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Einfluss von Naturfaktoren und Flächennutzungswandel auf die genetische Struktur xerothermer Tierarten in der Region Trier

Betreuender: Juniorprofessor Dr. rer nat. Thomas Schmitt

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The influence of **natural factors** and **landuse changes** on the **genetic structure** of xerothermic animals in the region of Trier

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Declaration

I declare that this thesis, composed by myself and embodying work done by myself. This thesis has not been accepted in any previous application for a higher degree. All sources of references and quotations have been duly acknowledged.

I have personally designed laboratory work, collected and analysed data, developed the discussion of results; the idea of this work was developed by Junior Professor Dr. Thomas Schmitt, my supervisor.

May, 2006

Contributions

Supervised by:
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Cover and format designed by Micha Heigl and me.

Summary

The fragmentation of landscapes has an important impact on the conservation of biodiversity. The genetic diversity is an important factor for a population's viability, influenced by the landscape structure. However, different species with differing ecological demands react rather differently on the same landscape pattern. To address this feature, we studied ten xerothermophilous butterfly species with differing habitat requirements (habitat specialists with low dispersal power in contrast to habitat generalists with low dispersal power and habitat generalists with higher dispersal power). We analysed allozyme loci for about 10 populations (à 40 individuals) of each species in a western German study region with adjoining areas in Luxemburg and north-eastern France. The genetic diversity and genetic differentiation between local populations was discussed under conservation genetic aspects. For generalists we detected a more or less panmictic structure and for species with lower abundance and sedentarily behaviour the effect of isolation by distance. On the other hand, the isolation of specialists was mostly reflected by strong genetic differentiation patterns between the investigated populations. Parameters of genetic diversity were mostly significantly higher in generalists, compared to specialists. Substructures within populations as an answer of low intrapatch migration, low population densities and high population fluctuations could be shown as well. Aspects of landscape history (the historical distribution of habitats resulting of the presence of limestone areas) and the changes of extensive sheep pasturing and the loss of potential habitats in the last few decades (recent fragmentation) are discussed against the gained genetic data-set of the ten butterflies.

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1. Introduction

Artificial and natural islands

Anthropogenetic habitat fragmentation of previously continuous habitats has been a topic of growing interest (Frankham 1995, Saunders et al. 1991, Wilcox & Murphy 1985, Young et al. 1996). Isolation of large populations into several smaller isolated populations can alter both demographic and genetic factors, which lead to an increased risk of population extirpation (Goodman 1987, Harrison 1996, Lacy 1987, Lande & Lande 1988). Several theoretical and experimental studies have determined the potential effects of habitat isolation among populations, but inferring the effects of habitat fragmentation among natural populations can be a difficult task (Knutsen et al. 2000, Peacock & Smitch 1997).

Metapopulation (Hanski 1999) and island biogeography theory (MacArthur & Wilson 1967) assume that landscapes consist of habitat patches embedded in a matrix of non-habitats (Hanski 1991, Levins 1970). Island biogeography theory (MacArthur & Wilson 1967) has been extended to terrestrial habitat islands on the same assumption that the land surrounding habitat patches is like a sea (Janzen 1968). Landscapes that appear patchy, such as fragmented forestry land (Mc Carthy & Lindenmayer 1999), agricultural areas, or naturally patchy landscapes (Leisnham & Jamieson 2002) provide the necessary spatial element for metapopulations to function. However, if species that occur in the patches also have self-sustaining populations in the matrix then the landscape is not an island-sea mosaic, and metapopulation theory would be irrelevant. In a recent review of concepts associated with habitat fragmentation, Hanski (2001) emphasized that the matrix around habitat patches may not be like a sea around oceanic islands, and that research was need to asses the amount of species overlap between habitat patches and the surrounding land.

Knowledge of the proportion of species that are confined to habitat patches in fragmented landscapes can provide some insight into how generally important metapopulation theory and island biogeography theory might be. For example, Cook et al. (2002) found that 24% of plant species in experimental field patches were also found in the mowed matrix. When these species were removed from the analysis, the result conformed better to predictions from island biogeography theory, with more species on larger patches and on patches closer to a source for colonists. Species that are confined to habitat patches represent the shortlist of species to which dynamic population theory could apply. In contrast, studies of sedentary species with high ecological demands like the butterfly species *Maculinea alcon*, only 70% of 50 investigated, potential habitats in northern Germany were occupied (Habel et al., submitted) – a consequence of isolation? In all cases a simple fact of loss of biodiversity.

The features of landscapes that influence the population and community ecology of species including insects, are well known. The

proportion ratio of habitat edge to interior (Chen et al. 1995, Radeloff et al. 2000), the isolation of habitat fragments (Collinge 2000), patch area (Kruess & Tscharntke 2000), patch quality (Hanski & Singer 2001, Kuussaari et al. 2000), patch diversity (Gathmann et al. 1994, Varchola & Dunn 2001) and microclimate (Braman et al. 2000) all contribute to determining the abundance and richness of organisms on landscapes. More recently, ecologists have incorporated temporal changes in landscape structure (Onstad et al. 2001, Solbreck 1995), genetic change in insect populations (Ronce & Kirkpatrick 2001, Singer & Thomas 1996), and differential responses of predators and prey (Kruess & Tscharntke 1994, With et al. 2002) into their understanding of the spatial ecology of insects. Even with a growing awareness of the features of landscapes that contribute to variation in insect populations and communities, clear gaps remain in our understanding of the links between landscapes change, insect dynamics and among patch exchanges (gene flow). Mostly the abundance of an organism and it's level of connectedness is reflected by its genetic texture and variability (Figure 1.1).

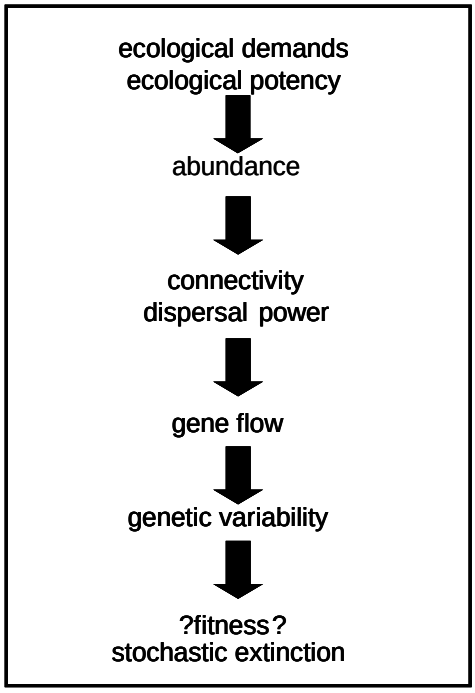


Figure 1.1: Relationship between the ecological potency of species and its extinction-probability. Starting with the width of the ecological amplitude, arrows are symbolising the effects for species condition.

The relationship between landscape pattern and population structure has become a major interest in landscape ecology (Fahrig & Merriam 1994, Gibbs 2001, Ramirez & Haakonsen 1999). Taylor et al. (1993) coined the term “landscape connectivity” to describe migration as a function of both, the mobility of an organism and the way in which the landscape facilitates or impedes movement of the organism among patches. The extent to which individual organisms in different habitat patches are genetically similar often depends, in turn, on the effective migration rate between patches (Ramirez & Haakonsen 1999). Thus, landscape connectivity plays a double role in that it is dependent on the interaction of landscape pattern and organism behaviour on the one hand, while it is a driver of population processes on the other hand (Goodwin 2003). However, landscape pattern and population structure do not necessarily change synchronously (Petit & Burel 1998, Purtauf et al. 2004). The impact of land management on species composition and population sizes in agricultural landscapes, for example, is often detectable only decades later (Burel 1992, Chamberlain et al. 2000, Wiens 1986). Less mobile organisms are assumed to integrate spatial changes over considerable periods of time (Burel et al. 1998). Because not every migration event leads to successful gene exchange, the response of population genetic structure may be particularly delayed. Accordingly, we consider the pattern of genetic similarity of a population in a dynamic landscape to be the cumulative outcome of varying gene flow and therefore of landscape connectivity dynamics during the past.

Conservation genetic studies have typically inferred the effects of habitat fragmentation by documenting patterns of genetic differentiation and levels of genetic diversity among potentially isolated populations (Harrisson & Hastings 1996, Oostermeijer et al. 1996, Young et al. 1996). Ideally, such studies should take additional factors into account like historical levels of isolation and recent differentiation among populations should be determined *a priori* (Bermingham & Avise 1986, Cunningham & Moritz 1998). Historical population structure can have a profound influence of the distribution of genetic variation among contemporary populations such that any observed differentiation may be the result of long-term isolation, rather than recent, anthropogenic fragmentation (Cunningham & Moritz 1998). Alternatively, a lack of differentiation among populations could be the result of shared ancestry among populations, rather than ongoing gene flow among them (Avise et al. 1987).

Also, levels of differentiation and genetic diversity among fragmented populations should be compared to populations thought to be undisturbed (Brawn et al. 1996, van Dongen et al. 1998, Jackson & Pounds 1979). Such comparisons have been effective at determining the effects of natural isolation among islands versus mainland populations (Baker et al. 1990, Brawn et al. 1996, Bates 2000, Vucetich et al. 2001). However, finding both fragmented and nonfragmented populations can be difficult among naturally occurring populations often because species are not of conservation concern until only a few isolated populations remain. Comparisons of

fragmented and no fragmented populations could be made among closely related species and at the intraspecific level; ecological or life history differences between species could have profound influences on the distribution of genetic variation.
Inferring the effects of habitat connectivity can be especially difficult for high gene flow species, tending to have relatively low levels of differentiation among populations in comparison to sedentary species (Waples 1998). The increased resolution provided by genetic hyper variable markers introduces the additional problem of potentially yielding statistically significant levels of differentiation among populations even when the biological relevance is questionable (Balloux et al. 2000, Goldstein et al. 1995, Hedrick 1999, Jarne & Lagoda 1996, Waples 1998).
Previous investigations have repeatedly demonstrated the influence of land-use pattern on species composition and population sizes (Petit & Burel 1998). Less often the effects of land use pattern were shown for population genetic structures (van Dongen et al. 1998, Coulon et al. 2004, Keyghobadi et al. 1999). To analyse the combined effects of species abundance, their mobility, the landscape pattern and landscape change during the past on population genetic structure, we chose ten relatively related xerothermophilous butterfly species and moths (belonging to one genus or at least family), but with differing ecological demands and dispersal abilities: *Melanargia galathea, Melitaea aurelia, Zygaena loti, Zygaena viciae, Zygaena carniolica, Thymelicus sylvestris, Thymlicus acteon, Thymelicus lineola, Aricia agestis* and *Cupido minimus* (Plate 2.1, 2.2). The study area was restricted to the rural landscape of south-west Germany and adjacent areas of France and Luxemburg (Figure 2.1).

The species-model

Abundance pattern in general reflect the ecological demands of species and therefore is the assumption of connectivity and metapopulation dynamics (Hanski 1999). Reduced abundance leads to isolation of the remaining populations, followed by reduced gene flow, increasing genetic differentiation and loss of genetic diversity (Holzhauer et al. 2005) often correlated with severe reductions of the fitness of individuals (Frankham et al. 2002, Hansson & Westerberg 2002, Reed & Frankham 2003).
Grasslands and especially semi-natural calcareous grasslands are among the most species rich habitats of Europe and therefore of special conservation interest (Swaay & Warren 1999, Wallis deVries et al. 2002). The traditional European agricultural landscape prior to the Second World War was rich of nutrient poor grasslands representing suitable habitats for many animals and plant species. Due to dramatic changes in agriculture, especially increased application of fertilizers and pesticides as well as mechanisation, these nutrient poor places of little productivity were dramatically shrinking in number and in size (Wallis de Vries et al. 2002). Therefore, the typical flora and fauna of these habitats has been disappearing from many parts of

Europe (Ellenberg 1992, Swaay & Warren 1999, Asher et al. 2001) and the distributions of species restricted to nutrient poor grasslands became more and more scattered (Wallis de Vries et al. 2002). This causes severe conservation problems (cf. Binot et al. 1998, Swaay & Warren 1999), because natural fragmentation given by geological and meso/microclimatic conditions today is reinforced by anthropogenic activities.

However the final consequence of the dramatic fragmentation of these habitats is still insufficiently understood. We know that species of these habitats have declined strongly all over Europe (Swaay & Warren 1999), but we do not know whether interconnection between habitats is disconnected on a local and/or regional scale for typical and common species of those grasslands. With our species-model we tested the landscape-permeability; a landscape therefore might be strongly fragmented for a habitat specialist with poor dispersal abilities and more or less continuous for a more generalist species with higher dispersal capacity. Generalist species with poor dispersal abilities as well as specialist species with high dispersal abilities might show some intermediate position (Figure 1.2).

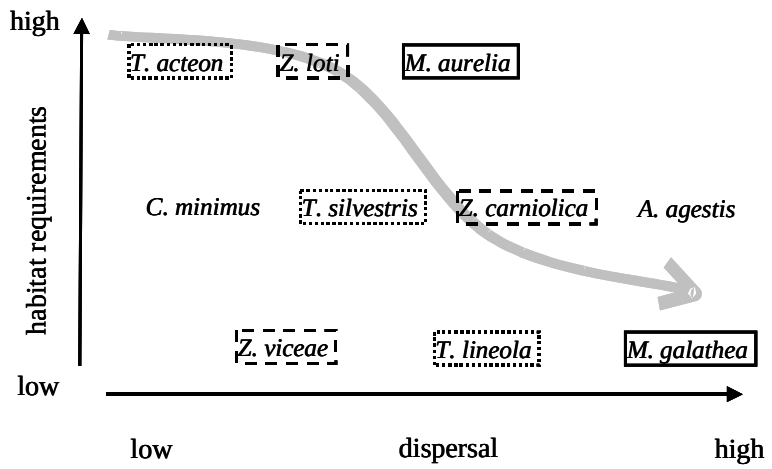


Figure 1.2: Species model; ten genetically analysed butterflies (*Thymelicus acteon, Thymelicus sylvestris, Thymelicus lineola, Melanargia galathea, Melitaea aurelia, Aricia agestis, Cupido minimus, Zygaena carniolica, Zygaena loti, Zygaena. viciae*). Classified by its species specific ecological demands (habitat requirements) (y-axis) and its dispersal ability (x-axis). The assumed genetic differentiation is symbolised by the grey arrow.

For species categorisations we followed Bink et al. (1992), based on observation data of many lepidopterologists to evaluate the species in their mobility and their habitat requirements. These notifications are valid for Central Europe. We enhanced the classification by personal experiences of lepidopterologists of the study region (Schmitt, Meyer, Weitzel, pers. comm.).
For comparability of our data we chose closely related species groups (within a genus e.g. *Thymelicus*) or at least within a family (e.g. Lycaenidae). We focused on the comparability of the chosen species pairs: at the interspecific level, the biology of their life histories between species, genera or families (mutation rate depends on generation rate) could have profound influences on the distribution of genetic variation. Therefore we compared species with similar biology.

In summary, this study will address the following questions in comparing the genetic data of these related butterfly-groups:

(i) Influence the fragmentation of habitats the exchange of individuals between patches?
(ii) How far are species adapted to naturally evoked patch isolation by geological or climatic conditions?
(iii) Suffer species in inbreeding because of high population fluctuations and low population densities in closed populations? And, are these processes reinforced by anthropogenous habitat fragmentation?
(iv) Is the ecological potency (abundance) responsible for gene flow between local populations?
(v) Are species genetically adapted to their dispersal strategy?

Regional effects of habitat fragmentation are only understandable in context with a broader understanding of e.g. phylogeographic aspects. Therefore, in Excursus I-V we discussed such topics that have relevance to our study.

2. Material and Methods

2.1. The study area

As study area, we selected the Rhineland-Palatinate and the Saarland (south-west Germany) with some adjoining areas in Luxemburg and north-east France (Figure 2.1). This region has several calcareous grassland areas (Negedank 1974) with populations of different xerothermophilous butterfly species (Kraus 1993, Schmidt-Koehl 1977). The limestone areas were mostly connected by flower rich meadows, fallow land and forest skirts (Kraus 1993, Schmidt-Koehl 1977). They could be devided in the Devon lime- and lacustrine area in the Saar-Mosel area, the lacustrine limestone area in the Eifel and the Mosel valley and limestone areas in the Bliesgau (Saarland) (Figure 2.1).

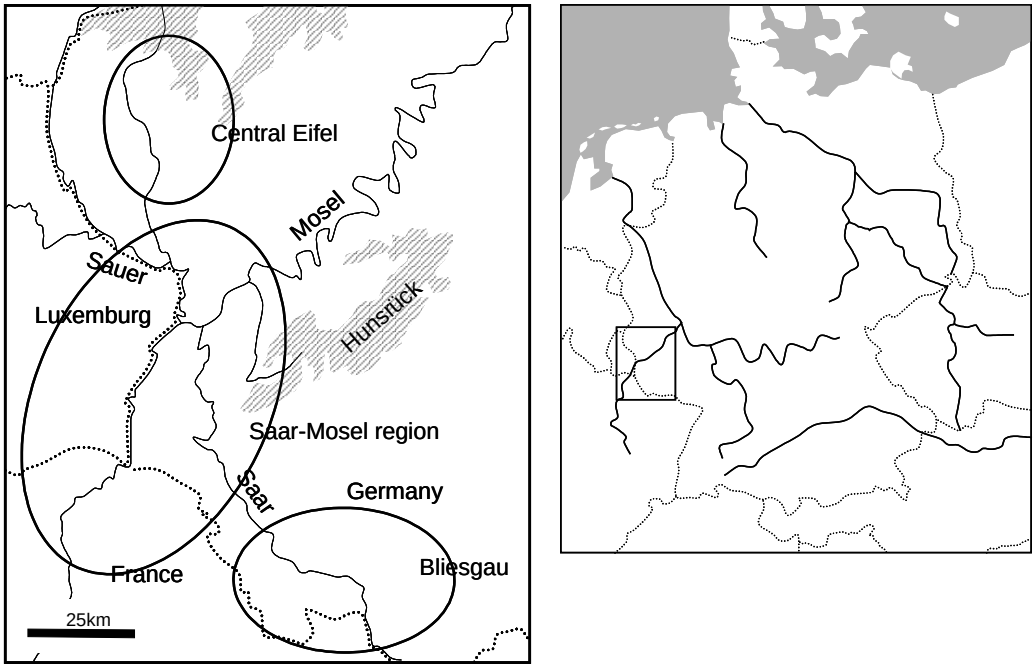


Figure 2.1: Study area in western Germany and adjoining France and Luxemburg. Three limestone areas (Central Eifel, Saar-Mosel region and Bliesgau) are marked.

Natural fragmentation and anthropogenic fragmentation

The study area is dominated by lacustrine limestone (Negedank 1974). The sample sites are within or at the border of this lacustrine limestone areas. Most of the investigated sites are typical calcareous grasslands, classified in submediterranean Brometalia erecti. This is further devided into the formation Mesobromion, which are characterized by low level of nutrient and a high species richness, often used by sheeping. Characteristic plants are *Bromus erectus* and orchid species (Wilmanns 1998).

In the Red List of endangered biotope types in Rhineland Palatinate, calcareous grasslands are highly endangered by anthropogenic loads of nutrient, intensive agricultural usage and the failure of extensive use, which leads to advanced succession and the destruction of these biotopes (Burggraaff & Kleefeld 1998). Thus, a strong reduction of these habitats have been observed during the last decades in our study area (Bielefeld 1985, Wenzel et al. 2005, Weitzel pers. comm.,).
Isolation of these investigated habitats can be caused by natural limiting factors like the geological condition (limestone presence/absence) and/or microclimatic factors and on the other hand by anthropogenic fragmentation. In the analysed genetic structures of the ten butterfly species, anthropogenic and natural habitat fragmentation were separately discussed.

2.2. The investigated study species

Ten butterfly species were chosen as the model organisms. They were selected by their different habitat requirements. These are reflected by their abundance pattern, different migration powers and their migration behaviour (Figure 2.2), different population densities and population fluctuations (Figure 2.3). An overview of all investigated study species is given in Table 2.1.

Two Nymphalids

Melanargia galathea is one of the most common species in the study region. This species comes across the region in most flower rich meadows, mostly in high densities, showing a high expansion potential (Vandewoestijne et al. 2004). Thus, this species is suitable as null method for the following butterfly species, especially of ones which are characterized by low migration power, small ecological potency and a sedentarily behaviour.

Melitaea aurelia is a common Nymphalid in Central Europe, building up high population densities, closely restricted to calcareous meadows in the study area (Weitzel, pers. comm.). This species is not found in agricultural used meadows, like *M. galathea* (Weitzel, pers. comm.). Due to the physical constitution of *M. aurelia* it shows a high mobility (Bink 1992).

Three Thymelicus species

Thymelicus acteon is strongly restricted to xerothermic habitats, which are naturally fragmented by climatic, geological and geomorphologic conditions. This skipper tolerates well to fragmentation of its habitats. In the cooler Central Eifel region, this species is exclusively found in special habitats like southern exposed Juniperus-heathlands. Compared with records of the 1960s to the 1970s, this species is actually increasing (Weitzel, pers. comm.).

Thymelicus sylvestris is closely related to *Thymelicus acteon*, but with a broader ecological potency and thus a broader distribution area, without gaps of presence in the landscape.

Thymelicus lineola represents a similar distribution pattern like *T. sylvestris*, widespread on waysides or meadow function as stepping stone of this species, interconnecting local populations to a continuum of this species in the landscape.

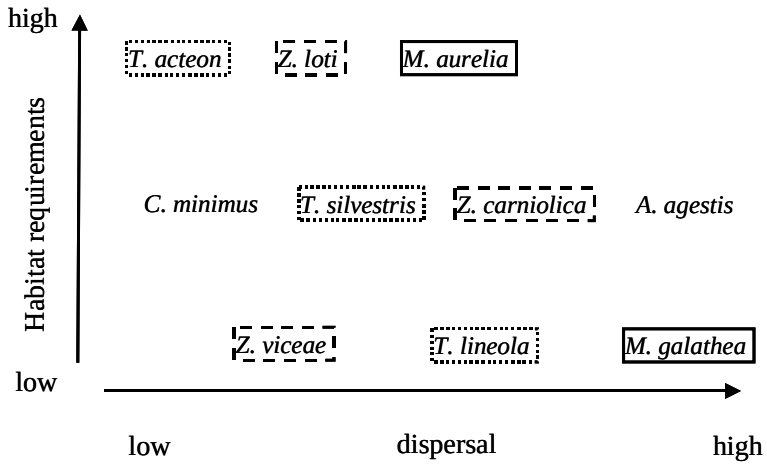


Figure 2.2: The investigated species classified by habitat requirements and dispersal behaviour. Classification by Bink (1992), Ebert & Rennwald (1991a,b) and personal observations. The corresponding species are accentuated by same frames.

Two Lycaenids

The Lycaenid *Cupido minimus* is a butterfly species with the lowest potential to migrate in correspondence to other butterfly species (c.f mark release recapture studies of Baguette et al. (2000)) and found on calcareous grasslands. Moving along edges, the actual agricultural intensification leads to disconnectedness of potential habitats.

Aricia agestis, an indicator species of warmer sites in Central Europe, is actually expanding its northern distribution border (England, Northern Germany, Denmark). The high dispersal power enables this species to colonise habitats like fallow land which are in an early successional stage. Comparing to *C. minimus*, this lycenid is less sedentary and shows strong migration power.

Three Zygaenids

In general, Zygaenids are characterised by low expansion potential and specific habitat conditions, concerning larval ecology and the requirements of imagos (Ebert & Rennwald 1991c) (Figure 2.3). Mostly, the butterflies can be observed sitting on flowers; they bridge only a few metres within their habitats, landing on the next nectar

source. Species of the genus Zygaena can be described as one of the lowest mobile Lepidoptera. Beside low migration potential, Zygaenids often show strong population fluctuations, including temporarily low density levels (Figure 2.3); this phenomenon was observed in the study area during the last few decades (Weitzel, pers. comm.).

Zygaena loti is closely restricted to calcareous grasslands. Because of strong population fluctuations, this species can occur frequently in periods, declining below the detection limit some generations later. During the last years, this species suffered local extinctions, probably due to climatic conditions in the study area (Weitzel, pers. comm., Werno, pers. comm.).

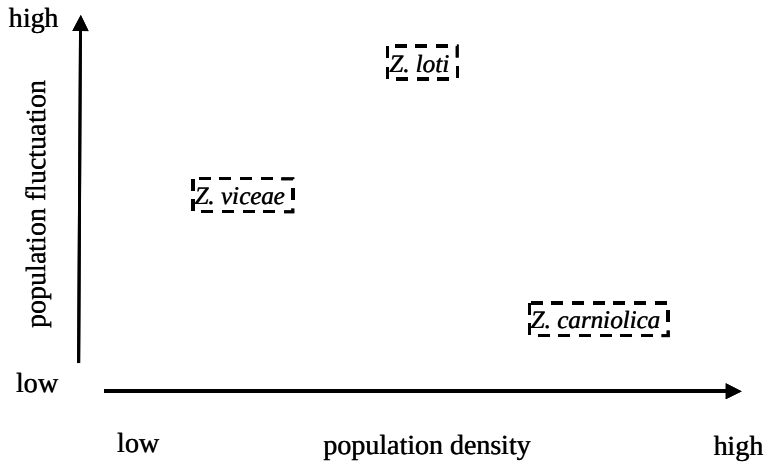


Figure 2.3: Population density and population fluctuation levels in the three investigated Zygaena species *Zygaena carniolica*, *Zygaena viciae* and *Zygaena loti* in the study area, classified by personal observations (Weitzel, pers. comm.).

Zygaena carniolica shows a similar attachment to a specific habitat type and occurs in similar abundance like *Z. loti*; mostly found in limestone areas in the study area (Weitzel, pers. comm.). This species was found in relatively high, constant population densities over the last decades, often >10.000 individuals recorded per site (Weitzel, pers. comm.).

Zygaena viciae is one of the smallest Zygaenid moths with wide ecological amplitude. This small species with low migration power occurs patchily in the landscape. This species represents a low density species with high fluctuations within local populations in the study area (Weitzel, pers. comm.). High fluctuations on low density levels could evoke genetic depressions within populations.

Melanargia galathea ♂
Linnaeus 1758

Melanargia lachesis ♂
Hübner 1790

Melitaea aurelia ♂
Nickerl 1850

Melanargia galathea ♂
Linnaeus 1758

Melanargia lachesis ♂
Hübner 1790

Melitaea aurelia ♂
Nickerl 1850

Zygaena carniolica ♂
Scopoli 1763

Zygaena loti ♂
(Denis & Schiffermüller 1775)

Zygaena viciae ♂
(Denis & Schiffermüller 1775)

Aricia agestis ♂
Denis & Schiffermüller 1775

Aricia agestis ♂
Denis & Schiffermüller

Cupido minimus ♂
Fuessly 1775

Cupido minimus ♂
Fuessly 1775

Cupido minimus ♀
Fuessly 1775

Plate 2.1: Analysed study species: *Melanargia galathea, Melanargia lachesis, Melitaea aurelia, Zygaena carniolica, Zygaena loti, Zygaena viciae, Aricia agestis, Cupido minimus.* Figures taken from Tolman & & Lewington (1997) and Naumann et al. (1999)

Thymelicus acteon ♂
Rottemburg 1775

Thymelicus acteon ♂
Rottemburg 1775

Thymelicus acteon ♀
Rottemburg 1775

Thymelicus lineola ♂
Ochsenheimer 1808

Thymelicus lineola ♂
Ochsenheimer 1808

Thymelicus lineola ♀
Ochsenheimer 1808

Thymelicus sylvestris ♂
Poda 1761

Thymelicus sylvestris ♂
Poda 1761

Thymelicus sylvestris ♀
Poda 1761

Plate 2.2: Analysed study species: *Thymelicus acteon*, *Thymelicus lineola*, *Thymelicus sylvestris*. Figures taken from Tolman & Lewington (1997).

