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A method for both mass and individual rearing of fungivorous astigmatid mites (Acari)

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Abstract. Several species of common fungi were assessed as food for fungivorous astigmatid mites. *Hypocrea nigricans, Botrytis cinerea* and *Flammulina velutipes* were generally good food sources for most mites examined. Fungal mycelia growing on PDA (potato dextrose agar) medium were not only nutritionally adequate but the system also maintained high humidity through the water-based agar medium. Among acarid mites, most species of Rhizoglyphinae could be reared easily with the method. Although filter-feeding histiostomatid mites do not feed directly on hyphae, some species were successfully maintained with the same method through multiple generations. Presumably, these mites obtained sufficient nutrition from the agar medium and fungal metabolites leaching into it. Most species ultimately produced dispersing heteromorphic deutonymphs on these media. Individual mites were also maintained in isolation within glass rings on fungal colonies. Using this technique, we were able to compare developmental periods, fecundity and survival periods of mites reared under different conditions.

Key words: Astigmata, fungivore, individual rearing, mycelium, rearing method, Rhizoglyphinae

Introduction

Many species of relatively desiccation-tolerant astigmatid mites which are pests in stored products and/or house dust have been successfully reared in the laboratory through their full ontogeny (Griffiths, 1964; Sinha, 1964; Hughes, 1976). Other rearing techniques have been developed for agriculturally important astigmatid mites which require higher humidity conditions such as *Rhizoglyphus robini* (Gerson *et al*., 1983; Shinkaji *et al*., 1988, 1991; Fashing and Hefele, 1991), and for histiostomatid mites which require essentially

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saturated conditions (Hughes and Jackson, 1958). Despite the utility of these techniques, many other astigmatid mites are still described only from either adults or heteromorphic deutonymphs but not both. Such descriptions limited to a single life stage can cause confusion in identification of the species and the group. Lack of such ontogenetic information makes phylogenetic study particularly difficult because whole character sets may be missing if the adult or deutonymph is unknown for an included taxon. Thus, in our efforts to develop a phylogenetic system for the Astigmata, methods for correlating these dimorphic life stages through laboratory rearing are of particular importance. As in the case of oribatid mites (Woodring and Cook, 1962), an artificial rearing method generally available for fungivorous astigmatid mites could solve this problem.

Numerous astigmatid mites feed readily on fungi and often show broad fungal food preferences (summarized in OConnor, 1984). However, few prior studies have employed acarid mites reared artificially on fungal mycelia (Rivard, 1961; Sinha, 1964; Solomon and Cunnington, 1964; Sinha and Whiteny, 1969; Thomas and Dicke, 1971; Bronswijk and Sinha, 1973; Okabe, 1993a). Dry yeast has also been shown to be an appropriate food source for laboratory-reared acarid mites such as *Acarus siro, Tyrophagus putrescentiae* and an undescribed species of *Histiogaster* (Mastumoto, 1961; Griffiths, 1966; Okabe, 1993a). In most prior studies of this type, laboratory rearings were used for biological and ecological studies but rarely for taxonomic ones, the studies of Hughes and Jackson (1958) and Griffiths (1966, 1970) being exceptional. Since individual field collections of the type typically used to describe new species normally yield series of specimens taken from identical environmental conditions, laboratory rearing also provides the opportunity to vary conditions to test the degree of phenotypic variation in a species. For example, both *A. siro* (Griffiths, 1966, 1970) and *R. robini* (Gerson and Capua, 1982) developed to smaller size and had relatively shorter idiosomal setae when reared under conditions of lowered food quality.

