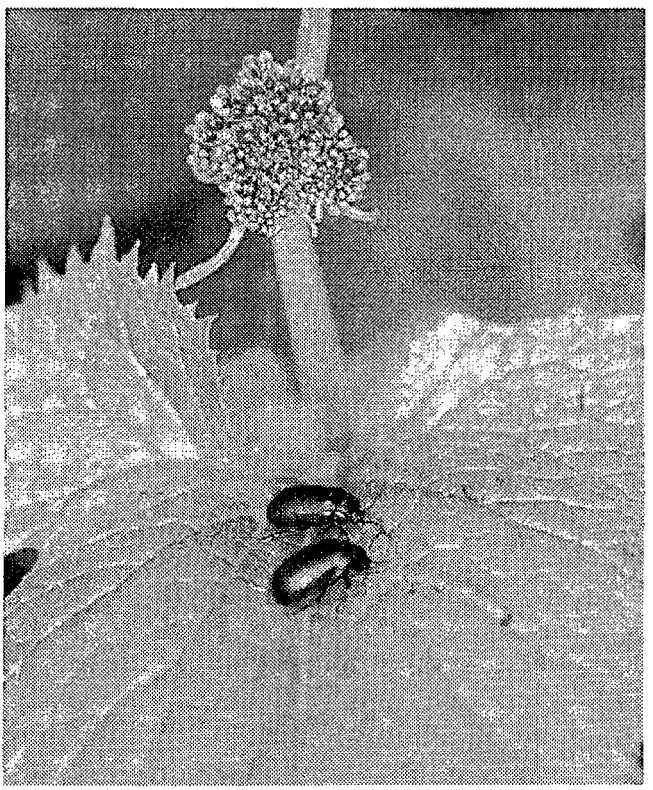
Steffi Knoll

Spatial population structure of an alpine leaf beetle



Verlag Langewiesche-Brandt

Thèse

Présentée à la Faculté des Sciences de l'Université de Neuchâtel pour l'obtention du grade de Docteur ès Sciences.

angefertigt am Laboratoire d'ecologie animale et entomologie, Institut de Zoologie, Université de Neuchâtel, 2007 Neuchâtel, Suisse

bei Prof. Dr. M. Rowell-Rahier

Examen de thèse: 25. 6. 1997
Jury: Prof. Dr. M. Rowell-Rahier (Univ. Neuchâtel), Prof. Dr. L. Keller (Univ. Lausanne), Prof. Dr. S. Menken (Univ. Amsterdam), Prof. Dr. C. Mermod (Univ. Neuchâtel)

ISBN 3-7846-1217-2. Printed in Germany.

Meinen Eltern zum 120 ten

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IMPRIMATUR POUR LA THÈSE

Spatial population structure of an alpine leaf beetle

de Mme Stefanie KNOLL

UNIVERSITÉ DE NEUCHÂTEL

FACULTÉ DES SCIENCES

La Faculté des sciences de l'Université de Neuchâtel sur le rapport des membres du jury,

Mme M. Rowell-Rahier (directrice de thèse),
C. Mermod, S. Menken (Amsterdam) et
L. Keller (Lausanne)

autorise l'impression de la présente thèse.

Neuchâtel, le 7 juillet 1997

Le doyen:

R. Dändliker

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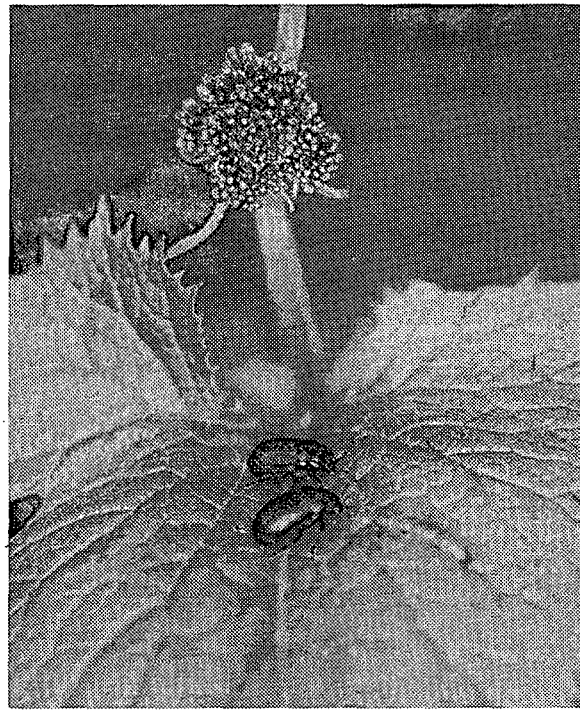

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CONTENTS:

SPATIAL POPULATION STRUCTURE OF AN ALPINE LEAF BEETLE:

INTRODUCTION AND THESIS OUTLINE

To better understand what are the patterns of diversity in nature and what are their critical ecological and evolutionary determinants, we need to focus our attention on the question how ecology, demography and the genetic population structure influence each other in a heterogeneous and ever changing world (Endler, 1992).
The population structure of a species, "..such matters as numbers, composition by age and sex, and state of subdivision..." (Wright and Provine, 1986) is a primary determinant of a species' ecology, it's genetic variability and thereby it's evolutionary potential. A spatial population structure, a subdivision in space, is a characteristic of almost all species (Roderick, 1996). As soon as we talk of spatial structure we are confronted with the question of scale: Where exactly can we observe structure? Dispersal and gene flow determine the geographic scale of population dynamics as well as genetic differentiation. If dispersal is limited and gene flow is small, strong geographical variation can occur over short distances and the form and dynamics of ecological processes can become quite complex (Gilpin and Hanski, 1991, Endler, 1992).
In this thesis I investigated the spatial population structure of an alpine leaf beetle, *Oreina cacaliae* Schrk. The great success of leaf beetles (Coleoptera, Chrysomelidae), as measured in species numbers, is explained by their adaptation to phytophagy, to a life on plants. How and why the variability of plants influences and determines the variability of phytophagous insects is one of the central questions in the study of insect plant interactions (Schoonhoven, 1996; Denno and McClure, 1983). The concept of coevolution as proposed by Ehrlich and Raven in 1964 gave a theoretical and methodological framework to approach this question, linking ecological (host plant use) and evolutionary (phylogeny) aspects, and has spawned much research in different systems, revealing not one mode of coevolution, but a variety of possible and realized interactions (Thompson, 1994, Menken, 1996).

The leaf beetle genus Oreina

In the genus *Oreina* a puzzling amount of variation was found, whatever characteristics were studied. Not only feed the different species of the genus on host plants of two distant plant families, the Asteraceae and the Apiaceae, they also use two different modes of chemical defense and display a wide variety of life history strategies (details in Dobler *et al.*, 1996). For their defensive secretions, some species produce autogenuously toxins (cardenolides) and others are able to sequester plant secondary compounds (pyrrolizidinalkaloids) from their host plants and some do both (Pasteels *et al.*, 1996). Moreover, it could be demonstrated that the very diverse composition of the defensive

secretions is - at least in *Oreina gloriosa* - heritable but varies according to the physiological state of the beetles (age, paired vs virgin females; Eggenberger, 1993). In leaf beetles generally autogenuous defense is thought to be the ancestral mode of defense and sequestration is thought to be the derived state (Dobler *et al.*, 1996). That sequestration could be the more effective defense mode was demonstrated in studies of the predation behaviour of non-native birds on *Oreina* beetles with different modes of defense (Rowell-Rahier *et al.*, 1995).
The reproductive strategies in the genus range from oviparity to viviparity, including all intermediates, whereby vivparity evolved apparently twice, once with matrotrophy and once without (Dobler and Rowell-Rahier, 1996). Based on an independent phylogeny of the genus *Oreina* (Dobler *et al.*, 1996; figure 1), it was postulated that in this genus opportunistic host plant switches to available hosts, including to distantly related and chemically dissimilar plants, within a given restrictive habitat are favoured over host tracking across unfavourable habitat. The low fidelity in host plant use was explained by a combination of ecological and life history constraints. In the high altitude habitats, where most *Oreina* beetle live, host plant availability in space and time might be an important limiting factor. A release of at least some host plant imposed constraints, on the other hand, is secured by externally feeding larvae, who can actively change host plants, and by the autogenuous defense mode independent of plant compounds. A further credit of any chemical defense should be a reduction of predation pressure.

Oreina cacaliae

The species *O. cacaliae* is viviparous, feeds on Asteraceae and relies solely on the sequestration of pyrrolizidin alkaloids for its defense (figure 1). *O. cacaliae* adults are found in high densities on patches of their host plants *Adenostyles alliariae*, *Senecio nemorensis-fuchsii* and *Petasites albus* (all Asteraceae). Patches vary in size from several square m to 1 km² and are characterized by a continuous plant cover, single plants intermingling their leaves. Adults and larvae feed on the same host plants in the same patches. *O. cacaliae* has overlapping generations and a long life cycle. Larvae are laid directly on their food plants in early summer, while the adults are already around since several weeks. Larvae are laid in aggregations directly on the host plants, up to 60 larvae per female in laboratory studies (Dobler, 1996). The first two instars are unconspicuously black and often stay on the underside of host plant leaves, possibly for microclimatic reasons like water regulation. The third and fourth instar are aposematic, as are the adults, and characterized by a shiny and black abdomen and a brilliantly orange pronotum. Density of larvae per plant is constantly diminishing and the fourth instar shows extensive movement behaviour (Conconi and Nessi, unpublished data). About 5 weeks after larviposition began, most larvae left the host plant to go into the soil for pupation, but there is a great variance in larval developmental time and larvae are still seen end of september, at the end of the adults season. *O. cacaliae* have been observed overwintering as larvae and as adults (Kippenberg, pers. communication; Knoll, pers. observation). About two weeks after the larvae largely disappeared, newly emerged beetles occur in high abundances at the patches, their soft elytra take about 48 hours to harden. Males of the previous generation are now seen mating with still soft females of the next generation. Mating takes place all over the season and mate guarding is

observed.
Oreina beetles are regarded as being sedentary and of low vagility (Rowell-Rahier, 1992). This is based on mark and recapture studies of *O. gloriosa* (Eggenberger and Rowell-Rahier, 1991) and the fact that they are not seen flying during the season. On the other hand, we have now several independent observations of *Oreina* beetles, namely *O. cacaliae*, flying in high numbers at warm and sunny days in early spring or late summer, at a time when their host plants have not yet emerged or are already withered (Conconi, D., S. Dobler, B. Hägele, N. Kalberer, pers. communication and Knoll, pers. observation).

Thesis outline

Within the genus *Oreina*, *O. cacaliae* is regarded as the evolutionary most successful species, based on it's high abundances in the field and it's derived mode of defense (figure 1). Therefore I focused my attention on *O. cacaliae* in my investigation of the population structure of *Oreina* leaf beetles. Furthermore I concentrated on the study of the spatial genetic population structure, which should reflect past and present evolutionary forces acting in the system. The aim of my study was (1) to quantify intraspecific variation in *Oreina* leaf beetles (chapter I and II), (2) to search for an explanation of the observed pattern in possible correlations with ecological and environmental parameters, such as host plant, altitude and geographic location (chapter II) (3) to assess the scale where population structure can be observed in the species (chapter III) and (4) to investigate independently the within patch demography of *O. cacaliae* (chapter IV) in order to (5) understand the population dynamics of this species (discussion in chapter III).

The theoretical framework for an interpretation of an observed spatial genetic structure is found in population genetic theory and metapopulation theory.

Theory

In theoretical ecology as well as in population genetics, populations were regarded as large homogenuous units for a long time - for the ease of modelling as well as for the ease of studying populations in the field (Endler, 1992). Exceptions to this overall view were the work of Andrewartha and Birch (1954) who stressed the importance of large fluctuations in the abundance of local populations and of Wright, who stressed the importance of a subdivided population structure for the overall distribution of genetic variability in a species. Wright not only developed methods to detect different levels of structuring, he also developed with his shifting balance theory a framework to understand the evolution in a species with a subdivided population structure (reprints of most important papers in Wright and Provine, 1986).
The shifting balance theory: Wright assumes that each species is living in a multidimensional "fitness landscape" with several local peaks. Populations are driven by selection in the direction of such peaks and in equilibrium each population is "trapped" on a local fitness maximum (peak), which might be quite different from the overall fitness maximum possible for this species. Evolution in the framework of the shifting balance theory can then be seen as a three step process: a subdivided population structure

consists of populations of sufficiently small size, so that the effects of genetic drift overwhelm selective pressures and thereby remove populations temporally from a local adaptive peak. Second, selection acts to bring the populations back to a - maybe different - adaptive peak and third, better adapted populations (the ones on a higher peak) have a fitness advantage and spread their genes into the species gene pool with a higher frequency than less adapted ones. This last step requires migration between populations and greater reproductive success for migrants coming from better adapted populations. In this theory populations are at least for certain times out of the equilibrium state, where a balance between selection, drift, mutation and gene flow keep the populations stable. Wright was the first to mention, that under such non-equilibrium conditions genetic variance between populations might be enhanced and that fitness differences between populations might lead to interdemic selection

The concept of metapopulations: In theoretical ecology, the fact that populations are structured into local entities and the effects thereof have received increased attention only recently (Gilpin and Hanski, 1991; but see references therein for the historical roots). In a metapopulation the level of stability (equilibrium) is moved from the local populations to a higher entity, the metapopulation, which differs in it's dynamics markedly from the dynamics of one single, local population. The focus of attention was more on possible and realized outcomes of the processes of extinction and colonization than on the cohesive effects of migration between groups (Gilpin and Hanski, 1991). Often the term metapopulation is associated with rapid turnover rates (frequent extinctions and colonizations), but "probably all species persist as a metapopulation at an appropriate scale" (Endler, 1992). Against this theoretical background, the scale dependence - spatial and temporal - of most ecological phenomena has moved in the foreground of current ecological research (Nürnberger and Harrison, 1995, Peterson and Denno, 1997b, Levin, 1992).

Slatkin (1977) was the first to incorporate extinction and colonization events in existing population genetic models assuming a subdivided population structure. He concluded that in species with frequent extinctions (and colonizations) of local populations, genetic drift will not have the time to fix neutral alleles and extinction and colonization thus work as a homogenizing force like gene flow. More recent studies, however, have found that the effect of colonization and extinction events on the distribution of genetic variance does not only depend on the frequency of population turnover, but also on the ratio of migration between existing populations vs founding of new populations, on the relatedness of immigrants (migrants or founders) and on the temporal variation of population parameters like population size (see discussion in chapter III and references therein).

Population structure of phytophagous insects

Since the introduction of allozyme electrophoresis as an easy method to screen fast the distribution of allele frequencies within a species, the population structures of hundreds of insect species (including many phytophagous species) have been investigated and promoted our understanding on how selection and genetic drift act on the evolution of these species (recent review in Roderick, 1996) A recent extensive review addresses

explicitely the population structures of phytophagous insects and identifies several life history traits - foremost dispersal ability -that may play a role in determining these structures (Peterson and Denno, 1997a). Surprisingly little detailed studies were done with Chrysomelidae species sofar (chapter I). Generally only few studies have explicitely investigated the level at which structuring occurs (chapter I and III and references therein). This is surprising, since the statistical methods for such an analysis were available before the rise of allozyme electrophoresis (Wright's F- statistics, Wright 1931) and the importance of putting in evidence the level at which differentiation is manifested has been stressed repeatedly (as detailed in Rank, 1992). For the studies, where such a hierarchical investigation was done, levels of differentiation found were often unexpected from the ecology or biology of a species (references in Roderick, 1996). I think that the framework of metapopulation theory combined with theory about non-equilibrium populations and the effects of population turnovers might help to explain otherwise paradox patterns. So far only few empirical studies were explicitely addressing the effects of population turnover and those were all done with species having a high turnover rate (as discussed in chapter IV). Some studies of phytophagous insects have explained the observed patterns as in agreement with metapoulation theory (Eber and Brandl, 1994; references in Peterson and Denno, 1997a, this thesis), but studies explicitely addressing the effects of extinction and colonization on the genetic population structure of phytophagous insects are rare (but see McCauley, 1989). It is fascinating to speculate about a possible link of plant and insect ecology expressed not only in a link of plant and insect demography but also in the distribution of their genetic variability. Host plant architecture (as an indication of host plant life history strategies) has been demonstrated to influence the genetic population structure of phytophagous insects (Peterson and Denno, 1997a). The need for independent informations from demographic and life history studies to fully understand the effects of population structure is long recognized (Slatkin, 1985, Nürnberger and Harrison, 1995), but rarely fulfilled. We have so far discussed only the effects of a spatial population structure. A structuring of a population due to kinship and family structure can have profound effects on the evolution of a species. This was investigated in detail for social hymenopteran insects, mainly in the context of the evolution of altruistic behavior (review in Keller, 1997). In Chrysomelidae, *Plagiodera versicolora* with its subsocial behavior of larval aggregation, group feeding, group defensive displays and postures ("coaxely", Wade, 1994) is to my knowledge the only species, which was studied in regard of the degree of relatedness in subgroups (McCauley *et al.*, 1988).

Dispersal and its effects on population structure

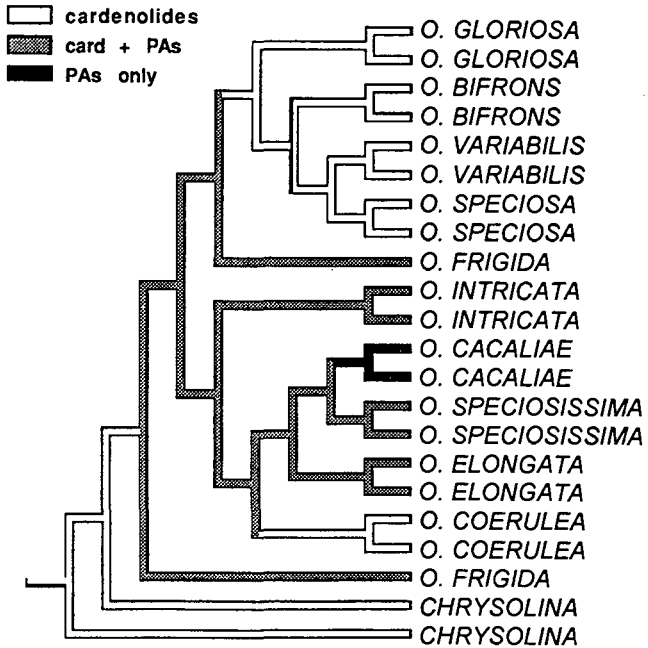
The study of spatial population structures is inevitably linked to the study of the dispersal ability and of the realized dispersal (Powell, 1976). Approaches for the study of dispersal respectively gene flow have followed two approaches, actual "direct" observations vs "indirect" inferences from the pattern of allelic distribution (Slatkin, 1985, Roderick, 1996). Individuals likely disperse greater distances than can be reliably detected with the present method of mark and recapture studies (chapter IV, Slatkin 1985, Peterson and Denno, 1997a). For example, wind borne migration might be a common mean of insect long distance dispersal, but "in spite of recent advances we know little or nothing of the

migratory behaviour of the overwhelming majority of species that travel in the winds in vast numbers" (Gatehouse, 1997). Studies of dispersal are further complicated by a possible variation in time, by possibly infrequent occurrence or by possible dispersal polymorphisms. Investigations about the effects of such variances in dispersal frequencies and dispersal polymorphisms have just begun (Olivieri *et al.*, 1990, Crespi and Taylor, 1990, Peterson and Denno, 1997a), though their existence and/or effect is often inferred from the observed genetical patterns (McCauley *et al.*, 1981, Chapuisat *et al.*, 1997, chapter III).

Studies of species with a subdivided population structure have attracted considerable interest, because with ongoing fragmentation of our landscape due to a growing human population, more and more species find themselves divided into locally restricted subgroups. Fragmented populations may show levels of genetic variability that are indicative of their former widespread distribution, rather than their presently constrained migratory patterns, as was shown for the grasshopper *Trimerotropis saxatilis* (Gerber, 1994, 1996). In discussions it is important to differentiate between, on the one hand, species, that find themselves today in an innaturally, anthropogenically fragmented landscapes and might not have the appropriate dispersal strategies, and, on the other hand, species, that live naturally in discontinuous and heterogeneous landscapes - as does the here studied genus *Oreina* - and had a long time to evolve appropriate dispersal strategies.

Next page:

Figure 1: Phylogeny of the genus *Oreina* (Dobler *et al.*, 1996). Mapped on the phylogeny is the mode of chemical defense that the species uses - either biosynthezising cardenolides or sequestering pyrrolizidinalkaloids or both. The species feeding exclusively on Apiaceae form a clade (*O. gloriosa*, *O. bifrons*, *O. variabilis* and *O. speciosa*); the other species feed either exclusively on Asteraceae or on plants from both families.


cardenolides
card + PAs
PAs only
O. GLORIOSA
O. GLORIOSA
O. BIFRONS
O. BIFRONS
O. VARIABILIS
O. VARIABILIS
O. SPECIOSA
O. SPECIOSA
O. FRIGIDA
O. INTRICATA
O. INTRICATA
O. CACALIAE
O. CACALIAE
O. SPECIOSISSIMA
O. SPECIOSISSIMA
O. ELONGATA
O. ELONGATA
O. COERULEA
O. COERULEA
O. FRIGIDA
CHRYSOLINA
CHRYSOLINA

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Chapter I

Knoll, S., M. Rowell-Rahier, P. Mardulyn and J. M. Pasteels. 1996.
Spatial genetic structure of leaf beetle species with special emphasis on alpine populations. in
Jolivet, P. H. A. and M. L. Cox (eds.):
Chrysomelidae biology, vol. 1: The Classification, Phylogeny and Genetics.

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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

Chrysomelidae Biology, vol.1: The Classification, Phylogeny and Genetics, pp. 379-388
Edited by P.H.A. Jolivet and M.L. Cox

structure of leaf beetles. The Colorado potato beetle was the first to attract attention and is still one of the most thoroughly investigated species (Jacobson and Hsiao, 1983, Zehnder *et al.* 1992). Nevertheless, as a crop pest species, it may be subjected to different selection pressures than are natural populations of other leaf beetles. In this chapter we will present data on the spatial population structure of non-pest chrysomelid beetles with special reference to alpine species.

Chrysomelid biology and possible effects on population structure

Most chrysomelid beetles are specialist herbivores and feed on the same host plants as larvae and adults. They generally have a high fecundity and, in some taxa, may have overlapping generations. Since mature females are heavy and not very mobile, migration may be temporally restricted. The beautiful colours of many chrysomelids are often warning colours that indicate chemical defenses. The beetles frequently show an aggregative distribution, further reinforced by host plant patchiness.

All species considered in this chapter are oligophagous or monophagous in the geographic region in which they were studied. We concentrated here on the amount of genetic variation occurring at different geographic scales.

Gene flow might be impeded at different scales for different reasons. Geographic barriers such as rivers or mountain ridges often prevent gene flow between contiguous regions. Also, geographic separation alone might lead to isolation by distance.

For herbivorous insects, which feed as larvae and adults on the same host plant, the patchy distribution of the plant alone might be enough to split them into small groups subjected to strong genetic drift. This effect, of course, might be antagonized by migration providing gene flow. If, however, even siblings do not migrate, but stay close together, differentiation due to family groups might be observable at a very small scale.

Genetic differentiation in leaf beetles, studied with a hierarchical sampling design

We know of four studies specifically investigating genetic differentiation of leaf beetle populations with a hierarchical sampling design at different levels.

McCauley *et al.* (1988) reported the geographic and temporal pattern of genetic variation among larval groups of the introduced willow leaf beetle, *Plagiadera versicolora* (Laich.). They gave the F_{ST} among individual trees, among localities (3 km to 30 km apart) and among regions (Illinois and Virginia, distance ca. 500 km).

Rank (1992) investigated the population structure of *Chrysomela aeneicollis* Schaeffer with a similar design. He calculated for this montane species of the Californian Sierra F_{ST} values among single trees (less than 150 m apart), among patches of trees (less than 5 km apart) and among drainages (less than 40 km apart, equivalent to localities in other sudies).

Carstens (1994) in her study of *Phratora vitellinae* (L.) made a comparison of the population structure of a species occurring in regions with different geomorphological features. She calculated F_{ST} between localities and between regions, thereby comparing one alpine region, several lowland regions and one region in Finland which is characterized by extensive water basins.

S. Knoll and M. Rowell-Rahier (unpublished data) studied the population structure of *Oreina cacaliae* (Schrk.), a European alpine species. In contrast to the other species mentioned, *O. cacaliae* feeds not on willows but on perennial herbs of the genera *Ade-*

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

Table 1. Summary of studies of genetic differentiation in leaf beetles, done with a hierarchical sampling design. All F_{ST} values are averages over variable loci, details of calculations are given in the references (see text)

Species	hierarchical level	F_{ST}	SE	sign.	no of loci
Plagiodera versicolora	between trees within different localities (several meters apart)	0.008	0.006	ns	3
sampled on 5 Salix spp..		0.024	0.008	ns	3
(S. alba, S. interior		0.041	0.022	ns	3
S. caroliniana,		0.006	0.003	ns	3
S. nigra, S. babylonica)		0.098	0.010	**	3
		0.008	0.004	ns	3
		0.015	0.002	**	3
		0.037	0.031	ns	3
	between localities within regions (3 km to 30 km apart)	0.012	0.001	**	3
		0.003	0.002	ns	3
	between regions (ca. 500 km apart)	0.057	0.030	ns	3
Chrysomela aeneicollis	between trees (2 m to 150 m apart)	significant F_{ST} for several loci reported			
sampled on 2 Salix spp..	between localities within drainages (0.3 km to 5 km apart)	0.012	0.001	***	5
(S. orestera, S. boothi)		0.010	0.002	**	5
		0.037	0.004	**	5
	between drainages (10 km to 40 km apart)	0.135	0.041	*	5
Phratora vitellinae	between localities within alpine regions	0.053	0.004	-	3
sampled on 4 salix spp.:	between localities within flatland regions	< 0.011	0.012	-	3
(S. myrsinifolia, S. purpurea,	between flatland regions	0.021		-	3
S. glauca, S. elaeagnas)					
and on *Populus tremulus*					
Oreina cacaliae *	between host plant patches within localities (1 to 3 km apart)	0.138	0.026	***	24
sampled on *Adenostyles alliariae,*		0.110	0.020	***	24
Senecio fuchsii, Petasites albus	between localities within alpine regions (50 km to 200 km apart)	0.070	0.008	***	24
		0.066	0.033	***	24
	betwcen mountain ridges (50 km to 1300 km apart)	0.094	0.019	***	24

* analysis of preliminary data set

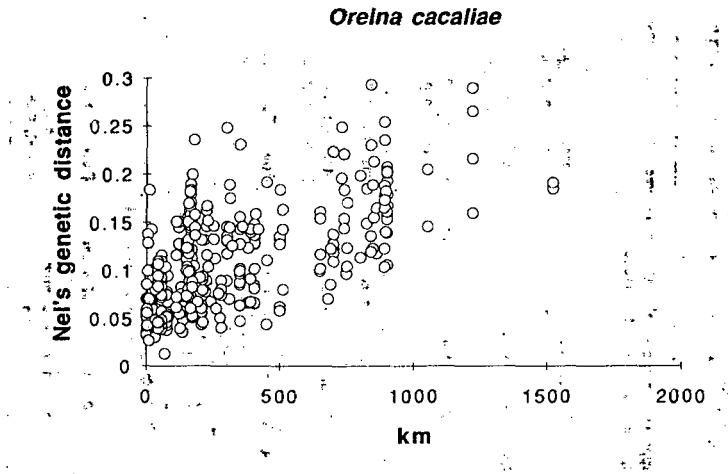

Fig. 1. Correlation between geographic distances (bee-line, km) and genetic distances (Nei's genetic distance, Nei, 1972) for *Oreina cacaliae* localities (Mantel test (*e.g.* Manly, 1985) with 500 permutations: r=0.652, p=0.004).

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 seperated 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,

Table 2. Differentiation between populations of leaf beetle species, studied with a non-hierarchical design. F_{ST} values for *Gonioctena* species are calculated with GENEPOP (Raymond and Rousset, 1995) from genotype frequencies and are all highly significant, F_{ST} values for *Oreina* species are calculated from published allele frequency tables using BIOSYS-1 (Swofford and Selander, 1981)

Species plants	F_{ST}	no. of variable loci	no. of samples	collected in	approximate range of distances between populations; host species sampled and references		
Gonioctena viminalis	0.573	10	2	Central Alps, Vosges	220 km	*Salix caprea, Salix aurito*	Mardulyn and Pasteels (in prep.)
Gonioctena olivacea	0.140	15	3	Belgium, Denmark, Portugal	700-2400 km	*Sorothamnus scoporius*	Mardulyn and Pasteels (in prep.)
Gonioctena quinquepunctata	0.255	10	3	Vosges, Black Forest, Denmark	50-1000 km	*Sorbus aucuparia*	Mardulyn and Pasteels (in prep.)
Gonioctena pallido	0.097	9	3	Vosges, Austrian Alps, Central Alps	100-230 km	*Corylus avellana, Salix caprea*	Mardulyn and Pasteels (in prep.)
Gonioctena interposita	0.140	7	2	Austrian Alps, Italian Alps	60 km	*Alnus viridis*	Mardulyn and Pasteels (in prep.)
Oreina cocaliae	0.234	6	4	Vosges, Southern and Central Alps	200-800 km	*Senecio fuchsii, Petasites albus, Adenastyles alliariae, Adenostyles leucophyllo*	Rowell-Rahier (1992)
Oreina speciosissima	0.051	6	3	Western and Central Alps	150 km	*Adenostyles alliariae, Petasites albus*	Rowell-Rahier (1992)
Oreina speciosa	0.193	6	2	Southern and Central Alps	500 km	*Chaerophyllum hirsutum*	Rowell-Rahier and Pasteels (1994)
Oreina gloriosa	0.137	3	5	Central Alps (Valais)	20-50 km	*Peucedanum ostruthium*	Eggenberger and Rowell-Rahier (1991)

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

Nm, the number of individuals exchanged between populations per generation, is a measure of gene flow (Slatkin, 1985, 1987). From F_{ST} one can estimate Nm by Wright's formula: $F_{ST}=1/(1+4Nm)$ (Wright, 1931). There are other methods available calculating Nm 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 $Nm < 1$ indicate serious impediment of gene flow (Slatkin, 1985, 1987) favouring population separation. This value of $Nm<1$ 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

that family groups (or closely related individuals) are being sampled. Only one study (McCauley *et al.*, 1988) compared groups from different years and they found significant differences for the same tree between different sampling times. This indicates temporal structuring which is expected when mating is random but sampling is not. However, since they sampled larval groups with a demonstrably high degree of relatedness (McCauley *et al.*, 1988) and not adults, such a result might not be surprising. For *C. aeneicollis* and *O. cacaliae*, such a small scale differentiation is more difficult to explain. For both studies adults were sampled and comparisons between years are still lacking. If the studies report differentiation between cohorts, which did not disperse much since their larval stage and if there is later in the life cycle, before mating, a migration phase, this observed differentiation might have no effect at all on the overall population subdivision.

For *O. cacaliae* unpublished mark and recapture data show increased movement (walking) during a very short time period (less than 14 days) in spring. However, observed movement is generally very limited (average no more than 3 m/day), and, furthermore, mark and recapture studies have the inherent disadvantage of not being able to show long distance dispersal. The relatively high F_{ST} values (respectively low Nm), which indicate some limitations to gene flow, argue against long distance dispersal. There are anecdotal observations of mass flight for *Oreina* species in very early spring, but normally these beetles are rarely seen flying. We do not yet know whether, when and how far beetles of the genus *Oreina* migrate.

According to Wade (1994), *P. versicolora* is also rarely observed flying before mating and generally has a low adult vagility. Its larvae aggregate in groups with sometimes high genetic relatedness (McCauley *et al.*, 1988) up to the the last instar, which enters into a "lonely wandering phase" before pupation. We observe similar larval behaviour for *O. cacaliae* in the field (presently studied) and the same is reported for *C. aeneicollis* (Smiley and Rank, 1986). Thus it seems probable that all these species have no extensive migration phase and closely related individuals stay close together and mate again. Wade (1994) used the term **kin group genetic structure** in this context, and such a kin group genetic structure is in agreement with the small scale differentiation shown here.

On a broader geographical scale – the scale at which most population structure studies are made, looking for differentiation between localities several tenths or hundreds of km apart – we can see clearly that for chrysomelid beetles **mountains are very effective barriers** to gene flow. *G. olivacea* is the only lowland species we are aware of that shows a high differentiation between populations. However, with only three populations, we cannot speculate, whether, for example, different host plant distribution patterns or sampling closely related individuals at a very small scale can be linked to this differentiation.

More heterogeneity and high differentiation in topographically heterogeneous areas is also reported for other insects (Liebherr, 1988; Mesaros and Tucic, 1995; Howard and Waring, 1991). The strong effect of geographic barriers as well as indications for isolation by distance could be an indication of a distribution following the stepping stone model (Hartl and Clark, 1989; Slatkin, 1985).

F_{ST} values as high or higher than those reported here for chrysomelid species are in insects otherwise only found in hymenopterans (and explained by their strong social organisation) or for very immobile insect species (for example Collembola, cave dwelling beetles etc.) or for species with highly isolated populations (for example Bilton, 1993; Crouau-Roy, 1989; Frati *et al.*, 1992; McCauley and Eanes, 1987; King, 1988).

Here, we concentrated on population differentiation possibly linked to geographical

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.

Acknowledgements

We are grateful to Nathan Rank and Heather Carstens, who made their unpublished data available to us. We thank Bernd Hägele, Giorgio Bertorelle, Marien de Bruyne and Pelle Ingvarsson, whose comments on a previous version improved the manuscript and Paul Flook, who corrected the English. Martine Rowell-Rahier and Steffi Knoll thank the SNSF (grant no. 31-33669.92) and the SANW, Jacques M. Pasteels and Patrick Mardulyn the Communauté française de Belgique (A.R.C. 93-3318) for financial support.

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CHAPTER II

DISTRIBUTION OF GENETIC VARIANCE AND ISOLATION BY DISTANCE IN TWO LEAF BEETLE SPECIES: *OREINA CACALIAE* AND *OREINA SPECIOSISSIMA*

Manuscript

We investigate the distribution of genetic variance in two closely related *Oreina* leaf beetle species, *Oreina cacaliae* and *Oreina speciosissima*. Populations of these alpine beetles were sampled in mountainous areas of Western Europe, the total sampling area ranges from the Pyrenees to the Czech republic. Allozyme electrophoresis of 21 (*O. cacaliae*) respectively 16 (*O. speciosissima*) loci revealed high genetic variability as expressed in an extremely high percentage of polymorphic loci (only one monomorphic locus for both species) and high heterozygosities. No overall linkage disequilibrium was found. We observed extensive heterozygote deficits in several samples, this is reflected in high F_{IS} values and high overall inbreeding coefficients (F_{IT}) of 0.349 (*O. cacaliae*) respectively 0.503 (*O. speciosissima*). The overall inbreeding coefficient was mainly attributable to within population differentiation. We explain the high heterozygote deficits by a combination of inbreeding resulting in kinship groups and a sampling effect over several such kinship groups.
No explanation of the observed patterns could be found in the host plant use or altitudinal location of the samples. For *O. cacaliae*, we found isolation by distance; in *O. speciosissima* not. Gene flow estimates were in the range of Nm = 0.8 to Nm = 1.5. The sample from the Pyrenees clustered in a UPGMA - cluster analysis separately from other populations, no other indication for distinct genetic lineages could be found.

INTRODUCTION

Evolution does not work on "panmictic species", but on often genetically different, structured populations, demes, families or individuals. While the patterns of a species' genetic structure are easily documented with modern molecular techniques, it is often a complex task to identify the causes of structuration, far more the consequences thereof (Slatkin, 1985; Lewontin, 1991; Roderick, 1996).

Historic, ecological and demographic processes are reflected in the genetic population structure of a species. Historically, vicariance events may lead to a biogeographic splitting of a species into two isolated and independently evolving lineages. Ecologically, intrinsic habitat discontinuities in space or time can lead to effective isolation at very small scales (Roderick, 1996 and references therein). For phytophagous insects plant patches represent such habitat discontinuities in space, that have been demonstrated as being responsible for population structuring (Rank, 1992; Guttmann *et al.*, 1989). Extinction and colonisation dynamics of local patches can profoundly influence spatial structuring and consequently the distribution of genetic variance within and between different populations of a species (Wade and McCauley, 1988; Harrison and Hastings, 1996; Olivieri *et al*, 1990). A herbivore generally has to develop certain adaptations to be able to cope with a new host plant - to overcome plant defence mechanisms or to adapt to a different nutritional quality of the new host or to different natural enemies on the new host (Futuyma and Keese, 1992). Thus, different host plants (or, more generally, "differential use of habitat") may also invoke divergence. Finally, demographic processes ultimately mould the genetic population structure of a species. Population sizes determine the effectiveness of genetic drift and selection, and migration is the prerequisite for gene flow. Recent reviews have also stressed the influence of different life history strategies and mating systems on the genetic population structure (Avise, 1994).

In the leaf beetle genus *Oreina* (Coleoptera, Chrysomelidae, Chrysomelinae) all of these factors have been proposed as being responsible for species divergence (Dobler *et al.*, 1996). Being mostly alpine species, these beetles must have undergone major habitat shifts during the last glaciation. Currently they live not only in a geomorphologically and climatically very diverse environment, within this habitat they occur in locally subdivided groups on their host plant patches. They are generally oligophagous on perennial herbs in the families Apiaceae and Asteraceae. *Oreina* species are aposematic, signalling that the beetles are chemically defended. The defensive secretions of most species consist of autogenously produced cardenolides, but some species developed the ability to sequester plant secondary compounds (Pyrrolizidine alkaloids) and use them for their own defence. One single species within the genus, *Oreina cacaliae* (Schrk.), has totally lost the ability to autogenously produce cardenolides and relies solely on the sequestration of plant derived compounds (Hartmann *et al.*, in press). Sequestration is regarded as an evolutionary derived trait within Chrysomelids (Dobler *et al*, 1996). In spite of such an intimate association to their host plants - beetles live and feed as larvae and adults on the same species - host shifts seem to occur frequently within the genus. It has been hypothesised that limited dispersal abilities, externally feeding larvae and the possibility of an autogenous defence independent of the host plant may have favoured host plant switches over host tracking in the evolution of the genus (Dobler *et al.*, 1997). The

selected type of chemical defence is thus seen as being a consequence of host shifts and not vice versa (Pasteels *et al.*, 1996).

Previous work has shown that there is considerable genetic variation within and between *Oreina* populations (Eggenberger and Rowell-Rahier, 1991, Rowell-Rahier, 1992). However, the limited number of populations in these studies could not provide an explanation for the observed patterns.

