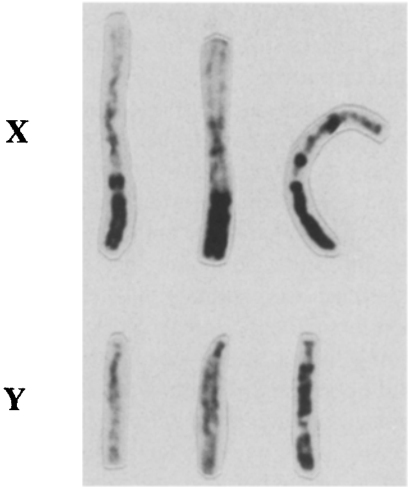

Figure 2. Replication mode of the Y chromosome of *M. cabrerae*. Sex chromosomes in the same column come from the same cell.

some does not start until the euchromatic portion of the X chromosome has replicated (fig. 2). Using 5-azacytidine treatment it has been shown that the euchromatic and heterochromatic portions of the X chromosome of *M. agrestis* may be influenced by two independent regulatory sites that control the timing of replication[11]. Our results suggest that replication of the Y chromosome may be controlled by a regulatory site functionally similar to that which may control replication of the heterochromatic portion of the LRX in XX individuals, as the Y chromosome starts its replication later than the X, but completes replication sooner.

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Microclimatic origin of inhaled air affects olfactory navigation of homing pigeons

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Abstract. Earlier experiments led to the conclusion that homing pigeons are able to deduce positional information from atmospheric odours they perceive at the site of release. Here we show that this ability is significantly reduced, if the inhaled air does not originate from the free airspace in an open landscape but from a close-to-the-ground level of a forest or a maize field.
Key words. Orientation; navigation; homing; pigeons; olfaction; microclimate; atmospheric trace compounds.

Many experimental findings strongly suggest that the homing of pigeons from unfamiliar areas is substantially based on positional information deduced from the local atmosphere by olfaction[1,2]. However, nothing is known on the chemical nature of the trace gases apparently involved and on their distribution in the airspace. A small analytical step to approach this problem is to ask whether every volume of air at a given location contains the same quality and amount of navigational information irrespective of its immediate environment. Does it make a difference whether the birds inhale and smell ambient air at the ground or at a higher altitude, in a closed forest or over open grassland, inside or outside a building, etc.?

Here we report on experiments that aimed to ask for possible differences in initial orientation as depending on the pigeons' preceding exposure to air from inside a forest versus air from open land, and on exposure to air from the ground of a maize field versus air from above open land.

Materials and methods

The forest experiments. Pigeons were transported from the loft to the release area on the top of a van in two airtight wooden boxes ventilated with air sucked through a charcoal filter as described earlier[3,4]. One of the boxes – containing the FOREST birds – was deposited (approx. 30 cm above the ground) in a fairly dense forest 2–5 km from the future release site. Details of the environment were different in the ten release areas used, but generally the sites tended to be in a more or less distinct valley, were always in an extended area of high trees (predominantly spruce, at some sites also deciduous trees) and often in the midst of more or less dense undergrowth. The other box – containing the OPEN pigeons – was subsequently transported to a site in the open field, usually on somewhat higher ground, which was either identical with the release site or up to 2 km from it. Here the van remained with the container on its top. Also this box was shadowed and visually shielded from the sky. At both sites the charcoal filters were simultaneously replaced by pervious paper filters or short curved tubes allowing free airflow. Ventilation with natural ambient air, at a flow rate of 200–300 l/min, lasted for 3 hours. Thereafter, again simultaneously on each container, the filters were re-installed. The van with the OPEN box went back to the forest site, picked up there the FOREST box, and all the pigeons together were brought to the release site, where the containers were unloaded and positioned for release, which started 30–60 min after the period of exposure to unfiltered air. The experiments were conducted from 18 July to 20 October in 1985–1989.

