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Source: Zoological Science, 25(12) : 1212-1219

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.25.1212

ZOOLOGICAL SCIENCE **25**: 1212–1219 (2008)

Fluorescent Pigment Distinguishes Between Sibling Snail Species

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Traditional taxonomy of shell-bearing molluscs does not generally use soft-body coloration. However, the land snails *Bradybaena pellucida* and *B. similaris* have been distinguished only on the basis of the color of the soft-body visible through the shell. Thus, the taxonomic status of the two species has traditionally been questionable. We found that dense spots of pigments embedded in the dorsal mantle are responsible for the yellow coloration of *B. pellucida*. Similar spots in *B. similaris* are white and less densely aggregated in whorls further from the apex, and the brown color of the hepatopancreas is visible through the shell. The yellow pigments of *B. pellucida* seep out with mucus from the body in natural and laboratory conditions. The two species became externally indistinguishable after 30 days of laboratory feeding, because the yellow spots disappeared in *B. pellucida* and the color of the hepatopancreas changed from dark brown to pale brown in both species. Irradiation with ultraviolet A demonstrated that the yellow pigment of *B. pellucida* fluoresces. Adult specimens of the two species were distinct in penial microsculpture, with F_1 hybrids intermediate in form. Populations of the two species differed significantly in allelic frequencies at four allozyme loci. Therefore, *B. pellucida* and *B. similaris* are morphologically and genetically distinct. The fluorescent yellow pigment distinguishes *B. pellucida* from *B. similaris* under natural conditions despite its environmental dependence.

Key words: mantle color, penial sculpture, hybrid, allozyme, *Bradybaena*, Pulmonata, Gastropoda

INTRODUCTION

Coloration of the soft body is not generally used as a key trait in the taxonomy of shell-bearing molluscs, largely because living or fresh specimens are necessary to examine the color, and color variation is not as readily preserved as structural variation in the shell or soft body (Stum et al., 2006). In slugs, mantle coloration cay vary genetically (Williamson, 1959) or depending on food within species and thus has confused the taxonomy of closely related species (Reise, 1997; Jordaens et al., 2001). Concerning mantle coloration of pulmonate snails, however, almost no cases of acquired change and only a few examples of genetic variation have been explicitly examined (Cain, 1959; Murray, 1963; Cain et al., 1968; Wolda, 1969; Richards, 1973, 1978), perhaps because in general shells attract far more immediate attention.

Differences in mantle coloration between *Bradybaena pellucida* Kuroda and Habe (Habe, 1953) and *B. similaris* (Rang, 1831) provide opportunities for biological studies of mantle color, because of their suitable characteristics for field and laboratory studies (Asami et al., 1997b; Asami and Asami, 2008). *Bradybaena similaris* (Férussac, 1821), which has frequently been cited for that species, is a nomen nudum (Cowie, 1997). *Bradybaena pellucida* was originally described as differing slightly from *B. similaris* in having a taller shell with wider whorls, a thinner ostracum, and a shiny pale-yellowish periostracum, through which the hepatopancreas appears bright yellow near the apex, while exhibiting no clear differences in the genitalia (Habe, 1953). However, the distinct yellow of *B. pellucida* is not the color of the hepatopancreas but of the dorsal mantle (Asami et al., 1997b; Seki et al., 2002). In contrast, *B. similaris* exhibits the colors of the internal organs through the nearly transparent mantle with some white portions at the apex (Fig. 1A). The color of the integument of the soft body when extended outside the shell is whitish to pale light brown in *B. pellucida* but darker brown in *B. similaris*. The color of the periostracum is often pale whitish yellow in *B. pellucida* but brownish in *B. similaris* (Fig. 1B). Shell differences are inconsistent, however, because of variation within each species. The two species also have similar genetic systems controlling the shell color and banding polymorphism (Asami et al., 1993, 1997a; Asami and Asami, 2008). Thus, *B. pellucida* and *B. similaris* have been reliably distinguished only by mantle color. However, when *B. pellucida* is fed on artificially composed food (Asami and Ohbayashi, 1999), it loses the yellow mantle color and becomes difficult to separate from *B. similaris* (Asami et al., 1997a, b). Thus, it has been questionable whether the two species are genetically or morphologically distinguishable from each other.

