GENETIC DIVERSITY IN HEADWATER-SPECIFIC MAYFLIES BASED ON THE MITOCHONDRIAL 16S RRNA GENE SEQUENCES

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Abstract

Headwaters are unique components of catchments as they usually support a taxonomically and ecologically unique fauna. This paper, focuses on an endemic Japanese dipteromimid mayflies, which have possibly very limited dispersal ability, and are restricted to headwaters. Its ecological relationships (its dispersal ability) and genetic variation were discussed and compared with some other mayfly species living in other water systems. Dipteromimid mayflies showed greater interpopulation genetic distances than those of any other of the mayfly species examined.

Key words: mayfly; Dipteromimidae; genetic property; genetic diversity; headwater area.

Introduction

Headwaters, the streams that make up the beginnings of rivers, are unique components of catchments as they usually support a taxonomically and ecologically unique fauna. However, since headwaters are generally narrow and have a fluctuating course, they have received little attention from ecologists and their benthic fauna, including insects, is poorly studied (Ota and Takahashi 1999). This paper, focuses on an endemic Japanese dipteromimid mayflies *Dipteromimus tipuliformis* and *D. flavipterus*, which have possibly very limited dispersal ability, and are restricted to headwaters (Tojo and Matsukawa 2003). Its ecological relationships (its dispersal ability) and genetic variation (genetic distance within and between populations in the mitochondrial 16S rRNA gene sequences) were discussed and compared with some other mayfly species (related ameletid and siphlonurid mayflies, and mayflies, e.g., ephemerid, polymitarcyid mayflies) living in other water systems.

Materials and Methods

Two dipteromimid mayflies, *Dipteromimus tipuliformis* MaLachlan (33 individuals from 13 localities) and *D. flavipterus* Tojo and Matsukawa (3 individuals from one locality), were used in this study (Fig. 1). Sequence data of 27 *D. tipuliformis* and all *D. flavipterus* individuals of which were referred from Tojo and