The maize-field experiments. The pigeons were transported in two filter boxes as in the previous series, however immediately to the release site. This was at the edge of a large field of maize (*Zea mays*) which had grown up at that time to a height of approx. 2–3 m, usually in green condition. Upon arrival the containers were unloaded and the charcoal filters replaced by flexible aluminium tubing (diameter 5 cm). The tube installed on the MAIZE box was laid over a length of 20–30 m on the ground of the maize field were it ended with a free opening, mostly more than 20 m from the edges of the field. The tube of the OPEN box was bent upwards and ended at a height of 2–4 m in the free air. Airflow was held fairly equal in the two boxes; between the experiments it varied in the range of 150–300 l/min. It continued for 1 or 2 h before, and was maintained during, the hours when the pigeons were individually released. Thus, in contrast to the forest experiments, each pigeon was taken for release immediately from the experimental condition with the birds exposed to different kinds of natural air. The experiments were conducted from 29 August to 14 October in 1986–1991.

General procedures. For release, the pigeons were individually taken from the containers, and immediately afterwards one puff of anaesthetizing Xylocain spray was given into each nostril[3,4]. After a rest of at least 5 min in an open crate, the birds were tossed into the air and then followed up with binoculars (10 × 40). The bearings observed at the moment of vanishing are the data used for analysis. Pigeons from the two treatment groups were released in alternation.

With two exceptions, as mentioned below, the pigeons were housed in a loft at Andechs, approx. 35 km SW of Munich. Their experience varied from none to six, in a few cases more, preceding homing flights. As a rule, all the participants of one release had made also the preceding releases in common; if not, birds with identical or similar experience were equally allotted to the two containers used in each experiment. Releases were pairwise corresponding in that they were conducted at a similar distance but opposing direction, and with either the same birds in immediate succession within several days or with pigeons having a comparable background of experience. The birds had never before been released at, or in the vicinity of, the respective release site.

Results and discussion

Initial-orientation data of the 34 FOREST and MAIZE releases are presented in detail in figure 1. As criteria indicating the relationship to the home direction, the homeward components $\bar{c}_H$ of the mean vectors are shown in figure 2 ($\bar{c}_H = \bar{a} \cos \bar{\alpha}_H$; $\bar{a}$ means length and $\bar{\alpha}_H$ deviation from home of the mean vector). As shown in the upper parts of the two graphs, these components are very variable and rather closely correlated in the two groups participating per release ($r = 0.76$ in each series). This kind of variability – more comprehensively shown in figure 1 – is quite common in pigeon orientation. To a considerable degree it is based on directional tendencies that apparently have little or nothing to do with the birds' awareness of their position with respect to home ('home-independent orientation', HIO; including general preference for a certain compass direction, possible responses to topographical features, etc.)[8,9]. Consequently, the mean homeward component resulting from a single release cannot be taken as an immediate measure for the pigeons' estimation of the direction towards home. Owing to interfering HIO-based directional preferences, $\bar{c}_H$ may be high, although positional information is weak or incorrect, and it may be low, although positional information is fairly good. Whether or not the initial bearings indicate home-related orientation at all, can be concluded only when looking at a number of symmetrical releases as a whole, in which HIO effects can be expected to be balanced[2,9].

Proceeding this way, it turns out that overall $\bar{c}_H$ is clearly above zero only in the OPEN groups. The low average level of the homeward component even there is not unusual, if one considers that the birds were a) only little experienced or inexperienced in homing, b) prevented from smelling ambient air during transportation, c) deprived of the sense of smell during the take-off, and d), in the case of OPEN vs FOREST, again disabled to smell natural air following the 3-h exposure and thus up to a few hours before release. (Probably it is because of this

