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Arachnology

# Scavenging by brown recluse spiders

The brown recluse spider (*Loxosceles reclusa*) is a threat to humans and establishes huge populations in urban habitats throughout central North America[1,2] — more than 2,000 of these spiders were recorded in a single house in Kansas[3]. What do these spiders eat in order to build and maintain such numbers? Here I combine laboratory prey-choice experiments with observations of the behaviour of *L. reclusa* in houses to show that this spider prefers dead, scavenged prey over live prey. This finding may explain how immense populations of these spiders can flourish even in adverse conditions.

All spiders consume live prey and they have evolved a diverse array of adaptations to enhance its detection and capture. Some spiders also supplement their diets with unusual food sources such as pollen[4], nectar[5] and insect eggs[6]. However, spiders would not be expected to prefer dead prey, given the stimulus of catching live prey that results from their extreme sensitivity to even the slightest movement of their victim.

Bites from brown recluse spiders (Fig. 1) can constitute a medical emergency in humans, inflicting slow-healing necrotic wounds and extensive tissue damage[1,2]. The same powerful paralysing venom that causes these effects in humans also allows the spider to immobilize its prey and avoid being injured itself[7]. Unlike most wandering spiders, which use vision, stealth and strength to capture prey, *L. reclusa* attacks, retreats and then returns to feed at its convenience[7]; it has relatively poor vision[7] and does not readily respond to substrate or airborne vibration.

I investigated the predatory behaviour of *L. reclusa* in 71 homes in Kansas and did not see spiders catching live prey — indeed, the spiders actually fled from potential prey. However, I witnessed the spiders locating and consuming dead prey, without prior attack, in more than 25 houses.

For the prey-choice experiments, I placed adult male and female *L. reclusa* ($n = 147$) into individual plastic boxes ($12 \times 17 \times 6$ cm) and fed them a variety of prey, both dead and alive, once a week, and then starved them for two weeks. The spiders were subsequently presented with equally sized live and dead prey (dead prey was killed by freezing at $-80$ °C). Spiders were kept in the dark and observed under low light every hour for evidence of prey choice, which was verified by fang penetration and feeding for 5 min or longer.

In these experiments, 81.4% of *L. reclusa* chose dead over live waxmoth larvae (*Achroia grisella*; $n = 59$), 75.6% chose dead over live domestic crickets (*Acheta domestica*; $n = 41$) and 97.6% chose dead over live yellow mealworm larvae (*Tenebrio molitor*; $n = 41$). Overall, spiders chose dead prey in 84.4% of trials ($\chi^2 = 72$, $P \ll 0.001$), indicating a clear preference for scavenging dead prey.

To test the effects on the spiders of eating dead rather than live prey, I used three groups of ten spiders that had been respectively given: old dead crickets that had been envenomated and partially digested by other *L. reclusa* two weeks previously; German cockroaches (*Blattella germanica*) that had been dead for at least a month; and *B. germanica* that had been killed 24 h earlier with Cessco-5E insecticide (0.5% pyrethrin). All spiders consumed the prey within 24 h of its being introduced, and showed their resilience in that no obvious negative effect was manifest over the ensuing 10 months, during which a regular feeding regime was followed.

The feeding preferences that I observed in urban habitats are consistent with my findings with captive spiders, indicating that *L. reclusa* actively searches for dead prey and ignores live prey. Spiders will even remain motionless and allow their prey to walk over them without attacking it, such is the extent to which they prefer dead prey. In five *T. molitor* trials, spiders attacked and killed live prey but did not eat it, yet consumed prey killed by freezing. Capture of live prey (15.6%) was often the result of prey walking into spiders rather than of active hunting.

The sequence of predatory behaviour shown by *L. reclusa* (attack, retreat and feed later) means that prey may escape before it is relocated. Spiders sometimes leave partially eaten carcasses that other spiders subsequently find and consume. It is likely that dead prey provides an easily accessible source of nutrition without incurring the additional costs or risks associated with attacking and manipulating live prey.