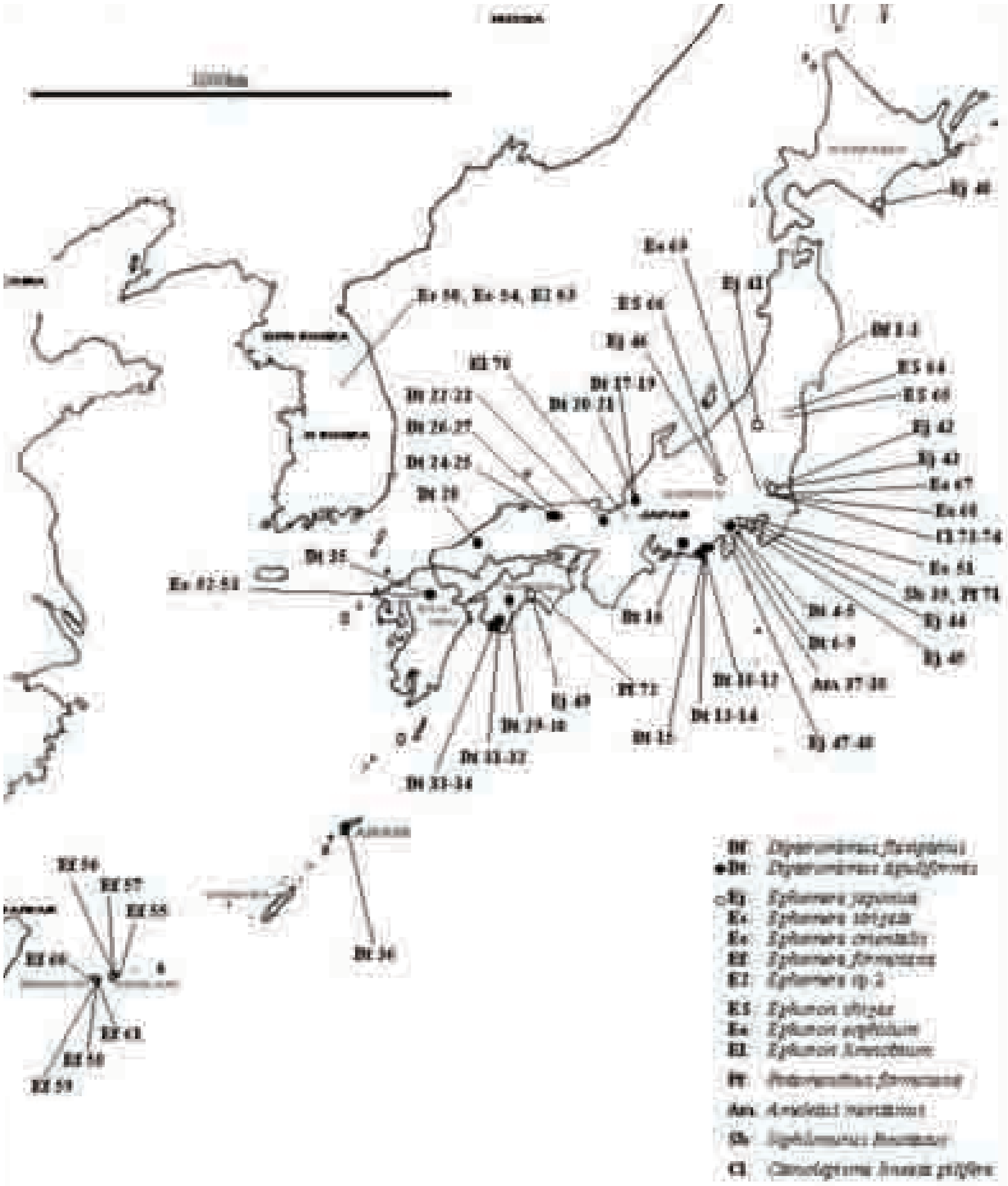

Figure 1. Sampling localities of mayflies examined.

Matsukawa (2003: DDBJ/AB110236-110266). Some related mayflies of Ameletidae and Siphronuridae and some burrowing mayflies of Ephemeridae, Polymitarcyidae and Potamanthidae, which have been studied for molecular analyses, were used as control for the analysis of genetic diversity. A sequence data of *Ephemera* sp. (from Immenburg, Bonn, Germany) was referred from GenBank (AF266047; Misof et al. 2001) and two individuals of silverfish were used as outgroup. Adults and nymphs were fixed with pure ethanol for molecular examination.

DNA Analyses by Sequencing of the Mitochondrial 16S rRNA Region. DNA was extracted from the specimens and purified using the DNeasy Tissue Kit (QIAGEN, Hilden). The 16S rRNA genes were amplified by a PCR method using as the forward primer 5'-TTACGCTGTTATCCCTAA-3' and the reverse primer 5'-CGCCTGTTTATCAAAAACAT-3'. PCR products were purified with Microcon Kit (MILLIPORE, Massachusetts). The purified DNA was sequenced directly by an automated method using the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences, New Jersey) on an automated sequencer (ABI PRISM 377 Genetic Analyzer; Perkin Elmer/Applied Biosystems, California).

Sequence data were aligned using Clustal W (Thompson et al. 1994, 1999) and phylogenetic analyses were performed by the neighbor-joining (NJ) method (Saitou and Nei, 1987), implemented using PHYLIP version 3.57 (Felsenstein 1995). The NJ analyses employed using Kimura's two-parameter method (Kimura 1980) and confidences of branches were assessed by 1,000 bootstrap resamplings. The significant difference in relationships between pairwise genetic distance (uncorrected p-distance) of individuals and geographic distance of their collection site was tested by Mantel test.

