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Influence of Culture Bed Substratum on Reproduction of *Oncomelania quadrasi*, the Snail Host of Schistosomiasis in the Philippines

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Abstract

Paddy and non-paddy soils, planting soils, oystershell for chicken feed, and other materials on the market such as Betacalc, Pearlcalc and beads were examined to obtain the most suitable and easily available materials for the culture bed of *Oncomelania quadrasi*. Cultures using Kofu soil or the gray soil of paddy fields inhabited by *O. nosophora* were the most suitable for the reproduction of *O. quadrasi*. In addition to this soil, surface calcareous soil and fine-grained soil derived from igneous rock with high calcium content were also suitable for oviposition of the snail. Neutral gray paddy soil with a particle size of less than 37 μm and a clay content of 25.0–37.5% was also suitable for snails to reproduce, whereas fine-grained soil containing no calcium and of a low pH value or with high calcium content and high pH value inhibited reproduction of the snail.

Key words: breeding; culture bed; *Oncomelania quadrasi*; reproduction; *Schistosoma japonicum*; soil texture.

Introduction

Oncomelania quadrasi, the intermediate host of *Schistosoma japonicum* in the Philippines, is amphibious in behavior and deposits eggs surrounded by mud skin, which, in some cases, fail to hatch even under optimum conditions of temperature, humidity and light. The failure in hatching seems to occur in cases where the physical and chemical properties of soil in the habitat are unsuitable for the formation of mud skin around eggs as in the case of *Oncomelania nosophora*, the snail host of the parasite in Japan (Nihei, 1978a, b; Nihei *et al.*, 1993). Reproduction experiments of *O. quadrasi* in cultures with soil collected from Bohol Island showed that deposited eggs and hatched young snails are markedly different in number between cultures provided with soils from different locations, and that soil conditions seriously affect the number of eggs and young snails produced (Nihei *et al.*, 1994). In this paper, an attempt was made to enhance fecundity of the snail kept in the breeding system devised by Nihei *et al.* (1996) and to examine the suitability of different soils for culture bed, the mixtures of these soils, planting soils, and materials on the market.

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Materials and Methods

Snail and bed materials

Snails used were collected from Bohol Island in the Philippines and then maintained in the laboratory. Mature male and female snails, 3.0–4.0 mm and 4.0–5.0 mm in shell length respectively, were used in the experiments.

Soil and bed materials

Soil samples used as culture bed were collected from a paddy field inhabited by *O. nosophora* in the western area of the Kofu Basin in Yamanashi Pre-

fecture (Kofu soil), and from some non-habitat paddy fields in Japan. Kofu soil was used as the control soil in the experiments and soils from the other locations were typical in texture and type in Japan. All the soil samples were air-dried and sieved through fixed meshes after collection and examined for physical and chemical characteristics (Table 1).

In addition to the soil, various kinds of materials available on the market were used: different kinds of planting soil, glass-beads with particle sizes of 37–63 μm and 177–250 μm, crushed oystershell for chicken feed, powdered nacre of pearl-oyster shells with a particle size of 70–400 μm, "Betacalc" or calcium carbonate with a size of less than 50 μm with a mean of 2.2 μm, "Pearlcalc" or calcium monohydrogen phosphate with a diameter of less than 75 μm with a mean of 37.9 μm (Mikimoto Pharmaceutical Co., Mie, Japan), and others pre-

