

# Genetic structure of two pseudoscorpion species living in tree hollows in Sweden

T. Ranius\* & P. Douwes

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## Abstract

*Genetic structure of two pseudoscorpion species living in tree hollows in Sweden.*— Two saproxylic pseudoscorpions, *Larca lata* and *Allochernes wideri*, were compared in an analysis of genetic structure in southern Sweden. *Allochernes wideri* is a relatively widely distributed species that occurs in single-standing trees and in small tree hollows, while *L. lata* is on the Swedish red list and confined to larger assemblages of very old trees with hollows containing large amounts of wood mould. In *A. wideri*, the polymorphism of PGM was used, whereas in *L. lata* the variation for PGI was studied. The genetic differentiation between trees within a site was low for both species, indicating that the migration between nearby trees is considerable despite the fact that phoretic dispersal has only been occasionally observed in these species. Between sites, situated four to 900 km from each other, the genetic differentiation was small both in *A. wideri* and *L. lata* with no difference between the species, when considered on the mainland only. The small differentiation suggests the habitat was fragmented recently (100–170 years ago). The relation between the rate of migration and long-term population survival and the risk of mis-interpretation due to selection for alleles is discussed.

Key words: *Allochernes wideri*, Allozymes, Dispersal, Habitat fragmentation, *Larca lata*, Phoresy.

## Resumen

*Estructura genética de dos especies de pseudoescorpión que viven en los huecos de árboles en Suecia.*— Se comparan dos pseudoescorpiones saproxílicos, *Larca lata* y *Allochernes wideri*, del sur de Suecia mediante un análisis de su estructura genética. *Allochernes wideri* es una especie de distribución relativamente amplia que se encuentra en árboles aislados y en pequeños huecos de árboles, mientras que *L. lata* aparece en la lista roja sueca y se encuentra confinado en grandes agrupaciones de árboles muy viejos cuyos huecos contienen gran cantidad de moho. En *A. wideri* se empleó el polimorfismo de PGM mientras que en *L. lata* se estudió la variación por PGI. La diferenciación genética entre árboles de un mismo lugar fue baja para ambas especies, indicando que la migración entre árboles cercanos es considerable aun cuando sólo se observó dispersión forética ocasionalmente en ambas especies. Entre zonas situadas a una distancia de 4 a 900 km, la diferenciación genética fue escasa en ambas especies, *A. wideri* y *L. lata*, sin ninguna diferencia entre las mismas cuando se consideró únicamente la zona principal. Esta pequeña diferenciación sugiere que el hábitat se fragmentó recientemente (100–700 años antes). Se discute la relación entre la tasa de migración y la supervivencia de la población a largo plazo y el riesgo de una mala interpretación debida a la selección de los alelos.

Palabras clave: *Allochernes wideri*, Aloenzimas, Dispersión, Fragmentación del hábitat, *Larca lata*, Foresis.

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Thomas Ranius & Per Douwes, Lund Univ., Dept. of Zoology, Helgonav. 3, SE-223 62 Lund, Sweden.

\* New address for corresponding author: Thomas Ranius, Swedish Univ. of Agricultural Sciences, Dept. of Entomology, P. O. Box 7044, SE-750 07 Uppsala, Sweden.

E-mail: [thomas.ranius@entom.slu.se](mailto:thomas.ranius@entom.slu.se)

## Introduction

Most species are associated with a habitat that is more or less patchy, resulting in a population structure with areas of high abundances separated by areas in which the species is rare or absent. In the last few centuries, human activities have caused many habitats to become subdivided into patches which are much smaller and more isolated than in the primaevial landscape (e.g. ANGELSTAM, 1992; HARRISON & FAHRIG, 1995). Especially for those species that have evolved in a spatially more continuous habitat, fragmentation into smaller, isolated populations may increase susceptibility to extinction both for genetic and demographic reasons (NILSSON & ERICSON, 1997).

