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Ecology of Sensing

With 100 Figures

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Introductory Remarks

For good reasons we think of the brain as the master organ shaping the behavior of an animal. Yet both the central nervous system and behavior are at the mercy of sufficient and adequate sensory information. This information is provided by sensory systems responding to different forms of energy and representing the biological interfaces between the environment and the behaving organism.

As has been known for a long time, there is a considerable degree of filtering, biasing, and distortion of the theoretically available information, starting way out in the sensory periphery and continued at the various stages of central nervous processing. Many kinds of foveae and distorted cortical or other sensory maps (to name just two examples) reflect the sophistication in the evaluation of the biologically significant aspects of complex stimulus patterns. Much of this filtering must have evolved under the constraints of the specific physical and chemical conditions prevailing in the natural species-specific habitat of an animal, in the marine environment of a planktonic crustacean in the same way as in the desert environment of a scorpion. As a consequence of evolution, in many cases the functional properties of sensory systems nicely match at least some of the properties of the stimulus patterns encountered under behaviorally relevant conditions. The detailed spatial and temporal characteristics of these stimulus patterns may often be of frightening complexity. However, the information actually used by the animal for the guidance of its behavior has often proved to be much simpler.

Sensory ecology in its widest sense deals with the acquisition of information and the ways in which an organism responds to sensory information to organize its interaction with its environment. The information addressed here informs about actual environments and habitats as experienced by an animal in its own individual life. It differs from genetically stored information, which reflects the environments experienced by past generations.

The ideas behind *Ecology of Sensing* are not new, and the reader is particularly referred to the seminal book by Dusenbery (1993). However, with all the knowledge and understanding now available of the basic principles at work in many sensory organs, and considering the much-increased appreciation of the need to experiment and observe under natural conditions, the ecology of sensing is more likely than ever before to have a great future. Among the first advocates of sensory ecology was Jakob von Uexküll who published his *Umweltlehre* and his *Umwelt und Innenwelt* der Tiere as early as 1909. Von Uexküll always stressed the uniqueness of the sensory worlds in which different animal species find themselves living and, as a consequence, underlined the importance of knowing the particular features in the environment relevant for them.

The present book is an outcome of a symposium organized by us in Vienna in 1999. Its 16 chapters exemplify the diversity of the problems to be dealt with when we consider the different constraints and opportunities which are associated how the geomagnetic field, an omnipresent environmental factor, serves different animal groups as a reliable indicator of direction in oriented behavior. Unfortunately, the primary processes of magnetoreception are still rather obscure. This is different in electroreception, which is the topic of the contribution of Gerhard von der Emde. He introduces us to the electrosensory world of the weakly electric fish and the remarkable adaptations of the electrosensory system to the conditions met by the fish in its freshwater habitat.

Clearly, this book can provide only examples of a topic in sensory biology which is as rich and varied as are both the sensory systems used by different animals and the habitats in which they are living.

Many thanks go to the Austrian Academy of Sciences for making the Symposium from which this book originated both possible and pleasant. Its generous support is gratefully acknowledged. The cooperation of the authors and the help and patience of Dr.D.Czeschlik and the editorial staff of Springer-Verlag are much appreciated.

Wien and Lofer, May 2000 *Friedrich G. Barth*

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I.2 Why Sensory Ecology Needs to Become More Evolutionary – Insect Color Vision as a Case in Point

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Abstract
Many of the subtleties in the evolutionary tuning of sensory systems still escape us. Insect color vision is a typical case. While we know much about its mechanisms, the abundant adaptive explanations of its components are often ornate speculations. We advocate using an evolutionary approach to understand why different animals see the world in different colors. Such an approach must include (1) phylogenetic analyses, which should help identify patterns of adaptation, constraint and history; (2) molecular studies, to predict how plastic the relevant genes will be in the face of particular selective pressures; (3) assessments of interindividual variance, to see if the raw material for evolution exists; (4) a consideration of pleiotropic effects, where selection on visual pigments may be affected indirectly through correlated characters; (5) biogeography, to explore if populations living in different visual habitats have adapted to those differences; (6) a consideration of random evolutionary processes; (7) selection experiments, to test for heritability of traits and to simulate the influences of strong directional selection; (8) fitness tests: to show that a trait is adaptive, we must show empirically that this trait confers greater fitness to its bearers, compared with conspecifics which lack this trait.