Bradybaena pellucida is endemic to Japan and is distributed mainly in the western region as well as on the southern Boso peninsula and some other places in the cen-

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doi:10.2108/zsj.25.1212

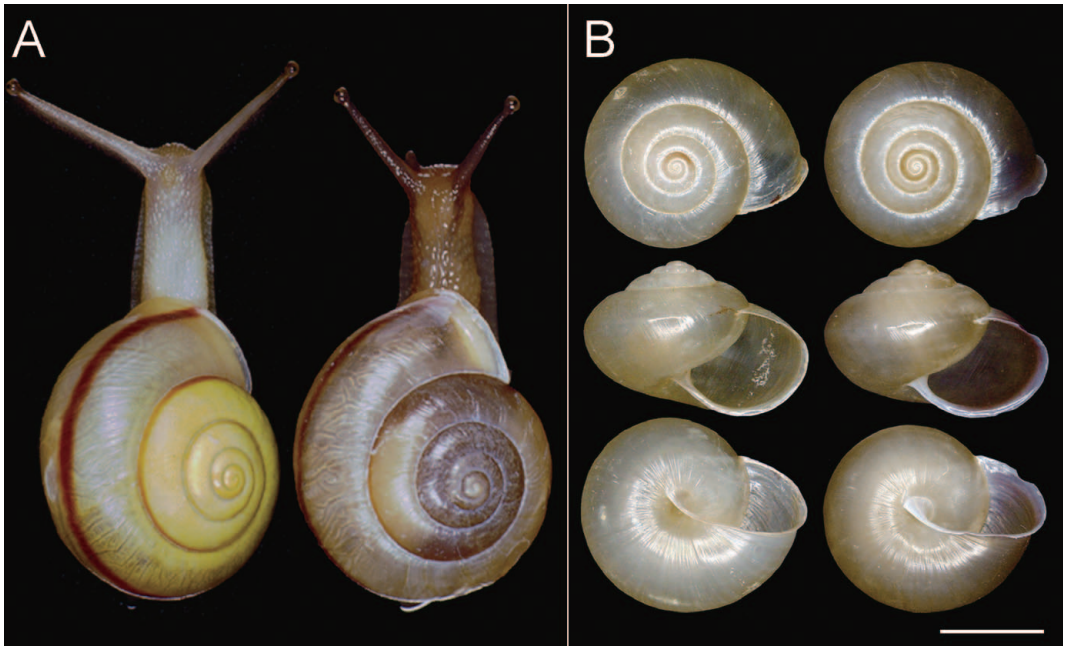

Fig. 1. External morphology of *Bradybaena pellucida* (left in each panel) and *B. similaris* (right in each panel). **(A)** Living adults of the light-brown banded morphs. **(B)** Empty shells of adults of the light-brown unbanded morphs; scale bar, 5 mm.

tral part of Honshu, while *B. similaris* is cosmopolitan and inhabits most of the islands of Japan (Komai and Emura, 1955; Asami et al., 1997b; Seki et al., 2002). Mixed colonies of *B. similaris* and *B. pellucida* are found where their distributions overlap, although their patchy distributions may be negatively associated with each other (Asami et al., 1997b; Seki et al., 2002).

We examined differences between *B. pellucida* and *B. similaris* in mantle pigmentation, penial morphology, and allozymes. This paper demonstrates that the fluorescent secondary metabolite, which *B. pellucida* acquires from the natural environment, corroborates the genetic distinction of the two species.

MATERIALS AND METHODS

To examine mantle color and genital morphology, we collected live adults and juveniles (6 to 7 mm in diameter) of *B. pellucida* at Shiroyama, Tateyama, and of *B. similaris* at Miyama, Funabaashi, Japan (Fig. 2). For the survey of allozyme variation, we collected samples from three single-species populations of each species, including the above two sites on the Boso Peninsula (Fig. 2).

Within two days after collection, we removed the shells of 20 adults and 20 juveniles of each species and examined the dorsal mantle under a dissecting microscope. In addition, we fed 20 adults and 20 juveniles of each species food composed of powdered oatmeal, egg shell, and dry cat food under controlled laboratory conditions (16 h light/8 h dark, 25°C) for 30 days, according to the protocol of Asami and Ohbayashi (1999). Then we examined the dorsal mantle in the same way as described above.

Because of the conspicuous brightness of the yellow pigmentation of *B. pellucida*, we tested whether the yellow substance emits fluorescence under ultraviolet A radiation by using a UV fluorescent lamp (Blacklight, NEC, FL20S-BLB; peak wave length: 360 nm) in the dark. For each species, we examined (1) the dorsal mantle of 20 adults and 20 juveniles within two days of collection, (2) the dorsal mantle of 20 adults and 20 juveniles that were fed in the laboratory for 30 days, and (3) the empty shells of 20 adults.

