

Vision in the nocturnal wandering spider *Leucorchestris arenicola* (Araneae: Sparassidae)

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SUMMARY

At night the Namib Desert spider *Leucorchestris arenicola* performs long-distance homing across its sand dune habitat. By disabling all or pairs of the spiders' eight eyes we found that homing ability was severely reduced when vision was fully abolished. Vision, therefore, seems to play a key role in the nocturnal navigational performances of *L. arenicola*. After excluding two or three pairs of eyes, the spiders were found to be able to navigate successfully using only their lateral eyes or only their anterior median eyes. Measurement of the eyes' visual fields showed that the secondary eyes combined have a near full (panoramic) view of the surroundings. The visual fields of the principal eyes overlap almost completely with those of the anterior lateral eyes. Electroretinogram recordings indicate that each eye type contains a single photopigment with sensitivity peaking at ~525 nm in the posterior and anteriomedian eyes, and at ~540 nm in the anteriolateral eyes. Theoretical calculations of photon catches showed that the eyes are likely to employ a combination of spatial and temporal pooling in order to function at night. Under starlit conditions, the raw spatial and temporal resolution of the eyes is insufficient for detecting any visual information on structures in the landscape, and bright stars would be the only objects visible to the spiders. However, by summation in space and time, the spiders can rescue enough vision to detect coarse landscape structures. We show that *L. arenicola* spiders are likely to be using temporal summation to navigate at night.

Key words: nocturnal navigation, visual field, electroretinogram, spectral sensitivity, temporal summation, spider navigation.

INTRODUCTION

Leucorchestris arenicola Lawrence (Araneae: Sparassidae) spiders are found in the Namib Desert where, like many other desert-living animals, they are strictly nocturnal (Nørgaard et al., 2006a). During daytime the spiders avoid the lethal temperatures on the desert surface by staying in burrows dug into the sand. The burrows are silk-lined tunnels that can be occupied for several months (Henschel, 1990). At night, hunting and searches for mating opportunities are the main reasons for the spiders to leave their burrows. Adult females and immature spiders of both sexes occupy territories of about 3–4 m radial distance around their burrows and predominantly limit their surface activity to this area. Adult males, however, will often venture out on long searches for females and mating opportunities. These searches can take them several tens of metres away from their burrows (Nørgaard et al., 2003). Having left the safety of their burrows, the spiders are then faced with the task of returning to their point of departure (the burrow). Both sexes and all life stages are capable of doing so *via* nearly straight-line trajectories at the end of often circuitous excursions on the desert surface. In adult males, homeward walks of more than 100 m have been recorded (Nørgaard, 2005). Even though the 3–4 m homing distances of females and immature spiders are not as remarkable as the homing distances covered by the adult males, they are still greater than those found for any previously studied spider (e.g. Seyfarth et al., 1982; Görner and Class, 1985; Dacke et al., 1999; Ortega-Escobar and Muñoz-Cuevas, 1999). The problems involved in homing over

distances of 3–4 m (adult females and immature spiders) and several tens of metres (adult males) are essentially the same insofar as neither can be done by pure ideothetic navigation alone (Benhamou et al., 1990). Homing over distances of the order performed by both sexes of *L. arenicola* spiders therefore suggests that external cues are used for minimising the detrimental effect of the inevitable accumulation of errors during (ideothetic) navigation over long distances (Benhamou et al., 1990). Prior studies, mostly done on adult male *L. arenicola* spiders, have excluded several external cues as being necessary for the spiders during homing. The sun, the moon and the polarized light patterns in the sky are not necessary for the spiders to return to their burrows (Nørgaard et al., 2006a). Nor is the direction of gravity, i.e. slope of substrate, or audible cues, i.e. vibration beacons or olfactory stimuli (Nørgaard et al., 2003; Nørgaard et al., 2007; Nørgaard, 2005). The diurnal eusocial hymenopterans, e.g. bees and ants, are among the most impressive and well-studied arthropod navigators, and vision plays a key role in the strategies they employ to avoid accumulating navigational errors (for reviews, see Wehner, 1982; Wehner, 1992; Wehner and Srinivasan, 2003). Although *L. arenicola* spiders are nocturnal and predominantly active during the moonless times of the night (Nørgaard et al., 2006a), a navigational strategy based on vision should not be excluded straightaway. Insects, with their compound eyes, have been shown to be able to navigate under very dim light conditions (Warrant, 2004). Unlike insects, spiders have single-lens camera-type eyes. The comparatively larger lens diameter

of this eye design should allow for photon catches high enough to be useful in low light intensity situations (Nilsson, 1990). Hence, vision could possibly be used by *L. arenicola* spiders even at night when the animals navigate back to their burrows in the dark.

Leucorchestris arenicola is a large spider. Adults can weigh up to 5 g, and their leg span when standing often exceeds 10 cm. Females are generally slightly heavier than males because of their larger opisthosoma, while the males have longer legs. Like most spiders, *L. arenicola* has eight eyes forming an anterior and posterior row on the carapace, with both rows being composed of four eyes (Fig. 1A). The eyes of spiders can be classified into four pairs according to their relative positions on the carapace: the anterior median eyes (AMEs), the anterior lateral eyes (ALEs), the posterior median eyes (PMEs) and the posterior lateral eyes (PLEs; Fig. 1B). The AMEs are referred to as the principal eyes, whereas the other three pairs are termed secondary eyes. The AMEs differ from the other eye pairs in morphology and development (see Blest, 1985). They contain an everse retina with the light-absorbing parts, the rhabdoms, projecting towards the lens, and there is no reflecting tapetum behind the retina. Furthermore, in many spiders the retinae of the AMEs have muscle attachments to control the direction of vision (Foelix, 1996). In the secondary eyes (ALEs, PMEs and PLEs), the retina is inverse and lined by a reflecting tapetum; there is no muscular movement of the retina.