Although many astigmatid mites are fungivorous, few prior workers have attempted to rear these mites on a diet of living fungal mycelia (e.g. Sinha, 1964). We tested several species of fungi to see if different taxa of astigmatid mites could be maintained or reared through their ontogeny on these diets. A brief test using dry yeast was also conducted to compare its acceptability as a rearing medium. We also investigated the utility of living fungal mycelia as suitable media for isolation and rearing of individual mites. For these studies, mites were collected in the field in Japan, Michigan (USA) and in Central America or received alive from colleagues; feeding trials were conducted in both of the authors' laboratories. Taxa utilized in this study are summarized in Table 1, including collection and voucher information. Voucher speci-

Table 1. Suitability of four fungal food sources for various astigmatid mites, and collection information of the mites

Mite species	Voucher no	Collection locality	Collection substrate	*Botrytis cinerea*	*Hypocrea nigricans*	*Flammulina velutipes*	Dry yeast
Histiostomatidae							
Histiostoma humidiatus	97-1300-039[a]	Japan	Rotting cabbage root	++	++	+	+
Winterschmidtiidae							
N. Gen. 1, sp. 1	98-0428-001[a]	Japan	*Anterhynchium flavomarginatum micado*	−			
Acaridae							
Acarus siro	97-0728-001	USA, MI	Poultry litter				++
Tyrophagus putrescentiae	98-1008-001[a]	Japan	Mushroom cultivation medium	++	++	++	++
Acotyledon paradoxa	97-0625-007	USA, MI	Horse manure	++			
Viedebanttia sp. 1	96-0810-002	USA, MI	*Xylaria polymorpha*	++		+	
Sancassania sp. 1	96-1200-011	Japan	Scarabaeid beetle	++		+	
Sancassania sp. 2	96-1200-012	Japan	Green onion	++		+	
Sancassania sp. 3	96-0726-001	USA, LA	*Calosoma sayi*	++		+	
Sancassania sp. 4	98-1004-001	USA, MI	Forest soil	++		+	
Sancassania sp. 5	98-0309-001	USA, FL	*Peltotrupes youngi*	+			

Table 1. (*continued*)

Mite species	Voucher no	Collection locality	Collection substrate	*Botrytis cinerea*	*Hypocrea nigricans*	*Flammulina velutipes*	Dry yeast
Rhizoglyphus rotundatus	96-0907-009	USA, MI	*Cantharellus infundibuliformis*	++		+	
	98-0913-001	USA, MI	*Pseudopolydesmus branneri*	++		+	
Rhizoglyphus robini	96-1200-013	Costa Rica	Oil palm seed		++		+
	96-1105-121	Japan	Green onion	++		+	
Rhizoglyphus sp. 1	96-0907-006	USA, MI	*Coriolus hirsutus*	++		++	
Rhizoglyphus sp. 2	96-1200-001	USA, MI	*Tyromyces* sp.	++		++	
Rhizoglyphus sp. 3	96-1105-132	Costa Rica	Beetle galleries	++			
Bromeliaglyphus sp. 1	96-1105-206	Costa Rica	*Metamasius dimidiatipennis*	++			
Passaloglyphus rosickyi	96-1105-198	Costa Rica	*Passalus punctiger*	++			
Boletoglyphus ornatus	98-0913-002	USA, MI	*Ganoderma applanatum*	−		−	
Histiogaster carpio	99-0817-001	USA, OH	Sap flux of elm tree	+			++
Histiogaster arborsignis	96-0728-001	USA, AK	Beetle gallerles	+		−	
Histiogaster sp. 1	97-0619-066	USA, MI	Under bark	++		+	
	96-1200-007	Japan	Lab. culture	++		+	

Table 1. (*continued*)

Mite species	Voucher no	Collection locality	Collection substrate	*Botrytis cinerea*	*Hypocrea nigricans*	*Flammulina velutipes*	Dry yeast
Histiogaster sp. 2	96-1200-010	Japan	Sap flux of elm tree	++	++	++	++
Histiogaster sp. 3	96-1105-100	Costa Rica	Sap flux of *Maclura*	++		+	
Naiadacarus sp. 1	96-0826-007	Guatemala	Bromeliad	++			
Schwiebea elongata	96-1200-011	Japan	Swimming pool	++	++	++	++
Schwiebea sp. 1	96-1200-008	Japan	Lab. culture	++		++	
Schwiebea sp. 2	96-0907-001	USA, MI	Rotting log	++			
Schwiebea sp. 3	96-0728-001	USA, AK	Beetle galleries	++			
Schwiebea sp. 4	96-0809-001	USA, MI	Under bark	++			
Schwiebea sp. 5	96-0810-001	USA, MI	Under bark	++			
Schwiebea sp. 6	96-1200-005	USA, MT	*Lycoperdina ferruginea*	++			
Calvoliella sp. 1	96-1013-001	USA, MI	*Blerkandera adusta*	−		−	
N. Gen. 1, sp. 1	96-0826-007	USA, MI	*Lycoperdon pyriforme*	++		++	

[a]The voucher no has OKB at the beginning; others with BMOC.
++: mites reproduced for multiple generations, +: mites underwent some reproduction, −: mites did not reproduce.

mens are deposited in the Museum of Zoology, University of Michigan, Ann Arbor.