Genetic differentiation measurements might provide an understanding of the population structure and migration, which are important in conservation work (STACEY et al., 1997). The impact of decreased habitat patch size and increased isolation on population genetics have, among invertebrates, mainly been studied on butterflies and moths (e.g. VAN DONGEN et al., 1998; HOOLE et al., 1999; MEGLÉCZ et al., 1999; CLARKE & O'DWYER, 2000; FIGURNY-PUCHALSKA et al., 2000), while other taxa have been less studied (see however VOGLER et al., 1993; KNUTSEN et al., 2000; JONSSON, 2002 on beetles).

The present study considers the genetic differentiation in two pseudoscorpion species that are associated with tree hollows. One of the species, *Larca lata* H. J. Hansen (Pseudoscorpionida, Larcidae), is only found in larger assemblages of ancient, hollow oaks, whereas the other, *Allochernes wideri* C. L. Koch (Pseudoscorpionida, Chernetidae), has a broader habitat and occurs at more localities (RANIUS & WILANDER, 2000). Old oaks occur mainly in old-growth deciduous forests and pasture woodlands, and in Europe both these habitats have decreased severely in the last few centuries (HANNAH et al., 1995; KIRBY & WATKINS, 1998). In Sweden, the major decrease of old oaks occurred 100–170 years ago (ELIASSON & NILSSON, 1999). A rare and endangered saproxylic fauna, mainly consisting of beetles, flies and pseudoscorpions, is associated with tree hollows (SPEIGHT, 1989). It seems that only one genetic study on a rare saproxylic invertebrate has been performed (JONSSON, 2002) and that was not on a species associated with tree hollows. Tree hollows are expected to be a stable habitat, with narrow fluctuations in microclimate and nutrient supply. In a living hollow tree, partly decomposed wood inside the trunk is surrounded by growing sound wood, resulting in a continuous nutrient supply for the saproxylic fauna. The daily microclimate fluctuation is much smaller in a trunk hollow than at the surface of the trunk (KELNER-PILLAULT, 1974; PARK & AUERBACH, 1954). This might cause inhabiting species to have narrow population fluctuations, which decrease their extinction rate in comparison with other invertebrate populations

of the same size (RANIUS, 2001). Therefore it is possible that these species have relictual distributions, with small, isolated populations surviving in small remnants over long periods after the habitat density has become too low to allow long-term metapopulation persistence (RANIUS, 2000).

Many pseudoscorpion species disperse phoretically, which means that they hitch-hike with other animals, usually insects. There is circumstantial evidence that both *L. lata* and *A. wideri* perform phoretic dispersal, but it is not known how frequent this behaviour is (RANIUS & WILANDER, 2000). As *L. lata* is confined to larger assemblages of hollow trees, whereas *A. wideri* occurs also in single trees far from other hollow trees, it has been suggested that *L. lata* has a more restricted colonization ability (RANIUS & WILANDER, 2000).

In this study, the degree of genetic differentiation was examined in order to estimate the extent of genetic drift and migration. Being the less abundant species and inhabiting a narrow ecological range *L. lata* was expected to have lower population sizes within a given area and lower frequency of migration, which would give rise to higher levels of genetic differentiation than for *A. wideri*.

## Material and methods

### Sampling

Sampling was designed to determine the degree of genetic differentiation at two geographic levels: 1. Samples were taken from trees situated 100–700 m from each other in Bjärka-Säby (fig. 1). About 30 individuals of *A. wideri* were then sampled from each of three trees and individuals of *L. lata* from four trees. 2. Samples were taken from sites situated four to 900 km apart, all within southern Sweden (fig. 1). *Allochernes wideri* was sampled from ten, and *L. lata* from six sites (table 1 and 2). A total sample of about 30 individuals of each species was taken from one to five hollow trees at each site, except at Bjärka-Säby where larger samples were taken as all those at the tree level were pooled when used as a sample at the site level.

The sampling was carried out from 1995 to 1999. It was performed by sieving wood mould, and in the field the fine fraction was spread out on a white sheet where the pseudoscorpions were searched for. The pseudoscorpions were stored alive before transfer to a freezer. Electrophoresis was performed within two years of sample collecting.

