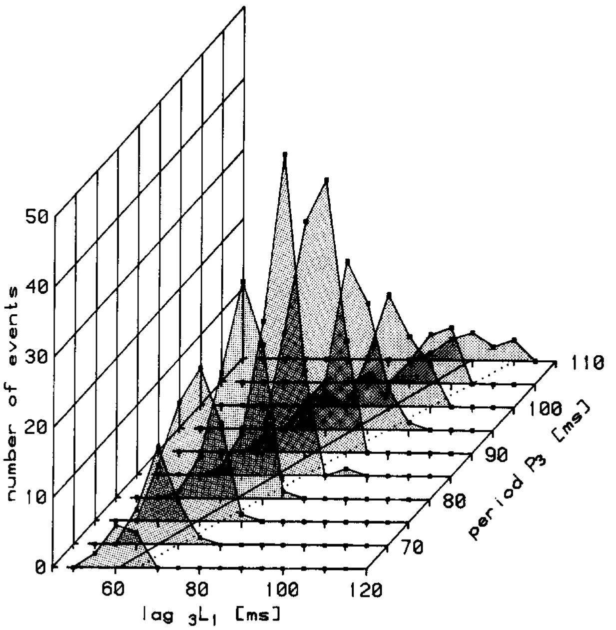

Fig. 3. Number of steps of a certain period and lag (P_3, $_3L_1$). Same set of data as in Fig. 2. The dotted line represents tripod coordination with $_3L_1 = P_3$, the drawn curve regression. From these data there is no evidence for the existence of two different gaits in an overlapping speed range for the fly

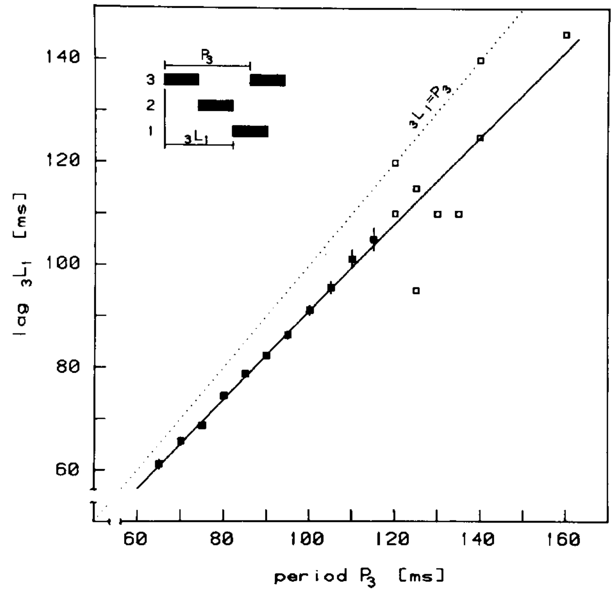

Fig. 2. Metachronal lag ($_3L_1$) versus period of the hindleg (P_3) for 10 flies and a total of 526 metachronal waves. See insert for the definition of $_3L_1$ (swing phases black). Closed symbols represent mean values of $_3L_1$ for a given mean period, open symbols single events. Error bars: standard error of the mean where this is larger than the symbol. Regression curve: $\langle {}_3L_1/[\mathrm{ms}]\rangle = 1.20 \cdot (P_3/[\mathrm{ms}])^{0.94}$

Table 1. Linear regression and correlation parameters for the phase dependence on period for contralateral ipsisegmental legs in the period range used for continuous straight walking

legs	*y-int*	*slope* [1/ms]	*r*	*n*	*mean phase*
L1:R1	0.469	$2.284 \cdot 10^{-4}$	0.055	289	0.489
R1:L1	0.533	$-3.238 \cdot 10^{-4}$	−0.079	289	0.504
L2:R2	0.521	$-3.735 \cdot 10^{-4}$	−0.087	292	0.489
R2:L2	0.480	$2.944 \cdot 10^{-4}$	0.070	296	0.505
L3:R3	0.501	$-1.405 \cdot 10^{-4}$	−0.031	295	0.489
R3:L3	0.479	$2.480 \cdot 10^{-4}$	0.053	293	0.501

y-int, ordinate intercept; *slope*, regression coefficient; *r*, correlation coefficient; *n*, number of observations. In all cases confidence intervals include slopes of either sign already at a level of confidence between 40% and 70%

represents a power function regression with $\langle {}_3L_1/[\mathrm{ms}]\rangle = 1.20 \cdot (P_3/[\mathrm{ms}])^{0.94}$ and a correlation coefficient of $r = 0.890$ calculated on the original data pairs. Reasons for the choice of a higher order regression are given in the Discussion.

A deviation of the mean lag $\langle {}_3L_1\rangle$ from tripod gait would also occur if datapoints would represent a mixture of two distinct gaits one being a perfect tripod coordination. Therefore, the distribution of steps of a certain period and lag (P_3, $_3L_1$) is shown in Fig. 3. (P_3 and $_3L_1$ are measured in discrete units of frame duration, i.e. 5 ms.) Data are distributed Gaussean-like around the respective mean value of $_3L_1$ (regression curve shown as solid line) and there is no significant evidence for the existence of two distinct gaits in *Drosophila* from these data.

