Transmission of ocular media in labrid fishes

Ulrike E. Siebeck[*] and N. Justin Marshall

Vision, Touch and Hearing Research Centre, University of Queensland, Brisbane, Queensland 4072, Australia

Wrasses (Labridae) are the second largest family of fishes on the Great Barrier Reef (after the Gobiidae) and, in terms of morphology and lifestyle, one of the most diverse. They occupy all zones of the reef from the very shallow reef flats to deep slopes, feeding on a variety of fauna. Many wrasses also have elaborately patterned bodies and reflect a range of colours from ultraviolet (UV) to far red. As a first step to investigating the visual system of these fishes we measured the transmission properties of the ocular media of 36 species from the Great Barrier Reef, Australia, and Hawaii, California and the Florida Keys, USA. Transmission measurements were made of whole eyes with a window cut into the back, and also of isolated lenses and corneas. Based on the transmission properties of the corneas the species could be split into two distinct groups within which the exact wavelength of the cut-off was variable. One group had visibly yellow corneas, while the corneas of the other group appeared clear to human observers. Five species had ocular media that transmitted wavelengths below 400 nm, making a perception of UV wavelengths for those species possible. Possible functional roles for the different filter types are discussed.

Keywords: Labridae; ocular media; transmission; vision; yellow cornea

1. INTRODUCTION

The wrasses (Labridae) are the second largest fish family on the Great Barrier Reef after the family Gobiidae. Wrasses are diurnal carnivores that live in all zones of the reef from the shallow reef flat to the deeper reef slopes. Their feeding strategies and prey items show a high diversity, which is reflected in a large variety of body and mouth shapes (Choat 1969). The varied diet and habitat pose a number of different visual problems for the members of the family. Finding a planktonic particle in the water column is quite a different visual task from detecting small crustaceans on coral branches or detecting a fish in a high spatial frequency reef background.

Many wrasses are beautifully coloured. The spectrum of colours reflected includes wavelengths from 300 to 800 nm (Marshall 2000). Thus it is pertinent to ask if the high variability of behavioural tasks is reflected in the wrasses' visual system and vice versa. For instance, are those wrasses which transmit ultraviolet (UV) light to the retina making use of these wavelengths, and if so how does their behaviour differ from other non-UV sensitive species?

Measurements of ocular media transmission characteristics are a first step in an investigation of the visual system of fishes (Thorpe *et al.* 1993), because ocular media can act as a filter that limits the spectrum that reaches the photoreceptors. A recent study assessing the possibility of UV vision in 210 species of coral reef fishes, for example, showed that even in fishes with UV coloration, UV wavelengths are often absorbed before they reach the retina (Siebeck & Marshall 2000).

It may be advantageous for a fish exposed to high-intensity illumination to block UV radiation from reaching the retina, because UV radiation is known to cause photo-oxidative damage (Zigman 1971). Another advantage of UV-absorbing filters is that in certain illumination conditions, short wavelength-absorbing ocular media increase the contrast between prey items and the background (Douglas *et al.* 1998). This is principally due to the removal of highly scattered short wavelengths, which can corrupt the image. On the other hand the perception of UV wavelengths may be useful for colour communication between con- and heterospecific fishes with UV coloration. It has also been shown that planktivorous fishes are often UV sensitive because the contrast of small objects against a light-scattering background may actually be increased by extending sensitivity into the UV (Loew *et al.* 1993).

2. MATERIAL AND METHODS

Fishes were caught around the Heron Island and Lizard Island Reefs, Great Barrier Reef, Australia, around Hawaii and on the reefs around Key Largo, FL, USA. The fishes were caught using hand-nets and a barrier net. Specimens were kept in the aquaria of both research stations in Australia and the National Undersea Research Center in Key Largo until they were used for the measurements (maximum of one to two days). Prior to measurement each fish was killed with an overdose of MS222. The eyes were enucleated as soon after death as possible to avoid artefacts of tissue degradation and measured immediately (Douglas & McGuigan 1989).

