RESEARCH ARTICLE

The stimuli evoking the aerial-righting posture of falling pea aphids

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ABSTRACT

Some wingless insects possess aerial righting reflexes, suggesting that adaptation for controlling body orientation while falling through air could have preceded flight. When threatened by natural enemies, wingless pea aphids (*Acyrthosiphon pisum*) may drop off their host plant and assume a stereotypic posture that rotates them in midair to land on their feet. The sensory information triggering aphids to assume this posture has so far been unknown. We subjected aphids to a series of tests, isolating the sensory cues experienced during free-fall. Falling aphids assumed the righting posture and landed upright irrespective of whether the experiments were carried out in the light or in complete darkness. Detachment of the tarsi from the substrate triggered the aphids to assume the posture rapidly, but only for a brief period. Rotation (mainly roll and yaw) of the body in air, in the light, caused aphids to assume the posture and remain in it throughout rotation. In contrast, aphids rotated in the dark did not respond. Acceleration associated with falling or airflow over the body per se did not trigger the posture. However, sensing motion relative to air heightened the aphids' responsiveness to rotation in the light. These results suggest that the righting posture of aphids is triggered by a tarsal reflex, but, once the aphid is airborne, vision and a sense of motion relative to air can augment the response. Hence, aerial righting in a wingless insect could have emerged as a basic tarsal response and developed further to include secondary sensory cues typical of falling.

KEY WORDS: Aerial descent, Flight control, Optic flow, Rotation, Tarsal reflex

INTRODUCTION

Some flightless animals possess the ability to control body orientation while falling through air (Dudley et al., 2007; Dudley and Yanoviak, 2011; Jusufi et al., 2011; Yanoviak et al., 2005; Yanoviak et al., 2010; Yanoviak et al., 2011). Such ability should be particularly adaptive for arboreal creatures, which are more likely to fall down from a high perch, either intentionally or inadvertently (Yanoviak et al., 2005). The ability to control body orientation while falling through air has the adaptive advantages of controlled descent and landing. Therefore, reflexes responsible for aerial righting might have evolved in arboreal insects prior to the development of flight, and could have served as the precursors for flight control mechanisms (Dudley et al., 2007; Dudley and Yanoviak, 2011; Yanoviak et al., 2009).

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While reptiles and cats can use the inertia of counter-rotating limbs to rotate their body in air, smaller insects are more likely to right their body using air resistance on their appendages (Jusufi et al., 2011). The latter can be achieved statically, i.e. the insect assumes an aerodynamically favourable posture and remains in it throughout the fall, allowing air resistance on the appendages to rotate the body and maintain it right-side-up (von Buddenbrock and Friedrich, 1932; Ribak et al., 2013). Such a static righting mechanism eliminates the need for sensory feedback during righting itself and therefore may require only a trigger for activation and termination.

Pea aphids (*Acyrthosiphon pisum*, Harris 1776) (Fig. 1A) are small sap-sucking insects (Hemiptera: Aphididae) that have an alate (winged) morph and an apterous (wingless) morph (Dixon, 1998). The apterous morph may evade predation (Nelson, 2007; Obrycki and Kring, 1998), parasites (Chau and Mackauer, 1997) or incidental ingestion by mammalian herbivores (Gish et al., 2010; Gish et al., 2011) by dropping off their host plant and free-falling towards the ground. While dropping helps to avoid the immediate danger on the plant, it subjects the aphid to new perils on the ground, including ground predation, desiccation and loss of feeding sites (Losey and Denno, 1998; Östman et al., 2003; Roitberg and Myers, 1979). We recently showed that upside-down aphids, dropping from a perch at least 20 cm high, land right-side up >90% of the time (Ribak et al., 2013). To right their body during a fall of 20 cm, aphids must rotate in midair to the upright orientation within less than 0.17 s. This is done by assuming a stereotypic 'righting posture', in which the antennae point forwards and up and the hindlegs extend backwards, sideways and dorsal relative to the body (Fig. 1B). In this righting posture, the body is aerodynamically stable only when the ventral side of the aphid is pointing down. Thus, regardless of the initial orientation of the falling aphid, air resistance rapidly rotates the body in midair to a right-side-up orientation (Ribak et al., 2013). Such aerial righting may enhance the aphids' probability of landing on and clinging onto lower leaves of the host plant and hence avoiding the hazardous ground.