### Electrophoresis

Horizontal starch-gel electrophoresis was used to investigate allozyme variation. The electrophoresis technique used has been described by SELANDER et

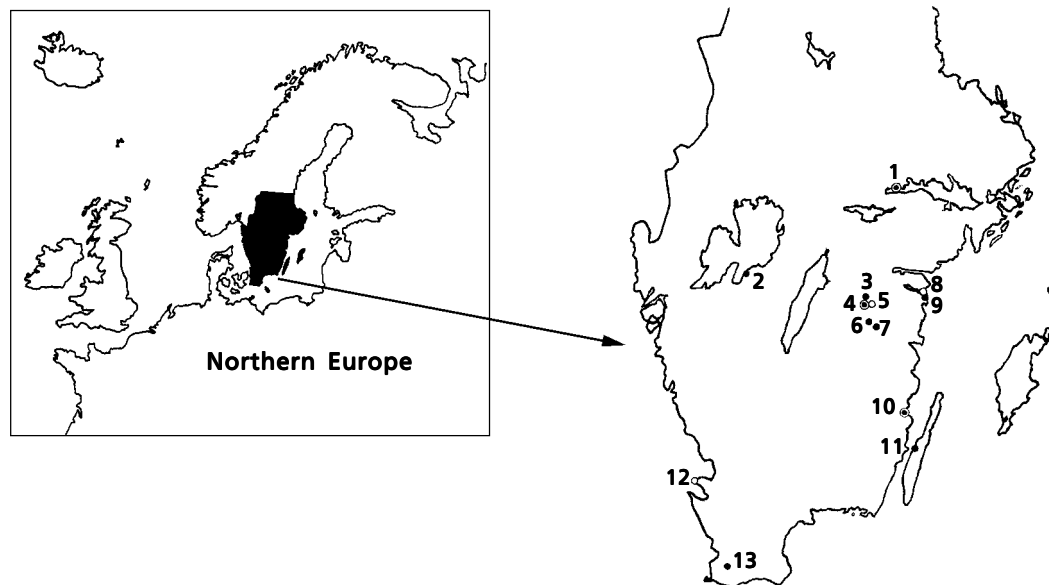


Fig. 1. Sampling sites in Sweden: ● Sites where *A. wideri* samples have been taken; ○ Sites where *L. lata* samples have been taken; ◐ Sites where both *A. wideri* and *L. lata* samples have been taken. Localities: 1. Strömsholm; 2. Kinnekulle; 3. Långvassudde; 4. Bjärka-Säby; 5. Grebo; 6. Tjärstad; 7. Kättilstad; 8. Djursö; 9. Sankt Anna; 10. Strömserum; 11. Halltorp; 12. Hallands Väderö; 13. Yddingen.

Fig. 1. Áreas de estudio de Suecia: ● Áreas donde se tomaron muestras de *A. wideri*; ○ Áreas donde se tomaron muestras de *L. lata*; ◐ Áreas donde se recogieron muestras de ambas *A. wideri* y *L. lata*. Localidades: 1. Strömsholm; 2. Kinnekulle; 3. Långvassudde; 4. Bjärka-Säby; 5. Grebo; 6. Tjärstad; 7. Kättilstad; 8. Djursö; 9. Sankt Anna; 10. Strömserum; 11. Halltorp; 12. Hallands Väderö; 13. Yddingen.

al. (1971). The loci of six enzymes were screened: ME (malic enzyme),  $\alpha$ -GPD ( $\alpha$ -glucosephosphate dehydrogenase), MDH (malate dehydrogenase), PGI (phosphoglucose isomerase), PGM (phosphoglucomutase) and IDH (isocitrate dehydrogenase) in specimens from Bjärka-Säby of both species. ME,  $\alpha$ -GPD and MDH had scorable activity in none or only a few individuals, and no variation was observed among scorable individuals. Therefore, these loci were not further used in this study. PGI, PGM and IDH had scorable activity in all individuals and were thus assessed in all samples. Sufficient material was normally obtained from each individual to load two starch gels, but some small nymphs did not give any visible bands. To control for possible between-gel artefacts, specimens from at least two sites were run in each gel.

#### Statistical analyses

The statistical package POPGEN vers. 1.31 (by F. Yeh, R. Yang & T. Boyle) was used to calculate allele frequencies, expected and observed

heterozygosity and F-statistics.

