

SPATIAL GENETIC STRUCTURE OF LEAF BEETLE SPECIES WITH SPECIAL EMPHASIS ON ALPINE POPULATIONS

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Abstract

In order to examine the amount of genetic variation in leaf beetle species occurring at different geographical levels, we compared F_{ST} values, calculated in several studies as a quantitative measure of population structuring. In three studies which investigated small scale differentiation, genetic differentiation could be detected between beetle groups feeding on neighbouring host plants (trees) or host plant patches (perennial herbs) less than 1 km apart. Beetles sampled in topographically diverse regions showed generally higher differentiation than those sampled in lowland regions, though there are exceptions. At large geographic distances (>300 km) some differentiation is always detectable.

Introduction

Geographic variation in allele and genotype frequencies is found between local populations of nearly all species. Such a genetic population structuring can be caused by various evolutionary factors, for example local adaptation by natural selection, genetic drift in small isolated populations or non-random mating because of family structures or isolation by distance. However, population structuring can only develop if gene flow, the homogenizing force of evolution, is impeded by barriers or geographical distance. An understanding of the spatial genetic structuring of a species can be the first step in identifying the forces driving its evolutionary history – and in understanding how today's ecological and life history factors form and maintain such a population structure.

Descriptive statistics for the degree of population structure are available in the form of Wright's F-statistics (Wright, 1978; Hartl and Clark, 1989). These coefficients subdivide the genetic variance present in a species into several components. The term F_{ST} thereby gives the proportion of the overall genetic variance which is attributable to the substructuring into groups. With a hierarchical sampling design, F_{ST} can be calculated for different levels of subdivision, for example between single host plants, between local populations or between broader geographical regions. Since one can obtain an estimation of gene flow from F_{ST} (Wright, 1931, 1978; Slatkin 1985, 1989), such a design can also indicate the amount of gene flow present between these groups.

Since the introduction of allozyme electrophoresis methodology to population genetics in the 1960s, hundreds of studies of the population structure of various species have been conducted. Only a few of these, however, have investigated the population

nostyles, *Senecio* and *Petasites* (all Asteraceae, Senecioneae). They sampled host patches of maximally 50m x 50m and compared the F_{ST} between these patches within localities (less than 3 km apart) with F_{ST} between different localities (20 km to 300 km apart) and between different mountain ridges (50 km to >1000 km apart). Table 1 gives the F_{ST} found at the different levels in these studies.

All these studies have in common the fact that they found genetic differentiation already at the lowest hierarchical level investigated. McCauley *et al.* (1988) and Rank (1992) reported significant F_{ST} between beetle groups from different trees; in *O. cacaliae* we found significant differences between host patches less than 3 km apart.

The additional variance due to structuring between localities (the next highest level investigated) can be even smaller than this low level differentiation (for example *O. cacaliae*, *P. versicolora*).

At this level – the differentiation between localities within regions – Carstens (1994) study showed no variation between localities of lowland regions and between localities separated by water barriers. However, the alpine localities are well separated. The F_{ST} value among the alpine populations, geographically no further apart than 100 km, is even higher than the F_{ST} among all other populations from Central Europe to Northern Scandinavia. Therefore she concluded that for *P. vitellinae* on the European continent, mountains appear to be the only major barrier to gene flow.

This is consistent with a comparison of the F_{ST} values for different species from mountainous and lowland areas. The two alpine species, *C. aeneicollis* in America and *O. cacaliae* in Europe, and *P. vitellinae* in its alpine regions have higher F_{ST} within comparable geographic regions than the species occurring at lower altitudes; *P. versicolora* in America and *P. vitellinae* in lowland regions of Europe.

On a larger geographical scale, for alpine as well as lowland species, there is some additional differentiation due probably to an isolation by distance effect.