While metachronal lag is weakly depending on period, there is no such dependence for the phase relation between contralateral (ipsisegmental) legs. They stay in exact antiphase over the entire period range used for continuous straight walking. Linear regression and correlation parameters are given in Table 1. In none of the cases the slope is of statistical significance: The confi-

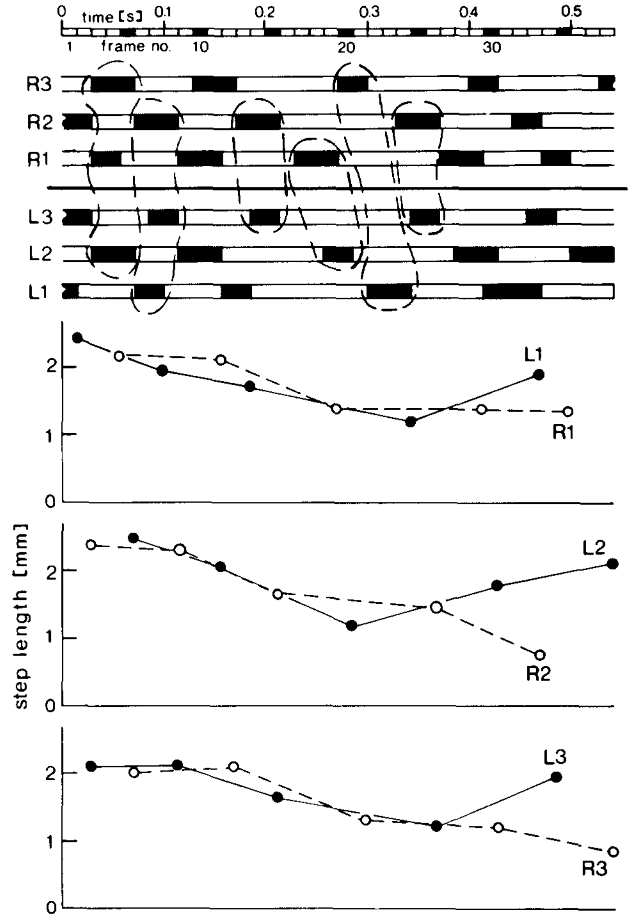

Fig. 4. Episode of straight decelerated walking with transient tetrapod coordination prior to a 36° right turn beginning with frame no. 27. Step lengths denoted at the end of their respective swing phases (black bars). Note that deceleration is achieved by reducing both step length and step frequency

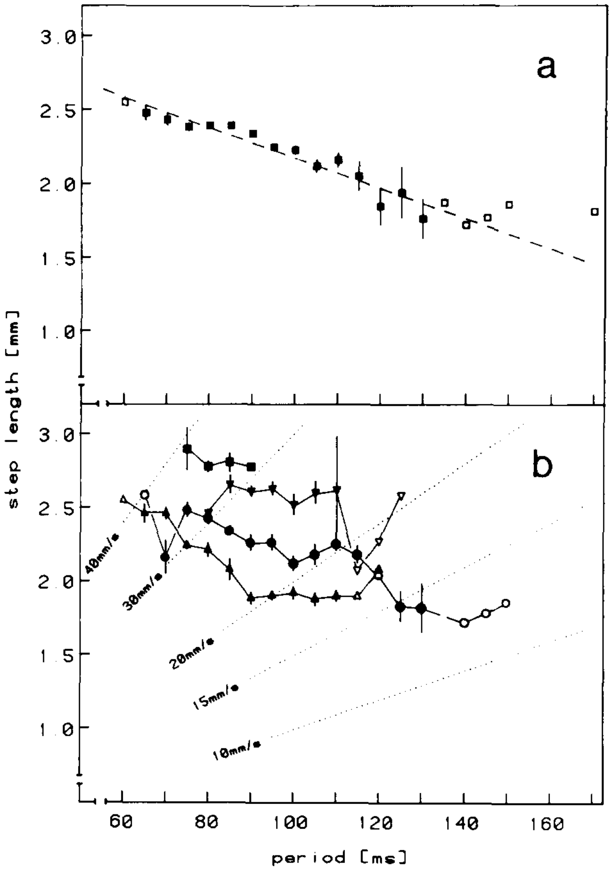

Fig. 5a, b. Dependence of step length upon period during straight walking. **a** Average dependence: Closed squares represent mean step length for a given period averaged over all legs of 10 flies ± SEM (vertical lines), where this is larger than the symbol. Open squares denote single events. Total number of steps is $n = 1736$. **b** Data of 4 individuals illustrating variability of step length. Conventions as indicated for **a**. $n = 200$–300 each. Dotted lines connect combinations of step length and period leading to the same walking speed

dence intervals include slope values of either sign already on a level of confidence between 40% and 70%.