We raised 20 juveniles of *B. pellucida* and of *B. similaris*, collected from Shiroyama and Miyama, respectively, to maturity in individual plastic containers (62×50×25 mm) and allowed them to reproduce in 10 interspecific pairs, according to the protocol of

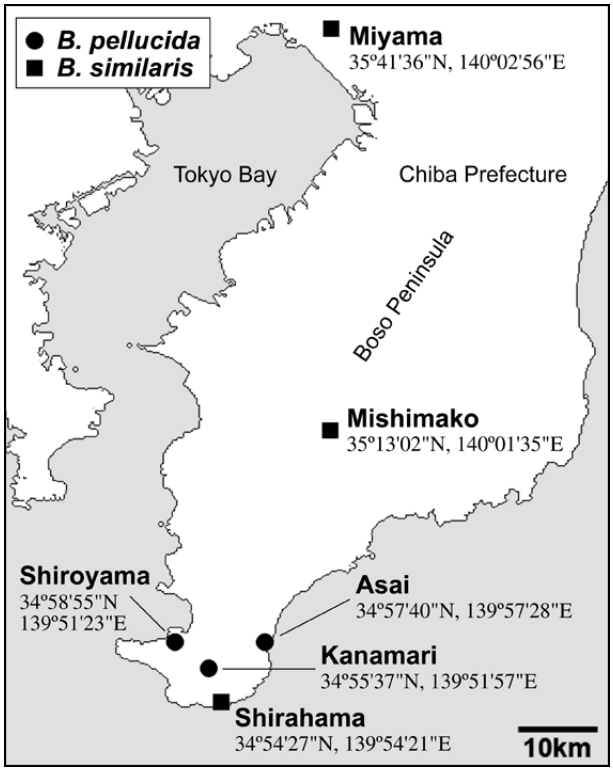

Fig. 2. Collection sites for *Bradybaena pellucida* and *B. similaris* in Chiba Prefecture, Japan.

Asami and Ohbayashi (1999), except for using sand as the oviposition substrate. We raised five offspring of each of four interspecific pairs to maturity. After examining mantle color and genital morphology, we confirmed that the offspring were interspecific hybrids rather than progeny produced by selfing, on the basis of their heterozygotic allozyme profile at the *Pgm* locus (Table 1). As controls, we raised to maturity five offspring of each of four intraspecific pairs of each species under the same laboratory conditions as the hybrids.

We dissected the genital system of 20 adults of each of the two species collected from the wild, and 20 adults of each species and the F_1 hybrids obtained from the laboratory crosses. We fixed the genitalia in 70% ethanol. Under a dissecting microscope, we examined possible differences in the gross morphology of the genitalia and of the sculpture of the inner wall of the penial tube, opened in a consistent orientation by using a razor blade.

A piece of hepatopancreas of each living specimen was homogenized in an equal volume of cold 0.01 M Tris-HCl buffer (pH 6.8) and centrifuged at 10,000×*g* for 10 min at 4°C. The supernatant was electrophoresed on a horizontal starch gel using four buffer systems for 11 enzymes (Table 1). Allozymes were observed by the staining methods of Murphy et al. (1996). Based on the electrophoretic patterns, we estimated allelic frequencies at 12 putative loci.

RESULTS

Pigment spots

In both *B. pellucida* and *B. similaris*, all adults and juveniles collected from the wild had many spots, each 0.01–0.02 mm in diameter, in the dorsal mantle underlying the shell (Fig. 3). The spots of *B. pellucida* were bright yellow, whereas those of *B. similaris* were white; both types were visible through the translucent shells. The pigment spots were only in the dorsal mantle, not in other parts of the mantle or in other organs. In most adults of *B. pellucida* collected from the wild, the yellow spots coalesced, covering the dorsal surface of the spire, which appeared thick and homogeneously yellow. Thus, the color of the hepatopancreas was not visible through the shell in living adult *B. pellucida*. The yellow spots were sparse in the outer whorls. The hepatopancreas color of adult *B. pellucida* was visible along the narrow zone between the whorls only when the shell was removed (Fig. 3A). In contrast, the white spots of *B. similaris* were dense only at the apex and sparsely distributed in the dorsal part of the spire mantle. Thus, *B. similaris* mainly exhibited the dark brown color of the hepatopancreas through the mantle and the shell (Fig. 3B).