In a preliminary analysis of the alpine species *O. cacaliae*, the F_{ST} values found among localities within one mountain ridge (ranging from 0.066 to 0.070) are comparable to those found by Carstens (1994) for *P. vitellinae* in alpine regions. However, between several mountain ridges, the Pyrenees, Central and Western Alps, the Voges and the Black Forest, there is considerable additional variation, as expressed by an F_{ST} of 0.090, implying that the distance and/or differences between the mountain ridges are causing more variation. For *O. cacaliae* this pattern is also reflected in a high correlation between genetic and geographic distances (Fig. 1, Mantel test (for example Manly, 1985) with 500 permutations, $r=0.652$, $p=0.004$; level of localities). Rank (1992) reported high differentiation between high elevation localities, not further than 50 km apart.

The two species sampled in lowland regions, *P. versicolora* in America and *P. vitellinae* in Europe, show no or only very little differentiation within regions. At a larger geographical scale, however, some differentiation is detectable.

Differentiation between populations of leaf beetle species, studied in a non-hierarchical design

Other studies, mainly conducted in order to construct phylogenies, reveal allozyme allele frequencies of leaf beetle populations. If two or more populations of one species are included in these studies, F_{ST} values can be calculated. We did so for several species of the genus *Oreina* (data extracted from several publications, see table 2) and *Gonioctena* (data from Mardulyn & Pasteels (in prep.)). We calculated F_{ST} values only

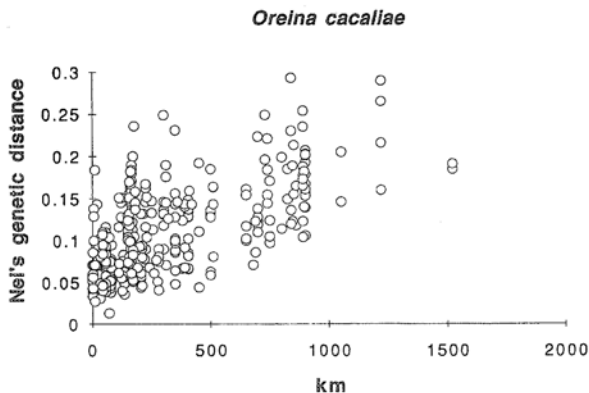


Fig. 1. Correlation between geographic distances (bee-line, km) and genetic distances (Nei's genetic distance, Nei, 1972) for *Oreina cacaliae* localities (Mantel test (e.g. Manly, 1985) with 500 permutations: $r=0.652$, $p=0.004$).

for species with more than 15 individuals per population to assure a reasonable estimation of allele frequencies. For the study of within species gene variability, differences in allele frequencies are normally the most important source of information, not the absence or presence of certain alleles (Swofford & Berlocher, 1987). To show these differences, larger sample sizes are necessary. The F_{ST} values given in Table 2 reflect the differentiation between two to five populations of the respective species.

The *Oreina* species show high F_{ST} values, ranging from 0.051 to 0.234 (table 2). All *Oreina* species presented here are true alpine species. The distribution of two of them, *O. cacaliae* and *O. gloriosa* (F.), is extremely patchy with very high local abundances. *O. speciosissima* (Scop.) is much less abundant and at a local scale more evenly distributed (unpublished mark and recapture data). *O. speciosa* (L.), though rare, seems to be patchy at a local scale, but for this species field observations are only anecdotal. The relatively low F_{ST} of *O. speciosissima* could also be attributable to its preference for slightly lower altitudes around 600-800 m, compared to the other species which prefer altitudes around 1400 m. At higher altitudes, populations are more likely to be isolated from one another by the unsuitable habitats of (a) the higher mountain ridges and (b) the lower valleys. Furthermore *O. speciosissima* is, to our knowledge, the only one of the four species which accomplishes its life cycle within one year, *O. gloriosa* and *O. cacaliae* having overlapping generations.

Much like the *Oreina* species, F_{ST} values for *Gonioctena* species are generally high. Although the species studied are not as strictly alpine as the *Oreina* species, most populations were collected in mountainous areas or the collecting sites were at least separated by major mountain chains. It seems that in this genus, too, mountains can be effective barriers to gene flow and calculating pairwise F_{ST} values can predict between which populations/locations the barriers to gene flow are to be expected.

