before cell breakdown, and (ii) in individual cells and slides there may be both: areas having free acid phosphatase and areas having intact lysosomes. Our evidence suggests that acid phosphatase is liberated at the moment of cell breakdown by rupture of the lysosomes.

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Codominance of Visual Pigments in Hybrid Fishes

Abstract. *Visual pigments of lake char and brook char (Salmonidae) are based on two different proteins. Both proteins are present in first-generation hybrids between these species and they segregate in second-generation and backcross hybrids, as expected of a single-factor difference. This first genetic study suggests that shifts observed in the absorption spectra maxima of visual pigments are related to substitutions of amino acids in the visual proteins.*

The visual pigments are proteins conjugated with a carotenoid prosthetic group known variously as vitamin A aldehyde, retinene, or retinal (*1*). The absorption spectra of visual pigments depend in part upon the prosthetic group, which is either retinene-1 or retinene-2. Either retinene can combine with the appropriate protein (opsin) to form a visual pigment. Hence, for every opsin, there is a pair of visual pigments; a mathematical relation between the wavelengths of maximum absorbance (λ_{max}) of such pairs has been described by Dartnall and Lythgoe (*2*). Many spectrally distinct visual pigments have been examined, and two series have been described, one based on retinene-1 (*2*) and another on retinene-2 (*2, 3*). The differences within each series are believed to depend on variations in the structure of the opsins (*4*). However, little is known about opsins as proteins because their low concentration and insolubility make standard biochemical study difficult (*5*).

The visual pigments of the trout-like fishes called chars (family Salmonidae, genus *Salvelinus*) have recently been investigated by the method of partial bleaching (*6*). The brook or speckled char, *Salvelinus fontinalis* (Mitchill), has a retinene-1 pigment with a λ_{max} of 503 ± 1 mμ. The lake char, *S. namaycush* (Walbaum), has a different retinene-1 pigment with a λ_{max} of 512 ± 1 mμ. In addition, they also have the corresponding retinene-2 pigments. Fertile hybrids between these species can be produced by artificial fertilization and these hybrids are called "splake" (from speckled × lake). The first-generation hybrids (F_1 splake) appeared to have both parental retinene-1 pigments, together with the corresponding retinene-2 pigments (*6*). This evidence suggested that these pigments are inherited with codominance, in the same way as human M,N blood antigens are, but the hypothesis was uncertain because the visual pigments were studied only in retinal extracts from several animals and not in individual fish. Furthermore, the analysis of four visual pigments mixed together in the same extract is extremely difficult.

We have obtained additional material, including F_1 splake, second-generation hybrids (F_2 splake), and the backcross progeny of F_1 splake and brook char parents (*7*). The retinas of individual dark-adapted fish were frozen in 4-percent potassium alum solution and then stored at −20°C until used. Each opsin can be represented in two forms (as the retinene-1 and retinene-2 pigments), but the analytic difficulty this presents was avoided by bleaching the visual pigments and then regenerating them in the form of the retinene-1 pigment only (*8*). The frozen retinas were thawed, centrifuged to remove the alum solution, and washed twice with distilled water and once with 0.15*M* phosphate buffer (*p*H 6.5). They were extracted with 0.5 ml of freshly prepared 2 percent digitonin and treated with high-frequency sound to disrupt the visual cells. After centrifugation the supernatant extract was bleached exhaustively with orange light (610 mμ), which is not absorbed by the products of bleaching. To the bleached extract was added 0.02 ml of a solution of 11-*cis* retinene-1 in digitonin (*9*), and regeneration proceeded in darkness for 2 hours at 25°C. The regenerated extract was mixed with 0.02 ml of 0.8*M* neutral hydroxylamine solution to inactivate any excess retinene and was buffered with 0.05 ml of saturated sodium borate (final *p*H, 8.3 to 8.8).

Table 1. Frequency distribution of opsins in parental and hybrid chars. Each opsin is represented as the retinene-1 pigment.

Number of fish		
503_1 pigment	$503_1 + 512_1$	512_1 pigment
	Brook char	
7	0	0
	Lake char	
0	0	19
	F_1 splake	
0	12	0
	F_2 splake	
4	8	3
	Backcross (F_1 × brook char)	
8	7	0

The extracts were analyzed by the method of partial bleaching (*10*). In every experiment the photosensitive pigment was bleached in three stages (Fig. 1). The difference spectra of the regenerated retinene-1 pigments of both brook and lake char are identical to the difference spectra of the retinene-1 pigments obtained in separate experiments performed without prior bleaching and regeneration (Fig. 2). This shows that 11-*cis* retinene-1 is the visual isomer in these species. The experiments with retinal extracts of hybrid fish were all performed in the same way. The procedure gives three criteria for determining whether each extract contains a mixture of the two retinene-1 pigments (λ_{max} 503 and 512 mμ), or either pigment alone. As a consequence of the difference in λ_{max} of these pigments, the 512_1 pigment is more sensitive to red light. An exposure to red light bleaches extracts of