(a) *Transmission measurements of whole eyes, lenses and corneas*

For the measurement of the whole eye, a window was cut into the back of the eye and the eye was then placed into a black velvet eye holder. Spectral transmission curves (300–800 nm) were obtained using a fibre optic spectrometer (S-2000; Ocean Optics Inc., Dunedin, FL, USA) and a pulsed xenon light

Table 1. *Transmission characteristics of 36 wrasse species*

(The species are sorted according to lens T50 values starting with the highest cut-off. For species with yellow corneas both the T25 and the T75 values are given for both whole eyes and corneas. For species with clear corneas T50 values were given for whole eyes, lenses and corneas. Species were from five different locations: He, Heron Island and Li, Lizard Island (Great Barrier Reef, Australia); Fl, Key Largo (Florida Keys); Ca, California; Ha, Hawaii. Behavioural notes from Randall *et al.* (1997). Habitat categories: C, coral; G, grassy sea beds; kelp, kelp forests; L, lagoon; open, open water column; Rf, reef flat; Ro, rocky outcrops or rubble; sh, shallow water; Sr, seaward reef. Food categories: B, benthic; c, crustaceans; fi, fish; g, gastropods; inv, invertebrates; m, molluscs; para, parasites; plan, planktivore; omni, omnivore; carn, carnivore.)

origin species	whole eye T25–T75 or T50 (nm)	lens T50 (nm)	cornea T25–T75 or T50 (nm)	behavioural notes	
				habitat	food
species with yellow corneas					
He *Gomphosus varius*	425–492	433	348–445	L, Sr, C; 1–30 m	Bc, fi, m
He *Thalassoma lunare*	432–485	431	386–489	L, C; 1–20 m	Binv
He *Coris gaimard* (juvenile)	—	430	347–493	Rf, Sr; 1–50 m	Binv, m, c
Fl *Halichoeres garnoti*	423–496	430	360–495	C, Ro; sh–de	inv
He *Halichoeres hortulanus*	420–558	430	414–489	L, Sr; 1–30 m	m, c, g
Li *Cheilinus chlorourus*	430–500	429	350–520	C, G; 2–30 m	Binv
He *Cheilinus diagrammus*	433–500	427	348–499	L, C; 1–120 m	fi
Ha *Thalassoma dupery*	428–495	429	380–495	C; 1–21 m	Binv, para
He *Anampses geographicus*	424–508	427	350–502	C; sh	Binv
He *Cheilinus trilobatus*	425–502	427	383–492	L, Sr; 1–30 m	Binv
He *Epibulus insidiator* (brown phase)	427–495	426	398–495	C, L, Sr; 1–40 m	fi, c
He *Halichoeres melanurus*	444–509	426	390–509	C; 2–15 m	Binv
He *Halichoeres ornatissimus*	424–515	426	392–513	C, L, Sr	Binv, c, m
He *Halichoeres prosopeion*	430–485	426	385–485	C, Ro; 5–40 m	Bm
He *Anampses neoguinaicus*	428–480	425	387–480	C; sh	Binv
He *Thalassoma lutescens*	427–485	425	382–514	Sr, L; 1–30 m	Binv
He *Hemigymnus fasciatus*	422–494	424	386–497	C, Rf; 1–20 m	Bm, c
He *Macropharyngodon choati*	421–510	424	390–496	C, Sr; 1–30 m	omni
Fl *Halichoeres bivittatus*	405–490	423	342–500	C, Ro; sh	Binv, fi
He *Hemigymnus melapterus*	422–493	422	342–515	Rf, L, Sr; 1–30 m	inv
He *Stethojulis strigiventer*	416–483	422	413–480	G, L; to 6 m	carn
He *Choerodon fasciatus*	396–493	397	387–500	C; 1–40 m	m, c
He *Choerodon cyanodus*	390–495	383	390–493	C, L, Sr; 4–40 m	m, c
He *Bodianus diana*	427–500	380	424–500	C, Sr; 6–25 m	Binv
Fl *Bodianus rufus*	413–503	377	395–504	C, Ro	c, para
Ha *Pseudocheilinus tetrataenia*	400[a]	370	368–455	Sr, C, Ro; 6–44 m	omni
Fl *Lachnolaimus maximus*	390–495	368	390–495	C; sh	m, c
species with clear corneas					
Ca *Oxyjulis californica*	435	435	not found[b]	kelp, Ro; sh	inv, para
Fl *Thalassoma bifasciatum*	434	432	387	G, C; sh	para
He *Hologymnosus doliatus*	423	422	424	C, Ro; to 30 m	fi, c
He *Labropsis australis*	419	422	388	C; sh	para
He *Labroides dimidiatus*	403	414	361	L, C; sh	para
He *Cirrhilabrus punctatus*	—	378	not found[b]	C, Ro	plan
He *Choerodon venustus*	402	375	398	C, Ro	Bm, c
Fl *Clepticus parrae* (juvenile)	380	371	342	C, open	plan
Ca *Semicossyphus pulcher*	414	370	414	Ro, kelp	m, c

[a] Measurement possibly taken through clear window of the cornea as the transmission curve resembled that of an eye with a clear cornea.