Having eight eyes, the spiders are able to assign and optimise different eyes to different visual tasks, e.g. navigation and prey detection. Such specializations have been shown to occur in several species (e.g. Dacke et al., 2001). In the present account we explored the possibility that vision is involved in the impressive homing abilities of *L. arenicola*, and whether all or only some of the four pairs of eyes are involved in homing navigation. We also investigated how the different eyes might be tuned to their visual tasks.

MATERIALS AND METHODS

Behavioural experiments

The significance of vision in the homing of *L. arenicola* was investigated in the field by occluding selected combinations of pairs of eyes, by covering the eyes with layers of non-toxic black paint (Plaka). The eye position on the spiders' carapace suggested that the lateral eyes (ALEs, PLEs) could provide the spiders with wide visual fields. These four eyes were therefore first chosen as one group of eyes to be occluded ($N=9$). The PMEs with their dorsal and upward-pointing position and the forward-pointing AMEs were treated as another group ($N=9$). In another group of spiders, all eyes were covered ($N=14$). This initial experimental setup was completed by including an untreated group, in which the spiders did not receive

any treatment except for being caught and released ($N=10$). In a sham-operated group, the spiders were provided with dots of paint on the carapace but the eyes were left open ($N=7$). After these experiments had been performed, the function of the median eyes (AMEs and PMEs) and that of the lateral eyes (ALEs and PLEs) were tested separately. In these experiments, either the AMEs ($N=7$) or the PMEs ($N=7$) were left open alone; the same was done with two groups of spiders that could rely on only the ALEs or the PLEs ($N=14$ in both groups).

The field experiments were carried out on the higher flood plains along the northern side of the ephemeral Kuiseb River near the Gobabeb Training and Research Centre in the central Namib Desert ($23^{\circ}33'S$; $15^{\circ}02'E$). This area is only flooded by the Kuiseb River in years with exceptionally high rainfall. It is characterized by open areas interrupted by *Acacia erioloba* and *Faidherbia albida* trees. This area was chosen because of the large population of *L. arenicola* spiders it contained. The spiders were located in the early morning hours by following their night-time tracks in the sand surface that led to their burrows. Then the spiders were dug out. Immediately after capture, the eyes of the spiders were carefully covered with black paint. To ensure that this treatment did render the eyes opaque, the procedure was tested on the exoskeletons from moulted spiders, and light transitioning through the lenses was observed under a microscope. After this procedure, the spiders were released and induced to build a new burrow at the location at which they had been caught. This was done simply by placing the spiders under a translucent cage. Unable to escape and exposed to daylight, the spiders promptly built a new burrow. In nearly all cases, the spiders remained at the location after the cage had been removed. Details of the spiders' activities and movements were monitored by reading the tracks in the sand (Nørgaard et al., 2006b). The furthest measurable distance from the burrow and the number of returns to the burrow made by each spider were recorded. The number of returns made by each spider in the different groups was used as a measure of their ability to navigate homeward.

Optics

In the laboratory the spiders were kept on a 12 h:12 h dark:light cycle at a temperature between 22 and 24°C. The optics of the eyes were studied ophthalmoscopically (Nilsson and Howard, 1989), exploiting the effect of the reflecting tapetum (Fig. 2A). The visual fields of the lateral eyes and the PMEs were measured by means of a goniometer. Since the AMEs of *L. arenicola* have no tapetum, the visual fields of these eyes cannot be obtained in the same way as for the secondary eyes. Instead, their focal length was measured by employing the hanging drop technique (Homann, 1928), and the shape of the retina was determined by histological sectioning. In the focal length measurements, both AME lenses from three dead adult spiders (two males and one female) were used. In each of the six eyes the shape of the visual field was then determined by measuring the angles from the nodal point to the edges of the retina. Video recordings were used to establish how the spiders carried their prosomas while walking, so that the measured visual fields could be plotted onto a sphere, the equator of which coincided with the horizon skyline. In the secondary eyes the receptors appeared as dark dots against the bright background of the tapeta. The inter-receptor angle ($\Delta\phi$) of these eyes was calculated by measuring the linear distance between the receptors and comparing this to a scale with a line of points of known angular separation. F -numbers of the AMEs were calculated by applying the equation $F=f/D$, where f is the focal length and D the lens diameter. The optical geometry and receptor dimensions were determined from semithin



Fig. 1. (A) Frontal view of an adult male *L. arenicola* spider showing the eye position on the carapace. (B) Schematic illustration of the eye arrangement. ALE, anterior lateral eye; AME, anterior median eye; PLE, posterior lateral eye; PME, posterior median eye.