The ability of the pea aphids to right their body during a short fall (<0.17 s) suggests that rapid reflexes may be involved in assuming the righting posture. However, the sensory cues that trigger the response are unknown. Do aphids assume the righting posture in response to loss of contact between their legs and the solid substrate (i.e. a tarsal reflex, *sensu lato*) (see Binns, 1977; Dudley, 2000; Fraenkel, 1932; Pringle, 1938), or do they sense changes in their orientation, acceleration or speed while falling through air? The distinction between the two types of cue is interesting because it reflects differences in adaptation of the sensory system for detecting a fall through air. While the first option may be regarded as a terrestrial response, as it depends on (not) sensing the ground, the second indicates an ability to sense motion and/or orientation through air. The latter is considered to be a basic requirement for controlled flight and gliding (Daniel et al., 2012; Dudley et al., 2007;
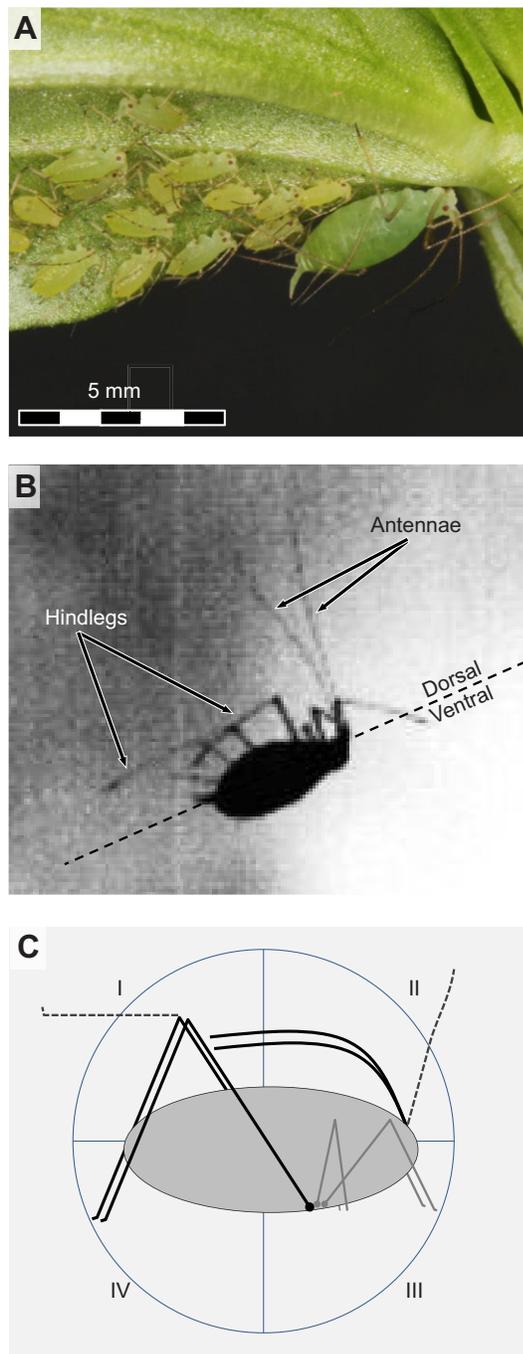
Fig. 1. Falling pea aphids. (A) A picture of pea aphids, *Acyrthosiphon pisum* (adult female and nymphs) on the lower side of a leaf. (B) A picture of an aphid falling right-side up after completing aerial righting. Note the stereotypic position of the hindlegs and the antennae. (C) A schematic drawing showing the criteria used to score whether the aphid is in the righting posture. An aphid was scored as being in the posture when the distal tips of both hindlegs moved from their position at rest (quartile IV) to the righting posture (quartile I). Curved and straight thick, black lines represent the antennae and hindlegs, respectively. Dashed lines show the position of the tibia and antennae in the righting posture.
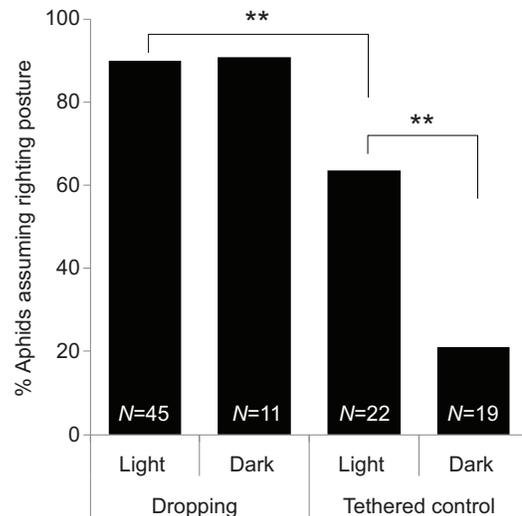

Fig. 2. The proportion of aphids assuming the stereotypic righting posture in the light, in the dark and during tethered drops. For a description of the tethered control, see Materials and methods. The light–dropping column shows data taken from Ribak et al. (Ribak et al., 2013). **Significant difference ($P<0.01$; Z-test for proportions).

Dudley and Yanoviak, 2011; Fraenkel and Pringle, 1938; Taylor and Krapp, 2007; Wigglesworth, 1972).

Wingless pea aphids are secondarily flightless (Hille Ris Lambers, 1974), i.e. wing loss in aphids is derived from a winged lineage. Furthermore, in aphids, flight-capable alate (winged) morphs and apterous (wingless) morphs can be found in the same genetic clone. Therefore, the basic mechanism for flight control exists in at least some members of the colony. Such flight control mechanisms depend on vision, sensing motion relative to air and/or sensing the rotation of the body in air (Daniel et al., 2012). The question that arises is whether the remarkable aerial righting capability of flightless pea aphids is due to an ability to detect body motion through air (i.e. a remnant of flight control reflexes, which persists in alate morphs) or is a more basic reflex associated with terrestrial locomotion.

To explore these questions, we designed a series of controlled experiments in which we distinguished between the various sensory cues (and their combinations) that might be used for detecting a fall and triggering the stereotypic righting posture.