[b] No cut-off could be found within the measured wavelength band (300–800 nm).

source (PX-2; Ocean Optics), or with Sub-Spec (Andor Technology, Oriel Instruments, Belfast, N. Ireland, UK) (Siebeck & Marshall 2000). All samples were measured in air (Douglas & McGuigan 1989). Whole eyes and lenses were always measured through the centre. After the measurement of a whole eye, the lens and cornea were isolated and rinsed in freshwater to remove traces of blood and vitreous. In the case of yellow-pigmented corneas several spots on the cornea were measured.

All transmission curves were normalized so that they reached 100% transmission at 700 nm (Douglas & McGuigan 1989). To characterize the species with clear corneas, the conventional measure, the wavelength at which 50% of the maximal transmittance (T50) was reached was determined for whole eyes, lenses and corneas by using a linear regression (Douglas & McGuigan 1989; Siebeck & Marshall 2000). For species with yellow corneas the T50 was only determined for lenses, for the
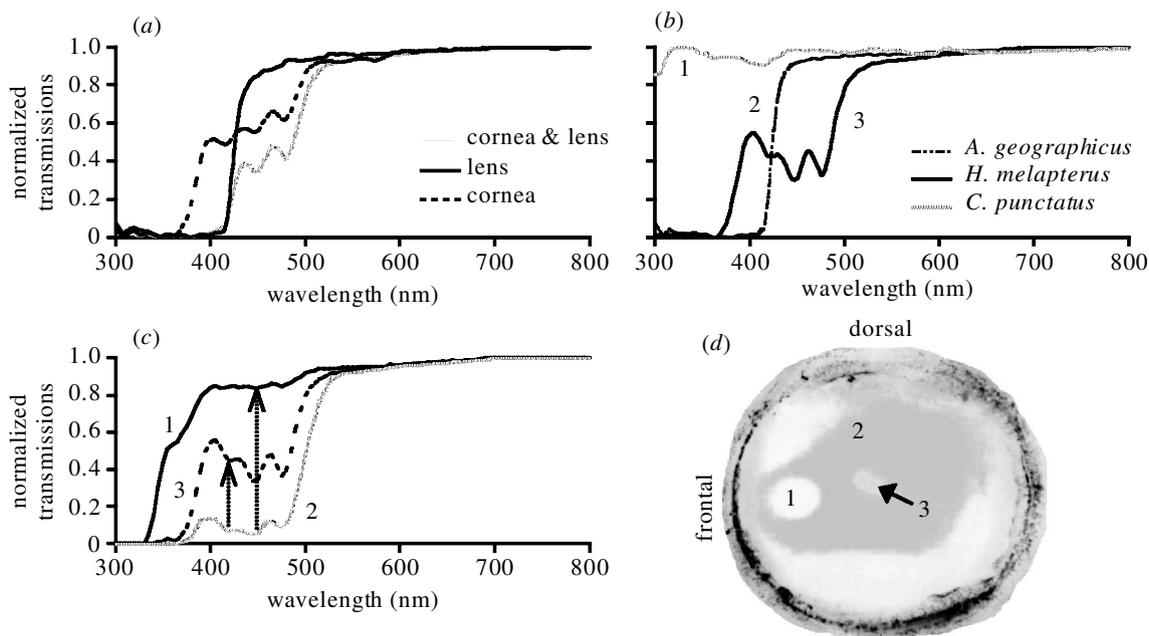
Figure 1. (*a*) Ocular media transmission of *Cheilinus trilobatus*. The graph demonstrates how the combination of the transmission of the lens and the cornea make up the whole-eye transmission. The combination of the lens T50 at 427 nm with the cornea T25 of 398 nm leads to a whole-eye T25 of 425 nm. At longer wavelengths the cornea influences the whole-eye transmission as it has a T75 of 492 nm, at which point the lens has already reached its maximum transmission. (*b*) Three typical corneal transmission curves, categories 1, 2 and 3. Category 1 only occurred in two species and had no cut-off in the measured wavelength band (300–800 nm). Category 2 occurred in nine species with clear corneas. Category 3 is typical for yellow corneas with carotenoid pigment as it has three intermediate maxima. (*c*, *d*) Example of a yellow cornea with patchy pigment distribution (*Hemigymnus melapterus*). Curves 1, 2 and 3 in (*c*) are measurements taken in the spots 1, 2 and 3 of the cornea in (*d*). There is a large difference between the transmission in the clear central or frontal window (1) and the dorsal densely pigmented area (3). The less-densely pigmented area in the centre of the cornea has intermediate transmission characteristics. The denser the pigment the lower the transmission between 400 and 500 nm.