Key words Color vision, evolution, insects, visual ecology, visual pigments

1 Introduction

One major focus of sensory ecology has long been the question why many animals see the world through color receptors so different from our own. Traditionally, the field has concentrated on adaptive explanations. We are used to thinking that if there were differences between species, this must reflect adaptations to different photic environments, whereas if animals share similar color receptors, they must live under similar selective pressures. Fish dwelling in progressively deeper habitats possess rods whose sensitivity is more and more shifted into the blue, thus matching the changing spectral distribution of the ambient light in deep water (Lythgoe 1972; Hunt et al. 1996; Douglas et al. 1998). Other presumed cases of adaptive spectral tuning include humans, whose trichromatic systems might have evolved as a response to frugivory (Mollon 1989), and bees, whose receptors were suggested to be evolutionarily tuned for flower color coding (Chittka and Menzel 1992). Such intuitively appealing stories of adaptation easily find their way into textbooks. Studies which do not find an obvious match between sensory traits and the environment do exist (Crandall and Cronin 1997; Fleishman et al. 1997), but they often achieve much less fame. Even in deep-sea fish, which have long been held as classical examples of adaptive visual pigment tuning, the situation is far from resolved. Fish which live in similar photic environments, but belong to different taxa, often have different visual pigments, whereas closely related fish species sometimes have similar visual pigments, even if they inhabit different light habitats (Douglas et al. 1998). Turning to bees, the notion that pollinator color vision is tuned to floral colors is compromised by the finding that arthropods living under entirely different visual conditions, such as the beach isopod *Ligia* (Hariyama et al. 1993), the freshwater-dwelling bug *Notonecta* (Bruckmoser 1968), nocturnal hawkmoths (White et al. 1994), and the larval ocelli of some Lepidoptera (Ichikawa and Tateda 1982) have similar sets of color receptors. On the basis of such difficulties, Goldsmith (1990) concluded that phylogenetic and molecular constraints might play more important a role in determining the wavelength positioning of color receptors than is good for any pan-adaptionist scenario. We wish to reiterate this warning, and to add several additional ones.

Our most important caveat is that to show that a trait is adaptive, we must demonstrate that it has an impact on fitness (Endler 1986; Reeve and Sherman 1993). What is the evolutionary significance of a model, for example, which shows that for a given visual task, one set of color receptors is 5% better than another? If this really translates into 5% more lifetime reproductive success, the effect of selection will probably be significant over evolutionary time. On the other hand, it is just as possible that 5% improved performance in some criterion will be absolutely irrelevant to fitness. To our knowledge, there is not a single study in sensory ecology that resolves this problem. Such fitness tests are challenging, but should be possible. In what follows, we will lay out a research agenda that include several steps towards such tests. We hope this treatise will stimulate a more evolutionary approach to sensory ecology, and a better understanding of why many animals see the world in colors so differently from ourselves.

2 Uses and Limitations of Model Calculations

Ten years ago, one of us (L.C.), in collaboration with R. Menzel, set out to identify the adaptive significance of bee color vision. The idea was to generate a theoretically optimal color vision system for the task of flower color coding, and to compare this with the system really implemented in bees. We had considerable

reasons for optimism. First, Menzel and his coworkers had established a database that included the color receptor sensitivity functions of a large variety of hymenopteran species (Peitsch et al. 1992) and insects in general (Menzel and Backhaus 1991). This database suggested that insects could, in principle, produce pigments with values of maximum sensitivity (λ_{max}) anywhere from 320 to 630 nm, and that the number of color receptor types varied widely between species. This, in combination with the fact that insects occupy a very wide range of visual environments, made studying the sensory ecology of their color vision look promising. Second, Backhaus (1991) had just developed a physiological model of bee color vision, which allowed quantitative predictions of the similarity of flower colors, and of flowers and their background. Such a model is an essential tool to measure the quality of a color vision system and, to date, such models are still not available for any animal species besides bees and humans. Third, unlike many other animals, bees seemed an ideal study subject because the relevant visual tasks are comparatively easily identified: bees obtain their food from flowers, and so selection should favor color vision systems that allow for swift detection of flowers and reliable identification of the most rewarding species (Chittka 1997).

Spectral sensitivity functions of color receptors have roughly Gaussian characteristics, and the exact shape of the curve can be easily predicted if the λ_{max} is known (Stavenga et al. 1993). Our evolutionary model calculations consisted of moving three color receptor sensitivity curves along the wavelength scale. For each theoretical combination of receptors thus generated, the quality of the color vision system for flower color coding was determined. The result was striking: the optimal color receptors generated by the evolutionary model invariably occurred at λ_{max} = 330, 430, and 550 nm, which is very close the most common λ_{max} really found in flower-visiting bees (Chittka and Menzel 1992). This result was independent of whether we varied one, two, or three photoreceptors. It was also independent of the particular set of flowers used (Chittka 1996a).

Since the optimal set of color receptors might also depend on the particular kind of opponent coding in the brain, the mode of this processing, too, was varied – the result remained unchanged (Chittka 1996a). An engineer could hardly design a better receiver for flower colors than the color vision system of bees. Does this, however, mean that flower colors indeed drove the evolution of bee color vision? This is an attractive notion for sensory ecologists. It is joined by other studies in which correlations between the results of model calculations and reality were taken as evidence for adaptation (Lythgoe and Partridge 1989). Thus, many colleagues took our finding to mean that bee color vision indeed adapted to flower colors, although we explicitly stated in the discussion of the original paper that this is not necessarily the case (Chittka and Menzel 1992).

Indeed, there are several complications. Models are useful to generate hypotheses of optimality, but a correlation between a model and biological traits does not resolve how these traits evolved. Using models to reject a hypothesis of evolutionary causality is much more straightforward. Had the optimal color receptors derived from the model calculations been different from the ones found in "real animals", then this would have indicated that evolution has not optimized the photoreceptors according to the same criteria. At the very least it would mean that there are other, more important, criteria, or that evolutionary constraints might have hindered the animal from evolving along the same lines as the model calculations. The fact that the calculations arrive at similar color vision systems as nature is tempting, but it does not necessarily imply that one has found the criterion which has driven the evolution of bee color vision. In fact, sets of color receptors similar to those of bees occur in animals that occupy entirely different ecological niches.

