

## Ethological isolation and genetic diversity

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Adults of *Leptophlebia marginata* (Ephemeroptera) exhibit two modes of swarming behavior: the swarming individuals of the first type orientate to trees and the individuals of the second to horizontal objects paler than the environment, e.g. stones or patches of lichen. The two types of swarms occur synchronously at the same locality. No morphological differences have been detected in individuals with different swarming habits. Electrophoretic analysis showed, however, clear-cut differences in the allele frequencies at two loci *a-Gpdh* and *Idh-2*, in the two populations. This indicates that the populations are reproductively isolated. The genetic distance between these populations is short in comparison with other comparable populations. This is mostly due to the low mean heterozygosity in *L. marginata*.

### I. Introduction

Areal swarming is a widespread rhythmic aspect of insect behavior. The swarming of Diptera is, in particular, well documented (e.g. McAlpine & Munroe 1968, Downes 1969). Recently the author Savolainen (1978) has studied the swarming of Ephemeroptera, the May flies. The adults of these insects are extremely short-lived, and it is generally held that swarming constitutes an effective mechanism for bringing the sexes together for copulation.

Swarms of closely related species of May flies may orientate to different objects, swarm markers, or they may have different swarming times. Differences in swarming behavior can

be considered to constitute an effective isolating mechanism. In central Europe e.g. *Ephemera vulgata* swarms in the morning and *E. danica* in the afternoon (Müller-Liebenau 1960).

Savolainen (1978) found, in studying the swarming of *Leptophlebia marginata* at a locality in Kaavi, central Finland (62° 51' N, 28° 52' E), that this species exhibited two different modes of behavior with regard to swarming sites and swarm markers: some swarmed beside trees in a fashion exhibited by individuals of this species elsewhere. In a dense forest these May flies swarm at the level of tree tops. Some again, even inside the forest, swarmed at a low level (0.2 to 1.5 m) above swarm markers paler than the environment, such as stones, patches of lichen and pieces of

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grey-and-white cardboard. They swarmed only above markers exposed to direct sunlight.

Savolainen studied these behavioral differences by transferring individuals orientating to horizontal markers to Matkonsaari, a locality some 10 kilometers away, where these marked individuals still continued swarming over pale markers; again specimens orientating to tree tops from this locality persisted in their former behavior, when transferred to Munaharju. No morphological differences could be established between *L. marginata* individuals swarming in different fashions: yet they appear to be two reproductively isolated sympatric populations. To test, whether there is gene flow between these populations, we have collected a material of about 400 *Leptophlebia marginata* May flies from different populations in Finland and studied their genetic constitution by electrophoresis.

## II. Material and methods

**May flies.** Swarming males of *Leptophlebia marginata* were captured with an insect net and brought alive to the laboratory, where they were kept in deep freeze for a couple of weeks before electrophoresis. Collections were made in early June in 1976 and in 1977.

The following populations were sampled

<i>L. marginata</i> orientating to trees	
locality	number of individuals
Kaavi, Matkonsaari	20
Kaavi, Munaharju	20
Kaavi, Näлкölämpi	28
Kaavi, Mietunlahti	44
Riistavesi, Lautta-aho	20
Kuopio, Kolmisoppi	33
Kuopio, Vuorilampi	6
Hyrnsalmi, Seitenjärvi	28
Taivalkoski, Valkeinen	7
Kuusamo, Jäkälämutka	28
Kuusamo, Särkikangas	17
Salla, Savilampi	16
<i>L. marginata</i> orientating to horizontal markers	
locality	number of individuals
Kaavi, Munaharju	86
Kaavi, Vääräpuro	6
Kuusamo, Haaralampi	30

Many of these localities are described in detail by Savolainen (1978).

**Laboratory techniques.** The May flies were homogenized in a drop of distilled water in a plastic tissue homogenizer attached to a "Multifix" laboratory stirrer. The starch gel electrophoresis technique used is described in detail by Saura et al. (1979). The following enzymes were successfully assayed: adenylate kinase (Adk), aldolase (Aldo), esterase (Est), fumarase (Fum), glutamate-oxaloacetate transaminase (Got),  $\alpha$ -glycerophosphate dehydrogenase ( $\alpha$ -Gpdh), hexokinase (Hk), hydroxybutyrate dehydrogenase (Hbdh), isocitrate dehydrogenase (Idh), lactate dehydrogenase (Ldh), malate dehydrogenase (Mdh) malic enzyme (Me), phosphoglucumutase (Pgm), 6-phosphogluconate dehydrogenase (6-Pgdh), phosphoglucose isomerase (Pgi), superoxide dismutase (Sod) and triosephosphate isomerase (Tpi). Our conventions to designate gene loci coding for enzymes and their alleles are given e.g. by Suomalainen et al. (1979). Individuals from an *Ephemera vulgata* population were used as controls in this study; the electrophoretic mobility of *L. marginata* allozymes are expressed in relation to these controls.