Pure tetrapod coordination, where at all times at least 4 legs are in contact to the ground and legs recover in diagonal pairs, has not been seen in continuous straight walks. It may transiently been reached, however, during deceleration episodes prior to turns or to a complete stop, e.g. when the fly is aiming at a pointlike source of food. In Fig. 4 such a change in coordination is shown prior to a 36° turn to the right.

Drosphila is very reliable in its coordination. In more than 2000 single steps of 15 flies neither doubled nor omitted swing phases were observed except for the halting of hindlegs in tight turns. The number of metachronal waves has been found to be equal for both body sides even during turns. At any time at least three legs are in contact with the ground.

Frequency-dependence of step length and swing phase duration

During walking, changes in the speed of forward movement can be obtained by altering step frequency or step length. Variability of either one of these parameters would be sufficient, but from deceleration episodes like the one shown in Fig. 4 it becomes evident, that *Drosophila* can alter both concomitantly. This is true not only of relatively rapid changes in walking speed but also of walks at different but constant speeds. In spite of a considerable flexibility in combining step length and frequency the two parameters are not independent in the fly. High step frequencies often are associated with long strides and low frequencies with small ones.

The mean dependence of step length upon period for 10 flies is shown in Fig. 5a. Data are averaged over the steps of all legs since obviously for straight walking, legs belonging to one tripod subset ought to step with equal strides. Not selfevident but evaluated from the data, this is also true for legs belonging to opposite tripod subsets provided that speed is constant. Each step is classified according to the stepping period of the respective leg. The latter value is a multiple of 5 ms (frame-to-frame interval). Linear regression of mean step lengths for given mean periods yields a slope of $-1.03 \cdot 10^{-2}$ mm/ms and an ordinate intercept of 3.20 mm, the

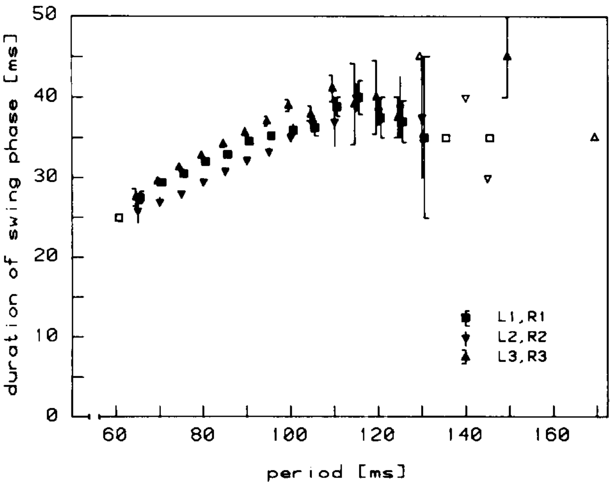

Fig. 6. Swing phase duration as a function of period. Same set of data as indicated for Fig. 2. Closed symbols represent mean values, averaged over both ipsisegmental legs, ±SEM, where this is larger than the symbol. Open symbols denote single observations

correlation coefficient of the averaged data being $r_{av} = -0.965$. The (anti-) correlation is slightly higher if period is measured as the duration of the swing phase – in which the stride is performed – plus the duration of the preceding stance phase (shown in Fig. 5) rather than adding the duration of the subsequent one ($\tilde{r}_{av} = -0.916$).

While the mean frequency-dependence of step length will be an important feature to distinguish possible walk-related mutant strains from wild-type on the average, it may give a misleading impression of rigidity of the interdependence. Intra- as interindividually flies are rather flexible in combining step length and period. The direct correlation between data pairs without prior classification according to period yielded coefficients between $r = -0.152$ and $r = -0.502$ for individual flies and of $r = -0.218$ for the pooled data of the 10 flies shown in Fig. 5a.

Examples of interindividual differences are given in Fig. 5b. Dotted lines mark combinations of step lengths and periods leading to equal walking speeds. Only to a small part differences are due to the variation in body size. Normalizing step lengths accordingly does not significantly reduce the interindividual differences. There is also a great deal of flexibility within the individual fly in combining step length and period for a certain walking speed. One fly was observed, which completed two walks at the same speed but with mean step lengths differing by 0.2 mm.

For all legs the mean swing phase duration increases linearly with increasing step period, up to a period of approximately 110 ms. Beyond this level, the mean time used for recovery shows an insignificant downward trend (all legs; 51 steps; $r = -0.086$) with a slope of $-5.02 \cdot 10^{-2}$ mm/ms and a mean duration of 38.6 ± 0.4 ms.

Within the period dependent range middle and hindlegs show the same slope but middle legs recover 3–4 ms faster on the average. The dependence of foreleg swing phase duration on period is less steep than for the other pairs of legs. Averaged data are shown in Fig. 6; regression parameters are given in Table 2. Since there is no significant difference in the swing phase duration between contralateral legs of the same segment those data are pooled.

