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Enzyme electrophoresis shows that *Heptagenia dalecarlica* Bengtsson and *H. sulphurea* (Müller) (Ephemeroptera: Heptageniidae) are bona species

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Heptagenia dalecarlica and *H. sulphurea* are a species pair, the adults of which are difficult to distinguish; likewise the species diagnostic criteria of the nymphs have been considered doubtful. Population samples from these two species, and from *H. fuscogrisea* and *H. joernensis* were studied with enzyme electrophoresis. The results show that *H. sulphurea* and *H. dalecarlica* have taxon-specific alleles indicating that they are closely related but reproductively isolated species. They are only distantly related to the other two *Heptagenia* species examined.

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The species pair *Heptagenia sulphurea* (Müller, 1776) – *H. dalecarlica* Bengtsson, 1912 exhibits a notorious taxonomic problem in the Fennoscandian ephemerid fauna (Saaristo & Savolainen 1980). The distribution of *H. sulphurea* covers all of Europe. Bengtsson (1912, 1917) described *H. dalecarlica* as a species close to *H. sulphurea*. Both taxa are easily distinguished from other *Heptagenia* species (e.g. Landa 1969, Kimmins 1972, Macan 1979).

The nymphs of *H. dalecarlica* are easily told from the corresponding stages of *H. sulphurea* on the basis of the reverse asymmetry of the mandibles while the winged stages are very similar. Ulfstrand (1968a, b) argued that the winged stages cannot be distinguished, and expressed doubts whether the reverse asymmetry of the nymphal mandibles is a valid species criterion. Saaristo & Savolainen (1980) redescribed the two species and showed that both nymphs and winged forms can be reliably distinguished. Zurwerra et al. (1987) made a survey of European Heptageniidae using biochemical markers and argued that *H. dalecarlica* and *H. sulphurea* are good species. Kluge (1987), using morphological criteria and extensive material came to the opposite conclusion. He claimed that there is within and between population variation in the diagnostic criteria and that distinguishing any single individual is therefore impossible.

Even when the existence of two species, *H. sulphurea* and *H. dalecarlica*, is not directly challenged (e.g. Puthz 1978), only the Scandinavians have acknowledged *H. dalecarlica* as a bona fide species. This has given rise to inconsistencies in species descriptions, drawings and distribution data (Saaristo & Savolainen 1980, for details).

H. sulphurea nymphs live in flowing water, including swift rapids (e.g. Tiensuu 1935, Puthz 1978, Saaristo & Savolainen 1980). The nymphal habitat of *H. dalecarlica* is less well known. In the material of Saaristo & Savolainen (1980) the central Finnish samples come from rocky exposed lake shores and the northern samples from running waters or lake shores. According to Ulfstrand (1968a, b, 1969), *H. sulphurea* preferres lake outlets while *H. dalecarli-*

ca is more abundant in other lotic sites and has a wider local distribution range. Yet, differences in habitat preferences cannot be used to distinguish the species.

This study aims at resolving the relationship of the two taxa, *H. dalecarlica* and *H. sulphurea* with enzyme electrophoresis and to establish their genetic distance. As a reference we have used two other species occurring in Fennoscandia, *H. fuscogrisea* (Retzius, 1783) and *H. joernensis* (Bengtsson, 1909). The latter species has recently been considered a member of the North American genus *Nixe* (Flowers 1986).

Materials and methods

Mayflies were collected from the following localities in central and northern Finland (the uniform grid coordinates, Grid 27°E, are given in parentheses):

1. North Häme, Karstula (696:39)
2. North Häme, Vesanto (698:45)
3. North Karelia, Kaavi (697:59)
4. Inari Lapland, Ivalo (759:52)
5. Inari Lapland, Utsjoki (774:49)

Sampling localities and sample sizes are as follows:

H. dalecarlica, from localities 2, 4 and 5, a total of 214 specimens
H. sulphurea, from locality 1, a total of 41 specimens
H. fuscogrisea, from localities 2 and 3, a total of 98 specimens
H. joernensis, from locality 2, a total of 67 specimens.

Horizontal starch gel electrophoresis and enzyme assays were carried out as described by Saura et al. (1979). The following twelve loci were scored: Esterase-2 (Est-2), Fumarase (Fum), α-Glycerophosphate dehydrogenase (α-Gpdh), Hexokinase (Hk), Isocitrate dehydrogenase (Idh), Lactate dehydrogenase (Ldh), Malate dehydrogenase (Mdh-1 and -2), Malic enzyme (Me), Phosphoglucose isomerase (Pgi), Phosphoglucomutase (Pgm) and Superoxide dismutase (Sod). A number of other assays were tried but the results were not reproducible.