Table 1 Analysis of paddy and non-paddy field soils used

Collection site	Soil type or parent material	Color	Texture	pH	P_2O_5* (mg)‡	Fe* (ppm)	Mn† (ppm)	MgO† (mg)	K_2O† (mg)	CaO† (mg)	NO_3-N (mg)	NH_4-N (mg)
[paddy field soil]												
Akita	peat soil	brownish black	clay loam	5.5	10	50	10	1≧	70	100	1>	1
Yokohama	Kanto loam	brown	clay	6.7	0	10	0	1	35	75	1>	0
Tono	muck	black	clay loam	5.9	0	10	25	1>	100	75	1>	5
Kofu 1	alluvial fan	gray	sandy loam	6.0	0	5	10	1	85	75	1>	0
Kofu 2	paddy soil	gray	sandy loam	6.0	15	10	10	1	100	200	1>	0
Mikatahara 1	diluvial upland	dark grayish yellow	clay	4.9	10	50	5>	1	20	50>	0	0
Mikatahara 4	diluvial upland	dark grayish yellow	loam	6.0	10	5	0	1	35	100	1>	1>
[non-paddy field soil]												
Mikatahara 7	diluvial upland (red yellow soil)	brown	clay	4.0	0	10	0	1>	70	0	0	1>
Mikatahara 8	diluvial upland	dull reddish	clay	3.9	0	4	5	1	100	0	1>	1
Mikatahara 9	diluvial upland	brown	clay loam	4.3	0	3	10	1	70	0	0	1>
Fukushima 1	limestone top soil	brownish black	loam	7.0	0	5≧	10	1	35	400	1>	1≧
Fukushima 2	limestone subsoil	grayish yellow	sand	7.7	5≧	5>	0	1	35	75	1>	0
Fukushima 3	forest subsoil	dull yellowish brown	sand	4.8	0	5>	15	1	70	0	0	0
Fukushima 4 (Mt. Azuma-kofuji)	volucano-geneous sand	dull yellow	sand	4.4	0	5	0	1≧	20	0	1>	0

* Available; † Exchangeable; ‡ mg/100g soil.

pared by mixing the above materials after sieved or crushed and sieved.

Water

Tap water dechlorinated by standing overnight or distilled water was used for rearing of snails.

Rearing methods and examination of snails

To examine oviposition, adult snails were kept in shallow unglazed clay pots, 9 and 4 cm in diameter and depth respectively, provided with a soil layer about 1 cm deep on the bottom. Cultures were covered with a lid and placed in a shallow plastic tray with some water. To each culture, 3 male and 5 female snails were transferred and fed with 30 mg of the powdered food weekly (Matsuda, 1969). One month later, parent snails were removed and another month later, the hatched young snails were counted under a stereoscopic microscope. Cultures in trays were placed in a room at 25°C and illuminated by room light from 06:00 to 18:00.

Analysis methods of soil

Soil samples were analysed to make clear the ecological conditions of the snail. Soil color was determined with the standard soil color chart, pH with the in situ soil pH meter (model SPAD-PHA-120, Fujiwara Seisakusho Co., Tokyo, Japan), soil texture by the finger method used in the field survey or the sedimentation method in still water, and chemical property by the soil nutrient tester, "Dr. Soil" or a soil rapid tester (Fujiwara Seisakusho Co., Tokyo). The collection sites of soil samples were surveyed by global positioning system (GPS) for landform, and water condition, vegetation and land use of the sites were also investigated.

Scanning electron microscopy of mud skin of eggs

Fresh mud skin, not fixed, was coated with platinum palladium on a slide glass and observed with a scanning electron microscope (Type S-900, Hitachi Seisakusho Co., Tokyo, Japan) to determine the size, morphology and property of component particles.

Results

Influence of culture bed soils on reproduction of snails

Different soils were compared on the suitability for culture bed. Experiments were carried out from the viewpoint of pH and Ca content of soil which seem to correlate with the oviposition of *Oncomelania* snails.

1) Rice paddy soils

Three kinds of rice paddy soils typical in texture and type in Japan were used as the culture bed: brownish black lowland paddy soil collected from a river in Akita City, lowland soil derived from Kanto loam or volcanic ash soil at the Tama Hills in Yokohama City, and muck soil at Tono City in Iwate Prefecture. Cultures using these 3 kinds of soils produced average numbers of juvenile snails of 14, 3 and 1 respectively, which were far smaller than in those using the control, Kofu soil. The pH values and calcium content of these soils ranged from 5.5 to 6.7, and 75 to 100 ppm respectively (Table 2, Exp. 1).

Soil samples from 2 paddy fields along a river that dissects through the red yellow acid terraces or Mikatahara Terrace in the west area of Shizuoka Prefecture showed pH values of 4.9 and 6.0 and a calcium content of less than 50 and 100 ppm, although these samples were derived from similar parent material. Cultures using these soils produced 54 and 119 snails respectively on average.