The density and distribution of spots in the mantle varied among individuals from the wild. The yellow spots of juvenile *B. pellucida* from the wild were less dense than those of adults; thus, their hepatopancreas color was visible through the shell. In the habitat of *B. pellucida*, the typical yellow color was not detectable in most juveniles smaller than 4 mm. In the adults and juveniles collected, however, the yellow spots were too dense around the apex to examine whether the white spots were also present.

Under laboratory conditions, all the wild adult and juvenile specimens of *B. pellucida* began to lose the yellow color from the first day of the experiment, while the white spots of *B. similaris* showed no detectable change. In the containers in which adult or juvenile *B. pellucida* were kept individually, mucus trails on the moist paper towel lining the bottom were often translucent yellow, especially at the beginning of laboratory feeding, suggesting that the yellow pigment seeps out of the body, presumably with the mucus. We confirmed that this also occurs in the wild when individuals crawl on white tissue paper.

Although individuals varied in how fast the yellow color disappeared, all adults and juveniles of *B. pellucida* completely lost the yellow pigment within 30 days. However, we found no detectable sign of changes in feeding or other behavioral activities. By removing the shells on the 30th day of feeding, we found only white spots in the dorsal mantle in both *B. pellucida* and *B. similaris* (Fig. 3). These spots were as sparse as the spots in the mantle of *B. similaris* before the feeding experiment. The color of the hepatopancreas itself drastically changed from dark brown to pale brown in both *B. pellucida* and *B. similaris* (Fig. 3). Because of the changes in the amount of yellow spots and the hepatopancreas color, the adult and juvenile specimens of the two species became indistinguishable from each other with respect to the soft-body coloration underlying the shell.

Under 360-nm ultraviolet (UVA) radiation, all the living adults and juveniles of *B. pellucida* collected from natural habitats emitted whitish fluorescence, which was visible to human eyes in the dark. By UVA irradiation of the soft body without the shell and of a sheet of dorsal mantle removed from the body, we confirmed that only the yellow portion of the dorsal mantle emits the fluorescence. The yellow pigments found with mucus trails on paper towel were also fluorescent, even after the paper towel and mucus had

Table 1. Enzymes and loci analyzed, and buffer systems used in starch-gel electrophoresis.

Enzyme	Locus	E.C.	Buffer system	Citation
Sorbitol dehydrogenase	*Sdh*	1.1.1.14	Discontinuous Tris-citrate	a
Lactate dehydrogenase	*Ldh*	1.1.1.27	Tris-borate-EDTA pH9.1	b
Malate dehydrogenase	*Mdh-I, II*	1.1.1.37	Tris-maleate	a
Isocitrate dehydrogenase	*Idh*	1.1.1.42	Tris-maleate	a
Purine-nucleoside phosphorylase	*Pnp*	2.4.2.1	Discontinuous Tris-citrate	a
Aspartate aminotransferase	*Aat*	2.6.1.1	Tris-citrate	c
Fructose-bisphosphatase	*Fbp*	3.1.3.11	Tris-borate-EDTA pH9.1	b
Cytosol aminopeptidase	*Cap*	3.4.11.1	Tris-citrate	c
Mannose-6-phosphate isomerase	*Mpi*	5.3.1.8	Tris-borate-EDTA pH9.1	b
Phosphoglucoisomerase	*Pgi*	5.3.1.9	Tris-borate-EDTA pH8.0	c
Phosphoglucomutase	*Pgm*	5.4.2.2	Tris-citrate	c

Citations: a, Selander et al. (1971); b, Werth (1985); c, Shaw and Prasad (1970).