Table 2. Linear correlation and regression parameters for the dependence of swing phase duration on period in the range from 65 to 110 ms

legs	*y-int* [ms]	*slope*	*r*	(r_{av})	*n*	*n'*
L1, R1	14.05	0.223	0.517	(0.989)	555	16
L2, R2	7.62	0.273	0.586	(0.997)	567	19
L3, R3	10.15	0.282	0.557	(0.987)	565	16

y-int, ordinate intercept; *slope*, regression coefficient; *r*, correlation coefficient on original data pairs; r_{av}, correlation coefficient on the averaged data as shown in Fig. 6; *n*, number of observations in the period range 65 ... 110 ms; *n'*, number of observations in the period range beyond 110 ms

Footprints

When walking in the horizontal plane flies produce 6 separate lines of footprints provided that the longitudinal body axis is aligned to the direction of propagation. Middle legs produce the outermost tracks, forelegs the innermost, hindlegs falling in between. An example for the symmetrical pattern is shown in Fig. 7a.

The transversal separation of the points of ground contact becomes important in fast walking, where *Drosophila* often uses strides longer than its body size. For instance middle legs swing anteriorly to the point of ground contact of the forelegs and legs would become entangled without transversal separation. The same consideration applies to the hindlegs: They swing to a point anterior and medial to the middle legs' point of ground contact.

Most often an asymmetrical pattern of footprints is observed. While middle legs still produce separate outer tracks, the footprints of fore- and hindlegs of one side fall close together yielding a common trail (since they are separated in time, they cannot get entangeled). In these cases the legs on the opposite body side produce 3 evenly spaced tracks in the medial-to-lateral order of foreleg, hindleg, middle leg.

The asymmetry is correlated with an average misalignment of the longitudinal body axis in relation to the direction of propagation of usually 2–3° towards the side where footprints of fore- and hindlegs fall together. A preferred direction of the misalignment for the wildtype strain used is likely since 12 of a total of 20 flies examined show the deviation to the right, 4 flies walked mainly symmetrically and 4 individuals showed deviation to the left. Individual flies always show asymmetry to the same side but they can also walk symmetrically for short episodes. A preferred direction of asymmetry may eventually account for the asymmetric outcome of behavioral experiments on walking flies in sym-

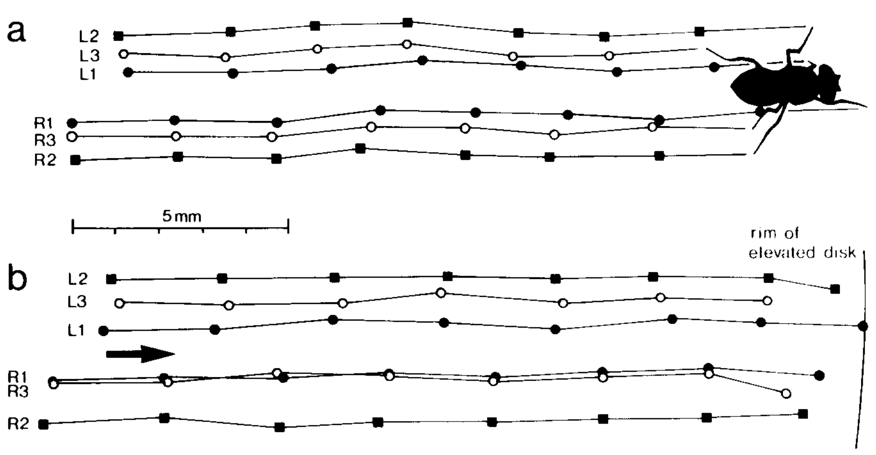

Fig. 7a, b. Patterns of footprints from 2 different flies. In **a** the longitudinal body axis is aligned to the direction of propagation and the pattern of footprints is symmetrical. The fly is redrawn from film. Tripod subset L3-R2-L1 is at the end of its stance phase and ready to swing.
b Walk with average misalignment of the longitudinal body axis and the direction of propagation by 3° to the right

metric setups (multiple-Y maze; M.H., unpublished observation).

Starts, breaks, stops

Patterns used for initiating and ending a walk can provide insight into the organization of the underlying network. This may especially apply to short interrupts in movement found relatively frequently in *Drosophila* walks.

Five starts from a resting position were filmed and in all cases flies initiated walking with a turn. In one case the change in walking direction was immediately corrected back to the initial direction of the longitudinal body axis at rest by means of a S-shaped curve. Flies usually show tripod coordination right from the beginning of a walk, but also starts with 4 legs in stance phase were observed. Walking, then, is accelerated and rapidly shifts to tripod coordination within about one metachronal wave of swing phases. As an example for tight turning, the walking sequence in Fig. 11 is at the same time a start from a resting position.

Drosophila shows two distinct halting behaviors. Flies can either stop abruptly or decelerate over the time of about one metachronal wave. The latter case is observed as a target-approach behavior and coordination as well as reduction of step length resemble the data shown for the deceleration episode in Fig. 4. An example for a sudden stop is given in Fig. 8. No reduction in step length can be detected prior to the stop. In the case shown, legs were rearranged to a symmetrical resting position 130 ms after the stop by means of backward directed swing phases.