*L. reclusa* is an opportunistic feeder rather than an obligate predator or obligate scavenger, but it prefers dead over live prey. In an environment such as a house, opportunists and scavengers have an advantage over more selective predators because their feeding requirements are more easily met[8]. Insects are attracted to houses by light, food and shelter, as well as for other unknown reasons, but they can easily die there from starvation, desiccation, overexertion, pesticide exposure or other causes. I conclude that scavenging contributes significantly to the diet of *L. reclusa* and may be important for the survival of this species in natural habitats, as well as being beneficial to them when they are in close proximity to humans.

**Figure 1** Still watches: the brown recluse spider *Loxosceles reclusa* prefers prey that is motionless or already dead, feigning indifference itself to avoid a struggle with living prey.

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COMMUNICATIONS ARISING

Biophysics

# Is rhodopsin dimeric in native retinal rods?

The model of vertebrate rhodopsin that has emerged from a combination of traditional biophysical techniques[1–6] defines it as an archetypal monomeric receptor protein that is randomly dispersed and freely diffusing in a fluid lipid matrix in the rod-disc membrane. A quite different arrangement has been demonstrated by Fotiadis *et al.*[7], however, who use atomic force microscopy (AFM) to reveal rows of rhodopsin molecules packed as dimers in isolated murine disc membranes at twice the surface density estimated from previous *in situ* measurements[3,4,6]; lipids are probably excluded from these rhodopsin packs[8]. We contend that these 'dimers' are in fact long double rows of equally spaced proteins, packed in partially ordered microcrystalline arrays, and suggest that the arrangement of irregular packs of protein and patches of pure lipid with a free-floating border may be an artefact resulting

from the conditions used in sample preparation for AFM.

Fast, transient, flash-induced photodichroism[1] first revealed the rapid rotational diffusion of rhodopsin *in situ*, and the kinetics of flash-bleaching recovery[2] indicated that rhodopsin undergoes rapid lateral diffusion in intact rods. Its random distribution in the membrane was evident from X-ray and neutron diffraction[3–5] and was confirmed by electron microscopy[6]. We therefore question whether the structures seen by Fotiadis *et al.*[7] in an osmotically burst disc membrane represent a native disc structure, or whether they can be explained by separation of the proteins and lipids during the 15-h incubation at 0 °C in 2 mM Tris–HCl in the preparation of samples for AFM[8].

A long exposure at low temperature will alter the native disc membrane structure in rods isolated from a warm-blooded vertebrate. In-plane X-ray diffraction revealed long ago that cooling cattle rods to 5 °C for only 15 min induces a partial phase transition of the lipids and a correlated segregation of the proteins[4]. By contrast, no phase separation in frog rods was ever observed by X-ray[4] or neutron[5] diffraction.

Low-temperature investigation of flash-induced transient photodichroism (R.C., unpublished results) has confirmed that frog rhodopsin can still undergo rapid rotational diffusion at temperatures close to 0 °C. Transient photodichroism cannot reliably distinguish between freely rotating dimers and monomers, but the packed rows of rhodopsin seen in the AFM images of Fotiadis *et al.*[7] are incompatible with rapid rotational diffusion. And if rhodopsin were oligomerized into rows *in vivo*, a stable or slowly decaying flash-induced dichroism would have been detected long ago in human vision and in isolated vertebrate retinae[1].

Electron-microscope images of snap-frozen, freeze-etched frog rods[6] were able to resolve rhodopsin monomers in a random array, with no evidence of dimers, although the rods had been incubated at 4 °C for 1 h. After digestion of these fragmented rods with phospholipase C, however, disc-membrane phospholipids were seen to segregate into droplets, whereas the packing of rhodopsin changed into concentrated, row-like arrays[6]. It is possible that these could be the equivalent of the double-row arrays that were observed by AFM[7].