Table 2.1: Sample sites; name of site, region, GPS-coordinates of each site, >: patch size >15ha, <: patch size <15ha.; numbers of collected individuals are supplied by the sampling-date in parenthesis. Abbreviations: D, Germany; L, Luxemburg; F, France, RLP: Rhineland-Palatinate, SL: Saarland; ce: Central Eifel, sm: Saar-Mosel region, bg: Bliesgau.

name of site	region	GPS	size	*M. galathea*	*M. aurelia*	*Z. viciae*	*Z. caniolica*	*Z. loti*	*T. sylvestris*	*T. acteon*	*T. lineola*	*C. minimus*	*A. agestis*
D-RLP-Niederehe	ce	50.19 6.45	<	18 (18-VII-2003)									
D-RLP-Lissendorf	ce	50.19 6.37	<	40 (18-VII-2003)				40 (24-VI-2005)					40 (3-VIII-2005)
D-RLP-Schönecken	ce	49.10 6.28		40 (10-VII-2003)			38 (10-VII-2003)	40 (20-VI-2005)	40 (10-VII-2003)		39 (10-VII-2003)		
D-RLP-Weinsheim	ce	50.15 6.29	<	40 (10-VII-2003)	40 (24-VI-2006)	40 (10-VI-2003)			40 (10-VII-2003)		39 (10-VII-2003)	40 (10-VII-2003)	
D-RLP-Ingendorf	sm	49.55 6.28	<		40 (16-VI-2004 17-VI-2005)		40 (1/7-VII-2003)	40 (16/17-VI-2004)	38 (06-VII-2004)	20 (7/8/12-VIII-2004)	40 (06-VII-2004)		40 (12-VIII-2004 27-VII-2005)
D-RLP Dockendorf	sm	49.55 6.28	<	40 (1-VII-2003)									
D-RLP-Ourtal	sm	49.52 6.19	<	40 (16-VII-2003)					32 (06-VII-2004 21/28-VII-2004)	17 (21/28-VII-2004 11-VIII-2004)	44 (06-VII-2004 21/28-VII-2004)		
D-RLP-Echternacher-brück	sm	49.49 6.26	>	40 (9-VII-2003)	40 (17-VI-2005)	40 (9-VII-2003 6/16-VII-2004)			40 (6/28-VII-2004)	19 (21/28-VII-2004 11-VIII-2004)		40 (20-VII-2003)	
D-RLP-Bettingen	sm	49.57 6.24	<	40 (7-VII-2003)	40 (16-VI-2004)	40 (7-VII-2003 6/16-VII-2004)	40 (1/7-VII-2003 17-VII-2005)		40 (1/6-VII-2004)	20 (7/8/12-VIII-2004)	40 (06-VII-2004)		
D-RLP-Trier	sm	49.45 6.4	<										40 (19-V-2004)

name of site	region	GPS	size	*M. galathea*	*M. aurelia*	*Z. viciae*	*Z. caniolica*	*Z. loti*	*T. sylvestris*	*T. acteon*	*T. lineola*	*C. minimus*	*A. agestis*
D-RLP-Igel	sm	49.43 6.33	<										40 (29-VII-2004 26-VII-2005)
D-RLP-Nittel	sm	49.40 6.27	<										40 (29-VII-2004 24-VII-2005)
D-RLP-Wasserliesch	sm	49.43 6.33	>	40 (24-VI-2003)	40 (8/11-VI-2004)	40 (25-VI-2003 7-VII-2003 16/17-VII-2004)	40 (25/30-VI-2003)		40 (28-VI-2004 5/18-VII-2004)	22 (24-VIII-2004, 2/4-VIII-2004)	24 (28-VI-2004 4/5/18-VII-2004)	40 (24-VI-2003 19-V-2004)	
D-SL-Perl	sm	49.28 6.24	>	39 (11-VII-20)	40 (11-VI-2004)	40 (11/17-VII-2003)	40 (11-7-2003)					40 21-V-2004 (19-VI-2005	40 (20-V-2004 27-VII-2005 2-VIII-2005)
D-RLP-Freudenburg	sm	49.33 6.32	>	40 (18-VII-2003)	40 (11-VI-2004)	40 (26-VI-2003)		36 (26-VI-2003 11-VI-2004)	39 (17-VII-2004 23-VII-2004)		41 (17-VII-2004 23-VII-2004)	40 (20-V-2004)	
F-Montenach	sm	49.25 6.24	>			40 (17/22-VII-2004)	40 (28-VI-2003)	40 (21/23-VI-2005)		19 (17-VII-2004 23-VII-2004)		40 (8-VI-2004 2-VIII-2005)	
F-Clouange	sm	49.16 6.32	>				40 (5-VII-2003)	40 (16-VI-2003 8-VI-2004)					
F-Lorry	sm	49.0 6.06	>	40 (17-VI-2003)									
L-Junglinster	sm	49.43 6.15	<				40 (1-VII-2004)						
L-Niederanven	sm	49.39 6.16	>	32 (13-VI-2003)		40 (20-VI-2004)	40 (13-VII-2004)	40 (13/16-VI-2003)	39 (20-VII-2004 05-VIII-2004)	29 (05-VIII-2004)	20 (20-VII-2004 05-VIII-2004)		40 (5-VIII-2004)

name of site	region	GPS	size	*M. galathea*	*M. aurelia*	*Z. viciae*	*Z. caniolica*	*Z. loti*	*T. sylvestris*	*T. acteon*	*T. lineola*	*C. minimus*	*A. agestis*
L-Rosselange	sm	49.16 6.05	>	40 (16-VI-2003)	40 (8/17-VI-2004 19-VI-2005)								
L-Schifflange	sm	49.30 6.01	>	36 (19-VI-2003)									
D-SL-Niedergailbach	bg	49.08 7.12	>	39 (27-VI-2003)	40 (20-VI-2004)	40 (27-VI-2003 19-VII-2004 26-VI-2005)	40 (27-VI-2003 19-VII-2004)		35 (19/21-VII-2004)	20 (17-VII-2004, 03-VIII-2004)	34 (19/21-VII-2004)	40 (24-V-2004)	40 (3-VIII-2004)
D-SL-Mimbach	bg	49.13 7.18	>	40 (27-VII-2003)			40 (27-VI-2003 19-VII-2004)		35 (19/21-VII-2004)		40 (19/21-VII-2004)	40 (24-V-2004)	
total numbers				644	360	320	438	276	418	166	361	320	320

2.3. Genetic analysis

Electrophoresis

We analysed a total of 3623 individuals of the ten study species from 6 to 17 populations sampled in the Rhineland-Palatinate and the Saarland (south-western Germany), Loraine (north-eastern France) and Luxemburg (see Table 2.1). Butterflies were sampled at meadows during its species-specific flight period (starting in April with *Cupido minimus*, ending in August with the third generation of *Aricia agestis*) of the years 2003, 2004 and 2005. The individuals were netted in the field, frozen alive in liquid nitrogen and stored under these conditions until analysis.
Half of the abdomen of each individual was homogenised in Pgm-buffer by ultrasound and centrifuged at 17,000 g for 3 min. For allozyme electrophoresis, we used cellulose acetate plates applying standard protocols (Hebert & Beaton 1993, Richardson et al. 1986). We analysed under species-specific conditions the allozyme loci. For specific electrophoretic conditions see Table 2.2.

Table 2.2: Analysed enzyme systems; electrophoresis conditions for the different enzymes analysed for ten butterfly species (*M. galathea*, *M. aurelia*, *Z. viciae*, *Z. loti*, *Z. carniolica*, *T. sylvestris*, *T. lineola*, *T. acteon*, *C. minimus*, *A. agestis*). Buffer, running time (min.) and homogenate applications for each species e.g. genus is specified. Abbreviations: TC: Tris-citrate pH = 8.2 (Richardson et al. 1986), TG: Tris-glycine pH = 8.5 (Hebert & Beaton 1993), TM: Tris-maleic acid pH = 7.0 (adjusted from pH = 7.8 (Richardson et al. 1986). All buffers were run at 200V. [1]: not scorable for *Z. carniolica*, [2]: not scorable for *Z. loti*, [3]: not scorable for *Z. viciae*.

locus	EC-Nr.	*M. galathea*	*M. aurelia*	*Zygaena*	*Thymelicus*	*C. minimus*	*A. agestis*
Pk	2.7.1.40	TM, 1, 40	TM, 3, 40	-	TC, 3, 30	TC, 3, 30	TC, 3, 30
G6pdh	1.1.1.49	TC, 2, 30	TC, 1, 45	TC, 3, 40	TC, 2, 30	TC, 3, 40	TC, 3, 40
6Pgdh	1.1.1.44	TC, 3, 45	TC, 3, 40	TC, 3, 40[3]	TC, 3, 45	TM, 3, 45	TM, 3, 45
Idh	1.1.1.42	TC, 3, 45	TC, 3, 40	TC, 2, 40	TC, 3, 45	TM, 3, 45	TM, 3, 45
Aat	2.6.1.1	TM, 3, 40	TC, 45, 3	TM, 3, 40	TG, 3, 40	TC, 4, 40	TM, 4, 40
Mdh	1.1.1.37	TC, 2, 40	TC, 3, 40	TC, 2, 40	TC, 2, 40	TC, 2, 40	TC, 2, 40
Pgi	5.3.1.9	TM, 1, 40	TG, 30, 1	TG, 1, 40	TG, 1, 30	TM, 1, 40	TM, 1, 40
Gpdh	1.1.1.8	TG, 4, 30	TC, 40, 3	TM, 3, 40	TM, 4, 40	TM, 4, 30	TM, 4, 30
Fum	4.2.1.2	TC, 3, 45	TC, 45, 4	TG, 3, 45	TG, 3, 40	TC, 3, 45	TC, 3, 45
Me	1.1.1.40	TM, 3, 40	TC, 3, 40	TM, 3, 30	TG, 3, 40	TC, 3, 40	TC, 3, 40
Gapdh	1.2.1.12	TC, 4, 40	TC, 50, 5	TC, 3, 30	TC, 4, 40	TC, 4, 40	TC, 4, 40
Acon	4.2.1.3	TC, 3, 45	TC, 3, 40	TC, 2, 30	TC, 3, 45	TC, 3, 45	TC, 3, 45
Hbdh	1.1.1.30	TG, 3, 40	TG, 3, 40	TM, 3, 45	TG, 3, 30	TG, 4, 30	TG, 3, 30
Apk	2.7.3.3	TG, 1, 30	TG, 1, 50	TC, 1, 45[2;3]	TG, 3, 30	TG, 3, 30	TG, 3, 30
Pgm	5.4.2.2	TM, 2, 30	TG, 2, 45	TC, 2, 30[1]	TG, 1, 40	TG, 3, 40	TG, 3, 40
Mpi	5.3.1.8	TC, 4, 25		TM, 3, 30[3]	TC, 4, 30	-	-
Pep (Phe-Pro)	3.4.11/13	TG, 3, 30	TC, 3, 40	-	TM, 3, 30	TG, 4, 25	TG, 3, 25
Pep (Leu-Gly-Gly)	5.4.11/13	-	-	--	TG, 2, 30	-	-

Statistics

The alleles were labelled according to their relative mobility, starting with "1" for the slowest. Allele frequencies, Nei's standard genetic distances (Nei 1978) and parameters of genetic diversity (i.e. mean number of alleles per locus *A*, expected heterozygosity H_e, total percentage of polymorphic loci P_{tot} and percentage of polymorphic loci with the most common allele not exceeding 95% P_{95}) were computed with G-Stat (Siegismund 1993).
AMOVAs, hierarchical genetic variance analysis, tests of Hardy Weinberg equilibrium and linkage disequilibrium were calculated with Arlequin 2.000 (Schneider et al. 2000).
Phenograms (using neighbour joining (Saitou & Nei 1987) and UPGMA were constructed with Phylip (Felsenstein 1993). Principal Components Analyses (PCA) based on the allele frequencies as data matrix was performed using the varimax factor rotation method and ANOVAs were calculated using the Kruskal-Wallis algorithm (calculation with STATISTICA).
Differences between means were analysed by Friedmann ANOVAs, Wilcoxon matched pairs tests or Mann-Whitney U tests and Sign-tests, using STATISTICA. Correlations between geographical and genetic distances were tested by Pearson correlation using XLSTAT.

2.4. Analysis of population ecology

Mark release recapture (applied in 3., M. galathea)

For mark release recapture-studies we chose a part of our study area, located south-eastern of the city of Bitburg. The selected area of four keuper hills with barren calcareous grasslands (Figure 3.4 in 3.1) are nature reserves (Jungbluth et al. 1995), maintained by extensive sheep pasturing. The distances between the studied sites varied from 0.6 km to 3.5 km.
Butterflies were netted, marked, released and recaptured in summer 2004. Each capture adult was sexed and marked with a number and a letter (X, O, S, W, A; the latter two are two sub patches approximately the equal in size) on the underside of one of the hind wings, using a Staedtler Lumcolor 313 permanent OH pen. Immediately thereafter, the specimen was release at the point of capture. For each individual, sex and exact date (i.e. day and time) of first capture were noted. For all recaptures, we noted the individual's code, the patch of first capture and the sex and exact date of recapture. 12 to 15 MRR campaigns were performed at every patch from 1st of July to 3rd of August of the year 2004 for representing the respective flight period. Population size (*N*) was estimated using the method of Jolly–Seber (Krebs 1999). As our data reveal a very limited number of interpatch migrations, we could apply models for the calculation of the number of individuals assuming closed populations (Model A of the program JOLLY) without committing major errors. Distances between capture

points, both within (sub patch W and A) and between the sites were observed.

3. Low genetic differentiation and high dispersal ability coincide in the widespread butterfly species *Melanargia galathea*

3.1. Results

Electrophoretic results

A total of 644 individuals of *M. galathea* from 17 populations sampled in Germany, France and Luxemburg were analysed by allozym electrophoresis. All enzyme loci had banding patterns consistent with known quaternary structures. Most loci were inherited autosomally, but 6Pgdh, Pgm and Me were located on the Z chromosome, so that hemizygous females only have a single copy. Therefore, we only found one allele in all analysed females for these loci whereas at least some heterozygote males were observed.

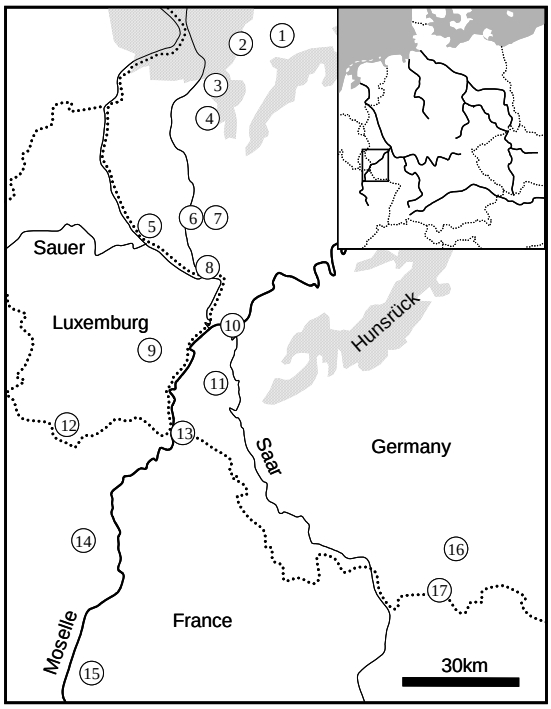


Figure 3.1: The geographical location of the 17 sample stations of *Melanargia galathea* in Germany, France and Luxemburg. Dotted lines: country borders, elevations above 500 m a.s.l. are hatched. 1: Niederehe, 2: Lissendorf, 3: Weinsheim, 4: Schönecken, 5: Ourtal, 6: Bettingen, 7: Dockendorf, 8: Echternacherbrück, 9: Niederanven, 10: Wasserliesch, 11: Freudenburg, 12: Schifflange, 13: Perl, 14: Rosselange, 15: Lorry, 16: Mimbach, 17: Niedergailbach.

Eight of the analysed 17 loci were polymorphic (allele frequencies see Appendix), but 10 loci (Idh1, Idh2, Mdh1, Mdh2, G6pdh, Aat1, Gpdh, Fum, Gapdh, Acon) had only one single allele. Deviations from Hardy Weinberg after Bonferoni correction were detected in the following sites: Wasserliesch: Pgi, Weinsheim: Pgi, Ourtal: Aat2, Echternacherbrück: Pgi, Aat2, Bettingen: Pgi, Perl: Pgi, Freudenburg: Aat2, Niederehe: Aat2, Niedergailbach: Mimbach: Ingendorf: Niederanven: Aat2, Pgi. No general linkage disequilibrium was observed for any locus. Therefore, further analyses were performed using standard algorithms in population genetics.
We obtained the following results for four population genetic parameters calculated: mean number of alleles per locus (A): 2.00 (±0.11 s.d.), ranging from 1.83 to 2.17; expected heterozygosity (H_e): 16.8% (±1.2 s.d.), ranging from 15.1%-19.1%; total percentage of polymorphic loci (P_{tot}): 38.6% (±2.39 s.d.), ranging from 33.3% to 44.4%; and percentage of polymorphic loci with the most common allele not exceeding 95% (P_{95}): 36.9% (±2.8 s.d.), ranging from 33.3% to 38.9% (see Table 3.1 for detailed data).

Table 3.1: Five parameters of genetic diversity for all populations analysed of *Melanargia galathea*: mean number alleles per locus (A), percentage of expected heterozygosity (H_e), percentage of loci with the most common allele not exceeding 95% (P_{95}) and total percentage of polymorphic loci (P_{tot}). ce: Central Eifel, sm: Saar-Mosel region, bg: Bliesgau, Sampling stations: see Figure 3.1.

site	area	A	H_e [%]	H_o [%]	H_o/H_e	P_{95} [%]	P_{tot} [%]
Niederehe	ce	1.83	16.8	15.8	0.94	33.3	33.3
Lissendorf	ce	2.00	16.2	13.4	0.83	33.3	38.9
Schönecken	ce	2.17	17.0	14.7	0.86	38.9	38.9
Weinsheim	ce	2.11	19.1	16.6	0.87	38.9	38.9
Ourtal	sm	2.00	16.2	14.4	0.89	33.3	38.9
Dockendorf	sm	1.94	16.4	13.9	0.85	38.9	38.9
Echternacherbrück	sm	2.17	16.9	14.1	0.83	38.9	38.9
Bettingen	sm	1.89	17.7	14.8	0.84	38.9	38.9
Wasserliesch	sm	2.00	15.9	13.4	0.84	38.9	38.9
Perl	sm	2.00	16.7	14.4	0.86	38.9	38.9
Freudenburg	sm	2.00	15.3	15.0	0.98	38.9	38.9
Lorry	sm	2.00	16.8	14.2	0.85	33.3	38.9
Niederanven	sm	2.06	18.3	15.8	0.86	38.9	44.4
Rosselange	sm	2.17	18.2	17.2	0.95	38.9	38.9
Schifflange	sm	1.89	15.5	13.4	0.86	33.3	38.9
Niedergailbach	bg	1.89	15.1	14.4	0.95	38.9	33.3
Mimbach	bg	1.89	18.9	13.7	0.72	33.3	38.9
mean		2.00	16.9	14.7	0.87	36.9	38.6
(± s.d.)		(±0.1)	(±1.2)	(±1.1)	(±0.1)	(±2.8)	(±2.4)

No significant differences for all analysed parameters of the genetic diversity were found between the three defined limestone sub-regions (ANOVA: all p>0.05). Correlations between the parameters of genetic diversity and the size of the patch was not significant (*p*-values: *A*: 0.045, H_e: 0.078, H_o: 0.070, P_{95}: 0.903, P_{tot}: 0.879). Cluster analyses based on neighbour joining and UPGMA revealed similar topologies; the first is shown in Figure 3.2.

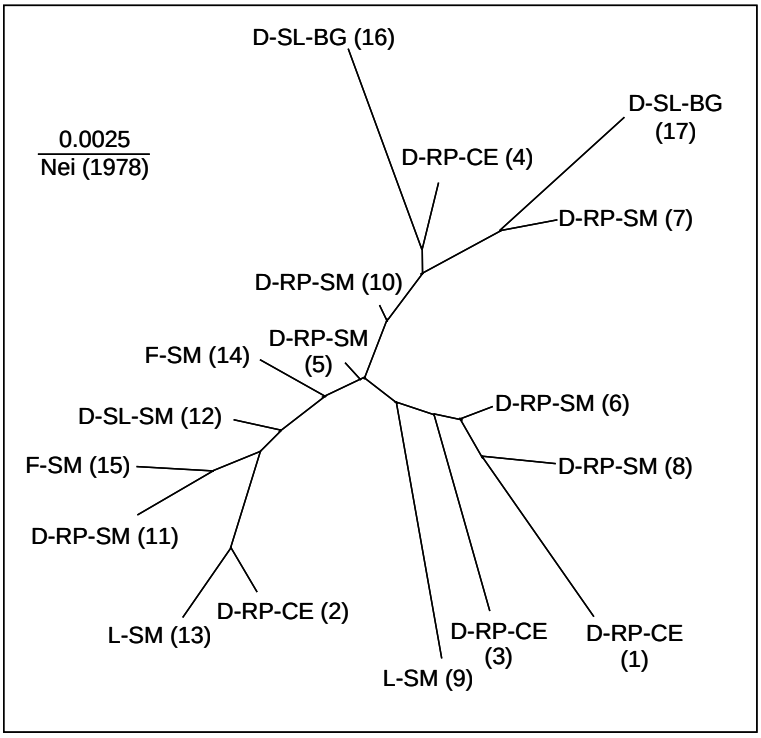


Figure 3.2: Neighbour-joining dendrogram based on the genetic distances (Nei 1978) of 17 populations of *M. galathea;* Abbreviations: D: Germany, L: Luxemburg, F: France, RP: Rhineland-Palatinate, SL: Saarland, CE: Central Eifel, SM: Saar-Mosel region, BG: Bliesgau. Numbers in parenthesis: sample sites see Figure 3.1.

The total genetic variance among populations contains 0.052 (p<0.001), among individuals within populations 0.203 (p<0.001) and within individuals 1.313 was found for *M. galathea* (total variance: 1.569) Thus, 3.3% of the total genetic variance were found among populations (F_{ST}). The genetic variance within individuals (F_{IS}) was 13.4%. A significant correlation between the geographical distances and the respective genetic distances (Nei 1978) of the populations of *Melanargia galathea* (r^2=0.225; Mantel test: p=0.000) were detected (Figure 3.3).

MRR results

We marked 3857 individuals (2984 males; 873 females). 604 individuals (453 males; 147 females) were recaptured at least once during one of the following campaigns (i.e. an overall recapture ratio

of 18.7%; (minimum: 10.5%. maximum: 25.1%). The detailed data for the four studied sites are presented in Table 3.2. Based on these data, we estimated medium population size ranging from 263 to 527 individuals per patch (Table 3.2). Thus, densities per ha ranged from 91 to 370 individuals per hectare (Table 3.2). One male survived at least 26 days. Over 50% of the recaptured individuals in A and W changed between these two sub patches. 13 movements of individuals between sites were observed (Figure 3.4). 12 individuals migrated 0.7 km from O (with a smaller population than the receiver population) to X and one butterfly the other direction. 3.3% of the recaptured individuals moved between patches. The isolated patches W, A and S neither acted as donor nor as receiver for any of the other studied sites.

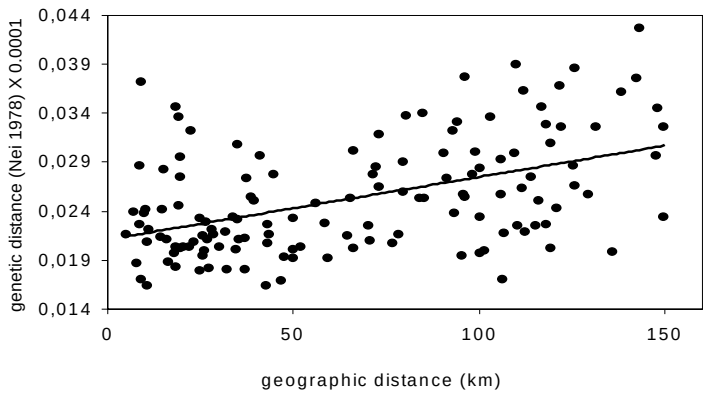


Figure 3.3: Correlation between the geographical distances and the respective genetic distances (Nei 1978) of the populations of *Melanargia galathea* (r^2=0.225; Mantel test: p=0.000)

Table 3.2: Number of marked and recaptured individuals of *Melanargia galathea*. Based on these data, the total number of individuals per patch over the whole flight period was estimated using JOLLY, Modell A (for closed populations).

parameter	X	O	S	W	A	W&A
patch size	2.5	1	3	4.5	2.2	6.7
marked individuals	830	619	550	630	546	1176
recaptured in site	208	116	128	66	86	152
recapture ratio [%]	25.1	18.7	23.3	10.5	15.8	13.15
estimated medium pop size	351.65	370.23	273.52	527.02	263	395
standard deviation	130.57	196.34	44.04	117.68	277.37	147.53
density (ind./ha)	141	370	91	117	120	118.5

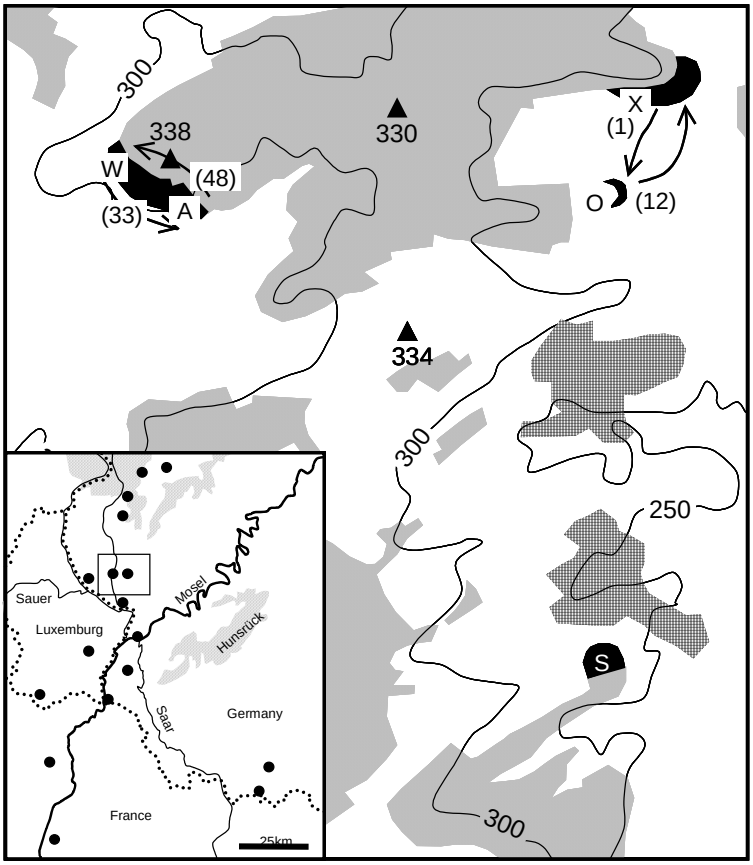


Figure 3.4: Map of the study area with the four study sites of *Melanargia galathea*; grey: forest, black crossed: villages, black: Keuper-Scharren, arrows indicate migrations, numbers at arrows symbolize the migrating individuals.

3.2. Discussion

Occupancy and connectivity of habitats

The micro distribution of *Melanargia galathea* is strongly influences by the distribution of biotic and abiotic conditions like preferred nectar sources (like *Centaurea spp*.). the presence of their larval habitat (often unshadowed, dry Mesobromion, Xerobromion and Arrhenatherion) and the need for particular i. e. warm microclimatic conditions (south facing slopes) (cf. Baguette et al. 2000, Vandewoistijne 2005). Patch occupancy indicates that a large potential of *M. galathea*-sites are mostly occupied in western Germany (Wenzel et al. 2006). which is similar to studies of *M. galathea* in Belgium (Vandewoistijne 2004) and differ from sedentary butterfly species like *Maculinea alcon* (Habel et al. 2005. Wallis de Vries 2004). *Melitaea cinxia* (Thomas et al. 2001) and *Coenonympha tullia* (Dennis & Eales 1997). Reduced mobility and high isolation of suitable habitats lead to a better part of patches rest unoccupied (Harrison 1991, Hanski & Gyllenberg 1993). Although *M. galathea*

make demands concerning habitat quality, the species is also present in linear structures as road verges and abandoned railway tracks, representing stepping stone biotopes and supporting permanent breeding populations (see also Munguira & Thomas 1992). These structures enable a higher permeability of the agriculturally intensively used matrix (Hanski 1999).
Strong dispersal capacity of species appears to ensure higher patch occupancy than does high density. Connectivity of populations is therefore a key ecological process linking metapopulations (Hanski 1999). This can be measured directly by mark release recapture (MRR) and indirect via gene flow.

Movements and ages

In our MRR-study we observed movements between favourable habitat patches. However a high proportion of marked individuals were residents. Nevertheless, movements of more than 7 km have been observed in other studies. In contrast to other marked butterflies like *Polyommatus corridon* (Schmitt et al. 2005) and *Maculinea alcon* (Wynhoff et al. 1996, Maes 2004), we detected strong intrapatch movements, by spliting a patch in two parts. *Polyomatus corridon* showed comparably high population densities to *M. galathea*, but in contrast very low intrapatch-movements (Schmitt et al. 2006). Low diameters of dispersal of most individuals of a population to be less than 50 m were observed by many butterflies (Wynhoff et al. 1996, Stettmer et al. 2001a,b). This could influence the genetic variability and processes of genetic differentiation in populations. Lifespan and mobility of an organism influences a successful colonisation of an empty habitat. The lifespan of *M. galathea* at an average amounts only 1.9 days in a single, but a long generation-time in a year. This is a relatively long flight periode (up to six weeks) comparing to other butterfly species like *Maculinea* species (Settele et al. 1995, Meyer-Hozack 2000). Short-term disturbances like bad weather conditions can be absorbed by the generation-turnover during the long enduring flight period.

Genetic diversity and differentiation

The low genetic differentiation (F_{ST}: 3.3) between the analysed *M. galathea*–populations reflect the high dispersal rate and high abundance (low distances between populations). The percentage of the total genetic diversity is typical of species characterised by a high dispersal capacity and high density populations like *Polyommatus coridon* and *Proclossiana eunomia,* (Vandewoestijne et al. 2004, Baguette 2003, Schmitt et al. 2002, Nève unpubl.).
The natural fragmentation by the geological expanse of limestone areas lead to a fragmentation in three limestone areas in our study area. We could detect no genetic differentiation nor between these regions, neither between other geographical scales – so called clines. The distances between analysed populations averages 65.77km (vary

from 4.9km to 149km); a significant effect of `isolation by distance´ was detectable. This corresponds to seven molecular genetically analysed *M. galathea* populations in Belgium on an equal regional scale. In our study area we analysed other butterfly species by the same molecular techniques for a better understanding how abundance patterns and dispersal ability influence the phenomenon of gene flow and genetic differentiation. The phenomenon of `isolation by distance´ we could detect by species with intermediate exchanges between populations (gene flow) and intermediate sedentarity (gene drift) – like *Thymelicus sylvestris* (see chapter 6).