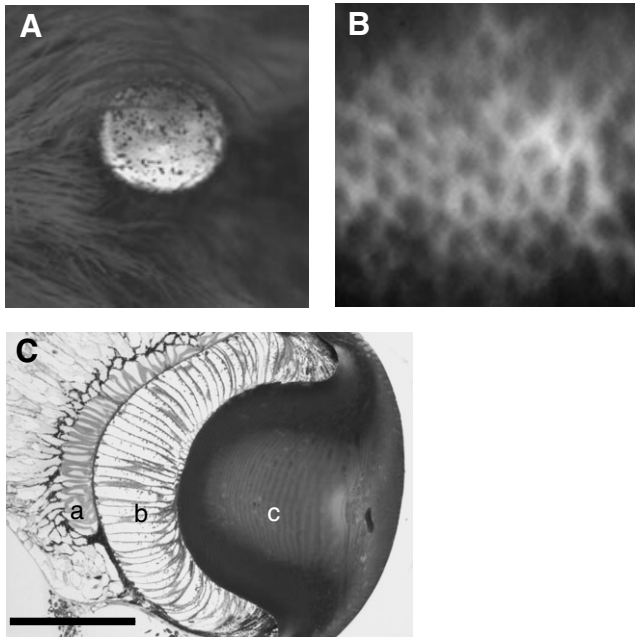


Fig. 2. (A) Left posterior lateral eye of an adult female *L. arenicola* showing tapetal glow. (B) Retinal mosaic of a posterior median eye. The individual receptors appear as black dots. (C) Horizontal and longitudinal section through a female AME; (a) rhabdoms, (b) vitreous body, and (c) lens (scale bar, 200 μm).

(2.5–3.0 μm) sections of eyes fixed in aldehyde (glutaraldehyde and paraformaldehyde) and embedded in plastic (see Nilsson and Ro, 1994). Horizontal and vertical sections were taken from the eyes of female *L. arenicola* spiders (Fig. 2C). The inter-receptor angle ($\Delta\phi$) of the AMEs was calculated using the equation $\Delta\phi = s/f$ (radians), where s is the receptor separation determined using the histological sections and f is the focal length (Land, 1997).

Spectral sensitivity

The spectral sensitivities of the spiders' eyes were determined using standard methods of electroretinogram (ERG) recordings (e.g. Barth et al., 1993). The spiders were fixed in a goniometer, which allowed for precise angular adjustment of the spiders' eyes under the illuminating ophthalmoscope (xenon-arc lamp Osram 75W XBO). $V\text{-log}I$ curves were obtained in dark-adapted eyes by using neutral density filters covering a range of 8 log units in 0.5 log unit intervals. Fourteen interference filters (Oriol) with half-widths of 20 nm were used in 30 nm steps spanning a spectral range of 350 nm to 740 nm. Stimuli were 15 ms rectangular flashes separated by 1 min. The ERG recordings were made by placing a glass microelectrode close to the edge of the corneal lens such that the electrolyte established direct contact with the eye. A thin, sharply pointed silver wire inserted into a leg joint membrane formed the reference electrode. The recorded signal was amplified 1000 times with a DAM 50 amplifier (World Precision Instruments, Sarasota, FL, USA) using a 1 Hz highpass filter, a 300 Hz lowpass filter and a 50 Hz notch filter (World Precision Instruments). All ERG data were transferred directly to a computer using a Data Translation 12 bit USB A/D converter and Scope software version 2.2.0.30 (Data Translation, Marlboro, MA, USA). The spectral sensitivity curves obtained from the ERGs were compared with theoretical curves (Govardovskii et al., 2000). Since the spiders under natural conditions are nocturnal, the specimens used in the ERG experiment were all dark adapted. Four adult spiders (one male and

three females) were used for the ERGs. The procedures for each eye type were repeated twice on each spider, giving a total of eight $V\text{-log}I$ and spectral curves for each type of eye. All spiders survived the treatment.

Temporal summation

While walking, the spiders often interrupt their walking paths and stay motionless for some time. In order to examine whether or not *L. arenicola* employs extended temporal summation as a strategy to allow for reliable night vision, the duration of these stance phases was recorded and correlated with the relative ambient light intensity. The rationale for this experiment was that if the spiders used temporal summation, a negative correlation between stance duration and ambient light intensity should exist: the less light available, the longer the stance phases should be. This question was tested in the field by video recording the spiders as they left their burrows (Sony DCR-TRV60E digital video camera). As males leave their burrows more often than female and immature spiders, only males were used in this experiment ($N=12$). The video camera can record with infrared (IR) light, which is invisible to the spiders, as the only light source. The field of view of the camera covered an area of approximately 2 m \times 2 m. This area was illuminated by the IR light source of the camera and in addition by two IR-LED clusters each containing 15 LEDs (peak wavelength 940 nm). Male spiders had previously been placed at the location of the recording (method described above). The video recordings yielded data about both the duration and the time of occurrence of the stances. As the camera recorded at 30 frames s^{-1} , the duration of the stances was calculated from the number of frames during which the spiders did not move. Stance duration was correlated with the existing relative ambient light intensity measured by means of a Research Radiometer ILT 1700 and a SHD033 detector (International Light Technologies, Peabody, MA, USA). The detector was pointed at a white paper surface at an angle of 45° from a distance of 20 cm. Each measurement was averaged over 1 min using a 2 Hz sampling frequency. All light intensity data were normalised to the highest measured value, i.e. setting the highest measured value to be equal to 100 in order to achieve a measure of relative ambient light intensity. A video recording of one or more stances at a specific ambient light intensity was termed a recording event. For technical reasons the relative ambient light intensity recording had to be made approximately 150 m from the location of the video recording.

Statistical procedures

The Kolmogorov–Smirnov test was used to test for Gaussian distribution of the data and Bartlett's test was used to test for homogeneity of variances. When two variables were tested, Student's unpaired t -test was used. When three variables were tested, the data were analysed by one-way ANOVA followed by the Tukey–Kramer *post-hoc* test. In one instance the data did not pass the Bartlett's test and therefore the non-parametric Kruskal–Wallis test and Dunn's multiple comparisons *post-hoc* test were applied.