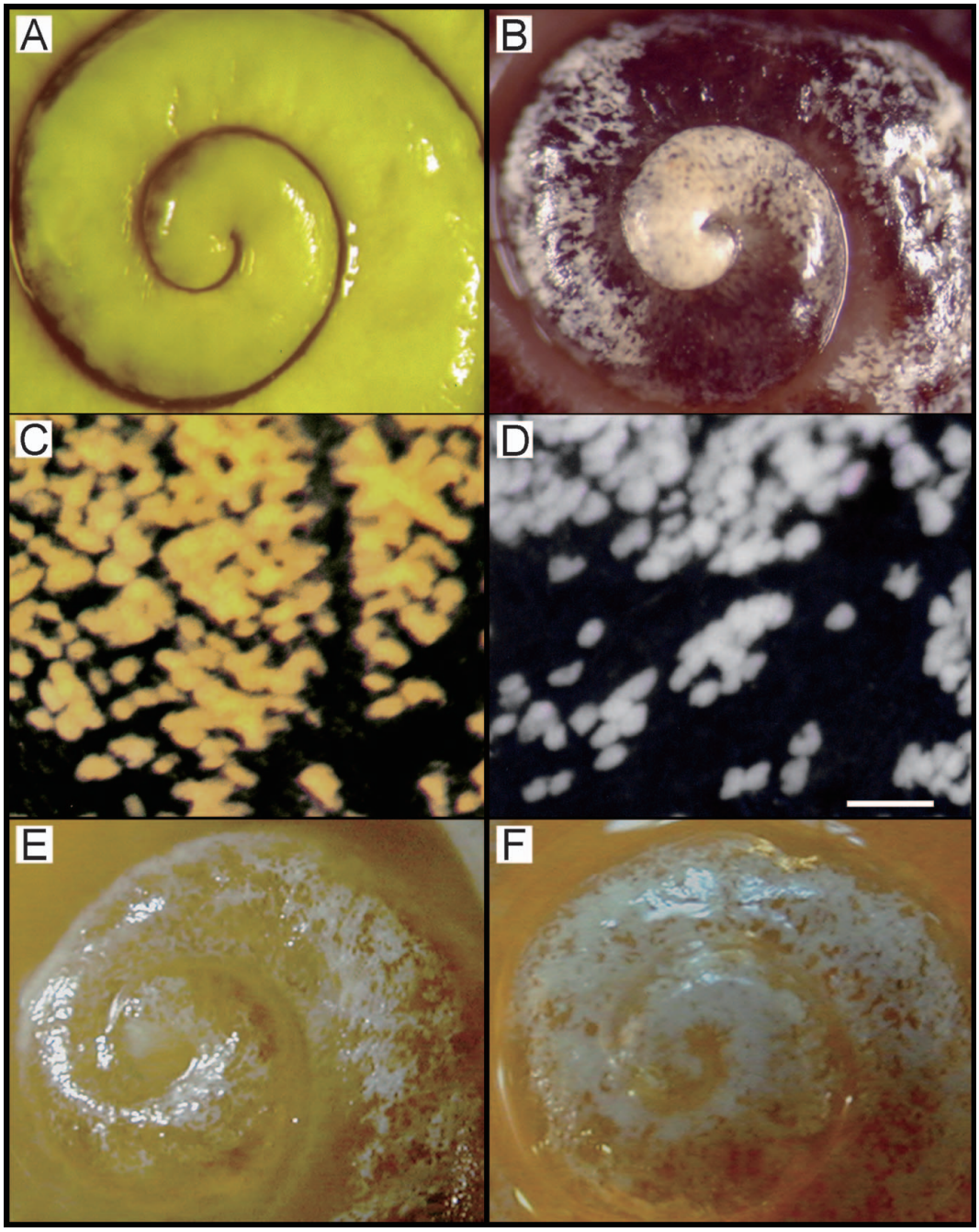

Fig. 3. Environment-dependent changes in mantle and hepatopancreas coloration in adult *Bradybaena pellucida* (left panels) and *B. similaris* (right panels). **(A, B)** Dorsal view of the spire without the shell before laboratory feeding. **(C, D)** Spots embedded in the mantle before laboratory feeding; scale bar, 0.05 mm. **(E, F)** Dorsal view of the spire without the shell on the 30th day of laboratory feeding.

dried. In contrast, we detected no fluorescence in the soft bodies of *B. similaris* or of *B. pellucida* that had lost its yellow pigment. Empty shells of the two species exhibited neither yellow pigment nor fluorescence. None of the 20 F_1 hybrids, which were raised in the laboratory, acquired the yellow pigment nor emitted fluorescence.

Genital anatomy

There was no detectable difference in the gross morphology of the genitalia between *B. pellucida* and *B. similaris*. However, the patterns of microsculpture inside the penial tube were different between the two species, though they varied little among conspecific adults. The penial sculpture of *B. pellucida* (Fig. 4) is characterized by rhomboidal pustules that are separated by straight, thin furrows and cover more than half the internal wall from the verge of the vas deferens (upper edge in Fig. 4). At the verge, several thin, obscure longitudinal pilasters are present. Some of these are partly zigzag crenulated. The discrete pustules fuse to form thick longitudinal pilasters along about one-third