Results and Discussion

Dipteromimid mayflies, *Dipteromimus tipuliformis* and *D. flavipterus* were used for examining genetic relationships with related ameletid, siphlonurid mayflies and controlled burrowing mayflies, and silverfishes as outgroups. The neighbor-joining (NJ) dendrogram derived from Kimura's (1980) distance matrix from aligned sequences is shown in Figs. 2 and 3. The monophyly of the dipteromimid mayflies (and other examined mayfly groups) could be strongly supported (the bootstrap proportion [BP] of Dipteromimidae = 82%; [BP] of *D. tipuliformis* = 96%; [BP] of *D. flavipterus* = 100%). As for the in-group, *D. tipuliformis* has high intraspecific variations and some local populations are distinguished on the basis of genetic differentiation (the subclusters were well separated geographically; Figs. 2 and 3). On the other hand, other mayfly groups: Ephemeridae and Polymitarcyidae could be also strongly supported as monophyly at species level (e.g., [BP] of *Ephemera japonica* =84%; [BP] of *E. formosana* = 100%; [BP] of *E. orientalis* =97%; [BP] of *Ephoron shigae* and *E. eophilum* = 100% all), but they have not so high intraspecific variations than dipteromimid mayflies.

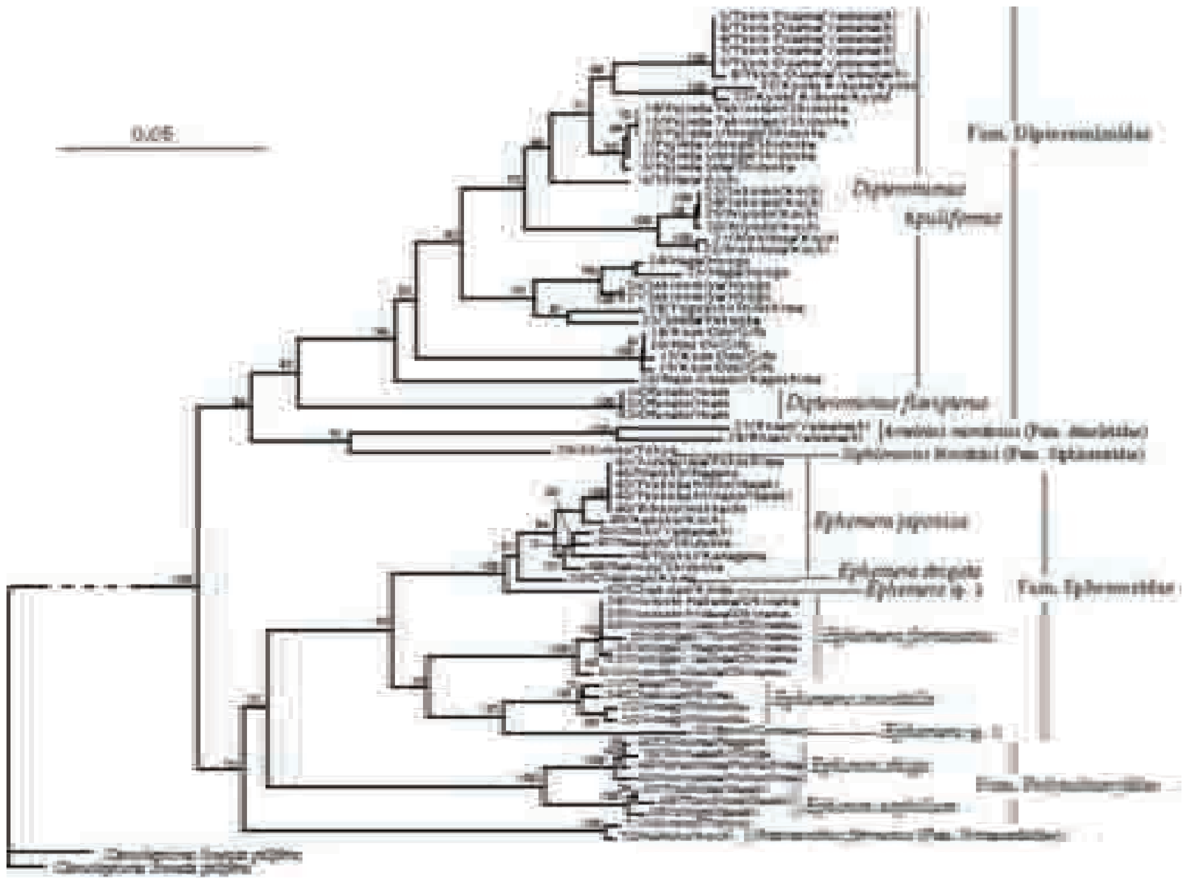

Figure 2. Neighbor-joining (NJ) dendrogram using mitochondrial 16S rRNA gene sequences of mayflies, based on Kimura's 2-parameter genetic distance matrix (Kimura 1980), with two silverfish specimens of *Ctenolepisma lineata pilifera* as outgroups. Bootstrap values for 1000 replicates are indicated at major nodes. Sample number and population information are given in Fig. 1.