Eighteen breaks between 25 ms and 0.4 s have been observed in walks of different flies. Neither body nor legs are moving during such an event even when leg cycles are interrupted during swing phase (4 cases). Legs then stand still off ground and finish recovery after the pause (Fig. 9a). Coordination before and after such a short break is best described by comparing it to the pause function of a cassette recorder. Both resume at exactly the point where they had been stopped.

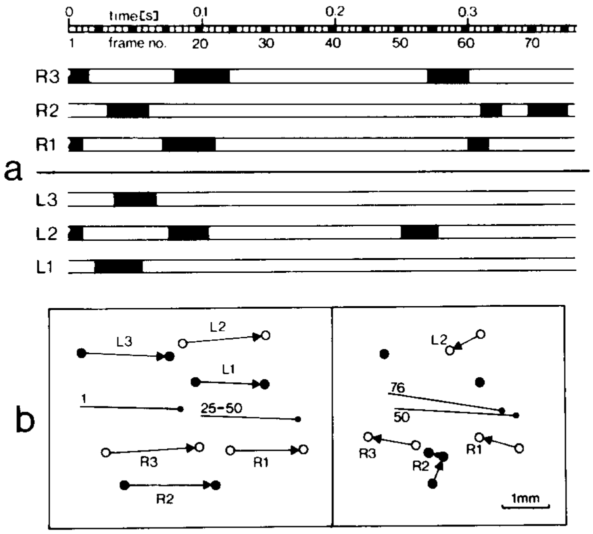

Fig. 8a, b. Rearrangement of legs to a symmetrical resting position by means of backward directed recovery strokes 130 ms after stopping. **a** Temporal pattern of swing phases. Conventions indicated in the legend of Fig. 1. **b** Direction of strides for swing phases shown in (**a**). For certain frames position of the fly is indicated by the longitudinal body axis, head is denoted by small filled circle

Frequently the direction of a walk is changed after the break. Figure 11 represents a further example of a pause marking the transition between successive phases of tight turning. During 114 ms (frame no. 11–18) all legs are in contact to the ground and there is no detectable movement of the body.

In Fig. 9b the occurrence of interruptions is summarized in a schematized diagram of normal coordination. In 3 of 18 observations the metachronal wave on the left (f) and in 13 observations on the right body side (a–d) is completed. In two more cases R3 and R2 have completed recovery and R1 is interrupted therein (e). The occurrence of the R3 swing phase belonging to the successive metachronal wave (d) might depend on whether at the time of interruption the coordination is closer to a tripod or tetrapod pattern.

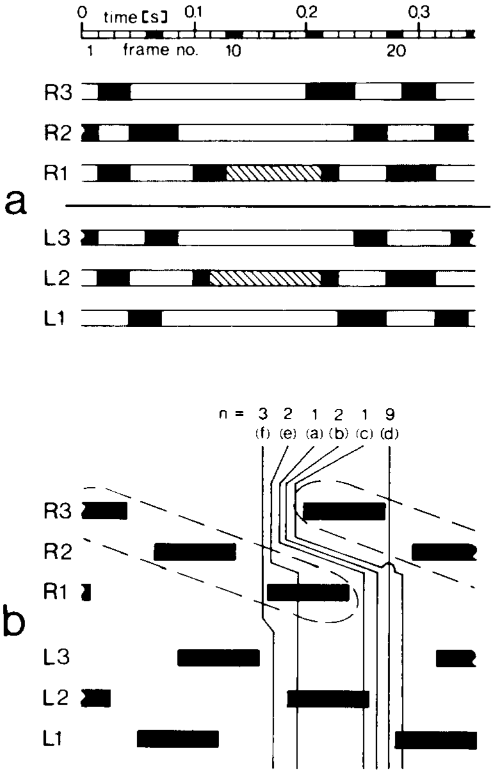

Fig. 9 a. A break in walking interrupts recovery strokes of R1 and L2. Swing phases are indicated black, hatched areas denote legs held up in the air. No movement of the body occurs during the break. **b** Cutting lines of the observed interrupts in a schematized diagram of the normal coordination (slower walking; $_3L_1 < P_3$). Within a certain swing or stance phase the partition is arbitrary and is not intended to show the actual timing. *n*, number of events; small letters refer to the text

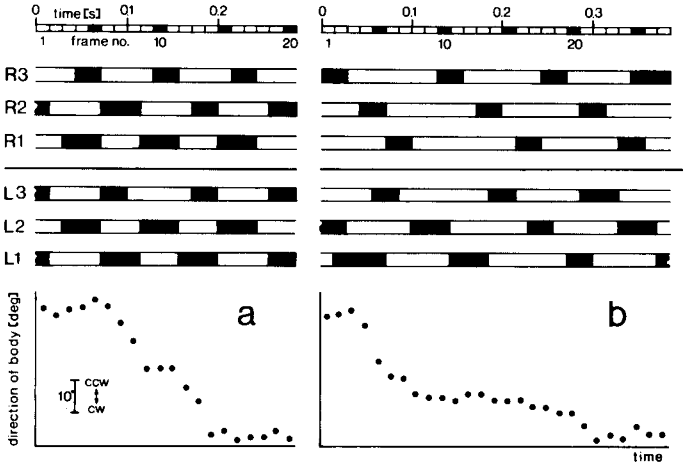

Fig. 10a, b. Two examples of different temporal patterns of swing phases during turns by approx. 40° to the right. In the lower graphs for each frame the direction of the longitudinal body axis is given in relation to a fixed axis of the walking surface. During rapid walking **a** the tripod coordination is not affected by turning and body sides remain rigidly coupled. During slow walking **b** coordination can get completely metachronal and body sides appear to be only loosely coupled