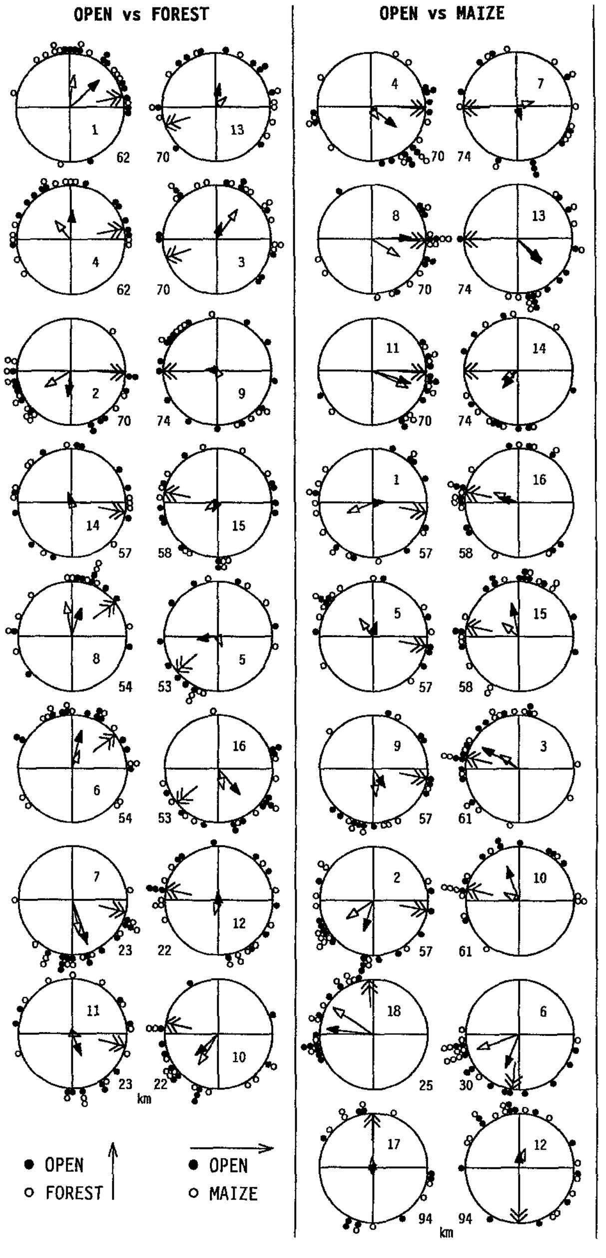

Figure 1. Initial orientation in two series of experiments. The peripheral dots on each diagram symbolize the vanishing bearings of individual pigeons released in succession on one day. Arrows indicate direction and length of the mean vectors calculated from these bearings[5] (maximum length 1 = radius). The direction towards home is given by the double-headed arrow, the distance in km by the number outside the circle. Inside the circle is the rank number of $\Delta\bar{c}_H$ according to the abscissae of figure 2. North is at the top. The two releases shown on the right side at the bottom were conducted with pigeons from a loft near Würzburg, all the others with pigeons housed at Andechs.

latter circumstance that $\bar{c}_H$ values are lower in A than in B of fig. 2.) An average homeward component around 0.2 in symmetrical releases is common in Germany even in completely untreated pigeons if they are inexperienced[10,11]; in untreated experienced birds $\bar{c}_H$ ranges most frequently between 0.3 and 0.4[10-14]. Owing to the weak and noisy performances under control conditions, detection of possible influences of more subtle interferences can be expected only on the basis of a broader statistical analysis.

Despite its low level, homeward orientation of the OPEN pigeons is statistically clearly significant. This results not only from first-order calculations (see right-hand numerals in fig. 2) but also from various second-order tests taking means per release as units ($n = 16$ and 18). The $\bar{c}_H$ values symbolized by filled columns in the upper parts of figure 2 are, on average, above zero with chance probability $p < 0.01$ under the t-test and $p < 0.015$ under the Wilcoxon test[7] in each series; pooling all releases ($n = 34$) results in $p < 0.001$ (Wilcoxon test). The mean of the 34 vectors differs from zero with $p < 0.01$ under the less specific Hotelling's test[5]. Even if only the mean directions per release are considered (neglecting vector length), the V-test[5] yields $p < 0.01$. In contrast, the values of FOREST and MAIZE, as well as of both these groups pooled, are not significantly different from zero under all the second-order tests applied.