Finally, close inspection of the model results reveals that the color receptors of bees are only nearly optimal. For example, the optimum long wave receptor for coding flowers is at λ_{max} =550 nm, whereas the visual pigments of bee green receptors are maximally sensitive at 540 nm. Since the modeled optima are fairly broad, performance of the real receptors is only 2% below the theoretical optimum for flower coding. This discrepancy is small when considering the large range over which the receptors were varied, but what causes it? If bee visual pigments can be freely tuned, why have they not achieved a perfect match with floral colors? One possibility is that there are tradeoffs with other visual activities. For example, it has been assumed that the bee green receptor is optimally matched to green foliage, and might thus serve as a background detector (Menzel 1979); but leaves also reflect most strongly at 550 nm (Chittka 1996a), so that, again, the theoretical optimum is at longer wavelengths than the peak sensitivity of the bee green receptors.

We are confident that there are other adaptive explanations that might be tried, and eventually, one might be successful: but we also wish to warn that trying a large number of adaptive explanations can lead to speculation. Gould and Lewontin (1979) caricaturized this approach in the following terms: "If one adaptive argument fails, try another" and "In the absence of a good adaptive argument... attribute it to imperfect understanding of where an organism lives or what it does." We are sure that some of our readers will recognize their own thinking in these words. We do not wish to discourage sensory ecologists to continue searching for adaptive explanations where at present they seem hard to find. In what follows, however, we list a number of tools that should make this search less speculative.

3 Phylogenetic Studies

One possible reason why animals in different ecological contexts have conserved traits is phylogenetic constraint. For example, there is little reward in searching for the adaptive significance of why bees have six legs, because leg number is evolutionarily conservative in insects. Mapping traits on an established phylogenetic tree will reveal if the trait is variable within a given taxon, and whether the search for adaptations will be worth our time (Brooks and McLennan 1991; Harvey and Pagel 1991). It is this simple evolutionary reasoning that is absent in many studies of sensory ecology. Often, each species was regarded as an entity

that could freely vary all its traits in all directions, and its history seemed to be of minor importance. Some physiologists, in fact, dismissed the possibility of evolutionary constraints entirely: ".... there is also the possibility that the evolutionary history of a species might be important in determining its visual pigments .... but taking a broader view .... the evolutionary explanation is not helpful." (Lythgoe 1972). Quite often, this view has led workers to detect biological adaptations where, in fact, there were none (see Chittka and Dornhaus 1999 for examples).

To test if flower signals drove the evolution of bee color vision, it must be shown that the ancestors of bees possessed different sets of color receptors prior to the advent of the flowering plants. To this end, we have to evaluate members of arthropod taxa whose evolutionary lineages diverged from those of bees before there were flowers. A phylogenetic analysis reveals that the values of peak sensitivity in the Crustacea and Insecta fall into three distinct clusters around 350, 440 and 520 nm (Chittka 1996b). The few insect species in which one of the three types is absent (*Periplaneta* and *Myrmecia*) represent secondary losses. Red receptors show up irregularly in both the Crustacea and Insecta; they have evolved several times convergently. The photoreceptor wavelength positions of UV, blue, and green receptors are surprisingly conserved in the Mandibulata. We conclude that the Cambrian ancestors of extant insects and crustaceans possessed UV, blue, and green receptors. Insects were well preadapted for flower color coding more than 500 million years ago, about 400 million years before the extensive radiation of the flowering plants that started in the mid-Cretaceous (100 million years ago).

To be sure, there *are* differences of 20-30 nm between the measured peak sensitivities within each cluster of insect color receptors. This means that our analysis does leave open the possibility of fine-tuning of receptors to particular visual tasks in each species. For example, microspectrophotometry reveals that the long wave pigment of fireflies differs by 12 nm in λ_{max} between nocturnal and crepuscular species, a difference which can be explained by the specific requirements posed by detecting and identifying conspecific flashes under different visual conditions (Cronin et al. 2000). Unfortunately, however, the data in many other studies were collected by electrophysiological measurements and are therefore noisy, so we do not yet understand whether the differences between many species can be attributed to measurement error, different methods, or actual variation between species.

At first sight, however, considering the large variety of light habitats and feeding habits of insects, it is surprising how little variation there is. If traits are conserved within a taxon, although we have reasonable grounds to predict that they should differ on the basis of optimality arguments, then we must also take the possibility of phylogenetic constraint seriously. Similar phylogenetic studies performed on other sensory traits, or on color vision systems in other animals, may reveal a different pattern; but it is through phylogenetic studies that we can decipher patterns of adaptation and constraint, convergence, and homology.

4 Molecular Tuning, Constraints, and Adaptation

To understand how easily visual pigments can be matched to the colored environment, it is necessary to look at their molecular structure, the mechanisms of tuning, and the genes that encode the pigments. For example, we would expect that color receptors readily adapt if small genetic changes (e.g., single mutations) cause large changes in spectral sensitivity. We predict slower adaptation if most mutations are adaptively neutral. Or if several mutations are necessary in conjunction to alter spectral sensitivity, this may mean that any functional changes require a comparatively improbable sequence of mutation events.