## III. Results

There were 18 monomorphic enzyme loci in the material. A locus is considered to be monomorphic, when the frequency of the most common allele exceeds 0.99. The following loci were monomorphic: *Adk-2*, *Aldo*, *Est-2*, *Fum*, *Got*, *Hk-3*, *Hbdh*, *Idh-1*, *Idh-2*, *Ldh*, *Mdh-1*, *Mdh-2*, *6-Pgdh-1*, *Pgm*, *Sod-1*, *Sod-2*, *Tpi-1* and *Tpi-2*.

It should be noted that even though *Idh-2* was considered to be monomorphic, it was, however, evidently monomorphic, for a different allele, either *Idh-2<sup>84</sup>* or *Idh-2<sup>85</sup>* in May flies orientating to different markers. The alleles were difficult to tell from each other, and eventual polymorphism could not be reliably determined.

There were only five polymorphic loci in the material, namely *Est-1* (with four alleles; the proportion of heterozygotes — on the assumption of a Hardy-Weinberg equilibrium — was 0.186). There were two alleles segregating at

*Me*; the expected proportion of heterozygotes was 0.396. The number of alleles at *Pgi* was four; the expected proportion of heterozygotes was 0.397. Both in *Me* and *Pgi* the northern samples, that is, samples to the north of the Kuopio—Kaavi level, were considerably less heterozygous than the southern samples. The locus labeled *6-Pgdh-2* was clearly polymorphic, but the sample size for this locus was so small that we have not included the two *6-Pgdh* loci in the heterozygosity estimations.

The most interesting locus is, however, *α-Gpdh*. There are three alleles at this locus. Their distribution in the *L. marginata* populations is clearly correlated with swarming orientation; as evidenced in the tabulation:

Orientation to	Sample size	Alleles			Proportion of heterozygotes
		93	95	98	
Horizontal markers	242	0.314	0	0.686	0.362
Trees	514	0.002	0.002	0.996	0.008

The Haaralampi population from Kuusamo differed from the other populations orientating to horizontal markers in being monomorphic for *α-Gpdh*<sup>98</sup>. When this population is excluded, the proportion of heterozygotes is as high as 0.481 with a standard deviation 0.04 in the populations orientating to horizontal markers.

*Idh-2* is also correlated with swarming behavior, so that *Idh-2*<sup>85</sup> is, at least in the southern populations, restricted to individuals orientating to horizontal markers.

The conclusion emerging from the results is that there are two reproductively isolated synchronously swarming populations. The absence of morphological differentiation as well as the fact that there are no good frequency differences at the other loci, suggests that we are here confronted with an interesting problem of speciation.

#### IV. Discussion

The average degree of heterozygosity per locus per individual *Leptophlebia marginata* is 0.058.

This value is based on heterozygotes at 21 loci, and is, if anything, low for an insect. Powell (1975) has compiled data of insect heterozygosities, and his estimates of mean heterozygosity are of the order of 0.16 for the class Insecta. The Ephemeroptera are, as a group, relatively little studied and it may be misleading to generalize this result.

The importance of aerial swarming as an isolating mechanism is a relatively little discussed topic. Lindeberg (1964) has suggested the extreme; i.e. that swarming behavior is as good a taxonomic criterion as any morphological characteristic. Savolainen (1978) has presented evidence that even females of at least certain Ephemeropterans orientate to the same swarm markers as males. So swarming behavior, at least theoretically, constitutes an effective isolating mechanism.

Few authors have described differences in the swarming behavior within a species. Harker (1953) suggested that the short life span and swarming behavior of May fly adults constitute an important isolating mechanism. Kosova (1967) has described three modes of swarming of *Palingenia sublongicauda* in the delta of the Volga. She also suggests that swarming behavior constitutes an important isolating mechanism. Savolainen (1978) has, in addition to the cases described here, reported differences in the swarming behavior of *Leptophlebia vespertina*.

The number of described species of the order Ephemeroptera does not exceed 2000, so that speciation has not been very extensive in this old insect order. We have, however, found apparent ethological isolation between synchronous and sympatric populations.

We shall briefly try to quantify the differentiation process by the distance method devised by Nei (1972). The distances reported are based on the Kaavi, Munaharju populations, and the following loci are not included in the calculations (as a result of inadequate data): *Aldo*, *Hbdh*, *Got*, *Ldh* and the two *Tpi* loci. The genetic distance, *D* (Nei 1972) between the two populations at Munaharju, is 0.02, if the *Idh-2* locus is omitted from calculations. If we assume that the populations, in fact, are homozygous for different alleles at this locus, we get a *D* = 0.10. The genetic distances are

very small in comparison with the *D*'s of sibling species of insects with range from 0.35 upwards, as discussed by Lokki et al. (1979). In this case the low genetic distance values here are due to the low mean heterozygoties at the two populations.

Yet the differences in the allele frequencies at the *α-Gpdh* and *Idh-2* loci indicate that the two sympatric populations do not interbreed in the nature. Whether the isolation is as complete in the northern populations as at Munaharju remains to be demonstrated. At this stage we can not exclude the possibility of gene flow through other populations. Studying behavioral aspects in relation to genetic differentiation appears to offer a promising approach to many population genetic problems.

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