In this study, we present the results of a macrogeographic study on the distribution of genetic variability of two species of the genus *Oreina*, *O. cacaliae* and *O. speciosissima*. The two are sister species; they differ in their ecological requirements, but not in their life history. Our first aim was, using allozyme electrophoresis, to report and compare the genetic variability of these two species, one with a rather broad, the other one with a rather restricted ecological niche (concerning host-plant use, geographical range and type of chemical defence). Second, we looked for an explanation of the observed patterns, potential causes of structuration. Therefore, on the one hand, we try to identify possible distinct genetic lineages, reflecting major, past or present, barriers to gene flow. On the other hand, we search for correlations between the observed genetic structure and ecological characteristics of the samples, trying to evaluate the relative importance of different host plants, different geographical locations and different climatic regimes (as suggested from the large altitudinal range of the sites of these alpine beetles). Furthermore, we use colour as a morphological character as well as the results of a more detailed morphometric study by S. Gallusser (1996) to assess, whether there is an agreement of observed patterns in the allozymatic genotype and morphological phenotype.

MATERIALS AND METHODS

The study organism

O. cacaliae and *O. speciosissima* are two sister species (Coleoptera, Chrysomelidae, Chrysomelinae). Their biogeographic range reaches from Northeastern Spain through southern France all over the Alps. It includes the Apennine in the South and the Middle European mountain ridges like the Vosges and the Black Forest in the North, and extends into the Karpats and the Czech and Slovakian mountains (Kühnelt, 1984). *O. speciosissima* is common in a broader altitudinal range than *O. cacaliae*, but both species are recorded between 500m and 3000m. They occur sympatrically, feeding on the same host plant patches of perennial herbs, but *O. speciosissima* has a broader host plant spectrum; like *O. cacaliae* it feeds on species of *Adenostyles*, *Senecio nemorensis-fuchsii* and *Petasites albus*, but it also accepts *Cirsium spinosissimum* and *Doronicum grandiflorum* (all Asteraceae). Being abundant early in the spring and late in the summer, *O. speciosissima* appears to avoid the peak densities of *O. cacaliae* in June/July. Both species are ovoviviparous and have overlapping generations. Overwintering males and gravid females occur in early spring; larvae are laid from spring until early summer directly on the host plants. Larvae develop in four larval stages to adults either in the same year, or, if they are late, overwinter as L4 in the soil. *O. speciosissima* larvae , to our knowledge, always develop from larvae into adults within the same season.

As many chemically defended chrysomelids, all species of *Oreina* are aposematic and *O. cacaliae* and *O. speciosissima* show a considerable colour polymorphism. Colour still is often used in the identification of subspecies or races (e.g. Kühnelt, 1984), although only the aedeagus is considered as a reliable character for species determination (Bourdonné and Doguet, 1991). The heritability of colour polymorphism, even though documented for other leaf beetles (Fujiyama and Arimoto, 1988; Vasconcellos-Noto, 1988) is unknown for any species of the genus *Oreina*. Within populations individuals of both species show only minor colour variations. Sympatric species of *Oreina* often show the same colour morph within one population. Since the colours are thought to be warning signals to possible predators (birds), this could be mimicry and adaptation of individuals to the local dominating colour form and consequently be under strong selective control (Vasconcellos-Noto, 1988).

Sampling

The sampling of populations of the two *Oreina* species was conducted mainly in summer 1993; some additional populations were sampled during the summers of 1994 and 1995. The total sampling area ranged from the Central Pyrenees to the Czech republic (figure 1). This represents the western half of both species' range; however, no *O. speciosissima* could be found in the Pyrenees.

One sample always consists of beetles randomly taken from only one host plant patch - a patch being defined by continuous plant cover. Adult beetles were picked by hand from their host plants, brought alive to the laboratory and stored in liquid nitrogen until further analysis. Samples included in this macrogeographic analysis were always more than 5 km apart. Since host plant patches can be very large (up to 1 km²) and beetles very abundant (chapter V, densities from 0.8 - 3 beetles m²), we sometimes sampled only a fraction of the patch (the sampled area rarely exceeded 100 m²). Former studies (Rowell-Rahier, 1992) have documented high F_{IS}-values for both species, therefore we sampled the supposedly smallest possible "random mating unit" (beetles at the same host plant patch) in order to avoid sampling over more than one neighbourhood (thereby creating a Wahlund effect (Hartl and Clark, 1989)). We sampled from three different host plant species (*A. alliariae*, *S. fuchsii* and *P. albus*). All patches sampled were clearly dominated by one of these species (and were classified as such), even when all three plant species were present in the area. The location of each patch was marked on 1:25000 topographic maps and is given together with host plant species and colour of the beetles as patch characteristics (table 1).

Next page:
Figure 1: Sampling sites of *O. cacaliae* and *O. speciosissima*

Sampling sites of *Oreina cacaliae* and *Oreina speciosissima*

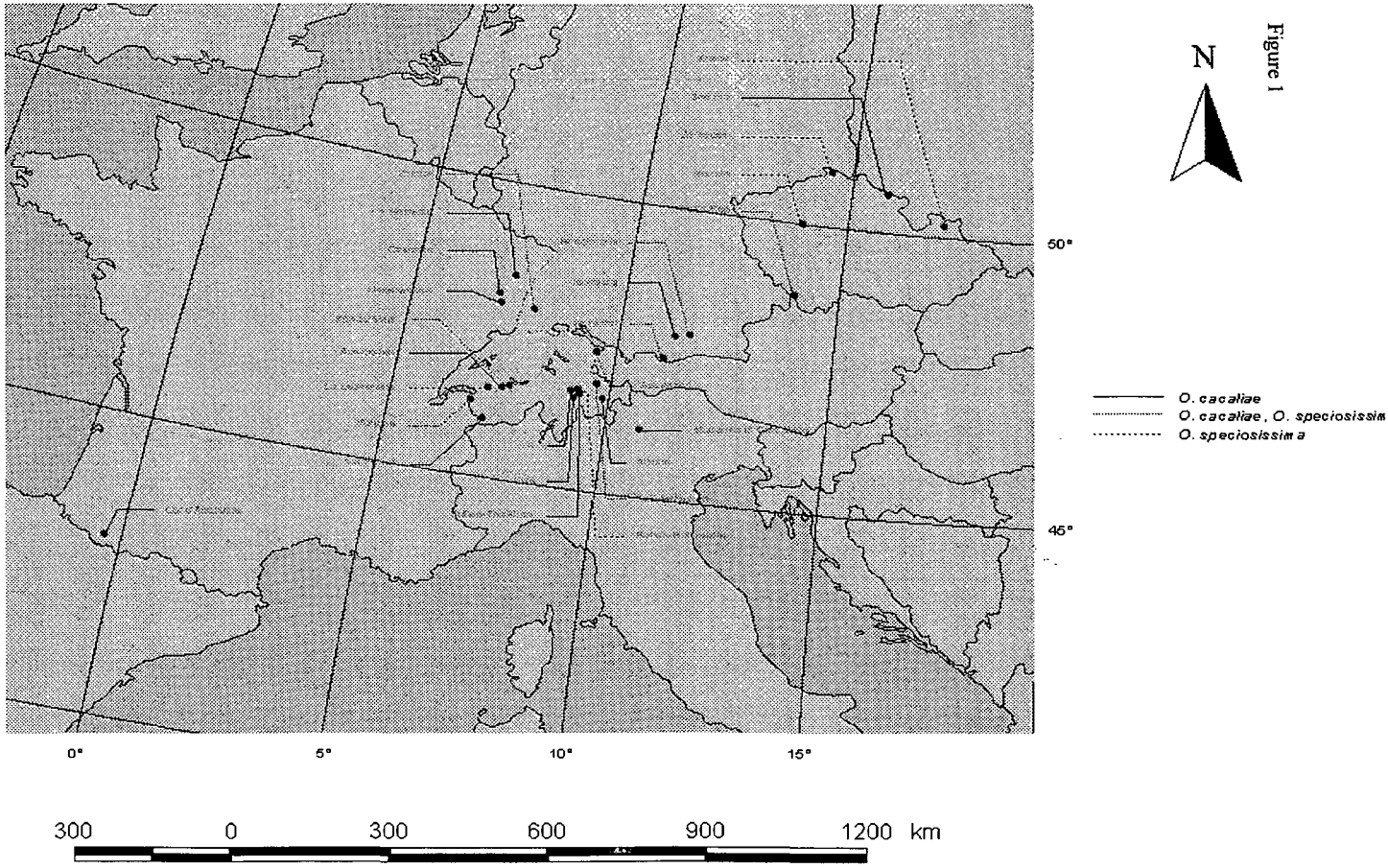


Figure 1

Table 1a: Location and environmental and ecological parameters of the sampling sites: altitude in m, colourtype (as body colour/stripe colour) for *O. cacaliae*

Sample	altitude	latitude	longitude	host plant	colour type
Adelboden	1500	46.28.10	7.32.10	*Adenostyles*	blue-green/-
Albula	1820	46.38.30	9.49.20	*Senecio*	blue/-
Appenzell	1300	47.16.00	9.27.48	*Adenostyles*	blue/-
Cascade	950	48.05.00	7.05.00	*Adenostyles*	green/blue
Col Aubrisque	1100	42.57.00	1.40.00	*Adenostyles*	green/blue
Ferret	1614	45.55.20	7.05.50	*Adenostyles*	blue-green/-
Hirschbach	1400	47.50.00	11.40.00	*Adenostyles*	green/blue
Hohwald	650	48.25.00	7.20.00	*Adenostyles*	green/blue
Höllental	1100	47.25.00	11.05.00	*Petasites*	green/blue
Kandersteg	1490	46.28.15	7.39.20	*Adenostyles*	blue-green/-
Lieserwasen	850	48.57.00	6.50.00	*Senecio*	green/blue
Madonna	1825	46.13.00	10.49.00	*Adenostyles*	blue/-
Morgins	1400	46.13.55	6.47.30	*Adenostyles*	green/blue
Nova Pec	850	48.45.00	13.55.00	*Senecio*	green/blue
Safien Th.	1700	46.37.50	9.16.35	*Adenostyles*	blue/-
Schneekoppe	1200	50.35.00	15.50.00	*Senecio*	green/-
Tschiertschen	1860	46.47.55	9.37.00	*Adenostyles*	blue/-
Ochsenalp	1920	46.48.45	9.38.55	*Adenostyles*	blue/-
Vals	1600	46.36.00	9.09.10	*Adenostyles*	blue/-
Vrin	1450	46.40.10	9.05.40	*Adenostyles*	blue/-
Zastler	1170	47.53.30	8.00.20	*Petasites*	green/blue

Table 1b: Location and environmental and ecological parameters of the sampling sites: altitude in m, colourtype (as body colour/stripe colour) for *O. speciosissimo*

Sample	altitude	latitude	longitude	host plant	colour type
Appenzell	1300	47.16.00	9.27.48	*Adenostyles*	green/blue
Boubin	990	49.56.00	13.50.00	*Senecio*	green/red
Höllental	1100	47.25.00	11.05.00	*Petasites*	green/blue
Hirschbach	1400	47.50.00	11.40.00	*Petasites*	green/blue
Kiental	1350	46.38.00	7.45.00	*Adenostyles*	green/blue
Kralov	700	50.10.00	17.20.00	*Senecio*	green/blue
Altvater	700	50.10.00	17.20.00	*Petasites*	green/blue
La Lecherette	1150	46.27.00	7.06.55	*Petasites*	green/blue
Morgins	1400	46.13.55	6.47.30	*Adenostyles*	green/blue
NovaPec	850	48.45.00	13.55.00	*Senecio*	green/red
Safien-Rainmatte	1416	46.42.20	9.19.30	*Petasites*	green/blue
Tschiertschen	1730	46.48.05	9.36.50	*Adenostyles*	green/blue
Hörnli	2300	46.45.50	9.36.50	*Cirsium*	black/-
Vrin	1450	46.40.10	9.05.40	*Adenostyles*	green/blue
Zamecek	750	50.60.00	13.60.00	*Senecio*	green/red
Zastler	1170	47.53.30	8.00.20	*Petasites*	green/blue

Allozyme electrophoresis:

For allozyme electrophoresis, beetles were dissected and the thoracic muscles were homogenised in a 0.1M Tris-EDTA buffer with Mercaptoethanol, pH 7.0. We scored the following 15 enzyme systems in standard horizontal starch gel electrophoresis (12% Sigma starch, for details of the procedure and recipes see Hillis and Moritz, 1990): ACOH (*Aconitase Hydratase* EC4.2.1.3), DDH (NADH-*Diaphorase*), MDHP (*Malate Dehydrogenase* EC 1.1.1.40), GPI (*Glucose-6-phosphate Isomerase* EC 5.3.1.9.) on TCA (0.001M Tris-citrate buffer, pH 6.7); AAT (*Aspartate Aminotransferase* EC 2.6.1.1. (2 loci)), PEP (LA) (*Peptidase* EC 3.4.-.-. (2loci)), ARK (*Arginine Kinase* EC 2.7.3.3. (3 loci), IDH (*Isocitrate Dehydrogenase* EC 1.1.1.42 (2loci)), EST (*Esterase*) on TCB (0.05 M Tris citrate buffer, pH 8.7) and FUMH (*Fumarate Hydratase* EC 4.2.1.2.), FDH (*Formaldehyde Dehydrogenase* EC 1.2.1.1.), GAPDH (*Glyceraldehyd-3-phosphatedehydrogenase* EC 1.2.1.12), G3PDH (*Glycerol-3-phosphate Dehydrogenase* EC 1.1.1.8.), SOD (*Superoxide Dismutase* EC 1.15.1.1. (2loci), TPI (*Triose-phosphate Isomerase* EC 5.3.1.1.), AO (*Aldehyde-oxidase* (2loci)) on EBT (0.2M Tris borate buffer, pH 8.6). For *O. cacaliae* 21 loci could be consistently scored, for *O. speciosissima* 16 loci (table 2). Banding patterns of all reported enzyme systems followed the ones reported in the literature (Hillis and Moritz, 1990).

Statistical analyses:

Genetic variability:

Allele frequencies were calculated using the program GENEPOP version 2.0 (Raymond and Rousset, 1995). We tested for linkage disequilibrium and for Hardy-Weinberg equilibrium. Associations of genotypes between loci were tested for each population separately (Option 2 of GENEPOP) and a sequential Bonferroni correction for multiple comparisons was used (Rice, 1989). To test for Hardy-Weinberg equilibrium, we present results of the "probability test", which corresponds to the exact test for Hardy Weinberg equilibrium (option 1, suboption 2 of GENEPOP). Whenever possible (not more than four alleles at a locus) the complete enumeration (Louis and Dempster, 1987) was used to calculate exact p values, otherwise a Markov chain method as proposed by Guo and Thompson (1992) was used to estimate, in 100 batches and 1000 iterations per batch, the p values and standard errors. A sequential Bonferroni correction for multiple comparisons was used (Rice, 1989).

As measures of the genetic variability we calculated the number of alleles per locus, percentage of loci polymorphic and the unbiased heterozygosity estimate as well as observed heterozygosity, using the program BIOSYS-1 (Swofford and Selander, 1981).

F-statistics were calculated with FSTAT (Goudet 1995) according to the formulas given in Weir and Cockerham (1984). This gives unbiased estimates of the F-statistics, that indicate the amount and partitioning of genetic variance within (F_{IS}) and between (F_{ST}) samples. Significance levels were determined in 10000 permutations.

Searching for possible causes of structuration

To identify possible distinct genetic lineages, we constructed a dendrogram, based on UPGMA with Rogers modified distance (Rogers, 1972).

We tested a possible relationship between - as dependent variables - the observed genotype and one morphological trait (colour) and - as possible explanatory factors - host plant association and geographical and altitudinal isolation (nonparametric Mantel tests, e.g. Manly, 1985). The following five distance matrices were used: the genetic distance matrix (Rogers modified genetic distance), a "colour-type" matrix, the geographic distance matrix, the altitudinal distance matrix and a "host plant" matrix. For the "colour-type" matrix we coded the observed colours in distinct classes (3 types for *O. speciosissima*: green with red stripes, green with blue stripes and black; and 4 types for *O. cacaliae*: green, green with blue stripes, blue-green, deep blue) and coded the distance between samples with beetles of the same colour as 0 and the one between samples with beetles of different colours as 1. In the same way the "host plant" matrix coded the distance between patches dominated by the same plant species with 0, and the one between patches dominated by different plant species with 1. Altitude as well as latitude and longitude of the sampling sites were recorded from 1:25000 topographic maps and the distance matrices were calculated thereof. For each patch these characteristics are given in table 1. Mantel tests were performed with the program R (Legendre and Vaudor, 1991). We report the r-values (interpretable as a correlation coefficient) and the probabilities of a correlation, resulting from 10000 permutations.

To account for correlations of the explanatory matrices (geographic distance, altitude and host plant), we conducted partial Mantel tests following the method of Smouse *et al.* (1986) whenever appropriate.

Isolation by distance was further tested as described by Slatkin (1993). In this model a negative slope in the regression of log (Nm) plotted against log transformed geographic distance indicates isolation by distance; the slope gives an estimate of the degree of differentiation with increasing geographic distance and the intercept of the regression equation is an indicator of the neighbourhood size (the smallest random mixing unit). Nm-values as a measure of gene flow (Slatkin and Barton 1989) were calculated with the private allele method and adjusted to sample sizes (Barton and Slatkin 1986) and compared to those calculated from F_{ST}-values (Wright, 1951).

RESULTS:

Genetic variability

Allele frequencies:

Allele frequencies for all samples are given in the appendix C, table 1. For both species only one locus of all the ones tested was not polymorphic (95% criterium) in at least one sample (Arg3 in *O. cacaliae* and IDH2 in *O. speciosissima*). All loci except GAPDH for both species and FDH and MDHP for *O. cacaliae* were also fixed in at least one sample, some samples were fixed for different alleles (table 2).

Table 2: Numbers of populations fixed (frequency of the most common allele p = 1) or polymorphic (frequency of the most common allele p < 0.95; 95% criterium) for different loci.

	O. speciosissima		*O. cacaliae*	
Locus	fixed	polymorphic	fixed	polymorphic
SOD2	12	4		
ACOH	7	9	4	15
AO1	1	15	1	18
ARK3	14	1	18	0
DDH	9	5	2	18
FDH	7	7	0	19
FUMH	6	10	11	5
GAPDH	0	15	0	21
AAT	2	14	1	19
IDH1	10	3	18	1
IDH2	14	0	19	1
PEP(LA)1	1	14	7	13
PEP(LA)2	5	10	1	20
GPI	2	14	13	6
SOD1	14	1	2	15
TPI	1	14	4	15
EST			1	20
ARK4			10	7
ARK2			11	10
AO2			1	20
G3PDH			1	20
MDHP			0	20

We did not find a latitudinal, longitudinal or altitudinal cline for any of the alleles with an overall frequency of p>0.10.

Overall a mean of 6.14 alleles per locus was found in *O. cacaliae* and of 5.87 alleles per locus in *O. speciosissima*; when excluding rare alleles these numbers decreased to 2.19 and 2.13 respectively.

Linkage disequilibrium:

O. speciosissima: 22 combinations of loci out of 158 possible tests gave a significant p-value at the 5% level, after applying sequential Bonferroni proccdures only one, FUM&AAT in Safien Rainmatte, still was significant.

The 22 at the 5% level significant loci-combinations included 3 combinations occurring twice: AO1&AAT, FUM&TPI and GAPDH&AAT). Seven occurred in the population Safien Rainmatte, three in Morgins, in 4 populations two significant combinations were found, in 4 populations 1 and in 5 none.

O. cacaliae: Out of 1987 possible tests, the following 6 gave a significant deviation from random distribution of genotypes after applying sequential Bonferroni corrections: in Ferret DDH1&TPI, in Kandersteg ACOH&Arg2, in Zastler DDH1&TPI and in Lieserwasen G3PDH&GAPDH, ACOH&LA2 and G3PDH&MDHP. At the 5% level (without Bonferroni corrections) 127 more tests were significant. The most often observed loci combinations were GAPDH&TPI and ACOH&GAPDH, both occurring four times; 10 combination occurred three times, and 11 twice. Again most of these results occurred in Lieserwasen (18), 17 in Ferret, 15 in Kandersteg, 11 in Ochsenalp and

10 in Cascade. In all other populations less than 8 tests gave p values below 0.05.

Hardy-Weinberg equilibrium:

O. speciosissima: With the exact test 29.36% (31 out of 126 possible tests) were significantly different from Hardy-Weinberg expectations after applying Bonferroni correction. Overall, in 76.9% of the polymorphic loci genotypic distribution deviated from Hardy-Weinberg equilibrium. The loci, where no significant deviation could be found are ACOH, LA2 and GPI. Not surprisingly, the overall loci, overall populations test showed highly significant deviation from Hardy-Weinberg equilibrium (χ^2=infinity, df=176, p=highly sig.).

O. cacaliae: With the exact test 13.58% (39 out of 287 possible tests) were significantly different from Hardy-Weinberg expectations after applying Bonferroni correction. Overall, in 52.38% (11 out of 21) of the polymorphic loci genotypic distribution deviated from Hardy-Weinberg equilibrium. Again, the overall loci, overall populations test showed highly significant deviation from Hardy-Weinberg equilibrium (χ^2=infinity, df=432, p=highly sig.).

Table 3a: Variability measures and F_{IS}-values for *O. cacaliae*. H.unb.: unbiased estimate of Heterozygosity; H.dc: observed Heterozygosity; % loci: % loci polymorphic (95% criterium); F_{IS} -values.

Population	H.unb.	H.dc	% loci	F_{IS}
Adelboden	0,337	0,257	80,95	0,270
Albula	0,331	0,224	66,67	0,368
Appenzell	0,305	0,274	76,19	0,102
Cascade	0,252	0,169	71,43	0,301
Col Aubrisque	0,222	0,155	57,14	0,284
Ferret	0,358	0,283	80,95	0,164
Hirschbach	0,286	0,203	76,19	0,348
Hohwald	0,299	0,203	66,67	0,276
Höllental	0,256	0,178	57,14	0,328
Kandersteg	0,339	0,244	66,67	0,282
Lieserwasen	0,278	0,215	57,14	0,222
Madonna	0,197	0,124	52,38	0,377
Morgins	0,345	0,369	71,43	-0,104
Nova Pec	0,138	0,103	33,33	0,391
Safien Th.	0,291	0,221	66,67	0,265
Schneekoppe	0,234	0,175	47,62	0,241
Tschiertschen	0,243	0,198	57,140	0,206
Ochsenalp	0,365	0,300	71,430	0,186
Vals	0,298	0,285	61,900	0,094
Vrin	0,328	0,265	61,900	0,195
Zastler	0,272	0,246	61,90	0,111

Genetic variability measures:

As measures of genetic variability we calculated number of alleles per locus, percentage of polymorphic loci, and unbiased heterozygosity as well as observed direct count heterozygosity (Avise, 1994). The number of alleles per locus found in single samples was the only measure of genetic variability dependent of sample size and therefore not taken into consideration. Percentage of polymorphic loci ranged in *O. cacaliae* from 33.33% in Nova Pec (Czech Republic) to 80.95% in Adelboden (Central Switzerland) and Val Ferret (Western Switzerland), in *O. speciosissima* from 31.3% in Zamecek (Czech Republik) to 81.3% in Safien Rainmatte (Eastern Switzerland). Unbiased heterozygosity values ranged in *O. cacaliae* from 0.138 (Nova Pec) to 0.365 in Ochsenalp (Eastern Switzerland), in *O. speciosissima* from 0.140 in Appenzell (Eastern Switzerland) to 0.370 in Safien Rainmatte (table 3a,b).

As for single alleles, no significant correlation of any measure of genetic variability with geographic location or altitude could be found.

F-statistics:

O. speciosissima: We observed a large range of F_{IS}-valucs in the different samples, ranging from -0.072 to 0.637 (table 3b). The overall inbreeding coefficient was very high, we found a value of F_{IT} of 0.503±0.056. This was mostly attributable to the within-population component (F_{IS}=0.350±0.042), although there was also a considerable among populations differentiation (F_{ST}=0.236±0.043).

Table 3b: Variability measures and F_{IS}-values for *O. speciosissima*. H.unb.: unbiased estimate of Heterozygosity; H.dc: observed Heterozygosity; % loci: % loci polymorphic (95% criterium); F_{IS} -values.

Population	**H.unb.**	**H.dc**	**% loci**	**F_{IS}**
Appenzell	0,140	0,084	37,50	0,478
Boubin	0,194	0,174	62,50	0,049
Höllental	0,175	0,097	43,75	0,415
Hirschbach	0,216	0,085	68,75	0,637
Kiental	0,303	0,149	62,50	0,517
Kralov 1	0,267	0,215	62,50	0,280
Kralov 2	0,180	0,128	50,00	0,277
La Lecherette	0,236	0,261	50,00	-0,072
Morgins	0,208	0,145	56,25	0,305
NovaPec	0,164	0,159	37,50	0,042
Safien-Rainmatte	0,370	0,197	68,75	0,466
Tschiertschen	0,342	0,183	62,50	0,487
Hörnli	0,268	0,197	56,25	0,301
Vrin	0,161	0,142	50,00	0,078
Zamecek	0,181	0,213	31,25	-0,060
Zastler	0,257	0,163	56,25	0,393

O. cacaliae: Again a great range of F_{IS}-values could be observed, ranging from 0.094 to 0.391 (table 3a). The overall inbreeding coefficient was somewhat lower than in *O. speciosissima*, (F_{IT}=0.349±0.024) and again mostly attributable to the within population component (F_{IS}=0.229±0.029), although there was also a considerable differentiation between populations (F_{ST}=0.155±0.028).
For both species different loci differed in their contributions to these values, some showing F_{ST}-values not different from 0 (figure 2a,b). The high values of IDH in *O. cacaliae* are only due to the single sample from the Pyrenees, removing this sample resulted in F_{IT}, F_{IS} and F_{ST} for IDH1 not different from 0. IDH1 is highly polymorphic in the Pyrenean sample (6 alleles) and monomorphic (95% criterium) in all other samples.

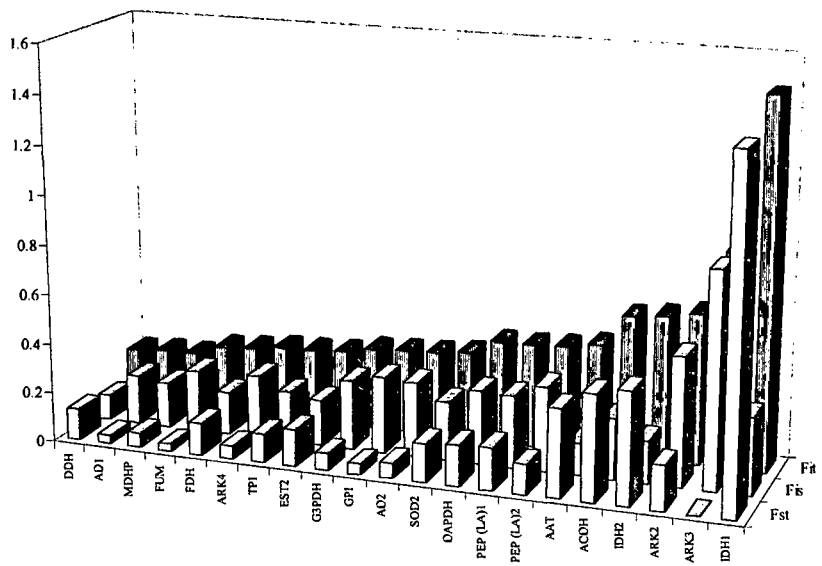


Figure 2a: F-statistics at the different loci for *O. cacaliae*. Loci are ordered by their F_{IT}-value, no colour indicate values not different from 0 (jackknifing over populations). The high values at IDH1 are due to the Pyrenean sample only (see text).

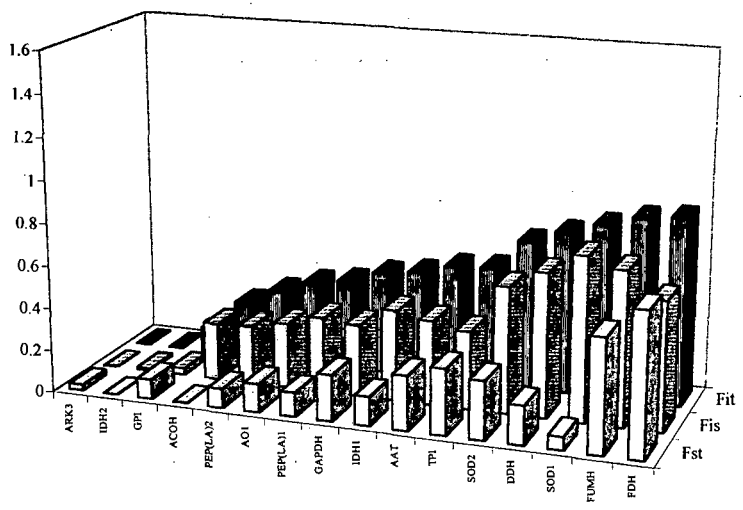


Figure 2b: F-statistics at the different loci for *O. speciosissima*. Loci are ordered by their F_{IT}-value, no colour indicate values no different from 0 (jackknifing over populations).

Searching for possible causes of structuration

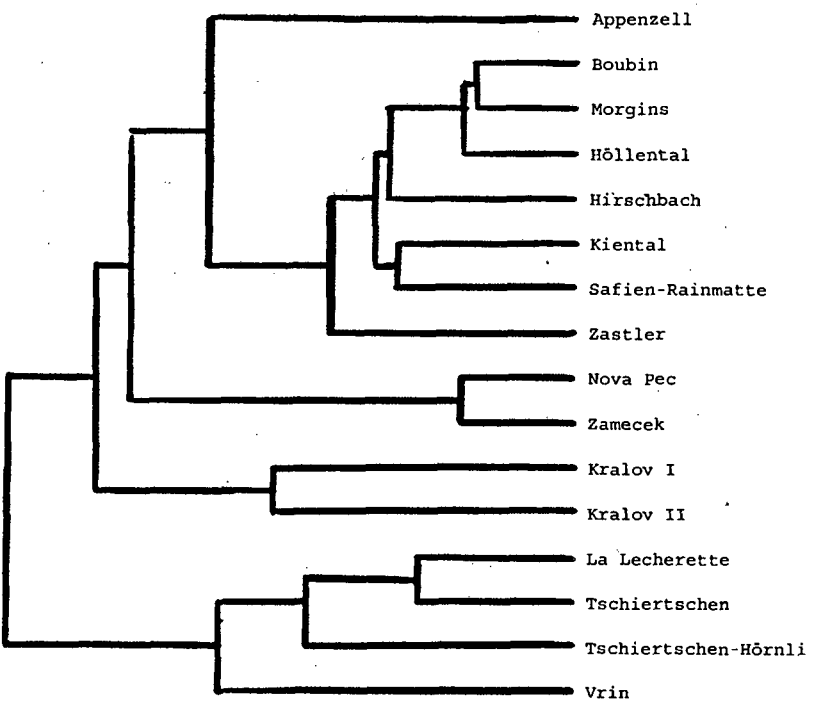
Cluster analyses:

The UPGMA dendrograms (figure 3) show only one interpretable cluster for *O. cacaliae*, the Pyrenean sample cluster is separated from the other populations. Another Pyrenean population, which was otherwise not presented here due to very small sample sizes, clustered together (unpubl. data, Appendix C, figure 1). No other major differentiation into subgroubs could be observed at the dendrograms of either species.

Next two pages:

Figure 3: UPGMA-cladogram, based on Rogers modified distance for a) for *O. cacaliae* and b) *O.speciosissima*


Adelboden
Morgins
Albula
Appenzell
Safien-Thalkirch
Tschiertschen
Ochsenalp
Hirschbach
Hohwald
Lieserwasen
Zastler
Vals
Vrin
Val Ferret
Kandersteg
Snezka
Höllental
Nova Pec
Madonna
Cascade
Col d'Aubisque


Appenzell
Boubin
Morgins
Höllental
Hirschbach
Kiental
Safien-Rainmatte
Zastler
Nova Pec
Zamecek
Kralov I
Kralov II
La Lecherette
Tschiertschen
Tschiertschen-Hörnli
Vrin

Mantel tests:

O. speciosissima: In the pairwise Mantel tests of the five distance matrices (genetic distance, colour, host plant, geographic distance, and altitude) all combinations were significantly correlated, except for the relationship between genotype and host plant (table 4). After removing the interaction of altitude and geographic distance, geographic distance was still positively correlated with colour-type(r=0.62049, p=0.002), but not with genetic distance (r=0.14401, p=0.14186) and altitude was not significantly correlated with either genotype (r=0.20973, p=0.13187) nor colourtype (r=0.25605, p=0.08192). Geographic distance was also positively related with colour type after removing host plant effects (r=0.62056, p=0.001), so we can conclude that there is a geographic component determining colour-type. However, there is still a positive host plant component on colour-type, even when geographic effects are removed (r=0.35584, p=0.003).

Table 4: Results of the pairwise Mantel tests, significant results are bold. For details of the different distance matrices tested see text.

O.speciosissima	**genotype**		**colourtype**		**km**		**altitude**	
	r	p	r	p	r	p	r	p
genotype								
colourtype	**0,403**	**0,002**						
km	**0,233**	**0,048**	**0,681**	**0,002**				
altitude	**0,277**	**0,042**	**0,431**	**0,002**	**0,378**	**0,007**		
host plant	0,114	0,071	**0,489**	**0,001**	**0,361**	**0,002**	**0,268**	**0,004**

O.cacaliae	**genotype**		**colourtype**		**km**		**altitude**	
	r	p	r	p	r	p	r	p
genotype								
colourtype	-0,012	0,452						
km	**0,511**	**0,004**	0,064	0,180				
altitude	-0,023	0,458	**0,430**	**0,001**	0,182	0,089		
host plant	0,071	0,336	-0,014	0,505	0,214	0,103	0,029	0,267

O. cacaliae: In the pairwise Mantel test, colour type was positively correlated with altitude and allozymatic genotype was positively correlated with geographic distance. No other combination showed positive correlations (table 4).

Isolation by distance and gene flow:

Slatkin's method (Slatkin, 1993) indicated isolation by distance for *O. cacaliae* ($\log(Nm) = 1.013 - 0.409 \log(km)$, $r^2 = 0.32$; figure 4a), but not for *O. speciosissima* ($\log(Nm) = -0.389 + 0.096 \log(km)$, $r^2 = 0.03$, figure 4b). This is analogous to the results obtained from the Mantel test, where a positive correlation between geographic distance and genetic distance could only be observed for *O. cacaliae*. The sample from the Pyrenees seems, again, to be more differentiated than could be explained by distance

alone, since a removal of this sample flattened the slope of the regression (log(Nm)=0.912-0.359log(km); r^2=0.23), whereas a removal of the other most distant sample from the Czech Republic did not (log(Nm)=1.063-0.433log(km), r^2=0.32).

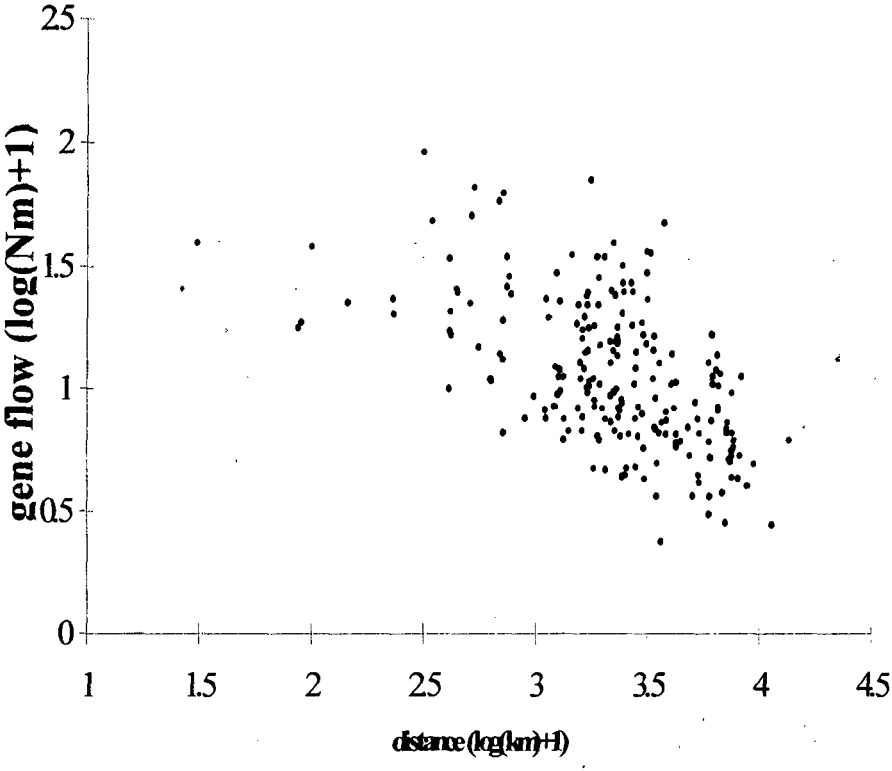


Figure 4a: Regression of pairwise estimates of dispersal against pairwise geographical distances for *O. cacaliae* - samples, indicating isolation by distance (Slatkin 1993). The regression equation is log(Nm)=1.013 - 0.409 log(km); r^2=0.32.

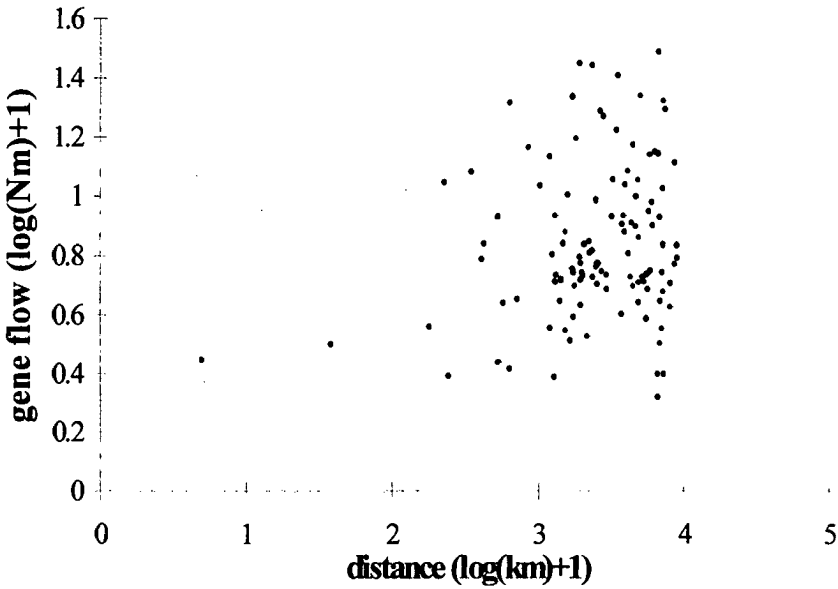


Figure 4b: Regression of pairwise estimates of dispersal against pairwise geographical distances for *O. speciosissima* - samples, indicating no isolation by distance (Slatkin 1993). The regression equation is log(Nm)=1.013 - 0.409 log(km); r^2=0.03.