Results

Identical electromorphs for α-Gpdh, Idh, Ldh, Mdh-1, Me and Sod were observed in *H. dalecarlica* and *H. sulphurea*, suggesting that identical alleles appeared to be fixed in both species.

The following loci were polymorphic: Est-2, Fum, Hk, Mdh-2 and Pgi. Est-2 was polymorphic in *H. sulphurea* with two alleles, of which the most common one was fixed in *H. dalecarlica*. In Fum *H. sulphurea* had a high frequency (0.83) of an allele not found in *H. dalecarlica*; the other allele present in *H. sulphurea* was almost fixed in *H. dalecarlica*, which had two other rare alleles. Hk had four alleles, one of which was abundant in *H. sulphurea* and in the Utsjoki population of *H. dalecarlica*; in addition *H. sulphurea* had a species-specific electromorph and *H. dalecarlica* two others, one of which predominated in the Vesanto and Ivalo populations and had a frequency of 0.17 at Utsjoki. Mdh-2 was monomorphic in *H. sulphurea* and polymorphic with three alleles in the three *H. dalecarlica* populations. Pgi was polymorphic with three alleles in both species: *H. sulphurea* was more variable than *H. dalecarlica*. In addition, Pgm, was polymorphic but *H. dalecarlica* and *H. sulphurea* did not have alleles in common. The species-diagnostic loci indicate that *H. dalecarlica* and *H. sulphurea* do not interbreed in nature.

Discussion

Genetic distance (D) was estimated according to Nei (1978). The genetic distances between *H. dalecarlica* and *H. sulphurea* ranged between 0.225 and 0.344. *H. dalecarlica - sulphurea* and *H. joernensis* did not have any alleles in common; *H. fuscogrisea* and *H. joernensis* were monomorphic for the same Fum allele; *H. fuscogrisea* and *H. dalecarlica - sulphurea* were monomorphic for the same α-Gpdh alleles. Otherwise the species did not share alleles at the loci studied. Dr Pekka Lankinen of the University of Oulu, Finland, informs us that nymphs sampled from a single locality (Oulanka, Kuusamo, grid number 736:60) can be distinguished through electrophoresis. He reports the same difference at Mdh-2 that we report here for *H. sulphurea* and *H. dalecarlica*.

Fig. 1 shows a UPGMA phylogenetic tree for the species *H. dalecarlica* and *H. sulphurea*. *H. dalecarlica* differs from *H. sulphurea* in the Pgm locus, at which the two species do not share alleles. Although their genetic distance is short, they are evidently good species that do not interbreed in the nature.

Zurwerra et al. (1987), who made an enzyme electrophoretic study of many species of Heptageniidae, also concluded that *H. sulphurea* and *H. dalecarlica* are good species. However, they used the most common allele only and did not study varia-

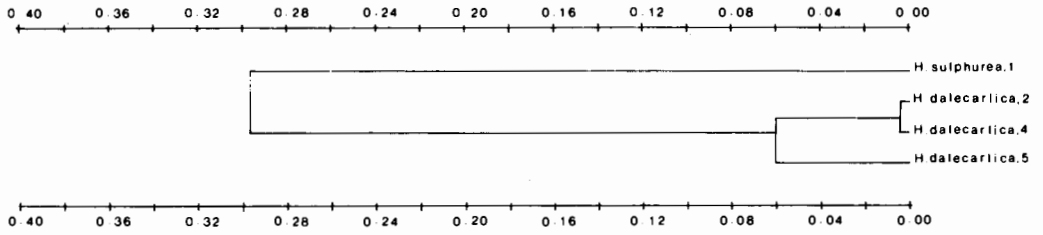

Fig 1. A dendrogram based on enzyme electrophoretic data showing the relationships between three populations of *H. dalecarlica* and a population of *H. sulphurea*. The scale is D, the genetic distance of Nei (1978).

tion within species. Kluge (1987) stressed the importance of variation within species in arguing that *H. sulphurea* and *H. dalecarlica* are identical species. Our electrophoretic data indicate that the two taxa are reproductively isolated.

As evidenced by the results, *H. fuscogrisea* and *H. joernensis* are only distantly related to each other and to the *H. dalecarlica - sulphurea* complex. The genetic distances between *H. fuscogrisea* and *H. joernensis*, and between each of these and *H. dalecarlica - sulphurea* all approach 1.0.

Zurwerra et al. (1987) argued, on the basis of electrophoretic data, that *joernensis* belongs to *Nixe*. They did not, however, have any reference material of that genus. Actually, *H. fuscogrisea* is also only distantly related to *H. sulphurea* and *H. dalecarlica*, and it differs substantially, both genetically and morphologically, from other *Heptagenia* (Landa 1969, Kimmins 1972, Macan 1979). More comparative data are required to settle whether *joernensis* properly belongs to *Nixe* or to *Heptagenia*.

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