corneas and whole eyes the T25 and T75 (25 and 75% transmission) were determined. The reasons for this are discussed in § 3(c).

3. RESULTS AND DISCUSSION

The ocular media of 36 species of wrasse were investigated. Table 1 lists the species according to the cut-off characteristics of the lenses. Labridae not only show a great diversity in diet and habitat but also the greatest variability of ocular media types in the families that have been investigated (Siebeck & Marshall 2000). Are these two factors linked?

(a) *Transmission characteristics of whole eyes*

The overall transmission of the whole eye is the combination of the transmission characteristics of the lens, the vitreous and the cornea. The filter that absorbs at the longest wavelength is the limiting filter of the whole eye. Therefore each individual ocular medium has to be measured to determine the filter responsible for the whole-eye cut-off. Vitreous humour was not measured in this study, but it was assumed that it did not significantly influence the overall transmission of the ocular media as either lens or cornea was found to be responsible for the transmission characteristics of the whole eyes. The shape of the whole-eye transmission curves therefore follows that of either one (cornea or lens) limiting filter, or a combination of the curves of both filters (figure 1*a*).

(b) *Transmission characteristics of lenses*

All lenses had smooth transmission curves with a single sharp cut-off (figure 1*a*). The spectral position of the cut-off was species dependent and varied between 370 nm (*Semicossyphus pulcher*) and 433 nm (*Gomphosus varius*). There are ten species with lenses that transmit UV light (table 1). Whether or not their whole eyes also transmit UV is dependent on the transmission properties of their corneas described below (§ 3(d)).

(c) *Transmission characteristics of corneas*

The curves of the corneas were smooth and generally (two exceptions) had a transition between maximal and minimal absorption at some point in the measured spectrum (300–800 nm) (figure 1*b*). The exact position of the transition and also the shape of the curve during the transition were both variable and species dependent. Based on the shape of the curve during that transition two distinct groups could be distinguished. In group 1, corneas were visibly yellow, and the slope of the curve was steep but with a step part-way through the curve (figure 1*b*, curve 3). Since more than one possible solution for T50 could be found due to the presence of the step, T25 and T75 values were determined instead. In group 2, corneas appeared transparent, and the slope of the curve was steep without the intermediate step (figure 1*b*, curve 2). There were two exceptions without a cut-off in the measured wavelength band (figure 1*b*, curve 1).

(d) *Group 1: species with yellow corneas*

Twenty-seven species belong to this group which is also characterized by yellow pigmentation of the corneas (table 1). In contrast to some other families with yellow corneas (Tetraodontidae, Balistidae and Monacanthidae) the pigment of labrid fishes is not retracted during low-light conditions (Kondrashev & Khodtsev 1984; Siebeck & Marshall 2000).

(i) *Distribution of the yellow pigment*

The distribution of the pigment was very patchy and the pattern varied between species. Most of the pigment was found in the dorsal and ventral parts of the cornea, therefore shading the eye from downwelling light and from light reflected off the sand or coral rubble on the bottom (Lythgoe 1979). Often the central areas of the corneas had less-pigmented or even unpigmented 'windows'. Since the density of the pigment was variable the transmission of a certain area depended on the density of the pigmentation in the measured area. In densely pigmented areas of the *Hemigymnus melapterus* cornea for example, wavelengths between 400 and 500 nm were transmitted to less than 10% (figure 1*c*, curve 2, and figure 1*d*, spot 2), while less-densely pigmented areas transmitted up to 60% in that wavelength band (figure 1*c*, curve 3, and figure 1*d*, spot 3). In the clear central area the transmission was more than 80% within that wavelength band (figure 1*c*, curve 1, and figure 1*d*, spot 1).