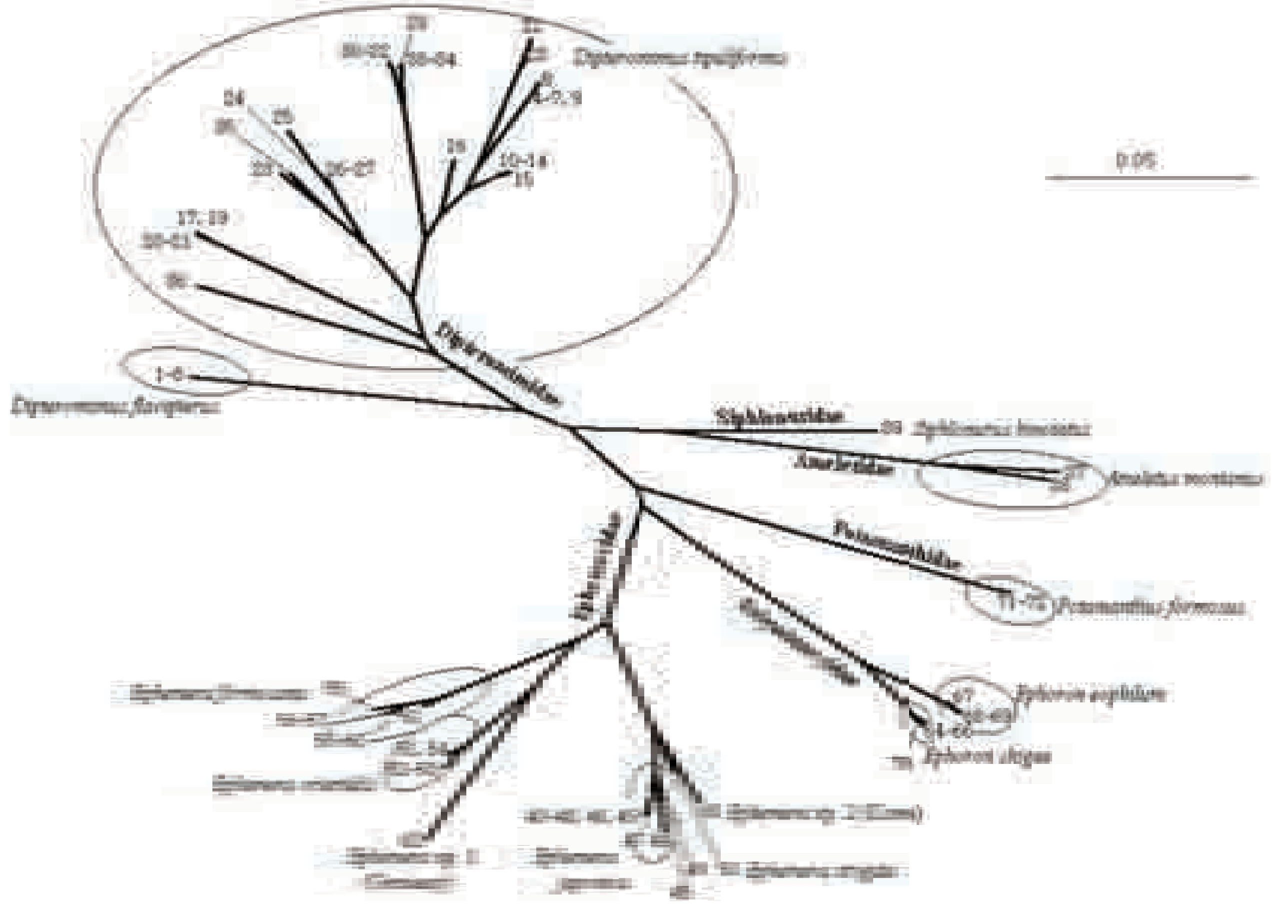

Figure 3. Unrooted NJ dendrogram of the mitochondrial 16S rRNA gene sequences from 72 mayfly specimens (see Fig. 1), based on the matrix as same as that of Fig. 2.

Figure 4 shows the relationships between pairwise genetic distance (uncorrected p-distance) of individuals and geographic distance of their sites in *D. tipuliformis* and *E. japonica*. Dipteromimid mayflies, especially *D. tipuliformis* showed greater intraspecific (interpopulation) genetic distances than those of *E. japonica* and any other of the mayfly species examined (not shown). Dipteromimid mayflies, ephemerid mayflies and the other mayflies examined have univoltine life cycles. We do not consider differences of the interpopulation's genetic