RESULTS
Effect of vision on aerial righting
Some insects rely on vision to detect self-motion relative to the surrounding panorama (Taylor and Krapp, 2007). Therefore, we first tested whether visual input affects aerial righting in aphids. Nearly all aphids that were dropped upside-down landed upright regardless of whether the experiments were carried out in the light or in the dark (90.5% and 95.2%, respectively). Using a high-speed camera and infrared illumination, we were able to confirm that 91% of the aphids falling in the dark indeed assumed the righting posture while in mid-air (Fig. 2). This high proportion is similar to that of aphids assuming the posture when dropped in the light [Fig. 2; from data in Ribak et al. (Ribak et al., 2013)]. However when dropped in the light glued to an insect pin (to evaluate tethering effects, experiment O in Table 1), a smaller percentage of the aphids (64%) were observed in the righting posture (Z-test for proportions – hereafter Z_P-test, $Z_P=2.6$, $P<0.01$). This suggested that the glued pin somewhat restricted the normal ability of the aphids to assume the posture. Consequently, we used the lower percentage (64%) observed in tethered drops as a baseline for comparison to the results in all other experiments (experiments B–N) in which the aphids were tethered to a pin. When the tethered dropping experiment was repeated in the dark (experiment O_{dark}) the percentage of aphids assuming the posture

Table 1. Summary of sensory cues eliciting the righting posture

| Experiment | Tested cues | Light | N | Available sensory cues | | | Acceleration | | Air flow | Percentage of aphids assuming the righting posture | Posing duration (ms) |
				Visual	Tarsal contact	Tarsal detachment	Vertical	Angular			
B	Acceleration	Light	20	+	+	−	+	−	−	0	NA
C	Acceleration without tarsal contact	Visible	20	+	−	−	+	−	−	20	NA
D	Acceleration with tarsal detachment	Visible	15	+	+	+	+	−	−	0	NA
E	Acceleration in air	Visible	20	+	+	−	+	−	+	0	NA
F	Tarsal detachment	Visible	30	+	+	+	−	−	−	60	40±5.2 (18)
F$_{dark}$		IR	19	−	+	+	−	−	−	53	112±33.4 (10)
G	Tarsal detachment with air flow	Visible	23	+	+	+	−	−	+	52	44±9.5 (12)
H	Yaw rotation without air flow or tarsal contact	Visible	21	+	−	−	−	+	−	10	494±101 (7)
I	Yaw rotation in air; without tarsal contact	Visible	21	+	−	−	−	+	+	52	>550^a (15)
I$_{dark}$		IR	21	−	−	−	−	+	+	5	66±8.3 (10)
J	Yaw rotation in air with tarsal detachment	Visible	20	+	+	+	−	+	+	80	>300^a (18)
J$_{dark}$		IR	19	−	+	+	−	+	+	32	77±31.6 (7)
K	Pitch rotation in air; without tarsal contact	Visible	20	+	−	−	−	+	+	11	>600^a (9)
K$_{dark}$		IR	20	−	−	−	−	+	+	10	248±99 (8)
L	Pitch rotation in air with tarsal detachment	Visible	20	+	+	+	−	+	+	75	422±82.6 (17)
L$_{dark}$		IR	20	−	+	+	−	+	+	50	160±53.4 (13)
M	Roll rotation in air; without tarsal contact	Visible	22	+	−	−	−	+	+	55	>600^a (19)
M$_{dark}$		IR	20	−	−	−	−	+	+	5	190±113 (8)
N	Roll rotation in air with tarsal detachment	Visible	18	+	+	+	−	+	+	83	728±105 (15)
N$_{dark}$		IR	18	−	+	+	−	+	+	61	272±110 (11)
O	Tethered dropping	Visible	22	+	−	−	+	+	+	64	NA
O$_{dark}$		IR	19	−	−	−	+	+	+	21	NA
−	Wind (from Gish et al., 2010)	Visible	−	+	+	−	−	−	+	NA	NA

^{a}Posing duration continued beyond the end of the movies.

Each row represents an experiment (letter coded B–O; in experiment A, aphids were simply dropped from forceps onto jelly, see Materials and methods and Fig. 2). Columns denote the sensory cues present in each experiment. N is the sample size. The proportion of aphids assuming the righting posture corresponds to aphids assuming the posture within 170 ms from stimulus onset. Posing duration (mean ± s.e.) corresponds to all aphids (*n*=sample size, in parentheses) that eventually assumed the posture.

(21%) was significantly lower than in the light (Z_P-test, Z_P=2.7, P<0.01, Fig. 2).

Table 1 summarizes the effect of isolated cues (experiments B–O) on assuming the stereotypic righting posture in pea aphids and the average duration that the aphids remained in the posture in the experiments that triggered them to respond. The various experiments can be seen in supplementary material Movie 1.

Falling acceleration

We examined whether the vertical acceleration, typical of a free fall, can trigger the righting posture. To distinguish between the falling acceleration and airflow over the body during a fall, we dropped aphids while they were enclosed in a transparent box (thus preventing a sense of motion relative to air, Fig. 3A). Dropping the aphids together with the substrate they were standing on (either inside the box or on a strip of transparent sheet without the box) did not trigger the righting posture (experiments B and E in Table 1). When the boxed aphids were tethered inside the box such that their legs were deprived of contact with a solid substrate (Fig. 3B), only 20% assumed the righting posture as the box dropped (experiment C in Table 1). This percentage was significantly lower (Z_P-test, Z_P=2.9, P=0.004) than that of aphids dropped tethered to an insect pin (experiment O in Table 1). When the boxed aphids were initially gripping a solid object and then pulled off it as they fell together with the box (i.e. adding a tarsal detachment cue to the experiment),