The combination of MRR- and molecular data support the assumption that: Low genetic differentiation and high dispersal ability coincide in the widespread butterfly species *Melanargia galathea.*

Excursus I

Genes in space and time: The influence of climatic changes in Europe shown on the *Melanargia galathea* / *M. lachesis* species complex

Beside regional effects of habitat isolation, phylogeographic studies deal with a larger scale in time and space.
About 45 million years ago, during the Eocene, the annual mean temperature of about 20°C declined to 15°C in the Pliocene (12-2 million years ago). The following Pleistocene is characterised by its cyclic warming and cooling periods (glacial/interglacial cycles). During the interglacials, the annual mean temperature was 15°C, during the glaciations 0°C (Hewitt 1996).
These changes in temperature led to a change from subtropics conditions with its vegetation in the Eocene to permafrost and steppe, dominated by *Poaceae*, *Chenopodiaceae* and *Artemisia* species in Central Europe (Hewitt 1996). Temperate species survived south of the European mountains (Alps, Carpathians and Pyrenees). Today, the southern slopes of the Alps is a region having many endemic species (Holdhaus 1954).
Phylogeographic studies reconstruct the history of species, their distribution patterns and the climate conditions (Hewitt 1996, 1999, 2000, 2001, Taberlet et al. 1998). Many genetic studies on different spatial and temporal scales, analysed by different genetic markers (microsatellites, mtDNA and allozymes) support that the climatic oscillation in the Pleistocene evoke spatial isolation over many 1000s of years. This had led to a manifestation of distinct alleles in these southern refugia. We tested the spatial and temporal dimension of these isolation effects on different butterfly species by allozyme electrophoresis.
Isolation of Mediterranean species in the southern European peninsulas during the cold glacial phases often evolves differentiation of several genetic lineages confined to the respective peninsulas (Hewitt 1996). There is good genetic evidence for multiple refugia in Iberia, Italy and the Balkan. We analysed the allozymes of the Marbled White butterfly *Melanargia galathea* and its sibling species *Melanargia lachesis*, distributed over the Iberian peninsula, where *M. galathea* is missing. We studied 18 allozyme loci of 1603 individuals from 41 populations of *M. galathea* distributed over a major part of Europe and one population (40 Individuals) of *M. lachesis* on the northern border of its distribution area. We obtained the following results:

(i) The *M. lachesis* sample from the southern Pyrenees was strongly genetically differentiated from *M. galathea* (F_{CT}: 0.312) (Figure I.1).

(ii) *Melanargia galathea* splits into two major genetic lineages (F_{CT}: 0.115), which both were found in postglacially invaded regions (Figure I.1). The further differentiation within these lineages was comparably low (F_{SC}: 0.028). The genetic diversity within populations was high compared to other butterfly species. The high genetic diversity within populations might be one reason for the recently observed expansions at the northern distribution limits. The overall genetic differentiation between populations was considerable (F_{ST}: 7.0%).

(iii) Cluster analysis discriminated three genetic groups in the Balkan: (i) a western flank in Ex-Yugoslavia, parts of eastern Austria and Hungary, (ii) an eastern flank with populations from Bulgaria and Romania (south of the southern Carpathians, eastern Carpathians) and (iii) the eastern Carpathian Basin (Figure I.2). Hierarchical variance analysis distributed 53% of the genetic variance between populations between these three groups.

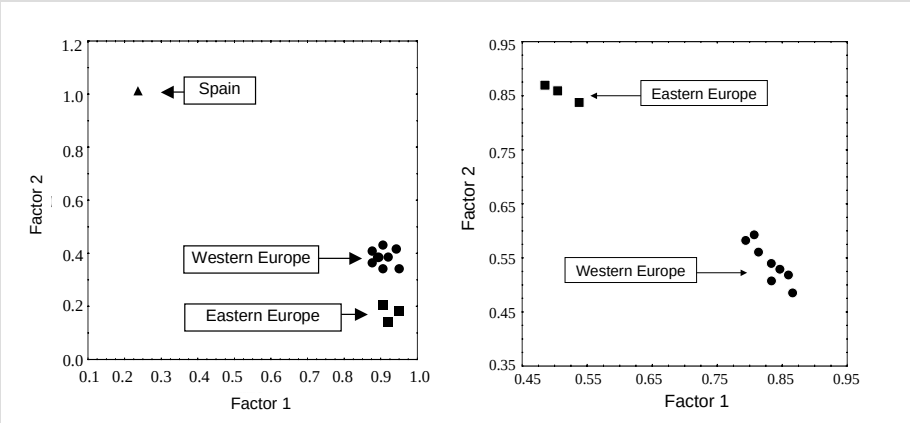


Figure I.1: Principal Components Analyses of *M. galathea* and *M. lachesis* (Spain); *M. lachesis* separated strongly from *M. galathea* on the first two factors, which explain about 93.7% of the total variance (factor 1: 87.4%, factor 2: 6.4%); a separate analysis based on samples of *M. galathea* only shows a clear separation of the eastern and western lineages on both of the two first factors, representing about 97.4% of the total variance (factor 1: 92.9%, factor 2: 4.3%).

These results let us propose that two refugia existed during the last glacial (Würm) in Italy and at the Balkan. The sibling species *M. lachesis* in the Iberian Peninsula evolved into a distinct species prior to this split. The Balkan was subdivided in three subrefugia. The eastern Carpathian Basin group might result from postglacial expansion from northern Greece through valley systems of the central Balkan peninsula, maybe even expanding westwards north of the western Balkan mountains reaching some parts of eastern Austria (e.g. Carinthia). Therefore, the Balkanic refugium of *M. galathea* might have been or not been continuous along the coastal areas of the Mediterranean, but must have been strongly genetically structured.

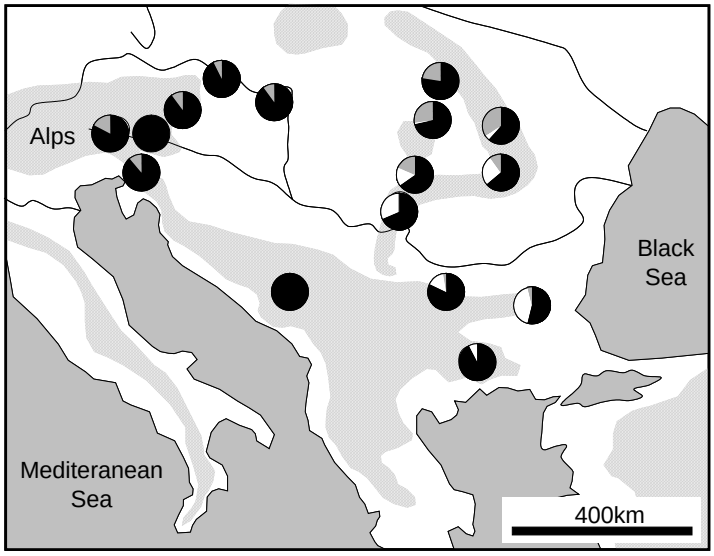


Figure I.2: Allele frequencies of the Hbdh (3-Hydroxyisobutyrate dehydrogenase) locus of the analysed populations of *Melanargia galathea* in south-eastern Europe. Allele 3: black, allele 4: white, all other alleles grey. Mountain regions above 1000 m a.s.l. are hatched.

Further details in:

Habel J C, Schmitt T, Müller P (2005) The fourth paradigm pattern of postglacial range expansion of European terrestrial species: the phylogeography of the Marbled White butterfly (Satyrinae, Lepidoptera), Journal of Biogeography, 32, 1489-1497.
Habel J C (2005) Auswirkungen vergangener klimatischer Veränderungen auf die aktuelle genetische Struktur von Organismen am Beispiel der Schachbrettfalter *Melanargia glalathea* und *Melanargia lachesis* (Lepidoptera: Satyrinae), Treffpunkt Biologische Vielfalt (Hrsg. BfN), 2005, 15-18.
Schmitt T, Habel J C, Zimmermann M, Müller P (2006) Genetic differentiation of the Marbled White butterfly *Melanargia galathea* account for glacial distribution patterns and postglacial range expansion in south-eastern Europe, Molecular Ecology 15,1889-1901.

4. Generalist versus Specialist: the genetic consequence of differing ecological demands

4.1. Results

A total of 718 individuals of the two Nymphalid butterfly species *Melanargia galathea* and *Melitaea aurelia* were collected in ten sites in western Germany (Figure 4.1) (358 individuals of *M. galathea*, 360 of *M. aurelia*) and were analysed by allozyme electrophoresis. Eight of the 16 analysed allozyme loci were polymorphic in both investigated butterfly species: the loci Pgi, Aat1, Aat2, Acon, Pep, Pgm, Mdh2 and Gapdh in *M. aurelia* and Pgi, Aat1, Pep, Pgm, Hbdh, Me, 6Pgdh and Mpi in *M. galathea*. The other loci had only one single allele. No remarkable deviations from Hardy-Weinberg equilibrium were detected. Only few cases of significant deviation exist after Bonferroni correction.

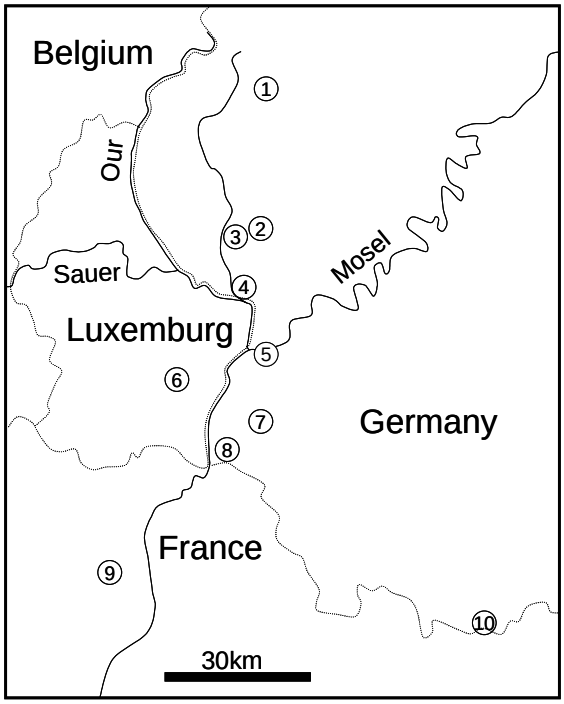


Figure 4.1: Geographical locations of the ten sample stations of *Melanargia galathea* and *Melitaea aurelia* in Germany, France and Luxemburg. Dotted lines: country borders. 1: Weinsheim, 2: Ingendorf, 3: Bettingen, 4: Echternacherbrück, 5: Wasserliesch, 6: Niederanven, 7: Freudenburg, 8: Perl, 9: Rosselange, 10: Niedergailbach.

We obtained the following results for five calculated population genetic parameter: The mean number of alleles per locus (*A*) was for *M. aurelia* 1.71 (±0.15 s.d.), ranging from 1.56 to 2.06, for *M. galathea* 2.02 (±0.10 s.d.), ranging from 1.89 to 2.17 and was lower in *M. aurelia*; the mean expected heterozygosity (H_e) amounts 10.4% (±1.90 s.d.) for *M. aurelia*, ranging from 5.9% to 13.2%, which is also

lower compared to *M. galathea* with a mean value of 17.0% (±0.01 s.d.), ranging from 15.1% to 19.1%; the mean percentage of the mean observed heterozygostiy (H_o) was also lower in *M. aurelia*, 9.3% (±1.98 s.d.), ranging from 8.1% to 11.6%, compared to *M. galathea´*s values of the observed heterozygosity of 15.0% (±0.01 s.d.), ranging from 13.4% to 17.2%. The mean percentage of polymorophic loci with the most common allele not exceeding 95% (P_{95}) was lower in *M. aurelia*: 28.7% (±8.43 s.d.), ranging from 18.8% to 43.8% and for *M. galathea* 37.8% (±2.00 s.d.), ranging from 33.3% to 38.9%. Mean of total percentage of polymorphic loci (P_{tot}) amounts 35.6% (±5.93 s.d.) for *M. aurelia*, ranging from 25.0% to 43.8% and for *M. galathea* 38.9% (±2.00 s.d.) ranging from 33.3% to 44.4%; Except the last parameter (P_{tot}), all analysed parameters of genetic diversity were significantly higher in *M. galathea* ($p<0.05$) (Table 4.2).
In both species, all analysed parameters of genetic variability showed no significantly higher parameters of their genetic diversity on large habitats (>15 ha) compared with smaller patches (p>0.05).

The total genetic variance among populations, among individuals within populations and within individuals was higher for *M. galathea* compared to *M. aurelia* (Table 4.1). For *M. aurelia*, 3.6% of the total genetic variance was found among populations, and thus is similar to *M. galathea* (F_{ST} 3.3 %). The F_{IS} values were relatively high for both species (*M. aurelia*: 8.8% and *M. galathea*: 11.7%) (Table 4.1).

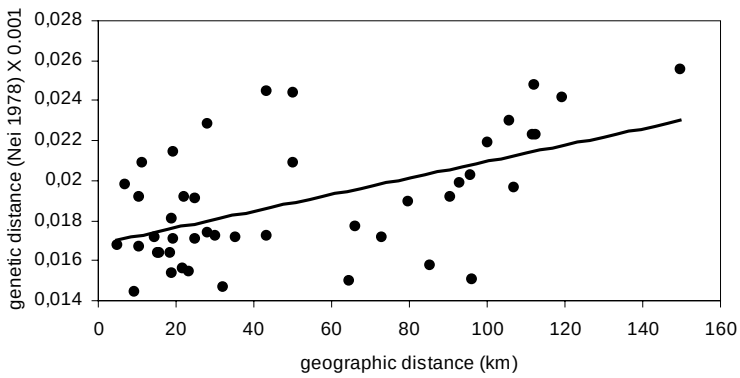
Table 4.1: Non-hierarchical variance analysis of *Melitaea aurelia* and *Melanargia galathea* of the ten analysed populations.

species	among populations	among individuals	within individuals	total variance
M. aurelia	0.031	0.073	0.762	0.866
M. galathea	0.051	0.178	1.344	1.573

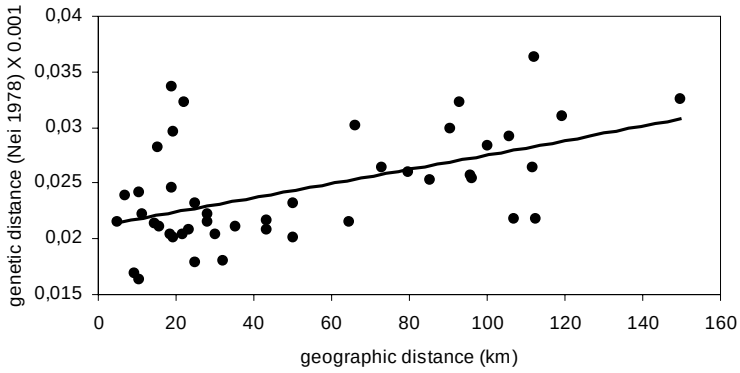
We detected an isolation by distance system in both species. The geographic distance explained 28.5% of the genetic variance in *M. aurelia* ($p = 0.001$) and 27.8% of the genetic variance for *M. galathea* (p=0.01) (Figure 4.2).

Table 4.2: Five parameters of genetic diversity for all populations analysed of *Melanargia galathea* and *Melitaea aurelia*: mean number alleles per locus (*A*), percentage of expected heterozygosity (H_e), total percentage of the observed heterocygostiy (H_o), Observed heterozygosity divided by the expected heterozygosity (H_O/H_e) indicating deviations of Hardy-Weinberg equilibrium, percentage of loci with the most commonest allele not exceeding 95% (P_{95}) and total percentage of polymorphic loci (P_{tot}). Abbreviations: ce: Central Eifel, sm: Saar-Mosel region, bg: Bliesgau, *M.a.*: *Melitaea aurelia*, *M.g.*: *Melanargia galathea*. Tests for significant differences are performed using Sign test.

Site	area	*A*		H_e [%]		H_O [%]		H_O/H_e		P_{tot} [%]		P_{95} [%]	
		M. a.	*M. g.*	*M. a.*	*M. g.*	*M. a.*	*M. g.*	*M. a.*	*M. g.*	*M. a.*	*M. g.*	*M. a.*	*M. g.*
Weinsheim	ce	1.88	2.11	11.4	19.1	10.1	16.6	0.89	0.87	37.5	38.9	31.3	38.9
Ingendorf	sm	1.56	1.94	10.2	16.4	8.1	13.9	0.79	0.85	37.5	38.9	31.3	38.9
Bettingen	sm	1.63	1.89	9.4	17.7	9.1	14.8	0.97	0.84	25.0	38.9	25.0	38.9
Echternacherbrück	sm	1.69	2.17	9.7	16.9	9.7	14.1	1.00	0.83	37.5	38.9	18.8	38.9
Wasserliesch	sm	1.56	2.00	5.9	15.9	4.8	13.4	0.81	0.84	25.0	38.9	18.8	38.9
Niederanven	sm	1.75	2.06	11.2	18.3	10.7	15.8	0.96	0.86	37.5	44.4	18.8	33.3
Freudenburg	sm	1.69	2.00	11.9	15.3	10.9	15.0	0.92	0.98	37.5	38.9	37.5	38.9
Perl	sm	1.75	2.00	10.9	16.7	10.6	14.4	0.97	0.86	37.5	38.9	31.3	38.9
Rosselange	sm	2.06	2.17	13.2	18.2	11.6	17.2	0.88	0.95	43.8	38.9	43.8	38.9
Niedergailbach	bg	1.56	1.89	10.2	15.1	8.1	14.4	0.79	0.95	37.5	33.3	31.3	33.3
mean (± s.d.)		1.71 (±0.15)	2.02 (±0.10)	10.4 (±1.90)	17.0 (±0.01)	9.3 (±1.98)	15.0 (±0.01)	0.89 (±0.07)	0.880. (±0.05)	35.6 (±5.93)	38.9 (±0.02)	28.7 (±8.43)	0.378 (±0.02)
Sign test (p)		0.004		0.004		0.004		0.751		0.113		0.026	



Melitaea aurelia



Melanargia galathea

Figure 4.2: Correlation between the geographical distances and the respective genetic distances (Nei 1978) of the populations of *Melitaea aurelia* (r^2=0.285; Mantel test: p=0.001) and *Melanargia galathea* (r^2=0.278, Mantel test: p=0.001).

4.2. Discussion

The two Nymphalid butterflies show similar genetic differentiation on the regional scale: *M. aurelia´*s genetic variance between populations measured by F_{ST} contain 3.6%, which is only marginally higher than in *M. galathea* (F_{ST}: 3.3%). Furthermore for both species we detected a significant isolation by distance system, explaining 28.5% (p=0.001) for *M. aurelia* and 27.8% (p=0.01) for *M .galathea*. These data reflect a significant ongoing exchange of individuals, especially between neighbouring sites. In contrast to the genetic differentiation, the genetic diversity is significantly higher in *M. galathea*. The mean number of alleles per locus (*A*), the expected and observed heterozygosity (H_e, H_o) and the percentage of loci with the most commonest not exceeding 95% (P_{95}) express significantly higher values for *M. galathea*.

Comparing the ecology of both species, we analysed a specialised butterfly in *M. aurelia*, exclusively restricted to jurassic and lacustrine limestone in our study area. The larvae are mostly feeding on *Melampyrum spec.* (Ebert & Rennwald 1991b). *M. galathea* is in contrast a rather common species in our study area present on meadows and verges. Regarding the ecological requirements, *M.*

galathea represents a broader ecological niche, leading to higher abundance and lower patchiness in landscape in comparison to *M. aurelia*. This lower specialisation on special life conditions evoke a broader genetic range for *M. galathea.* The contrast of this coherence lower genetic variability was found for the specialist *M. aurelia*. Both butterflies species appear constantly in high densities in past and present in our study area (Weitzel, pers. comm.).

Compared to *M. galathea*, in *M. aurelia* we detected significant lower genetic diversity consisting of only few alleles without rare ones. This constitution of few main alleles is an adaptation to prevent a loss of genetic diversity while stochastic population depressions or may be a result of colonisation processes combined by negative effects of genetic bottlenecks evoked by small founder populations. The low range between the minimum and maximal value of the mean number of alleles (*A*) over all investigated sites let us assume that the simple genetic pattern is a result of an evolutionarily process. *M. aurelia* survived in a historical patchily habitat situation evoked by geological conditions obviously well adapted on this habitat network. A recent genetic depression by anthropogenous habitat destruction would affect the genetic constitution in each habitat in a different intensity. This lead to a high variation in the values of analysed gene diversity, showing in *Zygaena lo*ti (see chapter 6). This species obviously is suffering under the present habitat deterioration. The genetic consequence of natural isolation, like in *M. aurelia* is studied in the butterfly species *Melitaea cinxia*: inbreeding experiments showed higher fitness, like longer flight period in naturally small fragmented populations with a history of repeated rounds of inbreeding in the past, compared to individuals deriving from a more continuous population structure (Haikola et al. 2001). Comparing sister species with differing ecological potentials, the genetic response was shown in bark beetles by Kelley et al. (2000): Generalist populations (*Dendroctonus ponderosa*) maintained approximately 10 times the genetic variation in mtDNA as the specialist populations (*D. jeffreyi*). This support the assumption that in general specialised species show this simple genetic design than generalist, according to our example. The low number of few alleles without infrequent ones found in *M. aurelia* is in accordance of genetic studies on further sedentary species of the genus *Maculinea*. This species, representing one of the most complex life cycle in butterflies (Thomas & Elmes 2001). Genetic analysis showed that small populations founded by some individuals have the same genetic constitution, compared to large metapopulation networks (Thomas et al. 1998, Wynhoff 2001). These species are characterized by a low gene diversity and a loss of heterozygosity (shown for *Maculinea alcon*, Gadeberg & Boomsma 1997). This small genetic range respond their complex life cycles. In the same time, this close adaptation might also be of high danger in stochastic extinction events, because of the failure to react to biotic and abiotic changes. However, studies in other butterfly species showed that genetic depressions evoked by isolation and sedentarily behaviour might be of prominent danger like for *Maculinea arion* and *Papiliao machaon,* the mobility of isolated populations is negatively influence by reduced

thorax length and consequently flight muscles (Thomas et al. 1998). Life span of *Maculinea teleius* and *M. nausithous* were shorter after relocation with the consequence of a lower potency to react during bad weather periods (Wynhoff 2001).

Based on the analysed F_{ST} estimates, we argue that the habitats are still sufficiently interconnected for both butterfly species, which is also reflected by a similar correlation between genetic and geographic distance. Genetic differentiation resulting from habitat fragmentation and the subsequent of geographical isolation of populations is well documented (Hall et al. 1995, Lacy & Lindenmayer 1995). Studies on three European skipper species analysed by the same genetic method in the same study area (see chapter 6) showed the genetic effect of low connectivity. The species specific genetic differentiation pattern were dependent on their abundance; whereas the commonest one (*Thymelicus lineola*) showed a panmictic genetic structure (low F_{ST}), the xerothermophilous specialist (*Thymelicus acteon*) was genetically differentiated (high F_{ST}) because of weak patch-connectivity (see chapter 6). This example shows, that in contrast to *M. aurelia*, *T. acteon* is not adapted to preserve its genetic diversity despite patchily occurrence. In both analysed butterflies, *M .aurelia* and *M. galathea*, we detected a similar correlation between genetic and geographic distance, which represents a limited dispersal mostly restricted on neighbouring populations. To estimate levels of habitat connectivity for a species, two techniques can be applied: (i) direct mark release recapture (cf. Vandewoestijne et al. 2004) and (ii) indirect via genetic analysis to detect gene flow gene flow between patches. Movement studies are mostly restricted to annual investigations, whereas gene flow estimates deal with averaged unobserved events during decades and may generate differing results, as shown in the grasshopper *Chrothippus parallelus* (Cooper et al. 1995) and in the butterfly *Heliconius charithonia* (Kronforst & Fleming 2001). Some populations of *M. galathea*, which were analysed by allozym electrophoresis, the dispersal additionally were studied by mark release recapture method (see chapter 3): the results coincide with the genetic data and showed low exchanges between patches, depending the geographical distance. This behaviour is reflected by the analysed isolation by distance system.

The genetic differentiation patterns are dependent on the mobility of the organisms and their genetic adaptation. In *M .aurelia*, despite of its patchily occurrence, the simple genetic texture enable this butterfly to show similar genetic constitutions in all investigated sites and thus low genetic differentiation. Strong interpatch differentiation may evolve by low dispersal combined by a failure of a genetic adaptation. In flightless Carabids even neighbouring populations may be genetically differentiated, documented for wingless ground beetles. They are very reluctant to cross paved roads (Mader 1984) and this isolation is strong enough for significant genetic differentiation among populations evolving within a few decades (Keller & Largiadèr 2003). Keller et al. (2005) detected similar patterns in the abundant ground beetle *Abax parallelopipedus* since the construction of a road some tens of years ago. How far the fragmentation is a recent event and

effects of genetic depressions has not yet manifested itself in the genetic variance of *M. aurelia* is improbably, because of its continuous equal low level of genetic variability. Time lacks of an genetic response to habitat changes are shown for grasshoppers (Chapco & Bidockca 1986, Chapco et al. 1992) and carabids (Assmann & Günther 2000), in which the genetic constitution resemble a pattern of historical distributed forest islands and effects of gene flow (or restricted gene flow) not yet manifested in the genome. However, the obtained genetic variability of *M. aurelia* and *M. galathea* represents evoluated species specific life behaviours. Their genetic *status quo* reveal connectedness and for *M. aurelia* a continuous low level of genetic diversity.

Excursus II

Africa to Europe
The African origin of “European” species

In Excursus I we discussed the genetic pattern of *Melanargia galathea* over Europe; typical refugia were detected in Southern Europe and postglacial recolonization routes could be identified by genetic information. Only few phylogeographic studies consider Northern Africa as the southern border of the Palearctic; this area were mostly not taken into account in genetic studies as potential refugia during glaciations or even differentiation centres for species, excluding few studies (Veith et al. 2004 and others).

Thermophilous species survived the ice ages in Mediterranean refugia around the Mediteranean (Hewitt 2001, Schmitt & Seitz 2001) and Northern Africa (Vila 2004). The Saharan desert was significantly contracted southwards during the glacial maxima of the Pleistocene (Glennie & Singhvi 2002, Hooghiemstra et al. 1992). A moister North Africa than today is further supported by paleoclimatic reconstructions for the last glacial maximum (Jedoui et al. 2002). Data by pollen records show, for instance, evidence for warm mixed forests at low altitudes in the region at the onset of the Holocene (Vila 2004 and references therein). Presence of moist-dependent ecosystems in both southern Iberia and Morocco throughout the Quaternary is demonstrated by the current occurrence of spots with Tertiary relicts like Macaronesian laurisilva vegetation (reviewed by Galan de Mera et al. 2003). The suitability of the North African habitat to host temperate fauna can also be inferred from warm mixed forests at high altitudes and temperate xerophytic woods / scrubs in the lowlands (Bennet et al. 2002).

We wanted to know, what happened with thermophilous species during the glaciations. We analysed the genetic relationship between Northern Africa and Europe in the butterfly species *Melanargia galathea*. We studied 18 allozyme loci of 943 individuals from 22 populations distributed over a major part of Europe, ranging from Southern France to Romania and the western part of Northern Africa (the Atlas Mountains) to Central Germany.

The African populations resemble the complete allelic richness found all over Europe and the genetic diversity of these samples is higher than for the European ones (Table II.1). Cluster analysis discriminated four European genetic groups of the butterfly *M. galathea*: (i) a western European lineage and (ii-iv) three eastern European lineages (see Exkurs I). However, the African samples did not form a separate cluster within a phenogram, but clustered randomly within the Balkan / south-eastern European groups. The genetic differentiation among the African populations (F_{ST}: 8.8%) was higher than the differentiation within any of the European lineages (F_{ST}: 2.6-5.5%).