Likewise, as one of the variables was found not to belong to a Gaussian distribution (data failed the Kolmogorov–Smirnov test) the non-parametric Spearman rank correlation was used in the test for correlation between relative ambient light intensity and stance duration.

RESULTS

Behavioural experiments

Covering selected eye pairs with black paint had a pronounced effect on the spiders' ability to find their way back to their burrows (Fig. 3). There was no difference between the untreated-group and the sham-

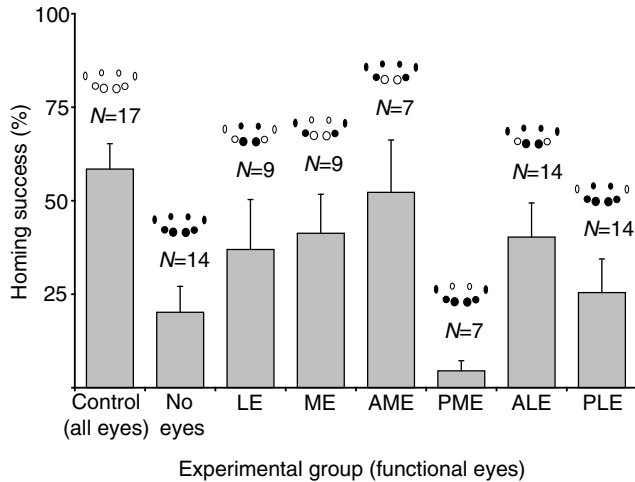


Fig. 3. Histogram illustrating the homing success of spiders with selected pairs of eyes left open in the eye-occlusion experiment. The control group combines untreated and sham-operated animals, i.e. spiders with all eyes functional. The no-eyes group contains spiders with all eyes covered. The LE group had all median eyes covered (AMEs and PMEs disabled), while the ME group had all lateral eyes covered (ALEs and PLEs disabled). The AME group had all eyes but the AMEs covered (ALEs, PMEs and PLEs disabled) and the PME group had the AMEs, ALEs and PLEs covered. The ALE and PLE groups had only the ALEs or PLEs functional, i.e. the AMEs, PMEs and PLEs or the AMEs, PMEs and ALEs were covered. The columns show means + s.e.m. of the number of successful returns made by each spider in the groups (N =number of spiders; normalized data). The schematic illustrations above the columns show which eyes were functional in the experimental group (filled circle indicates covered eye and open circle indicates open eye). All statistical analyses were performed on the absolute values (for details see Results).

operated group (Student's unpaired t -test, $P=0.8890$). Hence, for statistical purposes these two groups were combined and subsequently called the control group. Compared with the control group, covering all eyes (blind spiders) had a strong effect on the spiders. This group of spiders showed a severely reduced ability to return to the burrow after an excursion that took them more than 50 cm away from the burrow (Student's unpaired t -test, $P=0.0053$). None of the adult males ($N=5$) in the group of spiders with no functional eyes was able to return to its burrow. In a few cases ($N=6$), females and immature spiders in this group did manage to return to their burrows after very short excursions with maximal distances to the burrow being less than 50 cm. Irrespective of sex or life stage, excursions further from the burrows resulted in the spiders getting lost and establishing a new burrow elsewhere. Exclusively disabling either the lateral eyes or the median eyes did not significantly affect the spiders' homing ability compared with the control group (one-way ANOVA, $P=0.8797$). A comparison of the behaviour mediated by the upward-directed PMEs and the forward-directed AMEs with the control group showed that when using only their AMEs the spiders could home correctly (Kruskal-Wallis test, $P=0.0034$; Dunn's multiple comparisons test, $P>0.05$), while the PME spiders showed drastically diminished homing abilities (in both cases Dunn's multiple comparisons test, $P<0.05$). In fact, there was no significant difference between the PME and the blind spiders (Student's unpaired t -test, $P=0.3015$). When examining the homing performances of groups of spiders with only the ALEs or the PLEs functional, and including a control group, the results showed significant differences between the groups (one-way ANOVA,

$P=0.0115$). No difference, however, was found between the homing abilities of the ALE spiders (with only the ALEs left open) and the control group (Tukey-Kramer, $P>0.05$). Likewise, there was no difference between the ALE and PLE spiders (Tukey-Kramer, $P>0.05$), although the difference between the means of these two groups appeared to be rather large (Fig. 3). It was only between the PLE spiders and the control group that a significant difference was found (Tukey-Kramer, $P<0.05$).

In summary, the AMEs and the lateral eyes are both involved in nocturnal navigation. There was a slightly better (though not significantly better) homing performance if the spiders could use their forward-pointing AMEs and ALEs. The PMEs, however, are apparently not used for night-time navigation.

Optics

The video recordings showed that the walking spiders tilted their opisthosoma at an angle of 10–15° relative to the horizon. Taking this tilt angle into account, the measured visual fields of the secondary eyes and the computed visual fields of the primary eyes were plotted onto a sphere (Fig. 4). The visual fields of the ALEs and PLEs turned out to be similar in shape, both being horizontally elongated and, though overlapping slightly, providing the spiders with an extended view of the surroundings along the horizon. Only to the rear was there a gap of 40–50° in their combined field of view. The visual fields of the PMEs covered the remaining upper part of the hemisphere with little or no overlap with the lateral eyes. Thus, as the animal is always very close to the ground, the three secondary eye pairs provide the spider with an almost complete view of its surroundings. The visual fields of the AMEs were nearly circular and overlapped with each other (at least when both retinæ were pointing forward) and considerably with the ALEs. By observing the spiders under the microscope it became obvious that the AMEs are provided with muscles allowing for retina movements. The AME visual fields were plotted onto the sphere in an approximately forward-looking direction (Fig. 4). However, movements of the retina could considerably extend the effective visual fields of the AMEs.