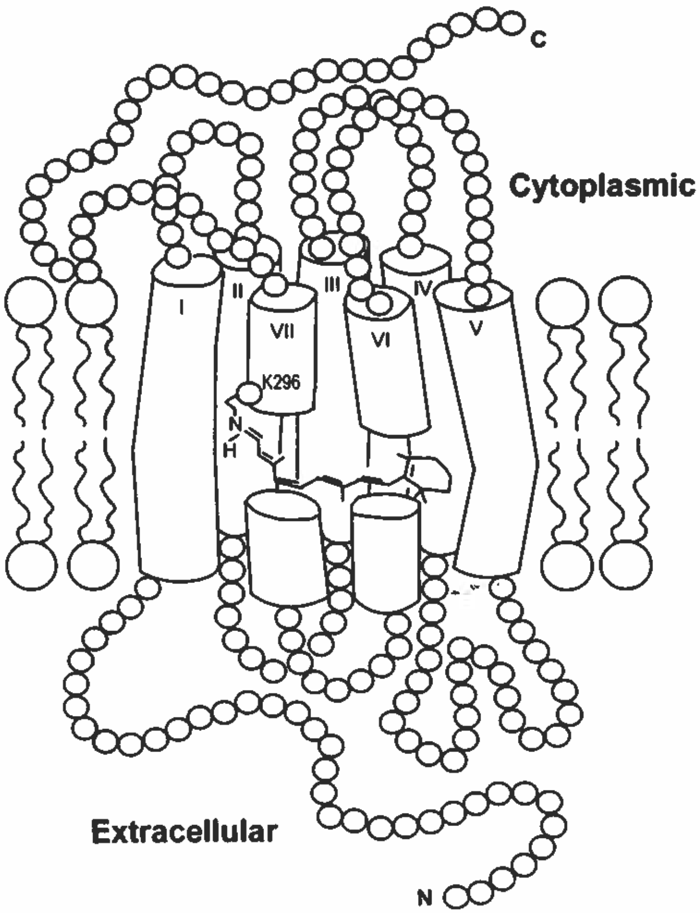

Fig.1. Diagram of an opsin bound to 11-*cis*-retinal at lysine 296. The seven transmembrane helices are numbered *I-VII*. Specific amino acid side-groups interact with the chromophore and shift its absorption maximum

We would expect particularly strong constraints, if pigments sensitive at two peak wavelength values are separated by adaptive valleys, so that, for example, two combined changes are necessary to alter a receptor's sensitivity, but each single mutation renders the pigment nonfunctional. We would also expect that pigment adaptations might be compromised by molecular spell-checkers that pit protein folding requirements against spectral tuning (Nakayama et al. 1998).

Each class of color receptor contains a distinct visual pigment, which consists of two components. One is the chromophore, retinal (or one of its congeners), which changes its configuration on absorption of a single photon (Seki and Vogt 1998). The other component is a protein moiety, the opsin (Fig.1). Opsins are integrated into the membrane of photoreceptive organelles of the receptors, and contain about 370 amino acids (Deeb and Motulsky 1996). They contain seven transmembrane helices, arranged in a quasicircle so that they form a pocket. This pocket holds the chromophore. Specific amino acids in the transmembrane helices oriented towards the center of the pocket (and, thus, interact electrostatically with the chromophore) are responsible for spectral tuning (Hope et al. 1997).

To understand how visual pigments in insects changed over evolutionary time, it is informative to evaluate the phylogeny of their opsins. To this end, we compared the amino acid sequences of the opsins of 54 species of arthropods, as well as different opsins found within the same animal species. The basis of such an analysis is that one groups together those proteins that are most similar; the nodes of the tree represent (hypothetical) ancestral opsins (Goldsmith 1990). It is immediately apparent that invertebrate opsins fall into distinct functional clades according to spectral sensitivity (Fig.2).

There is one cluster of UV pigments, a distinct group of blue pigments, a third group of long wave pigments, which includes pigments with peak sensitivity from green to red. Most interestingly, chelicerate and crustacean green sensitive pigments are more similar to insect green pigments than they are to UV and blue pigments, which confirms the phylogenetic analysis above: opsin clades diverged from one another before the major groups of arthropods diverged, and it is therefore likely that ancient arthropods already possessed (at least) UV and green visual pigments. Somewhat puzzling are the origins of two clades of blue-green (480 nm) pigments of *Drosophila* and the crab *Hemigrapsus sanguineus*. They might be the result of convergent evolution, or of several independent gene losses. On the basis of electrophysiology, not many insects are expected to have pigments that fall in the 480 nm spectral class, which favors the latter hypothesis. We advocate testing this hypothesis by looking for opsin pseudogenes to members of this clade.

Another important result of this comparative analysis is that rather large portions of opsin amino acid sequence can be exchanged while the spectral function remains surprisingly constant. Bee UV and *D. melanogaster* Rh3, which have nearly identical λ_{max} values (353 and 345 nm) differ by 36.6%, while the two *Limulus* sequences, which are thought to differ by 10 nm, differ by only 1%!

Of course, our argument is dependent on the assumption that differences between opsins do not represent cases of convergent evolution, possibly as a response to similar selective pressures.