Nm values, calculated as a measure of gene flow from the frequencies of "private alleles", were very similar for both species (1.33 for *O. speciosissima* and 1.51 for *O. cacaliae*). Nm values calculated from F_{ST} were both smaller, 0.81 for *O. speciosissima* and 1.36 for *O. cacaliae*. Values around one indicate rather limited gene flow - which is in accordance with the limited vagility reported for these beetles - but not small enough to support the idea of a permanent population differentiation into distinct genetic lineages (Slatkin , 1985).

DISCUSSION:

Distribution of genetic variance

O. cacaliae and *O. speciosissima* displayed a high genetic variability. Measures of genetic variability were in the same range for both species, not supporting the hypothesis of an enhanced genetic variability in the species with broader ecological niche. In comparison with studies of other chrysomelid species, the percentage of polymorphic loci in our species is very high; the heterozygosity values, however, are similar to those reported in Rowell-Rahier (1992) and for other Chrysomelidae (Eggenberger and Rowell-Rahier, 1991; Jacobson and Hsiao, 1983; Hsiao, 1989; McDonald *et al.*, 1985; Verdyck *et al.*, 1996).

Considerable genetic variation was found, both within and among populations. Moreover, the within-population component was always larger than the amount of genetic variation attributable to differentiation among populations (F_{IS}-values greater than F_{ST}-values). The high F_{IS}-values reported here correspond to the ones reported in an earlier study (Rowell-Rahier, 1992). On the other hand, the difference in F_{ST}-values between the two species suggested in this study could not be confirmed. We think, that the reported, much lower F_{ST}-value (0.051) for *O. speciosissima* in the previous study (Rowell-Rahier) is probably an artefact of the low number of samples (3). With a high amount of within population variance a significant amount of between population differentiation is expected (Wright, 1978).

The high F_{IS}-values in this study result from substantial heterozygote deficit in almost all populations. Often we found deviations from the Hardy-Weinberg equilibrium. This is neither the effect of one or two single loci (indicating selection on these loci, Slatkin 1985), nor do all loci show the same amount of heterozygote deficit, as would be the result of inbreeding or a Wahlund effect. A homogeneous heterozygote deficit over nine polymorphic loci was, for instance, observed in the cave dwelling beetles of the genus *Speonomus* (Crouau-Roy, 1988) and explained by inbreeding.

Three possible explanations for heterozygote deficits have been proposed (Crouau-Roy, 1988): the presence of null alleles, inbreeding, and a Wahlund effect (sampling over several random mating subgroups, Hartl and Clark, 1989). We exclude the possibility of null alleles, since no missing genotypes (homozygote null alleles) were found in the pattern expected in the gels revealing allozyme-genotype. Missing genotypes in our data set are always due to the missing of a whole gel run or staining.

Bilton (1992), in a study on the dytiscid beetle *Hydroporus glabriusculus*, found a similar pattern of high overall genetic variance mainly due to high F_{IS}-values. He attributed this to the sampling procedure conducted over several different aggregations. Our sampling procedure was explicitly designed to avoid Wahlund effects, sampling only small and continuous host patches, wherein the beetles were spaced evenly. Although the beetles do not disperse much (chapter IV), a substructuring of independent groups within these patches seems unlikely to us.

Inbreeding should result in a homogeneous effect at all loci, which was not found here. We explain the pattern found by a combination of inbreeding resulting in kinship groups and a sampling effect over several different closely related groups.

Similar arguments have been used for the treehopper *Enchenopa binotata* (Guttmann *et*

al., 1989). In this species, the fact of high differentiation among samples of nymphs on different branches of their host trees and high F_{IS}-values within these branches was explained by sampling over the offspring of only a few females. They discuss a possible sampling artefact, which implies that later in the season (before mating) there is dispersal and thereby a mixing of these sibling groups. We did not find smaller F_{IS}-values in populations we sampled just before larviposition, the moment, when most dispersal and mixing should have taken place. F_{IS}-values were not related to the date of sampling, thus we do not see our results as representing a structuring not significant for the population or indicating no inbreeding-like effects.
We can only speculate about the extent of inbreeding (mating with relatives) and whether this occurs by chance (due to limited dispersal) or by assortative mating. We have no information about mate choice or parentship in *Oreina* species. In the field we observe frequent mating up to the time of larviposition and we know from laboratory studies that these frequent mating do not result in more larvae laid (Dobler, pers. communication). Also, we observe frequently males of last years generation mating with newly hatched, still soft females. Overlapping generations, in combination with low vagility, should enhance the possibility and magnitude of inbreeding.
In conclusion we suggest, that in *Oreina* "host plant patch"-populations do obviously not mate at random and probably constitute a mix of more or less closely related kin groups, thereby offering the possibility of kinship selection (Wade, 1985).

Causes of population structuring

No major differentiation into subgroups could be detected for either species with the exception of the Pyrenean population of *O. cacaliae*. This distinction is probably due to the greater isolation of the Pyrenean mountain ridge. Morphologically, Kühnelt (1984) groups the beetles we sampled in the Pyrenees together with forms from the Vosges, Black Forest and Central Alps, distinct from a Pyrenean form "tussilaginis".
A correlation of population variability with environmental parameters is an indication of local adaptation and selection (Endler, 1977, Manly, 1985). For three explanatory factors, host plant, geographic distance and altitude, we tested for an influence on the population structure.
For the two species investigated here, one common result is, that obviously there is no host plant effect for either species. We see this in agreement with the results of a phylogenetic study of the genus, which documented low host fidelity and flexibility in host affiliations for the genus (Dobler *et al.*, 1996). Autogenous defence, in combination with aposematism should supposedly promote independence from the host plant. Since *O. cacaliae* has given up the possibility of autogenous defence, relying exclusively on sequestration of host secondary compounds, we assumed a closer association to its host as reflected in its smaller host plant spectrum. However, we found no indication for a host plant effect on the macrogeographic scale investigated here. At a microgeographic scale Kreslavsky *et al.* (1976) reported host races for *O. cacaliae* from a morphometric study based on length of elytrae. A morphometric study of 12 characters conducted in our laboratory on a subset of the populations presented here, ranked length of elytrae as not very informative for investigating differentiation between *O. cacaliae* or *O. speciosissima* populations. The most informative characters were the characters

measured at the aedeagus and the length of tarsae (Gallusser, 1996).
Also, for both species there is no indication for a greater differentiation at higher altitudes as has been proposed for several interspecific comparisons (Knoll *et al.* 1996, Liebherr, 1988).
For *O. cacaliae*, the observed population structure seems to be due to - and only due to - isolation by distance. The pattern of isolation by distance seems not to be due to selection and adaptation in geographically different areas, as indicated by the absence of any clinal pattern. Rather it seems to be imposed by limited gene flow and geographic distance in an otherwise homogeneous species as originally proposed by Wright (1978). In a recent review of population structure of sedentary species Peterson (1996) shows that isolation by distance is a general feature of the population structure of sedentary species on a macrogeographic scale.
The colour of *O. cacaliae* showed a strong correlation with altitudes. At higher altitudes beetles were dark blue. Melanism in high altitudes is a common feature of many insect species and commonly explained by providing better UV protection.
Colourtypes are often used in chrysomelids to identify "races" or "subspecies" (e.g. Kühnelt, 1984 for *Oreina*), but so far no genetic differentiation could be proven for colour forms (Verdyck *et al.*, 1996). We used colour-type as a morphological character and found no correlation with geographic distance. By contrast, in a detailed morphometric study of *O. cacaliae*, identical patterns were found for the morphological variation as for allozymatic variation (namely "isolation by distance" for both data sets). However, the study revealed that there is no correlation between the morphological data set and the allozymatic data set, although data were obtained from the same individuals. Geographic distance seems to be the factor influencing both of them independently (Gallusser, 1996).
For *O. speciosissima* none of the tested environmental factors (geographic distance, altitude, and host plant) were positively correlated with the genotype. However, colourtype was correlated to geographic distance and host plant. This might reflect a flaw in our sampling regime, the populations from the Czech Republic all belonging to a specific colour type (green with red stripes, found nowhere else), and the population from Hörnli being the only black one and the only one on *Cirsium spinosissimum* (table1). *O. speciosissima* showed no indication for isolation by distance and very low Nm-values, interpretable as a species not at equilibrium with virtually no ongoing gene flow (Slatkin, 1993). However, since the sampling for *O. speciosissima* populations was not as intensive as for *O. cacaliae*, it might not give the power to detect isolation by distance (Slatkin, 1993; Slatkin and Maddison, 1990). This seems more likely to us, since we cannot explain, why *O. cacaliae* should have reached an equilibrium state, whereas *O. speciosissima* has not.

Conclusion

Both species show comparable amounts of genetic variation and considerable population structuring. In *O. cacaliae* the observed structure can be explained by the isolation by distance model - limited gene flow in a sedentary species over larger scales - whereas for *O. speciosissima* no such explanation could be found. For both species, no host-plant effect can be granted.
Obviously, demographic processes play an important role in determining the distribution of genetic variation in *Oreina* species and should receive further investigation.

Acknowledgements:

We would like to thank B. Benrey, S. Dobler, J. Goudet, C. Liepert, J.M. Pasteels and T. Turlings for helpful comments on an earlier draft of the manuscript . C. Knoll and G. Schwarzbözl helped with sampling in the Pyrenees and H. Kippenberg kindly indicated *Oreina* sites. This work was supported by the Swiss National Science Foundation (grant no. 31-33669.92).

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CHAPTER III

HIERARCHICAL GENETIC STRUCTURE AND GENE FLOW IN *OREINA CACALIAE* (COLEOPTERA: CHRYSOMELIDAE)

Manuscript

We conducted a hierarchical sampling analysis - patches within localities, localities within regions, regions within total sampling area, ranging from the Pyrenees to the Czech Republic - in order to assess the scale of genetic differentiation in *O. cacaliae*.
With two methods, hierarchical F-statistics and spatial autocorrelation, we investigated at which scale differentiation can be observed. Hierarchical F-statistics revealed that most differentiation is attributable to the lowest hierarchical level, the level between patches. Only few additional differentiation can be observed at the level of localities (which was traditionally regarded as a "population"), but relevant additional differentiation can be observed between different regions, separated by geographic barriers or distances of more than 50 km. Spatial autocorrelation showed a positive autocorrelation in distance classes up to 100 km and a pattern corresponding to isolation by distance at higher distances. Differentiation between patches within one locality showed no indication for isolation by distance. No within patch structuration could be observed.
We suggest that the population structure of *O. cacaliae* is in agreement with the one expected in a metapopulation: independence of local populations (patches), genetic integrity at the level of the metapopulation (the regions or somewhat larger areas in our hierarchy) ensured by long distance gene flow and a pattern of isolation by distance in a species consisting of several, homogeneously distributed metapopulations.

INTRODUCTION

Most ecological patterns, mechanisms and processes have been found to be scale dependent (Levin, 1992). The genetic population structure of a species is an integral part of its ecology, reflecting the species evolutionary history as well as its life history. There are two contrasting attitudes in interpreting an observed genetic population structure (Avise, 1994, pp 26-34 and references therein; Johannesson and Tatarenkov, 1997). From an adaptionist point of view, assuming persistent populations in equilibrium and selection as the most important driving force of evolution, a subdivided population structure favours local adaptation to environmental differences. The scale at which structuration can be observed thereby determines the scale at which selection is acting and leads to stable polymorphisms. A more stochastic approach views populations as transient, in an intermediate state between colonization and extinction, and often not being in demographic and genetic equilibrium. This "nonequilibrium view of the dynamics of allele frequencies" (Mitter and Futuyma, 1983) is today regarded as more realistic (Nürnberger and Harrison, 1995, Ingvarsson, 1997). Recently developed theory investigates consequences of nonequilibrium-dynamics for the distribution of genetic variance among transient populations (Slatkin, 1977, Whitlock and McCauley, 1990, Gilpin, 1991, Whitlock, 1992b). Hanski and Gilpin (1991) distinguish in their "metapopulation terminology" three scales, a) a local scale at which individuals move and interact routinely with each other, b) a metapopulation scale, at which movement and gene flow between groups takes place, but infrequently and with a high risk of not finding an appropriate habitat, and c) a geographical scale, representing the species entire geographical range.

For phytophagous insects, the patchy distribution of host plants often leads to an effective geographic separation on a very local scale. It has been shown that movement between host plant patches is often restricted (McCauley, 1991). For two willow-feeding leaf beetle species, *Chrysomela aeneicollis* (Rank, 1992) and *Plagiodera versicolora* (McCauley *et al.*, 1988), genetic differentiation has been found already between single host trees. At the macrogeographic scale, we have studied the genetic variability of two *Oreina* leaf beetle species, feeding on perennial herbs (chapter II). We found high genetic differentiation between and among groups of beetles sampled on plant patches at least 10 km apart. Distribution of genetic variance was not related to host plant use, but for one of the species, *Oreina cacaliae*, the observed structure was in agreement with the isolation by distance model (Wright, 1943; Slatkin, 1993). This model predicts that spatially more distant populations are also genetically more differentiated. Such a pattern occurs if in a continuous population limited dispersal leads to gene flow frequencies that are positively correlated with geographic distance. Isolation by distance is reported for many phytophagous insects, however, not necessarily at all geographic scales (review in Peterson, 1996) or in all habitats (Britten *et al.*, 1995).

Oreina cacaliae (Schrk.) (Coleoptera, Chrysomelidae, Chrysomelinae) feeds as larva and adult on herbaceous plants of the genera *Adenostyles*, *Senecio* and *Petasites* (all Asteraceae, Senecioneae) in the palearctic mountains at altitudes between 500m and 3000m. It is viviparous and has overlapping generations, males of the previous generation are observed to mate with newly emerged females. The beetles are patchily

distributed at two levels. First, on a larger landscape scale, they are not found regularly throughout the distributional range of their host plants but very locally on some mountain hillsides and not on others. Second, within one hillside, they are patchily distributed as are their host plants. Patches are separated by unsuitable habitat. The beetles require a certain degree of humidity at their localities, but this dependence cannot fully explain their distribution and it is unclear what other causes may be responsible for this distributional pattern. Beetles found on a mountain hillside (up to 10 km^2) were traditionally regarded as a population. To avoid misinterpretations we will not use the term population and refer instead to such hillsides as localities, in contrast to patches or larger geographic regions. A patch is defined by a continuous plant cover with a more or less continuous beetle density and is clearly distinct from the surrounding habitat. In this study we did not take into account the level of single plants, since plants grow in dense patches very close to each other, their leaves usually intermingle and beetles change frequently between plants (chapter IV). Patch size varies from only one square meter to several hundred m^2 and patches are separated one from each other by at least 25 m of unsuitable habitat. A region corresponds to different mountain ridges (eg the Vosges, Black forest, Pyrennees etc.) and, within the alps, areas separated by natural barriers (eg the Rhone valley) or areas with a sampling gap in between of at least 50 km.

We assumed that the genetic population structure of *O. cacaliae* would reflect this hierarchy of abundance: patch - locality - region - whole species range. In this study we used two methods and a hierarchical sampling design to investigate at which spatial scale genetic structuring can be observed in this species. The genetic variance at different scales can be described and quantified with Wright's F-statistics (Wright, 1951) or with spatial autocorrelation (Sokal, 1978). The former partitions the observed genetic variance within and among previously defined subdivisions. The latter is more independent from the assumed population structure, reporting correlations among groups within the same - a priori defined - distance classes (Slatkin, 1985).

For *O. cacaliae* a wide range of and sometimes very high within-patch fixation indices (F_{IS}-values) have been reported (chapter II, also in Rowell-Rahier, 1992). It was suggested that high fixation indices could be due to within-patch structuring resulting from very low dispersal rates of these beetles (Knoll *et al.*, 1996), which can remain their whole life cycle on the same host plant species. Closely related individuals would then stay close together and mate, such that sampling over one patch would in reality be a sampling over several kin groups. We looked therefore for indications of population structuring among local patches (furtheron referred to as "fine scale population structure"). Additionally, we tested for a possible structuring within one patch.

Materials and methods

Allozyme electrophoresis:

To study the genetic population structure, we used allozyme electrophoresis. We report the allozymatic genotype of individuals as revealed in standard horizontal starch gel electrophoresis (Hillis and Moritz, 1990). Buffers and protocols are described in detail in chapter II. The following nine presumptive loci could be screened for all samples and were used in the analysis of the hierarchical population structure: AO (*Aldehyde-oxidase*), DDH (NADH-*Diaphorase*), FUMH (*Fumarate Hydratase* EC 4.2.1.2.), GAPDH (*Glyceraldehyd-3-phosphatdehydrogenase* EC 1.2.1.12) , AAT (*Aspartate Aminotransferase* EC 2.6.1.1.), IDH (*Isocitrate Dehydrogenase* EC 1.1.1.42 (2loci)), MDHP (*Malate Dehydrogenase* EC 1.1.1.40) and GPI (*Glucose-6-phosphate Isomerase* EC 5.3.1.9.). For the study of fine scale population structure among host plant patches and for the investigation of within patch structuring, we included 7 more loci into the analysis: ACOH (*Aconitase Hydratase* EC4.2.1.3), G3PDH (*Glycerol-3-phosphate Dehydrogenase* EC 1.1.1.8.), ARK (*Arginine Kinase* EC 2.7.3.3.), FDH (*Formaldehyde Dehydrogenase* EC 1.2.1.1.), PEP (LA) (*Peptidase* EC 3.4.-.-.), SOD (*Superoxid Dismutase* EC 1.15.1.1.) and TPI(*Triose-phosphate Isomerase* EC 5.3.1.1.).

Hierarchical population structure

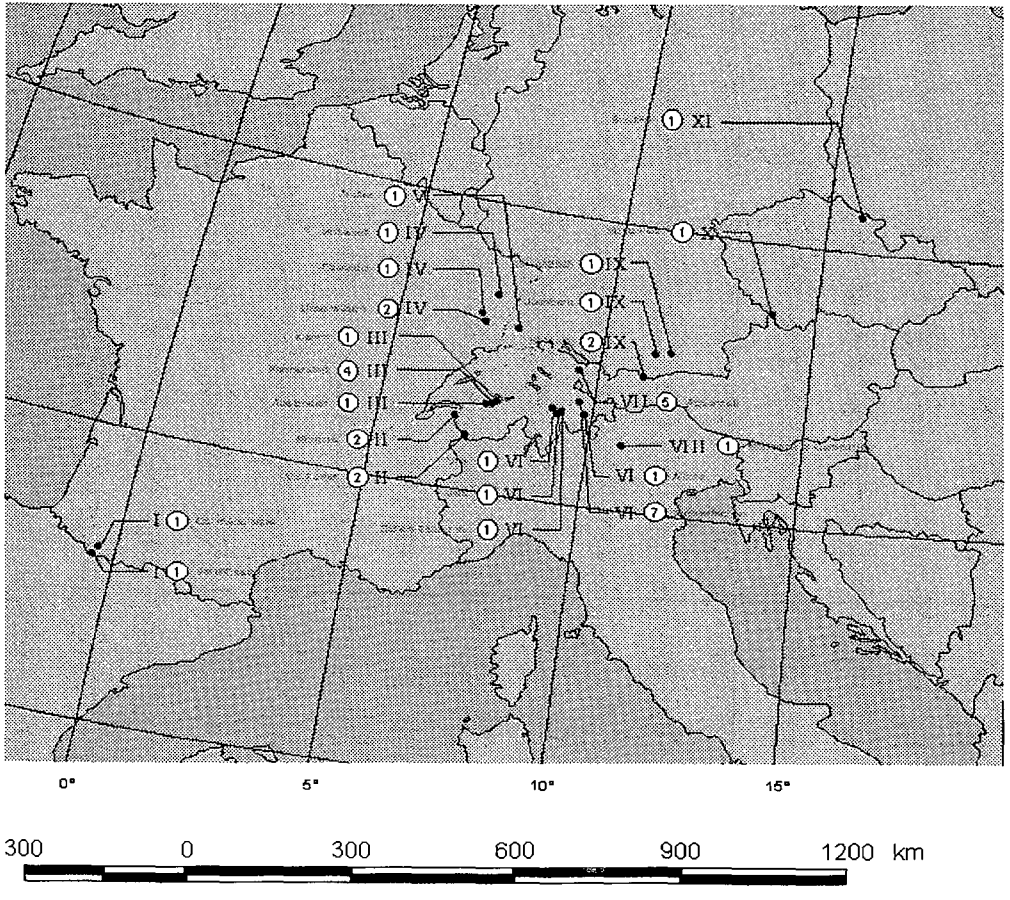
We conducted sampling over the whole western species range, from the Pyrenees to the Czech Republic. Whenever possible we sampled two patches - not further apart than 2.5 km - per locality. At four localities we sampled more than two patches. We grouped the samples according to the folllowing hierarchy: patches within localities, localities within regions, regions within total sampling area. All samples and their place in the hierarchy are illustrated in figure 1.

To partition genetic differentiation among the different hierarchical levels studied, we calculated hierarchical F-statistics as proposed originally by Wright (1951) using the program BIOSYS-1 with the option of hierarchical F-statistics (Swofford and Selander, 1981). Standard deviations were obtained by jackknifing over sampled patches and the probability of a significant differentiation (F-statistics >0) was tested with a one tailed t-test (degrees of freedom = number of patches - 1) (Rank, 1992). Because we have a highly unbalanced sampling scheme, we conducted two separate hierarchical analyses: the first one included only the localities, at which several host plant patches were sampled and only two hierarchical levels. We calculated the differentiation of patches within localities (F_{PL}) and of localities within total sampling area (F_{LT}); from here on we refer to this analysis as the two-level hierarchy. Thereby we assessed the importance of local structuring - the differentiation between host plant patches not further apart than 2.5 km - in comparison to any structuration at

Next page:

Figure 1: Map of *O. cacaliae*-localities. The number of patches sampled at each locality is given inside the circle, the latin numbers show the region, in which this locality was placed in the three level hierarchy (see legend next to map).

Hierarchical sampling regime



Shown are the localities sampled, number of patches sampled at each locality are indicated in the circle, roman numbers indicate the region according to the following list

I	:	**Pyrenees (F)**
II	:	**Western CH**
III	:	**Central CH**
IV	:	**Vosges (F)**
V	:	**Black Forest (D)**
VI	:	**Eastern CH**
VII	:	**Northern CH**
VIII	:	**Trentino (I)**
IX	:	**Bavarian Alps (D)**
X	:	**Bohemia (Cz)**
XI	:	**Riesengebirge (CZ)**

higher hierarchical levels. To assess differentiation at larger scales we conducted an analysis which included all sampled patches and used a three-level hierarchy: patches within localities (expressed by F_{PL}), localities within regions (expressed by F_{LR}) and regions within the total sampling area (expressed by F_{RT}).

Furthermore, we assessed the importance of genetic differentiation at different distance classes with multidimensional spatial autocorrelation (Oden and Sokal, 1986; Sokal *et al.*, 1987). We constructed a Mantel correlogram, using the genetic distance matrix (Rogers genetic distance, Rogers, 1972) and the following distance classes: 0-10km (class1: 41 pairs); 10.1-50km (class2: 49 pairs); 50.1-100km (class 3: 111 pairs); 100.1-150km (class 4: 336 pairs); 150.1-500km (class 5: 152 pairs), >500km (class 6: 120 pairs). The lowest distance class corresponds to all "patches within localities". The second one was chosen, because 30 km was reported as an important distance differentiating two scales in the study of gene flow in *Euphilotes enoptes* and 40-50 km are given for *Tetraopes tetraophthalmus* as the distance, below which gene flow regularly occurs (as in Peterson, 1996). Other distance classes were chosen to assure similar frequencies in all classes. For each distance class, we tested for a possible autocorrelation using the Mantel test and report the Mantel statistics (r) and p values resulting from 10000 permutations. Calculations were done with the program R (Legendre and Vaudor, 1991). The shape of the resulting correlogram gives indications of the underlying population structure (Sokal *et al*, 1989).

Fine scale population structure:

To further assess genetic differentiation among patches within one locality, three localities were sampled in detail. These were "classical" *Oreina* localities, where the beetles were abundant over a rather large area (several km²), including an altitudinal gradient, and were locally subdivided into groups living on host plant patches of differing size. We collected samples of beetles from several host plant patches within one locality. We sampled: at Appenzell 5 host plant patches (within an altitudinal cline of 150 m and an area of ca. 0.5 km²) ; at Tschiertschen 7 host plant patches (within an altitudinal cline of 400 m and an area of ca. 7.5 km²) and at Kandersteg 4 host plant patches (within an altitudinal cline of 250 m and an area of ca. 0.25 km²).

We were interested in the population differentiation at this lowest hierarchical level, the level of "patches within localities". Therefore we calculated separate F-statistics for three localities, using FSTAT (Goudet, 1995) which gives unbiased estimates of F-statistics and has the option to determine significance levels of these estimates in permutation procedures. Gene flow estimates between single patches as well as an overall within-locality estimate were derived from F_{ST}-values according to the formula $Nm=1/4F_{ST} - 0.25$ (Wright, 1951). Only at Tschiertschen we had a sample of more than five patches and were so able to get an independent estimate of overall gene flow (Nm) from the frequencies of private alleles (eg Slatkin and Barton, 1989). Also because of the limited number of patches sampled per locality, Slatkin's test for isolation by distance (Slatkin, 1993) was not possible at this local scale. We tested for a correlation of genetic differentiation (F_{ST}-values) and geographic distance with the Mantel test (eg Manly, 1986), significance was determined in 1000 permutations.

Within patch structuring

At one locality, Lieserwasen in the Vosges, we found a high within patch fixation index of F_{IS}=0.222 (chapter II) for one large patch and we had informations about the dispersal of *O. cacaliae* there (chapter IV). Thus, it seemed an appropriate patch to investigate within patch structuring. However, the pattern of patch distribution at Lieserwasen is somewhat peculiar (detailed description in chapter IV). The beetles were found in one large patch, on their host plants in a stripe (ca. 1.5km long) following both sides of a straight forest road. They did not disperse laterally into the forest. Beginning and end of this patch were clearly marked, although the host plants continued further along the road. Here we could not clearly determine host plant patches, since only few gaps existed in the plant cover which were normally bridged by single plants that were used by the beetles (results of mark and recapture experiments, chapter IV). At the beginning of June, shortly before larviposition, we sampled all 114 beetles present at this locality on their host plants and noted their exact position on a topographic map. From this map, positions were coded as [x,y] data and distances between single beetles could be calculated.

With spatial autocorrelation procedures (Sokal, 1978) we looked for a fine scale spatial structuring as revealed from the genotypes of the single individuals. We coded single locus genotypes for polymorphic loci (95% criterium) as proposed in Berg (1995) and Hossaert-McKey (1996): homozygotes of the most common allele were coded as 1, heterozygotes with the most common allele as 0.5 and homozygotes or heterozygotes of all other alleles as 0. The distance classes were chosen as follows to assure similar frequencies in all classes: <5m (class1), 5.1-20m (class 2), 20.1-50m (class 3), 50.1-100m (class 4), 100.1-200m (class 5), >200m (class 6). We report Moran's I for all distance classes and all 12 polymorphic loci; the probability of a spatial autocorrelation (unilateral test) was calculated for each distance class and a Bonferroni correction, taking into account the dependence of the 6 distance classes, was applied. Calculations were done with the program R (Legendre and Vaudor, 1991).

RESULTS:

We assessed the genetic population structure with allozyme electrophoresis, allele frequencies and sample sizes are given in Appendix D, table 1.

Hierarchical population structure

The two-level hierarchical F-statistics revealed significant differentiation at both levels, among patches within localities as well as among localities within the total sampling area (table 1). However, the differentiation at the lowest level (F_{PL}=0.133) was much larger than at the higher level (F_{LT}=0.032).

Table 1: Hierarchical F-statistics with the two-level hierarchy, including only localities, where at least two patches were sampled. For definitions of the hierarchy levels see text. Difference of the mean estimate from zero was tested with a one tailed t-test, *** indicate p<0.001.

	F_{PL}	F_{LT}
AO-1	0,106	-0,025
DDH1	0,153	0,019
FUM	0,039	-0,014
GAPDH	0,177	0,038
AAT	0,120	0,075
IDH2	0,081	0,011
PEP(LA)1	0,037	0,038
GPI	0,024	0,011
IDH1	0,055	-0,006
across loci	0.133^{***}	0.032^{***}
jackknifing over patches	0.132 ± 0.008	0.033 ± 0.005

Table 3: Hierarchical F-statistics with the three-level hierarchy, including all patches. For definitions of the hierarchy levels see text. Difference of the mean estimate from zero was tested with a one tailed t-test, *** indicate p<0.001; ** indicate p<0.0025.

	F_{PL}	F_{LR}	F_{RT}
AO-1	0,092	-0,016	0,004
DDH1	0,157	-0,001	0,071
FUM	0,043	0,000	-0,011
GAPDH	0,204	0,035	-0,002
AAT	0,144	0,000	0,155
IDH2	0,063	-0,015	0,091
PEP(LA)1	0,051	-0,008	0,016
GPI	0,031	0,001	0,004
IDH1	0,005	0,152	0,336
over all loci	0.144^{***}	0.01^{**}	0.064^{***}
jackknifing over patches	0.147 ± 0.007	0.005 ± 0.010	0.065 ± 0.005

Table 2: F-statistics at the local level; at Appenzell, Kandersteg and Tschiertschen. Difference of the mean estimate from zero was tested with permutation procedures, for F_{IS} permutting alleles within samples for F_{ST} permutting genotypes within total and for F_{IT} permutting alleles within total (Goudet, 1995). *** indicate p<0.001. .

	Appenzell			Kandersteg			Tschiertschen		
	F_{IS}	F_{ST}	F_{IT}	F_{IS}	F_{ST}	F_{IT}	F_{IS}	F_{ST}	F_{IT}
ACONH	-0,069	0,000	-0,069	0,692	0,081	0,665	0,192	0,053	0,147
G3PDH	0,381	0,146	0,274	0,229	0,068	0,173	0,078	0,077	0,000
AO-1	-0,019	0,001	-0,02	0,413	0,012	0,406	0,517	0,116	0,453
ARK3							1,000	-0,024	1,000
DDH1	0,317	0,045	0,285	0,429	0,216	0,272	0,090	0,040	0,052
FDH	0,419	-0,029	0,436	0,067	0,029	0,039	0,168	0,008	0,161
FUMH	0,009	0,058	-0,052	0,327	0,020	0,313	0,426	0,055	0,393
GAPDH	0,388	0,089	0,328	0,350	0,111	0,269	0,347	0,155	0,227
AAT	0,365	0,093	0,301	-0,005	0,006	-0,011	0,066	0,024	0,043
IDH2				1,000	0,133	1	0,665	-0,014	0,670
PEP(LA)1	0,207	0,042	0,172	0,226	0,014	0,215	0,207	0,045	0,170
MDHP	0,258	0,077	0,196	0,367	0,302	0,094	0,114	0,063	0,054
GPI	0,006	0,050	-0,047	-0,011	0,030	-0,043	0,277	-0,005	0,281
SOD2	0,250	0,105	0,162	0,303	0,110	0,216	0,259	0,065	0,207
TPI	-0,045	0,008	-0,053	0,418	0,184	0,287	0,208	0,096	0,124
IDH1				1,000	0,079	1			
over all loci	0.269***	0.061***	0.222***	0.328***	0.110***	0.246***	0.206***	0.066***	0.150***
jackkmifing over loci	0.274±0.051	0.062±0.020	0.226±0.051	0.327±0.063	0.110±0.030	0.244±0.065	0.205±0.044	0.066±0.016	0.148±0.037

The three-level hierarchy also showed that most differentiation occurs at the level of patches within localities (F_{PL}=0.144, table 3). The remainder was mainly due to a differentiation between regions (F_{RT}=0.064). The differentiation among localities within one region was very low (F_{LR}=0.010), but still significantly different from 0 when jackknifing over patches (table 3). Thus very high levels of small scale differentiation were found between groups of beetles not further apart than 3km, whilst there was almost no differentiation at the intermediate scale (comprising distances of 10-70 km) and a significant, intermediate differentiation at the macrogeographic scale (distances of more than 50 km).

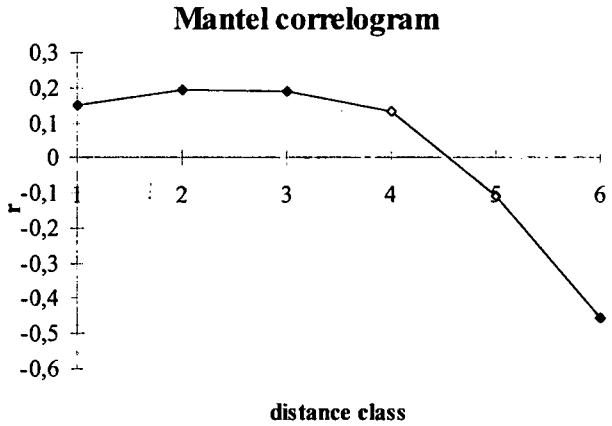


Figure 2: Mantel correlogram, showing spatial autocorrelation of *O. cacaliae* patches at different geographic distances: 0-10km (class1: 41 pairs); 10.1-50km (class2: 49 pairs); 50.1-100km (class 3: 111 pairs); 100.1-150km (class 4: 336 pairs); 150.1-500km (class 5: 152 pairs), >500km (class 6: 120 pairs), caclulated from the Rogers genetic distance matrix. Significance values were obtained in 10000 permutations, significant spatial autocorrelations are characterized by closed circles ($p<0.05$ after applying Bonferroni correction for six distance classes).

The same pattern appears in the results of the spatial autocorrelation (figure 2), although the distances at which "scales" change are larger than the ones of our predefined sampling hierarchy. The Mantel correlogram shows a positive spatial autocorrelation (as seen by a significant positive r) at the small distance classes up to 100 km, no significant autocorrelation in the intermediate distance classes from 100-500km and a negative spatial autocorrelation in the highest distance class >500km. Moreover, the Mantel statistics take very similar values in the three classes showing positive autocorrelation; if at all, the smallest distance class of <5km shows a lower value than the two following distance classes. The smallest distance class corresponds exactly to the hierarchy level "patches within a locality". In the following distance classes, pairs of localities in the same region and pairs of localities in different regions are found, because some of our arbitrary defined regions are not further apart than 50 km (eg Appenzell and Tschiertschen)

Fine scale population structure:

At all three localities, where more than two patches were sampled, a significant differentiation between host plant patches is observed (table 2). All three F_{ST}-values, however, were lower than the F_{PL}-values from the hierarchical analyses. At none of the localities we found a significant correlation between geographic distance and genetic differentiation (Mantel test: Tschiertschen r=-0.13, p=0.39; Kandersteg r=-0.74, p=0.07; Appenzell: r=0.70, p=0.07).

Gene flow estimates between individual patches, calculated from F_{ST}-values, are always larger than Nm=1, thereby indicating gene flow acting as a homogenizing force (Slatkin, 1985). Estimates range from Nm=1.1 to Nm=10.2 (table 4; excluding one comparison at Appenzell, where F_{ST}=0 and Nm therefore not defined) with local overall estimates of Nm=3.8 at Appenzell, Nm=2.0 at Kandersteg and Nm=3.5 at Tschiertschen. The estimation of Nm from the frequencies of private alleles at Tschiertschen gave a value of 2.6.

Within patch structuring

We could not find any indication for within patch structuring at the one patch tested, Lieserwasen. In none of the six distance classes did we find significant spatial autocorrelation for any of the 12 loci (figure 3).

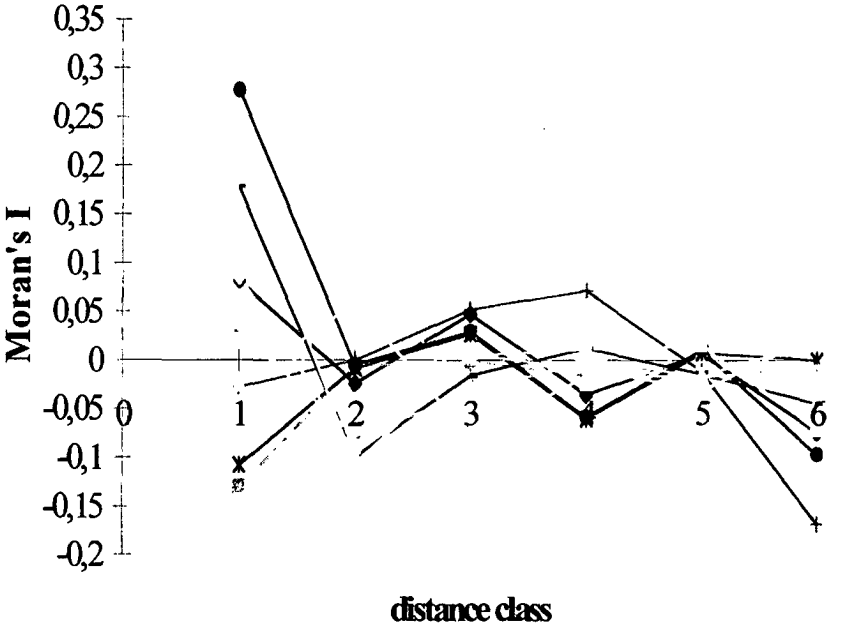


Figure 3: Within-patch spatial autocorrelation at Lieserwasen for 12 polymorphic loci. None of the Moran's I is significantly different from 0 (all $p>0.05$ after applying Bonferroni correction for six distance classes). Distance classes are as follows: <5m (class1), 5.1-20m (class 2), 20.1-50m (class 3), 50.1-100m (class 4), 100.1-200m (class 5), >200m (class 6).