The optical quality of the secondary eyes was sufficient to be able to clearly resolve the receptor mosaic when viewed through the ophthalmoscope. The fixed focus of the secondary eyes was consistently emmetropic (focused at infinity). Since the AMEs do not have a reflective tapetum, the optical quality of their lenses was evaluated by removing the lenses from spiders that had died recently, and subsequently the images formed by the lenses were examined under a microscope. The lenses of these eyes also proved to form good quality images at a distance corresponding to the position of the retina. The focal length of the AMEs amounted to $337\pm 2\ \mu\text{m}$ (mean \pm s.e.m., $N=6$) resulting in an inter-receptor angle of $\Delta\phi=2.5^\circ$ (see Materials and methods). The absolute sizes of the lenses varied, of course, with body size but the largest lens diameters were always found in the AMEs (Table 1). There were no apparent differences in eye positioning or lens size between male and female *L. arenicola*.

The retinæ of all pairs of secondary eyes proved to be of the simple type typical of sparassid spiders (Fig. 2B) (Land, 1985; Blest, 1985) with ophthalmoscopically determined $\Delta\phi$ values of $2.15\pm 0.03^\circ$ (ALEs), $2.10\pm 0.03^\circ$ (PLEs) and $3.34\pm 0.06^\circ$ (PMEs; mean \pm s.e.m., $N=40$ for all eye pairs). There was no indication that the tapetæ of the secondary eyes had polarization reflective properties as is the case in another wandering spider, *Drassodes cupreus* Blackwall (Dacke et al., 1999). The rhabdoms were generally shorter in the secondary eyes than in the AMEs (Table 1).

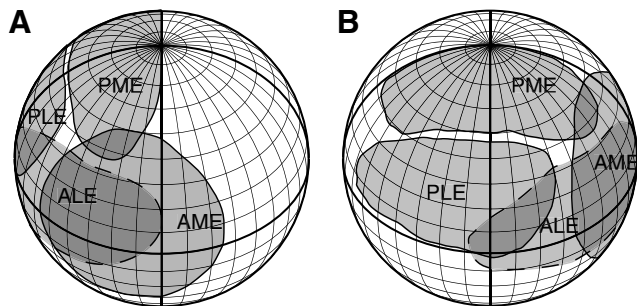


Fig. 4. (A) Frontal view of the visual fields of the four eyes on the right side of a *L. arenicola* spider. (B) Lateral view of the right-side visual fields. The equator defines the horizontal plane with the spider exhibiting its typical body posture. The dashed lines mark the boundaries of the ALE visual fields (averages from five spiders, goniometric measurements). The visual fields of the primary eyes are based on histological measurements of the shape of the retina (averages from two spiders).

ERG recording

All recorded ERGs from the eyes of *L. arenicola* revealed cornea negative potential differences similar to ERGs recorded from the nocturnal ctenid *Cupiennius salei* Keyserling (Barth et al., 1993). The spectral response curves of all eyes showed a single peak indicative of only one photopigment in the photoreceptors of each eye (Fig. 5). In the AMEs, PMEs and PLEs, these measured peaks were located at 500 nm, whereas the measured peak in the ALEs was at 530 nm. However, theoretical spectral sensitivity curves (Govardovskii et al., 2000) showed the best fit for peak values of 541 nm (ALE, $R^2=0.920$), 525 nm (AME, $R^2=0.915$), 529 nm (PLE, $R^2=0.922$) and 523 nm (PME, $R^2=0.901$), using the least sum of the square method (Fig. 5). The eyes of *L. arenicola* are thus sensitive in the green area of the spectrum, with the ALEs having their spectral sensitivity shifted to slightly longer wavelengths than the others. The minimum temporal half-widths of impulse responses in the ERGs were roughly 50 ms in all eyes (Table 1).

Temporal summation

During a 2 month experimental period, 62 stance durations from 12 spiders were recorded on 22 separate nights. Often, more than one stance occurred and was recorded during a recording event, i.e. at a certain ambient light intensity. The number of stances observed during each such recording event was 2.28 ± 0.36 (mean \pm s.e.m.). At times, the spiders were recorded both when they were leaving the burrow and when they were returning to it. In these cases the ambient light intensities during the leaving and returning phases always

differed and hence were subsequently treated as separate events. The resulting 62 data points scattered substantially. However, after disregarding five extreme outliers with stance durations between 1.78 s and 3.37 s, a correlation analysis of the remaining data points (57 stances) revealed a weak but significant negative correlation between stance duration and ambient light intensity (Spearman $r=-0.3148$, $P=0.0171$; 95% confidence interval, -0.5374 to -0.05113 ; Fig. 6). Hence, *L. arenicola* when stationary does collect light for durations of up to 1 s and thus can employ temporal summation as a means of improving night-time vision.

DISCUSSION

While adult *L. arenicola* males concentrate almost exclusively on finding mating opportunities, immature spiders and adult females are classical sit-and-wait predators, hunting by darting out of their burrows only when a suitable prey is detected by vibrations carried through the sand (Henschel, 1994). Although the homing distances can differ by orders of magnitude, both sexes and all life stages of *L. arenicola* must essentially solve the same navigational task of finding the way back to the safety of the burrow after each above-ground excursion. In the present account we report results from both sexes and all life stages of *L. arenicola*. In none of our experiments did we observe noteworthy differences between sexes and life stages.