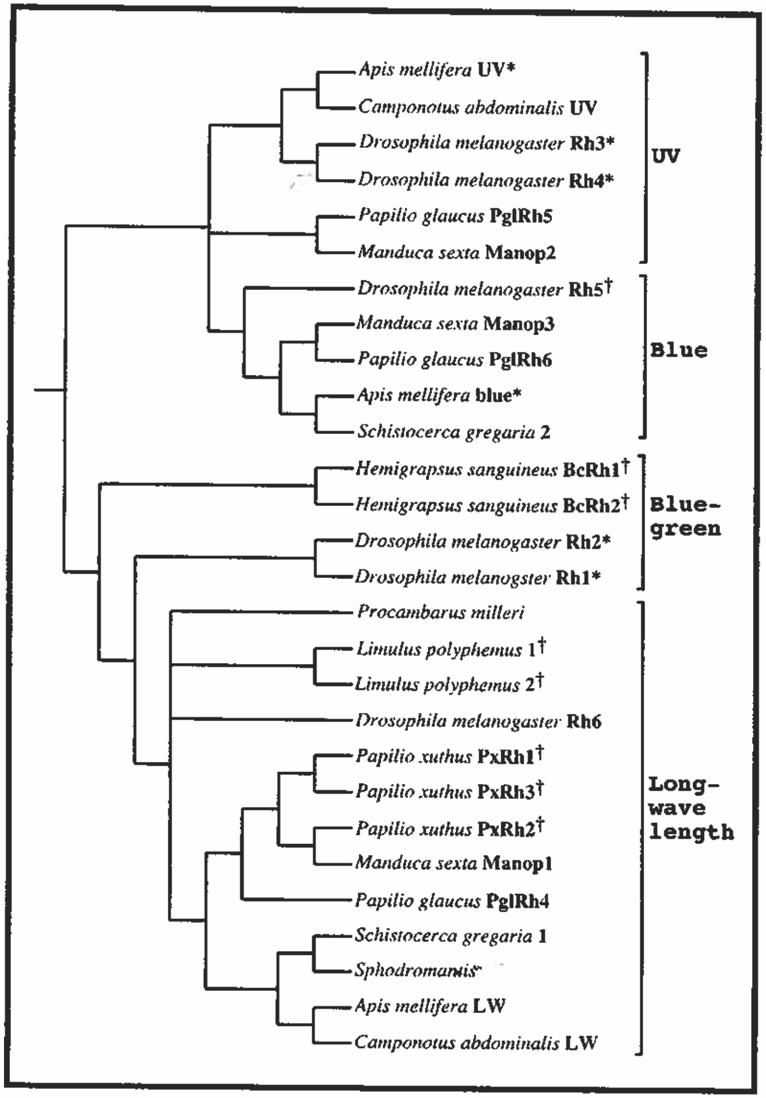

Fig. 2. Phylogeny of insect, chelicerate and crustacean opsins, based upon a maximum parsimony analysis of opsin amino acid sequences. Tree shown is simplified from the analysis of a larger data set of 54 opsin sequences (Briscoe 1998b). Only representative species from available orders or suborders are shown. *Brackets* indicate measured (*single asterix*) or inferred (*cross*) spectral properties of the visual pigments in each clade. Inferred spectral properties are based upon in situ hybridization or immunohistochemistry in combination with electrophysiological studies. References for measured spectra: *Apis mellifera* UV and blue, *Drosophila melanogaster* Rh1-Rh4 (see Briscoe 1999). References for in situ hybridization: *D. melanogaster* Rh5 (Papatsenko et al. 1997); Rh6 (Huber et al. 1997); *Hemigrapsus sanguineus* (Sakamoto et al. 1996), *Limulus polyphemus* 1 and 2 (Smith et al. 1993); *Papilio xuthus* PxRh1-3 (Kitamoto et al. 1998). Reference for immunohistochemistry: *D. melanogaster* Rh5 (Chou et al. 1996)

This, in turn, is dependent on the ratio of amino acid substitutions that are adaptively neutral to those that cause functional changes. If only a few amino acid substitutions are responsible for spectral tuning, then the overwhelming majority of amino acid changes used to reconstruct the phylogeny are going to reflect the actual history of the gene family.

Although we do not yet know how many amino acid residues are involved in spectral tuning of the insect visual pigments, we infer from research in vertebrates (Hunt et al. 1996; Neitz et al. 1991) that this number is comparatively small relative to the total number of amino acids of opsins. Therefore the pigments which fall into distinct clades are similar because they share a common ancestry, and not because natural selection has erased their history. This view is corroborated by the fact that opsins in the distinct clades have intron splice sites that are shared (Briscoe 1999).

In order to predict how easy it is to change spectral sensitivity as a response to specific selective pressures, it is necessary to see how many amino acid replacements are required to cause such changes. The majority of studies on spectral tuning have focused on the vertebrate visual pigments. These studies, which use mutagenesis to tease apart the amino acids relevant to the spectral properties of visual pigments, have made significant progress in furthering our understanding of the relationship between vertebrate opsin structure and function.

Almost all of the variation in cone pigment absorption spectrum has been accounted for: a mere one to nine specific amino acid substitutions are responsible for the 10-100 nm differences between the vertebrate cone pigments (Asenjo et al. 1994; Sun et al. 1997; Lin et al. 1998). This suggests that adaptive spectral tuning will be achieved relatively easy. On the other hand, previous studies have only looked at the ease of tuning between pigments that already exist in nature. These studies do not necessarily imply that spectral tuning in all spectral domains will happen as readily. For example, the fact that only three amino acid replacements are necessary to turn a human green receptor pigment (λ_{max}=530 nm) into a red receptor pigment (λ_{max}=560 nm) does not mean that generating a hypothetical pigment with λ_{max}=590 nm will require equally few changes.