(ii) *Influence of the cornea on the whole-eye transmission*

Corneas were always responsible for the T75 value of the whole-eye curves, but not necessarily for the T25 values because the lens T50 values were larger than the T25 values of the corneas in 88% of cases. Figure 1*b* shows the transmission curves for *Cheilinus trilobatus* as an example. The cornea has a T25 of 383 nm while the lens T50 lies at 427 nm. Therefore the whole-eye transmission curve initially follows the lens curve. The T75 of the cornea is at 492 nm at which wavelength the lens has already reached maximal transmission. Therefore the second part of the whole-eye curve follows the cornea transmission curve.

There are only four cases in which the corneas were responsible for both the T25 and the T75 of the whole-eye transmission (*Bodianus diana*, *Bodianus rufus*, *Choerodon cyanodus* and *Lachnolaimus maximus*). All of those species have UV-transmitting lenses, but due to the absorption of the cornea the whole eye of *B. diana* blocks UV. The exact whole-eye values of *B. rufus* are not explained by the cornea or lens transmission values. It is possible that the measurements of the whole eye were not taken in the same spot as the cornea measurements, but offset to more densely pigmented areas and that therefore the cut-off values of the whole eye were overestimated.

There are two more species with lens T50 values below 400 nm, *Choerodon fasciatus* and *Pseudocheilinus tetrataenia*. In *P. tetrataenia* the cornea and the lens cut-offs are much lower than the whole-eye cut-off. It is possible that here also the whole-eye measurement was overestimated.

The central areas of the eyes of *C. cyanodus*, *L. maximus* and potentially also *P. tetrataenia* and *B. rufus* might transmit some UV light while at the same time the other areas of the eyes are relatively shaded through the sunglass like yellow pigment. Anatomical studies are necessary to determine whether this pattern is reflected in the distribution of photoreceptor types and, as any one point in space is visualized through the whole cornea, whether the pattern is significant.

(iii) *Chemical composition of the yellow pigment*

The shape of the curves of the corneas of 23 species is indicative of the presence of carotenoid pigments. Between maximal and minimal transmission were three intermediate absorbance maxima at about 425, 440 and 480 nm, which have been described as typical for carotenoid pigments (Appleby & Muntz 1979).

The transmission curves of three species (*B. diana*, *B. rufus* and *P. tetrataenia*) did not have the three intermediate maxima. The chemical composition of the yellow pigment in those corneas has not yet been investigated.

(e) *Group 2: species with clear corneas*

There are nine species with corneas that appear transparent to humans and seven of them have transmission curves with a single smooth cut-off. The position of the T50 values was species dependent and ranged from 342 nm (*Clepticus parrae*) to 424 nm (*Hologymnosus doliatus*). The two examples of curves that did not have a cut-off in the measured wavelength band transmitted all wavelengths to about 90% (*Cirrhilabrus punctatus* and *Oxyjulis californica*) (figure 1*a*).

There are four species with clear corneas that have lens T50 values below 400 nm. UV transmission is only achieved, however, in two (*Clepticus parrae* and *Cirrhilabrus punctatus*) of the four species. The other two (*Choerodon venustus* and *Semicossyphus pulcher*) have lenses that absorb UV, leading to a whole-eye cut-off above 400 nm. It is interesting that the only two species with UV-transparent ocular media have a planktivorous life style. This finding supports the hypothesis that planktivorous species benefit from UV transmission and possibly UV vision (Loew *et al.* 1993). The reasoning being that of all wavelengths, short wavelength radiation is scattered most strongly, so that the contrast between tiny food particles that absorb UV and the light-scattering background is enhanced.

It is also interesting that four species out of the seven UV-blocking species with clear corneas are cleaners, at least at some stage in their lives (*Labropsis australis*, *Labroides dimidiatus*, *Oxyjulis californica* and *Thalassoma bifasciatum*).