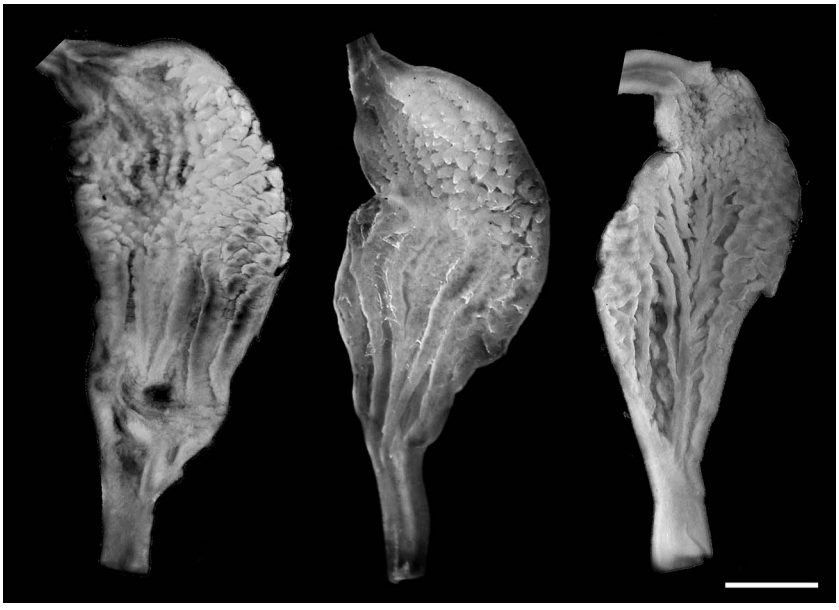

Fig. 4. Sculpture on the internal wall of the penis of adult *Bradybaena pellucida* (left), *B. similaris* (right), and an F_1 hybrid between the two (middle). The upper edge is the verge of the vas deference, and the lower edge continues to the genital orifice. Scale bar, 1 mm.

of the penis proximal to the genital orifice (lower edge in Fig. 4). The longitudinal edges of these pilasters are weakly crenulated, but not branched or fused to each other. The internal penial surface of *B. similaris* (Fig. 4) is mostly covered with pilasters, which run from the verge of the vas deferens to the genital orifice. These pilasters vary in width. In the part proximal to the vas deferens, the pilasters are fine, and mostly crenulated and compressed. In the major portion of the penial internal surface, the pilasters are branched and largely crenulated, fusing into a few major pilasters near the genital orifice.

All 20 F_1 hybrids examined were different from the

Table 2. Allele frequencies, heterozygosities (H), and F_{ST} values for the polymorphic allozyme loci in this study.

Locus	Allele	*B. pellucida*				*B. similaris*			
		Asai	Kanamari	Shiroyama	F_{ST}	Miyama	Shirahama	Mishimako	F_{ST}
Aat	*146*	0.000	0.000	0.000	–	0.068	0.000	0.000	*0.046*
	100	1.000	1.000	1.000		0.932	1.000	1.000	
	H	*0.000*	*0.000*	*0.000*		*0.126*	*0.000*	*0.000*	
Idh	*100*	0.825	0.963	0.950	*0.049*	1.000	1.000	1.000	–
	80	0.175	0.037	0.050		0.000	0.000	0.000	
	H	*0.289*	*0.071*	*0.095*		*0.000*	*0.000*	*0.000*	
Cap	*100*	0.025	0.019	0.000	*0.037*	0.270	0.567	0.500	*0.080*
	92	0.250	0.167	0.383		0.405	0.017	0.033	
	82	0.500	0.630	0.617		0.108	0.267	0.300	
	73	0.225	0.185	0.000		0.216	0.150	0.167	
	H	*0.636*	*0.541*	*0.473*		*0.704*	*0.585*	*0.631*	
Mdh-I	*100*	0.000	0.056	0.000	*0.025*	1.000	1.000	1.000	–
	64	1.000	0.944	0.967		0.000	0.000	0.000	
	39	0.000	0.000	0.033		0.000	0.000	0.000	
	H	*0.000*	*0.105*	*0.064*		*0.000*	*0.000*	*0.000*	
Mdh-II	*100*	0.675	0.630	0.467	*0.033*	1.000	1.000	1.000	–
	64	0.325	0.370	0.533		0.000	0.000	0.000	
	H	*0.439*	*0.466*	*0.498*		*0.000*	*0.000*	*0.000*	
Pgm	*100*	1.000	1.000	1.000	–	0.000	0.000	0.000	–
	45	0.000	0.000	0.000		1.000	1.000	1.000	
	H	*0.000*	*0.000*	*0.000*		*0.000*	*0.000*	*0.000*	
Mean heterozygosity		*0.227*	*0.197*	*0.188*		*0.138*	*0.098*	*0.105*	
Sample size		20	27	30		74	30	30	