Fig. 3. Illustration of the stimulation techniques. (A) An upside-down aphid dropped inside a transparent box during vertical acceleration tests (experiment B). The box (76×25×25 mm) was made of glass walls (a) and transparent PVC sheets (b) to seal the sides. Inside the box, a small stage (c) was glued to the centre of the ceiling to assist viewing of the aphid by the camera. The box with the aphid was held above the ground by two metal plates (d) on its top connected to a horizontal electronic magnet. (B) An aphid dropped in the same box but tethered upside-down without tarsal contact. (C) Tarsal detachment experiment. (D) Dropping aphids inside a dark crate by placing them on a slippery test tube (black) and rotating it above the field of view of the camera to drop the aphid (in grey). (E–G) Rotating the aphids using a DC motor about the yaw (E), pitch (F) and roll (G) axes.

none of the aphids responded by assuming the righting posture (experiment D in Table 1).

Tarsal detachment

When aphids were tethered upside-down with a small piece of paper to cling on to with their tarsi (Fig. 3C), more than 50% assumed the righting posture in response to having the piece of paper pulled from their grip (experiment F in Table 1). The results were similar whether the experiment was conducted in the light or in the dark (Z_P-test, Z_P=0.5, P=0.61, Table 1). The proportion of aphids assuming the righting posture also did not differ from that during tethered drops in the light (experiment O) (tarsal detachment in the light: Z_P-test, Z_P=0.3, P=0.79; tarsal detachment in the dark: Z_P=0.7, P=0.46). The mean (±s.e.m.) duration of posing among the aphids that assumed the posture was 40±5.2 and 112±33.4 ms in the light and in the dark, respectively (Table 1). Adding an updraft wind stimulus to this experiment, in the light, gave similar results for the proportion of aphids assuming the posture (compare experiments F and G in Table 1) and did not significantly prolong the mean posing duration (44±9.5 ms, N=12) compared with experiment F (Mann–Whitney U-test, U=107.5, Z=0.021, P=0.983).

Rotation

In contrast to controlled aerial descent, an insect unable to control its body orientation in air may swirl and rotate throughout the fall. To evaluate the effect of rotation through air, aphids were tethered to insect pins and rotated about the major body axes (Fig. 3E–G). When the tethered aphids were placed inside the transparent box (described in experiment B above and Fig. 3B) and the box was rotated about the yaw axis and in the light, the percentage of aphids responding to rotation (10%) was significantly lower than that during tethered drops (Z_P-test, Z_P=3.7, P<0.001; experiment H in Table 1). However, when the tethered aphids were rotated about their yaw axis, without the glass walls (experiment I), 52% of them assumed the righting posture and remained in it for extended durations (Table 1). In contrast, aphids rotated in the same way but in the dark (experiment I_{dark}) did not assume the posture (Table 1). Qualitatively similar results were obtained when the aphids were rotated about the roll axis (experiment M in Table 1; light, 55%; dark, 5%; Z_P-test, Z_P=3.5, P<0.001). When rotated about the pitch axis, the response of the aphids was low in both the light and dark conditions (11% and 10%, respectively; experiment K in Table 1).

When tarsal detachment (as in experiment F) and yaw rotation (as in experiment I) were combined (experiment J), the percentage of aphids assuming the righting posture was significantly higher in the light than in the dark (light, 80%; dark, 32%; Z_P-test, Z_P=3, P=0.002). In the dark, the percentage of aphids assuming the righting posture during yaw rotation plus tarsal detachment did not differ from the percentage observed in the tarsal detachment experiment (i.e. not different from 53% in experiment F_{dark}, Z_P-test, Z_P=1.3, P=0.19). When pitch and roll rotations were combined with tarsal detachment in the dark (experiments L_{dark} and N_{dark}), the results were not statistically different from those for tarsal detachment in the dark without rotation (pitch: Z_P-test, Z_P=0.2, P=0.85; roll: Z_P=0.5, P=0.62).