variations as originated from these life cycle differences. The dipteromimid mayflies, however, are endemic to headwater areas. These species possibly have very limited dispersal ability. This may be characteristic of headwater aquatic insects (not only dipteromimid mayflies). Other mayfly species and the other aquatic insects living in headwaters should be compared with those living in other water systems (cf. Waples 1995).

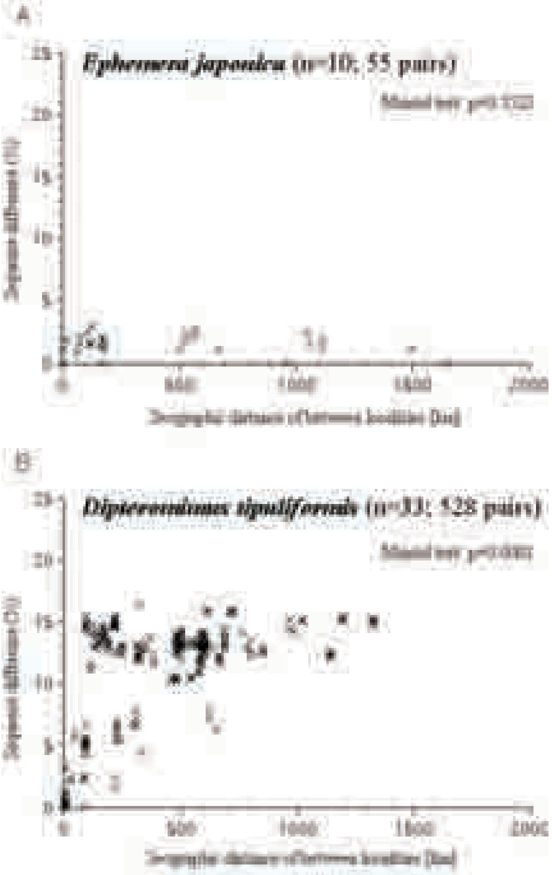

Figure 4. Relationships between pairwise genetic distance (uncorrected p-distance) of individuals and geographic distance of their collection sites. A: *Ephemera japonica* (N = 10; 55 pairs); B: *Dipteromimus flavipterus* (N = 33; 528 pairs).

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