DISCUSSION:

A hierarchical analysis of the genetic structure of *O. cacaliae* revealed most differentiation occurring among single patches and a very low differentiation at the intermediate scale, the level of localities within regions. Among the other studies of the population structure of leaf beetles, a similar pattern has been reported for *Plagiodera versicolora* (large small-scale differentiation and "surprisingly little differentiation at the scale of geographic regions" (McCauley *et al.*, 1988)), but not for *Chrysomela aeneicollis* (Rank, 1992), where high genetic differentiation was found at all levels and selection was invoked as an explanation for the observed patterns.
At the highest level investigated in this study of *O. cacaliae*, we found that there is a significant, though moderate additional differentiation between different regions, which is in agreement with the isolation by distance structure reported for *O. cacaliae* (chapter II). The shape of the Mantel correlogram corresponds very well to the pattern of the hierarchical F-statistics. However, the scale of the pattern seen in the Mantel correlogram is larger than the one of our pre-defined levels for the hierarchical F-statistics. Homogeneity within distance classes is seen up to distances of 100 km (the "plateau" of this Mantel correlogram) and a negative slope, crossing the ordinate and indicating isolation by distance (Sokal *et al*, 1989), is seen only at distances larger than 100 km (figure 2), with a significant negative spatial autocorrelation at distances over 500km. A very similar pattern was reported for Australian populations of the cactophilic *Drosophila buzzatii*: positive autocorrelation of similar magnitude in the lower distance classes (here up to 600 km) and then a continuous drop to negative correlations (Sokal *et al.*, 1987). Genetic drift and selection against a single environmental gradient was rejected as an explanation for this pattern and it was proposed that selection operating at different spatial scales is responsible for this population structure. The differing values of F_{ST}, we found at different loci, is also usually interpreted as an indication for selection. But as Sokal *et al.* (1987) for *D. buzzattii*, we cannot find a correlation between any environmental feature and one or more loci (chapter II), which would be the result of selection against an environmental gradient (Endler, 1977). We have no data to assess the importance of selection in our system and hesitate to invoke vague differential microhabitat selection working at such a small scale as studied here.
The close to zero differentiation between localities within regions indicates high levels of gene flow at the regional scale (between patches within one region). Similarly the "plateau" of the Mantel correlogram indicates a homogeneous population structure and thereby genetic integrity at distances up to 100 km. At the local scale, we find significant genetic differentiation between host plant patches not further apart than 2.5 km. Resulting gene flow estimates larger than 1 indicate ongoing or recent gene flow with a homogenizing effect on the population structure (Slatkin, 1985). Gene flow is obviously not restricted to neighbouring patches as shown by the lack of a correlation between geographical distance and degree of genetic differentiation (F_{ST}) at the local level. Indeed we do not have any indications for increased gene flow at the local level, between patches within one locality, in comparison to the next higher studied level, localities within regions. Patches from different localities are in no way more differentiated than the ones within one locality - resulting in similar gene flow estimates. Thus it seems that the level that was traditionally referred to as an *Oreina*-"population" has not much

significance for the population structure of *O. cacaliae*.
Long distance gene flow (up to 100 km) therefore seems to be the dominating form of gene flow in the system (not taking into account dynamics and matings within one patch). Modelling the consequences of long distance gene flow, Nicholson and Hewitt (1994) predict a "broad patchy mixing" of assorted genotypes, as we observe it in *O. cacaliae*. Their model was developped in the context of range expansion and introgression into previously uncolonized areas (as happening in the hybrid zones of *Chorthippus parallelus*). But it might be equally attributable to any colonization of vacant habitat. Vacant habitat in our system are uncolonized, but suitable plant patches. Until now, *Oreina* species were thought to have limited dispersal abilities and to be very sedentary (Eggenberger and Rowell-Rahier, 1991; Knoll *et al.*, 1996). The high genetic differentiation reported in macrogeographic studies and mark-recapture experiments supported this view (Rowell-Rahier, 1992; Eggenberger and Rowell-Rahier, 1991). Mark-recapture studies show high recapture rates and low movement-rates of the beetles and no movement between neighbouring host plant patches over one season (chapter V). Of course it is a known, common flaw of mark and recapture studies, that they are not able to detect long distance dispersal (Slatkin, 1985). *O. cacaliae* can fly and beetles were seen flying in masses at the end and at the beginning of the season, after their host plant withered in the autumn and before the host plant patches are freed from snow and plants emerge in spring (Dobler, S.; Conconi, D.; Kalberer, N., all pers. communication). The beetles' overwintering places are not known for sure, but they are observed emerging from under the bark of trees or old wood at their host plant patches (pers. observation) when host plants are already available. They overwinter in the soil if kept caged with their host plant in pots or if kept in the laboratory (Kippenberg, pers. communication and pers. observation). At one locality beetles were observed in autumn flying to a large cliff 200 m away from the nearest host-plant patch, and in spring they started flying from there when the host-plant patches were still covered by snow (N. Kalberer, pers. communication). One can hypothesize about a kind of dispersal polymorphism, where beetles which are not in diapause at a time when their host plants are not available, undertake long distance dispersal in search of other host-plant patches in a more favourable state. An induction of long distance dispersal is reported for other species, when they find themselves in a hostile environment (Coyne *et al.*). However, beetles which enter diapause before all host plants are withered and emerge from diapause at a time when host plants at their patch are already available have no need to disperse and may stay where they are. Thus we see *O. cacaliae* as both, sedentary and not limited in its dispersal ability.
Dispersal polymorphism have been demonstrated for many species and might be a common feature in insects (Denno, 1993). For several insect species trade-offs were demonstrated between flight capability and reproductive success (review in Zera and Denno, 1997). For *O. cacaliae* we can speculate about a trade-off between high fertility, when staying sedentary at the patch they are born in, and the risk of not finding a patch at all, when they stay at a place where there are - maybe only momentarily - no host plants. So far, consequences of dispersal polymorphism on population structure are rarely studied at the intraspecific level (but see Olivieri *et al.*, 1990, Peterson and Denno, 1997 and references therein)
The beetles' movement during the season is restricted to walking, they are very rarely

seen flying as long as their host plants are available. However, some individuals are found to disperse across a whole plant patch (chapter IV) and this might explain, why we have not found any within-patch structuring. This would have been expected, if we were sampling over family groups, as was stated in an earlier hypothesis (chapter II, Knoll *et al*, 1996). We sampled the beetles to test for within-patch structuring at a time, when all individuals should have dispersed maximally, shortly before larviposition. At the patch level the sometimes high fixation indices (chapter II) therefore can not be explained by a limited within-patch dispersal of the beetles, leading to random kin mating. In plants a spatial substructuring within populations is reported for several species (eg *Lathyrus sylvestris*, Hossaert-McKey *et al*, 1996; review in Heywood, 1991). We investigated in this study only spatial structuring. In mobile animals, social population structuring resulting in a genetic structure might go unnoticed. However, we cannot think of any reason for deliberate inbreeding - searching actively relatives for mating in a randomly dispersed group - and also the great heterogeneity of F_{IS}-values argues against an inbreeding mating system (Giles and Goudet, 1997). However, we may find an explanation for the high within patch fixation indices in the context of the distribution of genetic variance in a metapopulation.

We suggest that the observed genetic population structure of *O. cacaliae* reflects the one of a metapopulation with the three scales as defined by Hanski and Gilpin (1991). The level of patches is the local scale, "at which individuals move and interact with each other in the course of their routine feeding and breeding activities". The level of regions would be the metapopulation scale "at which individuals infrequently move from one place to another, [....] often with substantial risk of failing [...]". We argue, that at the (macro)geographical scale - the "species entire geographic range; the individuals have typically no possibility of moving to most parts of the range" - limited dispersal between metapopulations in equilibrium leads to the observed isolation by distance structure.

In metapopulation models, subpopulations are random and small subsamples, taken at different times from the whole metapopulation. Direct consequences of this sampling are an age structure within the system - with a gradient from young to old patches - and nonequilibrium dynamics in many patches.

The distribution of genetic variance within and between nonequilibrium populations was investigated with the following conclusions. Subpopulations from different age classes have different levels of within population differentiation (F_{IS}), due to the relative influences of founder events and continued migration between established subpopulations (Whitlock and McCauley, 1990; Whitlock, 1992b). Whether differentiation among subpopulations is increased or decreased compared to the level predicted from equilibrium conditions, depends further on the degree of relatedness of founding individuals; kin-structured migration leads to high population differentiation. The degree of differentiation is, of course, also dependent on population sizes in non-equilibrium subpopulations. Variance in population sizes further increases genetic variance among subpopulations (Whitlock, 1992a)

Thus a metapopulation-explanation of the population structure of *O. cacaliae* is in accordance with the lack of within-patch structuration, with the often high, but very variable within-patch fixation indices as well as with the sometimes high, but very variable differentiation between patches. Different F-values simply could be related to different ages of patches (in dependence of their population size). We have no

informations about the relatedness of dispersing individuals. However, since females are always mated before winter (Dobler and Rowell-Rahier, 1996) and consequently before the assumed dispersal period in autumn or spring, kin structured dispersal is highly possible for this species.
Nevertheless, there is a problem when *O. cacaliae* groups on single host-plant patches are seen as the "subpopulations" of a metapopulation. The term "metapopulation" implies extinction and colonization events. In consequence all studies testing the theory of the distribution of genetic variance within and among nonequilibrium populations were done with typical colonizing species (eg. in insects with mycophagous beetles (*Bolitotherus cornutus*, Whitlock, 1992; *Phalacrus substriatus*, Ingvarsson, 1997), in plants with *Silene* (*S. dioica*, Giles and Goudet, 1997; *S. alba*, McCauley *et al.*, 1995).
In 14 years studying *Oreina* and visiting at least some localities frequently (eg Lieserwasen, Appenzell, Tschiertschen), we observed only one patch extinction when it was totally destroyed by a newly built road (chapter IV). Otherwise patches are very persistent and beetles are predictably found on some of them, on others not. This is probably not due to an unsuitability of uninhabited patches themselves, as suggested by an experiment, whereby 30 individuals were transplanted to a non-used patch and beetles have been found on this patch ever since (Rowell-Rahier, pers. observation). However, the suitability of "empty patches" has never been formally tested. We expect some extinction events to occur due to the disappearance of the host plants - herbs as host plants are not the successional most permanent resource and in the mountains landslides destroying whole hillsides are common. However, these possible host-plant patch extinctions are by no means "frequent" and can not answer the question, why beetles on so many host-plant patches do not reach equilibrium conditions. Possible factors delaying equilibrium are a variance in reproductive success, which might have the same effect as frequent bottlenecks or kin group colonization (discussed in Whitlock and McCauley, 1990) and inbreeding effects, further increased by overlapping generations.
In conclusion, at different spatial scales we found marked differences in the genetic structure. We argue that the observed genetic structure - no spatial within patch structure, high genetic differentiation among patches and genetic integrity at the higher level of regions - indicates a metapopulation structure. Furthermore, subpopulations (patches) of one metapopulation inhabit an area with a diameter of about 100 km and rare long distance dispersal is the dominating form of gene flow between subpopulations. We believe that metapopulation models and theory are necessary to understand the dynamics of *O. cacaliae* population biology, even though extinction and colonization events are probably not frequent in this species.

Acknowledgements:

We wish to thank P. Ballabeni, B. Benrey and J. Goudet for helpful comments on the manuscript. H. Kippenberg kindly indicated *Oreina* sites. R. and A. Cheyne provided the sample from Madonna di Campiglio, J. and W. Knoll helped sampling in the Czech Republic and in the Bavarian Alps. This work was supported by the Swiss National Science Foundation (grant no. 31-33669.92).

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CHAPTER IV

WITHIN PATCH POPULATION SIZES AND MOVEMENT PATTERNS OF AN ALPINE LEAF BEETLE

Manuscript

With mark and recapture experiments we studied within patch population sizes, persistence rates and movement rates of *O. cacaliae* (Coleoptera, Chrysomelidae). We give estimations of population sizes and densities according to two models, one assuming an open population (Jolly-Seber model) and one assuming a closed population. We found only very little movement out of the study area and very high persistence rates. We therefore propose, that a patch represents an independent unit and that a closed population model is more appropriate for estimating population densities. For the two patches, where within patch movement was studied, we found it was low (with a mean of distance moved between first and last capture of 4.7m and 4.1m), but some beetles traversed the whole patch. For a subsample we studied differences between the sexes and found that females move faster and further than males. This is mainly attributable to one peak of movement at the time of larviposition.

INTRODUCTION

The distribution of herbivorous insects on a set of host plants might reflect the spatial dispersion of the host plant population (Kareiva, 1982, Stanton, 1982). The extent to which structuration of the host plant population causes structuration of the population of insects depends mainly on their movement strategies and their dispersal ability (Bach, 1980). Movement therefore is an important constraint in the organisation of plant herbivore systems (Kareiva, 1982). If movement is limited and locally independent subpopulations exist, the evolution in such a system will be mainly determined by the internal population dynamics of these subpopulations and the dispersal between them (Hansson, 1991; Hanski, 1991).

Mark and recapture experiments are the most common approach used to assess population parameters and movement strategies. Population parameters of interest are the size of the population, birth and death rates and immigration and emigration events. But although the question "how many are where" is maybe the oldest question in ecology, their are still no simple answers to it. A range of sophisticated statistical analyses have been developed to estimate population parameters from mark and recapture data (review in Seber, 1982; Lebreton, 1992), but they all depend - more or less - on often unrealistic assumptions. Furthermore, it is not possible to disentangle the effect of birth/immigration and death/emigration solely from mark and recapture data. Movement within a locally restricted study area can be often determined with reasonable accuracy. With increasing area, however, accuracy declines rapidly and for insects it is practically impossible to observe long distance movements.

Oreina cacaliae, an alpine leaf beetle (Coleoptera, Chrysomelidae) feeds and lives in all of its life stages on mainly two host plants, *Adenostyles alliariae* and *Senecio nemorensis-fuchsii*. *Petasites albus* is also a frequently used food plant for adult beetles, but it is of a lower quality as larval food plant (Dobler and Rowell-Rahier, 1996) and in the field only few larvae are seen on *P. albus*. Since *P. albus* is the first of the three host plants to emerge in early spring when adult beetles are seen in high abundance on this plants (Rowell-Rahier, pers. observation), we hyposethized, that *P. albus* is used as a transient food plant in times of food shortage, whereas later on beetles move to neighbouring *Senecio* or *Adenostyles* patches. All three plants are perennial herbs, belonging to the Asteraceae, tribe Senecioneae, and often occur sympatrically in mixed patches. They all grow in dense patches of varying sizes (from several square meters up to 2 square km), normally surrounded by forests, bare ground or, above the timber line, alpine meadows. *O. cacaliae* occurs on some of these patches in high numbers, but while its host plants frequently disperse into the forest, *O. cacaliae* is found only in clearings. The beetles, whose movement is restricted to walking during the season (Knoll and Rowell-Rahier, personal observation), can often change host plants without touching the ground, since leaves of one plant are regularly overlapping with the leaves of neighbouring plants. This is especially true for *A. alliariae*, whose broad leaves (ca 50 cm^2) form a platform about 70 cm above the ground. But also *S. nemorensis-fuchsii*- and *P. albus* plants often intermingle.

In this paper we report the variation in abundance and movement of adult *O. cacaliae* during one season. We studied six patches, which differ in their dominating host plant,

their surrounding vegetation and in altitude and are situated in different geographic localities, comprising different mountain ridges. We were interested in the population sizes of and densities at these patches as a basic characteristic of the populations of *O. cacaliae*. Furthermore, we report the residence time of the beetles within the patch as an indicator of patch persistence. We were especially interested in the extent of between patch movement. For two patches, one dominated by *S. nemorensis-fuchsii*, the other by *A. alliariae*, we investigated the movement patterns of individuals within these patches.

METHODS AND MATERIAL

Mark-recapture:

We estimated population size and movement of *O. cacaliae* with mark recapture experiments at three sites in a total of six patches (figure 1, table 1, and description of study sites below). These aposematic beetles are very conspicuous when they sit on their host-plants. They don't move much and don't show escape behaviour. Thus they can be easily picked by hand for marking. Beetles were marked individually with TippEx. We used a seven-point code (3 points on each elytra and one at the pronotum) and different colour each date for newly captured beetles.

Table 1: Mark and recapture censuses and ecological characteristic of the study patches. Given are the number of censuses taken and in parenthesis the number of censuses used for the estimation of the population size according to the model of an open and of a closed population respectively. Beetles marked refers to the total number of beetles marked at this patch and sightings are the total number of beetle sightings.

patch	Censuses	beetles marked	sightings	altitude	dominating plant	surrounding vegetation
Lieserwasen	18 (9,9)	584	1498	850	*S. nemorensis-fuchsii*	broadleaved forest
Appenzell	11 (7,5)	317	532	1300	*A. alliariae*	coniferous forest
TS1	9 (8,4)	366	478	1580	*P. albus*	mixed, open forest
TS2	13 (10,3)	172	451	1600	*S. nemorensis-fuchsii/ A. alliariae*	mixed, open forest
TS3	10 (10,3)	1143	2447	1680	*A. alliariae*	mixed, open forest
TS4	13 (12,5)	1012	1860	1760	*A. alliariae*	mixed, open forest

Beetles were carefully taken from their host plants, marked and immediately returned to the same leaf from which they had been removed. Handling induced an escape response

Next page:
Figure 1: Map of the three sites, where *O. cacaliae* patches were studied.

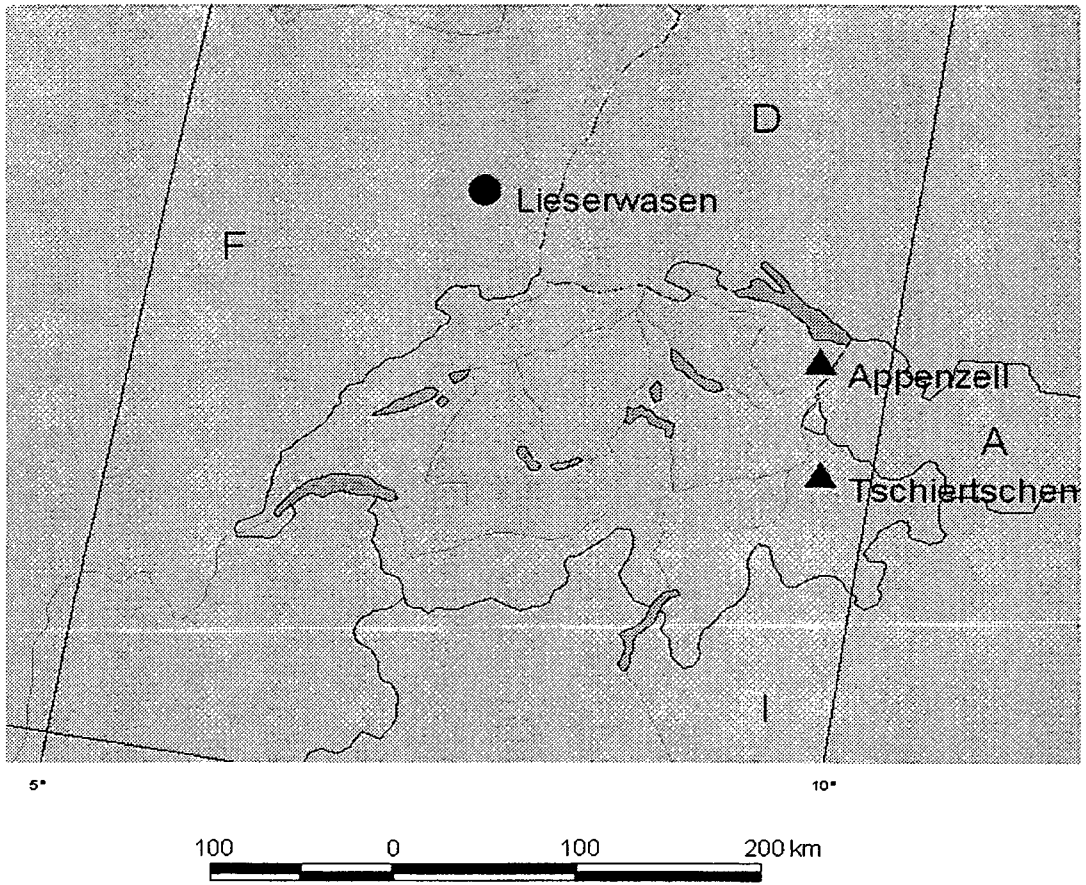


Figure1

● *O. cacaliae*

▲ *O. cacaliae*, *O. speciosissima*

in the beetles. If placed on the ground, they started walking for tenth of minutes in a straight line without taking notice of any host-plants in their way. Placed on their host-plants, however, they circled for two to three minutes, sometimes left the leaf they were placed at (especially on *S. nemorensis-fuchsii*), but rarely the plant (Knoll, pers. observation). Censuring at subsequent occasions could be mostly done without handling the beetles or the plants, except in some doubtful cases, when the beetle was partly hiding under neighbouring leafs or when mark loss was suspected. There was some loss of markings, but because of our seven point system we had in most of the cases the possibility to identify the beetle without doubt. If there was ambiguity, identity was assumed to be the one individual, which was at the least distance at the last encounter. At two patches, Lieserwasen and Appenzell, the plants where the beetles were found were individually numbered with a plastic band. Three times in the season, positions of all marked plants were noted on a map and later coded as [x,y] data. *Oreina* beetles are difficult to sex unambiguously without dissecting them, but sex was marked whenever possible (pairs, gravid females). However, only at one site, Lieserwasen, where we have information from dissections, sex of a larger number of beetles is known. In newly emerged beetles the elytra do not harden fully for about 48 hours and such individuals were recorded as "soft". At the four patches, where we did not map plants, we nevertheless always noted if two or more beetles were found on the same leaf (*Adenostyles* and *Petasites*) or on the same plant (*Senecio*). *Adenostyles* and *Petasites* form clones and it is not possible to recognise individuals within a patch without digging up the rhizomes.

Study sites:

In the summer of 1993 mark and recapture studies were conducted at three different sites (figure 1) in a total of six patches (table 1). At one of these sites we studied four patches at differing altitudes. We visited the sites regularly from early May, when beetles emerged from diapause, until beetles disappeared end of September. Visiting the sites in October confirmed the end of the season, no more beetles were seen then. Censuring was done only in good weather conditions - warm (>20°C) and not raining - when the beetles were active on their host plants. We always did our marking at the time of peak activity of the beetles. This was mostly between 10 am and 7 pm, except at some very hot days in July/August, when beetles were active already in the early morning hours and until late at night, but rested from ca 11am to 4pm.
At **Lieserwasen** in the Vosges (elevation 850m) we studied a patch dominated by *S. nemorensis-fuchsii*. The plants grow in a stripe 10 cm to 1 m wide along both sides of a forest road. Occasionally two other host plants of the beetles were also found, *P. albus*, growing in small patches of ca 1 m^2 and *A. alliariae*, seen as single plants along the road. In 14 years of visiting this patch, beetles have been found persistently only at a certain section along this road (ca 1.5 km, total patch area was estimated as 1500 m^2), although their host plants continue before and after this section. Additionally, there are two small (ca 15 m^2) patches dominated by *A. alliariae* nearby, ca 10 m distant from the road in the forest, where *O. cacaliae* occurs, too. Otherwise no *O. cacaliae* were found in a diameter of 2.5 km. In censuses we walked along the road and noted the position of each host plant, where a beetle was found. We also searched into the forest and especially at

the two *A. alliariae* patches for marked beetles. At June 7th we took all beetles seen at this date to the laboratory for another, genetic study (chapter III). This allowed us to sex these individuals by dissecting them and consequently this is the only patch where we have enough data to investigate possible sexual differences in movement patterns of this subgroup. Although the patch was censured further, all analyses (population size, persistence rate etc.) are done with only the censuses up to June 7th.

At **Appenzell** (above Sämtisersee, elevation 1300m), we studied a 300 m^2 patch, dominated by *A. alliariae*, in the lower section intermingled with *P. albus* and *S. nemorensis-fuchsii* plants. The patch consisted of a clearing in a coniferous forest. The host plants continued as understorey into the forest, but the beetles were abundant only at the clearing. However, the next patch of *O. cacaliae* was only 50 m below. At this site beetle patches are common over an area of ca. 0.5km^2 at clearings in the forest or at the edge of this forest, wherein *A. alliariae* grows as an almost continuous understorey. Apart from this forest, beetles are also found just at the other hillside, ca 500m away. In our censuses we always searched very carefully a diameter of about 20m outside the study patch and the wider surroundings. Once a month we spent an extra day at the site to search in the whole area for marked beetles outside the study patch.

At **Tschiertschen** (near Chur, Graubünden) we studied four patches to which we refer in the following as TS1 to TS4. They were located in a transect line at different altitudes. In Tschiertschen, beetles are abundant in separate patches over an area of about 8km^2. All three host plants are found in the area, however, most patches are dominated by *A. alliariae*. The censuring of the four patches was always done the same day; the order of censuses was changed randomly. About every three weeks we spent one or two extra days at the site to search intensively for marked beetles in the whole area.
TS1: This 80 m^2 patch is found at the lower end of the distribution of *O. cacaliae* at this site, at 1580 m. It is dominated by *P. albus* with a row of *S. nemorensis-fuchsii* at one side. This patch was nearly totally destroyed in the middle of the season (census at the 6th August) because a road was constructed and no more beetles were found at this site later.
TS2: This 100 m^2 patch is 300m away from TS1, at an elevation of 1600 m. It is the only patch studied at Tschiertschen not continuously covered by host plants. *A. alliariae* and *S. nemorensis-fuchsii* occur in approximately equal proportions with frequent gaps of ca 1m - 2m between small groups or single plants.
TS3: This 150 m^2 patch is found ca 500m away from TS2, at an elevation of 1680 m. It is dominated by *A. alliariae*, intermingled with few *S. nemorensis-fuchsii*. This patch is bordering on a patch of *Petasites paradoxus* (recorded as another food plant for *O. cacaliae*), however, beetles have been never found feeding on this plant.
TS4: This 500 m^2 patch is about 600m away from TS3, just below the timber line at 1760 m. Again, it is dominated by *A. alliariae*, intermingled with few *S. nemorensis-fuchsii*.
There was no other patch of host plants between TS1 and TS2, or between TS2 and TS3, but between TS3 and TS4 there were three other host plant patches with *O. cacaliae*.

Analyses:

Since we always sampled only the beetles sitting on their host plant, sampling intensities can give an indication as to what part of the population their number corresponds. The sampling intensity at a census is defined as the proportion of marked beetles seen at this census in comparison to the number of marked beetle around and seen either at this census or at some time later (as in Lederhouse, 1983) and was calculated for all censuses.

Models for the estimation of population sizes from mark and recapture data can be subdivided into two classes according to whether they assume a closed or an open population. In a closed population, population size doesn't change within the study period and there is no immigration/births or emigration/deaths. Since this is normally unrealistic, the a priori choice for calculating population size was the Jolly-Seber model (Seber, 1982) for open populations. It estimates not only population size (N), but gives also estimates of the residence rate (□) (according to Lawrence a better term for Jolly's survival rate (Jolly, 1965)) and the number of immigrants (B) between censuses. Calculations were done using JOLLY (Pollack *et al.*, 1990) according to the classical Jolly-Seber model. Residence time (t) was calculated according to the formula given in Lawrence (1988): $t=-\ln(\phi)^{-1}$.

However, since we had sometimes very low sampling intensities, high residence times and could observe no emigration (see results), we decided to estimate population size also according to a closed population model. We included in the data set only censuses done before the emergence of adults from the following generation, thereby we are sure to have no birth events (birth into the adult population is equivalent to the emergence of the following generation). According to Seber (1982), natural mortality does not bias the estimators as long as it occurs equally in the marked and in the unmarked part of the population. Calculations were done with the program CAPTURE (Otis *et al.*, 1978). We chose to give estimations of population size according to the model M_t of Otis *et al.*, which assumes equal capture probabilities for all beetles, but allows capture probability to vary with time for two reasons: first, this was the model which had lowest standard errors in estimates for most patches (but see Appendix E) and, second, we are convinced that in our patches capture probability varies with time. Since the activity of our beetles does depend on temperature (Knoll, pers observation), also capture probability will depend on and be affected by temperature, even though censuses were only done in favourable conditions.

At two patches, Lieserwasen and Appenzell, where we had individual plant positions, we calculated the distance moved (d_i, notation follows Scott (1973)) between sightings from [x,y] data as euklidean distances. Therefrom we obtained the distance moved per day (or the velocity between different censuses, v_i). Furthermore we calculated the total distance moved (D) for each beetle as the sum of all d_i and the range (R) of one beetles movement as the distance between its first and its last capture. In Lieserwasen the calculation of euklidean distances probably leads to a strong underestimation of longer distances moved, since we expect the beetles not to traverse through the forest but to follow the distribution of their host plants along the road, which has one major curve, changing direction at about 45° (figure 2).

With information about movement rates and density within a population, one can

estimate the size of the effective neighbourhood N (Wright, 1946), defined as a group of individuals within a continuous population, which regularly meet and might interbreed ("their gametes might come together", Wright, 1946). In Lieserwasen, where the habitat was consisted of one long stripe and thus had an essentially one dimensional distribution, we used the formula given for linear continuity: $N = 2\pi\sigma_d d_p$, whereby σ_d is the standard deviation of the dispersal function of the parents and d_p is the density of the parents. For habitat, we used the formula for area continuity: $N = 4\pi\sigma_d^2 d_p$. Both formulas are strictly valid only for an ideal population, where the dispersal distribution follows a normal distribution.

For an estimation of the neighbourhood size, we calculated σ_d as the standard deviation of D, the total distance moved by one beetle, and d_p was calculated from the Jolly-Seber population size estimate.

RESULTS

Abundance

The number of censuses, the total number of beetles marked and the number of sightings are given in table 1, the date of the censuses and the numbers of beetles found at the different patches are given in figure 2. Overall mean sampling intensity was 0.54, indicating that each time we sampled about half of the population. The variance in sampling intensity is related to climatic conditions; in June, which was cold and rainy in 1993, sampling intensities are generally lower than in May or July/August (Appendix E). Freshly eclosed adult beetles could be observed from mid June (Lieserwasen) until mid August (TS3 and TS4) (figure 2 and Appendix E). Altitude was obviously a factor influencing the phenology of the beetles, as can be seen at the four patches in Tschiertschen (figure 2). The lowest patch there, TS1, differs markedly in the pattern of abundance from all other studied patches, presenting peak abundance in early spring (217 beetles seen on plants in the patch), which drop soon to a constant, much lower level (around 35 beetles; figure 2). The beetles marked in this patch in the first census could not be found again, despite the effort in searching for them. At Lieserwasen, five of the beetles marked in 1993 were found again in 1994; however, over the winter they had lost all marks except one point and were not identifiable.

Table 2: Population estimates for different patches of *O. cacaliae* according to the Jolly-Seber model and assuming a closed population . Densities (beetles per m^2) are calculated by dividing the estimated population size by the patch area. Residence time t is calculated from ϕ.

	open population - Jolly Seber model				closed population model M_t	
Patch	**N±se**	**density/m²**	**ϕ±se**	**t**	**N±se**	**density/m²**
Lieserwasen	263±9	0,18	0.96±0.01	24,07	451±4	0,30
Appenzell	119±9	0,40	0.96±0.01	22,07	202±7	0,67
TS1	92±16	1,15	0.91±0.01	10,91	988±163	12,35
TS2	86±6	0,86	0.98±0.01	46,23	56±2	0,56
TS3	428±22	0,85	0.96±0.01	25,81	246±7	0,49
TS4	502±20	3,34	0.99±0.01	119,98	536±11	3,57

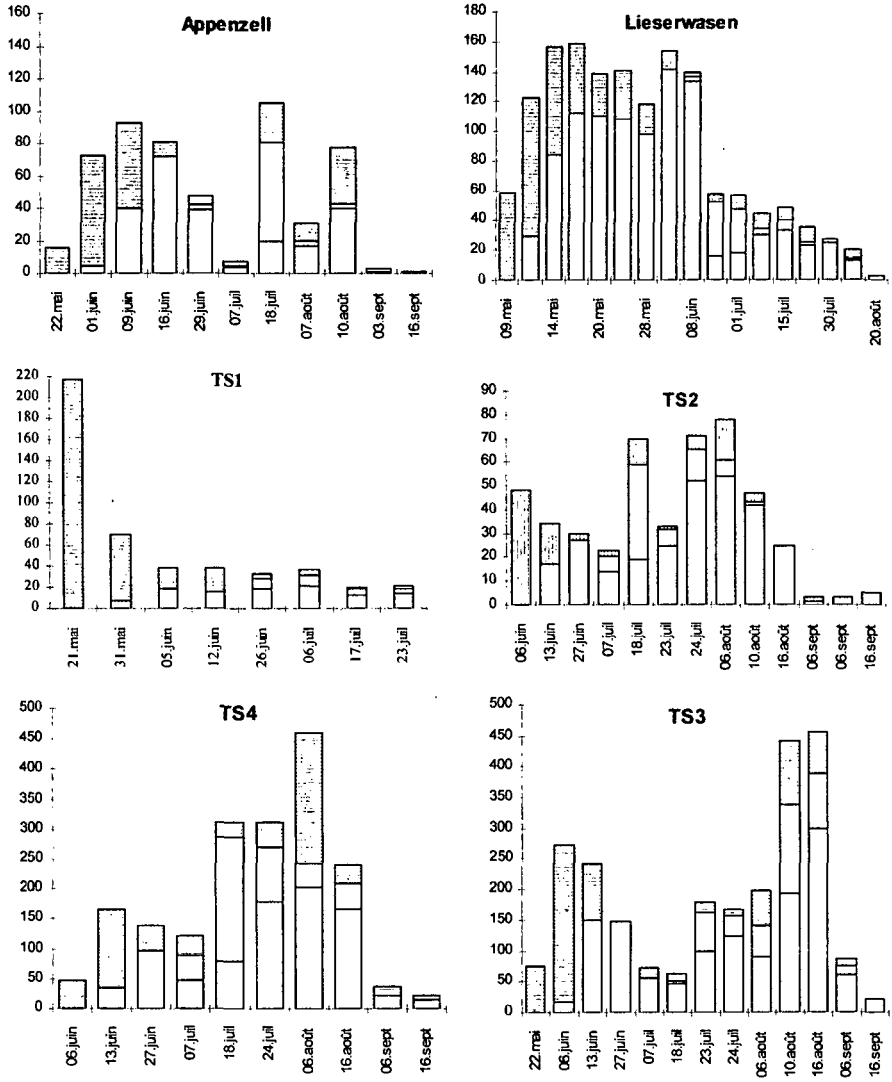


figure 2: Phenology at the different study patches. Total number of beetles seen at one date is divided into the number of first sightings with hard elytra (black), the number of first sightings of newly emerged beetles with still soft elytra (white) and the number of resightings (grey).

Population size

Estimates of mean population sizes using the open population model (Jolly-Seber) and the closed population model (M_t with varying capture probabilities each time) as well as resulting estimates of density per m^2 are given in table 2. Estimates according to the closed population model were always higher than those obtained from the Jolly-Seber model and had mostly lower standard errors (table 2). The variation of the population size with time according to the Jolly-Seber model is shown in figure 3 and follows the phenology of the abundance of the beetles (figure 2).the closed population model (M_t with varying capture probabilities each time) as well as resulting estimates of density per m^2 are given in table 2. Estimates according to the closed population model were always higher than those obtained from the Jolly-Seber model and had mostly lower standard errors (table 2). The variation of the population size with time according to the Jolly-Seber model is shown in figure 3 and follows the phenology of the abundance of the beetles (figure 2).

Residence time

Daily residence rates calculated according to the Jolly-Seber model (table 2) were always above 0.9 between single censuses (figure 4). The only patch where residence rates below 0.9 were calculated is patch TS1. However, after the mass disappearance of the beetles marked at the beginning of the season, estimates of population size and residence rate stabilised also at this patch. Mean residence rates and resulting residence times were highest at TS4, the highest patch just below the timberline in Tschiertschen, and lowest at TS1 and at Appenzell (figure 4)

Distribution of beetles per plants

Beetles changed plants frequently, on average 73.8% (Lieserwasen) and 94.6 % (Appenzell) of resighted beetles had changed host plant since the last sighting and in average 26.2 % (Lieserwasen) and 5.3 % (Appenzell) of the beetles remained on their host plants and did not move at all during a maximum of 62 days (Lieserwasen, mean = 5.2±0.3 days) and 38 days (Appenzell, mean = 15±4 days) respectively.

In Lieserwasen, 715 plants were recorded with one or more beetles and 483 plants in Appenzell (figure 2). In Lieserwasen, 57% of these plants were seen with a beetle only once, and only eleven plants were seen with beetles more than 6 times (counting each beetle as a sighting, not each date). The maximum is a plant which was recorded for 15 times, this is due to two beetles which stayed at this plant from May 11th until June 5th and 7th respectively. The one of the beetles that stayed on the plant until June 7th was a male, but we do not know the sex of the other one. In Appenzell, even more observations, 71.3%, were of plants recorded only once. The maximum there was a plant recorded six times, two plants were recorded 5 times and seven four times.

We know the distribution of beetles per plant from all six patches, since we always noted when more than one beetle was sitting on a plant (Appendix E). The maximum number of beetles observed in this study were 12 beetles sitting on a *Senecio* plant, but we also sampled once up to 50 beetles from one *Adenostyles*-leaf. On average the density of beetles per plant, however, was 1.08 beetles per plant and no indication for aggregation could be observed (Appendix E).

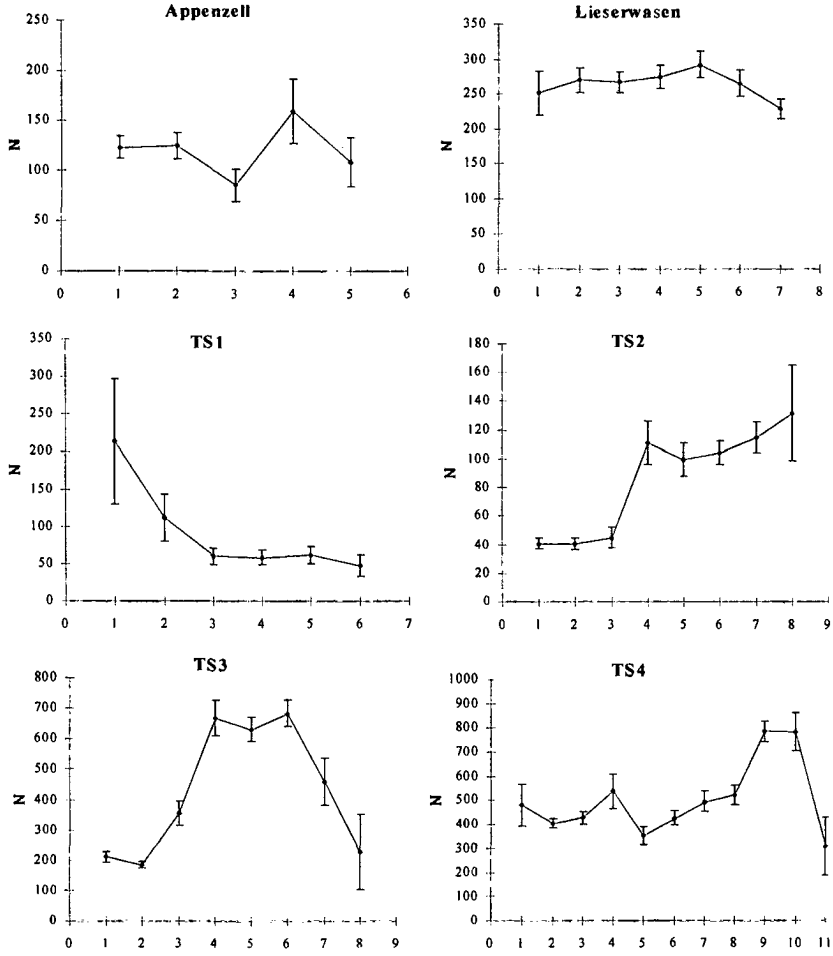


figure 3: Variation of population sizes at the different patches with the standard error, caleulated according to the Jolly-Seber model (Seber, 1982). The census interval is specific for each patch, dates of censuses are given in figure 2.