Soil with reduced pH values and calcium content showed poor fecundity of snails. In the case of Kofu soil, on the other hand, the mean number of young produced was 57 although the pH and Ca content were 6.0 and 75 ppm respectively (Table 2, Exp. 2).

2) Non-rice paddy soils

Red yellow soil samples from Mikatahara Terrace in Shizuoka Prefecture (Nos. 7, 8 and 9) were strongly acid, pH 3.9, 4.0 and 4.3 respectively, and contained no calcium. None of the cultures using these samples produced young snails, although those using Kofu soil produced 65 young on average (Table 3, Exp. 1).

Calcareous top soil (black loam) and subsoil from weathering calcareous crust of karst in the Abukuma Mountains in Fukushima Prefecture, subsoil from a non-calcareous slope of the same district (forest soil), and strongly sulfuric acid volcanogeneous sand from Mt. Azumakofuji in the same prefecture were used for culture beds. Cultures

Table 2 Influence of various rice paddy soils on reproduction of *O. quadrasi*

Locality of soil	pH	Ca* content (mg)	No. of snails hatched† Trial No. 1	2	3	Mean	95% confidence limit
(Exp. 1)							
Akita	5.5	100	20	12	10	14	20–8
Yokohama	6.7	75	3	3	2	3	4–2
Tono	5.9	75	3	0	0	1	3–0
Kofu 1	6.0	75	89	78	56	74	93–54
(Exp. 2)							
Mikatahara 1	4.9	50>	90	48	24	54	92–16
Mikatahara 4	6.0	100	181	108	68	119	184–54
Kofu 2	6.0	75	80	60	32	57	85–30

*Exchangeable CaO mg/100g soil.
†Three male and 5 female snails each were kept in pots.

Table 3 Influence of various non-paddy field soils on reproduction of *O. quadrasi*

Locality of soil	pH	Ca* content (mg)	No. of snails hatched† Trial No. 1	2	3	4	5	Mean	95% confidence limit
(Exp. 1)									
Mikatahara 7	4.0	0	0	0	0	–	–	0	
Mikatahara 8	3.9	0	0	0	0	–	–	0	
Mikatahara 9	4.3	0	0	0	0	–	–	0	
Kofu 1	6.0	75	89	54	53	–	–	65	89–42
(Exp. 2)									
Fukushima 1	7.0	400	165	165	149	–	–	160	170–149
Fukushima 2	7.7	75	49	49	23	16	2	28	46–10
Fukushima 3	4.8	0	94	53	50	–	–	66	94–38
Fukushima 4	4.4	0	27	4	4	–	–	12	26–0
Kofu 2	6.0	200	179	115	112	104	84	118	178–92

*Exchangeable CaO mg/100g soil.
†Three male and 5 female snails each were kept in pots.

using this soil produced average numbers of young snails of 160, 28, 66 and 12 respectively, whereas that with Kofu soil produced 118 snails. The number of young produced was significantly greater for the black loam with pH and Ca content of 7.0 and 400 ppm respectively than for Kofu soil, 6.0 and 75 ppm.

Cultures with the above subsoil produced a smaller number of young than those with Kofu soil; that is, on the subsoil of weathering calcareous crust, the number of young produced was smaller than on the control, Kofu soil although the pH value and calcium content were 7.7 and 75 ppm respectively, and in the case of forest soil, although the pH value was as low as 4.8, a good number of young were produced when parent snails were kept under good conditions.

Cultures using acid sand from volcanic Mt. Azumakofuji, on the other hand, produced an extremely small number of juvenile snails, but the sand differed in component from Mikatahara red yellow soil in which the CaO was lost and K_2O and Mn liquated out by weathering (Table 1; Table 3, Exp. 2).

Influence of culture bed composed of materials available on the market on reproduction of snails

To utilize the materials available on the market as culture bed, planting soil and standardized materials were examined for suitability as culture bed.

The materials used were as follows: Fujizuna basaltic sand less than 150 μm in particle size, pumice, pumice less than 150 μm in diameter, Kanumatsuchi or light clay and sand, Akadamatsuchi, crushed oystershell, sphagnum, river sand from the Tsukuba area less than 590 μm in size, Pearlcalc, Betacalc, various particle sizes of calcinated oystershell, activated charcoal and beads with different diameters.