In contrast to the large body of work that has led to the development of our current model of spectral tuning in the vertebrate pigments, only one study to date examines spectral tuning of insect opsins. Using a *Drosophila* transgenic expression system, Britt et al. (1993) studied the spectral properties of 13 chimeric opsins created by exchanging one or more transmembrane domains of the *Drosophila* Rh1 and Rh2 opsin genes. These chimeric opsin-encoding genes were introduced into a mutant *Drosophila* strain using P element germline transformation, and expressed in the R1-6 photoreceptor cells. The expression of the chimeric opsin genes in the R1-6 cells restored normal spectral sensitivity function to the mutant flies. Using electrophysiology and microspectrophotometry, Britt et al. (1993) measured changes in, respectively, the spectral sensitivities of the R1-6 photoreceptor cells and the absorption spectra of the chimeric visual pigments. This approach allowed them to monitor the effects of exchanging different Rh1 and Rh2 transmembrane domains on the spectral tuning of the opsins.

Britt et al. (1993) found that no single transmembrane domain was responsible for the 60 nm difference between Rh1 (480 nm) and Rh2 (420 nm). Exchange of a single Rh1 transmembrane domain (TM) for the corresponding Rh2 domain resulted in a 4-10 nm shift towards shorter wavelengths for most domains except TM 4, which resulted in an 11 nm shift to longer wavelengths. Replacement of almost all TM domains (TM2-7) was required to convert a Rh1 transgene into a Rh2-like opsin (436 nm). Unlike the three to seven amino acid residues responsible for tuning the primate red and green cone pigments (Neitz et al. 1991), which differ by 15-30 nm, the *Drosophila* Rh1 and Rh2 TM domains do not interact in an additive fashion. For example, replacement of only Rh1 TM6 (with a Rh2 TM6) results in a 12 nm shift to shorter wavelengths, and replacement of Rh1 TM7 alone results in a 4 nm in the same direction. However, replacement of both Rh1 TM6 and 7 simultaneously results in a 20 nm shift to longer wavelengths. The authors propose two mechanisms to account for spectral tuning, one of which is involved in large-scale or coarse spectral tuning, and the other in fine-scale tuning, as exemplified by the human red and green cone pigments. While fine tuning in the vertebrate pigments is apparently nearly additive in effect (Hunt et al. 1996), coarse tuning in the case of the *Drosophila* opsins occurs in a combinatorial manner, involves many more TM domains (Britt et al. 1993), and occurs over a larger evolutionary time scale. Finally, not all mutations in visual pigment genes are neutral or cause functional changes; some are downright deleterious. For example, three different missense mutations in the gene that codes for the human blue sensitive pigment all cause complete loss of the human short-wave receptor function, or even cell death (Deeb and Motulsky 1996).

Besides spectral fine tuning of existing photopigments, color vision systems can also evolve by changing the number of color receptor types. Although clearly the number of such types is conservative in many taxa, there is also some variation. For example, more than 40 species of Hymenoptera have 3 color receptor types most sensitive in the UV, blue, and green, but there are at least three species which have red receptors in addition (Peitsch et al. 1992). Such increases in color receptor types occur through gene duplication and subsequent spectral tuning (Briscoe 1999; Briscoe 1998a). How common are opsin gene duplications? The crab *Hemigrapsus sanguineus* (Sakamoto et al. 1996) and the horseshoe crab *Limulus polyphemus* (Smith et al. 1993) have unique opsin gene duplications, found in no other species so far. The problem is that we are not sure if these duplications are species specific, or whether they are more basal in larger taxonomic groups, such as genera or even orders. The butterflies *Papilio glaucus* and *Papilio xuthus*, for example, share two gene opsin gene duplications (Briscoe 1998a; Kitamoto et al. 1998); as do *Drosophila melanogaster* and *Drosophila*

pseudoobscura (Zuker et al. 1985; Carulli and Hartl 1992). All other insects that have been surveyed for opsins have been undersampled, so that we cannot be sure how constrained individual species are in terms of photoreceptor number.

So far we have emphasized changes in the opsin protein as a mechanism for the evolution of color vision. Arthropod chromophores come in five forms that are all derived from retinal. When extracted in ethanol, their absorption maxima differ by several nanometers: RAL1 (383 nm), RAL2 (400 nm), RAL3 (379 nm) and RAL4 (377 nm). Visual pigments reconstituted with the same opsin but different chromophores have slightly different absorption maxima (Seki and Vogt 1998). In nature, most species use only one chromophore but there are exceptions. In the firefly squid, for instance, there are two kinds of chromophore and one kind of opsin giving rise to three kinds of visual pigment (Seidou et al. 1990). Clearly, the particular chromophore used has an effect on spectral tuning, and in some cases, the opsins and chromophores may be coevolving.

5 Interindividual Variance

Variance between individuals is the raw material for evolution. This does not mean that the lack of such variance in extant species indicates the traits are not adaptive - in fact, if a trait is strongly favored by selection, it is likely that it becomes fixed in a population, and all variance might be eliminated (Endler 1986; Reeve and Sherman 1993). The results of many evolutionary "experiments" may no longer exist in our time, but variance is important, both for animals and for scientists studying adaptation. It allows populations to respond to ongoing changes in environmental pressures, and to colonize new habitats. Where there is lack of heritable variation, such changes cannot occur (Chittka 1997; Goldsmith 1990).