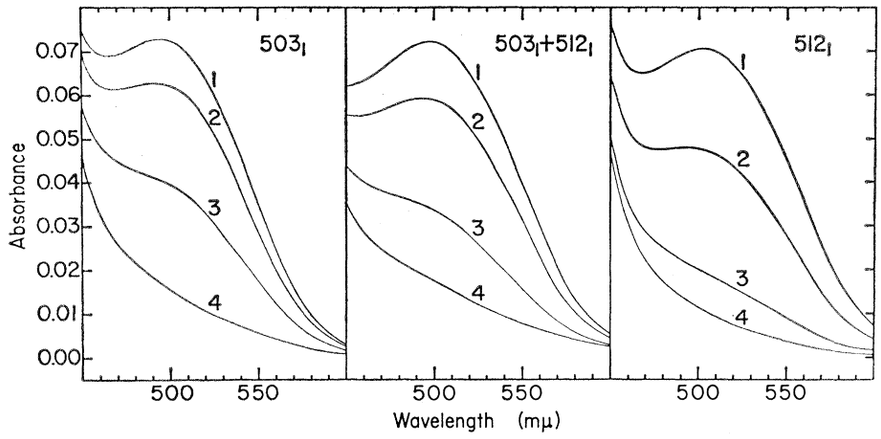

Fig. 1. Partial bleaching experiments with retinal extracts of three individual F_2 splake. Curve 1, absorbance spectrum of each extract following regeneration with 11-*cis* retinene-1; curve 2, after 2-minute exposure to 660-mμ light; curve 3, after 10-minute further exposure to 660-mμ light; curve 4, after 6-minute exposure to 610-mμ light. As indicated, the extracts contained a mixture of the retinene-1 visual pigments absorbing at wavelengths of 503 and 512, or either pigment alone. The proportions of the total pigment bleached at each stage reflect the greater sensitivity of the 512_1 pigment to 660-mμ light.

the 512_1 pigment more completely than it does the mixed extracts or those of the 503_1 pigment alone. (i) The nature of the extract is clearly indicated by the fractions of the total pigment that are bleached at each stage in the standard experiment (Fig. 1). (ii) Each extract can be assigned to one of the parental types or to an intermediate group (mixture) by comparing the total difference spectra, resulting from all bleaches taken together, with the difference spectra of the pure parental retinene-1 pigments (Fig. 2). (iii) The presence of either pigment alone or of a mixture of the pigments is shown by plotting the individual difference spectra resulting from each bleach. When either pigment is present alone, the difference spectra are all alike; with a mixture, the difference spectra shift in position as the experiment proceeds. The combination of these three criteria makes the final assessment secure.

The occurrence of the 503_1 and 512_1 pigments in individual brook and lake chars and the various hybrids is presented in Table 1. In the F_1 generation each individual contains both parental pigments. The F_2 splake have either visual pigment alone or a mixture of both. Backcrosses between F_1 splake and brook char contain either the 503_1 pigment or a mixture of 503_1 and 512_1 pigments. The results fit a ratio of 1 : 2 : 1 for the F_2 generation and 1 : 1 for the backcross.

The genetic data indicate that there is a single-factor difference between the visual pigments of brook and lake char. A lack of dominance is shown by the presence of both pigments in the heterozygotes. Most of the hybrid fish were immature and their sex was not determined, except for nine backcross animals, but the available data suggest that the inheritance is not sex-linked. The pattern of segregation is typical of a codominant allelic pair and extends this type of inheritance to the proteins of visual pigments. Multiple alleles probably exist, for the retinene-1 pigment of the closely related arctic char, *Salvelinus alpinus* (Walbaum), has a λ_{max} of 509 mμ (*6*). The visual pigments of hybrids between this species and other chars have not yet been examined. We suggest the genetic symbols Op^{503}, Op^{509}, and Op^{512} for the alleles that determine the opsins of *S. fontinalis*, *S. alpinus*, and *S. namaycush*, respectively. This nomenclature is based on the combination of the opsins with retinene-1 rather than retinene-2.

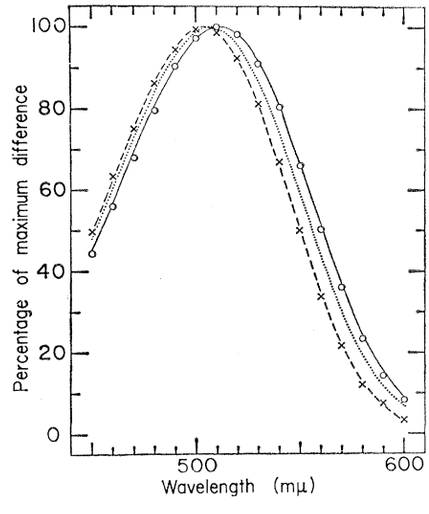

Fig. 2. Total difference spectra of regenerated retinene-1 visual pigments of three individual F_2 splake. Each curve is equivalent to the difference between curves 1 and 4 of Fig. 1, replotted on a percentage scale. Solid line, from an extract containing only the retinene-1 pigment absorbing at a wavelength of 512; dashed line, the 503_1 pigment alone; and dotted line, a mixture of these pigments. Open circle (○), difference spectrum of visual pigment 512_1 of lake char; crosses (×), difference spectrum of visual pigment 503_1 of brook char.