In the majority of nocturnal spiders, vision is believed to play a lesser role, or no role at all, in mediating behavioural responses (Foelix, 1996). However, our findings presented here show that *L. arenicola* requires vision in its nocturnal behaviour. It is active even at the darkest times of night (Nørgaard et al., 2006a) and yet appears to rely heavily on vision when returning to its burrow. All eyes except the PMEs were found to be used for this purpose, and by covering them all we were able to disrupt the spiders' homing abilities almost completely. On the other hand, the spiders were occasionally found to be capable of navigating successfully without vision over distances shorter than 0.5 m. This ability is most likely to be the result of the spiders being able to rely completely on ideothetic path integration when returning to the burrow over such short distances. Such non-visual returns from short distances were never found in adult males with all eyes covered. This is probably due to the fact that adult males most often walk much further than 0.5 m away from their burrows. Ideothetic homing over distances exceeding 0.5 m does not appear to be possible, and finding the burrow entrance by random searches is highly unlikely, especially for males returning from up to 100 m away.

The optical measurements of the visual fields of the spiders' eyes show that all eight eyes together provide a nearly full view of the spider's visual surroundings (Fig. 4). The horizontally elongated shapes of the visual fields of the lateral eyes provide these eyes

Table 1. Quantum catch, Q , in the four eye types under starlight conditions (per receptor and integration time)

	D (μm)	$\Delta\phi=\Delta\rho$ (degrees)	l (μm)	Δt (ms)	Q	Q_{pool}	C_{pool} (%)
AMEs	470 ± 2.3 ($N=8$)	2.5*	82 ± 0.8 ($N=8$)	56 ± 5.5 ($N=12$)	1.1 (0.5–1.9)	102 (45–173)	10 (8–15)
ALEs	330 ± 2.1 ($N=9$)	2.15 ± 0.03 ($N=40$)	48 ± 0.4 ($N=9$)	63 ± 3.3 ($N=12$)	0.5 (0.3–1.0)	47 (23–90)	15 (11–21)
PMEs	280 ± 1.3 ($N=8$)	3.34 ± 0.06 ($N=40$)	52 ± 0.2 ($N=8$)	48 ± 2.2 ($N=16$)	0.7 (0.4–1.2)	67 (33–108)	12 (10–18)
PLEs	430 ± 2.1 ($N=6$)	2.10 ± 0.03 ($N=40$)	47 ± 0.2 ($N=6$)	56 ± 2.6 ($N=12$)	0.7 (0.5–1.1)	67 (41–79)	12 (10–16)

Calculations (see Appendix 1) are based on the tabulated optical values: D , lens diameter; $\Delta\rho$, acceptance angle; l , rhabdom length; Δt , integration time; other values are given in Appendix 1 (means \pm s.e.m.). Integration time was taken as the half-width of electrophysiologically measured impulse responses. For ALEs (anterior lateral eyes), PMEs (posterior median eyes) and PLEs (posterior lateral eyes), which all have reflecting tapeta, the morphological rhabdom length l was doubled in the calculation. (AMEs, anterior median eyes.) Quantum catch for spatiotemporal pooling, Q_{pool} , assumes groups of $3 \times 3 \times 3$ rhabdoms in a hexagonal array, and a 10-fold increase in integration time. The minimum detectable contrast C_{pool} is calculated as the signal difference that can just overcome quantum noise (square-root of Q_{pool}). For Q and C values the ranges in parentheses are calculated from the measured minimum and maximum values of D , and the upper and lower bounds of standard deviation of $\Delta\phi$, l and Δt . *Calculated from anatomical data (see Materials and methods).

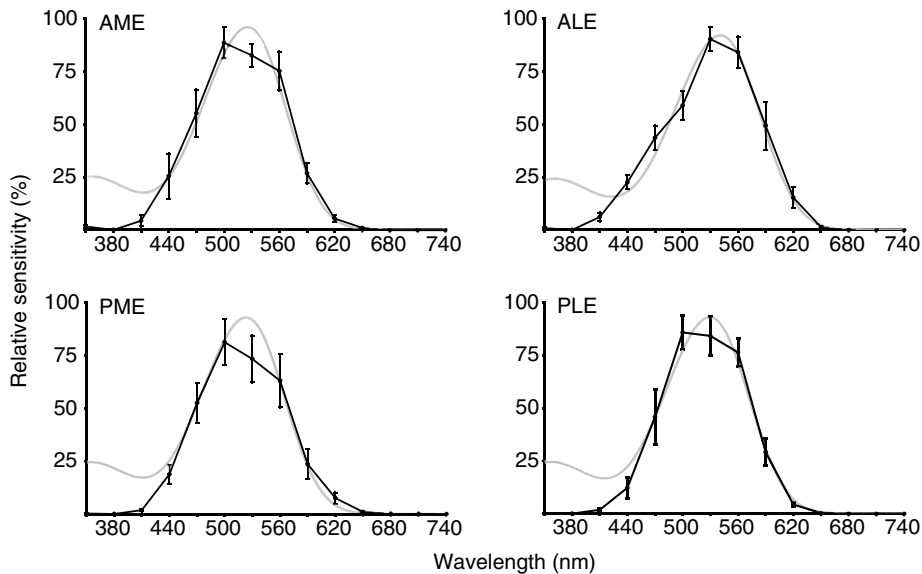


Fig. 5. Spectral sensitivity curves of the four eye pairs. The black lines depict the normalised measured values (means \pm s.e.m.). For the AMEs, PMEs and PLEs the peak value is 500 nm. In the ALEs the peak lies at 530 nm. The grey lines show theoretical spectral sensitivity curves for single photopigments fitted to the measured values using the least sum of squares method. The peaks of the theoretical curves lie at 525 nm (AME), 541 nm (ALE), 523 nm (PME) and 529 nm (PLE). The measurements were done on four adult spiders (one male and three females).

with a broad view of the horizon. Hence the lateral eyes might be involved in detecting features of the horizon skyline. The PMEs are apparently dedicated to covering the upper part of the visual hemisphere.