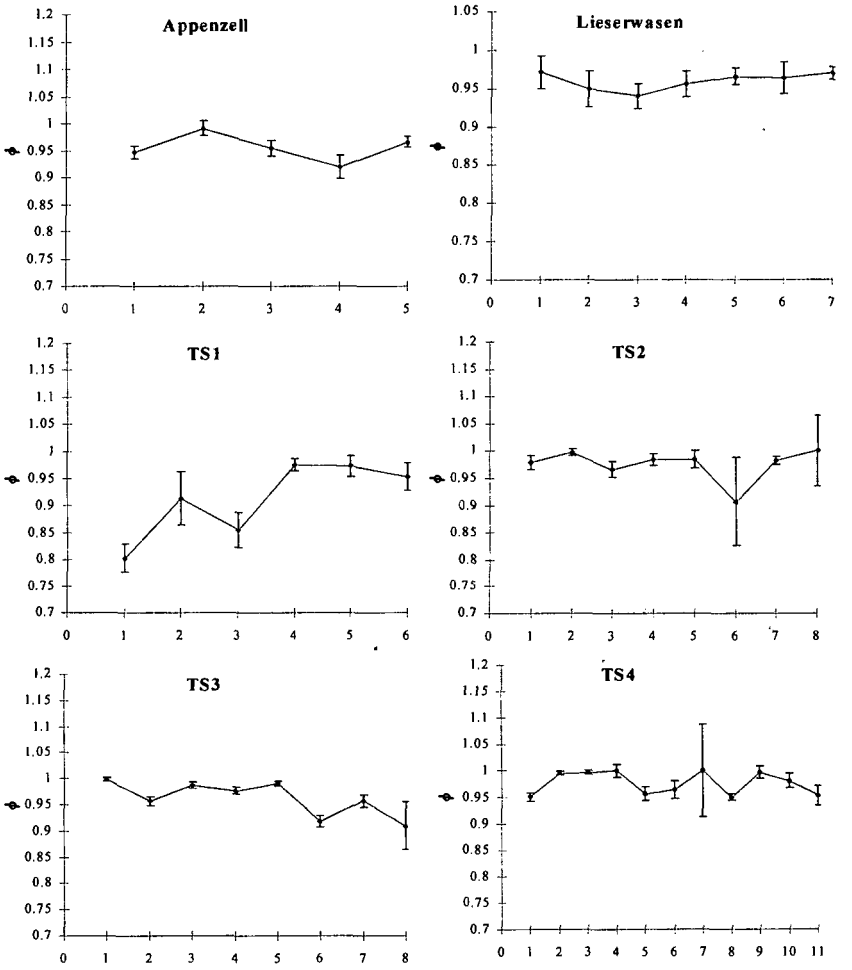


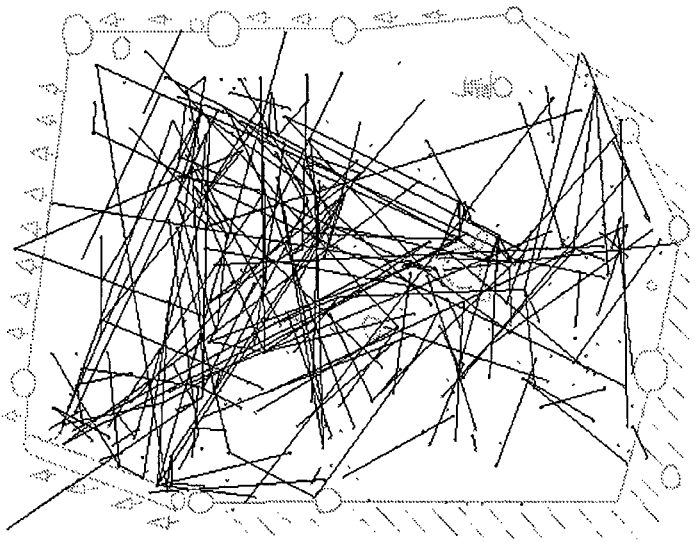
figure 4: Daily residence rates at the different patches with the standard error, calculated according to the Jolly-Seber model (Seber, 1982). The census interval is specific for each patch, dates of censuses are given in figure 2.

Next pages:
figure 5: Movement at a) Lieserwasen and b) Appenzell. Plotted are all plants, where beetles could be observed (points) and all movements during the whole season as calculated from Cartesian coordinates.

Figure 5a Lieserwasen

Lieserwasen

Figure 5b Appenzell



Appenzell

Movement between patches

During the whole summer, only few marked beetles were found outside the study patches. In Lieserwasen one dispersed ca 10m along a side road, outside the section, where beetles are abundant. Three others dispersed into a clearing, where otherwise no beetles, but host plants were found (figure 5a). In Appenzell, two beetles were found dispersing into the forest (less than 5 m; figure 5b) and one was found outside the study patch just below the cliff bordering the patch, ca 50 m away. In Tschiertschen a total of 27 beetles (corresponding to 1% of all marked beetles) were found outside their study patch, but of these, 24 of these beetles were found less than 5m outside the patch on single plants or dispersing into the forest. Thus 0.1% of all marked beetles were found further than 5m away from the patch they were marked in. No beetles were observed to change patches. No beetles were found outside of TS1. None of the beetles, which were marked there at the first census and disappeared afterwards could be observed anywhere in the area. TS2, the next closest patch was included in the census as we observed disappearance of beetles from TS1, but no marked beetle occurred there. One beetle was found outside of TS2 on a single *Adenostyles* ca 7 m away. Three beetles from TS3 were found more than 5 m away, one ca 10 m moving uphill and resting on a single plant, the two others were found downhill at a small clearing in otherwise dense coniferous forest, ca. 15 m away from the larger patch. At the clearing there were some *Adenostyles* plants and some other unmarked beetles as well. One marked beetle remained there from August 6th until September 6th, the other one was there seen only once.

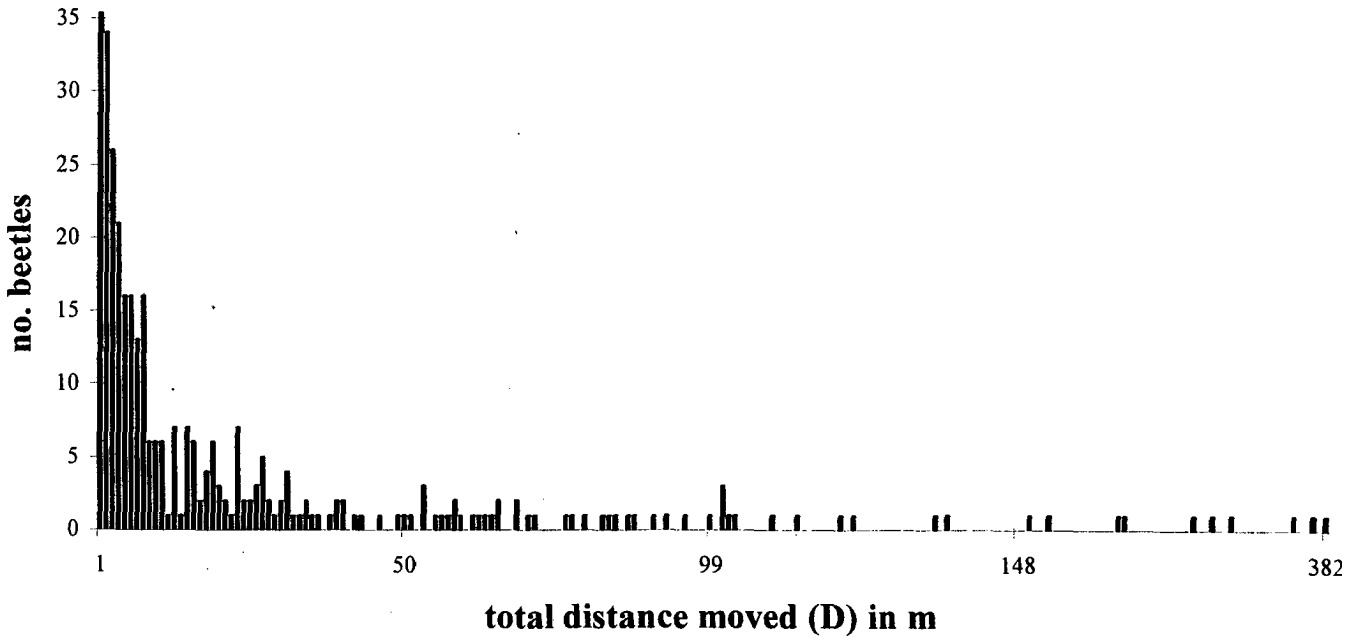


figure 6a: Frequencies of total distance moved D in Lieserwasen

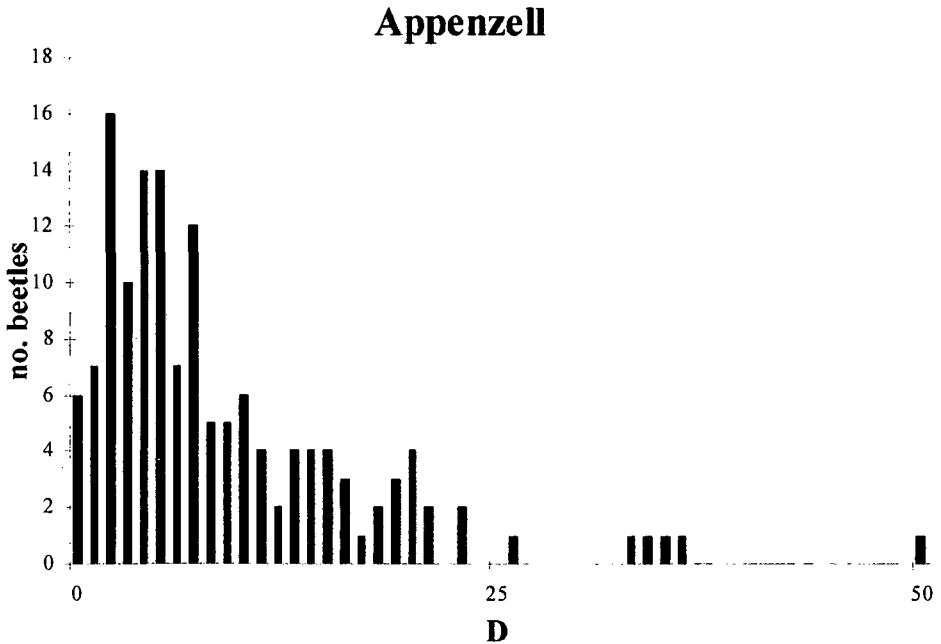


figure 6b: Frequencies of total distance moved D in Appenzell

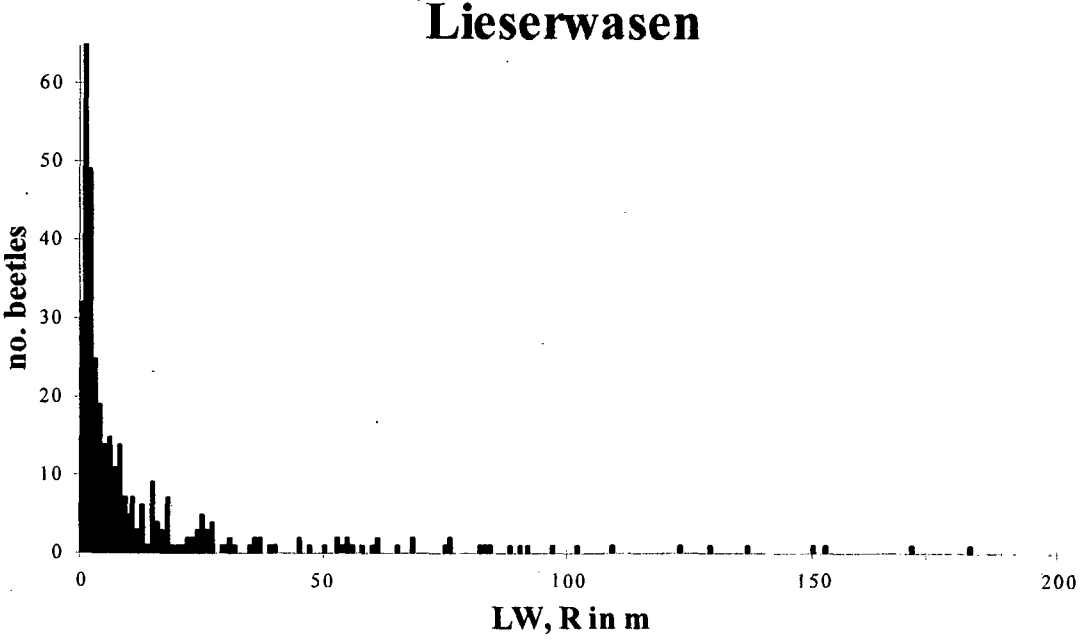


figure 7a: Frequencies of the range R moved (the distance between first and last encounter) in Lieserwasen

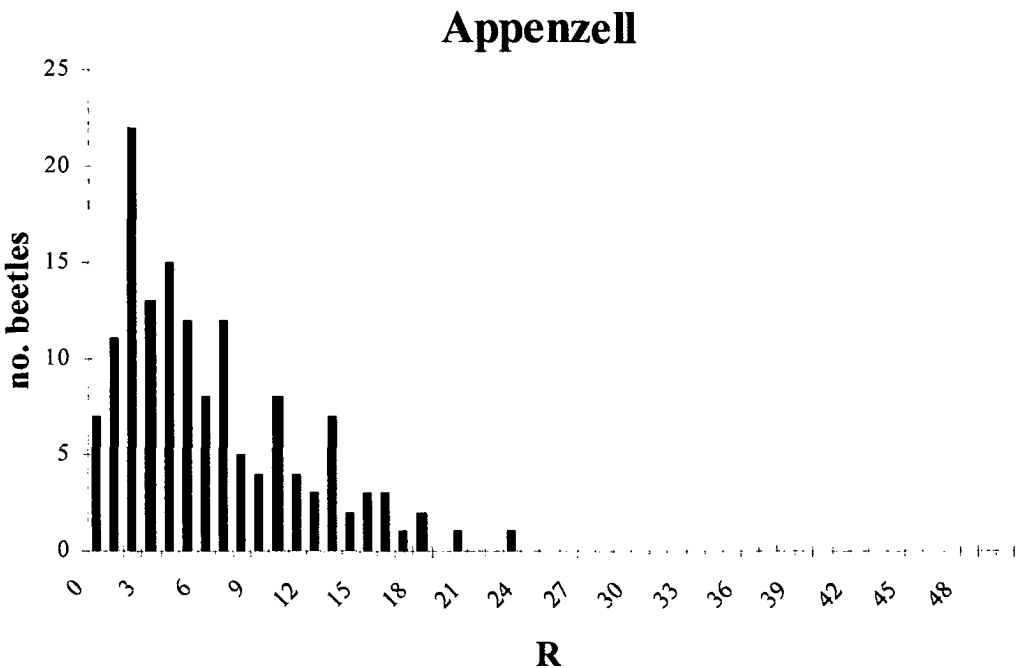


figure 7b: Frequencies of the range R moved (the distance between first and last encounter) in Appenzell

Movement within patches

At Lieserwasen and Appenzell we noted the position of the host plants on which we found beetles and were so able to calculate movement rates for each beetle resighted. In Lieserwasen we could observe movement of 366 beetles, in Appenzell of 144 beetles. The distribution of total distance moved (D), a measure of activity, and of the absolute distance moved (R), a measure of dispersal, was highly skewed in both patches (figures 6, 7) with some long distant dispersal (table 3). Since the distribution was highly left-hand skewed, we used a logarithmic transformation (ln(x+1)) for all the following statistics (table 3). In both patches, beetles traversed throughout the patch (figure 5a,b), but movement outside the patch was rare.

Table 3: Movement parameters and their descriptive statistics. D: total distance moved by one beetle, v_i daily moved distance (m/d), R range: distance moved between first and last capture, T days between first and last capture.

movement	Lieserwasen				Appenzell			
parameters	D	R	T	v_i	D	R	T	v_i
n	366,0	366,0	366,0	965,0	144,0	144,0	144,0	224,0
mean	7,3	4,8	21,5	0,6	5,6	4,2	27,3	0,3
std	3,1	2,7	16,5	1,0	1,5	1,3	15,0	0,3
min	0,0	0,0	0,3	0,0	0,0	0,0	3,0	0,0
max	381,3	181,1	103,0	151,6	12,6	12,2	107,0	3,8

Table 4: Difference between the sexes in the movement parameters. D: total distance moved by one beetle, v_i daily moved distance (m/d), R range: distance moved between first and last capture, T days between first and last capture.

movement	males				females			
parameters	D	R	T	v_i	D	R	T	v_i
n	63,0	63,0	63,0	237,0	78,0	78,0	78,0	210,0
mean	7,3	4,1	23,8	0,4	13,4	8,0	24,8	1,0
std	3,5	3,1	10,3	0,8	3,5	3,2	20,8	1,3
min	0,0	0,0	3,0	0,0	0,0	0,0	3,0	0,0
max	204,2	190,3	69,0	24,2	205,6	135,5	102,0	88,1

We recorded the sex of 165 beetles at Lieserwasen, most of these collected the 7th of June, but for 23 females and 1 male this was the first sighting (table 4). For the other 78 females and 63 males we were able to test for differences in their movement. Overall, there was a significant difference between the two sexes in the total distance moved (D; two tailed t test, $p<0.05$) as well as in the range (R) the beetles moved (two tailed t test, $p<0.05$). Females moved farther and more and they did so faster than males (difference D/T, two tailed t test, $p<0.05$).

Separated according to the different capture occasions, a difference in daily movement rates (v_i) between the sexes was significant (two tailed t test, $p<0.05$) only at three census intervals - including a peak in movement at recaptures on the 19th of May (figure 8).

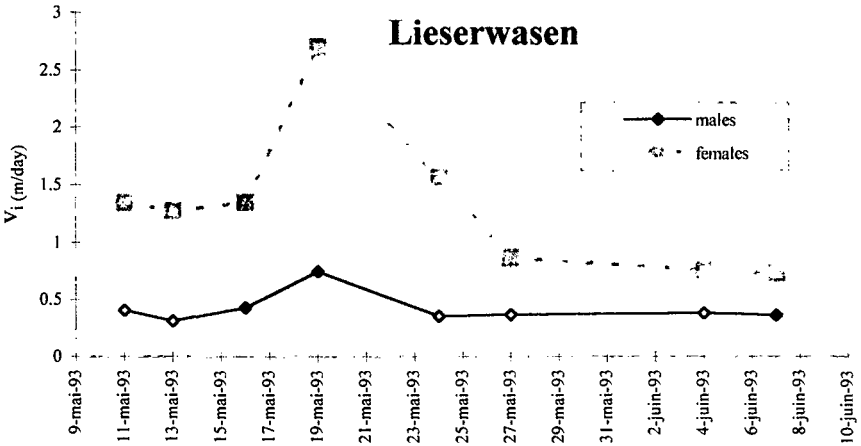


figure 8: Daily movement rates (v_i) at the different censuses for beetles with known sex at Lieserwasen. Filled symbols indicate a significant difference between the sexes at this date (two tailed t test, $p<0.05$).

Wrightian neighbourhood size

We estimated a neighbourhood size for Lieserwasen of N=1.9 beetles and for Appenzell of N=10.6 beetles. Using the densities of the closed population estimate instead of the one of the open population estimates, this changes to N=3.3 in Lieserwasen and N=17.7 in Appenzell.

DISCUSSION

For phytophagous insects, there does not yet exist a general theory of the responses to particular properties of host plant patches. Instead, present studies reveal a puzzling variety of possible responses (review in Kareiva, 1983), depending on the species studied. Different patch sizes, different host plant species, different architecture of host plant species, different surrounding vegetation and different phenology of the host plants are all reported to influence the distribution of phytophagous insects (Kareiva, 1982; Bach, 1988, Lawrence and Bach, 1989).

In this study on the leaf beetle *O. cacaliae* we estimated for six patches, which differed in many ecological parameters, within patch population sizes of between 80 to 500 beetles per patch, resulting in density estimations of 0.18 to 3.34 beetles/m^2. We estimated population sizes according to two models, one based on an open population and one based on a closed one. The choice of the right estimator depends highly on the choice of the right model (Lebreton, 1993). Unequal catchability can bias the more general model of Jolly Seber. If the newly caught beetles at the censuses before the emergence of the next generation represent not immigrants, as they are treated in the Jolly-Seber model, but are permanent members of the populations, the higher estimate of the closed model is to prefer. This does not apply to patch TS1, where the model M_t did not perform well (Appendix E).

Five of the patches studied showed more or less stable beetle abundance up to the time, when the next generation of beetles emerged. The phenology of the beetles at the patches, the time of first occurrence of *O. cacaliae* at the patch as well as the time when freshly eclosed adults caused peak abundance, were all highly dependent on altitude. However, we could not track beetles following this phenology in an uphill movement as was demonstrated for the butterfly *Euphilotes enoptes* and its perennial herbaceous host plant *Eriogonum compositum* (Peterson, 1997). In early spring *O. cacaliae* is found mainly on patches of *P. albus*, the first of it's food plants to emerge but an inferior host plant in terms of larval performance (Dobler and Rowell-Rahier, 1996). The beetles disappear from *P. albus* patches at about the same time when they start to feed on patches of *A. alliariae* and *S. nemorensis-fuchsii* patches. Thus, our hypothesis was that *P. albus* patches are used as a transient food resource until more suitable host plant patches are available and the beetles change to the next available patch. We had included one *P. albus* patch in our study. This patch, TS1, at the lower distributional range of *O. cacaliae* in Tschiertschen, differed markedly from all others since it showed steadily declining population sizes. Despite extensive effort we could not once demonstrate a change of host plant patches for the beetles disappearing from patch TS1.

Generally we could never observe between-patch movement. This is in accordance not only with the very high residence rates calculated for *O. cacaliae* in this study (except for TS1), but also corroborates the results from a previous study of the genetic population structure of *O. cacaliae*, which reported high differentiation and thereby restricted dispersal between neighbouring patches (chapter III).

Also the mean within patch dispersal is very low and corroborates the idea of these beetles being sedentary and of limited vagility. Above that, daily movement rates as well as total movement per beetle are very similar for the two patches studied in detail (Lieserwasen and Appenzell), despite their gross differences in area, shape and

dominating host plant. As in most studies of dispersal, movement parameters are not normally distributed but highly skewed in favour of the smaller distances with some individuals moving exceptionally far (Slatkin, 1985).
The only other field study on within patch population dynamics of *Oreina* leaf beetles was conducted with *O. gloriosa*, feeding monophagous on *Peucedanum osthrutium* (Eggenberger and Rowell-Rahier, 1991). For *O. gloriosa*, density was up to 13 beetles per m^2, this is much larger than our estimate here of up to 1.13 beetles per m^2 for *O. cacaliae*. Estimates of residence rates are in the same range, between 70-96% per week. Comparisons with studies on the movement behaviour of other leaf beetle species should be done with caution, since not only the natural history, but also the scale and intent of the studies differ markedly. *Acalymma vittatum* and *Diabratica undecimpunctata*, two closely related pest species of cucumbers, already showed marked differences in their movement behaviour in a common study, conducted with artificial host plant patches in an experimental garden (Lawrence and Bach, 1989). The two species differed in regard to colonisation rate as well as subsequent within and between patch movement rates. Although distances between single patches are not explicitly stated, the scale of their study is supposedly smaller than the one in our study with *O. cacaliae* and therefore does not allow comparison of residence rates and inter patch movement rates. The same can be said for a study conducted on two species of *Phyllotreta* leaf beetles feeding on crucifers (Kareiva, 1982). At a scale below 11m, frequent between patch movement and active patch selection according to host plant patch quality were found. A distinction of patches at a smaller scale than the one used in this study, does not seem realistic in regard of the population structure of *O. cacaliae*, since at both patches studied in detail, Lieserwasen and Appenzell, beetles are found to traverse the whole patch during one season.
We have no indication for any aggregation on individual plants within the patch (Appendix E), as is often reported for other leaf beetles (Morris *et al.*, 1996). Beetles changed plants frequently, certainly favoured by the dense and intermingling structure of the plant patches.
We studied only a fraction of the population of *O. cacaliae*, namely "the adults sitting on a host plant". The conspicuousness of these beetle in combination with their habit to sit for hours without moving and stay on the same host plant for sometimes over one month, gives raise to the assumption that one could estimate abundance by direct counting (Southwood, 1978). However, we showed that often only less than 50 % of the beetles are actually on the plants and our personal observations suggest that the percentage found depends mainly on temperature conditions.
High residence rates as found in this study for *O. cacaliae* are not unexpected for long-lived beetles which are restricted in their mobility. Of course, high residence rates are equivalent to high survival rates (for semantics see Lawrence, 1988). *Oreina* beetles are chemically defended and *O. cacaliae* depends for this defence on the sequestration of plant secondary compounds from it's host plants (Pasteels *et al.*, 1996). We have no information about natural enemies of *O. cacaliae*. The aposematic coloration points to visual oriented predators, namely birds. In trials with non-native birds a high effectiveness of the defensive compounds could be demonstrated (Rowell-Rahier *et al.*, 1995). In dissections of more than 1000 adult beetles from various sites we found a very low parasitation rate (ca. 0.3%) mostly with nematodes (Knoll, pers. observation). *O.*

cacaliae might have found it's "enemy free space" (Denno *et al.*, 1990) on it's host plants, whereby the host plant ensures chemical defence which in turn ensures reduced predation pressure and high survival rates. *P. albus* does not contain pyrrolizidinalkaloids, the plant secondary compounds that are sequestered by *O. cacaliae*, and this might be a reason for the low residence rates at the *P. albus* patch TS1.

A difference in movement rates between the sexes has been documented repeatedly (Lawrence, 1988, Mason *et al.*, 1995), but also several studies failed to show a difference in movement rates (for Chrysomelidae Strauss and Morrow, 1988). We found for *O. cacaliae* within patch movement that females move faster, farther and longer than males. A difference in the dispersal between the sexes can profoundly influence the population structure of a species (McCauley *et al.*, 1981). Our results here are surely only a first indication of sex biased dispersal strategy of *O. cacaliae*, since they are based only on a small subgroup of beetles (found on June 7th at the patch and sampled for dissection).

From the distribution of allele frequencies of allozymes we concluded that *O. cacaliae* represents a metapopulation with host plant patches as independent local subpopulations (chapter III). This suggestion of independence of patches is confirmed in our mark recapture experiments. We could not detect between patch movement and only few beetles were seen outside the study patches. Furthermore, residence rates within the patches and resulting residence times of up to 119 days are very high and very constant . Within patch movement was very variable (figures 6 and 7), but we could observe some individuals traversing a whole patch (figure 4). The neighbourhood sizes calculated are about one order of magnitude smaller than the census population size. Thus the through patch dispersers seem not to be of sufficient number to avoid further within patch structuring. We also suggested that only infrequent and long distance dispersal takes place between such patches. This is almost impossible to observe directly in mark and recapture experiments. Temporally restricted long distance movement can profoundly change predictions for population structuration obtained from direct observations (Mallet, 1986). We had also speculated that *O. cacaliae* undertakes long distance movement only at times, when their host plant is not available. Here, we censured the patches only during one season, and host plants were available at all six patches when beetles occurred. The one *P. albus* patch, TS1, might however represent the situation where host plants become unsuitable for the beetles (because they are unsuitable for larviposition or do not offer secondary plant compounds needed for defence) and they have to disperse. This could also be a reason for sex biased dispersal, males having no need to find plants suitable for larvae. The peak of female dispersal (figure 8) was at the time of larviposition. In conclusion, our mark and recapture studies back the notion of *O. cacaliae* patches as independent populations.

Acknowledgements:

We would like to thank Betty Benrey and Louis-Felix Bersier for comments on an earlier draft of this manuscript. This study was supported by a grant of the Swiss National Science Foundation (grant no31-33669.92).

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Appendix A

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à propos .....PLAYING WITH NUMBERS

The evolution in a species with a subdivided population structure is mainly determined by genetic drift. To quantify the effects of genetic drift one needs to know population size and gene flow, two population parameters notoriously difficult to measure in natural populations. However, since the interplay of small population size promoting genetic drift and gene flow opposing it leave their characteristic pattern in the genetic structure, a variety of so-called "indirect" methods have been proposed for the estimation of gene flow and/or population size. Indirect estimates are always based on the patterns of variation of allele frequencies. However, these methods base on idealized models and often cannot separate different alternative combinations of parameters resulting in the same genetic population structure. Thus it is of primary importance to compare them with "direct" estimates, obtained from ecological or demographical studies (Nürnberger and Harrison, 1995; Peterson and Denno, 1997). There are still only few comparisons of direct and indirect estimates. Most often these consist of a comparison of gene flow estimates (references in Slatkin, 1985, Roderick, 1996) with observed dispersal in a species. Many studies comparing direct and indirect population parameters were done with snails, which are a tempting study system because of their low mobility and conspicuousness (Johnson and Black, 1995). The same features made *O. cacaliae* look like a suitable system to study population size and dispersal directly as well as indirectly.
In the following we review some methods of indirect estimation of population parameters from allele frequencies and give estimations for *O. cacaliae* whenever possible (and even if it is not strictly appropriate, since not all assumptions of the model in question are met). Allele frequencies were obtained with allozyme electrophoresis as described in chapter II and III, detailed description of the study sites mentioned is also given in chapter III and IV.

N_em as a measure of gene flow

The most robust indirect estimate one can obtain is N_em, defined as the product of effective population size and migration rate. It has the inherent disadvantage that it is an estimation of a product and as such not easy to imagine. N_em is taken as a measure of gene flow and can be interpreted as the number of migrants exchanged per generation (Wright, 1970). There are two independent methods to estimate N_em, first from F_{ST} as proposed originally by Wright for an - idealized - island population. Second an estimation of N_em is possible from the frequency of rare allele with the rationale that rare alleles can reach high frequencies in local populations only if gene flow is restricted. We gave estimates of N_em as estimates of gene flow at several scales in chapter II and III. In our mark and recapture studies we tried to obtain direct estimates of dispersal/migration rates. However, since we could not observe any between patch movement, we cannot quantify migration rate but only conclude that it is low. With our estimations of population sizes and assuming that the direct observed

population size equals the effective population size at least in orders of magnitude, we can calculate migration rates from N_em. We took the overall $N_em = 3.54$ (chapter IV) from Tschiertschen (since we do not have genetic and mark and recapture data from the same patches) and lower and upper values of observed population sizes in Tschiertschen. For a population size of 80 we obtain a migration rate of 0.044 and for a population size of 500 a migration rate of 0.007.

Effective population size

Population size is a key parameter, if one wants to understand the evolutionary ecology and population biology of a species. In chapter IV we report "within patch population sizes" of *O. cacaliae* as estimated from mark and recapture studies. This represents an estimate of the individuals actually present in the study area (if we assume a closed population) or associated with the study area (if we assume an open population). The important parameter influencing the evolution of a species, however, is its effective population size, defined as the proportion of the population contributing to the next generations gene pools (more precisely as "the size of an idealized population which would give rise to the variance in gene frequency or the rate of inbreeding observed in the actual population under consideration", Caballero, 1994). The correct estimation of the effective population size is an active field of research in evolutionary ecology (Caballero, 1994, 1995; Nunney, 1995; Wang, 1996; Jorde and Ryman, 1995).
In the maybe simplest case the sexes differ in their contribution to the gene pool of the next generation. N_e depends then mainly on the less numerous sex and can be estimated from the number of mature males and females present as follows (Wright, 1938):

$$N_e = \frac{4N_mN_f}{(N_m + N_f)} \qquad \text{equation (1)}$$

Strictly this applies only to actually reproducing males and females, but since one can rarely determine their number, an approximation is often given from the percentage of males and females present. For *O. cacaliae* we can do so in Lieserwasen (to be exact: for the proportion of beetles sitting on plants the 7th of June) and obtain a N_e=141.69. This is in the same order of magnitude than our "uncorrected" estimates of N=[263;451] (chapter IV).
If genetic drift is the only cause of allele frequency shifts over time, one can estimate N_e from samples taken at different times. In the so called temporal method a variation of Wright's standardized variance (F) is the basis to relate observed changes in allele frequencies to N_e. A first approximation of N_e is given by t/2F if t is not large (t is the time of generations between the two samples). Since F has to be estimated from a sample, correction factors containing sample sizes have been included in order to get a more precise estimate of N_e (Waples, 1989).

There are different estimators of F but the most common used is F_c:

$$F_c = \frac{1}{K} \frac{\sum_{i=1}^{k} (x_i - y_i)^2}{\sum_{i=1}^{k} ((x_i + y_i)/2 - x_i y_i)} \qquad \text{equation (2)}$$

Waples (1989) generalized the former restrictive assumptions of the temporal model to only three remaining, namely that the observed variation is neutral, that migration between subpopulations is negligible and that the species has discrete generations. He gives formulas for the estimation of N_e under two different sampling regimes, called plan I and II. Plan I means sampling after reproduction or replacing the sampled individuals back into the population , they can contribute to the next generations gene pool. Plan II means sampling and thereby removing animals from the population before they reproduce. The formulas given for Plan I respectively plan II are:

$$\text{plan I. } N_e = \frac{t}{2(F_c - 1/S_0 - 1/S_t)} \qquad \text{equation (3)}$$

$$\text{plan II. } N_e = \frac{t}{2F_c - 1/S_0 - 1/S_t + 2/N_e} \qquad \text{equation (4)}$$

whereby F is calculated according to equation (2), S_0 and S_1 are the sample sizes at time 0 and time 1, t is the no of generations between the samples and N_e is the population size at the first sampling date.

Table 1: Values of $_{Fc}$ for two patches, one at Appenzell and one at Zastler (figure 1, chapter II). Calculations of F_c are according to equation (2) in the text, weighted mean F_c were calculated as $\Sigma K_j F_{cj} / \Sigma K_j$ (Waples 1989).

Appenzell	F_c	K
ACONH	0,0652	3
G3PDH	0,0256	6
AO-1	0,0033	2
DDH1	0,0284	4
FDH	0,1213	3
FUMH	0,0210	4
GAPDH	0,0163	6
AAT	0,0096	4
PEP(LA)1	0,0710	2
MDHP	0,0171	7
SOD2	0,0338	3
TPI	0,0142	4
mean	0,0307	

Zastler	F_c	K
ACONH	0,0276	3
G3PDH	0,0934	6
AO-1	0,0286	3
DDH1	0,0510	3
FDH	0,0970	3
GAPDH	0,3800	8
AAT	0,0841	4
MDHP	0,1094	7
SOD2	0,0423	4
TPI	0,0231	6
mean	0,1196	

We have samples from different years from one patch at Appenzell and one from Zastler (see map in chapter II). By allozyme electrophoresis as described in chapter II we obtained frequencies of 48 respectively 47 alleles from 12 respectively 10 polymorphic loci for these samples. In Zastler the samples were always taken at early spring, so we assume plan II (sampling before reproduction) appropriate. In Appenzell, samples were taken early summer, when larviposition had begun, but in a first approximation we also assumed plan II to be appropriate. We guess that the first two assumptions of the model, neutral variation and negligible migration, are be met in our system, but not the third. Nevertheless the above formulas should be an approximation also for species with overlapping generations (Waples, 1989). Values of F_c are given in table 1. t was assumed to be 1 generation. Calculation of N_e resulted then in a negative (!) estimation of N_e for Appenzell (N_e = - 110.011) and in an estimation of N_e = 7.22459 for Zastler. Neglecting the correction factors and taking t = 1.5, which seems reasonable for *O. cacaliae*, we obtain an estimate of N_e = 20.25 for Appenzell.

The influence of overlapping generations on the estimation population size with the temporal model has been studied in detail by Jorde and Ryman (1994). They conclude that a) the value of F_c is larger than what can be explained only by genetic drift over one year and b) F_c is not uniquely determined by the effective population size, but depends further on the age specific birth and survival rates when generations overlap. They give an estimator of N_e taking into account r, the growth rate of the population and generation length, two parameters wherefore we have no information for *O. cacaliae*. The concept of the neighbourhood was introduced by S. Wright (1946) to describe differentiation in a continuous population. Since per definition divergence can accumulate only beyond the neighbourhood area, the point at which divergence begins to increase with increasing distance can be taken as the extent of the neighbourhood (Selander and Kaufmann, 1975). Recently a method for estimating neighbourhood size from a pattern of isolation by distance has been suggested (Slatkin and Maddison, 1990), whereby the log(neighbourhood size) is given by the intercept of the regression line of the plot log(N_em) versus log(distance). For *O. cacaliae* we did so with the regression line reported in chapter II for all sites except the Pyrenees (since there is some extra differentiation associated with populations from the Pyrenees, chapter II) and obtained an estimate of neighbourhood size of N_e=7.94. This correspods very well to the neighbourhood sizes estimated from the variance of dispersal distances (chapter IV, Wright 1946): N_e=1.9362 beetles for Lieserwasen and N_e=10.6384 beetles for Appenzell.

In conclusion can be said that we have many indications for very small effective neighboorhood sizes (up to 20 beetles), one order of magnitude lower than the census population sizes (chapter IV). This is in agreement with the observed high within-patch structuration (chapter II, III). With decreasing effective population sizes the effects of genetic drift and inbreeding are increasing, whereas the effects of selection are decreasing and only weakly selected alleles become selectively neutral (Caballero, 1994). If the ratio N_e/N_c is markedly below 1, random genetic drift can be strong although the population is large. It was proposed that this could be a reason for extinction (due to a loss of genetic variation) in a demographically stable population (Nunney, 1995).

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Appendix B

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à propos .....SEX

Differences between the sexes in the dispersal ability or in realized dispersal have been observed in several insect specics and can be of importance for the population structure (chapter 1V). Sex differences have been mainly investigated in studies "directly" observing dispersal and have rarely been taken into account in studies of the genetic population structure. Genetic differences in the genetic population structure are only observable before reproduction takes place, when the dispersing sex should show an allelic distribution different from the one of the non dipersing one. Provided that a great proportion of immigrants comes from outside populations each generation. For *O. cacaliae* a significant difference in the distribution of genetic variance was found at populations sampled very early in the season (Rowell-Rahier, 1992). Males had significantly larger F_{IS} and F_{ST} values than females.

In our study of *O. cacaliae*, we found a significantly overall male biased sex ratio in the samples taken for electrophoresis (398 females, 654 males, χ^2=62.29657, $p<0.05$), this was also reported in Rowell-Rahier (1992). However, sex ratio varied from sample to sample (table 1). We know sex ratios at birth only from one *Oreina* species, *O. gloriosa*, where it is not different from a 50:50 ratio. A deviation from a 50:50 ratio in our samples could also reflect a behavioral difference. Males could be more intended to sit on their host plants and to be thus catchable.

An analysis of the distribution of genetic variance following the "modificated gender F-statistic method" proposed in Rowell-Rahier (1992) was done for Lieserwasen, the largest patch sampled in regard of area as well as beetles number sampled. Sampling was done before the emergence of the following generation. The F_{ST} between the sexes (or following the notation of Rowell-Rahier, 1992, the F_{gt}) was significantly different from 0 ($p<0.005$; permutting over genotypes within the total). Mean values of F_{ST} were low, with a 95% confidence interval of [0.008;0.033] (bootstrapping over loci, all analyses done with FSTAT (Goudet, 1995)).