Table 3. Nei's (1972) genetic distances, across the 12 loci examined, between populations of *Bradybaena pellucida and B. similaris.*

		Bradybaena pellucida			*B. similaris*	
		Asai	Kanamari	Shiroyama	Miyama	Shirahama
B. pellucida	Kanamari	0.0034				
	Shiroyama	0.0094	0.0065			
	Miyama	0.2320	0.2268	0.2512		
B. similaris	Shirahama	0.2380	0.2268	0.2602	0.0123	
	Mishimako	0.2333	0.2219	0.2550	0.0106	0.0003

parental species in penial sculpture (Fig. 4). Irregularly shaped pustules were present with a few finely crenulated pilasters in about one-third of the penial wall near the vas deferens. Thus, the area with the pustules was smaller in hybrids than in *B. pellucida*. More than half the area of the internal wall proximal to the genial orifice was covered with prominent pilasters. These pilasters were longer than those of *B. pellucida*, and thicker, less branched, and less crenulated than those of *B. similaris.* This pattern of penial sculpture in the F_1 hybrids appears intermediate between those of *B. pellucida* and *B. similaris*.

Allozyme variation

Of the 12 putative loci coding the 11 enzymes examined, six loci of five enzymes were polymorphic across the six populations of *B. pellucida* and *B. similaris* (Table 2). The two species shared the most common allele at two loci, *Aat* and *Idh*. At the other loci, however, allele frequencies differed markedly between species. At the *Cap* locus, the Cap^{82} allele was commonest in *B. pellucida*, whereas Cap^{92} or Cap^{100} was most frequent in *B. similaris*. At *Mdh-I*, the three populations of *B. similaris* were fixed for $Mdh\text{-}I^{100}$, which was rare in *B. pellucida*. Similarly, at *Mdh-II*, the three populations of *B. similaris* were fixed for $Mdh\text{-}II^{100}$, while the three populations of *B. pellucida* were polymorphic for two other alleles. All three populations of *B. pellucida* were fixed for Pgm^{100}, and those of *B. similaris*, for Pgm^{45}.

The mean heterozygosity was 11% in *B. similaris* and 20% in *B. pellucida*. The value of F_{ST} did not exceed 0.08 at any locus (Table 2). Mean genetic distances (Nei, 1972) across the 12 loci examined were 0.2384 between the two species and 0.0071 within species (0.0064 for *B. pellucida*; 0.0077 for *B. similaris*) (Table 3). The difference between the inter- and intra-specific genetic distances was statistically significant (Mann-Whitney test, P=0.0014). Twenty F_1 progeny obtained from the four interspecific pairs were all heterozygous for Pgm^{100} and Pgm^{45}, for which the parental populations of *B. pellucida* and *B. similaris*, respectively, were fixed, indicating that they were interspecific hybrids and not the result of selfing.

DISCUSSION

Fluorescent mantle pigment

The yellow pigments seep out of the soft body in natural habitats as well as in the laboratory. Thus, laboratory conditions are not the direct cause of pigment loss. Instead, the laboratory conditions probably prevented replenishment of the pigment, perhaps because the artificial food did not provide the necessary substance(s) for pigment production. Small juveniles of *B. pellucida* in the wild do not exhibit the yellow color at all or do so only weakly. This suggests that *B. pellucida* in the wild accumulates the yellow substance into spots while it grows.

We found no yellow spots in the mantle of *B. pellucida* that had lost the yellow color in the laboratory. Instead, sparse white spots were present, similar to the spots of *B. similaris*. In the middle of the laboratory feeding period, the remaining yellow spots of *B. pellucida* exhibited little color variation. Thus, the yellow spots disappeared instead of changing to the white spots, and the white spots of *B. pellucida* were probably present behind the dense yellow spots from the beginning of the experiment.

The difference in mantle coloration between the two species may result from differences in food and/or microhabitat. However, their habitat types largely overlap, and they sometimes occur together. Nevertheless, no specimen with yellow coloration has been found in *B. similaris* in thorough surveys across its distribution range in Japan (e.g., Komai and Emura, 1955; Asami and Ohba, 1982). This suggests that the environment-dependent mantle pigment of *B. pellucida* is an indication of a genetic difference from the sibling species, *B. similaris*.