Turning behavior

Different ways of changing the direction of walking are described for insects. In principle turning can be achieved by altering step length or step frequency between the in- and outside of a curve, the latter resulting in a different total number of metachronal waves for the two sides of the body (e.g. in the adult stick insect; Graham 1972). Turns can be achieved by specialized legs (e.g. reduction of the middle leg propulsion in first instar stick insect nymphs; Graham 1972) or by all legs concomitantly (e.g. third instar stick insect; Jander and Wendler 1978). Also reversal of the walking direction on the inside of tight turns is described, for example for the cockroach (Bell and Schal 1980) and the bee (Zolotov et al. 1975).

Drosophila uses different methods which can be ranked according to their translatory component superposed on the rotation. Common to all turning maneuvers is the constant number of metachronal waves for left and right body side. Tracks of freely walking flies appear to be composed of episodes of straight walking interrupted by quick turns.

Turns achieved by altering step length. Slight changes in the direction of a walk are obtained by changing step length at least on the inside of the curve and maintaining tripod coordination throughout the turn. For turns larger than about 20° also participation of the legs on the outside of the curve becomes apparent but their participation in turns of any size is likely.

For increasing angles of turning different temporal patterns of recovery strokes are observed depending on walking speed. During rapid walking the tripod pattern is not affected even for turning angles of up to 50° (Fig. 10a). Body sides remain rigidly coupled and exact antiphase of ipsisegmental legs is maintained. During slow walking (Fig. 10b) ipsilateral metachronal waves can be widely spaced in time (i.e. the period is getting longer than the duration of the wave) and body sides appear to be only loosely coupled. In the example shown in Fig. 10b metachronal waves are spread in time in such a way that in frames 8 and 9 only one leg (L2) is recovering. Nevertheless, in all cases observed the number of metachronal waves of left and right side was equal over the turn.

Turns achieved by pure rotation. This method of turning is found as a subunit of tight turns only and is used in two different variants. For one, a turn of approximately 50° to 90° often starts as pure rotation. Later rotation fades out and is superposed by an increasing translatory component. For tight turns ranging from approx. 90° to 180° the maneuver above is preceded by rotation with superposed backward translation (see below).

During pure rotation the directions of recovery strokes as well as of propulsion strokes are directed laterally and the turning point is located close to the abdominal tip. The transition between different phases frequently is marked by short breaks.

