to the monkeys would be reduced, when the demand for food is actually at its greatest.

Much attention has been focused on the ability of man to change and modify his environment, whereas the similar ability of nonhuman primates has gone unnoticed. The white-faced monkey, as an integral part of its environment, disperses plant seeds, pollinates flowers, limits the reproductive ability of plants, lowers the numbers of certain insects (*2*), and apparently changes the morphology of a few plant species in the surrounding forest.

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Luminescent Systems in Apogonid Fishes from the Philippines

Abstract. *Luminescence has been discovered in five apogonid fishes from the Philippine Islands. The luminescent organ systems, which are of two types, are morphologically different from the systems in the Japanese cardinal fish,* Apogon ellioti, *and in the apogonid genus* Siphamia. *Extracts of the organs all show a luciferin-luciferase type of reaction and cross-react with extracts of* Apogon ellioti, Parapriacanthus ransonneti, *and* Cypridina hilgendorfii.

The marine teleost family Apogonidae contains about a hundred small, shallow-water species widely distributed in the Pacific and Indian oceans, as well as a few widely distributed pelagic species. Before our study was undertaken six luminous species were known: *Apogon ellioti* and five species of *Siphamia* (*S. versicolor, S. majimai, S. elongata, S. cuneiceps,* and *S. roseigaster*). We obtained material from five additional luminous apogonids from Cebu in the Philippines during May and October 1968: *Archamia fucata* (Cantor), 58 specimens; *A. zosterophora* (Bleeker), 18 specimens; *A. lineolata* Cuvier and Valenciennes, 62 specimens; *Apogon striata* (Smith and Radcliffe), 23 specimens; *Rhabdamia cypselura* Weber, 624 specimens. We also obtained specimens of *Apogon ellioti* (*1*) and of another luminous species, *Parapriacanthus ransonneti,* of the family Pempheridae (*2*).

Luminous organs are similarly constructed and arranged in *Archamia lineolata, A. fucata, A. zosterophora,* and *Apogon striata,* which have the luminous body located at the juncture of the pyloric ceca and the intestine. Light is also emitted from the proximal portion of the intestine and the pyloric ceca. In a specimen of *Archamia lineolata* 55 mm in length, the luminous organ is 1.6 mm in diameter. The luminescence is visible externally through the translucent thoracic muscle.

The luminous organ of *Rhabdamia cypselura* is different; the distal ends of a pair of pyloric ceca are transformed into luminous bodies or ducts (Fig. 1c, *PHOT*). A pair of transparent lens-like organs (*L*), encircled with black pigment lie in the ventral-lateral wall of the body cavity (Fig. 1b and 1d, *L*). The luminous ducts are attached to the lens-like organs and through them light is transmitted to the outside. In a specimen 44 mm in length, the diameters of the lens-like organs and luminous bodies (*PHOT*) are 1.0 mm and 0.6 mm, respectively.

These two distinct systems of luminous organs each differ markedly from the system of *Apogon ellioti,* and to an even greater degree from that of *Siphamia,* in the five species of which the luminescence is continuous and appears to be due to the presence of symbiotic luminous bacteria in the photogenic organs (*3*). Hence at least four types of luminescent organ systems are developed in apogonids.

The luminescent organ system of *Apogon ellioti* consists of an oval-shaped, lemon-yellow organ (1 to 2 mm in diameter) connected by a duct to the second bend of the intestine (*1*). This organ (the thoracic or anterior luminous duct) lies in the ventral, translucent keel muscle, through which the light passes to the exterior. Toward the posterior lie a pair of small organs (the anal or posterior luminous ducts) attached to either side of the rectum by a pair of ducts.

Light is produced in *Apogon ellioti* through a luciferase-luciferin (enzyme substrate) type of reaction, involving the oxidation of luciferin by molecular oxygen. When a dark, cold-water extract (luciferase) and a hot-water extract (luciferin) of an organ are mixed, light results (*4*). When luciferin and luciferase from two different organs are mixed, a light-emitting cross-reaction occurs.