Rearing Methods

Mass rearing

Dry yeast

Dry yeast (*Saccharomyces cervesiae*), alone or in combination with wheat germ, has been successfully used as a rearing medium for many species of astigmatid mites (Griffiths, 1966; George, 1982), and we have successfully reared numerous other species using this medium as well (Table 1). In our experience, it was much easier to control the amount of yeast when using the powder (Wako Chemical Industries Ltd.) than compressed yeast in the form of a pill (Tanabe Seiyaku Co., Ltd.). *Tyrophagus putrescentiae* performed well on both powder and pills, while rhizoglyphine mites tended to excavate their food sources and had trouble with pills which were too small to allow them to burrow successfully. Pill formulations also tended to acquire contaminating fungi more readily than powdered yeast.

Because many fungivorous astigmatid mites prefer high humidity, maintaining adequate humidity can be difficult when dry yeast is used as a food source. Matsumoto (1961) originally used this method, placing a small container in a larger jar with fluid to control humidity for *T. putrescentiae*, and using different saturated salt solutions to maintain different relative humidity conditions. When contaminating mold grew on the yeast in our experiments, the mites were transferred to another beaker with fresh dry yeast after surface sterilizing individual mites in 70% ethanol for about 30 s. Seventy percent ethanol is not as toxic as the hydrochlorides used by Griffiths *et al.* (1959) as it only killed contaminating micro-organisms but never the mites (Okabe, 1999). New colonies were initiated when deutonymphs appeared in the original, using the same procedure as above.

Generally, mites tolerant of relatively low humidity, such as species of *Tyrophagus, Sancassania*, and *Histiogaster*, could be maintained without particular effort on dry yeast on filter paper or plaster. On the other hand, *Rhizoglyphus* and *Histiostoma* species were lost more often using this technique, because the high humidity needed to successfully rear these mites caused excessive mold growth. Populations of many species of stored product pests were maintained for many years at the Slough Laboratory of the British Ministry of Agriculture, Fisheries and Food using a mixture of dry yeast and wheat germ. Mold was controlled by sterilizing media with propylene oxide prior to use (George, 1982). The flammability and toxicity of this material, however, prevents its use in other than industrial settings.

Fungal mycelia

We also experimented with fungal mycelia for rearing astigmatid mites. A stock culture of *Botrytis cinerea* was obtained from the Forest Pathology Laboratory, and stock cultures of *Hypocrea nigricans* and *Flammulina velutipes* originating in the field were obtained from the Forest By-Products Laboratory of the Kyushu Research Center, Forestry and Forest Products Research Institute in Kumamoto, Japan. Each fungal mycelium was cultivated on PDA (potato dextrose agar) medium in a 9 cm Petri dish, and stock cultures were maintained under refrigeration on standard slants. Mites were collected in the field as either feeding stages in natural substrates or deutonymphs on phoretic hosts. Those mites were transferred from the substrate or host with a thin needle or a fine paintbrush to a mycelium. Mites collected in the field from fungal substrates were often covered with fungal spores or dust, and these were rinsed in 70% ethanol to minimize contamination. In one colony of *Naiadacarus* sp. we were not able to eliminate contaminating yeast-like micro-organisms, but this mite colony thrived through some generations. We suspect that although the fungus we gave it was not ideal for the species, an appropriate fungus or bacterium which passed through the mite gut could be cultivated on the original fungal culture.

Acarus, Tyrophagus and *Sancassania* mites seemed to prefer different conditions from most of the other species tested. These mites performed better on drier and/or more deteriorated material than on a fresh fungal colony.