For us, interindividual variance offers the possibility to study predictions of adaptation. One phenotype may be favored in one photic environment and another phenotype in a different one. Many physiologists, however, treated such variance as noise, which needed to be eliminated by averaging large numbers of measurements from different animals. Sometimes this may be legitimate. Physiological measurements are often so noisy that extracting any information at all is not possible without averaging, and strong deviations from expected observation will in fact often mean that the measurement is imperfect, for example in electrophysiology: but we may have lost much valuable information through such averaging! Could it be that the reason for much of the conservatism in arthropod color receptors exists because there simply is no variance between individuals of some of the species in question? A large number of scientists have worked on, e.g., the color receptors of honeybees, and the results differed within studies as well as across studies, but the debate about these differences mostly focused on the possible contributions of artifacts or different electrophysiological methods (Menzel et al. 1986). To be sure, both of these may add noise to the measurements, but unfortunately, the possibility that interindividual variance may also contribute has not been considered.

In some vertebrates, conversely, such variance exists and has been well quantified, for example in guppies (Archer et al. 1987) and primates including humans (Deeb and Motulsky 1996; Shyue et al. 1995). In invertebrates, to our knowledge, only a single published study reported intraspecific variance between the sequences of visual pigments. Ayala et al. (1993) sampled five Rh3 alleles from each of four species in the *Drosophila melanogaster* subgroup and three alleles from *D. pseudoobscura*, and found a single amino acid polymorphism in one of the five surveyed species. One of us (A.B.) also found intraspecific amino acid variation in some of the opsin loci from *Papilio glaucus*. We cannot be sure, however, if any of these naturally occurring variants differ in their spectral sensitivities. Clearly, we need more data.

6 Pleiotropy – Selection Through Correlated Characters

A possibility rarely considered by sensory ecologists is that a sensory trait under scrutiny may be favored indirectly, through correlated characters, that do not necessarily have anything to do with the selective pressures associated with the perception of sensory stimuli. For example, Hope et al. (1997) tested the possibility that the pigments of abyssal fish might be adaptations to resist denaturation by the elevated pressure in deep waters, rather than adaptations to the photic environment. They rejected this possibility, but it is an approach well worth considering. Crandall and Hillis (1997) suggested that rhodopsin might have a previously unrecognized function, possibly in the control of circadian rhythms. This conclusion was based on lack of apparent differences in the rate of molecular sequence evolution between the opsin homologues of blind subterranean crayfish and their "sighted" relatives. In another example of possible nonvisual function of opsin, Alvarez et al. (1996) found that *Drosophila* Rh2 opsin is expressed in the testes of male flies, in addition to the ocelli (Pollock and Benzer 1988). If opsins have such dual functions, then spectral sensitivity may sometimes be an effect of pleiotropy, i.e., selection on correlated characters that we have not recognized.

7 Adaptation, Genetic Drift, and Biogeography

It is a widespread misconception of non-evolutionary biologists that beneficial mutations will rapidly spread through a population and eventually wipe out the pre-mutation genotype. However, new mutations are frequently lost immediately upon their introduction because of stochastic processes commonly designated *genetic drift* (Gould and Lewontin 1979). This may cause considerable evolutionary inertia particularly in large populations, or if the adaptive value of the new mutation is relatively small. There are many cases where local populations are kept considerably below their adaptive peaks because of continued gene flow with a large parental population (Stanton and Galen 1997).

Island populations may adapt more readily to local conditions because gene flow with the parental population is disrupted (Emlen 1978). On the other hand, deleterious mutations may also be common on islands, especially where the populations started out from very few animals, or experienced occasional strong reductions in population size (*founder* and *bottleneck effects*) (Endler 1986). It is important to keep these points in mind when considering sensory adaptations.

Of course, a particularly strong case for adaptation could be made if we found biogeographical differences in color vision systems within species, and if we could link these differences to particular environmental pressures exerted on different populations of the species. Unfortunately, no studies in insects are available to date, but a few observations on humans are worth considering. Humans have a single amino acid polymorphism at position 180 in the opsin protein. 62% of white Caucasians have serine at this position, and their red receptor absorbs maximally at 557 nm. The remaining 38% have alanine, and their red receptors are maximally sensitive at 552 nm. This difference has been shown to be relevant in color discrimination, and males with Ser at residue 180 have higher sensitivity to red light (Deeb and Motulsky 1996). This is just the variation we need for selection to act on! Even more interestingly, this polymorphism is different in different human populations: it is 80% Ser; 20% Ala in African Americans and 84/16% in Japanese (Deeb and Motulsky 1996). Are these differences adaptive, however? We favor the notion that these differences are due to genetic drift. Likewise, Ayala et al. (1993), who discovered a single amino acid polymorphism in *Drosophila,* concluded that the alleles are evolving by selectively neutral processes.

Another finding from human color vision is worth consideration. On the tiny South Sea island Pingelap, 75 of the 700 inhabitants are totally colorblind: they have only rods for scotopic vision. This is the result of a classic bottleneck effect. In 1775 this island had almost 1000 inhabitants, when it was struck by a typhoon, which reduced the population of the island to 20 survivors, one of whom was the king. After a few generations, the population was almost back to its pretyphoon level. Unfortunately, the king was carrier of the gene responsible for color blindness, so that today one third of the population carries the recessive gene that is responsible for this defect, and more than 10% of the population is phenotypically color blind (Sacks 1997). In other human populations, the frequency of this defect is about 1 in 30 000.