The crucial criterion for a possible role of the air the pigeons could smell is the *difference* $\Delta\bar{c}_H$ between the groups exposed to air of different origin. This difference is largely independent of the absolute level of homeward directedness, certainly because HIO effects concern both groups in a similar way and irrespective of olfactory conditions[8,9]. In the lower parts of figure 2 $\Delta\bar{c}_H$ is ranked according to decreasing values. Under the null hypothesis – independence of the kind of inhaled air – the right half of the graph should be an inverse image of the left half, showing correspondingly increasing bars below the zero line. Actually, however, the sign of the difference is positive in 81% (FOREST) and 72% (MAIZE) of the releases. The difference is significant with $p < 0.01$ (A) and $p < 0.05$ (B), respectively. The parallel outcomes of the two series mutually support each other.

If the homeward components of the two series are pooled, thus giving a comparison between 'open field' versus 'dense vegetation', the OPEN value is higher than either the FOREST or the MAIZE value in 26 (76%) of the 34 releases ($p = 0.002$; sign test). The matched-paired differences are significant under the t-test as well as under the Wilcoxon test with $p < 0.001$.

Within a single release, the difference between the two groups is weakly significant ($p < 0.05$) in only three cases (fig. 2). Yet this is unimportant, because first-order significances are not necessary preconditions for the application of a decisive second-order test (see earlier discussions of this aspect in orientation-cage experiments with migrant birds[15,16]). Considering the background of stochastic noise and HIO, it is worthless to discuss the results of individual experiments.

Even on a second-order basis, however, using the mean homeward components per release as units, it is possible to make at least one further discrimination. If the releases are subdivided according to the direction of displace-