Latency and posing duration

In some of the experiments, we could measure the latency from stimulus onset to onset of the motor response by the aphids (Fig. 4), and the duration that they remained in the righting posture ('posing', Table 1). For roll, pitch and yaw rotations, the latency period tended to be longer in the absence of a tarsal detachment stimulus (Fig. 4,
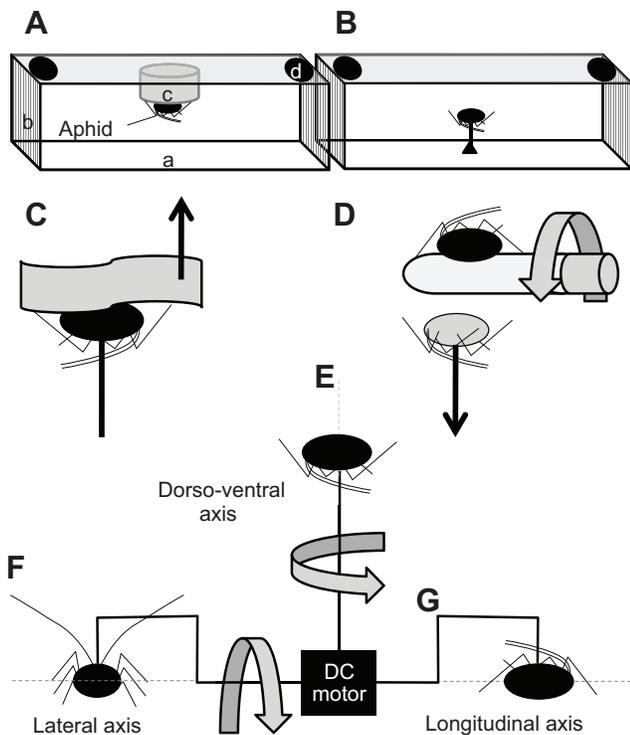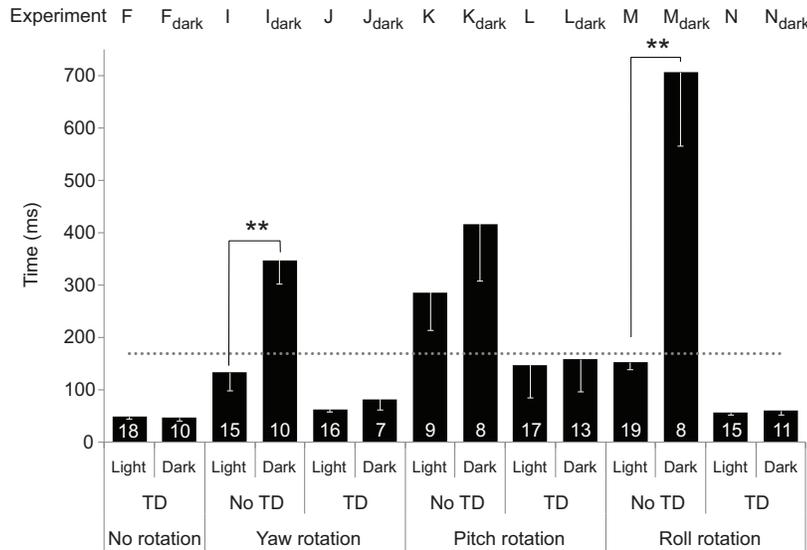
Fig. 4. Mean response time (latency) to triggering the righting posture. The vertical axis denotes the time from onset of the stimuli to onset of the response. The letters above the columns denote the experiment code in Table 1 and in the text. Numbers within the columns denote the sample size of all aphids that eventually responded to the stimulus. Error bars are −1 s.e.m. The dashed horizontal line denotes 170 ms from the onset of the stimulus, i.e. the time by which 90% of free-falling aphids had managed to right their body in air (Ribak et al., 2013). TD, tarsal detachment. **Significant differences between the latency in the light and in the dark ($P<0.01$, Mann–Whitney test).

Table 2). In these cases, darkness seemed to further lengthen the latency period compared with rotation in full light (Fig. 4).

Compared with the posing duration following tarsal detachment (experiments F and F_{dark}), the mean posing duration was much longer when the aphids were rotated about the roll, pitch and yaw axes in the light. In the dark, posing duration during rotation tended to be shorter than in the light (Table 1).

DISCUSSION
While falling aphids can potentially use diverse sensory information to detect a fall accurately, the response needs to be fast in order to allow them sufficient time to rotate before hitting the ground. We found that two unrelated stimuli can trigger the righting posture. First, detachment of the legs from the solid substrate (hereafter

'tarsal response'), caused the aphids to assume the posture quickly, but posing lasted for a short duration only. The second stimulus was rotation of the body (hereafter 'rotation response'). This stimulus resulted in a longer response time (latency) to trigger the aphids to assume the posture but kept them in the righting posture for extended durations. While the tarsal response elicited the posture irrespective of the light/dark conditions, the rotation response was drastically impaired in the dark. It therefore seems that the rotation response is a visually mediated response, possibly associated with the optic flow typical of rotation.

Although vision was found to trigger the righting posture in our experiments, the idea that it plays a substantial role in triggering the aerial righting posture during natural falls is questionable. This is because most aphids also managed to assume the righting posture

Table 2. Statistical significance of differences in posing duration and response latency between a tarsal detachment stimulus and stimuli involving rotations

Variable	Stimulus 1	Stimulus 2	Stimulus 3	Statistics		
				U	Z	P
Posing duration	Light	Tarsal detachment (F)	Yaw (I)	6	4.67	<0.001
			Yaw + tarsal detachment (J)	0	4.97	<0.001
			Pitch (K)	6	3.86	<0.001
			Pitch + tarsal detachment (L)	16	4.53	<0.001
			Roll (M)	6	5.02	<0.001
			Roll + tarsal detachment (N)	1	4.85	<0.001
	Dark	Tarsal detachment (F_{dark})	Yaw (I_{dark})	49.5	0.38	0.971
			Yaw + tarsal detachment (J_{dark})	24	1.07	0.283
			Pitch (K_{dark})	30	0.89	0.374
			Pitch + tarsal detachment (L_{dark})	55	0.62	0.535
			Roll (M_{dark})	39	0.89	0.929
			Roll + tarsal detachment (N_{dark})	37	1.27	0.205
Latency	Light	Tarsal detachment (F)	Yaw (I)	97	1.37	0.169
			Yaw + tarsal detachment (J)	76	2.35	0.019
			Pitch (K)	11	3.6	<0.001
			Pitch + tarsal detachment (L)	121	1.06	0.291
			Roll (M)	17.5	4.67	<0.001
			Roll + tarsal detachment (N)	112	0.83	0.405
	Dark	Tarsal detachment (F_{dark})	Yaw (I_{dark})	0	3.56	<0.001
			Yaw + tarsal detachment (J_{dark})	16.5	1.81	0.07
			Pitch (K_{dark})	0	3.56	<0.001
			Pitch + tarsal detachment (L_{dark})	32.5	2.02	0.044
			Roll (M_{dark})	0	3.56	<0.001
			Roll + tarsal detachment (N_{dark})	35.5	1.38	0.169