Patterns of genetic structure were revealed through the analysis of allele frequencies using F-statistics (HEDRICK, 1983). The most commonly used statistic,  $F_{st}$  is a measure of the extent to which subpopulations show spatial genetic heterogeneity.  $F_{st}$  values range from 0, suggesting lack of differentiation or panmixia, to 1, indicating fixation of alternate alleles and complete differentiation. Chi-square was used to test the significance of the allele frequency differences among populations:

$$\chi^2 = 2NF_{st}(k - 1) \quad \text{d.f.} = (k - 1)(s - 1)$$

(BAKER, 1981; BILTON, 1992).  $N$  is the total number of individuals,  $k$  is the number of alleles for the locus and  $s$  is the number of subpopulations. The gene flow between trees within a site was estimated from F-statistics (HEDRICK, 1983):

$$F_{st} = 1 / (4Nm + 1)$$

where  $Nm$  is the average number of migrants

between subpopulations per generation. This gene flow estimate is developed from an island model of population structure, with every subpopulation inhabiting an island equally accessible from every other, with a balance between genetic drift and migration. Although few populations actually conform to the assumption of this model it is useful as an approximation of the magnitude of gene flow, as dispersal has a strong correlation with the genetic structure in most populations (BOHONAK, 1999). The gene flow was not calculated at the site level, as the effects of genetic drift and migration were not considered to be in equilibrium there.

The genetic distance  $D$  (NEI, 1972) was calculated in both species at the site level for each pair of populations. The coefficient of correlation between  $D$  and the geographical distance was

calculated, and the statistical significance of the correlation was tested with a one-tailed Mantel test with 200 randomized values calculated (SOKAL & ROHLF, 1995).

## Results

In *A. wideri* only PGM was polymorphic, while in *L. lata* variation both in PGM and PGI was found. However, in *L. lata* the heterozygotes of PGM did not segregate properly and it was therefore impossible to score the genotypes unambiguously. It was therefore decided to omit this locus from further analysis. IDH was monomorphic throughout in both species and PGI was monomorphic in *A. wideri*.

At the PGM locus (*A. wideri*) four alleles were

Table 1. Allele frequencies at the PGM locus in populations of *Allochernes wideri*: Sample size. Number of individuals in the sample; Stand size. Roughly estimated number of hollow trees with wood mould;  $H_e$  Expected heterozygosity;  $H_o$  Observed heterozygosity; Frequency of alleles (Z. Very slow, S. Slow, M. Medium, F. Fast).

Tabla 1. Frecuencia de alelos en el locus PGM en una población de *Allochernes wideri*: Sample size. Número de individuos de la muestra; Stand size. Número estimado aproximado de huecos de árboles con moho de madera;  $H_e$  Heterocigosis esperada;  $H_o$  Heterocigosis observada; Frecuencia de alelos (Z. Muy baja, S. Baja, M. Media, F. Alta).

### A. Sites in southern Sweden situated 4–500 km from each other

Locality	Sample size	Stand size	$H_e$	$H_o$	Frequency of alleles			
					Z	S	M	F
Strömsholm	26	100–200	0.50	0.58	0.00	0.56	0.44	0.00
Kinneulle	27	50	0.48	0.48	0.00	0.33	0.65	0.02
Långvassudde	38	100–200	0.55	0.58	0.00	0.41	0.54	0.05
Bjärka-Säby	90	200	0.53	0.50	0.01	0.46	0.52	0.02
Tjärstad	29	1	0.61	0.52	0.03	0.19	0.57	0.21
Kättilstad	31	20	0.49	0.48	0.00	0.37	0.61	0.02
Sankt Anna	31	20–50	0.55	0.55	0.00	0.26	0.61	0.13
Strömserum	29	50–200	0.67	0.52	0.03	0.19	0.45	0.33
Halltorp	21	20	0.61	0.57	0.02	0.29	0.55	0.14
Yddingen	30	10–30	0.53	0.43	0.02	0.27	0.63	0.08

### B. Trees within the Bjärka-Säby site, situated 100–300 m from each other

	38		0.52	0.45	0.00	0.40	0.58	0.03
	27		0.52	0.56	0.02	0.41	0.57	0.00
	25		0.51	0.52	0.02	0.60	0.36	0.02

Table 2. Allele frequencies at the PGI locus in populations of *Larca lata*: Sample size. Number of individuals in the sample; Stand size. Roughly estimated number of hollow trees with wood mould;  $H_e$  Expected heterozygosity;  $H_o$  Observed heterozygosity; Frequency of alleles (S. Slow, M. Medium, F. Fast).