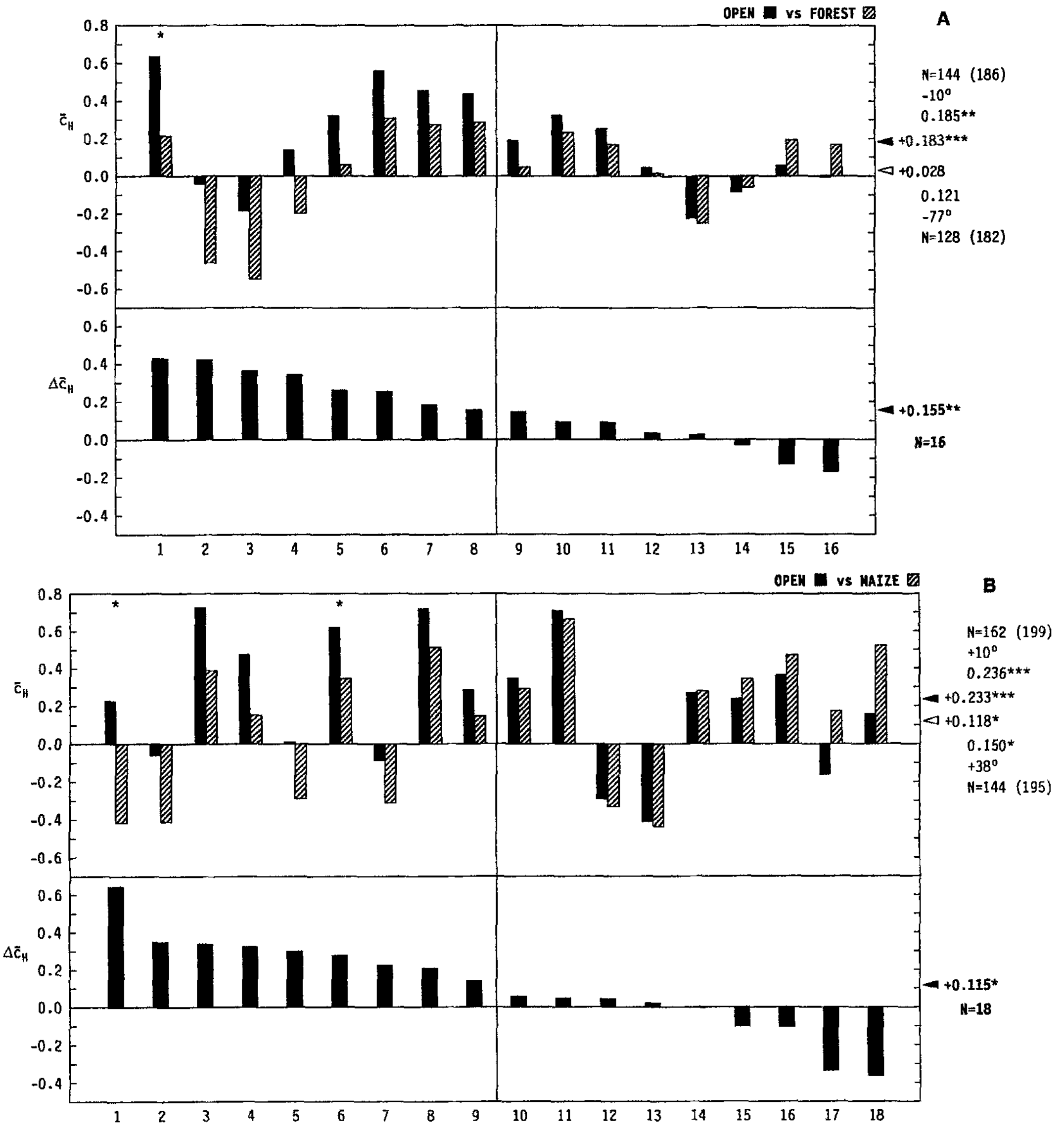

Figure 2. Mean homeward components $\bar{c}_H$ per release (top) and differences $\Delta\bar{c}_H$ between them (bottom) calculated from the data shown in figure 1. The releases are ranked and ordered on the abscissa according to decreasing $\Delta\bar{c}_H$. Numbers on the right side of the upper part in A and B show the numerical overall means ($\bar{c}_H$ at the arrowhead, $\bar{a}_H$, and $\bar{\alpha}_H$). Asterisks indicate first-order significance levels under the V-test ($\bar{c}_H$) and the Rayleigh test ($\bar{a}_H$)[5]: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, no symbol $p > 0.05$. N is 'reduced N', i.e. smallest sample size per release times number of releases, on which significance calculations are based (pseudo-pooling method[6]); in brackets the total number of bearings. On the right of the bottom parts second-order $\Delta\bar{c}_H$ is given, with asterisks indicating significance under the t-test and Wilcoxon test[7]. Significant differences within individual releases ($p < 0.05$) are marked by asterisks on top of the bars.

ment, it turns out that a dependence on the inhaled air appears quite clear at the sites west of home and only weak at the sites east of home (fig. 3). This holds true, in a similar way, for both series. If only the western sites are considered, pigeons exposed to 'open field air' were better than those exposed to 'dense vegetation air' in 14 out of 15 releases ($p < 0.001$, sign test; $p < 0.0005$, Wilcoxon test). The amounts of effect in the west and the east (right-hand column of WEST vs EAST in fig. 3) are significantly different with $p < 0.01$ (Mann-Whitney U-test, two-tailed[7]).