The size of the lenses of the eyes should allow for high photon catches. They are very probably an adaptation to the dim light conditions prevailing in the desert at the times of night when the spiders are active (Nørgaard et al., 2006a). The raw spatial resolution of the secondary eyes is sufficient for detecting the few apparent landmarks, e.g. grass hummocks, present in the spiders' habitat. Stars or star constellations near the horizon where they can be perceived by the AMEs, ALEs and PLEs could also potentially be used by the spiders to obtain compass information. In some spiders (*Agelena labyrinthica* Clerck and *Lycosa tarantula* Linnaeus), the AMEs have been found to detect polarized light, possibly for navigational purposes (Görner and Class, 1985; Ortega-Escobar and Muñoz-Cuevas, 1999). However, *L. arenicola* is fully capable of navigating home on moonless nights well after astronomical twilight when skylight polarization has entirely gone (Nørgaard et al., 2006a). The poor alignment of the rhabdomeres within the retinae of the secondary eyes is in accord with the fact that polarization cues are not available to *L. arenicola* during the times of night when the spiders are most active. The PMEs with their coarse spatial resolution and their dorsally orientated visual fields could be involved in predator detection.

The spectral sensitivity curves obtained from the ERG recordings indicate that all eyes are colour-blind. When fitting the data points to a theoretical opsin curve the results will always improve if the theoretical curve is the sum of several curves. Using a single opsin curve provided good correlations ($R^2=0.90-0.92$), though, which is why we conclude that all the eyes probably contain a single opsin only. The short receptors would not be expected to generate any broadening of the spectral sensitivities by self-screening, and the recorded functions should therefore be close to the spectral absorbance of the photopigments themselves (Warrant and Nilsson, 1998). The measured ERG responses of the AMEs, PLEs and PMEs show the best fit to opsins with peak sensitivity close to 525 nm, while the ALEs have the best fit to an opsin peaking at about 540 nm. The difference between the measured ERG values below 410 nm and the expected theoretical values is likely to be an effect of UV absorption by the lenses. It is interesting to note that the ALEs appear to have

a different photopigment peaking at 540 nm, although the difference in peak sensitivity is not large. The differences in the spectral sensitivities between the AMEs and the ALEs and their overlapping visual fields further strengthen the previous notion that these two pairs of eyes are involved in different visual tasks. Our results suggesting a single photoreceptor type in each retina are in contrast with the results from the eyes of jumping spiders (Blest et al., 1981), where two receptor types, a UV receptor and a blue receptor, are generally found. We do not have any data directly explaining this difference, but having only a single receptor type will in general increase the overall sensitivity, which may be highly beneficial to *L. arenicola* when operating at extremely low light intensities.

Even though all the eyes are of the camera type, and are larger than in most other spiders, lenses of less than half a millimetre will not catch much light under starlight conditions when the animals exhibit maximum above-ground activity (Nørgaard et al., 2006a). To elaborate on this problem, we used our measured values of lens diameter, retinal sampling, receptor length and integration time to calculate the photon catch per receptor and integration time in the different eyes under starlight luminance (Table 1, Appendix 1). The results show that in all eyes individual receptors detect roughly one photon per integration time. Because the statistical uncertainty (standard deviation) of photon arrival is the square root of the mean, counts of one photon do not provide any usable information at all. Using the highest possible temporal and spatial resolution allowed by the eyes, the spiders would therefore be completely blind to landscape structures in the starlit Namibian desert. Since the behavioural experiments showed that vision is necessary for successful homing during night excursions, the spiders must use spatial and/or temporal summation to overcome photon noise. A workable noise level is obtained only after each image channel has received an average of 50–100 photons in each count. Realistically, such summation cannot be achieved by spatial pooling alone, because this would almost entirely abolish spatial resolution. The same argument holds for using pure temporal summation, because this would extend the integration time beyond any known example and would render the animals completely blind even when walking slowly. Hence, a combination of temporal and spatial pooling would clearly be the best strategy, because it compromises neither spatial nor temporal resolution beyond realistic limits (Warrant, 1999; Warrant, 2004). If we assume spatial pools of seven receptors

(3×3×3 within a hexagonal array) and a 10-fold increase of integration time, the pooled visual channels would cover an acceptance angle of 6–10°, would allow for a shutter speed of about 0.5 s, and would be able to detect contrast levels as low as 10–20%. This would leave the animals with coarse but functional vision at starlight intensities.