Tabla 2. Frecuencia de alelos en el locus PGI de una población de *Larca lata*: Sample size. Número de individuos de la muestra; Stand size. Número estimado aproximado de huecos de árboles con moho de madera;  $H_e$  Heterocigosis esperada;  $H_o$  Heterocigosis observada; Frecuencia de alelos (S. Baja, M. Media, F. Alta).

A. Sites in Sweden, situated 7–500 km from each other

Locality	Sample size	Stand size	$H_e$	$H_o$	Frequency of alleles		
					S	M	F
Strömsholm	44	100–200	0.57	0.68	0.55	0.35	0.10
Bjärka–Säby	129	200	0.60	0.61	0.37	0.50	0.13
Grebo	49	130	0.62	0.57	0.52	0.28	0.20
Djursö	38	80	0.59	0.58	0.50	0.40	0.10
Strömserum	29	50–200	0.52	0.55	0.60	0.35	0.05
Hallands Väderö	27	50–100	0.33	0.19	0.80	0.00	0.20

B. Trees within the Bjärka–Säby site, 100–700 m from each other

	36		0.48	0.50	0.39	0.61	0.00
	30		0.65	0.77	0.35	0.43	0.22
	30		0.60	0.57	0.38	0.50	0.12
	33		0.64	0.63	0.35	0.45	0.20

found, one of which (Z) occurred in very low frequencies (< 5%, table 1). The three alleles at the PGI locus (*L. lata*) appeared in almost all samples except the rather frequent M allele, which was not found at Hallands Väderö (table 2).

The  $H_o$  did not deviate from Hardy–Weinberg equilibrium (table 2) when it was tested by  $\chi^2$  for each population ( $p > 0.05$ ). This suggests that the samples were taken from fairly panmictic populations. The genetic differentiation between the populations ( $F_{st}$ ) at sites was low in *A. wideri* and moderate in *L. lata* (table 3). However, without the Hallands Väderö population, the  $F_{st}$  in *L. lata* was considerably lower ( $F_{st} = 0.0241$ ), yet still significantly above zero. The genetic differentiation between trees was low but significant in *A. wideri*, whereas in *L. lata*  $F_{st}$  did not deviate significantly from zero (table 3).

In *A. wideri*, there was no significant correlation between Nei's genetic distance *D* and the geographic distance between sites ( $r = 0.03$ ), as 19.5% of the correlation coefficients

Table 3.  $F_{st}$  for four separate sets of samples: G. Geographic level; Np. Number of populations; Na. Number of alleles; ns. Not significant; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

Tabla 3.  $F_{st}$  para cuatro grupos de muestras separadas: G. Nivel geográfico; Np. Número de poblaciones; Na. Número de alelos; ns. No significativo; \*\*  $p < 0,01$ ; \*\*\*  $p < 0,001$ .

Species	G	Np	Na	$F_{st}$
<i>A. wideri</i>	Trees	3	4	0.0369**
	Sites	10	4	0.0481***
<i>L. lata</i>	Trees	4	3	0.0206 ns
	Sites	6	3	0.0761***

resulting from the Mantel test randomizations were higher than the observed correlation coefficient. In *L. lata*, the correlation between Nei's genetic distance  $D$  and the geographical distance between sites was positive ( $r = 0.49$ ) and statistically significant (3.5% of the correlation coefficients resulting from the Mantel test randomizations were higher than the observed correlation coefficients). The significant relation arose because Hallands Väderö was geographically the most isolated site, and its population was the genetically most deviant. Without Hallands Väderö, no significant relation was found ( $r = -0.48$ , 92.5% of the correlation coefficients from the Mantel randomizations were higher than the observed correlation coefficient).