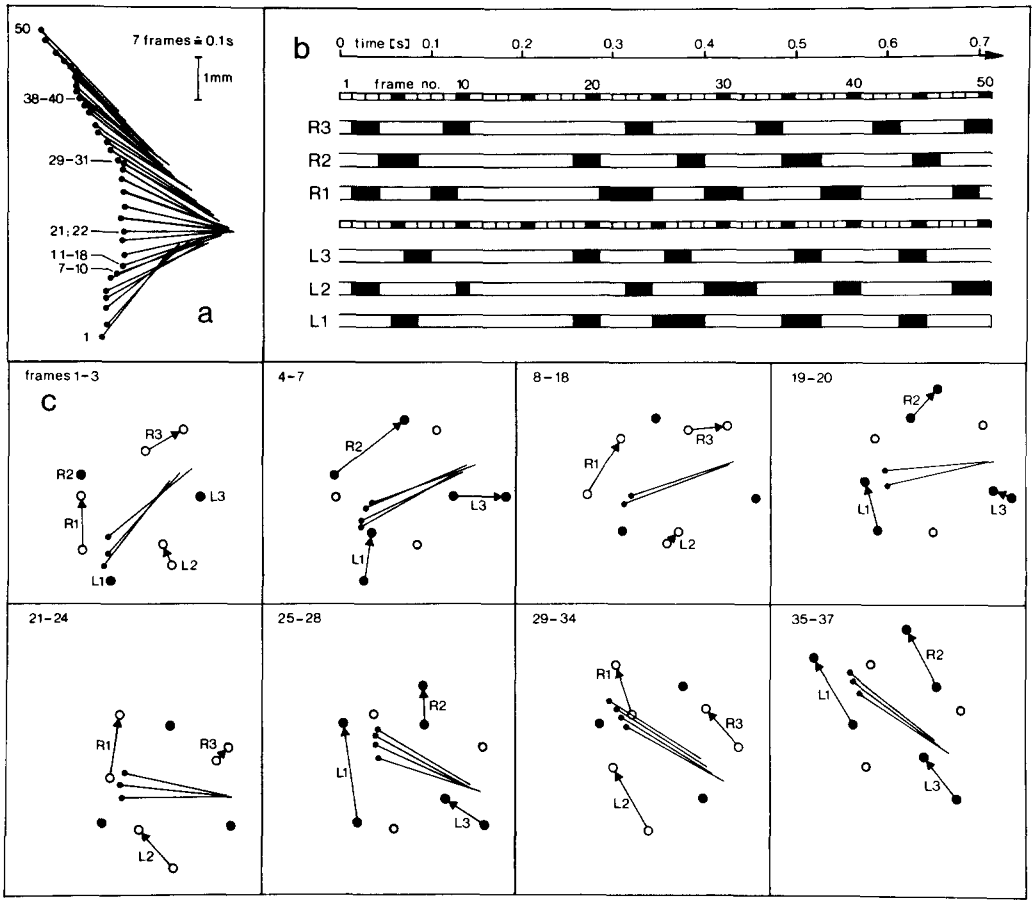

Fig. 11a–c. 100°-turn performed as a start sequence. **a** Movement pattern of the longitudinal body axis shown every 14.3 ms (i.e. 70 frames/s). Head denoted by filled circle, numbers indicate time in units of frames. The turn consists of 3 distinct phases with respect to the translatory component superposed on the rotatory motion: Frames 1–10 (*phase I*): Backward directed translation; frames 11–18: 114 ms break; frames 19–28 (*phase II*): Pure rotation; frames 29–50 (*phase III*): Forward translatory component with fading out rotation. **b** Coordination of legs. Conventions as indicated in the legend of Fig. 1. **c** Direction of strides in successive steps. Same scaling as in **a**. Footprints of legs belonging to one tripod subset are denoted by a common symbol (filled or open circles); frame numbers are indicated in the upper left corner. While 3 legs recover in the direction indicated by arrows, the respective tripod subset in stance phase accomplishes movement as indicated by the longitudinal body axis. As can be seen from **b**, tripods do not always recover synchronously

Turns achieved by reversal of walking direction. Reversal of walking direction is found in tight turns up to 180° as the first of 3 consecutive phases distinct in their translatory components. Propulsion strokes and direction of recovery strokes during this first phase are directed backwards for legs on the inside of the turn and laterally at the outside. Approximately one quarter of the overall turning angle is achieved during this first phase, one half during pure rotation and the remaining quarter in the third phase. The sequence is frequently used in front of obstacles.

In Fig. 11 a 100°-turn out of a resting position is shown with all the features of tight turning described above. Phase I, i.e. backward directed translation, is used from frame 1 to 10. After a break of 114 ms pure rotation can be observed for frames 19–28 (phase II). Beginning with frame no. 29 forward movement is increasing and the rotation fading out (phase III). Note that beginning with frame 29 legs recover in the desired final direction of propagation given approximately by the longitudinal body axis in frame 50.

Step lengths performed by hindlegs during pure rotation are very short (see Fig. 11) and can – within the accuracy of measurement – vanish completely. The smallest displacement in order to detect a swing phase was 0.1 to 0.2 mm. Very rarely also a swing phase of a middle leg can not be detected during pure rotation.

Discussion

Relation of the data to those of other walking insects

Leg coordination during straight walking in *Drosophila* is in close qualitative agreement with that described for the cockroach (Delcomyn 1969, 1971), locust (Burns 1973) and grasshopper (Graham 1978). All show a frequency-dependent deviation from perfect tripod coordination for walks at the lower end of their speed range. Graham (1977) adapted parameters of his model of leg coordination to period-dependent metachronal lag data obtained by the authors cited above. He showed that his model, originally derived for the stick insect, is also capable of describing the coordination of these insects. In the period range relevant for walking the curves can be considered to be power functions with positive exponents smaller than unity. Accordingly we have used a power function to fit the data in Fig. 2.

Linear regression yields a correlation coefficient of $r_{\text{lin}}=0.898$ which insignificantly differs from that obtained by power function regression ($r_{\text{exp}}=0.890$). Thus, the power function can be considered to be an equally good description of the data. Evidence for a nonlinear function is provided by the transient episodes of tetrapod walking if one considers them to represent the long-period end of the same function. Except for the identity of period and lag $_3L_1$ in a period-independent perfect tripod coordination only the nonlinear dependence of $_3L_1$ on period is compatible with the walking model proposed by Graham (1972, 1977). Undisturbed coordination, however, is not a critical test for the models proposed for leg coordination (cited in the Introduction) since all of them are able to describe the frequency-dependence of coordination in undisturbed intact animals. What can be said is that *Drosophila* is within the frame of intact walking described for other insects.

The existence of a second gait in an overlapping range of speeds is still possible even though it can be excluded for the set of data presented in this work. Environmental influences less startling than 'Buridan's paradigm' may elicit changes in walking behavior. Such an example is described for the first instar stick insect, where the rarely used gait II can be elicited by smearing leaf sap on the walking surface (Graham 1985). Accordingly, influences might exist for the fly leading to continuous walks at speeds lower than 1.5 cm/s.