Apogon luciferin will undergo a further light-emitting cross-reaction with the luciferase of both the luminous fish *Parapriacanthus ransonneti* and *Cypridina hilgendorfii,* a small marine ostracod crustacean (*4, 5*), two other species in which the luciferin-luciferase type of reaction has been demonstrated. Correspondingly, *Apogon* luciferase cross-reacts with *Parapriacanthus* and *Cypridina* luciferins. The luciferin and luciferase of *P. ransonneti* also give light-emitting cross-reactions with the luciferin and luciferase of *C. hilgendorfii* (*6*). Moreover, the chemical properties of the luciferins of *Cypridina, Apogon,* and *Parapriacanthus* are closely related or identical (*6, 7*).

The luminous organs of *Archamia fucata, A. lineolata,* and *Rhabdamia cypselura* were used in studies in vitro. Soon after the specimens were collected, the organs were removed, air-dried, and stored over $CaCl_2$. The organs were thoroughly dried under vacuum after they were returned from the Philippines. Crude luciferase was prepared from one to three organs ground in 3.5 ml of 0.1*M* sodium phosphate buffer, *p*H 6.8, in an all-glass homogenizer chilled in an ice bath. The homogenate was dialyzed overnight against 0.1*M* sodium phosphate buffer, *p*H 6.8, at 4°C and then centrifuged at 15,000*g* for 1 hour at 5°C; the supernatant was used at once. Crude luciferin was prepared from three luminous organs homogenized in 3.5 ml of boiling distilled water for 1 minute; the homogenate was then rapidly cooled in an ice bath and centrifuged at 15,000*g* for 5 minutes; the supernatant was used immediately.

When the organs were first ground in phosphate buffer, the extract exhibited a blue luminescence readily visible in the dark. The luminescence

lasted from several minutes to 4 hours, depending on the luciferin concentration. Dark extracts and crude luciferase solutions immediately became luminous when fresh luciferin (hot-water extract) was added, demonstrating the existence of a typical luciferin-luciferase system in all three species. The luminescence was dependent upon oxygen. Bubbling 99.99 percent argon through the extract extinguished the light in about 10 minutes (as observed after 30-minute dark-adaptation). Blowing air into the extract immediately restored luminescence to approximately its original intensity. This procedure was repeated several times.

The crude preparations of luciferin and luciferase from *Archamia fucata, Archamia lineolata,* and *Rhabdamia cypselura* were further used for cross-reaction studies with similar extracts from *Apogon ellioti* (from Kochi, Japan) and *Parapriacanthus ransonneti* (from Izu, Japan), and with highly purified luciferin and luciferase from *Cypridina hilgendorfii* (from Tateyama, Japan) (*8*). The luciferins and luciferases of *Archamia fucata, Archamia lineolata, R. cypselura, Apogon ellioti, P. ransonneti,* and *C. hilgendorfii* all cross-reacted, producing light. In addition, hot- and cold-water extracts were prepared from freshly dissected organs of *Archamia zosterophora, Archamia lineolata, Apogon striata, R. cypselura, Apogon ellioti,* and *P. ransonneti.* When these extracts were tested at Cebu, reciprocal cross-reactions were obtained between all pairs of these organisms.

Compounds that stimulate light emission from tissue extracts of luminescent systems from other organisms were tested on centrifuged extracts of dried organs from *Rhabdamia cypselura* (*9*). The most striking effect was obtained with fresh extracts (in phosphate buffer) slowly decaying in light intensity. Addition of either reduced nicotinamide-adenine dinucleotide (NAD) or reduced nicotinamide-adenine dinucleotide phosphate (NADP) caused an immediate, marked, and prolonged emission of light. The stimulating effect of reduced NADP appeared to be slightly greater than that of reduced NAD of equal concentration. Unreduced NAD or NADP had no effect on light intensity. Extracts of the thoracic light organ (dried or fresh) of *Apogon ellioti* (from Kochi, Japan), prepared by the same procedure, were either stimulated only slightly or not stimulated at all by reduced NAD and reduced NADP. Stimulation by reduced NAD has not previously been reported for the apogonid system, but extracts of the pempherid, *Parapriacanthus ransonneti,* have been observed to be slightly stimulated (*2*). Slowly decaying luminescent extracts of *Cypridina hilgendorfii* were not affected by these compounds. Adenosine triphosphate and reduced flavin mononucleotide also had no effect on any of the above extracts. No conclusions can be drawn concerning the effects of these cofactors on the luminescent systems of *Archamia fucata* and *Archamia lineolata* because of the low level of light intensity of the extracts.