The gene flow between trees in Bjärka-Säby was estimated from  $F_{st}$ -statistics (HEDRICK, 1983):

$$F_{st} = 1 / (4Nm + 1)$$

$Nm$  (the average number of migrants between trees per generation) was estimated to seven individuals for *A. wideri* and twelve for *L. lata*.

## Discussion

The small genetic differentiation between sites was not unexpected as until 150–200 years ago, old hollow oaks were a widespread habitat, probably occurring contiguously over large areas of southern Sweden (ELIASSON & NILSSON, 1999). The generation time of the study species is unknown, but for other pseudoscorpion species the life time is 2–5 years (WEYGOLDT, 1969; GÄRDENFORS & WILANDER, 1992). Thus, the reduction in connectivity and population sizes should not yet be fully manifested in the genetic variation between sites, as fragmentation of the habitat has occurred within the last 100 generations of the pseudoscorpions and the population size per site may be relatively large. Many threatened invertebrates would be expected to show patterns similar to *L. lata* in this respect; they suffer from habitat fragmentation and become extinct from small sites (RANIUS & WILANDER, 2000). However, because the local populations are often still relatively large it is difficult to detect genetic effects of fragmentation before the population has disappeared for other reasons.

In *L. lata*, the genetic differentiation was larger than in *A. wideri* only when the Hallands Väderö population was included in the analysis. Also the correlation between genetic and geographic distances between sites was dependent on that the Hallands Väderö population was included. This indicates that in *L. lata* the gene flow between Hallands Väderö, which is on an island, and the mainland might have been low or absent over a longer period than between the mainland populations. Thus, the larger differentiation between all sites in *L. lata* compared to *A. wideri*

does not suggest any difference in the migration rate between mainland populations.

Within the Bjärka-Säby site, the conditions would not be expected to have changed much over the past 150 years. Under the assumption that there is no selection that affects gene frequencies, the observed genetic variation between trees should therefore reflect the ongoing genetic drift, migration and extinction-colonization process. The extinction-recolonization turnover, which might take place in hollow trees within a stand, results in imprecise correlations between  $F_{st}$  and  $Nm$ , and most cases yielding too low estimates of  $Nm$  (WADE & MCCAULEY, 1988). In spite of this, the estimated migration rate was fairly high both in *L. lata* and *A. wideri* (12 and 7 tree<sup>-1</sup> year<sup>-1</sup>, respectively). From observations in the field the population size was estimated as being five to 500 individuals per tree, and the mean might be 50 individuals per occupied tree for both species (calculated from the field data set used in RANIUS & WILANDER, 2000). The relatively frequent migrations that seem to occur in both species are almost certainly performed by phoresy, even though it has infrequently been observed for these species. As there is a positive relation between occupancy per tree and stand size in *L. lata*, it has been hypothesized that the *L. lata* populations in stands conform to metapopulations, with each tree possibly sustaining a local population (RANIUS & WILANDER, 2000). The results from the present study suggest that the migration rate might be too high for metapopulation dynamics to be important at this scale, but as the estimates are very rough this hypothesis can not be rejected.

The underlying assumption of the  $F_{st}$  calculation is that there is no selection for any alleles. If selection maintains different alleles in high frequencies in different local populations, the  $F_{st}$  estimation indicates little or no gene flow even if it is actually large. Selection for heterozygosity, however, may generate similar gene frequencies for many local populations and the  $F_{st}$  calculation then suggests a gene flow larger than reality. The loci used in this study are for two metabolically adjacent enzymes, PGI (phosphoglucose isomerase) and PGM (phosphoglucose mutase). Both these loci have been found to experience natural selection in studies on butterflies (PGM: GOULSON, 1993; PGI: CARTER & WATT, 1988, indications of selection in both: CARTER et al., 1989). Also in a beetle, *Chrysomela aenicollis*, natural selection probably acts on PGI (RANK, 1992). A further genetic study based on a larger number of loci would therefore be interesting to control possible effects of selection, chance or history which may act on individual alleles in the pseudoscorpion species.

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