In each case, the relevant experiment is given in parentheses.

and right their body while falling in the dark (Fig. 2). The only exceptions to this finding were aphids dropped while tethered to a pin (experiment O), in which darkness significantly decreased the proportion of aphids assuming the righting posture compared to that when falling in the light. In these experiments, the aphids were picked up and released using an insect pin glued to their dorsal side. In such manipulation (which is unlikely to occur during natural falls), the cue of suddenly leaving a solid substrate was absent. Although light does not seem to be a necessary condition for aerial righting, the combination of tarsal detachment and specific visual cues (rotation) triggered both a fast and prolonged falling response compared with rotation in the dark and tarsal detachment in the dark or light (Table 1, Fig. 4). Hence, visual cues may contribute to prolonging the posing duration during a fall.

Our objective in this study was to determine whether the righting posture is evoked by the sensation of leaving the solid substrate or by a sense of motion through air, which would represent a form of adaptation that is closer to flight/gliding control. Interestingly, we found that these two options are not mutually exclusive. It seems that, while dropping in a short fall, aphids make use of the tarsal reflex to trigger the righting posture and no visual cues are needed. However, in the absence of tarsal detachment and with ample light, the response can be elicited and the posing duration prolonged by simply rotating the body through air. It thus seems that the tarsal reflex is responsible for the rapid righting ability of aphids, but that they may also possess additional mechanisms for sensing motion and body orientation relative to air, once airborne. In falling aphids, tarsal detachment occurs first and rotation relative to air comes later. The stereotypic posture rotates the aphid to the right-side-up orientation and then keeps it falling in the correct orientation. Hence, it makes sense for a falling aphid to freeze in the posture for as long as the body rotates.

Tarsal response

The falling response was not activated when the aphids were dropped together with the substrate they were standing on (experiments B and E), suggesting that leg contact has an inhibitory effect on the falling response. Such inhibition is known from tethered flying insects, where removal of tarsal contact resulted in immediate activation of the flight apparatus, whereas restoring tarsal contact terminated flight (Dudley, 2000; Fraenkel, 1932; Pringle, 1938). In winged aphids (*A. fabae*), while removal of tarsal contact only occasionally resulted in immediate flight, tethered aphids terminated their flight when tarsal contact was reapplied (Binns, 1977). We found that in the light, the transient detachment of the tarsi from the substrate caused 60% of the aphids to move their hindlegs to the righting posture. This proportion was similar to the response of aphids free-falling while tethered to a pin.

Aphids falling together with the air surrounding them (inside a box) did not assume the posture following detachment of tarsal contact (experiment D in Table 1). The discrepancy between this result and the tarsal detachment experiments outside the box (experiments F and G) may be explained by the isolation of the boxed aphid from the air surrounding the falling box. Another source of variance comes from the fact that in experiments F and G we pulled the piece of paper from the stationary aphids, whereas in experiment D it was the aphids that were pulled off the stationary substrate. Hence, experiment D included a downwards acceleration of the body (typical of free fall) that did not exist in experiments F and G. As the lack of response from the boxed aphids is in sharp contrast to the natural response of falling aphids, it seems that the tarsal reflex of the aphids in the box was inhibited by a missing second sensory cue that is present during natural falls. Isolating the aphids from the surrounding air did seem to inhibit their response to falling (compare experiments C and D with O in Table 1), while air flow relative to the body elevated their responsiveness to rotation (compare experiments I and H in Table 1). Hence, it seems that some sense of motion relative to air is required in order to allow the aphids to assume the righting posture and remain motionless in it (experiments H–J in Table 1).

Rotation response

Following the observation that the aphids responded to rotation of the body by assuming the righting posture, we verified that the observed movement of the hindlegs into the righting posture during body rotation was not the result of passive motion due to centrifugal force. This is not the case because (1) the legs of aphids rotated in the dark remained ventral and did not move dorsally, (2) the hindlegs of five tethered dead aphids rotated in the same way as the live ones remained ventral during body rotation (supplementary material Movie 1). Hence, the live aphids actively assumed the righting posture in response to sensory cues resulting from the rotation.

During natural dropping, tarsal detachment will always precede rotation in air and, therefore, vision will only affect the righting posture after it has already been evoked. The horizontal dashed line in Fig. 4 denotes 170 ms from the onset of the stimulus. By that time, 90% of the aphids should have righted their body during a fall (Ribak et al., 2013). We found that during such short falls the prolonged posing associated with body rotation is not required. Rather, the shorter tarsal response is sufficient for the aphid to assume the posture and evidently also to right the body, as demonstrated by the aphids dropped in the dark.

The fact that 90% of the falling aphids performed up to half of a full body revolution in less than 0.17 s (Ribak et al., 2013) implies a mean rotation rate of at least 2.9 revolutions s^{-1} (1044 deg s^{-1}) and mean acceleration of 12,456 deg s^{-2}. The maximum torque of our motor resulted in angular accelerations that were 2.3-fold lower, meaning that while rotations in our experiments reached comparable rotation rates to those of falling aphids, a longer time was needed to reach these velocities. Thus, it is possible that visual detection of rotation requires an angular velocity threshold that took some time to develop in our rotation experiments, in which the aphids were rotated by the motor from rest. Hence, the longer latencies observed in the rotational response compared with the tarsal response may be due to the experiment conditions. Alternatively, the difference in the latency could represent physiological differences between the two sensory systems.