Figure 3 might suggest that the amount of effect depends on the level of the OPEN results; one might assume that if it is low, it cannot be lowered much more by whatever treatment. This, however, is not generally true (see fig. 2, ranks 2, 3, 4 in A and ranks 1, 2, 5, 7 in B). Within the western and the eastern releases there is no correlation between $\bar{c}_H$ of the OPEN birds and $\Delta\bar{c}_H$ ($r = -0.13$ and $+0.14$). A possible interference of a consistently preferred compass direction, which in other cases apparently caused direction-dependent differences in the amount of effect of olfactory deprivation[2, 9], can be ruled out in this case: the pigeons did not generally prefer a stable compass direction (the length of the mean compass vector resulting from the 30 west and east releases together is 0.06 in OPEN and 0.05 in FOREST + MAIZE).

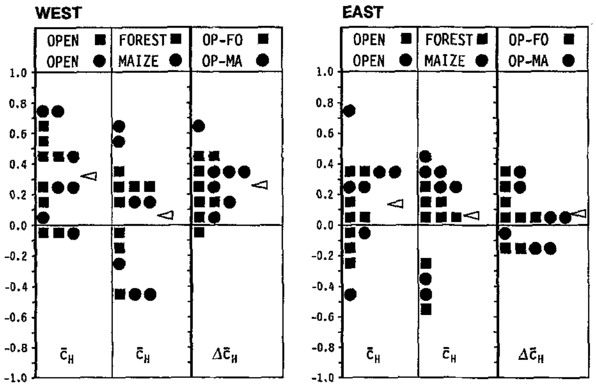

Figure 3. Frequency distributions of the mean homeward components resulting from the releases west and east of home. (All the experiments shown in figure 1 are included except of the lowermost four in its right half.) The ordinate stands for $\bar{c}_H$ or $\Delta\bar{c}_H$ as indicated. Arrowheads show arithmetic means.

The west-east aspect gains weight if the contrast to other experiments is considered in which open-field air conditions were compared with uninterrupted charcoal-filtered air conditions[2-4]. Pigeons from the same loft at largely the same sites showed clear and significant ($p < 0.01$) differences in homeward orientation according to these conditions irrespectively of whether they were released east or west of home (unpublished data). This confirms our assumption that air between dense vegetation is not completely emptied of navigational information, as charcoal-filtered air apparently is. If this is true, it seems very likely that some compounds are more concerned than others, and then one should not expect an equilibrated reduction of homeward orientation everywhere but different degrees of false orientation depending on the spatial relationship between release site and home site. Owing to the above-mentioned difficulties (noise, HIO), it is hardly possible to deduce from our data more than the tentative conclusion that the two types of vegetation tested in our experiments changed the physico-chemical composition of the air in such a way, that the birds' capabilities to recognize displacements to the west were clearly reduced, whereas the apparently less reliable information indicating eastward displacement was only little affected.

Our experiments strongly suggest that conditions of the immediate environment do exert some influence on atmospheric trace substances that are relevant to pigeon navigation. Microclimatic conditions, e.g. humidity and temperature, at the ground of a forest or a maize field are considerably different from those in the open airspace. In the latter there is much more air exchange due to winds[17]. Owing to the longer lasting presence of air quanta as well as to the metabolic activities of the vegetation itself, decomposition or conversion of relevant compounds, and adsorption at surfaces, may be enhanced in woods and cornfields. Other substances are produced there and hence are more concentrated than in the windy air outside. Either these substances themselves might be of concern or they might mask suitable odorants. As a result, the perceived spectrum of relevant compounds[8] may be modified in a manner that leads the birds to misjudgements of their position. (Accordingly, the air in a barn, where pigeons were confined for several days and apparently did not gather positional information sufficient to replace preceding impressions from the outward journey[18], may not have contained normal site-specific olfactory signals.)

If the pigeons' orientation varies depending on obvious dissimilarities of the atmospheric environment, it might also depend on less obvious environmental conditions which are not under the experimenter's control. This might be one more possible source contributing to the spatial and temporal variability characterizing initial orientation of pigeons.