All of these biophysical data are old, but remain valid. Until now, they have not been experimentally challenged or refuted. They exclude the occurrence of rhodopsin oligomers in native, unactivated, frog rods and provide evidence for the rapid phase separation of rhodopsin and lipids in mammalian disc membranes kept at low temperature.

Fotiadis *et al.* observe the same separation of rhodopsin and lipids when their samples are incubated at the higher temperature of 25 °C (see www.mih.unibas.ch/Nature/Fotiadis.html), but this is still below physiological temperature. Phase separation in a burst disc membrane may slow down under these conditions but will not be prevented. In osmotically disrupted cattle discs, for example, phase-separation artefacts were often visible in early X-ray patterns that required 15 h of exposure at room temperature[4].

We therefore maintain that native rhodopsin exists in monomeric form in the retinal rods of vertebrates. The double-row arrays seen in AFM[7,8] in isolated and osmotically disrupted mouse discs probably result from separation of the lipid phase from the proteins, which occurred rapidly at 0 °C and more slowly at 25 °C, during the long incubation and upon osmotic disruption of the discs.

Our contention excludes only constitutive oligomerization of non-activated rhodopsin in dark-adapted rods. After illumination, in an arrestin-mediated deactivation process, rhodopsin molecules may undergo pairing[9]. Oligomerization also occurs in other G-protein-coupled receptors[10] in the β-arrestin-mediated process of their inactivation and clustering in coated pits, but these processes are distinct from the oligomerization of non-activated receptors.

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*Fotiadis* et al. *reply* — Individual biological molecules can be imaged under physiological conditions by atomic force microscopy. Our results from AFM, supported by electron microscopy, revealed distinct rows of rhodopsin dimers and paracrystalline arrays in native murine disc membranes[1,2]. This supramolecular arrangement was also found for the light-activated form, opsin[1]. We counted 30,000–55,000 rhodopsin molecules per square micrometre, a packing density comparable to that measured by optical methods in amphibian discs[3]. From the lattice vectors describing the paracrystalline arrays, a maximum possible packing density of 62,900 rhodopsin molecules per square micrometre was calculated. The rhodopsin dimers seen in AFM topographs[2] have cytosolic protrusions separated by 3.8 nm, providing an ideal docking platform for arrestin, which has two binding grooves that are separated by 3.8 nm as well[1].

On the basis of data reported some 30 years ago, Chabre *et al.* are challenging our observations. Results from their and other[3] early experiments, which were all carried out on large ensembles of amphibian disc membranes, indicated that inactivated as well as activated rhodopsin diffuses freely as a monomer in the lipid bilayer. Chabre *et al.* now propose that the rhodopsin-packing arrangement that we observe by AFM[1,2] is induced by the segregation of proteins and lipids at low temperatures.

To respond to this criticism, we recorded electron micrographs as well as AFM topographs of rhodopsin paracrystals on native disc membranes that had been prepared and imaged at room temperature (see www.mih.unibas.ch/Nature/Fotiadis.html). Three different surface types could again be distinguished: rhodopsin paracrystals, rhodopsin rafts and the lipid bilayer; at increased magnification, rhodopsin dimers, the building block of the paracrystal, are clearly seen. This dimerization and higher-order organization of rhodopsin at room temperature argues against the claim by Chabre *et al.* that the packing arrangement we describe[1,2] is a result of low-temperature-induced protein–lipid segregation, particularly if we consider that the phase-transition temperature of the bovine-disc lipids is below 3 °C (ref. 4).

Freeze-fracture electron microscopy has also revealed paracrystalline rhodopsin arrays in *Drosophila* photoreceptive membranes[5] and in the plasma membrane of bovine-rod outer segments[6]. The concept of oligomerization in the presence or absence of ligands is generally accepted for many G-protein-coupled receptors[7], and rhodopsin is not an exception.

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