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Experimental infection of the digeneans to some congeneric snail species radiated in a single water system: Relative importance of local evolution and phylogenetic constraint

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ABSTRACT

To determine the relative importance of local adaptation caused by host–parasite coevolution and resource tracking by the parasites, the susceptibility of the freshwater snail genus *Semisulcospira* to the digenean parasite genus *Genarchopsis* was investigated experimentally. Four snail species endemic to the Lake Biwa system in Japan and two non-endemic species were investigated. All but one species was also tested for local variation in susceptibility.

Parasites were obtained from Takashima (mix population of *Genarchopsis gigi* and *Genarchopsis chubuensis*) and Nagahama (*G. chubuensis*). In endemic *Semisulcospira*, closely related specie pairs (*Semisulcospira habei* and *Semisulcospira niponica*, *Semisulcospira decipiens* and *Semisulcospira nakasekoae*) showed similar susceptibilities to parasites from both localities. *S. habei* and *S. niponica* were highly susceptible to parasites from Takashima, but were resistant to parasites from Nagahama. *S. decipiens* and *S. nakasekoae* showed moderate susceptibility to parasites from both localities. None of the endemic snail species showed a clear local variation in susceptibility. These results show that the susceptibility of endemic *Semisulcospira* to *Genarchopsis* is conservative and can be regarded as an example of resource-tracking. One of the non-endemic snails, *Semisulcospira libertina*, showed local variation in susceptibility. This variation was not related to the sympatry of the parasites used for the experimental infection, suggesting that it was not the result of local adaptation by parasites.

1. Introduction

Host–parasite coevolution is one of the more challenging topics of study in both evolutionary dynamics and the origin of biodiversity. Many experimental studies have been conducted to understand the dynamic nature of host–parasite associations [1–3].

Parasitic platyhelminthes are the dominant taxon of parasitic animals. They can affect host condition and population dynamics, but the effect is usually conspicuous in the case of taxa that increase the parasitic load within individual hosts, such as monogeneans on fish or amphibians, and larval digeneans in mollusks.

For larval digeneans, it has been demonstrated that the parasites can coevolve with their host mollusks within only a few generations [1,3–5]. In addition, local adaptation arising from coevolution is common [6–11]. These studies have led to the recognition that larval digeneans must contribute to the genetic diversity of their host species. However, larval digeneans are thought to contribute little to host speciation on a phylogenetic time scale. In typical cases, host species diverge allopatrically and parasites seem to track them, or switch hosts to novel taxa [12–13]. In fact, in his review, Little [14] reported that few studies have shown host–parasite coevolution under natural conditions. These differences between short-term experimental or field studies and longer biogeographical or phylogenetic studies suggest that different modes of evolution occur between small and large spatial or time scales.

The Lake Biwa water system in Honshu, Japan contains 15 endemic species of *Semisulcospira* Boettger (subgenus *Biwamelania* Habe) that diverged in a single, relatively small water system; they can be regarded as representing sympatric speciation [15–17]. Fossil records reveal that the diversification of endemic species started, at the latest 4 million years ago [18]. Prominent variation in karyotypes is one of the intrinsic factors that allowed sympatric speciation to occur [16,19,20]. Therefore, the lake represents a very interesting system for studying host–parasite relationships in mollusks and larval trematodes. In this system, it is possible to investigate variation in the host–parasite relationship on both small temporal and geographical scales (comparisons between local populations of the same species), and on a phylogenic scale (comparisons between different species) simultaneously, while excluding the effect of geographic isolation.

Twenty-eight digenean species from *Semisulcospira* have been recorded in the Lake Biwa water system, but species diversity is poorer in the lake than in its tributaries or outlet [21]. The total prevalence of endemic species, except for *Semisulcospira* (*Biwamelania*) *nakasekoae*

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Kuroda, is usually lower than non-endemic species, and it is suggested that this is caused by their tolerance to trematodes [21]. Only two genera of digeneans (*Genarchopsis* Ozaki and *Metagonimus* Katsurada) have widely parasitized *Semisulcospira* species in the lake [21]. The definitive hosts of the genus *Metagonimus* are birds and mammals, and those of *Genarchopsis* are freshwater gobies. In general, the parasites of *Semisulcospira* have higher migration rates than their hosts because they can be dispersed by their definitive hosts (vertebrates), while *Semisulcospira* are viviparous and have no planktonic stage. Under these conditions, it is expected that the parasites will adapt to their hosts locally [22]. This study focused on the genus *Genarchopsis* because they are autogenic and it is easy to interpret host–parasite relationships within a water system. The larval form of *Genarchopsis* parasitizes the stomach, gonads and midguts of *Semisulcospira* and sterilizes them, as do many other digenean species. Therefore, the fitness of infected snails becomes almost zero and the effect of infection must be serious in an evolutionary sense.

Our previous study showed that there are two species of *Genarchopsis* in the Lake Biwa water system [23]; *Genarchopsis gigi* Yamaguti is endemic to Lake Biwa and its outlet and *Genarchopsis goppo* Ozaki is distributed mainly in inlets. Recently, a part of *G. goppo*, which is distributed from Kinki District to Kanto District including Shiga Prefecture ("central Japan group" in Ref. [23]), was described as an independent species, *Genarchopsis chubuensis* Shimazu [24]. *G. gigi* diverged from a non-endemic linage of *Genarchopsis*, which includes *G. goppo* sensu *strico*, *Genarchopsis fellicola* and *G. chubuensis*, a considerable time before present [23]. This suggests that *Semisulcospira* snails in the Lake Biwa water system have long associated with *G. gigi*, and perhaps also with *G. chubuensis*, and have affected each other.