The results indicate that a variety of luminescent systems has evolved in Apogonidae. However, the origin of luciferin and luciferase is still not known. One possibility is that apogonid fishes make use of the luciferase-luciferin in the bodies of luminous food organisms, such as *Cypridina.* This possibility has been investigated most thoroughly for *Apogon ellioti. Cypridina* thus far have not been found in the stomachs of *A. ellioti,* although more than 1000 specimens have been examined. Furthermore, *Apogon* luciferase is not likely to come from ingested *Cypridina* (unless one assumes that it is modified after ingestion), since the chromatographic, immunologic, and kinetic properties of *Apogon ellioti* and *Cypridina hilgendorfii* luciferases are significantly different (*10*).

There are no records from the Philippines of *Cypridina hilgendorfii,* although the ostracod, *Cypridina noctiluca,* does occur. The latter is a small (2.0 mm long) pelagic species, in contrast to the larger *C. hilgendorfii* (3.0 to 3.9 mm long), which lives beneath the sand. It is not known whether the Philippine apogonids feed on such pelagic organisms as *C. noctiluca* to obtain their light-emitting components. Such a mechanism, however, would explain the cross-reactions between the Philippine apogonids and *C. hilgendorfii* since the luciferins and luciferases of *C. noctiluca* and *C. hilgendorfii*

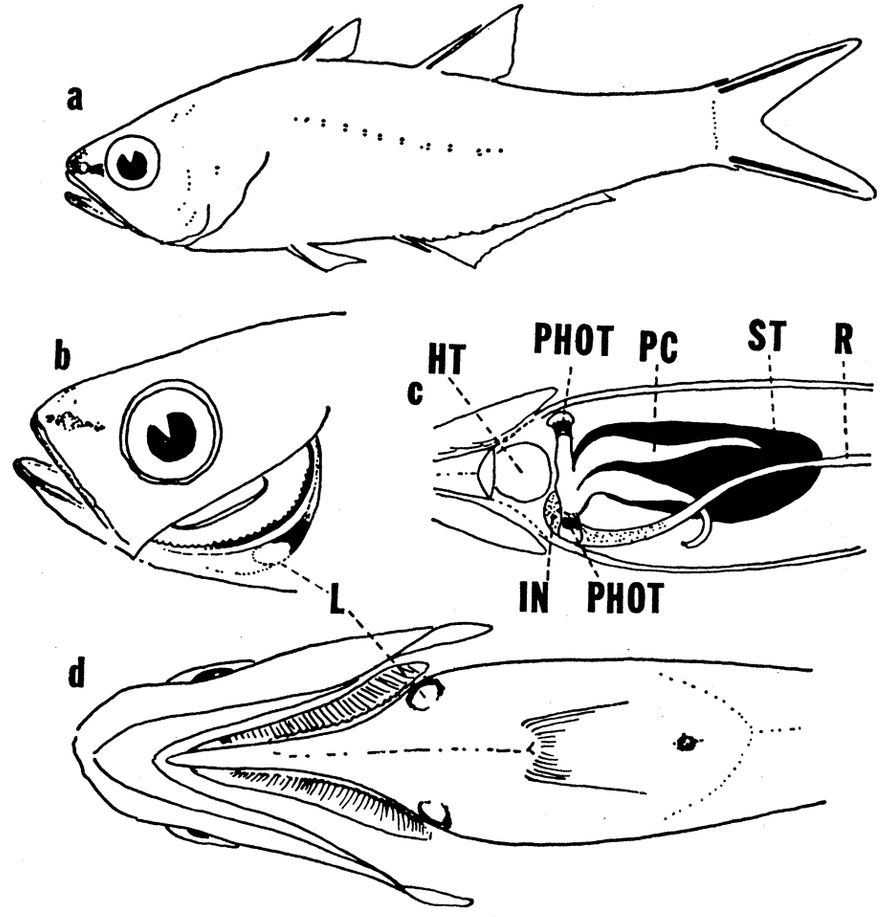

Fig. 1. (a–d) Diagram of *Rhabdamia cypselura* from the left lateral and ventral abdominal aspects showing the luminescent organ system. *PHOT,* luminous duct; *PC,* pyloric ceca; *L,* lens-like organ; *IN,* intestine; *R,* rectum; *ST,* stomach; *HT,* heart. Liver, reproductive organ, and swim bladder are not shown. Only the ends of two pyloric ceca are luminous; the intestine and rest of the pyloric ceca are nonluminous.