Although in the individual fly the dependence of step length on period is not rigidly fixed, clearly, walks at the upper end of the range of walking speeds are associated with longer strides than are those at the lower end. The same but weaker relation can be assumed to exist for the cockroach *Periplaneta americana* from the dependency of average walking speed on average frequency of leg movement observed by Delcomyn (1971). The slope is slightly increasing with increasing frequency which must be due to larger strides in fast walking. The opposite is observed for the stick insect first instar nymph (Graham 1972), where strides slightly increase with decreasing frequency of stepping.

The variability in step length found for *Drosophila* may express changes in the motivational state of the fly in its attempts to reach the black bars in 'Buridan's paradigm'. There are other parameters suggesting this interpretation. Walking speed in the paradigm is frequently changing after a turn at the water-filled moat. Equally variable is the number of successive runs between the black bars performed without a break. There are no obvious changes in the external conditions such as walking surface, temperature or illumination. It is not likely that changes are due to exhaustion since no trend could be detected.

With respect to the duration of swing phases *Drosophila* resembles the behavior described for the cockroach (Delcomyn 1971). In both species middle legs recover faster than the others. While in the cockroach the slope for the dependence on frequency is found to be equal for all legs, in *Drosophila* the forelegs show a less steep dependence on period than the others. By determining the dependence of swing phase duration on period in homeotic mutants it should be possible to decide whether differences between the leg pairs in *Drosophila* are caused by sole mechanical constraints (during part of the walking cycle middle legs are the only support on one side of the body) or by neuronal differences between the segments.

The asymmetry in the fly's pattern of footprints on left and right body side is correlated with an average misalignment of the longitudinal body axis in relation to the direction of propagation. The finding suggests that in *Drosophila* setting of a hindleg at the end of its swing phase might be carried out relative to the body rather than in relation to the point of ground contact of the ipsilateral middle leg. The opposite is true for the stick insect where Cruse (1979b) showed the dependence of the hindleg setting on the position of the ipsilateral middle leg.

The observed asymmetry in *Drosophila* might allow the determination of possible genetic factors of behavioral handedness by trying to seperate alleles of right or left handed walking. Other examples of asymmetry in walking are described in the literature. For slow walking adult stick insects Graham (1972) found ipsisegmental legs to recover almost always in a phase relation of 0.3 for right on left while the symmetrical alternative of 0.7 (right on left) occurred only rarely.

Obviously it is most important to an organism to be able to terminate locomotion instantaneously when further movement would be dangerous. Hence, one would not expect that a certain point in coordination has to be reached prior to a stop. The finding that legs can be interrupted during recovery (Fig. 9b, (a), (c), (e)) supports the above statement.

Except for some of the interruptions observed which may be caused by stimuli given unintendedly by the experimenter most of the events are spontaneous. This makes it tempting to speculate about a preferred breakpoint in the coordination of swing phases. Most of the interruptions occurred when all legs were in contact to the ground (14 of 18 cases, (b), (d), (f)). In 13 of 18 events the metachronal wave of the right body side is completed while this is true in only 3 cases (f) for the left body side. Even though it is not of statistical significance, it might reveal possible differences in the role of body sides for coordination.

Turning behavior of walking *Drosophila* has already been approached by Götz and Wenking (1973). By means of amputation experiments they showed in tethered walking flies that leg pairs of all segments participate in performing rotational movements. The turning behavior of *Drosophila melanogaster* is closest to that described for the bee *Apis mellifera* L. by Zolotov et al. (1975) since both show a hierarchy of turning methods. In contrast to the bee, however, in the fly the number of metachronal waves is always equal for both sides of the body and for all turning methods described. Pure rotation around the cerci followed by rotation with superposed translation and finally pure translation is also described for the escape behavior of the cockroach (Camhi and Levy 1988).

Relevance of data for an automated search for locomotor mutants

Using an automated system to determine the temporal pattern of swing phases it should be possible to isolate *Drosophila* locomotor mutants. The reliability of the normal coordination in wild-type flies will allow one to detect possible mutants omitting or adding recovery strokes. Within the (ipsilateral) metachronal wave of swing phases the recovery onset of the middle leg is highly conserved at a metachronal phase value around 0.5 even during turns (see Figs. 10, 11). Screening for this parameter will omit the necessity to restrict evaluation of data to sequences of straight walking. The same consideration applies to a screen for equal frequencies (equal number of metachronal waves) on left and right body side allowing the detection of putative disturbances in the cross body coordination. The turning behavior found for slow walking suggests that temporarily also wild-type flies can uncouple body sides.

In contrast to the parameters discussed above it is time consuming to address perturbations in step length. For obvious reasons the evaluation has to be restricted to episodes of straight walking. Additionally, the variability in wild-type flies necessitates a statistical treatment of a larger body of data for every single fly. Nevertheless, the parameter is most interesting since it might, e.g., allow the isolation of mutants defective in the proprioception of joint position. Using high speed motion pictures already two mutant strains have been found with distinct changes in the average dependence of step length on period. A computer-aided video system for a screen for locomotor mutants is presently constructed.

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