For our total sample, a plot of the F-statistics at different loci between the male population and the female population did not suggest any differences between the sexes (figure 1). Most comparisons of not only F_{IT}, but also F_{ST} and F_{IS} were distributed along the diagonal; with large differences in dispersal between the sexes, larger F_{IS}- and F_{ST}-values would be expected in the females. (all analyses were done with the data set used in chapter III including allelefrequencies at 9 loci). A comparison of the inbreeding values of all investigated patches showed at some patches very high F_{IS} values for females in comparison to males, but overall samples were distributed approximately even around the diagonale (figure 2).

A similar pattern is seen when looking only at the patches at Tschiertschen (figure 3), though there is a bias towards higher F_{IS} for the female part of populations. Caleulating measures of gene flow separately for both sexes with the private allele method of Slatkin (1985) reveals consistent differences in the overall sample as well as on the local level of populations in Tschiertschen. Nm values calculated for females are always lower (Nm = 0.45 overall and Nm = 1.16 in Tschiertschen) than for males (Nm = 1.32 overall and Nm = 2.04 in Tschiertschen).

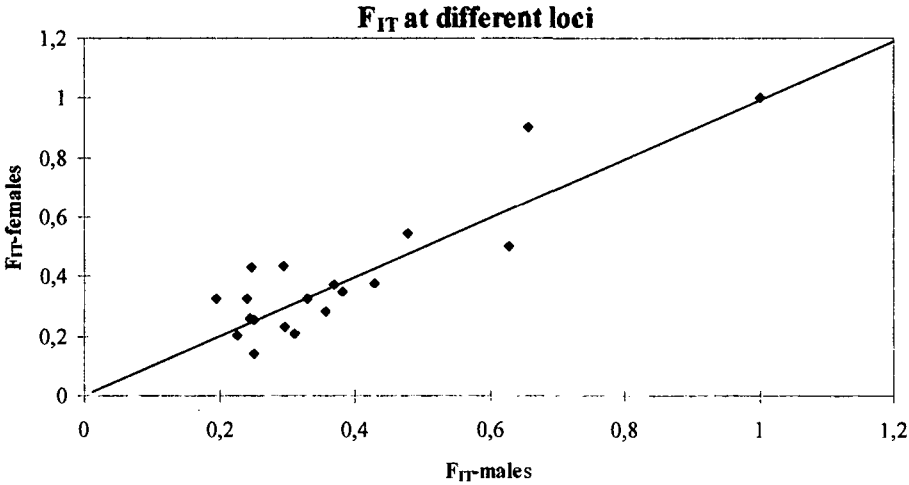

figure 1a: Comparison of F-statistics conducted with the male and female part of the populations, including all samples as reported in chapter III. a) F_{IT}-values for different loci

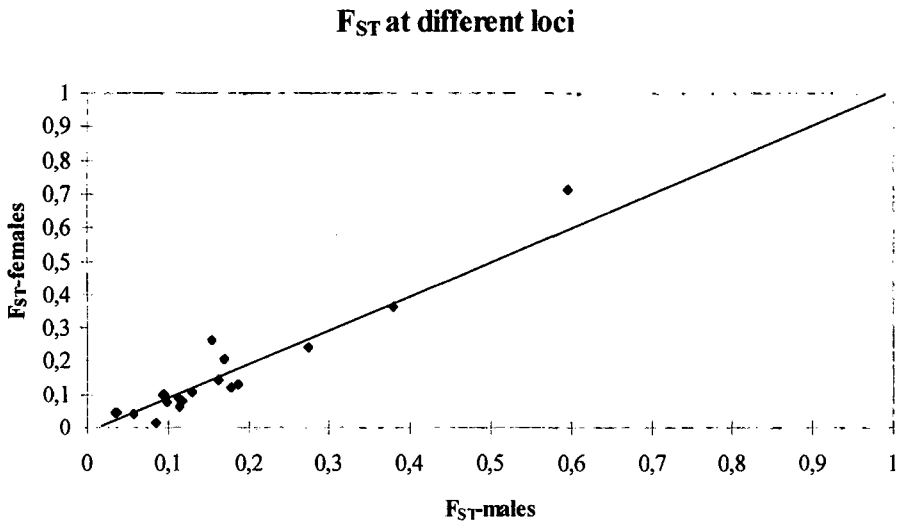

figure 1b: Comparison of F-statistics conducted with the male and female part of the populations, including all samples as reported in chapter III. b) overall F_{ST}-values for different loci

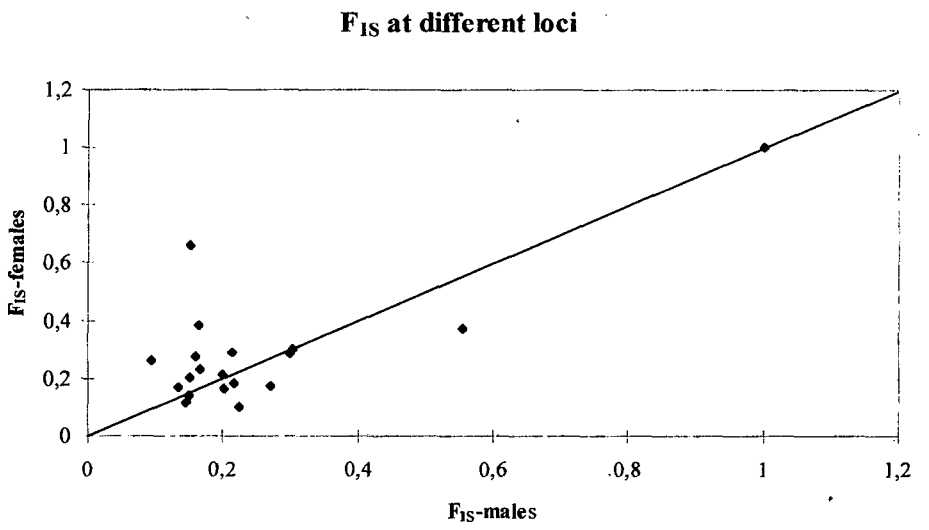

figure 1c: Comparison of F-statistics conducted with the male and female part of the populations, including all samples as reported in chapter III. c) overall F_{IS}-values for different loci.

Thus there are indications for a sex bias in between patch dispersal. Migration between patches (respectively immigration in one patch) must be frequent, to allow such a - even weak - pattern to be detectable, since such differences must be reestablished every generation, and should have disappeared after the emergence of the following generation.

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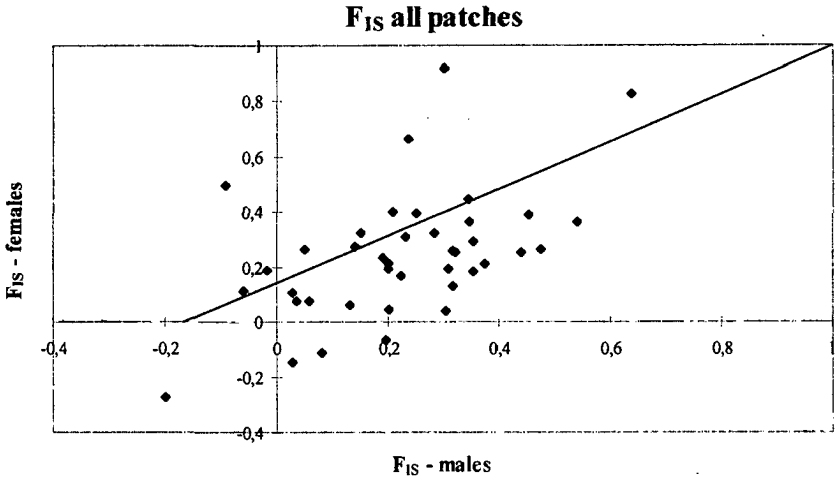

figure 2: Comparison of the F_{IS}-values for the male and female part of all sampled patches, where at least two females were sampled (mean F_{IS} over all loci). Please not that the sample size is sometimes very low (sample sizes according to table 1).

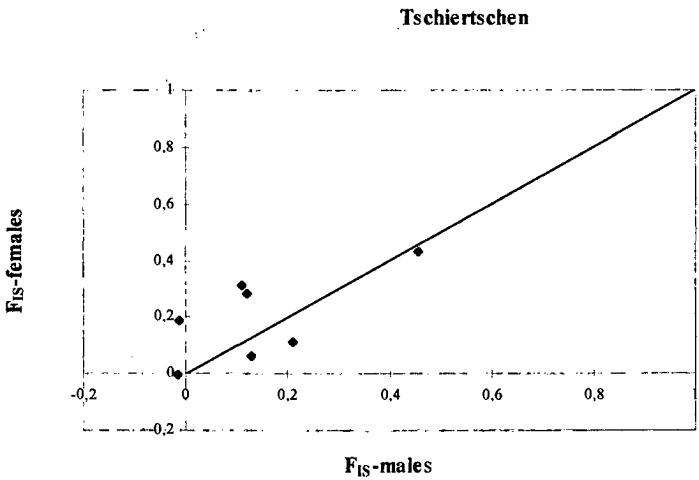

figure 3: Comparison of the F_{IS}-values for the male and female part of patches sampled in Tschiertschen (mean F_{IS} over all loci). Please not that the sample size is sometimes very low (sample sizes according to table 1).

Table 1: Sex ratio in different samples. Given are the total number of beetles dissected, the % females and the sampling period (I: early in the season, no larviposition, II larviposition but no "soft" beetles of the following generation, III mid to end of the season, the new generation has already emerged)

site/patch	total	%females	sampled
K95unten	12	0,00	I
Ko95andoben	12	0,00	I
Mg95-Morgins	10	0,00	I
Gustiberg	5	0,00	II
TS5	34	0,12	II
Ap5	25	0,12	II
Madonna1	25	0,16	?
TS7	18	0,17	II
TS11	23	0,17	II
Hirschbach oben	17	0,18	I
Flerret	16	0,19	I
Coben	25	0,20	I
Safien Thalkirch	43	0,21	III
Zastler - 95B	23	0,22	I
Kandersteg 1	18	0,22	I
TSO	26	0,23	II
TS1	25	0,24	II
ClAubrisque 3	35	0,29	II
Kandersteg KMU	10	0,30	I
Madonna2	10	0,30	?
Snezka 1 P	26	0,31	II
Ferret 3	20	0,35	I
ApA2	28	0,36	II
Nova Pec 1	13	0,38	II
Lieserwasen	149	0,39	II
Jochberg	5	0,40	?
Kiental oben	20	0,40	II
Ap3	29	0,41	II
TS6	29	0,41	II
Ap95oben	12	0,42	I
Morgins-oben	18	0,44	I
Hupfleitnjoch	6	0,50	II
Höllental	24	0,50	II
Vals	33	0,52	III
TS8	27	0,52	II
Vrin	17	0,53	III
Albula1	18	0,61	II
Ap4	15	0,67	II
Kandersteg-oben	51	0,67	I
Kandersteg-unten	14	0,71	I
Hohwald	28	0,71	I
Ap6	11	0,73	II
Adelboden	23	0,78	?
Ap2	24	0,92	II

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Appendix C

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in Appendix C you find:

table 1: Allele frequencies as used as data set in chapter II for a) *O. cacaliae* and b) *O. speciosissima*

figure 1: UPGMA cluster analysis, based on modified Rogers distance, of a data set with all patches which were investigated for *O. cacaliae*. Names are based on the ones given in the map (figure 1) of chapter III, but this analysis contains some patches which were omitted from the final analysis due to very small sample size. Please note the cluster of the Pyrenees patches Col d'Aubisque and Val d'Ossau.

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Table 1a: Allele frequencies as used as data set in chapter II for a) *O. cacaliae*

		Adelbodeu	Albula	Appenzell	Cascade	Col d' Aubrisque	Val Ferret	Hirschbach	Hohwald	Höllental	Kandersteg	Lieser-wasen
ACOH	n	8	9	22	29	26	37	18	14	25	42	113
ACOH	A	0.063	0.944	0.909	0.414	0.942	0.135	1	0.893	0.96	0.31	0.854
ACOH	B	0	0	0.023	0.569	0.038	0.054	0	0.107	0	0.202	0.137
ACOH	C	0	0.056	0	0	0.019	0.014	0	0	0.02	0	0.009
ACOH	D	0.938	0	0.068	0	0	0.797	0	0	0.02	0.488	0
ACOH	E	0	0	0	0	0	0	0	0	0	0	0
ACOH	F	0	0	0	0	0	0	0	0	0	0	0
ACOH	G	0	0	0	0	0	0	0	0	0	0	0
ACOH	H	0	0	0	0	0	0	0	0	0	0	0
ACOH	I	0	0	0	0.017	0	0	0	0	0	0	0
G3PDH	n	15	11	23	35	22	34	19	17	23	42	119
G3PDH	A	0.667	0.773	0.848	0.886	0.727	0.471	0.526	0.559	0.739	0.619	0.567
G3PDH	B	0	0	0.065	0.029	0.159	0.279	0.395	0.029	0.109	0.071	0.13
G3PDH	C	0.233	0.182	0.043	0	0.068	0.059	0.053	0.265	0.13	0.262	0.223
G3PDH	D	0.033	0	0.022	0	0.023	0.074	0.026	0.059	0	0.012	0.038
G3PDH	E	0	0.045	0	0	0.023	0	0	0.029	0.022	0	0.013
G3PDH	F	0	0	0	0	0	0	0	0	0	0	0.008
G3PDH	G	0	0	0	0	0	0	0	0	0	0	0
G3PDH	H	0.067	0	0	0	0	0	0	0	0	0.012	0.013
G3PDH	I	0	0	0	0	0	0	0	0	0	0	0
G3PDH	J	0	0	0	0	0	0	0	0.029	0	0	0
G3PDH	K	0	0	0.022	0.086	0	0.118	0	0.029	0	0.012	0.004
G3PDH	L	0	0	0	0	0	0	0	0	0	0	0.004
G3PDH	M	0	0	0	0	0	0	0	0	0	0.012	0
AO1	n	16	12	26	25	20	20	14	16	23	32	122
AO	A	0.844	0.75	0.75	0.98	0.85	0.7	0.857	0.688	0.87	0.766	0.77
AO	B	0.156	0.25	0.25	0.02	0.15	0.275	0.143	0.313	0.13	0.234	0.225
AO	C	0	0	0	0	0	0	0	0	0	0	0
AO	D	0	0	0	0	0	0.025	0	0	0	0	0.004
AO2	n	10	5	22	14	18	17	20	7	28	24	89
AO2	A	0.1	0.1	0.386	0.536	0.5	0.176	0.25	0.286	0.446	0.208	0.427
AO2	B	0.3	0.3	0.318	0.143	0.278	0.529	0.35	0.286	0.196	0.333	0.197

		Adelboden	Albula	Appenzell	Cascade	Col d' Aubrisque	Val Ferret	Hirschbach	Hohwald	Höllental	Kandersteg	Lieser- wasen
AO2	C	0.6	0.6	0.136	0.321	0	0.265	0.25	0.286	0.054	0.458	0.135
AO2	D	0	0	0.136	0	0.222	0.029	0	0.143	0.304	0	0.225
AO2	E	0	0	0.023	0	0	0	0.075	0	0	0	0.006
AO2	F	0	0	0	0	0	0	0.075	0	0	0	0
AO2	G	0	0	0	0	0	0	0	0	0	0	0
AO2	H	0	0	0	0	0	0	0	0	0	0	0
AO2	I	0	0	0	0	0	0	0	0	0	0	0
AO2	J	0	0	0	0	0	0	0	0	0	0	0.011
ARK2	n	7	9	18	8	2	13	8	8	7	32	99
ARK2	A	1	1	0.889	0.938	0	0.769	1	1	1	0.688	0.995
ARK2	B	0	0	0	0	0	0	0	0	0	0	0
ARK2	C	0	0	0.028	0.063	0	0	0	0	0	0	0
ARK2	D	0	0	0.083	0	1	0.231	0	0	0	0.313	0.005
ARK3	n	16	13	25	25	9	33	7	10	17	31	90
ARK3	A	1	0.962	1	1	1	1	1	1	1	1	0.989
ARK3	B	0	0	0	0	0	0	0	0	0	0	0.011
ARK3	C	0	0.038	0	0	0	0	0	0	0	0	0
ARK3	D	0	0	0	0	0	0	0	0	0	0	0
ARK4	n	13	11	23	24	8	29	6	9	17	28	73
ARK4	A	0.846	0.955	0.826	0.958	0.813	0.948	1	0.889	0.853	1	0.966
ARK4	B	0.115	0	0.174	0.042	0.188	0.052	0	0.111	0.147	0	0.027
ARK4	C	0	0.045	0	0	0	0	0	0	0	0	0.007
ARK4	D	0	0	0	0	0	0	0	0	0	0	0
ARK4	E	0.038	0	0	0	0	0	0	0	0	0	0
DDH1	n	10	9	27	30	12	39	12	10	12	28	100
DDH1	A	0.3	0.667	0.463	0.817	0.625	0.718	0.375	0.5	0.417	0.679	0.66
DDH1	B	0.35	0.278	0.463	0.117	0.375	0.167	0.583	0.45	0.583	0.304	0.31
DDH1	C	0	0	0.019	0	0	0.038	0	0	0	0.018	0
DDH1	D	0	0	0	0	0	0	0	0	0	0	0
DDH1	E	0.35	0.056	0.056	0.067	0	0.077	0.042	0.05	0	0	0.025
DDH1	F	0	0	0	0	0	0	0	0	0	0	0.005
EST2	n	8	11	24	29	13	30	3	16	18	37	121
EST2	A	0.563	0.364	0.229	0.828	0.577	0.217	0.833	0.406	0.417	0.649	0.475
EST2	B	0.438	0.364	0.604	0.017	0.077	0.433	0	0.531	0.083	0.203	0.07

		Adelboden	Albula	Appenzell	Cascade	Col d' Aubrisque	Val Ferret	Hirschbach	Hubwald	Höllental	Kandersteg	Lieser-wasen
EST2	C	0	0.273	0.083	0.155	0.231	0.233	0.167	0	0.389	0.027	0.298
EST2	D	0	0	0	0	0.115	0.05	0	0	0	0.027	0.095
EST2	E	0	0	0	0	0	0	0	0.031	0.111	0	0.062
EST2	F	0	0	0	0	0	0	0	0	0	0	0
EST2	G	0	0	0.083	0	0	0.067	0	0.031	0	0.041	0
EST2	H	0	0	0	0	0	0	0	0	0	0.054	0
FDH	n	7	5	21	30	7	31	7	15	26	41	81
FDH	A	0.429	0.6	0.762	0.967	0.143	0.403	0.786	0.733	0.596	0.378	0.63
FDH	B	0.429	0	0	0	0	0.274	0	0	0.038	0.329	0.049
FDH	C	0.143	0.4	0.238	0.033	0.857	0.323	0.214	0.267	0.365	0.268	0.321
FDH	D	0	0	0	0	0	0	0	0	0	0.024	0
FUMH	n	15	8	28	35	18	35	14	17	29	34	122
FUMH	A	0.933	1	1	1	0.917	0.986	1	0.882	0.931	0.985	1
FUMH	B	0	0	0	0	0	0	0	0	0.034	0.015	0
FUMH	C	0.067	0	0	0	0.083	0.014	0	0	0	0	0
FUMH	D	0	0	0	0	0	0	0	0	0	0	0
FUMH	E	0	0	0	0	0	0	0	0	0.034	0	0
FUMH	F	0	0	0	0	0	0	0	0.059	0	0	0
FUMH	G	0	0	0	0	0	0	0	0	0	0	0
FUMH	H	0	0	0	0	0	0	0	0	0	0	0
FUMH	I	0	0	0	0	0	0	0	0	0	0	0
FUMH	J	0	0	0	0	0	0	0	0.059	0	0	0
GAPDH	n	16	11	28	35	26	37	18	17	27	40	117
GAPDH	A	0.438	0.591	0.804	0.543	0.885	0.838	0	0.912	0.63	0.463	0.846
GAPDH	B	0.25	0.091	0.018	0.129	0.096	0.081	0	0	0.222	0.087	0.068
GAPDH	C	0.313	0.045	0.018	0.043	0	0.014	0.083	0	0.056	0.1	0.03
GAPDH	D	0	0	0	0.043	0	0.027	0	0	0.037	0.025	0
GAPDH	E	0	0.091	0	0	0	0	0.028	0	0	0.063	0.004
GAPDH	F	0	0.182	0.161	0.2	0.019	0.027	0.111	0.088	0.056	0.188	0.038
GAPDH	G	0	0	0	0.029	0	0	0	0	0	0	0
GAPDH	H	0	0	0	0	0	0	0	0	0	0	0
GAPDH	I	0	0	0	0.014	0	0.014	0.306	0	0	0.075	0.013
GAPDH	J	0	0	0	0	0	0	0	0	0	0	0
GAPDH	K	0	0	0	0	0	0	0	0	0	0	0

		Adelboden	Albula	Appenzell	Cascade	Col d' Aubrisque	Val Ferret	Hirschbach	Hohwald	Höllental	Kandersteg	Lieser- wasen
GAPDH	L	0	0	0	0	0	0	0.472	0	0	0	0
AAT	n	18	13	26	30	25	32	21	9	24	42	121
AAT	A	0.639	0.538	0.635	0	0.9	0.422	0.833	0.944	0.979	0.417	0.901
AAT	B	0.167	0	0	0	0.06	0	0	0	0	0	0.05
AAT	C	0	0.423	0.288	1	0.04	0.547	0.143	0	0.021	0.583	0.05
AAT	D	0	0	0	0	0	0	0	0	0	0	0
AAT	E	0	0.038	0.077	0	0	0	0	0	0	0	0
AAT	F	0.194	0	0	0	0	0.031	0.024	0.056	0	0	0
IDH1	n	18	12	28	31	17	38	22	17	22	42	123
IDH1	A	1	1	1	0.177	1	0.987	1	1	1	1	0.988
IDH1	B	0	0	0	0.71	0	0	0	0	0	0	0.008
IDH1	C	0	0	0	0.016	0	0.013	0	0	0	0	0
IDH1	D	0	0	0	0	0	0	0	0	0	0	0
IDH1	E	0	0	0	0.016	0	0	0	0	0	0	0.004
IDH1	F	0	0	0	0.016	0	0	0	0	0	0	0
IDH1	G	0	0	0	0.065	0	0	0	0	0	0	0
IDH2	n	8	12	17	30	2	20	6	12	16	40	117
IDH2	A	1	1	1	1	1	1	1	1	0.969	1	1
IDH2	B	0	0	0	0	0	0	0	0	0.031	0	0
IDH2	C	0	0	0	0	0	0	0	0	0	0	0
IDH2	D	0	0	0	0	0	0	0	0	0	0	0
IDH2	E	0	0	0	0	0	0	0	0	0	0	0
IDH2	F	0	0	0	0	0	0	0	0	0	0	0
LA1	n	18	13	28	35	23	38	21	17	26	42	115
LA1	A	0.917	0.885	0.929	0.943	0.935	0.934	1	0.941	0.904	0.952	0.722
LA1	B	0	0	0	0	0	0.066	0	0	0	0	0.017
LA1	C	0	0	0	0	0	0	0	0	0	0	0
LA1	D	0.083	0	0.071	0.043	0.065	0	0	0.059	0.096	0.048	0.257
LA1	E	0	0	0	0	0	0	0	0	0	0	0
LA1	F	0	0.115	0	0	0	0	0	0	0	0	0.004
LA1	G	0	0	0	0.014	0	0	0	0	0	0	0
LA2	n	17	11	18	24	11	4	21	10	16	32	107
LA2	A	0.735	0.318	0.333	0	0.773	0	0.738	0.25	0.438	0.578	0.379
LA2	B	0.265	0.227	0.472	1	0.227	0.75	0.238	0.75	0.563	0.328	0.607

		Adelboden	Albula	Appenzell	Cascade	Col d' Aubrisque	Val Ferret	Hirschbach	Hohwald	Höllental	Kandersteg	Lieser- wasen
LA2	C	0	0.455	0.194	0	0	0.25	0	0	0	0.031	0.009
LA2	D	0	0	0	0	0	0	0	0	0	0.047	0
LA2	E	0	0	0	0	0	0	0	0	0	0	0
LA2	F	0	0	0	0	0	0	0.024	0	0	0.016	0.005
MDHP	n	6	7	22	35	20	39	10	12	17	29	98
MDHP	A	0.583	0.643	0.727	0.5	0.4	0.654	0.6	0.792	0.471	0.81	0.633
MDHP	B	0.333	0.143	0.045	0.371	0.25	0.167	0.4	0.125	0.294	0.086	0.148
MDHP	C	0	0	0.023	0.014	0.125	0.051	0	0	0.118	0.034	0.092
MDHP	D	0	0	0.045	0	0	0	0	0.042	0.029	0	0.026
MDHP	E	0	0	0	0.043	0	0.038	0	0	0.029	0.017	0
MDHP	F	0	0	0	0	0.1	0	0	0	0	0	0.005
MDHP	G	0	0.143	0	0	0	0	0	0	0	0	0.005
MDHP	H	0	0	0	0	0	0	0	0	0	0.034	0
MDHP	I	0	0	0	0	0.075	0	0	0	0.029	0	0.005
MDHP	J	0	0.071	0.159	0.043	0.05	0.09	0	0.042	0.029	0.017	0.077
MDHP	K	0.083	0	0	0.029	0	0	0	0	0	0	0.01
MDHP	L	0	0	0	0	0	0	0	0	0	0	0
GPI	n	18	11	28	35	22	39	22	12	24	26	108
GPI	A	0.944	1	1	0.857	0.977	0.897	1	1	1	0.923	0.968
GPI	B	0	0	0	0.086	0	0	0	0	0	0	0
GPI	C	0	0	0	0	0.023	0.064	0	0	0	0.077	0.019
GPI	D	0.056	0	0	0	0	0	0	0	0	0	0.009
GPI	E	0	0	0	0	0	0	0	0	0	0	0
GPI	F	0	0	0	0.057	0	0.038	0	0	0	0	0
GPI	G	0	0	0	0	0	0	0	0	0	0	0
GPI	H	0	0	0	0	0	0	0	0	0	0	0
GPI	I	0	0	0	0	0	0	0	0	0	0	0.005
GPI	J	0	0	0	0	0	0	0	0	0	0	0
SOD2	n	8	10	23	23	7	20	8	6	15	30	95
SOD2	A	0.375	0.35	0.652	0.978	0.857	0.65	0.625	0.75	0.733	0.783	0.958
SOD2	B	0	0	0	0	0	0.05	0	0	0	0.083	0
SOD2	C	0	0.2	0.043	0	0	0	0	0.167	0	0	0
SOD2	D	0.625	0.05	0.304	0.022	0.143	0.2	0.375	0.083	0.267	0.133	0.042
SOD2	E	0	0.4	0	0	0	0	0	0	0	0	0

		Adelboden	Albula	Appenzell	Cascade	Col d' Aubrisque	Val Ferret	Hirschbach	Hohwald	Höllental	Kandersteg	Lieser- wasen
SOD2	F	0	0	0	0	0	0	0	0	0	0	0
SOD2	G	0	0	0	0	0	0	0	0	0	0	0
SOD2	H	0	0	0	0	0	0.1	0	0	0	0	0
TPI	n	8	13	28	30	23	39	17	12	29	37	113
TPI	A	0.813	0.885	0.875	0.867	1	0.769	0.912	0.792	0.828	0.973	0.973
TPI	B	0.063	0	0.036	0	0	0.077	0	0.125	0	0	0
TPI	C	0	0.038	0.054	0.017	0	0.064	0.088	0.083	0.086	0.014	0.013
TPI	D	0	0	0	0	0	0	0	0	0	0	0
TPI	E	0	0.077	0.036	0.017	0	0.026	0	0	0.069	0	0.013
TPI	F	0	0	0	0	0	0.013	0	0	0	0	0
TPI	G	0	0	0	0.033	0	0.013	0	0	0	0	0
TPI	H	0	0	0	0	0	0	0	0	0	0	0
TPI	I	0.125	0	0	0.067	0	0.038	0	0	0.017	0.014	0

		Madonna di C.	Morgins	Nova Pec	Safien-T.	Snezka	Tschier tschen 1	Tschier tschen 2	Vals	Vrin	Zastler
ACOH	n	20	8	6	42	26	27	24	24	8	25
ACOH	A	1	0.063	1	0.881	0.962	0.852	0.688	1	0.5	0
ACOH	B	0	0.25	0	0	0	0	0.063	0	0.5	0
ACOH	C	0	0	0	0	0.038	0	0	0	0	0
ACOH	D	0	0.688	0	0.119	0	0.148	0.25	0	0	0.12
ACOH	E	0	0	0	0	0	0	0	0	0	0
ACOH	F	0	0	0	0	0	0	0	0	0	0
ACOH	G	0	0	0	0	0	0	0	0	0	0
ACOH	H	0	0	0	0	0	0	0	0	0	0
ACOH	I	0	0	0	0	0	0	0	0	0	0
G3PDH	n	20	10	11	41	26	34	24	23	14	20
G3PDH	A	0.575	0.55	1	0.732	0.481	0.897	0.438	0.826	0.464	0.675
G3PDH	B	0.375	0.1	0	0.146	0.269	0.103	0.146	0.022	0.25	0.025
G3PDH	C	0	0.2	0	0.085	0.115	0	0.083	0.152	0.179	0.125
G3PDH	D	0.05	0	0	0.012	0.115	0	0.063	0	0.071	0.125
G3PDH	E	0	0	0	0.024	0	0	0.063	0	0	0
G3PDH	F	0	0	0	0	0	0	0	0	0	0
G3PDH	G	0	0	0	0	0	0	0	0	0	0
G3PDH	H	0	0	0	0	0	0	0	0	0	0

		Madonna di C.	Morgins	Nava Pec	Safien-T.	Snezka	Tschier tschen 1	Tschier tschen 2	Vals	Vrin	Zastler
G3PDH	I	0	0	0	0	0	0	0	0	0	0
G3PDH	J	0	0	0	0	0	0	0	0	0	0
G3PDH	K	0	0.15	0	0	0.019	0	0.208	0	0.036	0.05
G3PDH	L	0	0	0	0	0	0	0	0	0	0
G3PDH	M	0	0	0	0	0	0	0	0	0	0
AO1	n	15	9	5	30	15	25	22	25	15	15
AO	A	0.967	0.833	1	0.833	0.6	0.92	0.727	0.64	0.867	0.533
AO	B	0.033	0.167	0	0.167	0.4	0.08	0.273	0.36	0.133	0.4
AO	C	0	0	0	0	0	0	0	0	0	0
AO	D	0	0	0	0	0	0	0	0	0	0.067
AO2	n	11	3	6	31	11	20	11	22	15	18
AO2	A	0.182	1	0	0.371	0.227	0.4	0.455	0.523	0.367	0.361
AO2	B	0.364	0	0.583	0.387	0.227	0.425	0.182	0.136	0.4	0.278
AO2	C	0.455	0	0.333	0.194	0.091	0	0	0.182	0.233	0.056
AO2	D	0	0	0	0.048	0.455	0.175	0.227	0.159	0	0.306
AO2	E	0	0	0.083	0	0	0	0.045	0	0	0
AO2	F	0	0	0	0	0	0	0	0	0	0
AO2	G	0	0	0	0	0	0	0	0	0	0
AO2	H	0	0	0	0	0	0	0.091	0	0	0
AO2	I	0	0	0	0	0	0	0	0	0	0
AO2	J	0	0	0	0	0	0	0	0	0	0
ARK2	n	20	2	12	26	10	27	16	17	7	6
ARK2	A	0.75	0.75	1	0.904	1	0.87	1	1	0.786	1
ARK2	B	0	0	0	0	0	0	0	0	0	0
ARK2	C	0	0.25	0	0	0	0.019	0	0	0	0
ARK2	D	0.25	0	0	0.096	0	0.111	0	0	0.214	0
ARK3	n	20	10	6	33	16	28	26	2	7	25
ARK3	A	1	1	1	1	1	1	0.962	1	1	1
ARK3	B	0	0	0	0	0	0	0	0	0	0
ARK3	C	0	0	0	0	0	0	0	0	0	0
ARK3	D	0	0	0	0	0	0	0.038	0	0	0
ARK4	n	18	5	6	33	15	22	19	7	4	25
ARK4	A	1	0.8	1	0.985	1	1	1	1	1	1
ARK4	B	0	0.2	0	0	0	0	0	0	0	0

		Madonna di C.	Morgins	Nova Pec	Safien-T.	Snezka	Tschier tschen 1	Tschier tschen 2	Vals	Vrin	Zastler
ARK4	C	0	0	0	0.015	0	0	0	0	0	0
ARK4	D	0	0	0	0	0	0	0	0	0	0
ARK4	E	0	0	0	0	0	0	0	0	0	0
DDH1	n	15	10	6	15	26	20	26	14	7	25
DDH1	A	0	0.35	1	0.667	0.962	0.65	0.596	0.536	0.5	0.76
DDH1	B	1	0.55	0	0.233	0	0.35	0.346	0.393	0.429	0.18
DDH1	C	0	0.1	0	0	0.019	0	0.058	0	0	0
DDH1	D	0	0	0	0	0	0	0	0	0	0
DDH1	E	0	0	0	0.1	0.019	0	0	0.036	0.071	0.06
DDH1	F	0	0	0	0	0	0	0	0.036	0	0
EST2	n	18	4	4	25	21	33	17	23	15	21
EST2	A	0.944	0.125	0	0.58	0.357	0.288	0.176	0.543	0.233	0.214
EST2	B	0	0.875	0	0.34	0.262	0.318	0.5	0.261	0.267	0.071
EST2	C	0.056	0	0	0.08	0.214	0.242	0.029	0.174	0	0.643
EST2	D	0	0	1	0	0.167	0.061	0.265	0	0	0.071
EST2	E	0	0	0	0	0	0.03	0.029	0.022	0.033	0
EST2	F	0	0	0	0	0	0	0	0	0	0
EST2	G	0	0	0	0	0	0.061	0	0	0.467	0
EST2	H	0	0	0	0	0	0	0	0	0	0
FDH	n	17	10	12	24	25	27	23	22	12	24
FDH	A	0.882	0.45	0.375	0.563	0.98	0.611	0.565	0.432	0.458	0.563
FDH	B	0.029	0.3	0	0.042	0	0	0.043	0.068	0.083	0.125
FDH	C	0.088	0.25	0	0.396	0.02	0.389	0.391	0.477	0.458	0.313
FDH	D	0	0	0.625	0	0	0	0	0.023	0	0
FUMH	n	15	5	12	42	16	27	26	25	15	25
FUMH	A	1	1	0.958	0.988	0.969	1	0.904	1	1	1
FUMH	B	0	0	0	0	0	0	0.038	0	0	0
FUMH	C	0	0	0.042	0	0	0	0	0	0	0
FUMH	D	0	0	0	0	0.031	0	0.038	0	0	0
FUMH	E	0	0	0	0	0	0	0	0	0	0
FUMH	F	0	0	0	0	0	0	0	0	0	0
FUMH	G	0	0	0	0	0	0	0	0	0	0
FUMH	H	0	0	0	0	0	0	0	0	0	0
FUMH	I	0	0	0	0.012	0	0	0.019	0	0	0

		Madonna di C.	Morgins	Nova Pec	Safien-T.	Snezka	Tschier tschen 1	Tschier tschen 2	Vals	Vrin	Zastler
FUMH	J	0	0	0	0	0	0	0	0	0	0
GAPDH	n	20	6	12	42	21	34	19	25	15	25
GAPDH	A	0.825	0.833	0.417	0.714	0.786	0.838	0.789	0.14	0.333	0.92
GAPDH	B	0.125	0.083	0.042	0.095	0.19	0.088	0.132	0.22	0	0.06
GAPDH	C	0.05	0	0.542	0.012	0.024	0.029	0.026	0.02	0.167	0
GAPDH	D	0	0.083	0	0.024	0	0	0	0.14	0.2	0.02
GAPDH	E	0	0	0	0.036	0	0	0	0	0.067	0
GAPDH	F	0	0	0	0.119	0	0.044	0.053	0.48	0.2	0
GAPDH	G	0	0	0	0	0	0	0	0	0.033	0
GAPDH	H	0	0	0	0	0	0	0	0	0	0
GAPDH	I	0	0	0	0	0	0	0	0	0	0
GAPDH	J	0	0	0	0	0	0	0	0	0	0
GAPDH	K	0	0	0	0	0	0	0	0	0	0
GAPDH	L	0	0	0	0	0	0	0	0	0	0
AAT	n	20	10	6	42	26	28	22	25	14	25
AAT	A	0.025	0.45	0.917	0.488	0.173	0.571	0.409	0.28	0.536	0.78
AAT	B	0	0	0	0.167	0	0	0	0	0	0.06
AAT	C	0.975	0.4	0.083	0.321	0.827	0.321	0.409	0.52	0.429	0.16
AAT	D	0	0	0	0	0	0	0	0	0	0
AAT	E	0	0	0	0.024	0	0.107	0.068	0.2	0.036	0
AAT	F	0	0.15	0	0	0	0	0.114	0	0	0
IDH1	n	20	10	12	42	21	34	25	25	15	25
IDH1	A	1	1	1	1	1	1	1	1	1	1
IDH1	B	0	0	0	0	0	0	0	0	0	0
IDH1	C	0	0	0	0	0	0	0	0	0	0
IDH1	D	0	0	0	0	0	0	0	0	0	0
IDH1	E	0	0	0	0	0	0	0	0	0	0
IDH1	F	0	0	0	0	0	0	0	0	0	0
IDH1	G	0	0	0	0	0	0	0	0	0	0
IDH2	n	20	2	12	23	16	33	11	22	10	12
IDH2	A	0.7	1	1	1	1	1	1	1	1	1
IDH2	B	0.275	0	0	0	0	0	0	0	0	0
IDH2	C	0	0	0	0	0	0	0	0	0	0
IDH2	D	0	0	0	0	0	0	0	0	0	0