The species reared using the different fungal mycelia are listed in Table 1. As most colonies originated from field sites, it is not surprising that most represent undescribed species. Deutonymphs appeared sooner or later in most mite colonies we established. Difficulties in obtaining deutonymphs occurred only with some *Histiogaster* and the *Naiadacarus* populations. Although Griffiths (1966) documented that lack of B vitamins induced deutonymphal formation in *Acarus siro*, the exact environmental cue for deutonymphal formation remains unknown for most other species. We observed that the *Histiogaster* and *Naiadacarus* mites were able to survive in deteriorated colonies longer than the others. Therefore, the same nutritional shortage would not affect their deutonymphal formation. We did ultimately obtain two deutonymphs in a colony of the *Naiadacarus* mite between the peak in population density and its decline but no deutonymphs at all in the *Histiogaster*.

One advantage of using a specific fungal mycelium is that it is possible to avoid unnecessary contamination with another fungus or bacterium to some extent. Although contaminating fungi or bacteria introduced with field collected mites often proved stronger than the fungus inoculated and eventually killed the original, in a number of cases mites could increase in number before

a contaminating agent took over. After a mite colony is established, contamination may induce the population to produce deutonymphs by producing conditions unfavorable for its feeding stages.

Other alternatives

We attempted to rear some species of mites on PDA medium without a fungus, but the plates became heavily contaminated with more than one kind of microorganism. Mites performed poorly under these conditions and most colonies died out quickly.

Bot and Meyer's (1967) medium has been successfully used previously for *R. robini* (Fashing and Hefele, 1991). We were able to establish and maintain *R. robini*, a *Sancassania* species and one *Schwiebea* species on this medium but no others. We suspect that the methyl p-hydroxy benzoate used to inhibit fungal growth in this medium is unpalatable or toxic to some mites. We also attempted to rear some species collected as deutonymphs from insect hosts on dead or living insects. *Sancassania phyllophagiana* was successfully reared on dead individuals of the scarab beetle, *Phyllophaga anxia*, a typical phoretic host of this species in Michigan. It was difficult to rear some of those mites on fungi (e.g. *Sancassania* sp. 5) even though some congeners do well on the fungi. Their particular nutritional requirements may involve animal protein.

Individual rearing

An individual mite was also reared using fungal mycelia as food. A small piece of PDA medium containing mycelium was isolated by pushing a glass ring (1 cm diameter and 1.2 cm high) through the fungus and medium on a plate. All glass equipment was sterilized in a dry oven (1800°C, 1.5 h) before use. A mite egg or an adult was placed in a ring containing mycelium with a fine paintbrush. We transferred a mite to a fungal mycelium in a ring, then placed 10 such rings in a sterilized petri dish (9 cm in diameter and 1.7 cm in height). In order to maintain moisture, a cover slip was fixed onto the top of the ring with silicone lubricant. Although the amount of mycelium in a ring was sufficient to raise an acarid mite from egg to adult, sometimes the mite had to be transferred to a new ring because the agar dried out after about 5 days. Occasionally, contamination with another fungus occurred before a mite became adult despite the medium remaining hydrated. If a mite had a developmental period longer than the effective life of the fungal colony in the ring, it was necessary to renew it more often. Renewing every 5 to 7 days was typically required in these cases.

In studying the development of acarid mites under these conditions, the interval between observations was shorter than 12 h for many fungivorous astigmatid mites reared at 20 –25°C (Okabe, 1993b). Due to condensation, the

Table 2. Difference in developmental period in an undescribed *Schwiebea* species (sp. 1) reared on two different food sources at 25°C

Food source	Egg	Larva	Protonymph	Tritonymph	Egg to adult
B. cinerea	3.06 ± 0.48	1.98 ± 0.47	1.60 ± 0.38	1.95 ± 0.44	9.15 ± 0.78
Dry yeast	2.92 ± 0.31	2.28 ± 0.43	1.61 ± 0.40	2.00 ± 0.47	8.80 ± 0.89

$x \pm SD$, $n = 20$.
There was no significant difference between two food sources in each stage by the *t*-test.

cover slip was typically opened to observe mites. Because many fungivorous mites tend to hide under hyphae or burrow into the agar medium, observations must be conducted carefully. We often needed to remove hyphae around a mite using a thin needle, taking care not to damage the mite. Some mites may burrow into agar and live deep in a medium. These are typically species that were collected from fungal fruiting bodies or subcortical habitats in which the mites naturally burrow.