No juveniles hatched out in almost all the cultures using these materials except in those using Fujizuna basaltic sand, smaller beads, Tsukuba river sand and crushed oystershell, where the smaller number of snails hatched out compared to those using Kofu soil (Table 4).

Influence of particle size and texture of bed material on reproduction of snails

To examine the influence of particle size, cultures using Kofu soil, sandy loam, and oystershell with different particle sizes were compared to each other. Hatched juvenile snails were significantly smaller in number in cultures using sieved Kofu soil

Table 4 Fecundity of *O. quadrasi* in cultures with the bed composed of planting soils and different kinds of materials

Soil	pH	Ca*	Number of hatched young snails† Trial No.				95% confidence limit
			1	2	3	Mean	
Fujizuna basaltic sand, <150 μm in particle size	6.2	20	41	32	23	32	42–22
pumice, <150 μm in particle size	6.5	0	0	0	0	0	
pumice	6.5	0	0	0	0	0	
activated charcoal	4.8	50≧	0	0	0	0	
Pearlcalc	8.1	800	0	0	0	0	
Betacalc	9.6	5,000	0	0	0	0	
calcinated oystershell powder	8.4	4,000	0	0	0	0	
beads, 37–63 μm in diameter	9.8	0	3	1	0	1	2–0
beads, 177–250 μm in diameter	9.8	0	0	0	0	0	
river sand around Tsukuba, 590 μm>	6.8	0	1	0	0	0	
Kanumatsuchi (light clay and sand)	5.8	0	0	0	0	0	
Akadamatsuchi (red loam)	6.7	0	0	0	0	0	
oystershell	7.1	2,000	1	0	0	0	
sphagnum	6.5	0	0	0	0	0	
Kofu soil 1	6.0	75	125	107	89	107	127–87

*Exchangeable CaO mg/100g soil.

†Three male and 5 female snails each were kept in pots.

with particle sizes of 250–500 μm and 100–200 μm than in those using original Kofu soil (Table 5, Exp. 1). Instead of Kofu soil, sandy soil with a particle size of 180–250 μm or less than 37 μm was used. Cultures provided with the soil of the larger particle size produced the smaller number of snails but those with soil consisting of the smaller-sized particles produced almost the same number of snails. These results show that soil less than 37 μm in particle size was necessary for snails to reproduce (Table 5, Exp. 2).

To examine the influence of soil texture on reproduction of snails, different kinds of soil were divided, according to clay content, as follows: sandy loam containing 12.5–25% of clay, loam 25–37.5%, clay loam 37.5–50% and clay more than 50%. The experiments revealed that cultures using loam produced the greatest number of juvenile snails, showing that loam was the best for the culture bed (Table 6, Exp. 1 and 2).

Table 5 Influence of particle size of Kofu soil on reproduction of *O. quadrasi*

Particle size in diameter	Number of snails hatched* Trial No.			Mean	95% confidence limit
	1	2	3		
(Exp. 1)					
250–500 μm	38	22	18	26	38–14
100–200 μm	45	37	36	39	45–33
Kofu soil 1 (sandy loam)	89	78	56	74	93–55
(Exp. 2)					
180–250 μm	40	28	19	29	41–17
less than 37 μm	66	63	60	63	66–60
Kofu soil 1 (sandy loam)	92	77	35	68	101–35

*Three male and 5 female snails each were kept in pots.

Table 6 Influence of soil texture on reproduction of *O. quadrasi*

Soil texture	Number of snails hatched* Trial No.					Mean	95% confidence limit
	1	2	3	4	5		
(Exp. 1)							
loam†	210	165	158	153	111	159	203–116
Kofu soil 1 (sandy loam†)	98	38	21	17	–	44	103–0
(Exp. 2)							
loam†	90	86	69	–	–	82	130–69
clay loam†	71	67	38	–	–	59	79–39
clay†	49	12	7	–	–	23	49–0
Kofu soil 1 (sandy loam†)	72	54	50	–	–	59	72–45

*Three male and 5 female snails each were kept in pots.
†Clay content: sandy loam, 12.5–25%; loam, 25–37.5%; clay loam, 37.5–50%; clay, more than 50%.