Table II.1: Four parameters of genetic diversity for 22 populations analysed of *Melanargia galathea* in Europe and North Africa; mean number alleles per locus (*A*), percentage of expected heterozygosity (H_e), percentage of loci with the most commonest allele not exceeding 95% (P_{95}) and total percentage of polymorphic loci (P_{tot}). Abbreviations: M: Morocco, F: France, D: Germany, A: Austria, H: Hungary, SLO: Slovenia, SCG: Montenegro, RO: Romania; Lineages: a: Africa, w: western flank, ef: eastern flank, ecb: eastern Carpathian Basin, am: Adriatic-Mediterranean lineage.

population	lineage	*A*	H_e [%]	P_{95} [%]	P_{tot} [%]	samples	date of sampling
M	a	2.35	21.4	35.3	52.9	36	23-V-2005
M	a	2.29	16.3	35.3	52.9	36	26-V-2005
M	a	2.53	18.0	35.3	58.8	36	29-V-2005
M	a	2.41	20.2	35.3	64.7	36	29-V-2005
mean Africa (± s.d.)	a	2.40 (±0.10)	18.9 (±2.3)	35.3 (±0.0)	57.3 (±5.7)		
F	w	2.12	17.5	35.3	41.2	40	29-VII-2003
F	w	2.06	17.8	35.3	41.2	40	17-VI-2003
D	w	2.06	17.2	35.3	41.2	40	18-VII-2003
D	w	1.94	18.6	41.2	41.2	40	12-VII-2003
D	w	2.29	18.1	41.2	41.2	40	25-VII-2003
mean (± s.d.)	w	2.09 (±0.13)	17.8 (±0.5)	37.6 (±3.2)	41.2 (±0.0)		
A	ef	1.88	14.6	35.3	47.1	40	31-VII-2003
H	ef	2.29	17.4	35.3	41.2	40	13-VII-2004
SLO	ef	2.06	15.3	35.3	35.3	40	04-VII-2003
SCG	ef	1.88	14.5	35.3	35.3	12	10-VII-2003
mean (± s.d.)	ef	2.03 (±0.20)	15.5 (±1.3)	35.3 (±0.0)	39.7 (±5.7)		
RO	ecb	1.88	14.6	35.3	47.1	40	25-VII-2004
RO	ecb	2.12	18.6	35.3	41.2	40	23-VII-2004
RO	ecb	2.06	19.1	41.2	41.2	40	15-VII-2004
mean (± s.d.)	ecb	2.02 (±0.12)	17.4 (±2.5)	37.2 (±3.4)	43.2 (±3.4)		
RO	am	1.82	19.9	35.3	41.2	40	9-VIII-2004
RO	am	1.88	19.0	35.3	41.2	40	29-VII-2004
B	am	2.06	18.8	35.3	41.2	40	10-VIII-2004
RO	am	2.29	21.0	35.3	41.2	40	31-VII-2004
B	am	2.06	18.8	35.3	41.2	40	3-VIII-2004
B	am	2.06	17.3	35.3	35.3	40	7-VIII-2004
mean (± s.d.)	am	2.03 (±0.14)	18.8 (±1.2)	35.3 (±2.9)	40.5 (±2.0)		
mean Europe (± s.d.)		2.05 (±0.14)	17.8 (±1.7)	35.9 (±2.8)	40.5 (±2.8)		

The high genetic diversity and the relatively strong differentiation of the four African populations sampled in a comparatively limited area of the Atlas Mountains indicate that the most probable origin of this

species was Northern Africa with its sibling species *Melanargia lachesis* evolving in parallel in Iberia. Only during the Eem interglacial some 130 ky ago, this species most probably first colonised Europe. Since *M. lachesis* as sibling species already must have existed in the Iberian peninsula during this period, *M. galathea* should have reached Europe via Italy. The genetic differentiation in distinct groups in Europe only evolved during the following Würm glacial period. This data suggests, that many "European" thermophilous organisms may be of African origin.

Further details in:

Habel J C, Meyer M, El Mousadik A, Schmitt T (2006) Africa to Europe: The complete phylogeography of the Marbled White butterfly species complex *Melanargia galathea / lachesis*, (manuscript).

5. Not adapted to live in isolation: The genetic response to recent habitat fragmentation and low population densities in three Zygaenids

5.1. Results

We analysed allozymes of a total of 1034 Zygaenids (438 *Z. carniolica*, 276 *Z. loti* and 320 *Z. viciae*) from 14 sites (9 for *Z. carniolica*, 7 for *Z. loti* and 9 for *Z. viciae*) (see Figure 5.1). A total of 17 loci were scorable in *Z. carniolica*, 15 in *Z. loti* and 15 in *Z. viciae*. The majority of the analysed loci was polymorphic in all three species: Nine of the 16 loci in *Z. carniolica* (G6pdh, Aat1, Aat2, Pgi, Hbdh, Me, Mdh1, Mdh2, Pgm) twelve of the 15 loci in *Z. loti* (Idh, Gpdh, Aat2, G6pdh Aat1, Acon, Mdh2, Mdh1, Pgm, Hbdh, Me, Pgi) and eleven of the 15 loci in *Z. viciae* (Pgi, Me, Gpdh, Hbdh, Mdh1, Mdh2, Pgm, Aat1, Aat2, Idh, Acon). Many cases of significant deviation from Hardy-Weinberg equilibrium were detected. Cases of significant deviation of single loci in discrete samples were obtained for *Z. carniolica* (Perl: Aat2, Niederanven: Aat2, Me, Ingendorf: Aat1, G6pdh, Junglinster: Hbdh), for *Z. viciae* (Niederanven: Acon, Aat2, Idh, Wasserliesch: Acon, Aat2, Pgi, Hbdh, mdh1, Me, Bettingen: Acon, Aat1, Aat2, md1, Me, Pgm, Echternacherbrück: Acon, Aat2, Pgi, Pgm, Perl: Acon, Aat2, Pgi, Pgm, Montenach: Aat1, Aat2, Pgi, Pgm, Niedergailbach: Aat1) and for *Z. loti* (Clouange: G6pgdh, Mdh2, Freudenburg and Lissendorf for all polymorphic loci, Montenach for all polymorphic loci except Hbdh and Pgm, Niederanven: Mdh2, Ingendorf: Acon, Go2, Idh1, Pgm, Schönecken all except Hbdh). In general, no linkage disequilibrium exists. Therefore, standard algorithms of population genetic analysis could be applied.

The mean numbers of alleles per locus (A), the percentage of expected heterozygosity (H_e), the percentage of observed heterozygosity (H_o), the absolute percentage of polymorphic loci (P_{tot}) and the percentage of polymorphic loci with the most common allele not exceeding 95% (P_{95}) were calculated. These parameters vary considerably among species and their populations (Table 5.1).

Comparing the means of the five parameters of genetic diversity revealed significant differences between the three species (Table5.2). While between *Z. viciae* and *Z. loti* no significant differences exist, (all U-tests: $p<0.05$), these two species had significantly higher means than *Z. carniolica* in all parameters of genetic diversity (all U-tests $p>0.05$). Wilcoxon-tests over the means of all parameters of genetic diversity revealed no significant difference between *Z. loti* and *Z. vicea*, but significant differences were found between these two species and *Z. carniolica*.

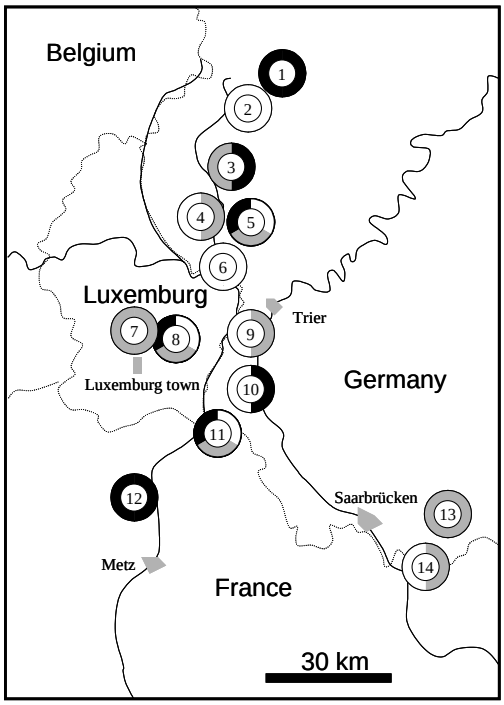


Figure 5.1: The geographical location of the 14 sample stations of three Zygaena species in Germany, France and Luxemburg. *Zygaena viciae* (white), *Zygaena carniolica* (grey) and *Zygaena loti* (black). Dotted lines: country borders. Abbreviations: 1: Lissendorf, 2: Weinsheim, 3: Schönecken, 4: Bettingen, 5: Ingendorf, 6: Echternacherbrück, 7: Junglinster, 8: Niederanven, 9: Wasserliesch, 10: Freudenburg, 11: Montenach, 12: Clouange, 13: Mimbach, 14: Niedergailbach.

Table 5.1: Five parameters of genetic diversity for all populations analysed of the three *Zygaena* species: mean number of alleles per locus (*A*), percentage of expected and observed heterozygosity (H_e and H_o), relation between H_o to H_e, percentage of loci with the most commonest allele not exceeding 95% (P_{95}) and total percentage of polymorphic loci (P_{tot}). Abbreviations: ce: Central Eifel, sm: Saar-Mosel region, bg: Bliesgau: D: Germany, L: Luxemburg, F: France.

5a: *Zygaena carniolica*

population	area	*A*	H_e[%]	*Ho*[%]	*Ho*/*He*	P_{95}[%]	P_{tot}[%]
Schönecken	ce	1.38	8.6	7.2	0.84	37.7	37.5
Niederanven	sm	1.50	10.8	9.6	0.89	31.3	37.5
Bettingen	sm	1.27	8.7	8.6	0.99	26.7	26.7
Junglinster	sm	1.44	10.2	10.0	0.98	31.3	37.5
Wasserliesch	sm	1.25	9.0	8.4	0.93	25.0	25.0
Montenach	sm	1.31	10.7	10.7	1.0	31.3	37.5
Römersköfchen	sm	1.38	9.2	7.8	0.85	31.3	31.3
Mimbach	bg	1.25	9.6	9.1	0.95	25.0	25.0
Niedergailbach	bg	1.31	7.0	5.9	0.84	25.0	31.3
mean (± s.d.)		1.34 (±0.09)	9.31 (±1.19)	8.60 (±1.48)	0.92 (±0.06)	29.4 (±4.31)	32.1 (±5.57)

5.1b: *Zygaena viciae*

population	area	*A*	H_e [%]	*Ho*[%]	*Ho*/*He*	P_{95}[%]	P_{tot}[%]
Weinsheim	ce	1.58	5.5	5.0	0.90	8.3	33.3
Niederanven	sm	1.67	14.1	12.8	0.91	41.7	58.3
Bettingen	sm	1.77	19.1	7.7	0.40	53.8	53.8
Echternach	sm	1.92	15.3	8.9	0.58	46.2	53.8
Freudenburg	sm	1.67	13.4	13.9	1.03	41.7	50.0
Perl	sm	1.85	14.9	8.4	0.56	53.8	53.8
Montenach	sm	1.86	19.5	6.3	0.32	42.9	57.1
Wasserliesch	sm	1.85	12.5	5.6	0.45	38.5	53.8
Niedergailbach	bg	1.59	6.7	4.9	0.73	25.0	50.0
mean (± s.d.)		1.75 (±0.13)	13.44 (±4.79)	8.20 (±3.28)	0.61 (±0.25)	38.8 (±14.39)	50.7 (±7.37)

5.1c: *Zygaena loti*

population	area	*A*	H_e [%]	*Ho* [%]	*Ho*/*He*	P_{95} [%]	P_{tot} [%]
Lissendorf	ce	2.33	22.8	9.4	0.41	60.0	60.0
Schönecken	ce	2.27	26.9	10.1	0.38	66.7	66.7
Clouange	sm	1.60	9.5	7.7	0.81	26.7	40.0
Freudenburg	sm	2.33	25.5	6.6	0.26	66.7	73.3
Montenach	sm	1.93	16.5	9.8	0.59	40.0	53.3
Niederanven	sm	1.73	14.0	12.2	0.87	33.3	60.0
Ingendorf	sm	1.80	15.5	12.4	0.80	40.0	66.7
mean (± s.d.)		2.00 (±0.31)	18.7 (±6.48)	9.7 (±2.14)	0.52 (±2.14)	47.6 (±16.53)	60.0 (±10.89)

Table 5.2: Means of five parameters of genetic diversity for all populations analysed of three Zygaenid species; Tests for significant differences are performed using Friedmann ANOVAs. Individual pairs were tested by U-test and significant differences ($p<0.05$) are indicated by different characters. Abbreviation: number of alleles per locus (*A*), percentage of expected heterozygosity (H_e), percentage of observed heterozygosity (H_o), total percentage of polymorphic loci (P_{tot}), percentage of polymorphic loci with the most commonest allele not exceeding 95% (P_{95}).

parameters	*Z. loti*	*Z. viciae*	*Z. carniolica*	p
A	2.00 [a] ±0.31	1.75 [a] (±0.13)	1.34 [b] ±0.09	0.005
H_e	18.7 [a] ±6.48	13.44 [a] (±4.79)	9.31 [b] ±1.19	0.066
P_{tot}	60.0 [a] ±10.89	50.7 [a] (±7.37)	32.1 [b] ±5.57	0.066
P_{95}	47.6 [a] ±16.53	38.8 [a] (±14.39)	29.4 [b] ±4.31	0.002

The size of all investigated habitats were classified in habitats larger than 15ha and smaller ones. There was no significant difference of the

parameters of genetic diversity between larger and smaller habitats for any of the three Zygaenid species (all p>0.1).

The analysed genetic structure of *Z. carniolica* and *Z. loti* showed no geographical pattern of the distribution of their genetic diversity. The three limestone areas (Bliesgau, Central Eifel and Saar-Mosel region were compared and genetic differences were tested by U-tests. Only in *Z. viciae*, the two populations located in the areas Bliesgau (bg) and Central Eifel (ce) had a significantly lower genetic diversity than the ones from the Saar-Mosel region. (Figure 5.2): U-tests (p): A: 0.038, H_e: 0.040, H_o: 0.040, P_{95}: 0.038, P_{tot}: 0.045.

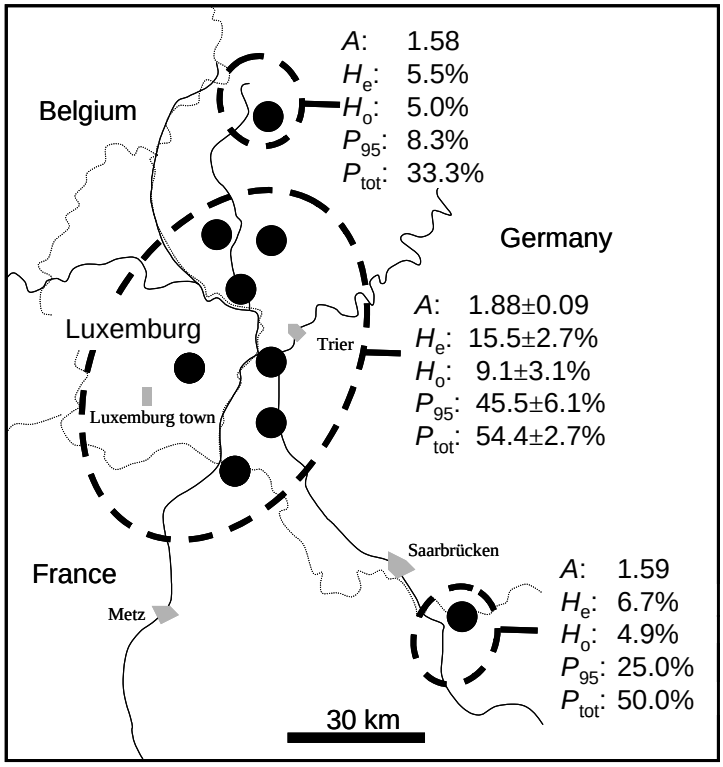


Figure 5.2: Five parameters of genetic diversity for all populations analysed of *Zygaena viciae*; mean number alleles per locus (A), percentage of expected and observed heterozygosity (H_e and H_o), percentage of loci with the most commonest allele not exceeding 95% (P_{95}) and total percentage of polymorphic loci (P_{tot}). For the mean values of seven populations from the Saar-Mosel region, standard deviations are given.

The total genetic variance among populations, among individuals within populations and within individuals was higher for *Z. viciae* and *Z. loti* compared to *Z. carniolica* (Table 5.3). The total genetic differentiation between populations was low for *Z. carniolica* and very high for the other two Zygaenids *Z. loti* and *Z. viciae*. An exact test for differentiation (Raymond & Rousset 1995) revealed a significant genetic structure within each of the three species (p<0.0001). The differentiation between populations was moderate in *Z. carniolica* (F_{ST}: 4.9%, p=0.001), higher for *Z. viciae* (F_{ST}: 5.8, p=0.001) and very high for *Z. loti* (F_{ST}: 13.6, p=0.001). The F_{IS} value was moderate for *Z.*

carniolica (7.8%). In the other two species, the F_{IS}-value unravel a very high level of inbreeding (*Z. viciae*: F_{IS} 29.0%, *Z. loti* 47.0%).

Table 5.3: Non-hierarchical variance analysis of *Zygaena carniolica, Zygaena viciae* and *Zygaena loti* of the analysed populations.

species	among populations	among individuals	within individuals	total variance
Z. carniolica	0.047	0.018	0.934	0.999
Z. viciae	0.092	0.437	1.069	1.598
Z. loti	0.218	0.652	0.736	1.606

The genetic distances among populations differ significantly ($p<0.05$) among the three species: *Zygaena carniolica*: 0.0196±0.0041, *Z. loti*: 0.0525±0.0217, *Z. viciae*: 0.037±0.01139.
We calculated phenograms based on Nei´s (1978) genetic distances for the three species. The tree topologies for the three Zygaenid species did not coincide with the geographical distributions of the sample sites and no isolation by distance was detected (Mantel test: *Z. carniolica*: r^2: 2.02%, p=0.41; *Z. loti*: r^2: 1.69%, p=0.601 and *Z. viciae*: r^2: 1.93%, p=0.406).

5.2. Discussion

Genetic differentiation and genetic diversity

Our genetic data shows moderate genetic differentiation and inbreeding values for *Zygaena carniolica*, but strong genetic differentiation and inbreeding effects for its relatives, *Zygaena viciae* and *Zygaena loti*. The detected F_{ST} values for *Z. viciae* (F_{ST}: 5.8%) and *Z. loti* (F_{ST}: 13.6%) are quite high, compared to genetic data on butterfly species obtained with the same analytic method and performed in the same region: *Melitaea aurelia* (F_{ST}: 3.6), *Melanargia galathea* (F_{ST}: 3.3), *Cupido minimus* (F_{ST}: 5.6%), *Aricia agestis* (F_{ST}: 3.9), *Thymelicus sylvestris* (F_{ST}: 1.6%) *Thymelicus acteon* (F_{ST}: 5.1%), *Polyommatus coridon* (F_{ST}: 1.4%) (Schmitt & Seitz 2002), *Polyommatus icarus* (F_{ST}: 0.4%, n.s.) (Schmitt et al. 2003) and the burnet moth *Aglope infausta* (10.3%) (Schmitt & Seitz 2004). Further molecular analysis on butterflies on a similar spatial scale in the species *P. coridon* (Krauss et al 2004), *Melitaea didyma* (Johannesen et al. 1997), *Parnassius mnemosyne* (Meglecz et al. 1997) and *Maculinea alcon* (Gadeberg & Boomsma 1997) also shows the high level of genetic differentiation among populations detected in these two burnet moth species. The mean values of genetic distances among populations underline, that *Zygaena carniolica*´s populations are low structured (0.0196±0.004), similar to other genetically analysed butterflies (see chapter 7), *Z. viciae* moderately (0.037±0.011) and *Z. loti* (0.053±0.021) very strong.
However, comparing the investigated levels of genetic diversity of *Z. viciae* and *Z. loti* with other Zygaenids like *Z. exulans* in the Pyrenees

and Alps (Schmitt et al. 2003), Z. *carniolica* appears in special position with it's simple genetic design. In comparison to further genetic studies on butterflies (see chapter 3, 4, 6, and 7) this genetic diversity is quite low and herein similar with sedentary species with special ecological adaptations, as e.g. the genus *Maculinea* (Wynhoff et al. 2001, Gadeberg & Boomsma 1997, Bereczki et al. 2005) and the Zygaenidae *Aglope infausta* (Schmitt & Seitz 2004).

Against the background of these results, the three analysed Zygaenids may be distinguished into two groups: (i) species with a simple genetic design, which leads to low genetic differentiation (low F_{ST}) and a continuous preservation of alleles in all populations (low standard deviations of the means of the parameters of genetic diversity) here represented by *Z. carniolica* and (ii) species with a primarily high genetic diversity of their populations and thus genetically not adapted to live in isolation, reflected by a high genetic differentiation (high F_{ST}), high amounts of genetic diversity in some populations and high standard deviations of the means of the parameters of genetic diversity, thus leading to strong inbreeding effects (high F_{IS}), here represented by *Z. viciae* and *Z. loti*.

In *Z. carniolica* we detected a lower number of alleles, which are evenly distributed over all populations. This burnet species is mostly restricted to calcareous grasslands which are often naturally fragmented. Therefore, *Z. carniolica* has to be adapted to live in fragmented environments. In the light of population ecology, the simple genetic design of this species might be a response which enables long-term survival in such a patchily environment. In other lepidopterans, with specific habitat requirements, similar genetic patterns were detected as in the Nymphalid *Melitaea aurelia* showing similar ecological requirements to *Z. carniolica* (see chapter 4). This species seems to be well adapted by its simple genetic texture to exist in isolated, closed populations, without a loss of genetic diversity. A further example of genetic adaptation to a fragmented occurrence is the lycaenid *Aricia agestis*. This species is genetically adapted by its relatively low genetic diversity to survive in a metapopulation system without deleterious loss of genetic diversity. This is a consequence of the frequent colonisation processes (see chapter 7). *Zygaena carniolica* appears mostly in high population densities, without strong population fluctuations. This behaviour combined with the simple genetic design might be the necessary prerequisite to live in isolation, surviving environmental stochasticity.

The ecological and genetic constitution of *Z. carniolica* is in contrast to *Z. viciae* and *Z. loti*, which show a high number of alleles and a generally high genetic diversity. However, a strong deviation from the mean values of genetic diversity was found for several populations (Table 5.1b, 5.1c). This constitution indicates stochasticity in population eroded parts of the original genetic diversity in some populations. Recent anthropogenetic habitat fragmentation aggravated the effect of the generally low vagility typical for all burnet moths (Zub 1996). In *Z. viciae* and *Z. loti* the complex genetic design coincides with broader ecological niches.

Actually, many habitats have disappeared because of advanced succession and/or agricultural intensification. Factors, like reduced gene flow because of a high degree of habitat isolation, and/or low population densities, evoked by high population fluctuations, have negatively influenced *Z. viciae* and *Z. loti* during the last decades. Actually, they may suffer an inbreeding depression. These effects might be evoked by different factors:

Small and isolated populations support a situation in which parents share their ancestors. The extend of inbreeding is related to the amount of ancestry that is shared by the parents of an inbred individual. Small isolated populations often are genetically less diverse than core populations, resulting in higher homozygosity of the isolated populations that may hamper their long–term survival (Billington 1991, Buza et al. 2000, Hudson et al. 2000, Jäggi et al. 2000, Madsen et al 2000, Pedersen & Leoschke 2001). The decrease in fitness of organisms owing to matings between relatives is well known from captive breeding and laboratory experiments (Joron & Brakefield 2003 with references therein). Especially *Zygaena loti* is characterised by their recent habitat isolation and high population fluctuations, which may reduce the populations affected below the detection limit (Naumann et al. 1999). In the past, such fluctuations were easily compensated by gene flow among patches. This historical situation of the widely distributed *Z. loti* is reflected by its high genetic constitution, the actual tendencies of habitat fragmentation may influence these species negatively. This species is known to be historically common in extensively used meadows in high densities (Weitzel, pers. comm.). The recent reduction of potential habitats may consequence a breakdown of an intact metapopulation-exchange and a loss of genetic variability.
Beside effects of isolation among distinct populations, *effects of Population subdivision within structured habitats* might also produce more or less isolated groups without panmictic mating behaviour. This phenomenon is known as `Wahlund effect´. Computer simulations show us, that subdivided populations rapidly lose variability of each sub-population (Lacy 1999). These effects of subdivision were mostly studied in sessile animals or plants (e.g. *Pithecellobium elegans*, Hall et al. 1995, *Siphonaria sp*., Johnson & Black R (1984)). However, even insects with the ability to fly often show restricted dispersal. mark release recapture studies showed low intrapatch movements, described as `homing behaviour´ like in *Maniola jurtina*, *Pyronia tithonus* (Conradt et al. 2001) and *Polyomatus coridon* (Schmitt et al. 2006). For Zygaenids, low migration power was described (Zub 1996). If low dispersal power is combined with low population densities and strong fluctuations (cf. den Boer 1981) like in *Z. viciae*, inbreeding effects may be established (cf. Johnson & Black 1984). *Zygaena viciae* shows the lowest level of population density of the three analysed Zygaenids. The number of individuals often fall below the detection limit. This might enforce bottleneck effects over decades with the result of high inbreeding (high F_{IS}). In contrast, the reduction of gene diversity by genetic drift is counterbalanced by one or a few

migrants per generation (Lacy 1999). If low migration within habitats create groups of subpopulations in Zygaenids, these might be detectable by an adapted sampling design. Combining the genetic data of each sample with its geographical coordinates of collection might clarify a potential substructure within a habitat. However all investigated habitats were sampled as panmictic mating populations.
A further source for inbreeding might be evoked by life history: *Non-random mating and the influence of diapause* refers to the degree of relatedness between mates relative to two mates chosen at random from a population. An individual is considered inbred if it's parents were more closely related than two randomly chosen individuals. A non-random mating as a possibility in the three studied species might be evoked by diapause behaviour, known for many Zyganids (Wipking 1988, 1992, 1995, Wipking & Kurtz 2000). This causes a situation of several small, parallel populations, existing in one habitat, creating temporal subpopulations evoking a failure of random mating (Hua et al. 2005, Naumann et al. 1999, Wipking 2001). However, comparing the results of analysed samples consisting of one generation, as well as of two or three generations, no difference in their inbreeding level were detected. Additionally, diapause also exists in *Z. carniolica*, which is not affected by such high inbreeding levels.
One last explanation for the observed deviations from Hardy Weinberg equilibrium might be *cryptic speciation*, as a result of allopatric differentiation and later secondary contact (see Excursus I and II). Many species were known to be separated in distinct refugia during glaciation periods. This isolation led to distinct evolution with the effect of a development of new taxa, semi taxa and genetic lineages (Hewitt 1996). Today, because of a postglacial range expansion these distinct lineages are found all over Europe. Often individuals from different populations show no morphological differentiation, but belong into different genetic lineages (Hewitt 1996). In the case of *Z. viciae* and *Z. loti* the high genetic inbreeding values may be a result of this phylogeographic phenomenon.

Conservation consequences

Despite mostly high population densities the genetic texture of *Z. carniolica* is quite simple. This might be understood as an adaptation to isolation: this simple genetic texture with a relatively low numbers of alleles is not strongly affected by genetic drift, even during phases of small population sizes.
In contrast, the two other Zygaenids, *Z. viciae* and *Z. loti* show a more complex genetic structure and a high genetic diversity, including rare alleles. Both species were common in the past and were not restricted to isolated habitat patches (Weitzel, pers. comm.).
Zygaena loti built up high population densities and was historically abundant, found on extensively used hay meadows (Weitzel, pers. comm.) This situation of interconnected populations has enabled the species to preserve the high genetic diversity by an exchange of individuals among patches. However, recent anthropogenic activities have led to habitat deterioration or even complete destruction of

habitats. The consequence of this development is a patchwork of isolated populations with a strongly reduced gene flow. The observed high genetic diversity in some few populations might be a relict of the normal genetic diversity a few decades ago. This underlines its former high abundance with strong connectivity among neighbouring populations, enabling a continuous gene flow. This structure safeguarded the high genetic variability. The observed high standard deviations of the all analysed parameters of genetic diversity and the high genetic variance within individuals reflect genetic erosion in *Z. loti* over the last few decades.

The genetic structure of *Z. viciae* with relatively high genetic diversity within populations and relatively low differentiation among populations. This seems to indicate a still viable metapopulation structure with sufficient gene flow among populations. However, a further deterioration of the habitat availability for this species might have fatal consequences on its survival and might cause rapid extinction due to strong losses of genetic diversity (Harrison& Hastings 1996, Keller et al. 2004).

Zygaena carniolica seems to be genetically well adapted for the life in isolated habitat patches so that this specialist might be the only one of the three Zygaenids not being endangered. Therefore, our study shows that not only the habitat specialist is vulnerable, but under the actual situation of severe habitat losses in Central Europe, more generalistic species might even be more endangered.

Table 5.4: Overview of the population density and the reaction on fragmentation of the species *Zygaena carniolica*, *Zygaena loti* and *Zygaena viciae* on these parameters. Abbreviations: -: no influence, +: moderate influence, +++: high influence; Arrows symbolize the hypothesized impact of ecological changes.

	Z. carniolica	*Z. loti*	*Z. viciae*
population density	+++	+++	+
natural fragmentation	+++	+	-
actual fragmentation	+++	+++	+

Excursus III

Eastern Europe meets Western Europe: Hybridisation of two lineages in *Zygaena carniolica*

One of the 11 analysed populations of *Zygaena carniolica* diverge significantly from the genetic constitution of all other ones. This population (site 9) is located at the south-western border of the study area (France-Lorrain) (Figure III,1). Six alleles (in the enzymes Pgi, Mdh1, Mdh2 and Me) were detected only at this site; thus the mean number alleles per locus (*A*) represents the highest value of all investigated populations of *Z. carniolica* (Table III,1). The genetic distance between this population and the others is very high (Table III,2).

This phenomenon might be explained as a result of the Pleistocene history of thermophilous species, which is already described in Excurs I and II. Zyganids are creating so called super species complexes, groups of species, closely related to each other. These semi species actually occur mostly parapatric over Europe. Accidentally, in analysing the samples of *Z. carniolica* we might have detected a border of two genetic lineages of *Z. carniolica* – which lead us again in the history of the Pleistocene.