		Madonna di C.	Morgins	Nova Pec	Safien-T.	Snezka	Tschier tschen 1	Tschier tscheo 2	Vals	Vrin	Zastler
IDH2	E	0	0	0	0	0	0	0	0	0	0
IDH2	F	0.025	0	0	0	0	0	0	0	0	0
LA1	n	20	10	12	42	21	26	25	25	14	25
LA1	A	0.975	0.8	0.917	1	0.929	1	1	1	1	1
LA1	B	0.025	0.15	0.083	0	0.071	0	0	0	0	0
LA1	C	0	0	0	0	0	0	0	0	0	0
LA1	D	0	0.05	0	0	0	0	0	0	0	0
LA1	E	0	0	0	0	0	0	0	0	0	0
LA1	F	0	0	0	0	0	0	0	0	0	0
LA1	G	0	0	0	0	0	0	0	0	0	0
LA2	n	15	5	12	27	15	19	9	25	15	5
LA2	A	0.4	0.4	0.5	0.444	0.067	0.395	0.444	0.42	0.2	0.1
LA2	B	0.6	0.6	0.458	0.556	0.667	0.605	0.556	0.58	0.8	0.9
LA2	C	0	0	0	0	0.267	0	0	0	0	0
LA2	D	0	0	0.042	0	0	0	0	0	0	0
LA2	E	0	0	0	0	0	0	0	0	0	0
LA2	F	0	0	0	0	0	0	0	0	0	0
MDHP	n	15	10	6	41	26	20	26	16	10	25
MDHP	A	0.933	0.8	0.833	0.707	0.865	0.975	0.635	0.719	0.5	0.46
MDHP	B	0.033	0	0	0.159	0.019	0.025	0.038	0.188	0	0.08
MDHP	C	0	0.05	0	0	0	0	0.154	0	0.35	0.04
MDHP	D	0	0	0.167	0	0	0	0	0.063	0	0.12
MDHP	E	0	0	0	0	0	0	0	0	0.1	0.02
MDHP	F	0	0	0	0	0	0	0.038	0	0	0.04
MDHP	G	0	0	0	0.11	0	0	0	0	0	0
MDHP	H	0	0	0	0	0	0	0	0	0	0
MDHP	I	0	0	0	0	0	0	0	0.031	0	0
MDHP	J	0.033	0.15	0	0.024	0.115	0	0.058	0	0	0.24
MDHP	K	0	0	0	0	0	0	0.038	0	0.05	0
MDHP	L	0	0	0	0	0	0	0.038	0	0	0
GPI	n	20	10	12	26	26	27	25	19	15	20
GPI	A	1	1	1	1	1	1	0.94	0.947	1	1
GPI	B	0	0	0	0	0	0	0	0	0	0
GPI	C	0	0	0	0	0	0	0	0.026	0	0

		Madonna di C.	Morgins	Nuva Pec	Safien-T.	Snezka	Tschier tschen 1	Tschier tschen 2	Vals	Vrin	Zastler
GPI	D	0	0	0	0	0	0	0.02	0.026	0	0
GPI	E	0	0	0	0	0	0	0	0	0	0
GPI	F	0	0	0	0	0	0	0	0	0	0
GPI	G	0	0	0	0	0	0	0	0	0	0
GPI	H	0	0	0	0	0	0	0	0	0	0
GPI	I	0	0	0	0	0	0	0	0	0	0
GPI	J	0	0	0	0	0	0	0.04	0	0	0
SOD2	n	10	9	12	24	26	28	22	11	12	21
SOD2	A	0.45	0.444	1	0.729	1	0.679	0.614	0.682	0.917	0.714
SOD2	B	0	0.444	0	0	0	0.089	0.091	0	0	0
SOD2	C	0.5	0	0	0.042	0	0.071	0.023	0	0.083	0.048
SOD2	D	0.05	0.111	0	0.229	0	0.161	0.227	0.318	0	0.238
SOD2	E	0	0	0	0	0	0	0	0	0	0
SOD2	F	0	0	0	0	0	0	0	0	0	0
SOD2	G	0	0	0	0	0	0	0	0	0	0
SOD2	H	0	0	0	0	0	0	0.045	0	0	0
TPI	n	20	10	11	42	21	34	26	23	12	25
TPI	A	0.95	0.6	1	0.917	0.548	1	0.692	0.891	1	0.84
TPI	B	0.025	0	0	0	0.452	0	0	0.022	0	0
TPI	C	0	0.05	0	0.012	0	0	0.077	0	0	0.02
TPI	D	0	0	0	0	0	0	0.038	0	0	0
TPI	E	0	0	0	0	0	0	0	0	0	0.1
TPI	F	0	0.25	0	0	0	0	0.115	0	0	0
TPI	G	0.025	0	0	0	0	0	0	0	0	0
TPI	H	0	0	0	0.012	0	0	0	0	0	0
TPI	I	0	0.1	0	0.06	0	0	0.077	0.087	0	0.04

table 1b: Allele frequencies as used as data set in chapter II for b) *O. speciosissima*

		Appen-zell	Boubin	Höllental	Hirsch-bach	Kiental	Kralov1	Kralov2	La Lech-erette	Morgins	Nova Pec	Safien-R.	Tschier tschen	Hörnli	Vrin	Zamecek	Zastler
ACOH	n	9	17	20	7	13	10	19	12	18	10	18	14	9	8	10	20
ACOH	A	0	0.059	0	0	0	0.05	0	0.083	0.056	0	0.028	0.107	0	0	0	0.025
ACOH	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.025
ACOH	C	0.944	0.941	1	1	1	0.95	1	0.917	0.944	1	0.944	0.857	1	0.938	1	0.925
ACOH	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ACOH	E	0	0	0	0	0	0	0	0	0	0	0.028	0.036	0	0.063	0	0
ACOH	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.025
ACOH	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ACOH	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ACOH	I	0.056	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AO1	n	13	17	19	16	18	5	18	8	18	7	20	6	5	8	9	23
AO1	A	0.538	0	0.316	0.313	0.417	0.2	0.028	0.125	0.083	0	0.125	0.333	0	0	0	0.283
AO1	B	0	0.059	0.026	0	0.028	0	0	0	0	0	0.075	0	0	0.125	0	0.022
AO1	C	0	0	0	0.094	0.028	0	0	0	0	0	0	0	0	0	0	0.022
AO1	D	0	0	0.053	0	0.083	0	0	0	0	0	0	0	0	0.063	0	0
AO1	E	0.115	0.294	0.158	0.188	0.167	0.5	0.306	0.438	0.306	0.5	0.35	0.5	1	0.813	0.444	0.609
AO1	F	0	0	0	0.031	0	0	0	0	0	0	0	0	0	0	0	0
AO1	G	0.346	0.471	0.395	0.375	0.278	0.3	0.667	0.438	0.611	0.5	0.15	0.167	0	0	0.333	0.065
AO1	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AO1	I	0	0.176	0.053	0	0	0	0	0	0	0	0.3	0	0	0	0.222	0
ARK3	n	9	14	7	12	10	10	5	15	5	9	10	14	9	8	13	6
ARK3	A	1	1	1	0.917	1	1	1	1	1	1	1	0.964	1	1	1	1
ARK3	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ARK3	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ARK3	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ARK3	E	0	0	0	0.083	0	0	0	0	0	0	0	0.036	0	0	0	0
DDH1	n	12	13	14	14	16	15	10	6	18	10	27	14	7	8	16	10
DDH1	A	0.875	1	0.964	0.929	1	1	1	1	0.944	1	0.722	0.964	0.929	1	1	1
DDH1	B	0	0	0	0	0	0	0	0	0.028	0	0	0.036	0	0	0	0

		Appen-zell	Buuhin	Höllental	Hirsch-bach	Kiental	Kralov1	Kralov2	La Lech-erette	Murgins	Nova Pec	Safien-R.	Tschier tschen	Hürnli	Vrin	Zamecek	Zastler
DDH1	C	0.042	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DDH1	D	0	0	0	0	0	0	0	0	0	0	0.278	0	0	0	0	0
DDH1	E	0.083	0	0.036	0.071	0	0	0	0	0.028	0	0	0	0.071	0	0	0
FUMH	n	18	6	20	14	18	14	9	10	20	10	25	14	6	7	5	20
FUMH	A	0	0	0	0.143	0.056	0	0	0	0	0	0.22	0	0	0	0	0.05
FUMH	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FUMH	C	0	0	0	0	0.111	0.464	0	1	0	0	0.08	0.75	0.833	0.929	0	0
FUMH	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FUMH	E	0	0	0	0	0	0	0.056	0	0	0	0	0	0	0	0	0
FUMH	F	1	1	1	0.857	0.833	0.357	0.944	0	1	1	0.68	0.214	0	0	0.5	0.95
FUMH	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0
FUMH	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FUMH	I	0	0	0	0	0	0.179	0	0	0	0	0	0.036	0.167	0.071	0	0
FUMH	J	0	0	0	0	0	0	0	0	0	0	0.02	0	0	0	0	0
GAPD	n	18	9	11	10	10	10	8	10	12	7	19	15	9	6	9	22
GAPD	A	0.139	0.722	0.455	0	0.15	0.4	0.188	0.65	0.708	0.571	0.316	0.3	0.278	0	0.667	0.091
GAPD	B	0	0.222	0.091	0.1	0.1	0	0.813	0	0.292	0.357	0.184	0	0	0.583	0.222	0.023
GAPD	C	0	0.056	0	0	0.1	0	0	0.05	0	0	0.026	0.2	0.056	0.25	0	0
GAPD	D	0	0	0	0	0	0	0	0	0	0.071	0	0	0	0	0.056	0
GAPD	E	0	0	0	0	0.15	0	0	0	0	0	0.132	0.067	0	0	0	0.182
GAPD	F	0.806	0	0.455	0.3	0.3	0.6	0	0.3	0	0	0.105	0.267	0.667	0	0.056	0.227
GAPD	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GAPD	H	0.056	0	0	0.6	0	0	0	0	0	0	0.105	0.133	0	0	0	0.045
GAPD	I	0	0	0	0	0.2	0	0	0	0	0	0.053	0.033	0	0.083	0	0.205
GAPD	J	0	0	0	0	0	0	0	0	0	0	0.079	0	0	0.083	0	0.091
GAPD	K	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GAPD	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.136
AAT	n	18	14	20	15	10	10	18	14	15	10	24	15	7	6	10	20
AAT	A	0	0.107	0.45	0.267	0.45	0.7	0.556	0.179	0.433	0.95	0.313	0.1	0.071	0.25	1	0.175
AAT	B	0	0.036	0	0	0.1	0	0	0	0	0	0.042	0.033	0	0	0	0.475
AAT	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

		Appen-zell	Boubin	Höllental	Hirsch-bach	Kiental	Kralov1	Kralov2	La Lech-erette	Morgins	Nova Pec	Safien-R.	Tschier tschen	Hörnli	Vrin	Zamecek	Zastler
AAT	D	0	0	0	0	0.05	0	0	0.179	0	0	0.021	0.3	0.429	0	0	0.025
AAT	E	0	0	0	0	0	0	0	0	0	0	0	0.033	0.214	0	0	0
AAT	F	1	0.857	0.55	0.733	0.4	0.3	0.444	0.643	0.567	0.05	0.625	0.433	0.143	0.75	0	0.3
AAT	G	0	0	0	0	0	0	0	0	0	0	0	0.1	0.143	0	0	0.025
IDH1	n	18	10	20	19	19	10	10	12	17	10	28	16	9	8	6	20
IDH1	A	0.972	0.95	1	0.737	1	1	1	0.958	1	1	0.982	1	1	1	1	0.7
IDH1	B	0.028	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IDH1	C	0	0.05	0	0.237	0	0	0	0	0	0	0.018	0	0	0	0	0.175
IDH1	D	0	0	0	0.026	0	0	0	0	0	0	0	0	0	0	0	0
IDH1	E	0	0	0	0	0	0	0	0.042	0	0	0	0	0	0	0	0.075
IDH1	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.05
IDH2	n	13	9	19	18	16	5	10	5	16	10	13	4	8	6	12	18
IDH2	A	0.962	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.972
IDH2	B	0.038	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IDH2	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IDH2	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IDH2	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IDH2	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.028
LA1	n	18	16	20	20	18	10	18	10	22	10	28	9	5	8	15	25
LA1	A	0	0	0	0	0	0	0	0	0	0	0.071	0.444	0.3	0	0	0.02
LA1	B	0.833	0.906	0.85	0.9	0.917	0.9	0.972	0.7	0.773	0.5	0.786	0.5	0.1	1	0.533	0.78
LA1	C	0.111	0	0	0.05	0	0	0	0	0	0	0	0	0	0	0	0
LA1	D	0	0	0.025	0	0	0	0	0	0	0.05	0	0	0	0	0.233	0
LA1	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LA1	F	0.056	0.094	0.125	0.05	0.083	0.1	0	0.3	0.227	0.3	0.143	0.056	0.6	0	0.2	0.2
LA1	G	0	0	0	0	0	0	0.028	0	0	0.15	0	0	0	0	0.033	0
LA2	n	17	14	16	19	15	7	9	9	14	8	27	8	5	8	6	21
LA2	A	0	0	0.031	0	0	0	0	0	0	0	0	0	0	0	0	0.024
LA2	B	0	0.25	0.094	0	0.067	0.143	0.056	0	0	0	0.167	0	0.1	0	0	0.048
LA2	C	0.971	0.714	0.875	1	0.7	0.786	0.944	0.778	1	1	0.759	0.625	0.7	1	1	0.643
LA2	D	0	0.036	0	0	0.233	0.071	0	0.056	0	0	0.074	0.125	0.2	0	0	0.286

		Appen-zell	Bnubin	Höllental	Hirsch-bach	Kiental	Kralov1	Kralov2	La Lech-erette	Morgins	Nova Pec	Safien-R.	Tschier tschen	Hörnli	Vrin	Zamecek	Zastler
LA2	E	0	0	0	0	0	0	0	0.167	0	0	0	0.25	0	0	0	0
LA2	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LA2	G	0.029	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GPI	n	13	17	13	14	12	15	20	15	17	10	28	14	9	8	16	16
GPI	A	0	0	0	0	0.167	0.2	0.275	0	0	0.05	0.054	0.071	0.222	0.125	0	0.125
GPI	B	0	0.059	0.038	0	0.042	0	0	0	0	0	0	0	0	0.063	0	0
GPI	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GPI	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GPI	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.031
GPI	F	1	0.853	0.923	0.857	0.667	0.8	0.725	0.767	0.588	0.7	0.625	0.786	0.667	0.5	1	0.781
GPI	G	0	0.088	0.038	0.143	0.125	0	0	0.233	0.412	0.25	0.321	0.107	0.111	0.313	0	0.031
GPI	H	0	0	0	0	0	0	0	0	0	0	0	0.036	0	0	0	0.031
SOD1	n	18	10	20	18	14	10	15	10	20	8	26	11	5	5	6	26
SOD1	A	1	1	1	0.972	1	1	1	1	1	1	1	0.864	1	1	1	1
SOD1	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SOD1	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SOD1	D	0	0	0	0	0	0	0	0	0	0	0	0.045	0	0	0	0
SOD1	E	0	0	0	0.028	0	0	0	0	0	0	0	0	0	0	0	0
SOD1	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SOD1	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SOD1	H	0	0	0	0	0	0	0	0	0	0	0	0.091	0	0	0	0
SOD2	n	9	10	10	15	10	9	10	5	10	10	18	4	7	7	10	21
SOD2	A	0	0	0	0	0.3	0	0	0	0	0	0	0	0	0	0	0
SOD2	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SOD2	C	1	1	0.9	1	0.4	1	1	1	0.8	1	0.444	1	1	1	1	1
SOD2	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SOD2	E	0	0	0	0	0.15	0	0	0	0.15	0	0.25	0	0	0	0	0
SOD2	F	0	0	0.1	0	0	0	0	0	0	0	0.222	0	0	0	0	0
SOD2	G	0	0	0	0	0.15	0	0	0	0.05	0	0.083	0	0	0	0	0
TPI	n	4	5	20	6	12	10	10	5	18	10	14	7	4	8	14	9
TPI	A	0.125	0.8	0.975	0.917	0.833	0.4	0.5	0.4	0.778	0.15	0.464	0.571	0.5	0.938	0.25	1

		Appen-zell	Boubin	Höllental	Hirsch-bach	Kiental	Kralov1	Kralov2	La Lech-erette	Morgins	Nova Pec	Safien-R.	Tschier tschen	Hörnli	Vrin	Zamecek	Zastler
TPI	B	0	0	0	0	0.083	0	0	0	0	0	0.071	0.071	0	0	0	0
TPI	C	0	0	0.025	0	0	0	0	0	0.083	0	0.107	0.071	0	0	0	0
TPI	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.071	0
TPI	E	0	0	0	0	0	0	0	0	0	0	0	0.071	0	0.063	0	0
TPI	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TPI	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TPI	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TPI	I	0	0.2	0	0	0.042	0.2	0.5	0	0.139	0.85	0.286	0	0	0	0.679	0
TPI	J	0.75	0	0	0	0.042	0.3	0	0.5	0	0	0.071	0.071	0.25	0	0	0
TPI	K	0.125	0	0	0.083	0	0.1	0	0.1	0	0	0	0.143	0.25	0	0	0
FDH	n	18	18	20	24	19	15	20	15	23	10	28	16	9	8	16	26
FDH	A	1	0.861	1	0.938	0.947	0.133	0.225	1	0.978	1	0.964	1	0.889	0.938	1	1
FDH	B	0	0	0	0.042	0.026	0.867	0.775	0	0.022	0	0	0	0.111	0	0	0
FDH	C	0	0	0	0.021	0	0	0	0	0	0	0.036	0	0	0	0	0
FDH	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FDH	E	0	0.111	0	0	0.026	0	0	0	0	0	0	0	0	0.063	0	0
FDH	F	0	0.028	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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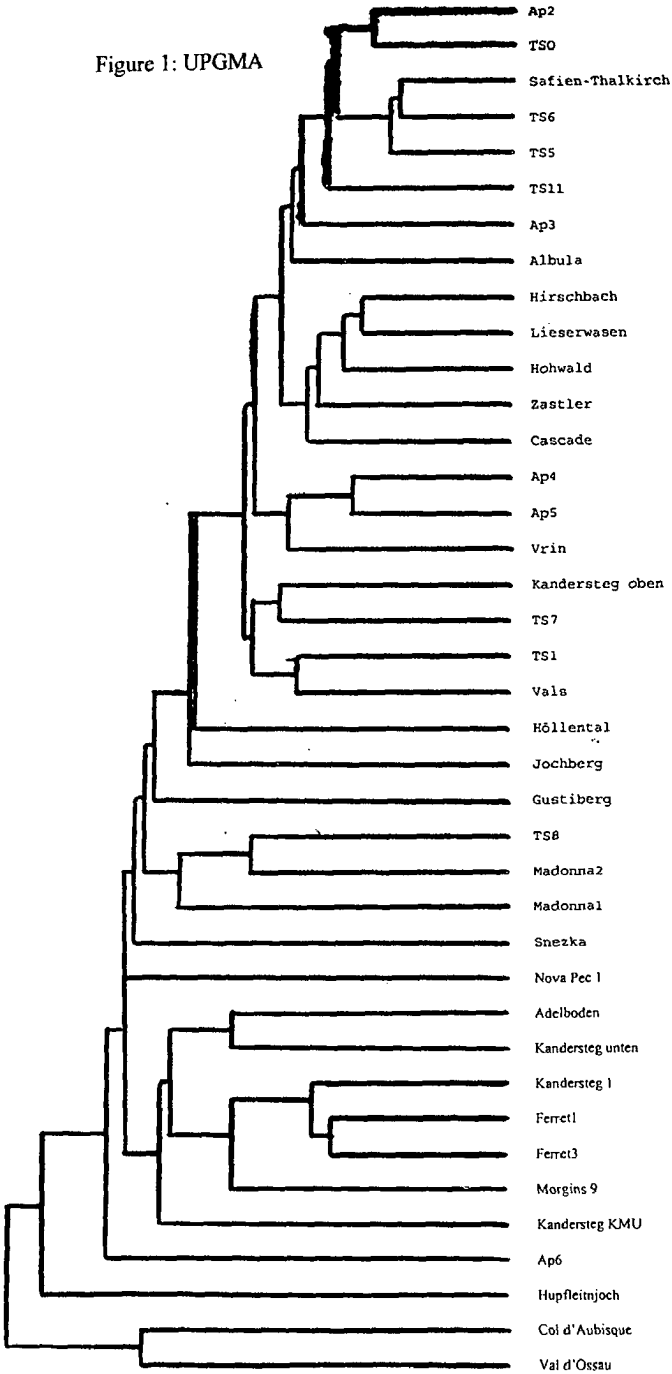
figure 1: UPGMA cluster analysis, based on modified Rogers distance, of a data set with all patches which were investigated for *O. cacaliae*. Names are based on the ones given in the map (figure 1) of chapter III, but this analysis contains some patches which were omitted from the final analysis due to very small sample size. Please note the cluster of the Pyrenees patches Col d'Aubisque and Val d'Ossau.

Figure 1: UPGMA



Appendix D

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in Appendix D you find:

table 1: Allelefrequencies as used as data set in chapter III for *O. cacaliae*. The names are the same as in the map (figure 1) of chapter III, except for the abbreviation TS for Tschiertschen. Numbers refer to different patches.

figure 1: Regression of pairwise estimates of dispersal against pairwise geographical distances for all *O. cacaliae* - samples. It is obvious that the isolation by distance effect can be observed only beyond distances between 50 and 150 km.

figure 2: Regression of pairwise estimates of dispersal against pairwise geographical distances for the loca scale ("fine scale structure" in chapter III) in a) Appenzell, b) Kandersteg and c) Tschiertschen. In Appenzell there is a trend for isolation by distance as could be noted already from the results of the Mantel test in chapter III.

figure 3: A map of the allelefrequencies at on locus - ACONH.

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Table 1: Allelefrequencies as used as data set in chapter III for *O. cacaliae*. The names are the same as in the map (figure 1) of chapter III, except for the abbreviation TS for Tschiertschen. Numbers refer to different patches.

		Appen-zell 1	Appen-zell2	Appen-zell 3	Appen-zell 4	Appen-zell 5	Höllen-tal	Hirsch-bach	Hupf-leitn	Joch-berg	Adel-boden	Kander-steg1	Kanders-teg2	Kander-steg3	Kander-steg4	Mor-gins	Val Ferret1	Val Ferret2	Ålbu-la	Safien-T.
ACON	N	21	32	14	11	5	18	14	2	5	8	18	9	47	9	8	16	23	9	42
ACON	A	0.643	0.781	0.75	0.682	0.9	1	0.893	1	1	0.063	0.139	0.222	0.34	0.278	0.063	0.094	0.152	0.944	0.881
ACON	B	0.143	0.125	0.179	0.227	0	0	0.107	0	0	0	0	0.333	0.181	0	0.25	0	0	0	0
ACON	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.022	0.056	0
ACON	D	0.19	0.094	0.071	0.091	0.1	0	0	0	0	0.938	0.861	0.444	0.479	0.722	0.688	0.906	0.826	0	0.119
ACON	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ACON	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ACON	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ACON	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ACON	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ACON	J	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ACON	K	0.024	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
aGDH	N	18	21	6	7	7	19	17	6	5	15	16	10	42	13	10	16	14	11	41
aGDH	A	0.556	0.286	0.917	0.714	0.714	0.526	0.559	0.333	1	0.667	0.656	0.5	0.619	0.308	0.55	0.625	0.393	0.773	0.732
aGDH	B	0.083	0.405	0.083	0	0	0.395	0.029	0	0	0	0.031	0.05	0.071	0.231	0.1	0.219	0.357	0	0.146
aGDH	C	0.139	0.071	0	0.286	0.286	0.053	0.265	0.667	0	0.233	0	0.25	0.262	0.385	0.2	0.063	0	0.182	0.085
aGDH	D	0	0	0	0	0	0.026	0.059	0	0	0.033	0.156	0	0.012	0	0	0.063	0.107	0	0.012
aGDH	E	0.056	0.238	0	0	0	0	0.029	0	0	0	0.125	0.1	0	0	0	0	0	0.045	0.024
aGDH	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
aGDH	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
aGDH	H	0	0	0	0	0	0	0	0	0	0.067	0	0	0.012	0	0	0	0	0	0
aGDH	I	0	0	0	0	0	0	0	0	0	0	0.031	0	0	0	0	0	0	0	0
aGDH	J	0	0	0	0	0	0	0.029	0	0	0	0	0	0	0	0	0	0	0	0
aGDH	K	0.167	0	0	0	0	0	0.029	0	0	0	0	0.1	0.012	0.038	0.15	0.031	0.143	0	0
aGDH	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0.038	0	0	0	0	0
aGDH	M	0	0	0	0	0	0	0	0	0	0	0	0	0.012	0	0	0	0	0	0
AO-1	N	24	25	15	6	7	16	16	6	5	16	6	10	37	5	9	10	11	18	30
AO-1	A	0.771	0.72	0.8	0.917	0.929	0.781	0.688	1	0.6	0.844	1	0.75	0.703	0.8	0.833	0.6	0.864	0.778	0.833
AO-1	B	0.229	0.26	0.167	0.083	0.071	0.219	0.313	0	0.1	0.156	0	0.25	0.297	0.2	0.167	0.35	0.136	0.222	0.167
AO-1	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AO-1	D	0	0.02	0.033	0	0	0	0	0	0	0	0	0	0	0	0	0.05	0	0	0
AO-1	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AO-1	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AO-1	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AO-1	H	0	0	0	0	0	0	0	0	0.3	0	0	0	0	0	0	0	0	0	0
Arg3	N	6	11	13	10	9	15	10	6	3	16	18	10	35	13	10	15	14	16	33
Arg3	A	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0.906	0
Arg3	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg3	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.094	0
Arg3	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DDH1	N	11	32	13	20	3	12	10	4	5	13	8	10	28	14	10	16	19	11	15
DDH1	A	0.364	0.469	0.385	0.3	0	0.375	0.5	1	0.8	0.308	0.938	0.4	0.679	0.179	0.35	0.875	0.684	0.545	0.667

		Appen-zell 1	Appen-zell2	Appen-zell 3	Appen-zell 4	Appen-zell 5	Höllen-tal	Hirsch-bach	Hupf-leitn	Joch-berg	Adel-boden	Kander-steg1	Kanders-teg2	Kander-steg3	Kander-steg4	Mor-gins	Val Ferret1	Val Ferret2	Älbu-la	Safien-T.
DDH1	B	0.364	0.422	0.615	0.625	1	0.583	0.45	0	0.2	0.423	0.063	0.4	0.304	0.607	0.55	0.125	0.158	0.409	0.233
DDH1	C	0.227	0.016	0	0	0	0	0	0	0	0	0	0	0.018	0	0.1	0	0	0	0
DDH1	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DDH1	E	0.045	0.094	0	0.075	0	0.042	0.05	0	0	0.269	0	0.2	0	0	0	0	0.158	0.045	0.1
DDH1	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0.214	0	0	0	0	0
FDH	N	13	21	8	13	6	7	15	6	5	7	15	8	42	10	10	15	12	5	24
FDH	A	0.538	0.429	0.5	0.5	0.667	0.786	0.733	0.75	0.8	0.429	0.567	0.25	0.381	0.55	0.45	0.333	0.458	0.6	0.563
FDH	B	0.038	0.024	0.125	0.038	0.083	0	0	0	0	0.429	0.1	0.313	0.333	0.1	0.3	0.333	0.167	0	0.042
FDH	C	0.423	0.381	0.375	0.462	0.25	0.214	0.267	0.25	0.2	0.143	0.333	0.438	0.262	0.35	0.25	0.333	0.375	0.4	0.396
FDH	D	0	0.167	0	0	0	0	0	0	0	0	0	0	0.024	0	0	0	0	0	0
FUM	N	24	32	15	12	5	14	17	6	5	15	16	9	41	15	5	16	24	8	42
FUM	A	0	1	1	0.917	0.9	1	0.882	1	1	0.933	0.969	1	0.976	0.9	1	0.969	1	1	0.988
FUM	B	0	0	0	0	0	0	0	0	0	0	0	0	0.012	0	0	0	0	0	0
FUM	C	0	0	0	0	0.1	0	0	0	0	0.067	0	0	0.012	0	0	0.031	0	0	0
FUM	D	0	0	0	0.042	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0
FUM	E	0	0	0	0.042	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FUM	F	0	0	0	0	0	0	0.059	0	0	0	0	0	0	0	0	0	0	0	0
FUM	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FUM	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FUM	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.012
FUM	J	0	0	0	0	0	0	0.059	0	0	0	0.031	0	0	0	0	0	0	0	0
G3PD	N	23	32	8	8	9	18	17	6	5	16	12	10	47	15	6	16	19	11	42
G3PD	A	0.783	0.672	0.563	0.313	0.333	0	0.912	0	0.4	0.438	0.833	0.15	0.457	0.533	0.833	1	0.711	0.591	0.714
G3PD	B	0.087	0.031	0	0.125	0.222	0	0	0.25	0.4	0.25	0.125	0.1	0.096	0.333	0.083	0	0.132	0.091	0.095
G3PD	C	0.022	0.016	0	0	0.111	0.083	0	0	0	0.313	0	0.2	0.096	0	0	0	0.026	0.045	0.012
G3PD	D	0	0	0	0	0	0	0	0.25	0	0	0	0.25	0.032	0	0.083	0	0.053	0	0.024
G3PD	E	0	0.016	0	0.063	0.056	0.028	0	0.5	0.2	0	0.042	0.3	0.053	0	0	0	0	0.091	0.036
G3PD	F	0.087	0.266	0.438	0.5	0.111	0.111	0.088	0	0	0	0	0	0.181	0	0	0	0.053	0.182	0.119
G3PD	G	0.022	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G3PD	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G3PD	I	0	0	0	0	0.056	0.306	0	0	0	0	0	0	0.064	0.133	0	0	0.026	0	0
G3PD	J	0	0	0	0	0.111	0	0	0	0	0	0	0	0.011	0	0	0	0	0	0
G3PD	K	0	0	0	0	0	0	0	0	0	0	0	0	0.011	0	0	0	0	0	0
G3PD	L	0	0	0	0	0	0.472	0	0	0	0	0	0	0	0	0	0	0	0	0
GOT	N	24	31	15	23	6	23	9	6	5	23	18	10	45	3	10	10	23	19	42
GOT	A	0.5	0.516	0.467	0.413	0.083	0.804	0.944	0.167	0.8	0.609	0.389	0.45	0.4	0.667	0.45	0.4	0.348	0.447	0.488
GOT	B	0.083	0	0.233	0.348	0.667	0	0	0	0	0.239	0	0	0	0.167	0	0	0	0.053	0.167
GOT	C	0.25	0.484	0.3	0.152	0.25	0.174	0	0	0.2	0	0.611	0.55	0.6	0.167	0.4	0.6	0.652	0.474	0.321
GOT	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GOT	E	0.042	0	0	0.087	0	0	0	0	0	0	0	0	0	0	0	0	0	0.026	0.024
GOT	F	0.125	0	0	0	0	0.022	0.056	0.833	0	0.152	0	0	0	0	0.15	0	0	0	0
IDH2	N	12	26	13	7	11	6	12	4	5	8	13	10	40	20	2	15	5	13	23
IDH2	A	0	1	1	1	1	1	1	1	1	0	0.923	0.8	1	1	1	1	1	0.923	0
IDH2	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IDH2	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IDH2	D	0	0	0	0	0	0	0	0	0	0	0.077	0.2	0	0	0	0	0	0	0

		Appen-zell 1	Appen-zell2	Appen-zell 3	Appen-zell 4	Appen-zell 5	Höllen-tal	Hirsch-bach	Hupf-leitn	Joch-berg	Adel-boden	Kander-steg1	Kanders-teg2	Kander-steg3	Kander-steg4	Mor-gins	Val Ferret1	Val Ferret2	Älbu-la	Safien-T.
IDH2	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.077	0
IDH2	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LAI	N	18	32	13	25	11	23	17	6	5	23	12	10	52	20	10	15	19	18	42
LAI	A	0.917	1	1	0.9	0.955	1	0.941	1	1	0.891	1	1	0.962	0.9	0.8	0.967	0.947	0.917	0
LAI	B	0.083	0	0	0	0.045	0	0	0	0	0	0	0	0	0	0.15	0.033	0.053	0	0
LAI	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LAI	D	0	0	0	0.1	0	0	0.059	0	0	0.109	0	0	0.038	0.1	0.05	0	0	0	0
LAI	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LAI	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.083	0
LAI	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ME	N	22	27	8	21	10	11	12	5	4	7	18	10	37	18	10	16	24	9	41
ME	A	0.795	0.685	0.875	0.619	0.4	0.636	0.792	0.7	0.375	0.643	0.639	0.4	0.851	0.278	0.8	0.625	0.583	0.5	0.707
ME	B	0.068	0.259	0	0.167	0	0.364	0.125	0.2	0.25	0.286	0	0.05	0.068	0.639	0	0.156	0.229	0.111	0.159
ME	C	0.068	0.019	0	0.048	0.25	0	0	0	0	0	0.083	0	0.027	0.083	0.05	0	0.063	0	0
ME	D	0	0	0	0	0	0	0.042	0.1	0	0	0.028	0	0	0	0	0	0	0	0
ME	E	0	0	0	0.167	0.1	0	0	0	0	0	0.056	0	0.014	0	0	0.094	0	0	0
ME	F	0	0.037	0	0	0.25	0	0	0	0.375	0	0.111	0	0	0	0	0	0	0	0
ME	G	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0.333	0.11
ME	H	0	0	0	0	0	0	0	0	0	0	0	0	0.027	0	0	0	0	0	0
ME	I	0	0	0.063	0	0	0	0	0	0	0	0	0	0	0	0	0	0.021	0	0
ME	J	0.068	0	0.063	0	0	0	0.042	0	0	0	0.083	0.45	0.014	0	0.15	0.125	0.104	0.056	0.024
ME	K	0	0	0	0	0	0	0	0	0	0.071	0	0	0	0	0	0	0	0	0
ME	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PGI	N	12	13	8	17	9	23	12	6	5	23	18	10	31	19	10	16	24	11	26
PGI	A	0	1	1	1	0.889	1	1	1	1	0.957	1	1	0.935	1	1	0.844	0.938	1	0
PGI	B	0	0	0	0	0.056	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PGI	C	0	0	0	0	0.056	0	0	0	0	0	0	0	0.065	0	0	0.156	0	0	0
PGI	D	0	0	0	0	0	0	0	0	0	0.043	0	0	0	0	0	0	0	0	0
PGI	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PGI	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.063	0	0
PGI	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PGI	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PGI	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PGI	J	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SOD2	N	13	18	12	11	7	9	6	4	5	8	16	8	30	8	9	5	11	16	24
SOD2	A	0.769	0.778	0.958	1	0.571	0.611	0.75	0.875	1	0.375	0.656	0.25	0.783	0.625	0.444	0.8	0.682	0.469	0.729
SOD2	B	0.154	0.167	0	0	0.429	0	0	0	0	0	0	0.063	0.083	0	0.444	0	0	0	0
SOD2	C	0.038	0	0	0	0	0	0.167	0	0	0	0	0.25	0	0	0	0	0	0.125	0.042
SOD2	D	0.038	0.056	0.042	0	0	0.389	0.083	0.125	0	0.625	0.344	0.188	0.133	0.375	0.111	0.2	0.136	0.031	0.229
SOD2	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.375	0
SOD2	F	0	0	0	0	0	0	0	0	0	0	0	0.188	0	0	0	0	0	0	0
SOD2	G	0	0	0	0	0	0	0	0	0	0	0	0.063	0	0	0	0	0	0	0
SOD2	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.182	0	0
TPI	N	19	27	8	7	9	18	12	5	5	8	15	10	41	15	10	16	24	17	42
TPI	A	0.868	0.926	1	1	0.889	0.917	0.792	0.6	0.6	0.813	0.633	0.7	0.976	1	0.6	0.906	0.771	0.853	0.917
TPI	B	0	0.019	0	0	0	0	0.125	0.2	0.2	0.063	0.133	0	0	0	0	0.063	0.083	0	0

		Appen-zell 1	Appen-zell2	Appen-zell 3	Appen-zell 4	Appen-zell 5	Höllen-tal	Hirsch-bach	Hupf-leitn	Joch-berg	Adel-boden	Kander-steg1	Kanders-teg2	Kander-steg3	Kander-steg4	Mor-gins	Val Ferret1	Val Ferret2	Älbu-la	Safien-T.
TPI	C	0.026	0.056	0	0	0	0.083	0.083	0.2	0.2	0	0.067	0	0.012	0	0.05	0	0.083	0.059	0.012
TPI	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.029	0
TPI	E	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0.042	0.059	0
TPI	F	0.053	0	0	0	0	0	0	0	0	0	0.133	0.2	0	0	0.25	0	0	0	0
TPI	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.031	0	0	0
TPI	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.012
TPI	I	0.053	0	0	0	0.111	0	0	0	0	0.125	0.033	0	0.012	0	0.1	0	0.021	0	0.06
IDH1	N	24	32	15	25	11	24	17	6	5	23	18	10	52	20	10	16	24	19	42
IDH1	A	1	1	1	1	1	1	1	1	1	0	1	1	1	0.9	1	0.969	1	1	0
IDH1	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IDH1	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0.031	0	0	0
IDH1	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IDH1	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IDH1	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IDH1	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

		TS1	TS2	TS3	TS4	TS5	TS6	TS7	Vals	Vrin	Col d'Aubisque	Val d'Ossau	Snezka	Zastler	Madonna	Nova Pec	Cascade	Lieser-wasen2	Hohwald	Lieser-wasen1
ACON	N	19	22	27	29	18	15	24	25	8	29	2	26	25	25	6	26	4	26	113
ACON	A	0.763	0.841	0.852	0.897	0.667	0.967	0.688	1	0.5	0.414	0	0.962	0.88	1	1	0.942	0.5	0.962	0.854
ACON	B	0.184	0	0	0	0	0	0.063	0	0.5	0.569	0.75	0	0	0	0	0.038	0.5	0	0.137
ACON	C	0	0	0	0	0	0.033	0	0	0	0	0.25	0.038	0	0	0	0.019	0	0.019	0.009
ACON	D	0.053	0.159	0.148	0.103	0.306	0	0.25	0	0	0	0	0	0.12	0	0	0	0	0.019	0
ACON	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ACON	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ACON	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ACON	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ACON	I	0	0	0	0	0	0	0	0	0	0.017	0	0	0	0	0	0	0	0	0
ACON	J	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ACON	K	0	0	0	0	0.028	0	0	0	0	0	0	0	0	0	0	0	0	0	0
aGDH	N	25	23	34	29	18	7	24	27	14	35	7	26	20	20	11	22	6	24	124
aGDH	A	0.78	0.739	0.897	0.707	0.722	0.429	0.438	0.852	0.464	0.886	1	0.481	0.675	0.575	1	0.727	0.417	0.729	0.569
aGDH	B	0.06	0	0.103	0.034	0	0.286	0.146	0.019	0.25	0.029	0	0.269	0.025	0.375	0	0.159	0	0.125	0.125
aGDH	C	0.1	0.152	0	0.155	0.111	0	0.083	0.13	0.179	0	0	0.115	0.125	0	0	0.068	0.417	0.125	0.226
aGDH	D	0.02	0.065	0	0.052	0.056	0.214	0.063	0	0.071	0	0	0.115	0.125	0.05	0	0.023	0.167	0	0.036
aGDH	E	0.04	0.022	0	0.052	0.111	0.071	0.063	0	0	0	0	0	0	0	0	0.023	0	0.021	0.016
aGDH	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.008
aGDH	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
aGDH	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.012
aGDH	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
aGDH	J	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
aGDH	K	0	0	0	0	0	0	0.208	0	0.036	0.086	0	0.019	0.05	0	0	0	0	0	0.004
aGDH	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.004
aGDH	M	0	0.022	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AO-1	N	24	9	25	13	6	17	22	31	15	25	6	15	15	20	5	21	6	24	128
AO-1	A	0.854	1	0.92	0.731	0.5	0.529	0.727	0.645	0.867	0.98	0.583	0.6	0.533	0.975	1	0.833	0.833	0.854	0.777