In *G. pallida* (L.), the F_{ST} value between the two alpine populations, 100 km apart,

is 0.122, and thereby higher than the comparison of either alpine population with the population from the Vosges (F_{ST} of 0.082 and 0.087), although the Vosges are further away (220 km and 230 km).

The two alpine populations of *G. interposita* (Franz & Palmen) show a similar degree of divergence as do the two alpine populations of *G. pallida* for comparable geographic distances.

The unexpectedly high F_{ST} for the two populations of *G. viminalis* (L.) is intriguing. It does not result from the divergence of one particular locus (which would suggest selection acting on this locus), but significant F_{ST} values were obtained for 5 out of 10 different loci. More populations of this species must be studied, to decide on the status of the Vosges and alpine populations.

G. quinquepunctata (F.) was collected at moderate (Vosges and Black forest) and low elevations (Denmark). An insignificant F_{ST} (0.080) was calculated for the samples of the Vosges and Black forest (only 50 km apart) and they must be considered to belong to one population. However, both these populations are clearly distinguished from the one in Denmark (F_{ST} values of 0.401 and 0.264 were calculated for a comparison with the Vosges and Black Forest populations respectively).

The overall F_{ST} for the three populations of *G. olivacea* (Forst) collected at lower elevation is rather low considering the distances that separate them. The Belgian population is more distant to that of Denmark (F_{ST} = 0.162, 700 km distance), than to that of Portugal (F_{ST} = 0.075, 2400 km distance). F_{ST} for the samples from Denmark and Portugal is 0.193, distance 2400 km. We have no explanation as to why the Danish population is so differentiated from the two others – other impediments to gene flow than geographical barriers or distances must be responsible.

Gene flow and possible barriers reducing it

N_m , the number of individuals exchanged between populations per generation, is a measure of gene flow (Slatkin, 1985, 1987). From F_{ST} one can estimate N_m by Wright's formula: $F_{ST} = 1/(1+4N_m)$ (Wright, 1931). There are other methods available calculating N_m from allelic data, but for simplicity and because great deviations between different calculations have rarely been observed (Slatkin, 1989) we did not do them here.

Values of $N_m < 1$ indicate serious impediment of gene flow (Slatkin, 1985, 1987) favouring population separation. This value of $N_m < 1$ corresponds to a $F_{ST} > 0.20$.

The only $F_{ST} > 0.20$ are from studies undertaken with less than five populations and sometimes large geographic distances between them. Only more detailed studies like those presented above allow recognition of possible barriers to gene flow.

We reported substructuring of chrysomelid beetle populations at two different levels.

First, whenever studied specifically, differentiation could be demonstrated between leaf beetles collected on single host plants or host plant patches. This is surprising, since the distances are of less than 1 km up to 5 km or, even between single, neighbouring trees. Even though some species seem to disperse only by walking (for example *Oreina* species), the beetles should be physically able to bridge gaps between suitable plants. Indeed they must be able to colonize new host plants, especially in the spring after overwintering in the soil. Additionally, some *Oreina* species might completely defoliate their herbaceous host plants during their life cycle and thus need to colonize a new host plant patch.

One explanation for this high genetic differentiation at such a small scale could be

features. Of course exist other barriers to gene flow. Different host plants or different distributions of host plants could be an important factor isolating chrysomelid beetle populations. We have deliberately not discussed the level of dietary specialisation, the distribution of major and secondary host plants or the dispersal ability of the beetles. The study of population structure of European chrysomelid beetles is still in its infancy and data on these ecological parameters are not complete for all the species mentioned. Factors influencing population structuring might be different for species within even one genus and much more so within such a large family as the chrysomelids. We are also aware that it is difficult to compare F_{ST} values from studies undertaken for different objectives. Such studies differ in the number of populations included, the number of loci studied, the methods of calculation of F_{ST} values and the sampling design employed. Nevertheless we think the results presented here reveal some patterns: most of all high levels of small scale differentiation, but also the effects of topography and of isolation by distance. We hope to stimulate further studies that might reveal how general these patterns are and which other factors are influencing population structuring in leaf beetles.

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