The distribution pattern of a species can usually be assigned to one of several `centres of distribution during the last glaciation. In the genus *Zygaena*, the genetic and morphological differentiation can be clearly demonstrated in the super species-complex of *Zygaena transalpina* (Hille & Naumann 1992); the authors described three taxa of the transalpina group (i) *transalpina hippocrepidis* (Atlanto-Mediterrannean faunal element), (ii) *transalpina transalpina* (Adriato-Mediterranean faunal element) and *Zygaena angelicae* (Ponto-Mediterranean faunal element). The speciation process has not been completed in all parts of this range – while the ecological and ethological character between these taxa is extremely advanced in some areas of secondary contact (e.g. the Schwäbische Alb); In contrast, there are other areas studied, where partial gene flow between *Z. translapina hippocrepidis* and *Z. angelicae* has been recognized by population genetic analysis (Naumann et al. 1999).

Against the background of the phylogeographic history, we analysed the hybridization area of two genetic lineages, which probably evolved isolated in two distinct refugia (one western and one eastern refugium). The detected exclusive alleles in site 9 are of high evidence. Wagner-Rollinger (1972) already described two subspecies (*ssp. modesta* and *ssp. gaumaisiensis*) of *Z. carniolica* in this study region by morphological characteristics. However, an exact verification by genetic analysis still failed.

For detecting genetic effects of regional habitat fragmentation, genetic patterns evocated by phylogeographic history may lead to a

falsification in the analysis, especially in a comparison to related species, which are not effected by the same admission. Therefore, population 9 was excluded in the regional studies on Zygaenids.

Table III.1: Four parameters of genetic diversity for all populations analysed of *Zygaena carniolica*: mean number alleles per locus (*A*), percentage of expected heterozygosity (H_e), percentage of observed heterocygosity (H_o), percentage of loci with the most commonest allele not exceeding 95% (P_{95}) and total percentage of polymorphic loci (P_{tot}). Sampling stations: 1: Schönecken, 2: Bettingen, 3: Ingendorf, 4: Junglinster, 5: Niederanven, 6: Wasserliesch, 8: Montenach, 9: Clouange, 10: Mimbach, 11: Niedergailbach.

site	*A*	H_e [%]	*H*o [%]	*H*o/*H*e	P_{95} [%]	P_{tot} [%]
1	1,38	8,6	7,2	0,84	37,5	37,5
2	1,27	8,7	8,6	0,98	26,7	26,7
3	1,38	9,2	7,8	0,85	31,3	31,3
4	1,44	10,2	10,0	0,98	31,3	37,5
5	1,50	10,8	9,6	0,89	31,3	37,5
6	1,25	9,0	8,4	0,93	25,0	25,0
8	1,38	10,7	10,7	1,00	31,3	37,5
9	**1,53**	**8,5**	**4,8**	**0,56**	**20,0**	**33,3**
10	1,25	9,6	9,1	0,95	25,0	25,0
11	1,31	7,0	5,9	0,84	25,0	31,3
mean (±s.d.)	1,38 (±0,10)	9,4 (±1,27)	6,9 (±2,09)	0,73 (±0,13)	0,11 (±4,81)	0,14 (±5,23)

Table III.2: Genetic distances between the ten investigated sites. 1: Schönecken, 2: Bettingen, 3: Ingendorf, 4: Junglinster, 5: Niederanven, 6: Wasserliesch, 8: Montenach, 9: Clouange, 10: Mimbach, 11: Niedergailbach

	5	10	2	4	8	11	3	1	6
9	**0.0425**	**0.0461**	**0.0473**	**0.0447**	**0.0419**	**0.0349**	**0.0328**	**0.0357**	**0.0470**
5		0.0218	0.0239	0.0204	0.0189	0.0193	0.0169	0.0169	0.0157
10			0.0143	0.0152	0.0140	0.0184	0.0201	0.0286	0.0225
2				0.0141	0.0151	0.0170	0.0226	0.0297	0.0245
4					0.0138	0.0165	0.0209	0.0265	0.0205
8						0.0170	0.0185	0.0250	0.0192
11							0.0165	0.0192	0.0225
3								0.0168	0.0203
1									0.0246
6									

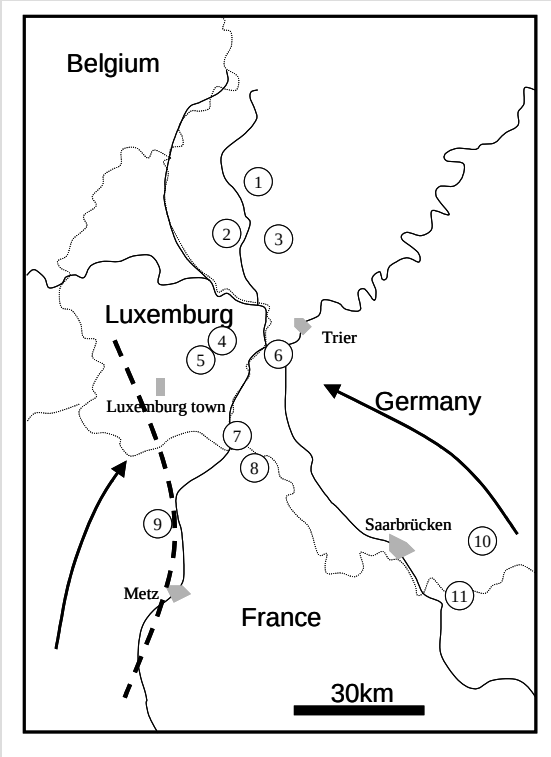


Figure III.1: The geographical location of the 10 sample stations of *Zygaena carniolica* in Germany, France and Luxemburg. Fine dotted lines: country borders, thick dotted line: Hypothetical hybrid zone of two European lineages of *Z. carniolica* symbolised as dashed line. Sampling stations: 1: Schönecken, 2: Bettingen, 3: Ingendorf, 4: Junglinster, 5: Niederanven, 6: Wasserliesch, 8: Montenach, 9: Clouange, 10: Mimbach, 11: Niedergailbach.

6. Strongly diverging population genetic patterns of three skipper species: isolation, restricted gene flow and panmixis

6.1. Results

A total of 1063 individuals of the three *Thymelicus* species were analysed by allozym electrophoresis: 457 *Thymelicus sylvestris*, 440 *Thymelicus lineola*, 166 *Thymelicus acteon* (sample sites see Figure 6.1). All 18 analysed allozyme loci of three *Thymelicus* species were polymorphic for all species with the exception of Me in *T. lineola*. Furthermore, polymorphisms in Gpdh of *T. sylvestris* were restricted to the out group sample from Bulgaria.

No remarkable deviation from Hardy-Weinberg equilibrium were detected. Only few cases of significant deviation exist after Bonferroni correction (*T. lineola*: 6Pgdh in Echternacherbrück; *T. sylvestris*: Aat1 in Wasserliesch and Bettingen, Hbdh in Ingendorf and Weinsheim; *T. acteon*: Idh1 in Wasserliesch, Bettingen and Echternacherbrück). In General, no linkage disequilibrium exists. However, some evidence of linkage between Pgi and Fum was found for *T. lineola*. As Fum is mostly monomorphic, this possible linkage has almost no consequences for further analyses. Therefore, standard algorithms of population genetic analyse could be applied.

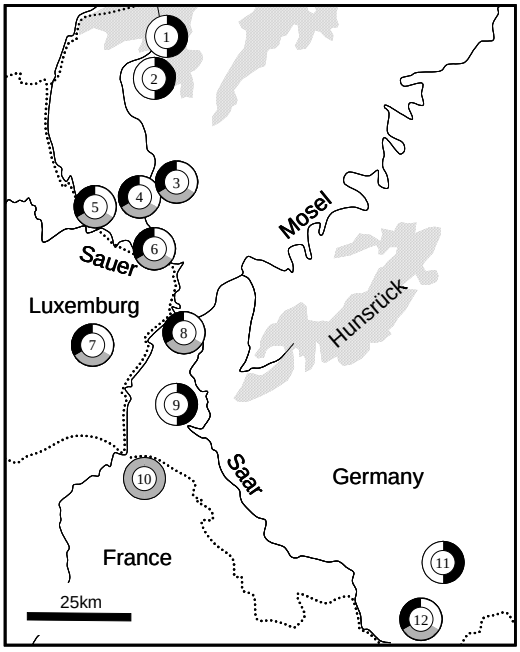


Figure 6.1: Location of the fourteen sample stations in western Germany and adjoining France and Luxemburg. 1: Weinsheim, 2: Schönecken, 3: Römersköfpchen, 4: Bettingen, 5: Echternacherbrück, 6: Ourtal, 7: Niederanven, 8: wasserliesch 9: Freudenburg, 10: Montenach, 11: Mimbach, 12: Niedergailbach. Collected samples on each site: white: *T. sylvestris*, grey: *T. acteon*, black: *T. lineola*.

The mean numbers of alleles per locus (*A*), the percentages of expected heterozygosity (H_e), the percentage of observed heterozygosity (H_o), the absolute percentage of polymorphic loci (P_{tot}) and the percentage of polymorphic loci with the most common allele not exceeding 95% (P_{95}) only refer to the first 20 individuals of each population (Table 6.1). These parameters vary considerably among populations.

Table 6.1: Five parameters of genetic diversity for all populations analysed of *Thymelicus acteon*, *Thymelicus lineola* and *Thymelicus sylvestris*: mean number of alleles per locus (*A*), percentage of expected heterozygosity (H_e), percentage of observed heterozygosity (H_o), total percentage of polymorphic loci (P_{tot}), percentage of polymorphic loci with the most commonest allele not exceeding 95% (P_{95}); Values in parenthesis refer to the first 20 individuals of the respective sample. The "average 20" gives the average of the first 20 individuals of each sample. The data for the out groups (Vallée de Glacier (F) and Kresna Gorge (BG) is given separately at the bottom of the tables.

6.1a: *Thymelicus acteon*

sampling site	*A*	H_e]%]	H_o [%]	P_{tot} [%]	P_{95} [%]
Wasserliesch	2.00 (2.00)	16.2 (16.6)	12.5 (12.8)	61.1 (61.1)	44.4 (44.4)
Niedergailbach	1.94 (1.94)	15.5 (15.5)	13.6 (13.6)	66.7 (66.7)	66.7 (66.7)
Ingendorf	1.72 (1.72)	10.9 (10.9)	10.0 (10.0)	61.1 (61.1)	27.8 (27.8)
Bettingen	1.67 (1.67)	11.3 (11.3)	9.3 (9.3)	55.6 (55.6)	38.9 (38.9)
Echternacherbrück	1.78 (1.78)	12.5 (12.5)	10.5 (10.5)	72.2 (72.2)	50.0 (50.0)
Ourtal	1.72 (1.72)	16.5 (16.5)	12.6 (12.6)	55.6 (55.6)	44.4 (44.4)
Niederanven	2.06 (2.00)	17.2 (16.7)	14.5 (13.5)	77.8 (77.8)	50.0 (55.6)
Montenach	2.17 (2.17)	18.9 (18.9)	17.5 (17.5)	77.8 (77.8)	66.7 (66.7)
Average ± s.d.	1.88 ±0.19	14.88 ±2.94	12.56 ±2.69	66.0 ±9.1	48.6 ±13.2
Average 20 ± s.d.	1.88 ±0.18	14.86 ±2.92	12.48 ±2.61	66.0 ±9.1	49.3 ±13.4

6.1b: *Thymelicus lineola*

sampling site	*A*	H_e [%]	H_o [%]	P_{tot} [%]	P_{95} [%]
Wasserliesch	2.00(1.83)	11.8(12.2)	10.0(10.1)	66.7(61.1)	33.3(38.9)
Freudenburg	2.39 (2.06)	12.9 (13.3)	12.2 (12.5)	72.2 (61.1)	38.9 (50.0)
Mimbach	1.83 (1.61)	8.5 (8.8)	8.6 (10.3)	55.6 (50.0)	27.8 (33.3)
Niedergailbach	2.06 (1.72)	10.0 (8.8)	9.3 (7.3)	61.1 (55.6)	27.8 (27.8)
Ingendorf	1.89 (1.50)	7.3 (6.2)	6.4 (5.8)	55.6 (38.9)	22.2 (27.8)
Bettingen	1.94 (1.72)	8.0 (7.5)	7.9 (7.0)	55.6 (44.4)	33.3 (27.8)
Echternacherbrück	2.39 (1.94)	10.5 (9.9)	9.4 (8.9)	77.8 (61.1)	33.3 (27.8)
Weinsheim	2.22 (2.00)	11.3 (11.9)	11.7 (12.2)	72.2 (66.7)	50.0 (55.6)
Schönecken	1.89 (1.61)	9.3 (9.0)	8.6 (8.4)	55.6 (44.4)	33.3 (38.9)
Ourtal	2.22 (1.83)	9.5 (8.6)	9.8 (9.2)	72.2 (50.0)	33.3 (38.9)
Niederanven	1.72 (1.72)	9.8 (9.8)	9.5 (9.5)	38.9 (38.9)	33.3 (33.3)
Average ± s.d.	2.05 ±0.23	9.90 ±1.67	9.40 ±1.62	62.1 ±11.3	33.3 ±7.0
Average 20 ± s.d.	1.78 ±0.17	9.64 ±2.11	9.20 ±2.07	52.0 ±9.7	36.4 ±9.4
Vallée de Glacier	2.06 (1.78)	14.5 (13.3)	12.5 (10.9)	72.2 (50.0)	38.9 (38.9)

6.1c: *Thymelicus sylvestris*

sampling site	*A*	H_e [%]	H_o [%]	P_{tot} [%]	P_{95} [%]
Wasserliesc	2.06 (1.83)	10.9 (11.3)	10.8 (11.7)	44.4 (33.3)	27.8 (27.8)
Freudenburg	2.11 (1.89)	11.6 (12.4)	11.2 (11.4)	50.0 (38.9)	27.8 (33.3)
Mimbach	1.83 (1.67)	9.7 (8.9)	9.6 (8.2)	50.0 (44.4)	27.8 (33.3)
Niedergailbach	2.22 (1.78)	13.3 (11.8)	14.4 (13.3)	61.1 (33.3)	27.8 (27.8)
Ingendorf	2.11 (1.83)	12.8 (13.5)	11.0 (10.8)	55.6 (50.0)	27.8 (38.9)
Bettingen	2.00 (1.72)	12.3 (12.1)	10.8 (9.3)	38.9 (33.3)	27.8 (27.8)
Echternacherbrück	2.39 (1.83)	12.8 (12.8)	10.7 (10.3)	77.8 (50.0)	33.3 (33.3)
Weinsheim	1.94 (1.72)	12.7 (14.4)	11.1 (12.6)	38.9 (38.9)	27.8 (38.9)
Schönecken	1.83 (1.67)	12.0 (11.5)	12.4 (11.3)	50.0 (44.4)	27.8 (33.3)
Ourtal	2.06 (2.00)	12.1 (11.4)	11.6 (10.8)	55.6 (55.6)	33.3 (33.3)
Niederanven	2.33 (1.89)	11.8 (10.5)	11.7 (10.7)	61.1 (50.0)	33.3 (27.8)
Average ± s.d.	2.08 ±0.18	12.00 ±1.01	11.39 ±1.22	53.0 ±11.2	29.3 ±2.6
Average 20 ± s.d.	1.80 ±0.10	11.87 ±1.47	10.95 ±1.41	42.9 ±7.9	32.3 ±4.2
BG Kresna Gorge	2.33 (1.78)	12.7 (11.8)	13.9 (12.8)	72.2 (55.6)	38.9 (33.3)

Comparing the means of the five parameters of genetic diversity revealed significant differences between the three species (Friedmann ANOVA: p= 0.022). While *T. sylvestris* and *T. lineola* showed no significant differences (Wilcoxon test: p= 0.50), these two species had significantly lower means than *T. acteon* (both Wilcoxon tests: = 0.043). The genetic diversities of out groups for *T. lineola* (Valleé des Glaciers, French Alps) and *T. sylvestris* (Kresna Gorge, south-west Bulgaria) were not significantly different from the means of the respective Central European samples (both Wilcoxon tests: p>0.10). Separate statistical tests on all parameters revealed no significant difference for the mean number of alleles per locus between the three species, but significance for the four other parameters. *Thymelicus lineola* had lower means than *T. sylvestris* and *T. acteon* for H_e and H_o, and the means for P_{tot} and P_{95} were higher in *T. acteon* than in the other two species (Table 6.2).

Table 6.2: Means of five parameters of genetic diversity for all populations analysed of three *Thymelicus* species excluding out groups. Tests for significant differences are performed using Friedmann ANOVAs. Individual pairs were tested and significant differences (p<0.05) are indicated by different characters. All data are given for the first 20 individuals of the respective sample. Abbreviation: number of alleles per locus (*A*), percentage of expected heterozygosity (H_e), percentage of observed heterozygosity (H_o), total percentage of polymorphic loci (P_{tot}), percentage of polymorphic loci with the most commonest allele not exceeding 95% (P_{95}).

	T. acteon	*T. sylvestris*	*T. lineola*	*p*
A_{20}	1.88 ± 0.18	1.80 ± 0.10	1.78 ± 0.17	0.5404
H_{e20}	14.86 ± 2.92^a	11.87 ± 1.47^a	9.64 ± 2.11^b	0.0183
H_{o20}	12.48 ± 2.61^a	10.95 ± 1.41^a	9.20 ± 2.07^b	0.0024
P_{tot20}	66.00 ± 9.10^b	42.90 ± 7.90^a	52.00 ± 9.70^a	0.0151
$P_{95\ 20}$	49.30 ± 13.40^b	32.30 ± 4.20^a	36.40 ± 9.40^a	0.0263

The size of the habitat influenced the genetic diversity of the respective population only for *Thymelicus acteon*, but not for the two other skipper species (Table 6.3). The Lulworth Skipper had significantly higher means for the mean number of alleles per locus (A), total percentage of polymorphic loci (P_{tot}) and percentage of polymorphic loci with the most commonest allele not exceeding 95% (P_{95}) and a marginally significantly higher mean for the observed heterozygosity (H_o) on habitats of 15 ha or more than on smaller patches.

Table 6.3: Influence of the habitat size on the genetic diversity of the *Thymelicus* species. The sample sites are distinguished into big and small ones. The p values of U-test are given in the Table.

parameters	*T. acteon*	*T. sylvestris*	*T. lineola*
A	0.024	0.646	0.118
H_e	0.101	0.068	0.78
H_o	0.053	0.31	0.23
P_{tot}	0.037	0.78	0.20
P_{95}	0.034	0.62	0.64

The total genetic variance was highest for *T. acteon*, intermediate for *T. sylvestris* and lowest for *T. lineola* (Table 2). An exact test for differentiation (Raymond and Rousset 1995) revealed a significant genetic structure within each of the three species ($p<0.0001$). The differentiation between populations of *T. acteon* was considerate (F_{ST} 5.1 %), low for *T. sylvestris* (F_{ST} 1.6%) and not significant for *T. lineola*. The F_{IS} values were relatively high for all species (15.5% in *T. acteon*, 4.7% in *T. lineola* and 5.0% in *T. sylvestris*). The two out groups showed a strong differentiation from the Central European samples (*T. sylvestris* F_{CT}: 7.7 % and *T. lineola* F_{CT}: 14.0%).

We calculated Nei´s (1978) genetic distance (means ± s.d.: *T. acteon*: 0.0402 ± 0.0096, *T. sylvestris*: 0.0176 ± 0.0023 and *T. lineola*: 0.0173 ± 0.0037). For better comparison between species we calculated these distances on the basis of the first 20 individuals of all populations as well (means ± s.d.: *T. acteon*: 0.0412 ± 0.009, *T. sylvestris*: 0.0324 ± 0.0026 and *T. lineola*: 0.0293 ± 0.0014). These means all differed significantly from each other (U-test: all $p<0.0001$). We calculated phenograms based on Nei´s (1978) genetic distances for the three species. The tree topologies for *T. acteon* and *T. lineola* did not coincide with the geographical distributions of the sample sites and no isolation by distance exist (both Mantel tests: $p>0.10$). In contrast, geographical and genetic data sets considered strongly for *T. sylvestris*; 20.30% of the genetic differentiation was explained by isolation by distance (Mantel test: $p<0.001$, Figure 6.2).

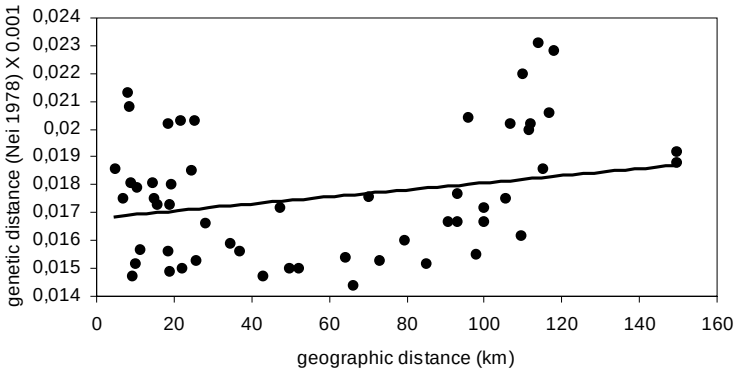


Figure 6.2: Correlation between the geographical distances and the respective genetic distances (Nei 1978) of the populations of *T. sylvestris* (r^2=0.203; Mantel test: p<0.001).

6.2. Discussion

Genetic diversity and differentiation

The population genetic diversity of the three analysed *Thymelicus* species was in the range of values typically observed for butterflies and moths (cf. Graur 1985, Packer et al. 1998, Vandewoestijne et al. 1999, Schmitt et al. 2002). None of the parameters analysed reached the average diversities found in the highly diverse butterfly family of the Blues (Lycaenidae) (e.g. Leliévre 1992, Peterson 1995, Marchi et al. 1996, Brookes et al. 1997, Schmitt & Seitz 2001, Schmitt et al. 2003), but the observed values were considerably higher than in genetically poor taxa as species of the genus *Yponomenta* (Menken 1987) or *Aglaope infausta* (Schmitt & Seitz 2004). In comparison with Nymphalids, the studied *Thymelicus* species showed population genetic diversities that were little less than in the common and widespread species of this family (e.g. Porter & Geiger 1995, Vandewoestijne et al. 1999, Schmitt et al. 2005), but considerably higher than in the rare and very localised taxa (e.g. Britten et al. 1994, Debinski 1994, Pelz 1995). The observed genetic diversity is in accordance with the commonness and broad distribution of *T. sylvestris* and *T. lineola*. However it is surprising that the localised *T. acteon* has similar or sometimes even higher genetic diversities of its analysed populations than the other skipper species.
The genetic differentiation between populations of the three species analysed vary considerably. Thus, the highest F_{ST} value and the highest mean genetic distance was found for *T. acteon*, which has a rather scattered island-like distribution in the study region. Comparable values were found in other regional studies for species with similarly scattered distribution patterns (Debinski 1994, Britten et al. 1994, Johannesen et al. 1996, Gadeberg & Boomsma 1997, Schmitt & Seitz 2004). Most probably, the gene flow between the studied samples of *T. acteon* is effectively impeded. On the contrary, the values for *T. sylvestris* and especially *T. lineola* were low or even

non-significant as typically found for other common and widespread species (Eanes & Koehn 1978, Hughes & Zalucki 1984, Daly & Gregg 1985, Goulson 1993, Korman et al. 1993, Bossart & Scriber 1995, Porter & Geiger 1995, Nibouche et al. 1998).
Comparing *T. sylvestris* and *T. lineola*, the genetic differentiation between populations of the latter is less than in *T. sylvestris*. This cannot be a function of habitat availability because (i) both species thrive on a wide variety of different grasslands (Ebert & Rennwald 1991, Asher et al. 2001) and (ii) co-occur in most of their habitats. However the dispersal ability (Bink 1992) differs between the two species with *T. sylvestris* dispersing worse than *T. lineola*. The latter species had a non-significant F_{ST} and the lowest mean genetic distance of the *Thymelicus* species. Most probably, *T. lineola* has a mostly panmictic population structure all over our study region, whereas the relatively weak but significant differentiation between the *T. sylvestris* populations suggest a metapopulation system with moderate to high gene flow rates between neighbouring populations.

Thymelicus and isolation by distance

Isolation by distance is a commonly observed phenomenon (cf. Peterson & Denno 1998). Depending on the dispersal ability of the analysed organisms and the respective habitat availability, this phenomenon was found at different geographical levels from the local (e.g. Pfenninger et al. 1996, Hamilton 1997, Raybould et al. 1998) to the continental scale (e.g. Chenoweth et al. 1998, Schmitt et al. 2003, Geiger & Shapiro 1992, Hellberg 1994). Referring to the regional level, isolation by distance is established at intermediate gene flow rates, mediating between isolated populations at low and panmictic continuous populations at high exchange rates. Gene flow itself depends on the combination of the dispersal capacity of a species and the availability and distribution of its habitat (Figure 6.3).
The high availability of suitable habitat patches combined with the relatively low dispersal capacity of *T. sylvestris* cause that only neighbouring habitat patches are located within the normal dispersal distance of the butterflies (Figure 6.4). Therefore, the genetic influence of one patch on another one decreases with distance. Over the generations, all patches are interconnected by gene flow with the geographical distances being responsible for the amount of genetic exchange, hereby leading to an isolation by distance system.

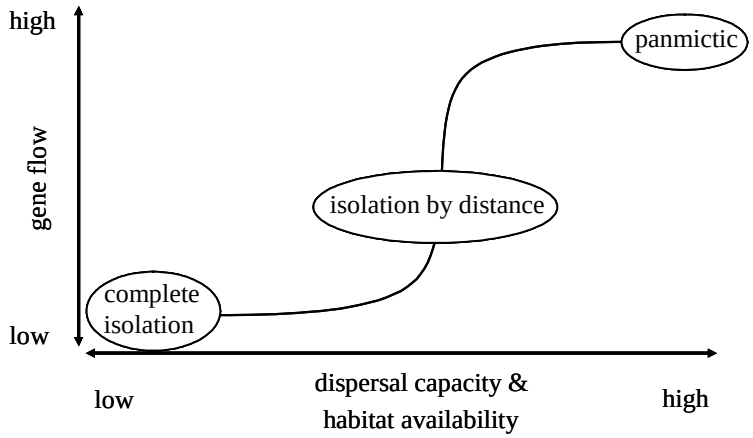


Figure 6.3: Isolation by distance is a phenomenon of transition. At a regional scale, this structure develops for species having an intermediate position conforming dispersal capacity and habitat availability (e.g. *T. sylvestris*). More mobile and/or more widely distributed species have higher gene flow rates resulting in panmictic structures (e.g. *T. lineola*). On the other hand, species with limited mobility and/or rather fragmented distribution have up to complete isolation between population impeding isolation by distance (e.g. *T. acteon*).

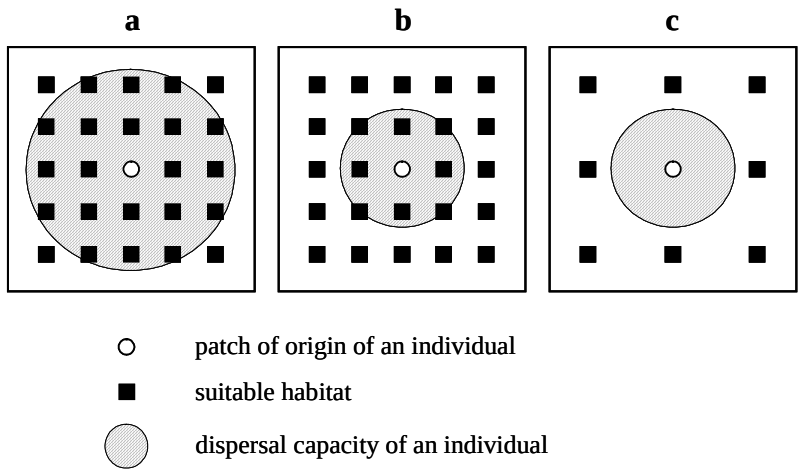


Figure 6.4: Dispersal power and habitat availability determine the genetic structure of species. (a) High dispersal capacity and high habitat availability result in intensive gene flow and a panmictic population structure (*T. lineola* Type). (b) Lower dispersal capacity in a landscape with high habitat availability reduces gene flow so that isolation by distance system will be establishing (*T. sylvestris* type). Limited habitat availability in combination with low dispersal capacity results in complete isolation with genetic drift acting independently in each single population (*T. acteon* type).

The situation of *T. acteon* differs from *T. sylvestris* insofar as the former is a habitat specialist restricted to dry and hot grasslands in Central Europe (Ebert & Rennwald 1991b, Settele et al. 1999). The

habitat availability for the Lulworth Skipper is therefore so restricted that the butterfly's dispersal capacity is not sufficient to establish gene flow over a network of habitats involving the whole study region; rather, even sub-structuring of the analysed samples is probable maybe explaining the relatively high F_{IS} value of 15%. Therefore, isolation by distance might be possible in well connected habitat networks on a local scale, but will break down on a regional level because of insurmountable hostile areas separating habitats (Figure 6.4c). As a consequence, the isolated populations develop independently resulting in relatively high rates of differentiation between samples without geographical information as in the case of *T. acteon* (Jäggi et al. 2000, Schmitt & Seitz 2002). This is underlined by the fact that small populations harbour less genetic diversity in our studied Lulworth Skipper populations, but not in the two other species.

As for *T. acteon*, no isolation by distance structure exist for *T. lineola*, but due to completely different reasons. First, the habitat availability of *T. lineola* is much higher than for *T. acteon*, and second, the dispersal power (Bink 1992) is higher than in the two other skipper species so that dispersing Essex Skippers might not only reach neighbouring patches but also more distant ones (Figure 6.4a) with the result of massive gene flow on a regional level.