Wingless pea aphids are secondarily flightless, with a winged morph also present in the population. Hence, the ability to detect body rotation while flying through air could have preceded the flightless lifestyle. While some flying insects rely on visual cues for deciphering their rotation relative to the surrounding panorama (reviewed by Taylor and Krapp, 2007), others can measure rotation directly with inertial sense organs such as the beating halteres in Diptera (Dickinson, 1999; Fraenkel and Pringle, 1938), the vibrating antennae of moths (Daniel et al., 2012; Sane et al., 2007) and perhaps also sensors on the flapping wings (Daniel et al., 2012). We show here that the response of wingless aphids to body rotation in air depends on the presence of light. This implies that the aphids predominantly respond to the rotational optic flow perceived by the eyes and not to the sense of rotation perceived by some other sense organs measuring body rotation (e.g. the Johnson's organ at the base of the antennae) (see Bromley et al., 1980; Johnson, 1956; Kring, 1972). Indeed, Bromley et al. (Bromley et al., 1980) suggested that

the mechano-proprioception sensory system, which is used to sense motion through air, is less developed in apterous aphids compared with alate. It is interesting to note that the visually mediated rotational response of wingless aphids was much more sensitive for yaw and roll rotations than for pitch. The reason for this is not clear and it would be of interest to determine whether flying aphids also respond differently to rotational optic flows about these axes.

MATERIALS AND METHODS

We used only mature *A. pisum* females taken from a colony reared at the University of Haifa. To observe the rapid response of the aphids, we used a high-speed video camera (Photron SA3, 1000 frames s^{-1}) allowing both slow-motion observation (supplementary material Movie 1) and timing of response at ±1 ms. First, we examined whether vision plays a role in aerial righting by dropping aphids in the light and in the dark (under infrared illumination), and determining the percentage of aphids landing upright (experiment A). We then delivered each one of the remaining cues (and combinations of these cues) in a series of experiments (experiments B–O), in which we scored whether isolated stimuli evoked the aphids to assume the stereotypic righting posture or not. An aphid was scored as 'assuming the righting posture' (Fig. 1B) if both hindlegs were moved from a position in quartile IV to quartile I (Fig. 1C) within 170 ms of the onset of the stimulus. This cut-off value (170 ms) was chosen based on our earlier results (Ribak et al., 2013) reporting that 90% of the aphids managed to right their body in air within the first 0.17 s of falling. In falling aphids, the antennae also participate in the righting posture by moving forwards relative to their typical position at rest (which is backwards along the dorsal side of the body, see Fig. 1A). However, in our experiments the antennae were often pointing forward prior to the onset of the stimulus, possibly due to an alert state associated with tethering. Therefore, we focused only on the hindlegs as an identifier of the stereotypic posture.

When possible (experiments F–N), we measured the latency between delivering the stimulus and the onset of hindleg motion towards the righting posture. The duration of remaining in the posture was also measured as the time interval during which both hindlegs remained motionless in the righting posture.

Dropping in light versus dark conditions (experiment A)

We released aphids using 'bristle forceps' (Ribak et al., 2013), from a height of 20 cm, onto a Petri dish containing a jelly-like solution (a mixture of 95% soybean oil and 5% white petrolatum, by weight). The aphids were released upside down with their longitudinal axis horizontal. The viscous jelly captured the orientation of the aphids at landing, enabling us to determine the proportion of aphids that landed upright. Experiments were carried out once in an illuminated room (1000 lx) and a second time in a dark room under infrared illumination. The light/dark conditions were alternated after every seven dropping trials until a total of 21 aphids had been dropped in each light/dark condition.

To confirm that the aphids falling in the dark indeed assumed the stereotypic righting posture, we used a test tube coated with a slippery substance (INSECT-a-SLIP, Bioquip Inc., CA, USA) mounted horizontally 20 cm above the ground. Aphids were placed individually on the upper surface of the tube and the entire set-up was covered by a large opaque crate to prevent external light from entering. A near-infrared (790 nm) floodlight placed inside the crate provided illumination visible to the high-speed camera, but beyond the visible spectrum of the aphids (Döring and Chittka, 2007). A crank handle, through one wall of the crate, was used to rotate the test tube manually (about the tube's long axis) from the outside, thus delivering aphids to the lower outer face of the tube, where they slipped and fell in the inverted position within the field of view of the camera (Fig. 3D). The high-speed films of the fall were inspected to score for falling in the stereotypic righting posture, or not.

Falling acceleration (experiment B)

To isolate acceleration from the sensation of air flow during a fall, aphids were dropped inside a sealed glass box (Fig. 3A). Inside the box, the aphids were placed on a platform, attached to the ceiling, so that they were upside-

down with respect to gravity. The box was secured from the top, 30 cm above ground, to an electromagnet. A switch disconnecting power to the magnet released the box to free fall, which isolated the aphid inside from sensing motion relative to air during the fall.