Influence of culture bed composed of soils and/or materials on reproduction of snails

When the culture bed was composed of crushed oystershell and its fine particles or crushed oystershell alone, young snails were hardly produced. Cultures provided with the bed consisting of Kofu soil and either of "Pearlcalc" and "Betacalc" produced the smaller number of young snails than those with Kofu soil alone (Table 7, Exp. 1). Cultures using sphagnum and small-sized crushed oystershell produced only a small number of juveniles (Table 7, Exp. 2). When crushed oystershell mixed with beads, less than 37 μm in particle size, or river sand was used as the bed substratum, the smaller number of young snails were produced than in the bed of Kofu soil (Table 7, Exp. 3).

Effect of the supplement of poorly productive bed soil with high calcium content was examined on the fecundity of snails. When limestone top soil (black loam) was supplemented with pumice or limestone subsoil, about a half the number of young snails were obtained only in the mixture of limestone top- and sub-soil, compared with the case of Kofu soil (Table 8, Exp. 1).

Even when any of the following, Pearlcalc, calcinated oystershell and Fujizuna basaltic sand

Table 7 Influence of mixed oystershell bed on reproduction of *O. quadrasi*

Materials mixed with mixture ratio in parentheses	Number of hatched snails* Trial No.					Mean	95% confidence limit
	1	2	3	4	5		
(Exp. 1)							
oystershell†	1	0	0	–	–	1	1–0
oystershell (75)†+fine shell powder (25)‡	0	0	0	–	–	0	
Kofu soil (75)+ Pearlcalc (25)	10	2	1	–	–	4	10–0
Kofu soil (75)+ Betacalc (25)	27	7	2	–	–	12	27–0
Kofu soil 1	61	43	40	–	–	45	63–27
(Exp. 2)							
sphagnum (50)+ fine grained oystershell§ (50)	19	10	9	–	–	13	19–7
Kofu soil 1	89	78	56	–	–	74	93–55
(Exp. 3)							
Fine-grained oystershell§ (57)+ beads (37–63 μm) (43)	35	28	17	15	12	21	30–13
Fine-grained oystershell§ (70)+ river sand (30)	32	27	6	3	3	14	27–2
Kofu soil 1	229	192	117	–	–	177	244–115

*Three male and 5 female snails each were kept in pots.
†Oystershell for chicken feed, less than 2 mm in diameter.
‡Coarse powder of pearl-oyster shell, 20 μm in mean particle size, the material of Pearlcalc.
§Oystershell, less than 63 μm in particle size.

Table 8 Influence of bed soil supplemented with materials of high calcium content on reproduction of *O. quadrasi*

Soil and materials with mixture ratio in parentheses	Number of snails hatched*				
	Trial No.				95% confidence limit
	1	2	3	Mean	
(Exp. 1)					
limestone topsoil (black loam) (25) + pumice (75)	11	7	3	7	12–2
limestone topsoil (50)+pumice (50)	24	16	0	13	27–0
limestone topsoil (50)+limestone subsoil (50)	65	63	58	62	66–58
Kofu soil 2	179	115	112	135	178–92
(Exp. 2)					
Mikatahara 8 (80)+Pearlcalc (20)	0	0	0	0	
Mikatahara 8 (80)+ calcinated oystershell powder (20)	0	0	0	0	
Mikatahara 8 (80)+Fujizuna (20)	0	0	0	0	
Kofu soil (70)+Fujizuna (30)	59	47	33	46	61–31
Mikatahara 1 (80)+Pearlcalc (20)	37	31	23	30	38–22
Mikatahara 1 (97)+Pearlcalc (3)	14	13	13	13	14–12
Mikatahara 1 (86)+calcinated oystershell powder (14)	–†	–†	–†	–†	
Kofu soil 1	89	54	53	65	88–32

*Three male and 5 female snails each were kept in pots.
†All the snails died because of the rottenness of bed material.

was added to Mikatahara Terrace Yellow soil No. 8 in order to regulate pH and calcium content, no young snails were produced. When the culture bed was made by mixing according to any of the following compositions as: Kofu soil and Fujizuna basalic sand, Mikatahara paddy soil No. 1 and Pearlcalc, and Mikatahara soil No. 1 and calcinated oystershell, only cultures with the bed of the former 2 compositions produced a good number of juvenile snails, whereas those with other compositions produced no young snails. In cultures using the last composition, all parent snails died out a few days after the start of the experiment because the bed material rotted away (Table 8, Exp. 2).