(f) *Possible advantages of yellow corneas*

(i) *Protection and a matched filter?*

In the shallow waters of the reef flat wrasses have to be able to cope with high-intensity radiation, including UV wavelengths. UV radiation is known to cause photo-oxidative damage not only in body tissues but also in the sensitive retinal tissues (Zigman 1971). It is therefore advantageous to have a pigment that absorbs those dangerous wavelengths before they reach the retina, thus protecting the sensitive retinal cells.

It is also possible that the yellow filter is mirrored by the environmental light field and the photoreceptor sensitivities in the different areas of the retina. In dorsal and ventral areas it reduces glare, chromatic aberration and also the danger of photo-oxidative damage, thereby

enhancing the quality of the retinal image and protecting the sensitive visual cells at the same time. Objects in the central retina will receive more light through the central clear patches of the cornea. The receptor sensitivities have to be determined to see if the distribution of different receptor types corresponds to the pattern of distribution of the yellow pigment.

(ii) *Contrast enhancement*

Another advantage of the yellow pigmentation is to enhance the contrast of prey items against a background, thus making it easier to detect and capture them. This mechanism is especially effective when the background is blue, as often occurs in the sea when objects are seen against down- or sidewelling light, similar to the mechanism that has been described for deep-sea fishes with yellow lenses (Douglas *et al.* 1998). The sensitivities of the photoreceptors are needed to confirm this. In the deep sea the difference of a blue bioluminescent signal and the blue downwelling background illumination is enhanced.

Yellow pigmentation leads to a loss of sensitivity in the short wavelength part of the spectrum. For some species much light below 500 nm is unavailable. This limitation, however, is perhaps not important for wrasses as they are highly diurnal, usually living in high-intensity light environments during the day. They are the first species to retire at night and among the last to resume activity in the morning (Randall *et al.* 1997). In other less-strongly diurnal families such as the Tetraodontidae, Monacanthidae and Ostraciidae a mechanism has been developed that allows the fishes to retract their yellow corneal pigment in low-light conditions, thereby increasing the sensitivity to short wavelengths and increasing their active time on the reef (Kondrashev & Khodtsev 1984).

REFERENCES

Appleby, S. J. & Muntz, W. R. A. 1979 Occlusable yellow corneas in Tetraodontidae. *J. Exp. Biol.* **83**, 249–259.

Choat, J. H. 1969 Studies on labroid fishes. II. A comparative study of the ecology of the Labridae and Scaridae. PhD thesis, University of Queensland, Australia.

Douglas, R. H. & McGuigan, C. M. 1989 The spectral transmission of freshwater teleost ocular media—an interspecific comparison and a guide to potential ultraviolet sensitivity. *Vision Res.* **29**, 871–879.

Douglas, R. H., Partridge, J. & Marshall, N. J. 1998 The eyes of deep-sea fish. I. Lens pigmentation, tapeta and visual pigments. *Prog. Ret. Eye Res.* **17**, 597–636.

Kondrashev, S. L. & Khodtsev, A. S. 1984 Light-dependent and humoral control of pigment transport in corneal chromatophores in marine fishes. *Zool. Jb. Physiol.* **88**, 317–325.

Loew, E. R., McFarland, W. N., Mills, E. L. & Hunter, D. 1993 A chromatic action spectrum for planktonic predation by juvenile yellow perch, *Perca flavescens. Can. J. Zool.* **71**, 384–386.

Lythgoe, J. N. 1979 *The ecology of vision.* Oxford, UK: Clarendon Press.

Marshall, N. J. 2000 The visual ecology of reef fish colours. In: *Animal signals. Adaptive significance of signalling and signal design in animal communication* (ed. Y. Espmark, T. Amundsen & G. Rosenqvist). Trondheim, Norway: Tapir Publishers. (In the press.)

Randall, J. E., Allen, G. R. & Steene, R. C. 1997 *Fishes of the Great Barrier Reef and Coral Sea*, 2nd edn. Bathurst, Australia: Crawford House Publishing.

Siebeck, U. E. & Marshall, N. J. 2000 Ocular media transmission of coral reef fish. Can coral reef fish see ultraviolet light? *Vision Res.* (In the press.)

Thorpe, A., Douglas, R. H. & Truscott, R. J. W. 1993 Spectral transmission and short-wave absorbing pigments in the fish lens. I. Phylogenetic distribution and identity. *Vision Res.* **33**, 289–300.

Zigman, S. 1971 Eye lens colour: formation and function. *Science* **171**, 807–809.