The results of this study touch on the relation between the structure of opsins and the spectral characteristics of visual pigments. Dartnall and Lythgoe (*2*) found that the known visual pigments do not form a continuous distribution with respect to λ_{max}, but cluster instead about certain wavelengths, and Bridges (*3*) demonstrated independently the existence of a series of "preferred positions" for retinene-2 pigments. Dartnall and Lythgoe (*2*) have suggested that the resonance of retinene is affected by the adjacent charge distribution on the opsin molecule. The experiments with hybrid chars show that a single-factor difference produces a sizable "jump" in λ_{max}, from 503 to 512 mμ. How might this genetic difference be expressed in the opsin molecule? Probably it is represented by the substitution of a single amino acid in a polypeptide. Possible specific mechanisms might be the alteration of a polar side chain of an amino acid or a changed spatial configuration of the opsin molecule. In either case, this substitution might disturb the resonant frequency of the retinene sufficiently to account for the observed change in λ_{max} of the visual pigment.

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Decision Theory in Studies of Discrimination in Animals

The work of Boneau, Holland, and Baker on wavelength discrimination in the pigeon (*1*) shows clearly that the tendency to respond to stimuli in which reward is never available is increased on trials immediately following reward. However, there was little if any decrease in the ability of the pigeon to respond *differentially* to closely spaced wavelengths, despite this general increase. The authors say they "can only describe the effect somewhat inexactly as a change in response bias."

I would like to suggest that their data are entirely consistent with an application of decision theory to the detection or recognition of stimuli in psychophysical experiments with human subjects. In this theory (*2*) the internal effects of stimuli are represented as Gaussian distributions of equal variance. When two stimuli differ physically by very little, their distributions overlap, and the observer is viewed as having to decide which distribution gave rise to his observation on a particular trial. In order to maximize expected value, he must weigh the probabilities and values of the outcomes and establish a criterion observation level, above which he always makes a certain response. The theoretical probability of response to a stimulus is given by the area under its distribution above the criterion. As the criterion varies, the probabilities of correct and incorrect responses covary according to a receiver-operating-characteristic (ROC) curve. This function has a parameter called d' which is equal to the difference between means of the distributions divided by their standard deviation. Invariance of d' under various experimental manipulations indicates that the discriminability of the stimuli has not changed, while the particular values of the response probabilities reflect the criterion level as well as discriminability.

If more than two stimuli are presented, as in the work of Boneau *et al.*, the theoretical probability of response to each stimulus is given by the area under its distribution which exceeds the criterion. If that criterion is lowered, response probabilities are increased. If these sets of response probabilities are plotted against each other, a curve of ROC form results, in which the parameter reflects the amount by which the criterion has changed.

In order to apply this theory to the data of Boneau *et al.*, I took the probabilities of responding to the stimuli in which rewards were never available from their Figs. 1 and 2 for trials immediately preceding ($T_0 - 1$) and immediately following ($T_0 + 1$) presentation of reward, and plotted them against each other. The results of this plot are shown in Fig. 1, together with a theoretical curve for a criterion change of 0.7 standard-deviation units.

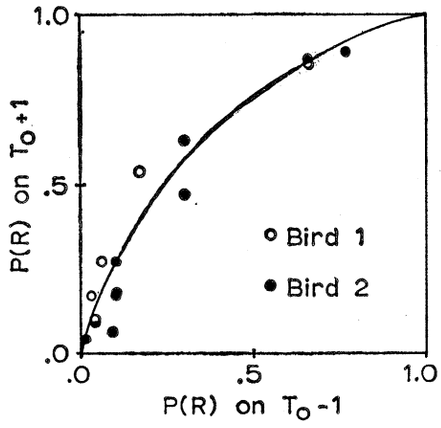

Fig. 1. Probability of response of two birds (*1*, figs. 1 and 2) to stimuli in which rewards are never available on trials immediately following reward ($T_o + 1$) plotted against probability of their response to the same stimuli on trials immediately preceding reward ($T_o - 1$). For description of theoretical curve, see text.

Both birds' data are reasonably well described by the curve.

It is by no means clear that decision theory is applicable in detail to operant discrimination experiments of this sort. In particular, we cannot evaluate rewards, the effort involved in responding, and the consequences of unrewarded responding in terms which make the maximization of expected value meaningful. However, the theory has been useful in integrating work on threshold processes in human psychophysics, and it may be fruitful in studies of animal discrimination performance as well. At the least, it may provide a technique for evaluating the effects of rewards on response bias.

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