cross-react (*11*). The present observations suggest that luciferin from fishes and *Cypridina*, whether derived vicariously or synthesized independently, may be more widely distributed as a common substrate than heretofore believed.

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Schizophyllum commune: Gene Controlling Induced Haploid Fruiting

Abstract. *A single gene,* fis+, *is essential for induced haploid fruiting in* Schizophyllum commune. *It appears to segregate independently of the incompatibility factors.*

In tetrapolar Basidiomycetes, such as *Schizophyllum commune*, reproductive morphogenesis can be envisioned as consisting of two kinds of development. (i) The series of reactions—nuclear migration, formation of clamp-connections, synchronous nuclear division, and so forth—that establishes the dikaryon, and (ii) the maturative changes in the dikaryotic mycelium that culminate in meiosis and sporulation in the fruiting bodies. The process of dikaryosis and its control by the incompatibility factors have been studied extensively (*1*), but the cellular mechanisms that underlie the development of fruiting bodies in dikaryotic mycelia are largely unknown. It is also uncertain whether the incompatibility factors play an essential role in the fruiting process.

Although the formation of fruiting bodies normally occurs as a corollary of dikaryosis, fruiting structures may develop from haploid mycelia under certain circumstances (*2–4*). A recent study showed that haploid fruiting is inducible by an exogenous chemical agent or agents (*4*). The inducing factor was first isolated from mycelial extracts of the imperfect fungus *Hormodendrum cladosporoides* (*Cladosporium*) and was later obtained from fruiting bodies of *S. commune* and *Agaricus bisporus*. Methods of extraction, partial purification, and a biological assay were established for the inducing substance or substances.

An initial survey showed that very few homokaryons of *S. commune* would respond to the fruiting-inducing substance or substances (FIS). These observations raised the question as to the extent and nature of the genetic contribution to FIS-induced fruiting. We now report evidence of a single gene that functions in FIS-induction of haploid fruiting in *Schizophyllum commune*.

When an FIS-inducible strain was crossed with a compatible, noninducible homokaryon and a number of the resulting offspring were exposed to FIS, inducibility and noninducibility showed a 1:1 segregation ($P = .3$ to .5 in cross 1 of Table 1). Each monosporus mycelium was tested in triplicate, and the inducible cultures usually fruited by the 5th day after the application of FIS to the margins of the 4-day old colonies. The pattern of segregation indicated a single-gene difference among the two classes of progeny. The hypothesis of a single gene with two alleles, henceforth symbolized *fis*+ and *fis*−, was tested in three additional crosses (Table 1).

If neither of the mated strains carried the *fis*+ allele, none of the progeny would be expected to respond to FIS. On the other hand, when both compatible homokaryons possess the *fis*+ allele, all of their progeny would be expected to fruit following exposure to FIS. Both of these expectations were realized. Inducibility by FIS can thus be added to the several genetic factors previously demonstrated to be important in the expression of haploid fruiting in *S. commune* (*2*, *5*).

The *fis* gene has segregated independently of the incompatibility factors in all tests conducted thus far (for example, $P = .3$ to .5 in cross 1 of Table 1). It is thus possible in compatible crosses between FIS-inducible and FIS-noninducible homokaryons to associate the *fis*+ allele with the incompatibility factors in any desired combinations.

Table 1. Mode of inheritance of FIS-induced haploid fruiting.

Mated strains	Offspring tested (No.)	*fis*+	*fis*−
A41 B41 fis+ × *A51 B51 fis*−	84	38	46
A41 B41 fis+ × *A51 B51 fis*+	30	30	0
A41 B41 fis+ × *A43 B43 fis*+	30	30	0
A41 B41 fis− × *A51 B51 fis*−	20	0	20

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