Conservation implications

Two of the three analysed skipper species (*T. sylvestris* and *T. lineola*) are widespread and rather common all over Europe and are not of conservation concern (Swaay & Warren 1999). The situation of *T. acteon* is completely different. This species is strongly declining in most countries of Europe and is listed in the European Red Data Book as vulnerable (Swaay & Warren 1999) making *T. acteon* a species of major conservation concern.

It is widely accepted that a species specifies level of genetic diversity is necessary for the viability of its populations (Frankham et al. 2002, Hansson & Westerberg 2002, Reed & Frankham 2003, Schmitt & Hewitt 2004) with many examples proffered (e.g. Saccheri et al. 1998, Westermeier et al. 1998, Bryant et al. 1999, Madsen et al. 1999, Meagher 1999, Rowe et al. 1999, Buza et al. 2000, Luijten et al. 2000, Ùjvàri et al. 2002). Therefore, the results of *T. acteon* seem to carry an optimistic message: The genetic diversity of the analysed population is as high as or even higher than for the two common skipper species. As even more southern samples of *T. sylvestris* and *T. lineola* did not have higher genetic diversity as the German *T. acteon* samples, major bottlenecks become rather unlikely for our studied *T. acteon* populations. At last some of these populations appear sufficiently large because of the maintenance of such a high level of genetic diversity. Therefore, complete extinction due to population genetic reasons is unlikely.

However, several considerations warn against untroubled optimism. The high F_{ST} value indicates that genetic exchange between habitats is rare or missing for *T. acteon*. Furthermore we detected genetic

impoverishment of the smaller populations as frequently observed for other organisms (Billington 1991, Buza et al. 2000, Hudson et al. 2000, Jäggi et al. 2000). This might even reduce the viability of the affected populations.

If one of the *T. acteon* populations becomes extinct (e.g. by management mistakes, stochastic events like parasitism, weather etc.) recolonisation from one of the other patches is almost impossible due to the high degree of habitat fragmentation. Therefore, the conservation status of *T. acteon* in our study region might be worse than its genetic diversities implies. The survival of each single population might be completely dependent upon the habitat management, and management mistakes might decrease biodiversity without realistic perspectives of rapid recovery by recolonisation. A strict conservation of all remaining calcareous grassland is absolutely necessary for the species survival and the development of new or restoration of former calcareous grassland would further safeguard its populations and the ones of other rare and endangered animal and plant species.

Excursus IV

Natural selection

High F_{IS} values and deviations from Hardy Weinberg equilibrium might be evidence of natural selection of alleles e.g. allele combinations by local adaptations. Therefore an exact look at the genotypic distributions of the enzyme phosphoglucosemutase (Pgm) in *Melanargia galathea* show, that the allele combination Pgm_{22} is underrepresented in all ten analysed populations; the expected and observed allele-frequencies of this locus differ significantly (Sign test: $p<0.000$) (Table IV.1). This could be affected by functional selection against the absence of the homozygote expression of this allele, which point toward a negative influence of this allele-combination. We did not study the functional coherence of ecological or ethological behaviour in *M. galathea*.

Allozyme variation in correlation to fitness was detected in further genetic studies on different insect species and were discussed against the influence of natural selection. The frequency of allozymes at Idh2 was correlated with the mean size of individuals in both sexes of the butterfly *Maniola jurtina* (Goulson 1993). Associations between flight activity depending on altitude and weather have been recorded with regard to Pgi allozyme frequencies in *Collias sp*. (Watt 1983). Pgm and Pgi frequencies in *Danaus plexippus* are as well influenced by natural selection (Carter et al 1989).

In *Colias* butterflies (*C. eurothyme* and *C. philodice eriphyle*) Watt (1977) detected different heat stabilities, maximal activities and Michaelis constants in genotypes. A polymorphism for phosphoglucose isomeras (Pgi) influences viability, flight time, mating success and fecundity in *Colias* butterflies (Watt 1977). Genotypes were selected against during extended periods of hot weather, others favoured by selection during periods of normal weather. The ability to fly is a critically important component of fitness in *Colias*, butterflies must fly to find food, to mate and to find oviposition sites. The body temperature of butterflies for flight is narrow 35°C to 39°C, but some butterflies are able to fly with body temperature outside this range, as low a 29°C and up to slightly over 40°C. In the study of Watt (1977) heterozygote individuals were more efficient over a broader range of temperatures than homozygotes were, indicating that heterozygotes were indicating that heterozyotes would fly over a greater range of temperatures than the other genotypes would (Watt 1983). The ability of heterozygotes to fly over a greater range of temperatures than homozygotes can gives heterozygotes advantages in foraging for food, in reaching oviposition sites, in males´ mating success (Carter & Watt 1988, Watt et al. 1983, Watt et al. 1986). Hovanitz (1948) found that the different wing colour morphs of *Colias sp*. are active at different air temperatures, so that samples of active individuals from the same site varied in morph frequency according to the weather. If the fitness of different morphs

varied according to the weather then short-term temporal heterogeneity of weather conditions could maintain polymorphisms, with fluctuations in morph frequencies from year to year depending the weather conditions during the flight period.

Table IV.1: expected (upper value) and observed (below value) allele-frequencies of the enzyme Pgm. Significance of the detected differences in both allele-values tested by Sign test.

Population Alleles	Pgm_{11}	Pgm_{12}	Pgm_{13}	Pgm_{14}	Pgm_{22}	Pgm_{23}	Pgm_{24}	Pgm_{33}	Pgm_{34}	Pgm_{44}
	0	2	2	0	**28**	5	0	3	0	0
Wasserliesch	0.10	3.15	0.65		**24.81**	10.24		1.06		
	4	1	5	1	**24**	0	0	5	0	0
Weinsheim	1.41	9.19	2.81	0.19	**15.01**	9.19	0.61	1.41	0.19	0.01
	1	3	3	0	**28**	3	1	0	0	0
Echternacherbrück	0.41	6.46	0.62	0.10	**25.44**	4.85	0.81	0.23	0.08	0.01
	1	1	3	0	**23**	9	0	2	1	0
Bettingen	0.23	4.20	1.28	0.08	**19.60**	11.90	0.70	1.81	0.21	0.01
	0	1	0	0	**12**	5	0	0	0	0
Lissendorf	0.01	0.83	0.14		**12.50**	4.17		0.35		
	0	5	1	1	**26**	6	0	1	0	0
Schönecken	0.31	5.51	0.79	0.09	**24.81**	7.09	0.79	0.51	0.11	0.01
	0	0	0	0	**34**	3	2	1	0	0
Freudenburg					**33.31**	4.56	1.83	0.16	0.13	0.03
	0	1	0	0	**33**	4	0	2	0	0
Niederehe	0.01	0.89	0.10		**31.51**	7.10		0.40		
	0	2	0	0	**34**	0	1	2	0	0
Ingendorf	0.03	1.82	0.10	0.03	**32.31**	3.64	0.91	0.10	0.05	0.01
	0	1	0	0	**29**	5	2	2	0	0
Perl	0.01	0.85	0.12	0.03	**27.92**	7.62	1.69	0.52	0.23	0.03
	1	0	4	0	**24**	7	1	3	0	0
Mimbach	0.23	4.20	1.28	0.08	**19.60**	11.90	0.70	1.81	0.21	0.01
	0	0	4	0	**23**	9	0	1	0	0
Niedergailbach	0.11	2.97	0.81		**20.44**	11.15		1.52		
	0	2	1	0	**26**	8	1	2	0	0
Ourtal	0.06	2.36	0.49	0.04	**24.81**	10.24	0.79	1.06	0.16	0.01
	1	0	2	0	**19**	4	1	4	1	0
Niederanven	0.13	2.69	0.94	0.13	**14.45**	10.08	1.34	1.76	0.47	0.03
	2	3	1	0	**25**	6	0	1	0	0
Rosselange	0.42	6.21	0.95		**22.90**	6.99		0.53		
	0	5	0	0	**26**	5	0	4	0	0
Lorry	0.16	3.88	0.81		**24.02**	10.07		1.06		
	0	0	0	0	**34**	1	0	1	0	0
Schifflange					**33.06**	2.88		0.06		
significance tested by Sign-test	n.s.	n.s.	n.s.	n.s.	$p<0.001$	n.s.	n.s.	n.s.	n.s.	n.s.

A further example of natural selection in butterfly genetics is shown in *Maniola jurtina*. Natural selection can favour heterozygous individuals, as shown in *Colias* species and *Maniola jurtina*, which

may affect low F_{ST} values; in that case, a high F_{ST} is probably not indicative of a high dispersal rate, but is more likely the result of selection acting on some of the polymorphic loci. Equal selection pressures may homogenize allele frequencies among patches (Goulson 1993). This was also hypothesised for lack of structure among populations of grasshoppers *Chortippus brumeus* (Gill 1981). The lack of Pgm allele across all *Stenobothrus lineatus* populations could suggest selection at this locus and therefore higher levels of gene flow than are actually present (Johannesen et al. 1999).

Removing the allele combination Pgm_{22} in our study, or the locus completely, did not alter the gene flow estimate. Thus, the lack of differentiation suggests high levels of patch connectivity for *M. galathea*.

7. Well adapted to be expansive or sedentary: The genetic response on dispersal behaviour in two Lycaenids

7.1. Results

A total of 562 individuals of the two Lycaenid butterflies *Aricia agestis* and *Cupido minimus* were collected during the summers 2003 to 2005 on the study area in western Germany (Figure 7.1) (294 individuals of *A. agestis* at 10 sites, 268 of *C. minimus* at 7 sites) and were analysed by allozyme electrophoresis. For *C. minimus*, all analysed loci (Me, G6pdh, 6Pdh, Aat1, Pgm, Mdh1, Mdh2, 6Pgdh, Idh1, Idh2, Pep, Pk, Apk and Pgi) were polymorphic, in *A. agestis* only 11 of 19 analysed loci (Mdh1, Mdh2, G6pdh, Pgi, Pgm, Aat2, Gpdh, Me, Hbdh, Pk and Pep) had more than one allele. Some remarkable deviation from Hardy-Weinberg equilibrium were detected for *C. minimus* and *A. agestis* after Bonferroni correction (*A. agestis*: G6pdh, Mdh1, Mdh2, Me in Perl, G6pdh, Aat2, Hbdh, Mdh1, Me, Pgm in Niedergailbach, G6pdh, Pgi, Hbdh, Me, Pgm in Igel, G6pdh, Aat2, Mdh2, Me in Lissendorf, G6pdh, Hbdh, Mdh2 in Niederanven, Me in Nittel and G6pdh, Hbdh, Idh1, Mdh1 and Me in Ingendorf; C. *minimus*: Pgi and Pep in Wasserliesch, G6pdh in Badstube and Pep in Hammelsberg).

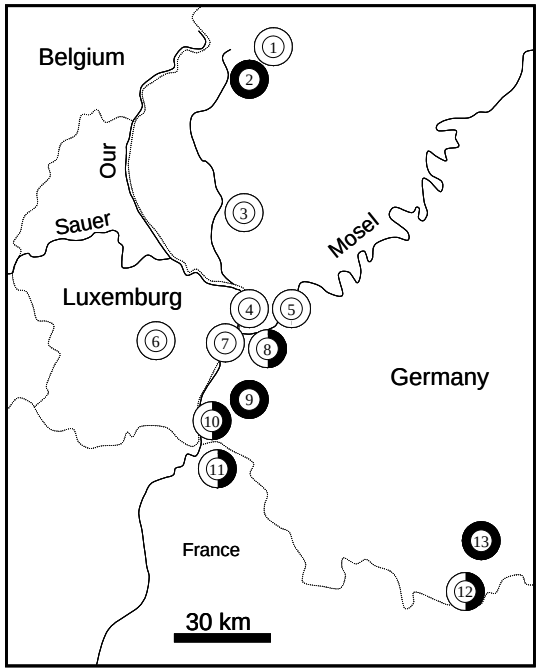


Figure 7.1: The geographical location of the 13 sample stations of *Cupido minimus* (black) and *Aricia agestis* (white) in Germany, France and Luxemburg. Dotted lines: country borders. 1: Lissendorf, 2: Weinsheim, 3: Ingendorf, 4: Igel, 5: Trier, 6: Niederanven, 7: Nittel, 8: Wasserliesch, 9: Freudenburg, 10: Perl, 11: Montenach, 12: Niedergailbach, 13: Mimbach.

All investigated parameters of genetic diversity (mean numbers of alleles per locus (*A*), percentages of expected and observed heterozygosity (H_e, H_o),), percentage of loci with the most commonest allele not exceeding 95% (P_{95}) and total percentage of polymorphic loci (P_{tot})) were higher in *C. minimus* (Table 7.1) than in *A. agestis*. Except for *A* and P_{95}, these differences between both species were significant. In average 2.12 (±0.10) alleles per locus were detectable for *A. agestis*, ranging from 2.00 to 2.28, and for *C. minimus* 2.24 (±0.22) ranging from 1.93 to 2.64 (p=0.245). The percentage of expected heterozygostiy is 15.7% (±1.5) in *A. agestis*, ranging from 13.6% to 17.8%, differing significantly (p=0.012) from the mean of *C. minimus*, 18.3% (±1.6), ranging from 16.2% to 19.8%. The percentage of observed heterozygosity (H_o) was also significantly higher in *C. minimus* (p=0.001) with a mean of 17.3% (±1.8) ranging from 15.1% to 19.3% and for *A. agestis* 12.1% (±0.9) ranging from 10.5% to 13.4%. The percentage of loci with the most common allele not exceeding 95% (P_{95}) differ not significantly in both species (p=0.481); in *A. agestis* the mean is 38.9 (±4.5), ranging from 33.3% to 44.4% and for *C. minimus* 42.9% (±10.1) ranging from 28.6% to 50.0%. Total percentage of polymorphic loci (P_{tot}) differed significantly in both species (p=0.007), with a mean value in *A. agestis* of 52.1 (±4.4), ranging from 44.4% to 55.6% and in *C. minimus* 73.5% (±16.4), with a minimum of 50.0% and a maximum of 100.0% (an overview of all values is given in Table 7.3).

Table 7.1. Overview of five parameters of genetic diversity for all populations analysed of *Aricia agestis* and *Cupido minimus*: mean number alleles per locus (*A*), percentage of expected heterozygosity (H_e), percentage of observed heterozygostiy (H_o), percentage of loci with the most commonest allele not exceeding 95% (P_{95}) and total percentage of polymorphic loci (P_{tot}). Tests for significant differences were performed using Mann-Whitney U-tests.

parameters	*Aricia agestis*	*Cupido minimus*	*p*
A	2.12 (±0.10)	2.24 (±0.22)	0.245
H_e	15.65 (±1.45)	18.26 (±1.57)	0.012
H_o	12.14 (±0.94)	17.31 (±1.82)	0.001
P_{tot}	52.1(±4.41)	73.47 (±16.35)	0.007
P_{95}	38.88 (±4.53)	42.86 (±10.10)	0.481

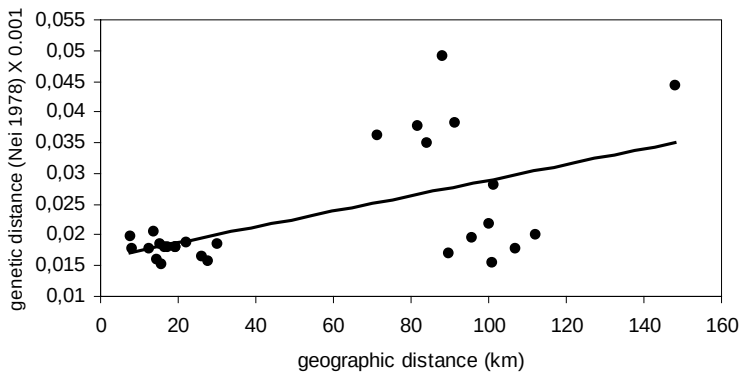
In both species, all analysed parameters of genetic variability showed no significant differences testing the habitats of 15 ha or more against the smaller ones (U-tests: all p>0.05)

The total genetic variance among populations, among individuals within populations and within individuals were higher for *C. minimus* compared to *A. agestis* (see Table 7.2). The differentiation among populations of *A. agestis* (F_{ST} 3.9%) was lower compared to *C. minimus* (F_{ST} 5.6 %). The F_{IS} values was relatively high for *A. agestis* (22.5%) and low for *C. minimus* (4.9%).

Table 7.2: Non-hierarchical variance analysis of *Aricia agestis* and *Cupido minimus* of the analysed populations.

species	among populations	among individuals	within individuals	total variance
A. agestis	0.057	0.315	1.090	1.462
C. minimus	0.075	0.062	1.220	1.357

We calculated phenograms based on Nei´s (1978) genetic distances for the two species. A significant isolation by distance in both species exist; this correlation of genetic distance and geographic distance was stronger detected in *A. agestis* (r^2=0.318; Mantel test: p=0.003) in comparison to *C. minimus* (r^2=0.218, Mantel test: p=0.031) (Figure 7.2). The mean of genetic distances in *C. minimus* was 0.0304±0.01033 and in *A. agestis* 0.02321±0.00961. The detected differences between both values of genetic distances was not significant (p=0.06).



Aricia agestis

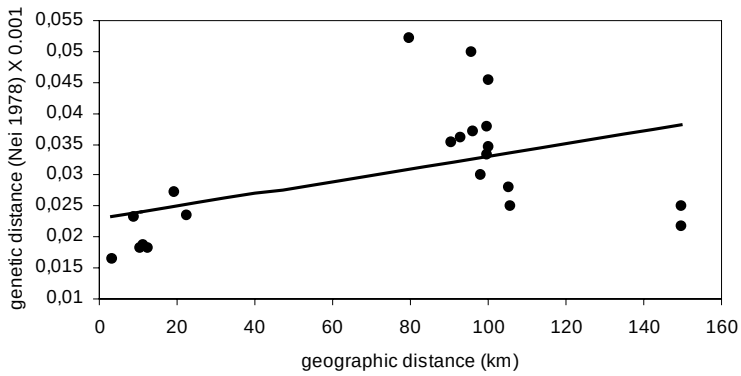


Figure 7.2: Correlation between the geographical distances and the respective genetic distances (Nei 1978) of the populations of *Aricia agestis* (r^2=0.318; Mantel test: p=0.003) and *Cupido min*imus (r^2=0.218, Mantel test: p=0.031).

Cupido minimus

Table 7.3: Five parameters of genetic diversity for all populations analysed of *Aricia agestis* and *Cupido minimus*: mean number of alleles per locus (*A*), percentage of expected heterozygosity (H_e), percentage of loci with the most commonest allele not exceeding 95% (P_{95}) and total percentage of polymorphic loci (P_{tot}); Abbreviations: ce: Central Eifel, sm: Saar-Mosel region, bg: Bliesgau, *A .a.*: *Aricia agestis*, *C. m.*: *Cupido minimus*; Tests for significant differences are performed using U-test.

site	area	*A*		H_e [%]		H_O [%]		H_O/H_e		P_{tot} [%]		P_{95} [%]	
		A. a.	*C. m*	*A. a.*	*C. m*	*A. a.*	*C. m*	*A. a.*	*C. m*	*A. a.*	*C. m*	*A. a.*	*C. m*
Lissendorf	ce	2.00		15.4		10.5		0.68		44.4		33.3	
Weinsheim	ce		2.29		18.8		18.8		1.00		78.6		50.0
Ingendorf	sm	2.17		16.8		11.6		0.69		55.6		38.9	
Igel	sm	2.22		16.7		12.0		0.72		55.6		38.9	
Trier	sm	2.06		15.0		12.3		0.82		50.0		38.9	
Niederanven	sm	2.06		15.4		12.7		0.82		55.6		44.4	
Nittel	sm	2.00		13.6		13.4		0.99		50.0		33.3	
Wasserliesch	sm		1.93		16.2		15.7		0.97		50.0		28.6
Freudenburg	sm		2.14		19.6		19.3		0.98		64.3		42.9
Perl	sm	2.28	2.36	14.5	19.7	11.9	18.5	0.82	0.94	55.6	85.7	38.9	50.0
Montenach	sm		2.64		19.8		18.4		0.93		100.0		57.1
Niedergailbach	bg	2.17	2.14	17.8	16.7	12.7	15.1	0.71	0.90	50.0	64.3	44.4	35.7
Mimbach	bg		2.21		17.0		15.4		0.91		71.4		35.7
mean (± s.d.)		2.12 (±0.10)	2.24 (±0.22)	15.65 (±1.45)	18.26 (±1.57)	12.14 (±0.94)	17.31 (±1.82)	0.78 (±0.11)	0.95 (±0.04)	52.1 (±4.41)	73.47 (±16.35)	38.88 (±4.53)	42.86 (±10.10)
U-test (*p*)		0.245		0.012		0.001		0.010		0.007		0.481	

7.2. Discussion:

The main objective of this study was to compare the genetic constitution of two butterflies, which represent completely different dispersal behaviours.

The two analysed species represent two contrary behaviours: while *A. agestis* is actually one of the most expansive butterflies with high dispersal ability (Bourn & Thomas 1993, Lewis & Bryant 2002), *C. minimus* is a very sedentary butterfly species. Very low migration rates for this species are reported by Bink (1992) and Weidemann (1988); Cowley et al. (2001) even ranked *C. minimus* as the most sedentarily butterfly in a comparison of 49 British butterflies. Mark release recapture experiments suggest that the majority of adults rarely move more than 40m with a maximum flight distance restricted to 762m and most of the individuals (91%) remained in their habitats (Baguette et al. 2003). Its single food plant and main nectar source *Anthyllis vulneraria* is mostly restricted to calcareous grasslands (Honnay et al. 2006). These ecological assumptions of a sedentary habitat specialist living in calcareous grasslands (Asher et al. 2001, Swaay 2002, Ebert & Rennwald 1991b) are responsible for their isolation.

In our study area, *Aricia agestis* exists in a metapopulation network with the ability to colonise new habitats; in contrast *Cupido minimus*, as one of the most sedentary species, built up very high densities but exists in mostly isolated populations. The genetic variations in both species characterise the difference in their life history: The genetic diversity in *C. minimus* is higher for all analysed parameters compared to the values of *A. agestis*. Except for the mean number of alleles (*A*), and the percentage of loci with the most commonest allele not exceeding 95% (P_{95}), all detected differences in genetic diversity differ significantly among these two species ($p<0.05$). The genetic differentiation among populations of *A. agestis* (F_{ST} 3.9%) was lower compared to *C. minimus* (F_{ST} 5.6 %). Both species built up an isolation by distance system, which is of higher importance in *A. agestis* ($r^2=0.318$; Mantel test: $p=0.003$) than in *C. minimus* ($r^2=0.218$, Mantel test: $p=0.031$).

The genetic texture of *C. minimus* is characterized by relatively high diversity and strong genetic differentiation between local populations in comparison to other Lycaenidae like *A. agestis*, *Polyomatus coridon* (Schmitt & Seitz 2002) and *Polyomatus icarus* (Schmitt et al. 2003). The F_{ST} value reflects a moderate exchange of individuals between neighbouring populations. The species is ecologically adapted to preserve a relatively high level of genetic variability, including some rare alleles, which occurs consistent in all analysed populations. This genetic constitution describes an adaptation of sedentarily behaviour, with low interpopulation connectivity to survive in a patchily habitat situation. To preserve genetic diversity without a continuous exchange of individuals may counterbalance high population densities. This affects a restricted gene flow, with stronger genetic differentiation between local populations, compared to *A. agestis*. *C. minimus* is classified from intermediate population densities (Bink 1992) reaching a maximum up to 500 individuals per hectare (Weidemann 1995).

However, the genetic diversity was not related to habitat size in the analysed populations. Studies showed that population densities are mainly affected by the cover of its larval food plant *Anthyllis vulneraria* (Krauss et al. 2004), which is also supported by modelling results (Leon-Cortes et al. 2003). This may explain a failure of genetic diversity and habitat size.

The behaviour of *A. agestis* is explained by a strong dispersal, shown in the fast expansion of its distribution area on the northern border of its distribution area (Asher et al. 2001, Heath et al. 1984). This butterfly increased 1.2-fold in the south of England and 4.9-fold further north, in central England. Such colonisation processes are mostly combined with founder effects evolving gene depressions. This phenomenon can indeed reduce the fitness, shown in many genetic studies (Hedrick & Kalinowski 2000, Keller & Waller 2002, Reed & Frankham 2003). Because of its colonisation behaviour a limited continuous exchange of neighbouring populations can be expected. In the case of *A. agestis*, its high migration power may lead to a compensation of such bottlenecks by such continuous exchanges between patches, which is underlined by the detected isolation by distance system. However relatively strong inbreeding effects were detectable compared to *C. minimus* and further butterfly species, analysed by the same marker system in the same study area like *M. galathea* (see 3.1), *M. aurelia* (see 4.1), *T. sylvestris* and *T. lineola* (see 6.1). This might be a consequence of founding new populations. The enhanced degree of isolation by distance in *A. agestis* underlines a geographically dependent gene flow among local populations. This suggestion of population dynamics on a local scale is in accordance with metapopulation simulations and further genetic analysis done in a study area in Britain with a similar spatial scale (Wilson et al. 2002, Wynne et al. 2003). However, in these populations, peripheral ones are genetically more differentiated from the central and well-connected populations of the network, presumably because of genetic drift and / or founder effects. This evoked a higher F_{ST}-value (F_{ST}: 9.3%) in Britain, the northern border of its distribution area, compared to the genetic differentiation of *A. agestis* in western Germany (F_{ST}: 3.9). This higher value in northern Europe could be affected by differing climatic conditions like temperature, which might influence migration potential. Consequently it can lead to lower gene flow and stronger genetic differentiation patterns. The higher F_{ST}-value in Britain are supported by mark-release-recapture studies in this area, which showed a main recorded movement less than 1 km (Wilson & Thomas 2002).

The genetic variation in both species characterise the differentiation in their life histories. We showed on *A. agestis* that metapopulation behaviour is characterised by low genetic diversity and a strong correlation of geographical and genetic distance. These affect a low genetic differentiation among local populations. In contrast, living in isolation as showing in *C. minimus* is indicated by higher genetic diversity, including rare alleles, a lower or complete failure of an isolation by distance system, and thus, a higher genetic differentiation among local populations.

Excursus V

A biodiversity hotspot

Comparing the analysed values of genetic diversity, we found one site, the Hammelsberg (Perl, Germany) with an outstanding genetic diversity. In the analysed butterfly species (*Melanargia galathea, Melitaea aurelia, Aricia agestis, Cupido minimus, Zygaena carniolica* and *Zygaena viciae*) most values of the five parameters of genetic diversity exceed the mean value. However only the total percentage of polymorphic loci (P_{tot}) differ significantly (Sign test: p=0.043). An exception of this trend is found in *M. galathea* (in all parameters) and in *A. agestis* (in the parameters H_e and H_o) (Table V.1).

Table V.1: Five parameters of genetic diversity for all populations analysed of the species *Melanargia galathea, Melitaea aurelia, Aricia agestis, Cupido minimus, Zygaena carniolica, Zygaena viciae*: mean number alleles per locus (*A*), percentage of expected heterozygosity (H_e), percentage of observed heterocygosity (H_o), percentage of loci with the most commonest allele not exceeding 95% (P_{95}) and total percentage of polymorphic loci (P_{tot}). Bold: Hammelsberg, numbers below in parenthesis: mean value of all samples analysed of the respective species. Significance was tested by Sign-test.

species	A	H_e [%]	*Ho* [%]	P_{95} [%]	P_{tot} [%]
M. galathea	**2.00** (2.0±0.1)	**16.7** (17.0±0.0)	**14.4** (15.0±0.0)	**38.9** (38.9±0.0)	**38.9** (37.8±0.0)
M. aurelia	**1.75** (1.7±0.1)	**10.9** (10.4±1.9)	**10.6** (9.3±1.9)	**37.5** (35.6±5.9)	**31.3** (28.7±8.4)
A. agestis	**2.28** (2.1±0.1)	**14.5** (15.6±1.4)	**11.9** (12.1±0.9)	**55.6** (52.1±4.4)	**38.9** (38.8±4.5)
C. minimus	**2.36** (2.2±0.2)	**19.7** (18.2±1.5)	**18.5** (17.3±1.8)	**85.7** (73.4±16.3)	**50.0** (42.8±10.1)
Z. carniolica	**1.44** (1.3±0.1)	**11.4** (9.4±1.2)	**12.0** (6.9±2.0)	**31.3** (29.4±4.3)	**37.5** (32.1±5.5)
Z. viciae	**1.85** (1.7±0.1)	**14.9** (13.4±4.7)	**8.4** (8.2±3.2)	**53.8** (38.8±14.3)	**53.8** (50.7±7.3)
Sign test(*p*)	0.371	0.683	0.683	0.075	0.043

Beside high values of genetic diversity, we detected a high amount of genetic differentiation to all other analysed populations in *Z. carniolica*.

We assume that the geographical position of this site, adjacent the Mosel river, has led to a situation of accumulation in gene diversity. Studies showed that the site Hammelsberg is of outstanding richness on taxa (Müller 1971, Nagel 1975,). With exact observations of lepidoterans during the last years over 1200 different species were observed, which is the highest number detected on a site in this region

(Werno, pers. comm.). This richness in taxa might be a result of high heterogeneity in habitat structures (Müller, pers. comm.), combined by a long duration of constant open vegetation on this site (Weitzel pers. comm., Meyer, pers. comm.).