		TS1	TS2	TS3	TS4	TS5	TS6	TS7	Vals	Vrin	Col d'Aubisque	Val d'Ossau	Snezka	Zastler	Madonna	Nova Pec	Cascade	Lieser-wasen2	Hohwald	Lieser-wasen1
AO-1	B	0.146	0	0.08	0.269	0.5	0.471	0.273	0.355	0.133	0.02	0.417	0.4	0.4	0.025	0	0.167	0.167	0.146	0.219
AO-1	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AO-1	D	0	0	0	0	0	0	0	0	0	0	0	0	0.067	0	0	0	0	0	0.004
AO-1	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AO-1	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AO-1	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AO-1	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg3	N	8	14	28	29	11	7	26	7	7	25	7	16	25	20	7	9	2	17	91
Arg3	A	1	1	1	1	1	1	0.962	1	0	1	1	1	1	1	1	1	0	1	0.989
Arg3	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.011
Arg3	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg3	D	0	0	0	0	0	0	0.038	0	0	0	0	0	0	0	0	0	0	0	0
DDH1	N	15	22	20	29	18	18	26	14	9	30	7	26	25	15	6	12	2	12	100
DDH1	A	0.6	0.386	0.65	0.603	0.583	0.417	0.596	0.536	0.5	0.817	0.286	0.962	0.76	0	1	0.625	0.5	0.417	0.66
DDH1	B	0.333	0.568	0.35	0.397	0.194	0.583	0.346	0.393	0.444	0.117	0	0	0.18	1	0	0.375	0.5	0.583	0.31
DDH1	C	0	0	0	0	0	0	0.058	0	0	0	0	0.019	0	0	0	0	0	0	0
DDH1	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DDH1	E	0	0.023	0	0	0.222	0	0	0.036	0.056	0.067	0.714	0.019	0.06	0	0	0	0	0	0.025
DDH1	F	0.067	0.023	0	0	0	0	0	0.036	0	0	0	0	0	0	0	0	0	0	0.005
FDH	N	18	23	27	28	18	1	23	22	12	30	7	25	24	17	7	8	2	26	81
FDH	A	0.778	0.5	0.611	0.589	0.639	1	0.565	0.432	0.458	0.967	1	0.98	0.563	0.882	0.286	0.25	0	0.596	0.63
FDH	B	0.083	0.087	0	0.054	0.056	0	0.043	0.068	0.083	0	0	0	0.125	0.029	0	0	0	0.038	0.049
FDH	C	0.139	0.413	0.389	0.357	0.306	0	0.391	0.477	0.458	0.033	0	0.02	0.313	0.088	0.143	0.75	0	0.365	0.321
FDH	D	0	0	0	0	0	0	0	0.023	0	0	0	0	0	0	0.571	0	0	0	0
FUM	N	20	23	27	29	18	25	26	27	15	35	7	16	25	20	13	23	6	31	127
FUM	A	0.95	1	1	1	1	0.82	0.904	1	0	1	1	0.969	1	1	0.962	0.891	0.917	0.935	1
FUM	B	0	0	0	0	0	0.12	0.038	0	0	0	0	0	0	0	0	0	0	0.032	0
FUM	C	0.025	0	0	0	0	0.02	0	0	0	0	0	0	0	0	0.038	0.109	0	0	0
FUM	D	0	0	0	0	0	0.02	0.038	0	0	0	0	0.031	0	0	0	0	0.083	0	0
FUM	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.032	0
FUM	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FUM	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FUM	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FUM	I	0.025	0	0	0	0	0	0.019	0	0	0	0	0	0	0	0	0	0	0	0
FUM	J	0	0	0	0	0	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0
G3PD	N	21	23	34	29	18	24	19	33	17	35	7	21	25	20	12	30	6	29	123
G3PD	A	0.143	0.565	0.838	0.828	0.556	0.313	0.789	0.212	0.353	0.543	0.071	0.786	0.92	0.825	0.417	0.9	0.333	0.655	0.837
G3PD	B	0.071	0.261	0.088	0.017	0.306	0.167	0.132	0.197	0.029	0.129	0	0.19	0.06	0.125	0.042	0.083	0	0.207	0.081
G3PD	C	0.143	0	0.029	0.052	0	0.313	0.026	0.061	0.147	0.043	0.071	0.024	0	0.05	0.542	0	0.083	0.052	0.028
G3PD	D	0.095	0.087	0	0	0.139	0.042	0	0.106	0.176	0.043	0	0	0.02	0	0	0	0.417	0.034	0
G3PD	E	0.024	0.022	0	0	0	0	0	0.015	0.088	0	0	0	0	0	0	0	0.083	0	0.004
G3PD	F	0.262	0.043	0.044	0.103	0	0.083	0.053	0.409	0.176	0.2	0.857	0	0	0	0	0.017	0	0.052	0.037
G3PD	G	0	0	0	0	0	0	0	0	0.029	0.029	0	0	0	0	0	0	0	0	0
G3PD	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G3PD	I	0.119	0.022	0	0	0	0.042	0	0	0	0.014	0	0	0	0	0	0	0.083	0	0.012
G3PD	J	0.095	0	0	0	0	0.042	0	0	0	0	0	0	0	0	0	0	0	0	0

		TS1	TS2	TS3	TS4	TS5	TS6	TS7	Vals	Vrin	Col d'Aubisque	Val d'Ossau	Snezka	Zastler	Madonna	Nova Pec	Cascade	Lieser-wasen2	Hohwald	Lieser-wasen1
G3PD	K	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G3PD	L	0.048	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GOT	N	18	23	28	29	18	15	22	29	15	30	7	26	25	25	6	27	6	26	122
GOT	A	0.361	0.478	0.571	0.466	0.306	0.333	0.409	0.31	0.533	0	0	0.173	0.78	0.02	0.917	0.889	0.917	0.981	0.902
GOT	B	0	0	0	0.052	0	0.2	0	0	0	0	0	0	0.06	0	0	0.074	0	0	0.049
GOT	C	0.472	0.261	0.321	0.397	0.583	0.467	0.409	0.517	0.4	1	1	0.827	0.16	0.98	0.083	0.037	0.083	0.019	0.049
GOT	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GOT	E	0.111	0.065	0.107	0.086	0.056	0	0.068	0.172	0.033	0	0	0	0	0	0	0	0	0	0
GOT	F	0.056	0.196	0	0	0.056	0	0.114	0	0.033	0	0	0	0	0	0	0	0	0	0
IDH2	N	13	19	33	21	11	6	11	22	10	30	7	16	12	20	13	4	5	16	121
IDH2	A	1	0.947	1	0.976	1	1	1	1	0	1	0.929	1	1	0.7	1	1	0.9	0.969	1
IDH2	B	0	0.053	0	0	0	0	0	0	0	0	0	0	0	0.275	0	0	0	0.031	0
IDH2	C	0	0	0	0	0	0	0	0	0	0	0.071	0	0	0	0	0	0	0	0
IDH2	D	0	0	0	0.024	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0
IDH2	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IDH2	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0.025	0	0	0	0	0
LA1	N	20	23	26	21	13	27	25	33	16	35	7	21	25	20	13	27	6	27	121
LA1	A	0.975	1	1	0.952	1	0.889	1	1	0	0.943	1	0.929	1	0.975	0.962	0.926	0.917	0.907	0.719
LA1	B	0	0	0	0	0	0	0	0	0	0	0	0.071	0	0.025	0	0	0	0	0.017
LA1	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LA1	D	0.025	0	0	0.048	0	0.111	0	0	0	0.043	0	0	0	0	0.038	0.074	0	0.093	0.26
LA1	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LA1	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.004
LA1	G	0	0	0	0	0	0	0	0	0	0.014	0	0	0	0	0	0	0.083	0	0
ME	N	15	23	20	29	18	18	26	23	12	35	6	26	25	20	6	23	5	18	98
ME	A	0.9	0.717	0.975	0.793	0.528	0.806	0.635	0.804	0.542	0.5	0.667	0.865	0.46	0.825	0.833	0.435	0.3	0.5	0.633
ME	B	0.1	0.065	0.025	0.069	0.139	0.167	0.038	0.13	0.042	0.371	0.333	0.019	0.08	0.075	0	0.239	0.2	0.278	0.148
ME	C	0	0.022	0	0.052	0	0	0.154	0	0.292	0.014	0	0	0.04	0.025	0	0.13	0.1	0.111	0.092
ME	D	0	0	0	0	0	0.028	0	0.043	0	0	0	0	0.12	0	0.167	0	0	0.028	0.026
ME	E	0	0	0	0	0.056	0	0	0	0.083	0.043	0	0	0.02	0	0	0	0.2	0.028	0
ME	F	0	0.022	0	0	0	0	0.038	0	0	0	0	0	0.04	0	0	0.087	0	0	0.005
ME	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.005
ME	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0
ME	I	0	0	0	0	0.139	0	0	0.022	0	0	0	0	0	0	0	0.065	0	0.028	0.005
ME	J	0	0.174	0	0.086	0.139	0	0.058	0	0	0.043	0	0.115	0.24	0.075	0	0.043	0	0.028	0.077
ME	K	0	0	0	0	0	0	0.038	0	0.042	0.029	0	0	0	0	0	0	0.1	0	0.01
ME	L	0	0	0	0	0	0	0.038	0	0	0	0	0	0	0	0	0	0	0	0
PGI	N	25	9	27	21	12	21	25	23	17	35	7	26	20	25	13	25	7	25	108
PGI	A	0.96	1	1	1	1	0.952	0.94	0.935	0	0.857	0.929	1	1	1	1	0.96	0.929	1	0.968
PGI	B	0.02	0	0	0	0	0	0	0	0	0.086	0	0	0	0	0	0	0	0	0
PGI	C	0	0	0	0	0	0.024	0	0.043	0	0	0	0	0	0	0	0.02	0	0	0.019
PGI	D	0	0	0	0	0	0.024	0.02	0.022	0	0	0	0	0	0	0	0.02	0.071	0	0.009
PGI	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PGI	F	0.02	0	0	0	0	0	0	0	0	0.057	0.071	0	0	0	0	0	0	0	0
PGI	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PGI	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

		TS1	TS2	TS3	TS4	TS5	TS6	TS7	Vals	Vrin	Col d'Aubisque	Val d'Ossau	Snezka	Zastler	Madonna	Nova Pec	Cascade	Lieser-wasen2	Hohwald	Lieser-wasen1
PGI	**I**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.005
PGI	**J**	0	0	0	0	0	0	0.04	0	0	0	0	0	0	0	0	0	0	0	0
SOD2	**N**	14	14	28	22	18	5	22	11	12	23	7	26	21	10	6	7	6	15	96
SOD2	**A**	0.357	0.536	0.679	0.591	0.639	0.3	0.614	0.682	0.917	0.978	0.929	1	0.714	0.45	1	0.857	0	0.733	0.958
SOD2	**B**	0	0	0.089	0	0	0	0.091	0	0	0	0	0	0	0	0	0	0	0	0
SOD2	**C**	0.393	0	0.071	0	0	0.5	0.023	0	0.083	0	0	0	0.048	0.5	0	0	0	0	0
SOD2	**D**	0.25	0.464	0.161	0.409	0.361	0.2	0.227	0.318	0	0.022	0.071	0	0.238	0.05	0	0.143	0	0.267	0.042
SOD2	**E**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SOD2	**F**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SOD2	**G**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SOD2	**H**	0	0	0	0	0	0	0.045	0	0	0	0	0	0	0	0	0	0	0	0
TPI	**N**	25	14	34	29	18	17	26	23	12	30	7	21	25	25	6	28	3	31	114
TPI	**A**	0.86	0.929	1	1	0.806	0.971	0.692	0.891	0	0.867	0.786	0.548	0.84	0.96	1	1	0.667	0.839	0.974
TPI	**B**	0	0.071	0	0	0	0	0	0.022	0	0	0.143	0.452	0	0.02	0	0	0	0	0
TPI	**C**	0	0	0	0	0.028	0	0.077	0	0	0.017	0	0	0.02	0	0	0	0	0.081	0.013
TPI	**D**	0	0	0	0	0	0	0.038	0	0	0	0	0	0	0	0	0	0.167	0	0
TPI	**E**	0.1	0	0	0	0	0	0	0	0	0.017	0	0	0.1	0	0	0	0	0.065	0.013
TPI	**F**	0.02	0	0	0	0	0	0.115	0	0	0	0	0	0	0	0	0	0.167	0	0
TPI	**G**	0	0	0	0	0.028	0	0	0	0	0.033	0.071	0	0	0.02	0	0	0	0	0
TPI	**H**	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TPI	**I**	0	0	0	0	0.139	0.029	0.077	0.087	0	0.067	0	0	0.04	0	0	0	0	0.016	0
IDH1	**N**	25	23	34	29	18	27	26	33	17	35	7	26	25	25	12	31	6	31	139
IDH1	**A**	1	1	1	1	1	1	1	1	0	0.271	0.571	1	1	1	1	1	0	1	0.989
IDH1	**B**	0	0	0	0	0	0	0	0	0	0.629	0.214	0	0	0	0	0	0	0	0.007
IDH1	**C**	0	0	0	0	0	0	0	0	0	0.014	0.143	0	0	0	0	0	0	0	0
IDH1	**D**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IDH1	**E**	0	0	0	0	0	0	0	0	0	0.014	0.071	0	0	0	0	0	0	0	0.004
IDH1	**F**	0	0	0	0	0	0	0	0	0	0.014	0	0	0	0	0	0	0	0	0
IDH1	**G**	0	0	0	0	0	0	0	0	0	0.057	0	0	0	0	0	0	0	0	0

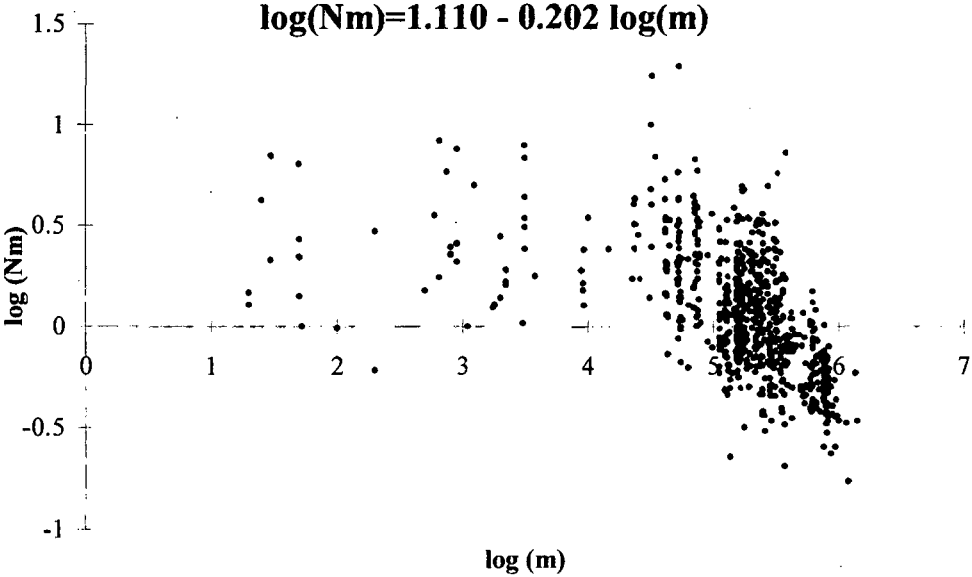


figure 1: Regression of pairwise estimates of dispersal against pairwise geographical distances for all *O. cacaliae* - samples. It is obvious that the isolation by distance effect can be observed only beyond distances between 50 and 150 km.

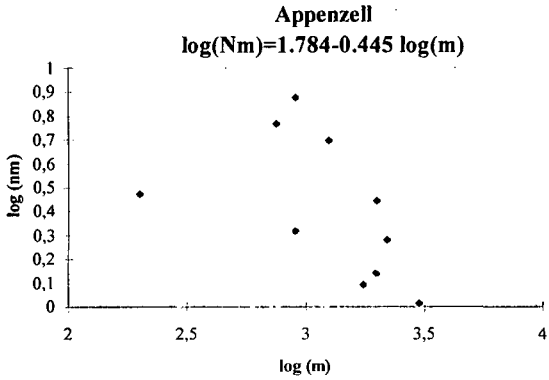


figure 2a: Regression of pairwise estimates of dispersal against pairwise geographical distances for the loca scale ("fine scale structure" in chapter III) in a) Appenzell. In Appenzell there is a trend for isolation by distance as could be noted already from the results of the Mantel test in chapter III.

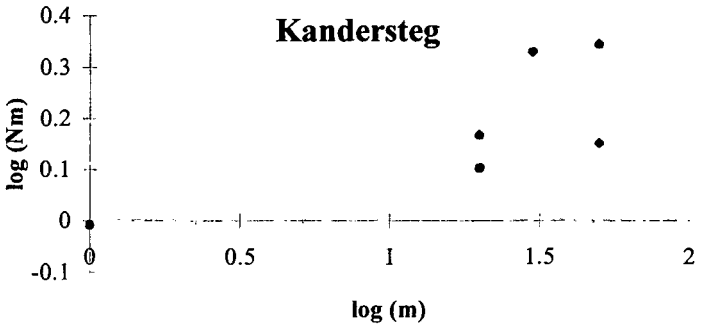


figure 2b: Regression of pairwise estimates of dispersal against pairwise geographical distances for the local scale ("fine scale structure" in chapter III) in Kandersteg

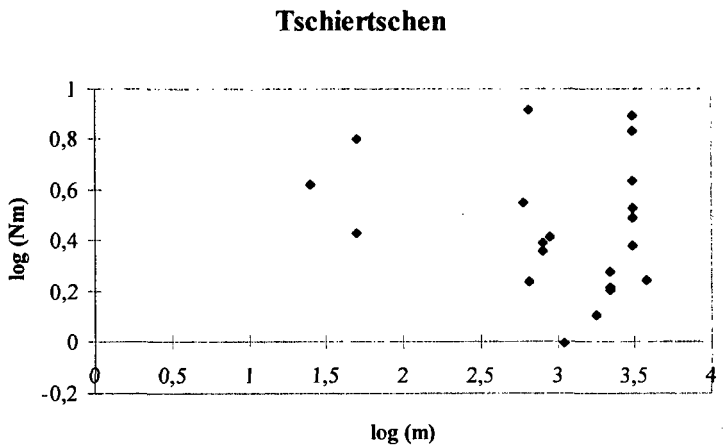
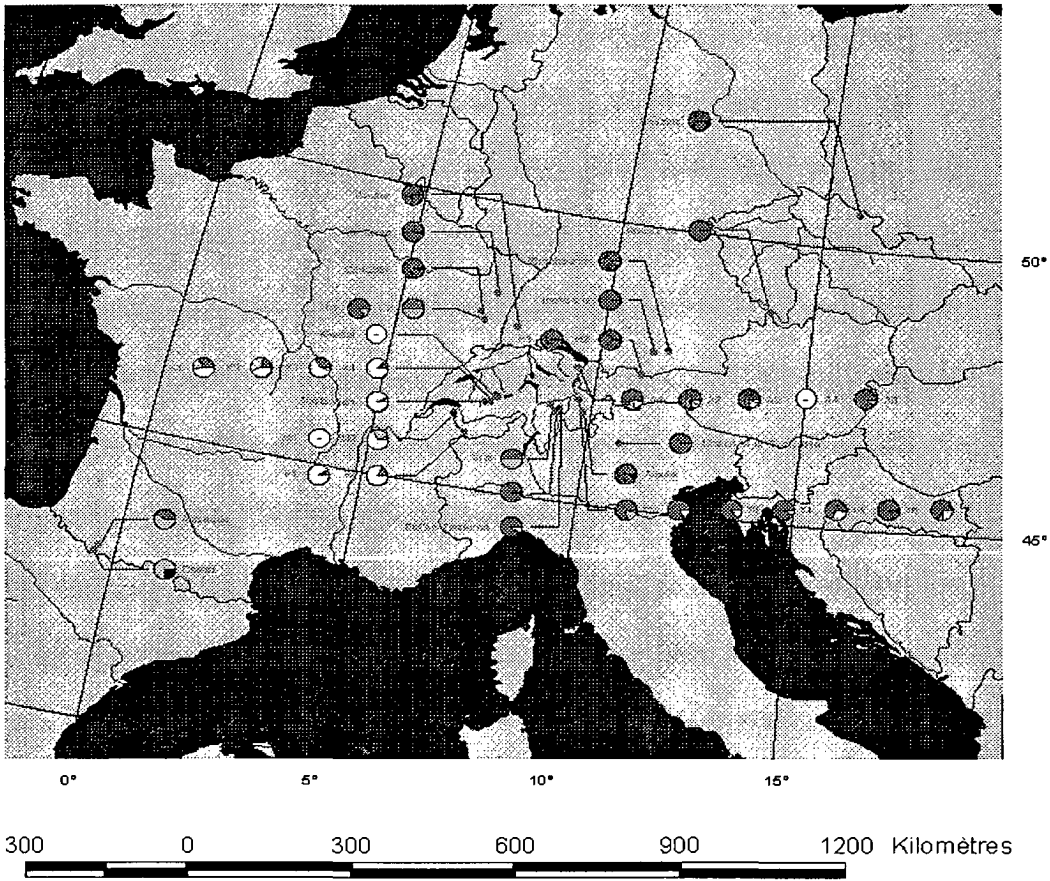


figure 2c: Regression of pairwise estimates of dispersal against pairwise geographical distances for the loca scale ("fine scale structure" in chapter III) in c) Tschiertschen.

Fréquence des allèles du locus ACON pour *Oreina cacaliae*



Code de couleurs des graphiques :

Allèle A :
Allèle B :
Allèle C :
Allèle D :
Allèle I :
Allèle K :

Figure 3: map of allele freq at 1 locus, ACOH

Appendix E

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Table 1: Sampling intensities (S_i) at the different sampling occasions.

TS4		TS3		TS2		LW		TS1		Ap	
period	**S_i**	**period**	**S_i**	**period**	**S_i**	**period**	**S_i**	**period**	**S_i**	**period**	**S_i**
1	0,77	1	0,47	1	0,79	1	0,52	1	0,79	1	1,00
2	0,75	2	0,61	2	0,74	2	0,58	2	0,29	2	0,74
3	0,34	3	0,44	3	0,50	3	0,60	3	0,73	3	0,66
4	0,46	4	0,14	4	0,61	4	0,51	4	0,64	4	0,55
5	0,49	5	0,17	5	0,33	5	0,48	5	0,35	5	0,62
6	0,68	6	0,42	6	0,68	6	0,42	6	0,45	6	0,28
7	0,52	7	0,34	7	0,69	7	0,72	mean	0,54	7	1,00
8	0,16	8	0,42	8	0,36	mean	0,55			mean	0,69
mean	0,52	9	0,56	mean	0,59						
		10	0,58								
		11	0,24								
		mean	0,40								

Overall mean sampling intensity: 0.535

Table 2: Population size calculated according to different models, all assuming a closed population. Calculations were done with the program CAPTURE, details of the models can be found in Otis *et al.* (1978): Statistical inferences from capture data on closed animal populations. Wildlife Monographs 62, 135 pp. Given are capture probability p, population size N with standard error and 95% confidence intervals. The models differ in the aspect, how they allow deviations from the condition of equal catchability. Model M_0 is the standard model, assuming equal catchability; model M_h allows for individual differences in catchability, Model M_b allows for behavioural response to captures (eg escape response after first capture) and model M_t allows for variation between different capture occasions. As detailed in chapter IV, we choose model M_t, because it showed in most patches low standard deviations and small confidence intervals. This is not the case for patch TS1, where surely Model M_t is not appropriate, and the model M_b, which otherwise is doing very badly (eg TS4) seems appropriate. This is caused by the mass disappearance of beetles from TS1 after the first census, which mirrors an escape response.

		Lieserwasen	Appenzell	TS1	TS2	TS3	TS4
no. trapping occasions		9	5	3	4	3	6
no animals captured		433	173	300	53	218	468
no. captures		1208	311	326	111	350	884
M_0	p	0,2974	0,2997	0,0826	0,4935	0,4441	0,2657
	N±se	451±4.8551	208±8.6123	1315±228.9996	56±2.3364	263±10.4121	555±13.147
	95%CI	[443;436]	[194;229]	[955;1870]	[53;64]	[264;288]	[532;584]
M_h	p	0,1875	0,2321	0,1903	0,4142	0,3788	0,2126
	N±se	715±56.664	267±19.909	570±23.3809	66±6.1925	307±14.1959	692±31.4129
	95%CI	[624;850]	[235;315]	[528;620]	[59;84]	[283;340]	[639;763]
M_b	p	0,1745	0,1697	0,7058	0,4107	0,0429	0,3244
	c	0,3436	0,339	0,0523	0,5273	0,5919	0,2511
	N±se	526±23.142	285±52.9362	307±3.6839	60±5.3553	1768±3134.8899	517±12.4745
	95%CI	[490;583]	[219;443]	[302;318]	[54;79]	[345;19106]	[497;547]
M_{bh}	N±se	497±20.285	178±3.7561	307±3.6838	60±5.3556	1860±3490.3059	476±3.5426
	95%CI	[467;550]	[174;191]	[302;318]	[54;79]	[344;21493]	[471;486]
M_t	N±se	451±4.6567	202±7.5582	988±163.088	56±2.037	246±7.7026	536±11.2708
	95%CI	[490;583]	[624;850]	[735;1388]	[53;63]	[234;265]	[517;562]

Table 3: Aggregation of beetles on single host plants at the six patches described in chapter IV. Given are the frequency that there were 1,2,3.... beetles on one host plant, the total number of beetles and the total number of plants, mean, variance and the dispersion index I_D, calculated according to Southwood, T.R.E. (1966): Ecological methods, Methuen, London. As can be already seen clearly from the frequencies, there was no aggregation on single host plants ($I_D > \chi^2$ (df = N_{plants}-1) in all patches at all dates)

TS1	1	2	N_{plants}	$N_{beetles}$	mean	var	I_D
6.1.93	66	2	68	70	1,0294	0,0290	1,8857
6.6.93	37	1	38	39	1,0263	0,0263	0,9487
6.13.93	38	0	38	38	1,0000	0,0000	0,0000
6.27.93	33	0	33	33	1,0000	0,0000	0,0000
7.7.93	33	2	35	37	1,0571	0,0555	1,7838
7.18.93	20	0	20	20	1,0000	0,0000	0,0000
7.24.93	18	2	20	22	1,1000	0,0947	1,6364
Total	245	7	252	259	1,0278	0,0271	6,6216

TS2	1	2	3	4	N_{plants}	$N_{beetles}$	mean	var	I_D
6.6.93	25	0	0	0	25	25	1,0000	0,0000	0,0000
6.13.93	29	2	0	0	31	33	1,0645	0,0624	1,7576
6.27.93	23	0	1	1	25	30	1,2000	0,5000	10,0000
7.7.93	15	4	0	0	19	23	1,2105	0,1754	2,6087
7.18.93	57	5	1	0	63	70	1,1111	0,1326	7,4000
7.23.93	22	4	1	0	27	33	1,2222	0,2564	5,4545
7.24.93	55	6	1	0	62	70	1,1290	0,1470	7,9429
8.6.93	70	1	3	0	74	81	1,0946	0,1690	11,2716
8.10.93	38	3	0	1	42	48	1,1429	0,2718	9,7500
8.16.93	28	0	1	0	29	31	1,0690	0,1379	3,6129
9.6.93	4	0	0	0	4	4	1,0000	0,0000	0,0000
9.16.93	1	0	0	0	1	1	1,0000	0,0000	0,0000
Total	367	25	8	2	402	449	1,1556	0,2249	78,0287

Table 3: Aggregation of beetles on single host plants at the six patches described in chapter IV. Given are the frequency that there were 1,2,3.... beetles on one host plant, the total number of beetles and the total number of plants, mean, variance and the dispersion index I_D, calculated according to Southwood, T.R.E. (1966): Ecological methods, Methuen, London. As can be already seen clearly from the frequencies, there was no aggregation on single host plants ($I_D > \chi^2$ ($df = N_{plants}-1$) in all patches at all dates)

TS3	1	2	3	4	5	6	7	8	9	12	N_{plants}	$N_{beetles}$	mean	std	var	I_D	no. obs.
6.6.93	35	2	1	1	0	0	0	0	0	0	39	46	1,1795	0,6014	0,3617	11,6522	39
6.13.93	117	20	3	0	0	0	0	0	0	0	140	166	1,1857	0,4421	0,1955	22,9157	140
6.27.93	104	12	2	1	0	0	0	0	0	0	119	138	1,1597	0,4691	0,2201	22,3913	119
7.7.93	107	6	1	0	0	0	0	0	0	0	114	122	1,0702	0,2890	0,0835	8,8197	114
7.18.93	248	22	5	1	0	0	0	0	0	0	276	311	1,1268	0,4115	0,1693	41,3215	276
7.23.93	235	22	2	5	0	1	0	0	0	0	265	311	1,1736	0,5904	0,3485	78,4051	265
8.6.93	322	23	5	8	4	1	0	1	0	1	365	461	1,2630	0,9788	0,9581	276,1258	365
8.16.93	145	23	7	1	0	0	1	1	1	0	179	240	1,3408	1,0283	1,0574	140,3750	179
9.6.93	34	2	0	0	0	0	0	0	0	0	36	38	1,0556	0,2323	0,0540	1,7895	36
9.16.93	19	2	0	0	0	0	0	0	0	0	21	23	1,0952	0,3008	0,0905	1,6522	21
Total	1366	134	26	17	4	2	1	2	1	1	1554	1856	1,1943	0,7020	0,4928	640,7823	1554

Table 3: Aggregation of beetles on single host plants at the six patches described in chapter IV. Given are the frequency that there were 1,2,3.... beetles on one host plant, the total number of beetles and the total number of plants, mean, variance and the dispersion index I_D, calculated according to Southwood, T.R.E. (1966): Ecological methods, Methuen, London. As can be already seen clearly from the frequencies, there was no aggregation on single host plants ($I_D > \chi^2$ ($df = N_{plants}-1$) in all patches at all dates)

TS4	1	2	3	4	5	6	7	N_{plants}	$N_{beetles}$	mean	var	I_D
6.6.93	181	23	3	0	0	0	0	207	236	1,1401	0,1502	27,1356
6.13.93	190	18	4	2	0	0	0	214	246	1,1495	0,2217	41,0732
6.27.93	151	18	1	0	0	0	0	170	190	1,1065	0,0957	14,6213
7.7.93	67	3	0	0	0	0	0	70	73	1,0429	0,0416	2,7534
7.18.93	57	2	0	0	0	0	0	59	61	1,0339	0,0333	1,8689
7.23.93	147	16	1	0	0	0	0	164	182	1,1098	0,1106	16,2418
7.24.93	140	10	2	0	0	0	0	152	166	1,0921	0,1107	15,3012
8.6.93	166	15	5	2	0	0	0	188	219	1,1649	0,2561	41,1096
8.10.93	289	38	15	5	1	1	0	349	441	1,2570	0,4492	124,3572
8.16.93	282	47	14	1	4	1	1	350	455	1,3194	0,5857	154,9190
9.6.93	62	4	1	0	0	0	0	67	73	1,0896	0,1131	6,8493
9.16.93	26	0	0	0	0	0	0	26	26	1,0000	0,0000	0,0000
Total	1758	194	46	10	5	2	1	2016	2368	1,1746	0,2841	487,4324

Appenzell	1	2	3	N_{plants}	$N_{beetles}$	mean	var	I_D
5.21.93	14	1	0	15	16	1,0667	0,0667	0,8750
5.31.93	69	2	0	71	73	1,0282	0,0278	1,8904
6.15.93	90	0	1	91	93	1,0220	0,0440	3,8710
6.28.93	76	3	0	79	82	1,0759	0,1480	10,7294
7.8.93	38	3	1	42	47	1,1190	0,1562	5,7234
7.17.93	93	3	1	97	102	1,0515	0,0702	6,4118
8.6.93	27	2	0	29	31	1,0690	0,0665	1,7419
8.9.93	65	3	2	70	77	1,1000	0,1471	9,2299
Total	472	17	5	494	521	1,0547	0,0721	33,6833

Table 3: Aggregation of beetles on single host plants at the six patches described in chapter IV. Given are the frequency that there were 1,2,3.... beetles on one host plant, the total number of beetles and the total number of plants, mean, variance and the dispersion index I_D, calculated according to Southwood, T.R.E. (1966): Ecological methods, Methuen, London. As can be already seen clearly from the frequencies, there was no aggregation on single host plants ($I_D > \chi^2$ ($df = N_{plants}-1$) in all patches at all dates)

Lieserwasen	1	2	3	4	5	N_{plants}	$N_{beetles}$	mean	var	I_D
5.8.97	54	3	0	0	0	57	60	1,0526	0,0508	2,7000
5.11.93	96	9	1	0	0	106	117	1,1038	0,1129	10,7436
5.13.93	154	8	0	0	0	162	170	1,0494	0,0472	7,2471
5.16.93	146	12	0	0	0	158	170	1,0759	0,0706	10,3059
5.19.93	106	12	3	1	1	123	148	1,2033	0,3600	36,5000
5.24.93	121	12	0	0	0	133	145	1,0902	0,0827	10,0138
5.27.93	86	12	0	0	0	98	110	1,1224	0,1086	9,3818
6.4.93	176	7	0	0	0	183	190	1,0383	0,0370	6,4842
6.7.93	106	4	0	0	0	110	114	1,0364	0,0354	3,7193
Total	1045	79	4	1	1	1130	1224	1,0832	0,0994	103,5654

Thanks to Merci Danke schön

Martine Rowell-Rahier, the ideal supervisor - anything I say more is less, merci, Martine.

Laurent Keller, Steph Menken and Claude Mermod for accepting to read and discuss this thesis and serve on my "jury de thèse".

Hugh Rowell for accepting to be my "Basel-supervisor" and for his manifold support.....

Francis Borel for all these maps.....
and Mahmoud Bouzelboudjen, for first explaining that everything is much more complicated but stating later that everything is possible with GIS.....

Betty Benrey for reading ALL chapters and providing very constructive critic on every "last minute" version - and for all these mexican dinners.....

Pierluigi Ballabeni and Ted Turlings for many stimulating discussions, criticisms and motivations and for understanding all my german remarks.....

Susanne Dobler, Bernd Hägele, Fredi Köpf and Jacques Pasteels for teaching me various aspects of chrysomelid biology, for discussions in all stages of this thesis and for being a friend.....

the members of the LEAE, who shared discussions, field trips, conferences, travaux pratique, lab meetings, sandwiches and champagner: Davide, Lucca, Nicole, Sandrine.

my family, not for their enthusiastic help collecting beautiful *Oreinas*, but for their never ending interest, support, understanding and love.....

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- Endless Forms: Species and speciation: a symposium in honor of Guy L. Bush, Monterey, USA, May 1996, poster
- Second European meeting of graduate students in evolutionary biology, Amsterdam, Feb. 1996, talk
- Fifth congress of the European society for evolutionary biology, Edinburgh, Scotland, Sept. 1995, poster*
- 9th Int. Symposium on Insect-Plant Relationships, Gwatt, Switzerland, June 1995, poster
- Zoologia 95: Interactions between genetics and ecology, Zürich, Switzerland, Mar. 1995, talk (Satellite symposium: Ecology, behaviour, evolution)*
- First European meeting of graduate students in evolutionary biology, Zürich, Mar. 1995, talk

b) without presentation:
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- IIIème cycle "Herbivory and plant secondary metabolites: ecological and evolutionary issues", Chateux d'Oex, Mar. 1995

- workshop (Priority Programme Environment, module biodiversity): "Effect of habitat fragmentation on plant and animal populations", Apr. 1995, Basel

- IIIème cycle "Evolution in structured populations", Lausanne, Sept. 1994

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SUMMARY

In this thesis I report on a study of the spatial population structure of an alpine leaf beetle, *Oreina cacaliae* Schrk. (Coleoptera, Chrysomelidae). An understanding of the population structure is an important step in identifying the forces driving the evolutionary history of a species - and in understanding how today's ecological and life history factors form and maintain such a population structure. I assessed the population structuring by studying genetic differentiation at different scales with a concurrent assessment of population dynamics and dispersal in the field. The studied *Oreina* beetles live in clearly recognizable patches - characterized by a continuous plant cover of intermingling host plants and by a more or less continuous beetle distribution (chapter IV). One central focus of this thesis is the question of the importance of such patches for the population structure.

The aim of my study was (1) to quantify intraspecific genetic variation in *Oreina* leaf beetles, (2) to search for an explanation of the observed patterns in possible correlations with ecological and environmental parameters, (3) to assess the scale at which differentiation can be observed and (4) to investigate independently within patch populations of *O. cacaliae* in order to (5) understand the population dynamics of this species.

In leaf beetles only few studies have addressed the question of population structure, but whenever studied, high differentiation was found already at the lowest level that was taken into consideration (review in chapter I). A higher between population differentiation is found in species living in topographic diverse regions than in species living in the lowlands (chapter I). In this study, allozyme electrophoresis revealed high genetic variability and high heterozygote deficits for two closely related *Oreina* leaf beetles(chapter II). I made the hypothesis that these high heterozygote deficits are caused by a combination of inbreeding due to the low vagility of these beetles and a sampling effect, due to sampling over several kin groups. This was later rejected based on a fine scale genetic study of within patch structuration, where no indication for such a structuration could be found and confirmed by the results of mark and recapture studies in the field, which revealed movement throughout the patches (chapter III and IV). For one of the species studied, *O. cacaliae*, I found an indication for isolation by distance, but not for the other, *O. speciosissima* (chapter II). No influence of host plant use could be detected and also no cline due to altitude, latitude or longitude (chapter II). With two methods, hierarchical F-statistics and spatial autocorrelation, I investigated at which scale differentiation can be observed. The hierarchy imposed was patch - location - region. I found high small scale differentiation at the lowest level looked at, genetic integrity of samples in an approximate radius of 100 km and a pattern of isolation by distance at larger distances (chapter III). With mark and recapture experiments I assessed within patch population sizes, patch persistence rates and movement rates in the field. Very high persistence rates and only very little movement out of the study area could be observed. Within the study area beetles frequently changed host plants and traversed the whole patch. An independence of patches was proposed (chapter IV).

I suggest that the population structure of *O. cacaliae* is in agreement with the one expected in a metapopulation: independence of local populations (patches), genetic integrity at the level of the metapopulation ensured by long distance gene flow and a pattern of isolation by distance in a species consisting of several, homogenuously distributed metapopulations (discussion chapter). We need further studies that address the mating behavior and a possible social organisation of *Oreina* leaf beetles, as well as studies on their ability for long distance dispersal to fully understand how such a population structure could arise and be maintained.