Table 2 shows the developmental period in one of the undescribed *Schwiebea* species (sp. 1) originally collected as a contaminant in a laboratory fungal colony and individually reared using this method. Okabe (1993a) also documented differences in developmental periods in *Histiogaster* sp. on three kinds of fungi. Table 3 shows the relationship between developmental period and temperatures. Fecundity, survival rate and longevity can also be investigated with this method (Okabe, 1993b). The developmental periods by the individual rearing supported the mass-rearing result that there was not much difference between *B. cinerea* and dry yeast as food sources for the mite. The life-cycle period became shorter when the temperature turned higher, as reported for many other astigmatid mites.

Table 3. Differences in developmental period in an undescribed *Schwiebea* species (sp. 1) reared at different temperatures, on a diet of *B. cinerea*

Temperature (°C)	Egg	Larva	Protonymph	Tritonymph	Egg to adult
15	9.43 ± 2.21	5.09 ± 1.22	4.48 ± 1.61	5.75 ± 1.24	24.87 ± 4.02
20	4.85 ± 0.13	2.36 ± 0.61	2.55 ± 0.57	2.50 ± 0.58	12.63 ± 0.99
25	3.06 ± 0.48	1.98 ± 0.47	1.60 ± 0.38	1.95 ± 0.44	9.15 ± 0.78
30	2.36 ± 0.34	1.67 ± 0.33	1.13 ± 0.26	1.40 ± 0.34	6.61 ± 0.61

$x \pm SD$, $n = 20$.

Discussion

We have been able to rear many species of mites in the families Acaridae and Histiostomatidae on fungal mycelia. Most mites did well on *Flammulina velutipes* as well as *F. umnosa* (Lasch.) Fr. and *F. carboraria* Fr. (Sinha and Whitney, 1969). In most acarid species tested, the mites consumed the fungus and were able to complete development through multiple generations before the cultures became contaminated. Contamination in these cases could have come from other fungi or micro-organisms from the surface of the mites' bodies or through spores or bacteria passing through the mites' guts in the case of colonies started with feeding stages of the mites. Filter-feeding histiostomatid mites in the genus *Histiostoma* were also easily reared, although in these cases, the mites were presumably feeding only on the agar medium containing fungal metabolites or on bacterial contaminants. These colonies typically developed to high densities once the fungus had disappeared from the plate. *Histiostoma* colonies also were not hampered as much by the development of bacterial contamination as were acarid colonies. Again, presumably the *Histiostoma* could feed on the bacteria while the acarid mites could not.

Many of our colonies originated with mites collected from fungal fruiting bodies, and certain species are clearly specialists in particular families or even species of fungi in the field. We observed that some of these species could be reared easily on mycelia of other species despite their apparent natural preference for other species. For example, the two species of *Viedebanttia* were both collected only from fruiting bodies of xylariaceous fungi in the genus *Xylaria.* Both were maintained for generations on other fungi, although deutonymphs formed in the laboratory colonies did not molt when placed on new media. Similarly, species in an undescribed acarid genus found only in fruiting bodies of gasteromycete fungi of the genus *Lycoperdon* also were reared through multiple generations on other species in the laboratory.

On the other hand, specialist species in the genera *Boletoglyphus* and *Calvoliella* failed to develop or reproduce on laboratory fungi. These mites appear to be obligate spore-feeders, with very elongate bodies specialized for inhabiting the spore tubes of their polypore fungal hosts. The one species of Winterschmidtiidae tested did not develop at all on fungi. Neither protonymphs nor deutonymphs of this species molted to the next stage on fungi. Although the natural food sources for this species are unknown, microscopic observation of immature feeding stages taken from host cells showed dark spots internally, suggesting these mites feed on non-liquid food, at least during their pre-adult stages.

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