If the spiders stop and collect visual information at regular intervals during night-time excursions, they could push the balance between spatial and temporal pooling towards more acute spatial vision. Indeed, when observing the spiders at night during their excursions, one immediately notices their habit of pausing for a while between walking short distances. Less than 2 m seems to be the usual distance travelled between two pauses (Nørgaard et al., 2003). Standing still between short intervals of walking would enable the spiders to engage in temporal summation and thus avoid too heavy a loss in spatial resolution. Indeed, when correlating the duration of the stances that the spiders make during their journeys with the relative ambient light intensity, it is likely that temporal summation is the method used to allow them to have night vision. At the lowest light intensities the measured stance durations approached 1 s. Such extended collection of light would allow the spiders even better spatial resolution than predicted by our theoretical calculations, albeit preventing the spiders from seeing moving objects. The rather high variance in the times the spiders spent stationary (Fig. 6) could result from the possibility that the spiders collect not only visual information when standing still. *Leucorchestris arenicola* relies heavily on substrate-borne information during prey capture, and is likely to do so for predator detection as well. Since detection of substrate vibrations is hindered when walking, the spiders are probably not only seeing but also 'listening' when standing still. An additional and probably the original reason for pausing is, of course, to catch breath: respiration in spiders is typically insufficient to sustain continuous fast locomotion (Foelix, 1996). Recording the pausing behaviour under laboratory conditions was not possible, and in field experiments many factors, e.g. the presence of other animals, cannot be controlled. If the spiders are indeed looking for landmarks, i.e. stationary objects, the loss of temporal resolution would not be too severe a handicap. Landscape snapshots at regular intervals could provide the spiders with the information necessary for fixing positions during their night-time excursions. Bright stars or star constellations could in this way also function as a compass

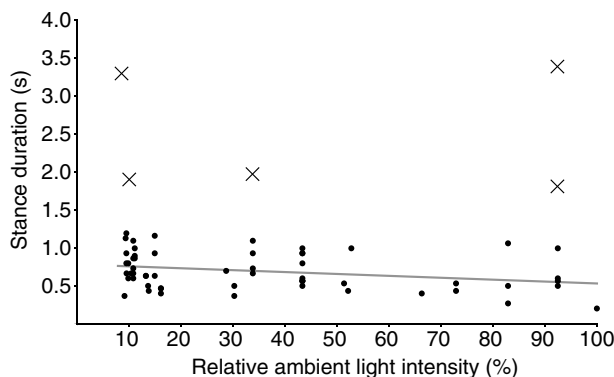


Fig. 6. Scatter plot showing the distribution of the 57 stance durations (black dots) as a function of relative ambient light intensity (for details see Materials and methods). The crosses show the five outliers excluded from the statistical calculation. The trend line shows the relationship between stance duration and relative ambient light intensity ($y = -0.0025x + 0.7779$, Spearman $r = -0.3148$).

guide for the spiders. However, this would imply that the spiders are able to compensate for the movements of the stars across the sky during excursions. The notion that the spiders rely heavily on temporal summation is further supported by the fact that they mostly, and when running always, use only six legs. During motion the front pair is pointing forward, possibly as mechanical 'antennae' making up for the diminished visual abilities.

Our data on eye design and performance in *L. arenicola* do not point towards any obvious division of labour between the different types of eye. The overlapping visual fields of the AMEs and ALEs, however, could suggest a division of visual tasks between these two pairs of eyes. The larger lenses of the AMEs allow for a slightly better resolution and/or contrast sensitivity than is the case with any of the secondary eyes, but the difference is marginal. The lack of a tapetum in the AMEs is largely compensated for by the longer rhabdoms in these eyes. Using the absorption equation for white light (Warrant and Nilsson, 1998) and assuming an absorption coefficient of 0.0067 per micrometre, the 82 μm rhabdoms of the AMEs absorb 19% of the incident light and the 48–63 μm rhabdoms of the secondary eyes (effectively doubled in length by the tapetum) absorb 22–27% of the incident light. These small differences in the photon capture efficiency seem only to diminish the effect of the slightly larger lenses in the AMEs. If any division of visual tasks between these eyes exists, this could perhaps be found in temporal resolution properties.

As far as the orientation of the visual fields of the various types of eye are concerned, the AMEs covering the forward field of view would be best suited to avoiding obstacles and guiding the final approach to the entrance of the burrow. The lateral eyes (ALEs and PLEs taken together) cover an almost circumhorizontal field of view and hence would be well suited to taking skyline snapshots. Their poor photon catch will effectively prevent them from acting as self-motion detectors during walking. The PMEs, even though mainly monitoring the sky, are apparently not necessary for night-time navigation. Being the smallest eyes, they are possibly involved only in warning of threats approaching from above, if the spider should be forced to leave its burrow during the day.

In conclusion, the present study has clearly shown that despite the nocturnal lifestyle of *L. arenicola*, vision must play an important role in the spiders' long-distance night-time navigational performances, but the particular way in which the spiders employ vision during homing remains to be resolved. Extended temporal summation appears likely to be the strategy that allows the spiders to have sufficiently high visual acuity during night-time hours. Hence, at this juncture, ideothetic path integration complemented by the use of visual landmark cues is the spiders' most likely mode of navigation.

APPENDIX 1

The quantum catch, Q , of single photoreceptors per integration time was calculated by multiplying the sensitivity equation (Land, 1981) with the quantum efficiency, integration time and ambient luminance (see Land and Nilsson, 2002; Warrant and Nilsson, 1998):

$$Q = \left(\frac{\pi}{4} \right)^2 D^2 (\Delta\rho)^2 \left(\frac{kl}{2.3 + kl} \right) q \Delta t \times I,$$

where D is the lens diameter, $\Delta\rho$ the acceptance angle of the receptor (in radians), k the absorption coefficient of the rhabdom, l the rhabdom length, q the quantum efficiency, Δt the integration time and I the ambient luminance. The value for k was taken as

0.0067 (see Warrant and Nilsson, 1998) and q was taken to be 0.4, based on an assumed transduction efficiency of 0.5 and transmission through the eye's optics of 0.8. The ambient luminance, Q , was taken for a starlit night: 10^{12} quanta m^{-2} sr^{-1} s^{-1} (see Land and Nilsson, 2002). The remaining values are given in Table 1.

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