Falling acceleration without tarsal contact/with tarsal detachment (experiments C and D)

We examined the effect of falling acceleration in the absence of contact between the tarsi and a solid substrate. Aphids were tethered upside-down by their dorsal side to an insect pin using a minute drop of glue (UHU, GmbH & Co., Baden, Germany) applied to their dorsal tergum. The pin was mounted vertically on the floor of the box described in experiment B, i.e. the aphid was tethered inside the box with its legs pointing upwards and without touching a solid substrate (Fig. 3B, hereafter experiment C). The box with the tethered aphid was dropped as in experiment B. In experiment D, we added the transition typical of detachment of the tarsi from the substrate (to represent the transition from a solid substrate, e.g. a leaf, to falling through air). We rotated the box by 90 deg and inserted a smooth metal rod (diameter 2 mm) through a 4 mm hole in the side (now top) wall of the box until the tip of the rod was within reach of the tethered aphid (see experiment D in supplementary material Movie 1). The aphid was allowed to cling to the rod, which remained in place while the box was released and dropped. Consequently, the aphid was pulled off the rod immediately prior to free falling together with the surrounding box.

Vertical acceleration through air (experiment E)

We examined whether subjecting the aphids to air flow elicited a falling response. As aphids resting on plants do not respond to wind by assuming the righting posture (Ben-Ari and Inbar, 2014; Gish et al., 2010), air flow cues were only tested in combination with other cues. To combine the vertical acceleration and air flow typical of falling, aphids were dropped vertically through air together with the substrate they were standing on – a strip (1.2×4.4 cm) of transparent sheet with a 1.2 g weight glued to the bottom. Holding the strip vertically by its upper end, we then released the sheet with the weight leading the fall, in a straight vertical path.

Tarsal detachment without/with updraft (experiments F and G)

To isolate tarsal detachment from falling acceleration, aphids were tethered upside-down and provided with a small piece of paper to cling on to with their legs (Fig. 3C). The paper was then abruptly pulled upwards using fine forceps (experiment F). In experiment G, the procedure was repeated while exposing the aphids to vertical airflow (2.0 m s^{-1}) from below (towards the aphid's dorsal side) coming from a vertical wind tunnel. The selected air speed (2.0 m s^{-1}) resembled the falling speed that an aphid would reach after falling 20 cm (Ribak et al., 2013). To examine how vision affected the response of aphids in experiment F, we replicated the experiment in the dark (hereafter experiment F$_{dark}$). The same experimental set-up was covered with a thick black curtain and filming was done using near-infrared light as described for experiment A, above.

Yaw rotation (experiments H–J)

To examine whether body rotation evokes the righting posture, aphids were first tethered upside-down inside the sealed transparent box as described above. The entire box was then mounted on a round plate and rotated with a DC motor about an axis aligned with the (vertical) tethering pin (experiment H, see supplementary material Movie 1). In experiment I, the tethered aphids were rotated the same way but outside the glass box, enabling them to sense the rotation relative to stagnant air (Fig. 3E). In experiment J, the procedure of experiment I was replicated but this time providing the aphids with a piece of paper to grip and then pulling away the paper simultaneously with commencement of rotation. In all three experiments the motor was turned on and the aphids started rotating at a mean (±s.e.m.) angular acceleration of 15.03±0.35 revolutions s^{-2} (N=42). The response of the aphid was filmed during the first second of rotation (mean turn rate ±s.e.m., 7.3±0.38 revolutions s^{-1}, N=42). Experiments I and J were replicated in the dark (experiments I$_{dark}$ and J$_{dark}$) in the light-sealed crate described above (experiment A).

Pitch and roll rotations (experiments K–N)

We examined the rotation about the pitch and roll axes as well. In experiment K, aphids were tethered as above but with the pin protruding laterally from the body (Fig. 3F). By bending the pin to 90 deg in three places, to create a crankshaft, we ensured that the rotation axis was aligned with the aphid's centre of mass. When the pin was held horizontal and rotated, the aphids pitched. In experiment L, pitch rotation was combined with tarsal detachment (as described above for yaw rotations). In experiment M the pin was aligned with the longitudinal axis of the body (Fig. 3G) and the aphids rolled. In experiment N, roll and tarsal detachment were combined. Experiments K–N were performed in the light and replicated in the dark under infrared illumination (K_{dark}–N_{dark}).

Tethered dropping (experiment O)

Aphids were dropped tethered to a pin as a control for glue and tethering effects (in experiments C, D and F–N). The aphids were held upside-down in air by the insect pin tethered to their dorsal abdomen and released from a height of 20 cm above the ground. This experiment was repeated once in the light (experiment O) and once in the dark (experiment O_{dark}) to evaluate the response with and without visual input.

Analysis and statistical procedures

In experiments I–K and M (in the light, rotation and tarsal+rotation), the aphids remained in the righting posture for an extended duration, which thus continued after the filming terminated. Therefore, in these experiments we could not measure when 'posing' ended and the values reported (Table 1) thus represent underestimated (minimal) durations.

Statistical differences in the proportions of aphids assuming the righting posture between different experiments were tested using a two-tailed Z-test for proportions with a 95% confidence level. Bonferroni correction was applied to account for multiple (5) comparisons (i.e. α=0.01). Because of a lack of normality, latency and posing durations were compared using a Mann–Whitney U-test.

Competing interests

The authors declare no competing financial interests.

Author contributions

Y.M., G.R., D.W. and M.I. designed the study, analysed the data and wrote the paper. Y.M. and G.R. conducted the experiments.

Supplementary material

Supplementary material available online at
http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.107490/-/DC1

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