We hypothesize that individuals, which used the Mosel valley as migration corridor colonised this site; in this way, populations might have evolved to high genetic diversity because of the accumulation of migrants. This effect of high genetic diversity is only found in species with stepping-stone-wise or leptokurtic migration behaviour. Species with a phalanx dispersal behaviour like in *M. galathea* lead to a panmictic gene structure, without populations of diversity deficit or diversity accumulation. In *Aricia agestis*, which was nearly extinct in the 1990s (Rote Liste Saarland) might have lost some of its genetic diversity, actually especially reflected by a low rate of heterozygosity.

Animals often use river-valley systems as migration routes. If individuals of these butterfly species have migrated along the Mosel valley from south and north, colonising this site, new generations at this site are enriched in their genetic constitution because of this hybridisation situation (cf. Arnold & Emms 1998, Arnold et al. 1999,). This addition of genetic variability could produce new recombinant genotypes, which is reflected by high A, H_e and H_o values in the hybrid populations. Such hybrid populations often show high fitness, as high as or higher than any of their parents (Grant & Grant 1996, Scribner 1993,). Hybrid genotypes that posses increased fitness in certain environments may enable the species to colonise new habitats by widening the range of environmental parameter that a single phenotype tolerates. Furthermore, hybridisation can act as the starting point for further evolutionary diversification in animals (DeMaras et al. 1992, Dowling & Secor 1997) and particularly in insects (Aubert et al. 1997, Bullini 1994, Mossakowski et al. 1990, Sperling & Harrison 1994).

8. Conclusion

We analysed ten butterfly (*Melanargia galathea*, *Melitaea aurelia*, *Thymelicus sylvestris*, *Thymelicus lineola*, *Thymelicus acteon*, *Aricia agestis*, *Cupido minimus*) and burnet moth species (*Zygaena carniolica*, *Zygaena loti*, *Zygaena viciae*) to investigate the influence of natural factors and land-use changes on the genetic diversity of these mostly xerothermophilous species. We question (i) how does fragmentation of habitats influence the exchange of individuals among patches? (ii) Are species adapted to natural patch isolation by geological or climatic conditions? (iii) Do species suffer from inbreeding because of high population fluctuations and low population density in closed populations and are these processes reinforced by anthropogenous habitat fragmentation? (iv) Is the ecological potential (abundance) responsible for gene flow among local populations? And (v) are species genetically adapted to their dispersal strategy?

(i) Isolation of habitats and its connectedness is shown in the butterfly *M. galathea* by mark-release-recapture studies and the genetic investigation of the same populations. Both analysis show inter- and intrapatch movements (small F_{ST}). Exchanges between patches are dependent on their geographic distance.

(ii) Historical habitat isolation by certain habitat requirements (geology, microclimate) is reflected by the abundance of this species. While *M. galathea* seems to be the most common studied species representing low genetic differentiation and high genetic variability, *M. aurelia*´s habitats are mostly restricted to limestone, and therefore are naturally fragmented. *Melitaea aurelia* is genetically well adapted to this patchily distribution situation by low genetic diversity, with the effect of lower risk to loose genetic diversity. The genetic differentiation is similar to *M. galathea*, which clarify the ability of this species to maintain its genetic constitution – even in isolated populations.

(iii) High population fluctuations and low population densities might evoke genetic bottlenecks, shown in Zygaenids (*Z. viciae*, *Z. loti*); this is reinforced by low intrapatch movements and diapause-behaviour, which effect a subdivided population structure within a habitat, reflected by high inbreeding effects (high F_{IS}). Stabilisation of the genetic constitution despite of the isolation of its habitats, shown in *Z. carniolica*, might be evoked by lower population fluctuations, higher population densities and its lower genetic diversity, consisting of a few main alleles, similar to the situation of *M. aurelia*.

(iv) The ecological amplitude of a species is reflected by its abundance pattern. This is the main trigger for the amount of gene exchange. Strong gene flow (panmixis) was detected for common species with high dispersal behaviour, like *T. sylvestris* and *T. lineola*, which is represented by low genetic differentiation between local populations (low F_{ST}); in contrast, low abundance species are characterized by gene drift, which leads to stronger genetic differentiation (high F_{ST}) and inbreeding effects (high F_{IS}) shown in the sedentary specialist *T. acteon*.

(v) The level of an exchange of alleles among populations, preventing gene drift and genetic depressions (as shown in *Z. viciae, Z. loti, T. acteon*), depends on the species specific dispersal behaviour. Comparing the genetic constitution of the expansive Lycaenidae *A. agestis* with one of the most sedentary species *C. minimus* let us propose that their genetics is well adapted in accordance to the species specific dispersal behaviour: while *C. minimus* shows a higher genetic diversity, this species is genetically stronger differentiated between populations (higher F_{ST}). Despite of the sedentary behaviour, the species preserve this genetic diversity, supported by high population's densities and low population fluctuations. In contrast, *A. agestis´* genetic constitution is restricted to few main alleles, which seems to be an adaptation for colonisation processes. These are always potential bottlenecks, which can evoke genetic depressions. On a regional geographic scale, this species has a metapopulation system, which is characterized by a constant extinction and colonisation turn over.

Summarising the results in the light of conservation genetics we conclude that species, which exist patchily, more or less isolated in landscapes evoked by naturally habitat fragmentation (geological and/or climatic conditions) seem to be well adapted to their situations (as shown in *A. agestis, M. aurelia, Z. carniolica*). Species, which are not adapted to habitat fragmentation often show higher genetic diversity (*Z. loti, Z. viciae*). These species are suffering under anthropogenous habitat fragmentation. During stochastic population fluctuations, rare alleles become lost, which are actually expressed by a deficit of these rare alleles and inbreeding effects (high F_{IS}).

9. Evolution…

Evolution, a term to express a continuous transition is also assignable in scientific studies, which are always in progress and never finished – thus, this study of ten butterflies offers many more new questions than answers:

(i) Our genetic data should also become analysed using alternative molecular techniques; many genetic studies showed differing results. This is consequentially because each marker system detect other gene loci. At the moment we verify our genetic data using a DNA-marker system (Amplified Fragment Length Polymorphism, AFLP).

(ii) Genetic patterns reflect the population situation of the last few decades. The actually fragmentation of species is still in process and might not yet be reflected by genetic patterns. Therefore, further analysis should follow a few decades later on the same study species in the same study area.

(iii) Sampling subdivided populations evoked high F_{IS}-values. Taking these subdivisions into account to verify our hypothesis, the geographical coordinates of each sampled individual should be recorded to analyse a possible genetic substructure within a habitat. This is planned for summer 2007.

(iv) Beside a hypothesized subdivision of the analysed Zygaenids, phylogeographic structures (e.g. cryptic speciation) will be tested, for example by using mtDNA (cytochrome-b). This analysis will be done in autumn 2006.

(v) The exceptional genetic position of the Perl, which revealed an exceptional genetic diversity for most of the genetic diversity for most of the analysed species is still in process and will be tested for further species in the next couple of years. The surrounding habitats will become implicated as well in the following analysis.

(vi) In Excursus I *M. galathea* and *M. lachesis* are described as two genetically distinct species. Both species normally occur allopatric. Some few sites are known, where both species exist in sympatry (Pyrenees). With genetic analysis we can clarify whether these species hybridize in these sites, which we will perform in summer 2007.

(vii) In Excursus II we demonstrate that the genetic information suggests that the origin of *M. galathea* is North Africa. We hypothesized that the butterfly crossed the Mediterranean via Sicily and colonised Europe. To verify this hypothesis, further samples from Tunisia, Sicily and Italy are needed to verify this assumption. During the next flight period of *M. galathea* we will collect samples in these regions.

... and more!

(biology means diversity, reflected by a never ending amount of questions).

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12. Appendix

Table 12.1: allele frequencies of all loci of all populations analysed of *Melanargia galathea, Melitaea aurelia, Zygaena carniolica, Zygaena viciae, Zygaena loti, Thymelicus sylvestris, Thymelicus acteon, Thymelicus lineola, Aricia agestis, Cupido minimus.*

Melanargia galathea

loci	alleles	Wasser-liesch	Weins-heim	Echter-nacher-brück	Bettingen	Hönsch	Schön-ecken	Eiderberg	Mösch	Römers-köpfchen	Hammels-berg
Idh1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0	0	0	0	0
Idh2	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
Mdh1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0	0	0	0	0
Mdh2	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
6Gpdh	1	0.013	0	0	0	0	0.038	0.038	0	0	0
	2	0.744	0.725	0.513	0.628	0.722	0.756	0.688	0.600	0.795	0.724
	3	0.205	0.275	0.461	0.372	0.278	0.205	0.250	0.386	0.167	0.276
	4	0.038	0	0.026	0	0	0	0.025	0.014	0.038	0
	5	0	0	0	0	0	0	0	0	0	0
G6pdh	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
Gapdh	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgi	1	0	0	0	0	0	0	0	0	0	0
	2	0.050	0.200	0	0.063	0.222	0.038	0	0	0	0.013
	3	0.050	0.100	0.025	0.013	0.028	0.125	0.213	0.162	0.095	0.051
	4	0.625	0.338	0.412	0.488	0.361	0.525	0.613	0.387	0.419	0.513
	5	0	0.025	0.075	0	0	0	0.025	0.013	0	0.090
	6	0.150	0.013	0.162	0.125	0.083	0.138	0.050	0.025	0.338	0.090
	7	0.075	0.213	0.112	0.087	0.167	0.100	0.075	0.325	0	0.026
	8	0.050	0.100	0.162	0.175	0.028	0.025	0.013	0.063	0.068	0.154
	9	0	0	0.050	0.050	0.111	0.050	0.013	0.025	0.081	0.064
	10	0	0	0	0	0	0	0	0	0	0
	11	0	0.013	0	0	0	0	0	0	0	0
Pgm	1	0.050	0.188	0.103	0.075	0.028	0.087	0	0.013	0.026	0.013
	2	0.788	0.613	0.808	0.700	0.833	0.788	0.913	0.887	0.910	0.846
	3	0.162	0.188	0.077	0.213	0.139	0.112	0.063	0.100	0.051	0.115
	4	0	0.013	0.013	0.013	0	0.013	0.025	0	0.013	0.026
	5	0	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0	0	0
	9	0	0	0	0	0	0	0	0	0	0
Pep	1	0	0	0	0	0	0	0	0.013	0	0
	2	0	0	0	0	0	0	0	0.013	0	0
	3	0	0	0	0	0	0.013	0.038	0	0.013	0.045
	4	0.893	0.913	0.943	0.865	0.714	0.947	0.925	0.975	0.800	0.879
	5	0.107	0.087	0.057	0.135	0.286	0.039	0.038	0	0.188	0.076
	6	0	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0	0	0

loci	alleles	Wasser-liesch	Weins-heim	Echter-nacher-brück	Bettingen	Hönsch	Schön-ecken	Eiderberg	Mösch	Römers-köpfchen	Hammels-berg
Pep	9	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0
	11	0	0	0	0	0	0	0	0	0	0
	12	0	0	0	0	0	0	0	0	0	0
Aat1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
Aat2	1	0.025	0	0.050	0	0.028	0.013	0	0	0	0
	2	0.050	0.075	0.100	0.115	0.028	0.213	0.213	0.090	0.064	0.176
	3	0.013	0.075	0.025	0	0	0.013	0	0.013	0	0
	4	0.250	0.387	0.200	0.346	0.389	0.138	0.262	0.282	0.231	0.257
	5	0	0.013	0	0	0	0.013	0	0	0	0
	6	0.313	0.087	0.313	0.346	0.333	0.375	0.400	0.333	0.397	0.351
	7	0	0.038	0.038	0	0	0	0	0	0.013	0
	8	0.350	0.325	0.237	0.192	0.222	0.237	0.125	0.282	0.295	0.216
	9	0	0	0.038	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0
	11	0	0	0	0	0	0	0	0	0	0
Gpdh	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
Fum	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Me	1	0.075	0.192	0.053	0.087	0	0.250	0.100	0.112	0.158	0.081
	2	0.925	0.808	0.947	0.913	1.000	0.750	0.900	0.887	0.842	0.919
	3	0	0	0	0	0	0	0	0	0	0
Hbdh	1	0	0	0	0	0	0	0	0	0	0
	2	0.350	0.375	0.262	0.287	0.250	0.262	0.463	0.488	0.300	0.459
	3	0.650	0.625	0.738	0.712	0.750	0.738	0.538	0.512	0.700	0.541
	4	0	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0	0	0
	9	0	0	0	0	0	0	0	0	0	0
Acon	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0
Apk	1	0	0	0	0	0	0	0	0	0	0
	2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	3	0	0	0	0	0	0	0	0	0	0
	N	27	40	37	40	16	39	39	39	40	37

Melanargia galathea

loci	alleles	Mimbach	Nieder-gailbach	Ourtal	Niederanven	Rosselange	Lorry	Schifflange
Idh1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0	0
Idh2	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
Mdh1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0	0
Mdh2	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0

loci	alleles	Mimbach	Nieder-gailbach	Ourtal	Niederanven	Rosselange	Lorry	Schifflange
6Gpdh	1	0	0	0	0	0	0	0
	2	0.757	0.808	0.770	0.450	0.613	0.688	0.486
	3	0.243	0.154	0.230	0.467	0.275	0.287	0.417
	4	0	0.038	0	0.083	0.112	0.025	0.097
	5	0	0	0	0	0	0	0
G6pdh	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
Gapdh	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgi	1	0	0	0	0	0	0	0
	2	0	0	0.171	0.297	0.213	0.050	0.106
	3	0.150	0	0.158	0.047	0.100	0.300	0.121
	4	0.587	0.462	0.500	0.297	0.438	0.412	0.545
	5	0	0.013	0	0	0.013	0	0
	6	0.087	0.449	0.026	0.094	0.038	0	0
	7	0.087	0.026	0.105	0.094	0.038	0.050	0.197
	8	0.050	0.026	0.026	0.109	0.100	0.125	0.015
	9	0.038	0.026	0.013	0.063	0.063	0.063	0.015
	10	0	0	0	0	0	0	0
	11	0	0	0	0	0	0	0
Pgm	1	0.075	0.054	0.038	0.063	0.105	0.063	0
	2	0.700	0.743	0.788	0.672	0.776	0.775	0.958
	3	0.213	0.203	0.162	0.234	0.118	0.162	0.042
	4	0.013	0	0.013	0.031	0	0	0
	5	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0
	9	0	0	0	0	0	0	0
Pep	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0.013	0.013	0.014
	4	0.778	1.000	0.889	0.903	0.872	0.962	0.944
	5	0.222	0	0.111	0.097	0.103	0.025	0.042
	6	0	0	0	0	0.013	0	0
	7	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0
	9	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	11	0	0	0	0	0	0	0
	12	0	0	0	0	0	0	0
	N	36	39	36	31	39	40	36
Aat1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
Aat2	1	0	0.013	0.014	0	0.038	0.100	0
	2	0.025	0.026	0.068	0.063	0.064	0.150	0.056
	3	0.013	0.013	0.014	0.016	0	0	0
	4	0.313	0.263	0.230	0.266	0.179	0.250	0.222
	5	0	0	0	0	0.013	0.013	0.014
	6	0.287	0.355	0.338	0.328	0.333	0.412	0.375
	7	0	0	0	0	0	0	0
	8	0.363	0.329	0.338	0.328	0.372	0.075	0.333
	9	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	11	0	0	0	0	0	0	0

loci	alleles	Mimbach	Nieder-gailbach	Ourtal	Niederanven	Rosselange	Lorry	Schifflange
Gpdh	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
Fum	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Me	1	0.363	0.167	0.056	0.031	0.075	0.087	0.083
	2	0.637	0.833	0.944	0.969	0.925	0.913	0.917
	3	0	0	0	0	0	0	0
Hbdh	1	0	0	0	0	0	0	0
	2	0.287	0.269	0.359	0.281	0.474	0.425	0.500
	3	0.712	0.731	0.641	0.719	0.526	0.575	0.500
	4	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0
	9	0	0	0	0	0	0	0
Acon	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0
Apk	1	0	0	0	0.016	0	0	0
	2	1.000	1.000	1.000	0.984	1.000	1.000	1.000
	3	0	0	0	0	0	0	0
	N	40	25	37	32	40	40	35

Melitaea aurelia

loci	alleles	Niedergailbach	Römersköpfchen	Rosselange	Wasserliesch	Weinsheim
6Gpdh	1	1.000	1.000	1.000	1.000	1.000
Acon	1	0	0	0	0.013	0
	2	0.775	0.961	0.825	0.975	0.988
	3	0.225	0.039	0.175	0.013	0.013
Fum	1	1.000	1.000	1.000	1.000	1.000
G6pdh	1	1.000	1.000	1.000	1.000	1.000
Gapdh	1	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0
Aat1	1	0	0	0.013	0	0.038
	2	1.000	1.000	0.950	1.000	0.950
	3	0	0	0.013	0	0.013
	4	0	0	0.025	0	0
Aat2	1	0	0	0.038	0	0
	2	0.900	0.975	0.925	1.000	1.000
	3	0.100	0	0.038	0	0
	4	0	0.025	0	0	0
Gpdh	1	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
	6	0	0	0	0	0
Pgi	1	0	0.013	0.013	0	0.087
	2	0.064	0	0.112	0	0
	3	0.154	0.150	0.250	0.026	0.025
	4	0.526	0.637	0.500	0.756	0.613
	5	0.038	0	0.013	0	0
	6	0.218	0.200	0.100	0.205	0.275
	7	0	0	0.013	0.013	0

loci	alleles	Niedergailbach	Römersköpfchen	Rosselange	Wasserliesch	Weinsheim
Hbdh	1	0	0.125	0.075	0.013	0.053
	2	0.938	0.806	0.538	0.833	0.645
	3	0.063	0.069	0.387	0.154	0.303
Idh1	1	1.000	1.000	1.000	1.000	1.000
Mdh1	1	1.000	1.000	1.000	1.000	1.000
Mdh2	1	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0
Me	1	1.000	1.000	1.000	1.000	1.000
Pep	1	0.013	0.112	0.050	0	0.063
	2	0.988	0.887	0.938	1.000	0.938
	3	0	0	0	0	0
	4	0	0	0.013	0	0
Pgm	1	0	0.038	0	0	0.027
	2	0.171	0.154	0.125	0.079	0.189
	3	0.829	0.679	0.875	0.882	0.649
	4	0	0.090	0	0.039	0.081
	5	0	0.038	0	0	0.041
	6	0	0	0	0	0.014
	N	38	39	40	38	37

Melitaea aurelia

loci	alleles	Niederanven	Bettingen	Freudenburg	Echternacherbrück	Hammelsberg
6Gpdh	1	1.000	1.000	1.000	1.000	1.000
Acon	1	0	0	0	0	0
	2	1.000	1.000	0.938	0.988	0.962
	3	0	0	0.063	0.013	0.038
Fum	1	1.000	1.000	1.000	1.000	1.000
G6pdh	1	1.000	1.000	1.000	1.000	1.000
Gapdh	1	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0
Aat1	1	0	0	0.090	0	0
	2	0.975	1.000	0.910	1.000	0.950
	3	0.025	0	0	0	0.050
	4	0	0	0	0	0
Aat2	1	0	0	0.025	0	0
	2	0.975	1.000	0.913	0.974	1.000
	3	0.025	0	0.063	0.026	0
	4	0	0	0	0	0
Gpdh	1	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
	6	0	0	0	0	0
Pgi	1	0.013	0	0.025	0.050	0
	2	0.064	0.013	0	0.050	0.090
	3	0.077	0.063	0.300	0.138	0.103
	4	0.462	0.587	0.450	0.463	0.603
	5	0	0	0	0	0.038
	6	0.346	0.338	0.225	0.300	0.167
	7	0.038	0	0	0	0
Hbdh	1	0.063	0.063	0.050	0.100	0.025
loci	alleles	Niederanven	Bettingen	Freudenburg	Echternacherbrück	Hammels-berg
	2	0.663	0.688	0.700	0.613	0.663
	3	0.275	0.250	0.250	0.287	0.313
Idh1	1	1.000	1.000	1.000	1.000	1.000
Mdh1	1	1.000	1.000	1.000	1.000	1.000

loci	alleles	Niederanven	Bettingen	Freudenburg	Echternacherbrück	Hammelsberg
Mdh2	1	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0
Me	1	1.000	1.000	1.000	1.000	1.000
Pep	1	0.013	0.025	0	0.013	0.050
	2	0.988	0.925	1.000	0.988	0.938
	3	0	0.050	0	0	0.013
	4	0	0	0	0	0
Pgm	1	0	0	0	0	0
	2	0.208	0.141	0.175	0.112	0.188
	3	0.653	0.795	0.800	0.875	0.762
	4	0.139	0.051	0.025	0.013	0.050
	5	0	0.013	0	0	0
	6	0	0	0	0	0
	N	36	39	40	40	40

Zygaena carniolica:

loci	alleles	Nieder-anven	Mimbach	Bettingen	Jung-linster	Monte-nach	Nieder-gailbach	Römers-köpfchen	Schön-ecken	Wasser-liesch
Apk	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
6Gpdh	1	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0
	3	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	4	0	0	0	0	0	0	0	0	0
Acon	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0
Fum	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
G6pdh	1	0.885	0.775	0	0.925	0.863	0.987	0.837	0.944	0.925
	2	0.115	0.225	0	0.075	0.138	0.013	0.162	0.056	0.075
	3	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0
Gapdh	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Aat1	1	0	0	0	0	0	0	0	0	0
	2	0.375	0.363	0.368	0.400	0.368	0.218	0.188	0.158	0.500
	3	0.625	0.637	0.632	0.600	0.632	0.782	0.813	0.842	0.500
	4	0	0	0	0	0	0	0	0	0
Aat2	1	0	0	0	0	0	0	0	0	0
	2	0.105	0	0	0.025	0.050	0	0	0.171	0
	3	0.842	1.000	1.000	0.975	0.950	1.000	1.000	0.829	1.000
	4	0	0	0	0	0	0	0	0	0
	5	0.053	0	0	0	0	0	0	0	0
Gpdh	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0
Pgi	1	0.112	0.162	0.147	0.175	0.237	0.090	0.162	0.066	0.200
	2	0.887	0.837	0.853	0.825	0.763	0.910	0.825	0.934	0.800
	3	0	0	0	0	0	0	0.013	0	0
	4	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0
Hbdh	1	0.013	0	0	0.025	0	0	0	0	0
	2	0.613	0.308	0.279	0.313	0.363	0.410	0.575	0.645	0.625
	3	0.375	0.692	0.721	0.663	0.637	0.590	0.425	0.355	0.375
Idh1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0
Mdh1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

loci	alleles	Nieder-anven	Mimbach	Bettingen	Jung-linster	Monte-nach	Nieder-gailbach	Römers-köpfchen	Schön-ecken	Wasser-liesch
loci	alleles	Nieder-anven	Mimbach	Bettingen	Jung-linster	Monte-nach	Nieder-gailbach	Römers-köpfchen	Schön-ecken	Wasser-liesch
	2	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0
Mdh2	1	0	0	0	0	0	0	0	0	0
	2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Me	1	0.038	0	0.088	0.112	0.038	0.051	0.050	0.066	0
	2	0.962	1.000	0.912	0.887	0.962	0.949	0.950	0.934	1.000
	3	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0
Mpi	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0
	N	40	40	34	40	40	39	40	38	40

Zygaena viciae

loci	alleles	Nieder-anven	Bettingen	Echternacher brück	Freudenburg	Hammels-berg	Montenach	Nieder-gailbach	Wasser-liesch	Weinsheim
Acon	1	0.029	0.026	0.028	0	0	0.013	0.014	0.013	0
	2	0.971	0.718	0.944	0.938	0.875	0.987	0.986	0.962	1.000
	3	0	0.256	0.028	0.063	0.125	0	0	0.025	0
	4	0	0	0	0	0	0	0	0	0
Fum	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
G6pdh	1	0	0	0	0	0	0	0	0	0
	2	0	0	0.030	0	0.053	0	0	0.158	0
	3	0.983	0.769	0.939	1.000	0.868	0.814	1.000	0.829	1.000
	4	0.017	0.231	0.030	0	0.079	0.186	0	0.013	0
Gapdh	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Aat1	1	0	0.013	0	0.150	0.013	0	0	0	0.094
	2	0.710	0.641	0.736	0.688	0.692	0.653	0.778	1.000	0.672
	3	0.274	0.282	0.250	0.162	0.192	0.319	0.208	0	0.219
	4	0.016	0.064	0.014	0	0.103	0.028	0.014	0	0.016
Aat2	1	0.037	0.300	0.056	0	0.179	0.309	0	0.182	0
	2	0	0	0	0	0	0.015	0	0	0
	3	0.574	0.450	0.639	0.637	0.500	0.338	0.667	0.682	0.609
	4	0.370	0.250	0.306	0.338	0.321	0.324	0.306	0.136	0.375
	5	0.019	0	0	0.025	0	0.015	0.028	0	0.016
Gpdh	1	0	0.075	0	0	0	0	0	0	0
	2	1.000	0.925	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	3	0	0	0	0	0	0	0	0	0
Pgi	1	0	0	0.029	0	0	0.026	0	0.013	0
	2	0.076	0.087	0.132	0.138	0.025	0.013	0	0.138	0
	3	0	0	0.029	0	0.025	0.105	0.014	0.075	0.016
	4	0.727	0.825	0.632	0.650	0.850	0.658	0.569	0.650	0.672
	5	0	0	0	0	0	0	0	0.100	0
	6	0.182	0.087	0.176	0.213	0.100	0.197	0.417	0.025	0.313
	7	0	0	0	0	0	0	0	0	0
	8	0.015	0	0	0	0	0	0	0	0
Hbdh	1	0.017	0	0	0	0	0	0	0.013	0
	2	0.138	0.200	0.292	0.235	0.284	0.176	0.288	0.237	0.224
	3	0	0	0	0.044	0	0.029	0.030	0	0.017
	4	0.707	0.712	0.542	0.588	0.514	0.471	0.621	0.395	0.638
	5	0.138	0.087	0.167	0.132	0.203	0.324	0.061	0.355	0.121
Idh1	1	0.038	0	0	0	0	0	0	0	0.016
	2	0.962	1.000	1.000	1.000	1.000	1.000	0.986	1.000	0.969
	3	0	0	0	0	0	0	0.014	0	0.016
Mdh1	1	0.242	0.450	0.403	0	0.282	0.184	0.148	0.167	0

loci	alleles	Nieder-anven	Bettingen	Echternacher brück	Freudenburg	Hammels-berg	Montenach	Nieder-gailbach	Wasser-liesch	Weinsheim
	2	0.758	0.550	0.597	1.000	0.718	0.816	0.852	0.833	1.000
	3	0	0	0	0	0	0	0	0	0
Mdh2	1	0.029	0.013	0.028	0.015	0	0.053	0.019	0	0
	2	0.926	0.988	0.958	0.985	0.975	0.947	0.981	1.000	1.000
	3	0.044	0	0.014	0	0.025	0	0	0	0
Me	1	0.297	0.175	0.153	0.025	0.075	0.167	0.056	0.025	0.031
	2	0.703	0.825	0.847	0.975	0.925	0.833	0.944	0.975	0.969
Pgm	1	0	0	0.042	0.013	0.051	0.344	0	0.028	0
	2	0.081	0.262	0.069	0.051	0.205	0.047	0.014	0.153	0.016
	3	0.903	0.625	0.875	0.897	0.692	0.594	0.886	0.764	0.952
	4	0	0.112	0.014	0.038	0.051	0.016	0.100	0.056	0.032
	5	0.016	0	0	0	0	0	0	0	0
	N	31	40	36	39	39	32	35	36	31