As in many other experiments on pigeon homing, there is some risk left that the real causal connections differ from those taken into account by the investigators. Slightly different conditions in the two containers, possibly not connected with the smelling of crucial substances, might have modified in some way the behaviour of the pigeons without actually interfering with the navigation process[8,9]. Considering all circumstances, however, including the west-east differences, we feel that there is little margin left for either uncontrolled side effects or chance events, or for alternative explanations. While such possibilities can, nevertheless, not be definitely excluded, it is obvious, on the other hand, that our results and interpretation fit very well into the catalogue of findings and conclusions supporting and illuminating the olfactory basis of pigeon navigation[1,2,8]. Thus, if the above interpretation is correct, we do not simply add one more 'effect' to the list of 'effects' described in the field of pigeon homing, whose causation and significance remain enigmatic. Rather, the results lengthen the list of coherent findings making olfactory navigation more and more credible.

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Behavioral selection in crayfish correlates with movement of a screening pigment in the eye

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Abstract. Light induces two contrasting behavioral responses in crayfish: attraction at low intensities and withdrawal at high intensities. The aim of our experiments has been to study whether screening pigments of the eye influence the selection of attraction or withdrawal responses. During illumination, screening pigments mask photoreceptor cells, reducing the gain of the visual system. Comparison of the time and light-intensity functions of pigment migration and of attraction and withdrawal responses suggest that pigment migration might influence the selection as well as the latency of the response.
Key words. Behavior; crayfish; vision; pigment; invertebrate; Crustacea.

The nervous system responds to sensory stimulation with different behavioral patterns. At present, not much is known about how mechanisms for selecting behavioral responses are structured. Crayfish are useful for this type of study, because of their wide behavioral repertoire and the great amount of information available about the physiology of their nervous system.

Field observations and behavioral recordings under laboratory conditions have shown that different light levels induce contrasting patterns of burrowing behavior in crayfish and in other crustacean species: low intensities induce an attraction towards light, whereas higher intensities produce a withdrawal response[1,2]. The attraction response is restricted to a range of 1 logarithmic_{10} unit of light intensities. Within this range, the latency of attraction (the time for the animal to emerge from the burrow after light onset) increases as a function of light intensity. A transition point exists where the attraction response is suppressed and a withdrawal reaction is produced. Withdrawal bears an opposite relation to light intensity: the latency (time for the animal to retreat to the burrow after light onset) becomes shorter as the intensity increases[1].

Behavioral and electrophysiological experiments suggest that attraction and withdrawal responses might be mediated by subsets of light-responsive sustaining neurons in the optic nerve[3] with low and high thresholds respectively[1]. Attraction is associated with the response of low-threshold sustaining fibers, whereas withdrawal is associated with the response of high-threshold sustaining fibers. In addition, the photoreceptor cells in the 6th abdominal ganglion[4] supply input to the withdrawal response[1,5]. At increasing light intensities, the rise in the latency of attraction correlates with the activation of high-threshold sustaining fibers. Therefore, the increase in the latency might be due to conflict over the selection of behavioral response, owing to the simultaneous activation of both inputs.

One possible mechanism underlying this complex behavioral integration might be the migration of the retinal screening pigments of the eye[6]. The migration of two different screening pigments modulates the amount of light arriving at the photoreceptor cells. Proximal pigment migration occurs as a response to background illumination. Light intensity determines the final position of proximal pigment without having any effect on the time course of migration[7,8]. By contrast, distal pigment migration requires the presence of the Distal Pigment Light-adapting Hormone[9]. Each pigment is known to modulate photon flux to the retinal receptors over a range of 1 logarithmic_{10} unit[9].

The present report concerns the relationship between light-intensity, the latency of the behavioral responses, and the migration of screening pigments.

Methods

In all experiments, adult crayfish *Procambarus clarkii* of either sex were used at intermolt. Crayfish were kept in individual chambers with a simulated burrow under controlled light: dark (12 : 12) cycles. During the light period, a white light of 1200 lx was applied.

Behavioral experiments. The design of the chambers and the methods used to record attraction and withdrawal responses have been described elsewhere[1,10]. In brief,