The purpose of this study was to determine whether the susceptibility of *Semisulcospira* to *Genarchopsis* varies among local populations, which would suggest the presence of local adaptation caused by host–parasite coevolution, or among species, which would suggest resource tracking by the parasites. Several species of *Semisulcospira* collected from more than one population were experimentally infected with *Genarchopsis* eggs from two localities in the Lake Biwa water system. Because *Semisulcospira* is viviparous and does not have a planktonic stage, any gene flow among local populations must be limited. Parasites that were allopatric with host snails were used, with a single exception, for the experimental infections; therefore, the data do not show any local adaptation of parasites (i.e. parasites are more infective to sympatric hosts than allopatric hosts). However, some local variation in susceptibility would be expected if host–parasite coevolution occurred on a generational scale. If the snails have interacted with the parasites on a phylogenetic time scale, variation in susceptibility would not be observed among local populations of the same species, but would be observed between species. The results are discussed from the viewpoint of ecological factors that affect host–parasite dynamics.

2. Materials and methods

2.1. Host snails

To conduct experimental infections, six species of *Semisulcospira* were collected from the Lake Biwa water system. All of the species were identified based on adult and newborn shell morphology according to Davis [15]. Four of the six species are endemic to this system (subgenus *Biwamelania*): *Semisulcospira habei* Davis, *Semisulcospira niponica* (Smith), *S. decipiens* (Westerlund) and *S. nakasekoae*. Kamiya et al. [17] showed that *S. habei* and *S. niponica* are relatively close to each other phylogenetically, by analyzing allozymes. Their close relationship is strongly supported by sequences of the ITS-1 region of ribosomal RNA [25]. Therefore, these species are termed the SBH + SBNi group. Similarly, a close relationship between *S.* (*B.*) *decipiens* and *S.* (*B.*) *nakasekoae* has been shown by both allozyme analysis [17] and ITS-1 sequences [25]. Therefore, these two species are termed the SBD + SBNa group. The remaining two species are non-endemic (subgenus *Semisulcospira*): *Semisulcospira libertina* (Gould) and *Semisulcospira reiniana* (Brot) type B [26].

Sampling locations are shown in Fig. 1. At least two sampling localities were used for each species to test for local differences in trematode susceptibility, except for *S. nakasekoae*, which was distributed only in the lake outlet (Uji River). *S. libertina* was not found in the lake, and snails were collected from small ditches or brooks near the lakeshore (northern and southern basins; Fig. 1) or from an inlet (Nagahama: the same locality as the sampling point for *G. chubuensis*).

2.2. Molecular identification of parasite species

The parasites used in this study were two species of the genus *Genarchopsis* that are distributed in the Lake Biwa water system; *G. gigi* and *G. chubuensis*. When the experimental infection program was initiated in 2007, the presence of two *Genarchopsis* species in this locality had not been recognized and *G. gigi* was regarded as a synonym of *G. goppo* sensu *lato* [27]. However, we now consider that *G. gigi* should be regarded as an independent species [23]. Therefore, a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) study was conducted to know the ratio of two species in the two sampling sites.

The parasites were obtained from two localities in Shiga Prefecture. One was along the shore of Lake Biwa, Imazu, Takashima City (35°24′21″N, 136°02′40″E) (Fig. 1). Here, *Genarchopsis* were obtained from the stomachs of the freshwater goby *Tridentiger brevispinis*. The other locality was an irrigation canal at Nishi-kouzaka and Higashi-kouzaka, Nagahama City (35°24′25″N, 136°19′20″E) (Fig. 1). Here, specimens of *Genarchopsis* were obtained from stomachs of the freshwater goby *Rhinogobius flumineus*.

The samples for the PCR-RFLP were collected in June and July 2011 for worms in *T. brevispinis* from Takashima, and in May and August in 2014 for worms in *R. flumineus* from Nagahama. The collected worms were rinsed in TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH = 8.0) and homogenized in 100 μL of 20 mM TNES buffer (Tris–HCL, 400 mM NaCl, 0.3% SDS, pH = 8.0) including 2.5 μg Proteinase K. The homogenates were incubated at 55 °C for 3 h to digest the proteins. The digested solutions were diluted 10-fold and provided as templates for PCR.

A partial sequence of the internal transcribed spacer 1 (ITS-1) and a short sequence of 5S of the ribosomal DNA were amplified using a primer set BD1 and 4S [28]. A PCR method was conducted using the same protocol as Urabe et al. [23]. The PCR products (9 μL) were incubated at 37 °C for 16–24 h with 2 U restriction enzyme (HaeII: New England BioLabs Japan Inc.) in ×10 NE buffer 4 and ×100 BSA. The DNA fragments were electrophoresed using 1.0% agarose gel and stained with Midori Green Direct (Nippon Genetics Co, Ltd.). The banding pattern was observed using Fas-Digi gel shooting system (Nippon Genetics Co, Ltd.) under 470 nm blue light.

The primer set BD1 and 4S produces the 747 bp PCR products for *G. gigi* and about the 746 bp PCR products for *G. chubuensis*. The PCR products of *G. gigi* have two restriction sites for HaeII [(A/G)GCGC/(T/C)], and three DNA fragments with close number of bases (261, 237 and 248 mer) were produced by HaeII treatment. The PCR products of *G. chubuensis* have one restriction site, and two DNA fragments (497 and 249 mer) were produced. Thus, the two species were distinguishable by the banding pattern of HaeII-treated PCR products.

2.3. Experimental infection

Experimental infections were conducted over five periods (Supplementary Table 1). Experiments using parasites from Takashima were conducted during periods 1–3 and 5; experiments using parasites from Nagahama were conducted during periods 3 and 4.