We do not rule out the possibility that adaptive differences in sensory systems exist between populations. In sticklebacks, for example, McDonald and Hawryshyn (1995) were able to link between-population differences with the light environment; but we wish to caution that not all differences between populations may be adaptive, and most likely random evolutionary processes can explain some of the differences between species as well. The most important message is that the data base is slim, and that we need more studies.

8 Selection Experiments

If we predict that animals will respond to different light habitats (or differently colored objects in their diet) by adjusting their spectral sensitivity, we must test this prediction by means of selection experiments. Such experiments are also necessary to separate phenotypic from genotypic variance. Given this importance, it is surprising that such studies are missing almost entirely in sensory ecology. To be sure, such experiments are demanding, but they are so essential to the reasoning of sensory ecology that we emphatically wish to encourage the taking on of such studies!

We are aware of only a single selection experiment on spectral sensitivity in guppies (Endler et al., subm.) and this experiment showed significant heritability for traits of the visual system. It also showed that animals may respond in multiple ways to the same selective pressure. For example, if animals are selected for higher sensitivity to long wavelengths, they may respond evolutionarily by adjusting their spectral sensitivity, by increasing their overall sensitivity, or by changing the relative strengths of postreceptor neuronal wiring (Endler et al., subm.).

9 Fitness Tests

If we cannot show that a trait confers greater fitness to its bearer, then we cannot claim that it is adaptive (Endler 1986). We must show, for example, that common wild-type animals indeed produce more viable offspring than do those with deviant traits, for example with a different set of color receptors. This is not trivial. Quite often, we will find that performance of one phenotype is better than another at a given task, but this may not have any impact on fitness. To return to the human red receptor polymorphism above, would we predict that someone with a red receptor with λ_{max}=557 nm will be able to *raise more children* than someone with a red receptor with λ_{max}=552 nm, even if it turns out that the person with λ_{max}=557 nm is slightly better at detecting red fruit? Probably not. Even the colorblind on the Island of Pingelap are able to detect and identify ripe fruits (Sacks 1997), so in conditions which are not strongly limiting, even strong deviations from the wild-type phenotype may not be selected against. In another example, Caine and Mundy (2000) were recently able to show that trichromatic marmosets were better at detecting orange fruit against a dappled foliage background than were their dichromatic conspecifics. Nevertheless, dichromats persist in the population.

Whether this occurs because of an unrecognized advantage of dichromats over trichromats at a task not related to frugivory remains to be tested. Without additional data, we also have to consider the possibility that the advantage of trichromats at detecting fruit is so small under natural conditions that it is irrelevant to fitness. The take-home message is that we need fitness tests in sensory ecology.

For such experiments, it is critical that animals be tested in environments which are realistic in terms of food distribution, predator and mate density, and abiotic parameters (Endler 1980). If we test the fitness of bearers of different color vision systems, e.g., bees with different color receptors, in an environment where detectability of flowers is not a limiting factor to search time, or where identification of the most rewarding flowers is not critical, then it is likely that we will find no fitness differences.

In this sense, pollinators such as bumble bees and solitary bees are ideal subjects because we need not worry about "building" an environment: they can forage in natural arrays of flowers, but because they raise all their offspring in one place, we can readily evaluate their fitness. The second prerequisite for fitness tests is that we find variance between individuals of a species in sensory traits. If there is such variation, we can either use naturally occurring phenotypes for fitness tests, or use strongly deviant phenotypes created by selection experiments.

If we do not find differences between individuals, we might consider creating phenotypes by experimental manipulation. This may be difficult in color vision systems (we cannot selectively paint over only one type of color receptor, or alter its spectral sensitivity), but in other sensory systems such manipulations appear feasible. An elegant alternative may be to test organisms in which mutants for sensory traits are available, such as *Drosophila melanogaster*. It is also feasible to create transgenic *Drosophila* with a modified repertoire of visual pigments (Britt et al. 1993), and to measure their fitness. Using these techniques, *Drosophila* has become a model to study the receptor-neuronal control of insect behavior; but these tools have not yet been used to study the role of such mechanisms for survival in nature. The difficulty, in this case, is to create an environment for *Drosophila* flies that is natural enough to allow for realistic tests of fitness. Unfortunately, however, we have not yet succeeded in creating transgenic animals more amenable to realistic fitness tests, such as bees.

10 Conclusion

We do not wish to abandon the notion that sensory systems are adaptive. Instead, we want sensory ecologists to consider constraint, evolutionary inertia, and random processes as possible alternatives to adaptive explanations, not to replace an adaptive scenario entirely. We are also emphasizing that if we want to demonstrate adaptiveness, it is not sufficient to show that sensory traits appear well (or even optimally) matched to the environment. Instead, we must show that animals carrying the sensory characteristics in question are fitter than those that do not. To do this, we must exploit heritable variation in natural populations, create new phenotypes by manipulation of existing traits or selection experiments, or use transgenic animals. Using such methodology, we may even eventually understand why bees have the color receptors they do, why most bee species lack red receptors, and why some other insects with entirely different life-styles have color receptors similar to bees.

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