Scanning electron microscopy of egg mud skins showed that the component particles were almost the same in size regardless of the materials, crushed oystershell and Kofu soil. In the case of Kofu soil, however, where the greater number of young snails were produced, component particles were diverse when it came to the kind of material such as soil particles, diatoms, plant seeds and others, and to shape such as granular, plate-like, rod-like, porous, angular, rounded, and so on. In the case of crushed oystershell, on the other hand, particles were composed of peeled pieces of the shell which were angular, plate-like even when the shell was crushed into clay-sized particles.

Discussion

Shallow unglazed clay pots were successfully used in the experiments on the oviposition of *O. quadrasi* as well as in those of *O. nosophora*, where the culture bed was composed of crushed oystershell (Nihei *et al.*, in press). Oystershell bed was convenient for *O. quadrasi* to be kept because the material was easily available on the market and autoclaved if necessary, but it was of limited use for the reproduction experiments.

Soil in the habitats and non-habitats of *O. quadrasi* in Leyte Island was not reported to be different from each other in chemical property (Pesigan *et al.*, 1958) but the present analysis of their data from the viewpoint of soil type revealed that both soil types differed from each other in texture and chemical property such as calcium content and pH. Although soil texture has not been reported to be an important factor for breeding of the snail, most of the different kinds of soil and material examined were unsuitable for snails to form mud skin around eggs, and further, the mixed soil with different textures and particle sizes was different in the suitability according to the chemical property such as pH and the morphology of particle, as shown by scanning electron microscopy.

From the viewpoint of texture, soil containing 25.0–37.5% of clay was better for *O. quadrasi* to be maintained in the laboratory than that with lower or higher clay content such as sandy loam or the mixture of clay loam and clay respectively.

In the case of *O. nosophora*, paddy soil, in general, is suitable for the culture bed because it contains the food of the snail which is carried by irrigation water. Non-rice paddy soils, on the other hand, are unsuitable for the snail to reproduce (Nihei, 1978a). In the present experiments, sandy loam was best for the culture bed although small-sized particles are necessary for snails to form mud skin around eggs. Eggs of *O. quadrasi* are smaller than those of *O. nosophora*, so the size of soil particles will influence more the formation of mud skin. Compared with *O. nosophora*, *O. quadrasi* preferred more fine-grained soil. In addition to the particle size, pH value and Ca content of the soil are considered to be important factors for the culture bed. Nevertheless, subsoil of weathering calcareous crust in the Abukuma Mountains was not suitable for the reproduction although it was 7.7 and 75 ppm in pH value and Ca content respectively, thus showing that the particle size is the more important factor for reproduction of snails than pH value and Ca content.

Generally, soil increases viscosity with the increase of clay content and this regulates the behavior of these snails. Humas content also has an influence on reproduction. Many of the fine soils contain a large amount of humas and, in the case of *O. nosophora*, soil containing too much or small amount of humas is unsuitable for reproduction and that containing 2–3% of humas is optimum (Nihei, 1978b).

For laboratory use, the materials for rearing snails need to be clean and should occasionally be sterilized. To meet this requirement many kinds of materials, that are clean and easily available on the market, instead of the soil, were examined for suitability for the culture bed. None of these materials was more suitable than Kofu soil.

The present experiments revealed that the chemical and physical properties of the culture bed regulated the reproduction of *Oncomelania* snails. When *O. quadrasi* is maintained in the laboratory, the culture bed should be composed of rather coarse and not too viscous a gray lowland paddy soil collected from flood plains of large rivers, which is air-dried and then passed through a sieve of 2-mm mesh. In addition to this soil, surface black loam, such as Fujizuna basaltic sand, from limestone areas such as limestone caves and basaltic soil derived from igneous rock, with a particle size of less than 2 mm and high calcium content can also be utilized as the culture bed although some of the fine-grained materials with high calcium content and a pH value of higher than 8 were unsuitable for the bed. Autoclaved Kofu soil mixed with crushed oystershell was also useful for the culture bed.

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