Zygaena loti

loci	alleles	Clouange	Freudenburg	Lissen	Montenach	Niederanven	Römers-köpfchen	Schönecken
Acon	1	0	0.029	0	0	0	0	0
	2	1.000	0.971	1.000	1.000	1.000	0.975	1.000
	3	0	0	0	0	0	0.025	0
Fum	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000
G6pdh	1	0.039	0.097	0	0	0.237	0	0
	2	0.961	0.774	0.846	0.975	0.762	1.000	0.738
	3	0	0.129	0.154	0.025	0	0	0.262
Gapdh	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Aat1	1	0	0	0.289	0	0	0	0.287
	2	1.000	0.758	0.711	1.000	1.000	1.000	0.688
	3	0	0.242	0	0	0	0	0.025
Aat2	1	0.039	0.059	0.139	0.141	0.025	0	0.211
	2	0.961	0.794	0.569	0.513	0.975	0.975	0.539
	3	0	0.029	0.264	0.231	0	0	0.184
	4	0	0.044	0.028	0.115	0	0	0.066
	5	0	0.074	0	0	0	0.025	0
Gpdh	1	1.000	1.000	1.000	1.000	0.950	0.825	1.000
	2	0	0	0	0	0.050	0.175	0
	N	38	34	39	40	40	40	40
Pgi	1	0	0.015	0	0	0.063	0.013	0
	2	0.039	0.242	0.064	0.450	0.138	0.050	0.575
	3	0.500	0.697	0.551	0.425	0.313	0.663	0.287
	4	0.132	0.045	0.269	0.125	0.488	0.275	0.075
	5	0.329	0	0.115	0	0	0	0.063
Hbdh	1	0.292	0.258	0.057	0.205	0.425	0.400	0.050
	2	0	0.030	0.114	0.115	0.013	0	0.700
	3	0.694	0.712	0.700	0.577	0.563	0.600	0.250
	4	0.014	0	0.086	0.090	0	0	0
	5	0	0	0.043	0.013	0	0	0
Idh1	1	0	0.176	0.105	0.075	0	0	0.150
	2	1.000	0.824	0.868	0.925	1.000	0.975	0.825
	3	0	0	0.026	0	0	0.025	0.025
Mdh1	1	0	0.212	0.090	0.075	0	0	0.150
	2	1.000	0.788	0.885	0.925	1.000	1.000	0.825
	3	0	0	0.026	0	0	0	0.025
Mdh2	1	0	0.132	0	0	0.038	0.125	0
	2	0.053	0.088	0	0	0	0	0.025
	3	0.947	0.779	1.000	1.000	0.962	0.875	0.950
	4	0	0	0	0	0	0	0.025
Me	1	0	0	0.103	0	0	0	0

loci	alleles	Clouange	Freudenburg	Lissen	Montenach	Niederanven	Römers-köpfchen	Schönecken
	2	0.053	0.250	0.103	0.025	0	0.077	0.262
	3	0.947	0.750	0.795	0.975	0.988	0.923	0.738
	4	0	0	0	0	0.013	0	0
Pgm	1	0	0.088	0.038	0	0.200	0.087	0
	2	1.000	0.809	0.885	0.887	0.800	0.600	0.725
	3	0	0.103	0.077	0.112	0	0.313	0.275
Mpi	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Thymelicus sylvestris

loci	alleles	Wasserliesch	Freudenburg	Mimbach	Niedergailbach	Römersköpfchen	Bettingen
Idh1	1	0	0	0	0	0	0
	2	1.000	1.000	1.000	1.000	1.000	1.000
	3	0	0	0	0	0	0
Idh2	1	0	0	0	0	0.013	0
	2	1.000	1.000	1.000	1.000	0.987	1.000
	3	0	0	0	0	0	0
	N	40	38	35	35	38	40
Mdh1	1	1.000	1.000	1.000	0.986	1.000	1.000
	2	0	0	0	0.014	0	0
	3	0	0	0	0	0	0
Mdh2	1	0	0	0	0	0.026	0
	2	0.962	0.962	0.957	0.971	0.961	1.000
	3	0.038	0.038	0.043	0.029	0.013	0
	4	0	0	0	0	0	0
6pg	1	0	0	0.016	0	0	0
	2	0	0	0	0	0	0
	3	0.987	1.000	0.969	0.986	0.972	1.000
	4	0.013	0	0.016	0.014	0.028	0
G6pdh	1	0	0	0	0	0	0
	2	0	0.014	0	0	0	0
	3	0.175	0.068	0.029	0.061	0.189	0.289
	4	0.750	0.797	0.853	0.879	0.784	0.605
	5	0.075	0.122	0.118	0.061	0.027	0.105
	6	0	0	0	0	0	0
Pgi	1	0	0	0	0	0	0
	2	0.013	0.026	0	0.014	0	0.025
	3	0	0.115	0.014	0.057	0.026	0
	4	0.063	0	0.071	0.071	0.118	0.050
	5	0.750	0.615	0.729	0.571	0.605	0.637
	6	0	0	0	0	0	0
	7	0.050	0.051	0.029	0.029	0.053	0.075
	8	0.112	0.192	0.157	0.257	0.197	0.200
	9	0.013	0	0	0	0	0.013
Pgm	1	0.038	0.013	0	0.043	0.039	0.013
	2	0.090	0.154	0.171	0.214	0.158	0.175
	3	0.872	0.808	0.829	0.629	0.789	0.813
	4	0	0.026	0	0	0.013	0
	5	0	0	0	0.114	0	0
Pep	1	0	0	0	0	0	0
	2	0	0.013	0	0.015	0	0.013
	3	1.000	0.962	0.986	0.956	1.000	0.975
	4	0	0.026	0.014	0.029	0	0.013
Aat1	1	0	0	0	0	0	0
	2	0.041	0.051	0	0.015	0.095	0.029
	3	0.541	0.615	0.729	0.636	0.378	0.629

loci	alleles	Wasserliesch	Freudenburg	Mimbach	Niedergailbach	Römersköpfchen	Bettingen
	4	0.122	0	0	0	0	0.029
	5	0.270	0.308	0.271	0.348	0.527	0.314
	6	0.027	0.026	0	0	0	0
	7	0	0	0	0	0	0
Aat2	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	1.000	1.000	0.986	0.971	1.000	1.000
	4	0	0	0.014	0.029	0	0
Gpdh	1	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0
Fum	1	0	0	0	0	0.014	0
	2	0.974	0.986	1.000	0.985	0.972	0.987
	3	0.026	0.014	0	0.015	0.014	0.013
	4	0	0	0	0	0	0
Me	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	1.000	1.000	1.000	1.000	1.000	1.000
Mpi	1	0	0.013	0	0	0	0
	2	1.000	0.987	1.000	1.000	1.000	1.000
	3	0	0	0	0	0	0
	4	0	0	0	0	0	0
Hbdh	1	0	0	0	0	0.029	0
	2	0	0	0	0.030	0	0.013
	3	0.938	0.949	0.926	0.864	0.900	0.885
	4	0	0	0	0	0	0
	5	0.025	0.013	0.029	0.076	0	0.026
	6	0.013	0.038	0	0	0	0
	7	0.025	0	0.044	0.030	0.071	0.077
Acon	1	0	0	0	0	0	0
	2	1.000	1.000	1.000	1.000	1.000	1.000
	3	0	0	0	0	0	0
Apk	1	0	0	0	0	0.013	0
	2	1.000	1.000	1.000	1.000	0.987	1.000

loci	alleles	Echter-nacherbrück	Weins-heim	Schönecken	Kresna Gorge	Nieder-anven	Ourtal
Idh1	1	0.025	0	0	0	0	0
	2	0.975	1.000	0.988	1.000	1.000	1.000
	3	0	0	0.013	0	0	0
Idh2	1	0	0	0	0.026	0	0
	2	0.987	1.000	1.000	0.974	1.000	1.000
	3	0.013	0	0	0	0	0
Mdh1	1	0.988	1.000	1.000	0	0	1.000
	2	0.013	0	0	0.026	0	0
	3	0	0	0	39	39	0
Mdh2	1	0	0	0	0.987	0.934	0
	2	0.913	0.962	0.962	0.013	0.039	1.000
	3	0.075	0.038	0.038	0	0.013	0
	4	0.013	0	0	39	38	0
6pg	1	0	0	0	0.013	0	0
	2	0	0	0	0.987	1.000	0
	3	1.000	1.000	0.962	0	0	1.000
	4	0	0	0.038	39	38	0
G6pdh	1	0	0.014	0	0	0.013	0
	2	0.014	0	0	0.069	0.079	0
	3	0.208	0.189	0.175	0.833	0.816	0.109
	4	0.736	0.689	0.750	0.097	0.092	0.828
	5	0.042	0.095	0.075	0	0	0.063
	6	0	0.014	0	36	38	0

loci	alleles	Echter-nacherbrück	Weins-heim	Schönecken	Kresna Gorge	Nieder-anven	Ourtal
Pgi	1	0	0	0	0.500	0.051	0
	2	0	0	0	0	0.013	0.016
	3	0.050	0	0	0.462	0.667	0.016
	4	0.013	0.100	0.112	0	0.064	0.063
	5	0.700	0.675	0.587	0.013	0.051	0.609
	6	0	0	0	0.013	0.141	0
	7	0.063	0.025	0.063	0	0.013	0.156
	8	0.162	0.200	0.213	39	39	0.141
	9	0.013	0	0.025	0	0.038	0
Pgm	1	0	0.038	0	0.949	0.808	0
	2	0.225	0.162	0.150	0.013	0.013	0.063
	3	0.762	0.788	0.850	0	0	0.922
	4	0.013	0.013	0	39	39	0.016
	5	0	0	0	0	0	0
Pep	1	0	0	0	0.872	0.987	0
	2	0	0	0	0.038	0	0.047
	3	0.988	0.975	0.975	39	39	0.922
	4	0.013	0.025	0.025	0	0	0.031
Aat1	1	0	0	0.013	0.692	0.397	0
	2	0.013	0.064	0	0	0	0
	3	0.628	0.462	0.551	0.256	0.564	0.453
	4	0	0	0	0	0	0
	5	0.359	0.474	0.436	0.051	0	0.547
	6	0	0	0	39	39	0
	7	0	0	0	0	0	0
Aat2	1	0	0	0	0.868	0.962	0.016
	2	0	0	0	0	0.038	0
	3	0.987	1.000	1.000	38	39	0.984
	4	0.013	0	0			0
	N	39	39	40	0.987	1.000	32
Gpdh	1	1.000	1.000	1.000	0.013	0	1.000
	2	0	0	0	39	39	0
Fum	1	0	0	0	1.000	1.000	0
	2	0.985	1.000	1.000	0	0	0.984
	3	0.015	0	0	0	0	0.016
	4	0	0	0	37	36	0
Me	1	0	0	0	0	0	0.016
	2	0	0	0	1.000	1.000	0
	3	1.000	1.000	1.000	39	37	0.984
Mpi	1	0	0	0	0.974	0.987	0
	2	1.000	1.000	1.000	0.013	0.013	0.984
	3	0	0	0	0	0	0.016
	4	0	0	0	38	39	0
	N	40	40	40	0.013	0	32
Hbdh	1	0.013	0	0	0.026	0	0
	2	0	0.013	0	0.897	0.949	0
	3	0.925	0.863	0.913	0	0	0.797
	4	0	0	0	0.051	0.026	0
	5	0.013	0.075	0.038	0	0	0.094
	6	0	0	0	0.013	0.026	0.016
	7	0.050	0.050	0.050	39	39	0.094
Acon	1	0	0	0	1.000	0.987	0
	2	0.987	1.000	1.000	0	0.013	1.000
	3	0.013	0	0	39	39	0
Apk	1	0.013	0	0	1.000	0.987	0
	2	0.988	1.000	1.000	39	39	1.000

Thymelicus lineola

loci	alleles	Echterna-cherbrück	Weinsheim	Ourtal	Schönecken	Nieder-anven	Out-group
Idh1	1	0	0	0	0	0	0
	2	0.987	1.000	1.000	1.000	1.000	1.000
	3	0.013	0	0	0	0	0
Idh2	1	0.013	0.043	0.012	0.064	0	0.154
	2	0.987	0.957	0.988	0.936	1.000	0.846
	3	0	0	0	0	0	0
Mdh1	1	0.988	1.000	0.988	0.987	1.000	1.000
	2	0	0	0.012	0.013	0	0
	3	0.013	0	0	0	0	0
Mdh2	1	0.013	0.051	0.012	0	0	0
	2	0.987	0.949	0.965	1.000	1.000	1.000
	3	0	0	0.023	0	0	0
	4	0	0	0	0	0	0
6pg	1	0.025	0	0	0	0	0
	2	0.900	0.949	1.000	0.910	1.000	0.738
	3	0.075	0.051	0	0.090	0	0.262
	4	0	0	0	0	0	0
G6pdh	1	0	0	0	0	0	0.079
	2	0.987	0.919	0.988	1.000	1.000	0.632
	3	0.013	0.081	0.012	0	0	0.289
Pgi	1	0	0	0	0	0	0
	2	0.025	0.013	0	0.013	0	0.150
	3	0.450	0.615	0.605	0.628	0.625	0.775
	4	0.450	0.346	0.337	0.346	0.250	0.075
	5	0.025	0.026	0.035	0.013	0	0
	6	0.050	0	0.023	0	0.125	0
Pgm	1	0.038	0	0.023	0.013	0.025	0
	2	0.026	0.038	0.058	0	0.075	0.095
	3	0.910	0.872	0.872	0.962	0.850	0.581
	4	0	0	0	0	0.025	0.135
	5	0.026	0.090	0.047	0.026	0.025	0.176
	6	0	0	0	0	0	0.014
Pep	1	0.038	0.038	0.023	0	0.025	0.087
	2	0.913	0.910	0.919	0.974	0.975	0.913
	3	0.050	0.051	0.058	0.026	0	0
Aat1	1	0.025	0.026	0.035	0.013	0.026	0
	2	0.887	0.910	0.942	0.908	0.947	0.988
	3	0.087	0.064	0.023	0.079	0.026	0.013
	4	0	0	0	0	0	0
Aat2	1	0	0.028	0.012	0	0	0.026
	2	0	0	0	0	0	0
	3	0.825	0.889	0.866	0.763	0.675	0.949
	4	0.013	0	0	0	0	0
	5	0.162	0.083	0.122	0.237	0.325	0.026
	6	0	0	0	0	0	0
Gpdh	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	1.000	1.000	1.000	1.000	1.000	0.987
	4	0	0	0	0	0	0.013
Fum	1	1.000	0.974	1.000	1.000	1.000	0.975
	2	0	0.026	0	0	0	0.025
	3	0	0	0	0	0	0
Me	1	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0

loci	alleles	Echterna-cherbrück	Weinsheim	Ourtal	Schönecken	Nieder-anven	Out-group
Mpi	1	0.013	0.013	0	0.013	0	0
	2	0.975	0.923	0.907	0.923	0.900	0.988
	3	0.013	0.013	0	0.026	0.075	0.013
	4	0	0	0	0	0	0
	5	0	0.051	0.093	0.038	0.025	0
Hbdh	1	0.013	0	0	0	0	0
	2	0	0	0.012	0	0	0.013
	3	0.988	1.000	0.965	0.974	1.000	0.988
	4	0	0	0.023	0.026	0	0
	5	0	0	0	0	0	0
Acon	1	0.013	0.013	0	0	0.075	0
	2	0.974	0.974	0.988	1.000	0.925	1.000
	3	0.013	0.013	0.012	0	0	0
Apk	1	0	0.038	0.024	0	0	0.013
	2	1.000	0.962	0.976	1.000	1.000	0.988
	N	40	39	42	39	20	40

loci	alleles	Römers-köpfchen	Wasser-liesch	Eider-berg	Mimbach	Bettingen	Niedergailbach
Idh1	1	0	0	0.012	0	0	0
	2	1.000	0.977	0.988	1.000	1.000	0.985
	3	0	0.023	0	0	0	0.015
Idh2	1	0.025	0.042	0.037	0	0.026	0.031
	2	0.975	0.958	0.963	1.000	0.974	0.969
	3	0	0	0	0	0	0
Mdh1	1	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
Mdh2	1	0.013	0.021	0.012	0	0	0
	2	0.962	0.958	0.976	0.988	1.000	0.956
	3	0.025	0.021	0.012	0.013	0	0.029
	4	0	0	0	0	0	0.015
6pg	1	0	0	0	0	0	0
	2	0.925	0.978	0.927	0.925	0.936	0.970
	3	0.075	0.022	0.073	0.075	0.038	0.030
	4	0	0	0	0	0.026	0
	N	40	23	41	40	39	33
G6pdh	1	0	0	0	0	0	0
	2	1.000	0.857	0.886	1.000	0.987	1.000
	3	0	0.143	0.114	0	0.013	0
Pgi	1	0	0.021	0	0	0	0
	2	0	0.063	0.024	0.025	0.026	0.015
	3	0.600	0.521	0.573	0.613	0.641	0.603
	4	0.375	0.396	0.378	0.363	0.282	0.338
	5	0.013	0	0	0	0.051	0.029
	6	0.013	0	0.024	0	0	0.015
Pgm	1	0.013	0	0.037	0.038	0.051	0.015
	2	0.013	0.042	0.061	0.025	0.013	0.044
	3	0.950	0.917	0.854	0.913	0.885	0.912
	4	0.013	0	0.012	0.013	0.026	0
	5	0.013	0.042	0.037	0.013	0.026	0.029
	6	0	0	0	0	0	0
Pep	1	0	0.042	0.013	0.013	0.026	0.045
	2	1.000	0.875	0.875	0.962	0.910	0.894
	3	0	0.083	0.112	0.025	0.064	0.061
Aat1	1	0	0.043	0	0	0	0
	2	0.962	0.913	0.951	0.925	0.947	0.953

loci	alleles	Römers-köpfchen	Wasser-liesch	Eider-berg	Mimbach	Bettingen	Niedergailbach
	3	0.038	0.043	0.049	0.075	0.053	0.047
	4	0	0	0	0	0	0
Aat2	1	0	0	0.013	0	0	0
	2	0	0	0	0	0	0
	3	0.875	0.739	0.700	0.762	0.908	0.758
	4	0	0	0	0	0	0
	5	0.125	0.261	0.287	0.237	0.092	0.242
	6	0	0	0	0	0	0
Gpdh	1	0.038	0	0	0	0.013	0
	2	0	0	0	0	0	0
	3	0.962	1.000	1.000	1.000	0.987	1.000
	4	0	0	0	0	0	0
Fum	1	1.000	1.000	0.986	0.987	1.000	1.000
	2	0	0	0.014	0.013	0	0
	3	0	0	0	0	0	0
Me	1	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0
Mpi	1	0	0	0.012	0	0	0
	2	0.975	1.000	0.915	0.962	1.000	0.956
	3	0	0	0.037	0	0	0.015
	4	0	0	0.012	0	0	0
	5	0.025	0	0.024	0.038	0	0.029
	N	40	23	41	39	39	34
Hbdh	1	0	0	0	0	0	0
	2	0	0.021	0	0	0	0
	3	1.000	0.979	1.000	0.988	0.974	1.000
	4	0	0	0	0	0.026	0
	5	0	0	0	0.013	0	0
Acon	1	0	0	0.012	0	0	0
	2	1.000	0.978	0.951	1.000	1.000	0.939
	3	0	0.022	0.037	0	0	0.061
Apk	1	0.013	0	0	0	0	0
	2	0.988	1.000	1.000	1.000	1.000	1.000
	N	40	24	41	40	39	34

Thymelicus acteon

loci	alleles	Wasser-liesch	Niedergail-bach	Römers-köpfchen	Bettingen	Echter-nacherbrück	Ourtal	Monte-nach	Nieder-anven
Idh1	1	0	0	0	0	0	0	0	0
	2	0.571	0.675	0.775	0.861	0.842	0.893	0.947	0.543
	3	0.333	0	0.025	0.056	0	0.107	0.053	0
	4	0.095	0.325	0.200	0.083	0.158	0	0	0.457
Idh2	1	0.023	0.050	0.025	0	0.026	0.063	0.026	0.034
	2	0.977	0.925	0.975	1.000	0.974	0.938	0.974	0.966
	3	0	0.025	0	0	0	0	0	0
Mdh1	1	1.000	0.950	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0.050	0	0	0	0	0	0
Mdh2	1	0	0	0	0	0	0	0	0
	2	0.977	0.800	0.950	1.000	0.895	1.000	0.947	0.897
	3	0.023	0.175	0.050	0	0.105	0	0.053	0.103
	4	0	0.025	0	0	0	0	0	0
6pg	1	0.977	1.000	0.975	1.000	1.000	0.969	0.974	0.983
	2	0.023	0	0.025	0	0	0.031	0.026	0.017
G6pdh	1	0.050	0	0	0	0	0	0.053	0
	2	0.950	1.000	0.974	1.000	0.974	0.969	0.895	0.983

loci	alleles	Wasser-liesch	Niedergail-bach	Römers-köpfchen	Bettingen	Echter-nacherbrück	Ourtal	Monte-nach	Nieder-anven
	3	0	0	0.026	0	0.026	0.031	0.053	0.017
	4	0	0	0	0	0	0	0	0
Pgi	1	0	0	0	0	0	0	0.105	0
	2	0.273	0.450	0.200	0.100	0.289	0.344	0.447	0.375
	3	0.568	0.500	0.625	0.775	0.579	0.625	0.368	0.446
	4	0.023	0.025	0	0	0	0	0	0.018
	5	0.136	0.025	0.175	0.125	0.132	0.031	0.079	0.161
Pgm	1	0.023	0	0	0	0	0	0	0
	2	0.841	0.900	1.000	0.925	0.895	1.000	0.895	0.845
	3	0.136	0.100	0	0.075	0.105	0	0.105	0.155
Pep	1	0.045	0.125	0	0.075	0.079	0	0.105	0.036
	2	0.932	0.825	1.000	0.925	0.921	0.750	0.868	0.875
	3	0.023	0.050	0	0	0	0.250	0.026	0.089
Aat1	1	1.000	1.000	0.975	1.000	0.947	1.000	1.000	1.000
	2	0	0	0.025	0	0.053	0	0	0
Aat2	1	0	0	0	0	0	0	0.158	0
	2	1.000	1.000	1.000	1.000	0.947	1.000	0.816	0.983
	3	0	0	0	0	0.053	0	0.026	0.017
Gpdh	1	0	0	0.025	0	0	0	0.053	0.052
	2	1.000	1.000	0.975	1.000	1.000	1.000	0.947	0.948
Fum	1	0	0	0	0.025	0.028	0	0	0
	2	1.000	1.000	1.000	0.975	0.972	1.000	0.944	1.000
	3	0	0	0	0	0	0	0.056	0
Me	1	0.705	0.900	0.800	0.778	0.974	0.531	0.688	0.786
	2	0.295	0.100	0.200	0.222	0.026	0.469	0.313	0.214
Mpi	1	0.023	0	0	0	0	0.063	0	0
	2	0.727	0.900	0.825	0.725	0.789	0.344	0.737	0.690
	3	0.227	0.100	0.175	0.275	0.211	0.469	0.237	0.293
	4	0.023	0	0	0	0	0.125	0.026	0.017
Hbdh	1	0.136	0.075	0.028	0.100	0.053	0.188	0.237	0.034
	2	0.864	0.925	0.972	0.900	0.947	0.813	0.737	0.914
	3	0	0	0	0	0	0	0.026	0.052
Acon	1	0	0.050	0	0.025	0	0	0	0
	2	1.000	0.950	1.000	0.975	1.000	0.933	1.000	0.983
	3	0	0	0	0	0	0.067	0	0.017
Apk	1	0	0.050	0	0.025	0	0	0	0
	2	1.000	0.950	1.000	0.975	1.000	1.000	1.000	1.000
	N	22	20	20	20	19	16	19	29

Aricia agestis

loci	alleles	Trier	Perl	Himms-klamm	Igel	Lissen-dorf	Nieder-anven	Römers-köppfchen	Nittel
Pk	1	0	0	0	0.038	0	0	0	0
	2	1.000	0.987	1.000	0.962	1.000	0.988	1.000	0.974
	3	0	0.013	0	0	0	0.013	0	0.026
Idh2	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Apk	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
6pg	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	4	0	0	0	0	0	0	0	0
Acon	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0
Fum	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

loci	alleles	Trier	Perl	Himms-klamm	Igel	Lissen-dorf	Nieder-anven	Römers-köppfchen	Nittel
G6pdh	1	0	0.013	0.075	0.013	0.050	0.053	0.013	0.013
	2	0.962	0.885	0.837	0.782	0.625	0.921	0.829	0.987
	3	0.038	0.077	0.063	0.205	0.313	0.026	0.092	0
	4	0	0.026	0.025	0	0.013	0	0.066	0
Gapdh	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Aat2	1	0	0	0	0.013	0	0	0	0
	2	0.872	0.897	0.750	0.872	0.913	0.895	0.846	0.850
	3	0.128	0.103	0.250	0.115	0.087	0.105	0.154	0.150
	4	0	0	0	0	0	0	0	0
Gpdh	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgi	1	0.063	0.039	0.038	0.026	0.175	0.132	0.013	0.064
	2	0.162	0.066	0.218	0.115	0.200	0.092	0.218	0.179
	3	0.387	0.539	0.410	0.372	0.350	0.382	0.462	0.462
	4	0.287	0.316	0.205	0.410	0.200	0.316	0.244	0.179
	5	0.100	0.039	0.128	0.077	0.075	0.079	0.064	0.115
Hbdh	1	0.013	0.014	0	0.014	0.044	0.039	0.026	0
	2	0.066	0.129	0.081	0.135	0.338	0.184	0.171	0.030
	3	0.895	0.814	0.905	0.797	0.588	0.776	0.776	0.924
	4	0.026	0.043	0.014	0.054	0.029	0	0.026	0.045
Idh1	1	1.000	1.000	1.000	1.000	1.000	1.000	0.962	1.000
	2	0	0	0	0	0	0	0.038	0
	3	0	0	0	0	0	0	0	0
Mdh1	1	0.025	0	0.013	0	0	0.108	0	0
	2	0.913	0.974	0.962	0.988	0.988	0.892	0.975	1.000
	3	0.063	0.026	0.025	0.013	0.013	0	0.025	0
Mdh2	1	0	0	0	0.013	0	0	0	0.013
	2	0.962	0.975	0.950	0.988	0.950	0.975	0.988	0.988
	3	0.038	0.025	0.050	0	0.050	0.025	0.013	0
Me	1	0	0.029	0	0	0	0	0	0
	2	0.051	0	0	0.026	0	0	0	0.029
	3	0.564	0.691	0.466	0.500	0.974	0.515	0.500	0.429
	4	0.385	0.279	0.534	0.474	0.026	0.485	0.500	0.543
Pep	1	0.122	0.054	0.175	0.066	0	0.050	0.050	0.125
	2	0.878	0.932	0.800	0.934	1.000	0.938	0.938	0.875
	3	0	0.014	0.025	0	0	0.013	0.013	0
Pgm	1	0	0.038	0.038	0.013	0.013	0	0.013	0.013
	2	0.063	0.077	0.038	0.141	0.087	0.013	0.224	0.050
	3	0.712	0.628	0.587	0.590	0.575	0.737	0.566	0.700
	4	0.162	0.192	0.250	0.141	0.275	0.145	0.158	0.162
	5	0.025	0.051	0.063	0.103	0.050	0.026	0.039	0.063
	6	0	0.013	0.025	0	0	0	0	0.013
	7	0.038	0	0	0.013	0	0.079	0	0
	N	40	39	40	39	40	38	38	40

Cupido minimus:

loci	alleles	Nieder-gailbach	Weinsheim	Montenach	Wasser-liesch	Eider-berg	Mimbach	Hammels-berg
Aat1	1	0	0	0.013	0.013	0	0	0
	2	0.981	1.000	0.925	0.962	0.974	0.950	0.961
	3	0.019	0	0.063	0.025	0.026	0.050	0.039
Pk	1	0	0.014	0	0	0	0	0
	2	1.000	0.986	0.988	1.000	1.000	1.000	0.987
	3	0	0	0.013	0	0	0	0.013
	N	26	37	40	40	39	40	38
Apk	1	1.000	1.000	0.974	1.000	1.000	1.000	0.987
	2	0	0	0.026	0	0	0	0.013
6pg	1	0.288	0.224	0.686	0.825	0.697	0.375	0.671
	2	0.712	0.776	0.314	0.175	0.303	0.625	0.329
G6pdh	1	0.904	0.934	0.975	0.975	0.949	0.962	0.987
	2	0.096	0.066	0.025	0.025	0.051	0.038	0.013
	3	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0
Gpdh	1	0	0	0.013	0	0	0	0.026
	2	1.000	0.974	0.950	1.000	1.000	0.988	0.974
	3	0	0.026	0.038	0	0	0.013	0
Pgi	1	0	0.013	0	0	0	0	0
	2	0.038	0.026	0.013	0.038	0.013	0.075	0.013
	3	0.500	0.526	0.538	0.363	0.474	0.500	0.605
	4	0.327	0.303	0.256	0.363	0.342	0.300	0.197
	5	0.096	0.132	0.141	0.237	0.132	0.112	0.132
	6	0.038	0	0.051	0	0.039	0.013	0.053
Idh1	1	1.000	0.987	0.988	1.000	1.000	1.000	1.000
	2	0	0.013	0	0	0	0	0
	3	0	0	0.013	0	0	0	0
	N	26	38	40	40	39	40	38
Idh2	1	0.981	0.947	0.897	1.000	0.885	0.962	0.921
	2	0.019	0.053	0.103	0	0.115	0.038	0.079
Mdh1	1	0.019	0	0.025	0	0	0.038	0.105
	2	0.981	1.000	0.975	1.000	0.974	0.962	0.895
	3	0	0	0	0	0.026	0	0
Mdh2	1	1.000	0.987	0.974	0.988	0.987	0.988	0.934
	2	0	0.013	0.026	0.013	0.013	0.013	0.066
Me	1	0.038	0	0.013	0	0	0	0
	2	0.962	0.934	0.938	1.000	1.000	1.000	1.000
	3	0	0.066	0.050	0	0	0	0
Pep	1	0.038	0.081	0	0.150	0.141	0.063	0.092
	2	0.885	0.703	0.903	0.700	0.667	0.800	0.789
	3	0.077	0.216	0.097	0.150	0.192	0.138	0.118
Pgm	1	0.021	0.041	0.064	0	0.051	0.013	0.039
	2	0.146	0.068	0.205	0.262	0.167	0.282	0.184
	3	0.458	0.392	0.397	0.262	0.385	0.615	0.474
	4	0.292	0.324	0.282	0.450	0.308	0.051	0.184
	5	0.083	0.176	0.051	0.025	0.090	0.038	0.118
	N	24	37	39	40	39	39	38

Lebenslauf

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1998 – 2000	Hochschule Zittau-Görlitz, Studiengang für Ökologie und Umweltschutz
2000 – 2003	Universität Lüneburg, Studiengang der Umweltwissenschaften (Schwerpunkte: Ökologie und Politikwissenschaften)
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