There is no record of similar fluorescent metabolites in other pulmonates, except for the case of fluorescent pigments found in the mucus of *Cepaea nemoralis* (L.) (J. Murray, pers. comm.). Although some banana slugs (genus *Ariolimax*) are known for bright yellow coloration (Gordon, 1994), whether their color variation is environmentally dependent to any degree is not known. The slug *Arion fasciatus* (Nilsson) lost yellow-orange mantle pigments when raised on carrot, lettuce, or paper, whereas *A. fasciatus* and *A. silvaticus* Lohmander raised on the European nettle *Urtica dioica* L. produced stronger pigmentation than wild individuals (Jordaens et al., 2001). In our preliminary feeding experiment, carrot, lettuce, or sweet potato did not prevent pigment loss in adult or juvenile *B. pellucida* collected from the wild or allow their offspring to acquire the pigment. *Bradybaena pellucida* is often found on the Japanese nettle *Boehmeria nipononivea* Koidz of the same family (Urticaceae) as the European nettle.

In natural as well as laboratory conditions, the yellow pigment seeps out with mucus from the body of *B. pellucida*. Similar examples are known in slugs; *Arion subfuscus* (Draparnaud) and *A. hortensis* Férussac are distinguished from other arionids by secretion of yellow-orange mucus (Kerney and Cameron, 1979), although it is unknown whether they lose mantle coloration in captivity. *Arion fasciatus* has colorless mucus in natural conditions, but it produces orange mucus when raised on nettles (Jordaens et al., 2001). Thus, the present case may not be unique to

B. pellucida among shell-bearing pulmonates, and similar examples may have simply been overlooked because of traditionally biased attention to shells.

Despite the continuous loss of pigment, *B. pellucida* accumulates the pigment, exhibiting a fluorescent yellow and making the dorsal view conspicuous, at least to human eyes. Whether there is any reason for the fluorescence and/or bright yellow of the dorsal mantle to be exposed through the shell is currently unknown. Exploration of a possible pigment function requires further studies of the ecology and evolution of *B. pellucida*. Chemical identification of the pigment is also necessary to understand how it is acquired from the environment.

Penial sculpture

Considering the close resemblance of the two species in shell and general genital morphology as well as in behavioral and reproductive traits, reflected in hybridization success, the discrete difference in the complex penial sculpture between *B. pellucida* and *B. similaris* is remarkable. Our result suggests that the patterns of penial sculpture have diverged faster than other morphological traits. The present study demonstrated that F_1 hybrids exhibit a penial anatomy intermediate between the distinct microsculpture of the parental species. Thus, the differences in penial sculpture between the two species may be polygenic or determined by one or a few genes that do not exhibit complete dominance.

The microsculpture of the penial wall is often useful to distinguish between closely related species of pulmonates (e.g., Emberton, 1991; Sutcharit and Panha, 2006). This suggests that penial sculpture evolves relatively quickly and thus may have a role in physical recognition of the copulation partner, although this aspect has so far attracted little attention. Because penial sculpture can function only after exposure of the penis (Asami et al., 1998), the evolutionary shift in sculpture may have generated a barrier for cryptic reproductive isolation that occurs during the process of copulation, instead of a post-copulatory prezygotic barrier (Howard, 1999).

Genetic distinction

The present allozyme survey was aimed at distinguishing the two species genetically and did not attempt exhaustive examination of allozyme variability across their ranges. Our allozyme analysis revealed that genetic distance at the 12 loci examined is significantly larger between *B. pellucida* and *B. similaris* than within either species. Differences between the species were most apparent at the *Pgm* and *Mdh-1* loci. Within each species, F_{ST} indicates that differences among the three populations were as little as 8% of the total genetic variation. Mean heterozygosity was a little higher in *B. pellucida* than in *B. similaris*, because the former was variable at four loci but the latter at two. Elucidation of population history by surveys of genetic variation at a sufficient number of localities over a larger geographic scale must await further research.

Our results demonstrate that *B. pellucida* and *B. similaris* produce viable F_1 hybrids, which can grow to maturity with no obvious breakdown. However, the strength of postzygotic isolation needs to be quantified by explicit evaluation of hybrid fitness. The current results highlight the importance of further investigation of introgression and reproductive isolation between the two species.

ACKNOWLEDGMENTS

We thank Kurt Jordaens, Georg Armbruster, James Murray, and Robert Cowie for comments, and Masatoyo Okamoto, Hiroshi Fukuda, Kiyonori Tomiyama, Naoko Asami, and Mao Asami for field collecting. This study was partly supported by JSPS Grants-in-Aid for Scientific Research to T.A.

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(Received August 7, 2008 / Accepted September 14, 2008)