Population Genetics of Butterflies

Gabriel Nève

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Institut Méditerranéen d'Ecologie et de Paléoécologie
UMR CNRS 6116
Case 36
Université de Provence
3 Place Victor Hugo
F-13331 Marseille Cedex 3
France
Email : gabriel.neve@univ-provence.fr

Nève, G. 2009, in press. Population Genetics of Butterflies.
***In* :**
The Ecology of Butterflies in Europe,
J. Settele, T.G. Shreeve, R.L.H. Dennis & H. Van Dyck (Eds)
Cambridge University Press, Cambridge.

Final version, 25 March 2008, updated 19 December 2008

Introduction

In a world where natural habitats are fast disappearing and where the landscape is intensively managed, even in nature reserves, it is important to understand the consequences of management and large-scale processes on populations. In this respect, studies of butterflies have been seminal in understanding how landscape structure affects populations (Ehrlich & Hanski 2004; Chapters D1 & D2). From a genetic perspective, the population structure of a given species in a particular landscape depends on a series of parameters : population size in each patch of habitat, movements between these patches, and the level of immigration and emigration to and from the system, together with their respective points of origin or destination. The two key factors determining the population structure of butterfly populations are the spatial distribution of their habitats and the ability of each species to disperse through the different components of the habitat matrix in the landscape. Different species may respond differently from the same change in habitat structure, depending on their movement ability and their habitat choice mainly, but not exclusively, determined by their choice of host plants.

When examining populations, it is important to define the scale at which processes occur. For example, post-glacial dispersal does not occur at the same spatial and temporal scales as emigration between habitat patches within a metapopulation. Depending on the scale, different population structures emerge. Population geneticists interested in spatial structure may ask different questions, which may be classified according to the scale at which they are tackled. These are: (1) Local scale - What are the causes of within population variation? Not two butterflies are exactly alike and how is this variation maintained? (2) Landscape scale - Are populations of a given species distinct entities within a landscape, or do they form a single genetic unit ? If populations are different within the landscape, what could be the causes of such differences? (3) Regional scale - To what extent do populations from different landscapes (i. e. river systems or mountain ranges) interact within a given region? (4)

Continental scale - Most species show differences of phenotypes between different regions. What are the causes of such differences?

At the local scale, population genetics theory recognises four parameters to explain the local makeup of any given population: mutation, genetic drift, migration and selection. Mutation is generally regarded as of minor importance in natural populations over short time scales. Genetic drift is the result of random changes of allelic frequencies due to the small size of an isolated population. Ultimately, drift may lead to loss or fixation of alleles. Migration involves movement of individuals originating in one population to another where reproduction occurs. This results in the immigration or emigrations of individuals to or from the population of interest. If different genotypes in a given population reproduce at different rates, then selection will be operating. This can be a major factor in shaping the population structure of organisms. The effect of selection or drift may be counterbalanced by migration processes but if the population is small and immigration is not occurring, genetic drift is likely to be an important factor. Thus the ability of butterflies to disperse is a key factor in shaping population structure. The individuals of some species rarely move more than a few hundred meters from their natal location, (e.g., *Cupido minimus*; Baguette et al. 1999 and *Plebejus argus*; Lewis et al. 1997), whereas the individuals of other species may move hundreds or thousands of kilometres (e.g., *Colias crocea*, *Cynthia cardui* and *Aglais urticae;* Roer 1968).

From behavioural and ecological studies, species have then been ranked according to their apparent dispersal ability (e.g. Thomas, 1984). Relationships between observed dispersal behaviour and gene flow are difficult to establish, as rare emigration events may have profound genetical consequences. However, the occasional foundation of a population far away from previously occupied patches may give some indication on the effective dispersal pattern. For example, intensive Mark-Release-Recapture (MRR) studies on *Proclossiana eunomia* gave the longest movement as 4 km, but colonisation movement on the same species was observed up to 6 km from established populations (Nève et al. 1996). Due to the difficulty of directly assessing long distance dispersal, other approaches are necessary and genetics may help in understanding current and past links between populations. For many species, even if they seem sedentary according to MRR studies, genetic approaches have demonstrated that population may be linked by migration. In the American checkerspot *Euphydryas anicia*, movement studies gave mean movement of 75 m for males, with a maximum of 1 km whereas genetic studies, using 9 allozyme loci, showed that the populations 2 to 58 km apart within the studied mountain peak system did not differ from each other (Cullenward et al. 1979). As a consequence care is needed in extrapolating observed movement patterns to genetic structuring.although both approaches provide complementary insights. The key review of Ehrlich & Raven (1969) demonstrated that when butterflies cannot move between populations, population differentiation occurs.

Genetics within populations

Population genetics aims to understand how populations change in genetic make-up through space and time. Ever since butterflies were scientifically described, it has been recognised that there is within-species variation. For example, wing patterns of *Parnassius apollo* vary greatly among mountain ranges, leading to the description of

numerous subspecies (e.g. Capdeville, 1978). Caution must be applied when looking at subspecies recognised only on the basis of morphological characters : in the Australian butterfly *Ogyris amaryllis* , the different subspecies were found to be less relevant than host plant choice in the partitionning of the among populations genetic variability (Schmidt & Hughes 2006). Individual aberrations, more or less frequent, were often formally named (e.g. Courvoisier 1907; review in Russwurm 1978), and their genetic basis have sometimes been described (Robinson 1990; Harmer 2000). How are these forms first described by ardent collectors related to the population structure of these butterflies ? The firsts to give a biological interpretation of individual variation were Ford & Ford (1930). They showed that, in *Euphydryas aurinia*, the individual variation was dependent on local population trends: during phases of population increase, phenotypic variation increased and included a series of aberrant individuals, whereas in periods of population stasis, the individuals were much closer to a uniform phenotype. This process was interpreted as an increase of genetic variability due to a decrease of selection during population increase phases. Thus, selection seems to play a key role in the variability of individuals within populations. The other key role is played by genetic drift, especially in small populations. Drift has two major impacts on populations : it is one of the main factors differentiating populations among which there is no gene flow, and through an increase in homozygosity of individuals it may have a deleterious effect on individual fitness. This was shown to be the case in at least two European butterflies of which populations had undergone bottlenecks : *Melitaea cinxia* and *Coenonympha hero* (see below).

Genetic differentiation among populations

The second level of variation is what happens among populations. For mountain species, numerous subspecies have often been described, and their distribution corresponds roughly to the distribution of mountain ranges (e.g. *Parnassius apollo*; Glassl 2005). In Europe, many species have a wide range and show wing pattern variation between northern and southern populations, such as the Mediterranean and northern subspecies of *Pararge aegeria* (Sbordoni & Foresterio 1985, Brakefield & Shreeve 1992), or variations within France of *Melanargia galathea* (Descimon & Renon 1975, Mérit 2000). These species are classically described as sedentary, moving at the most a few kilometres out of their habitats. For migratory species, such as *Aglais urticae* or *Vanessa cardui*, hardly any within Europe variation can be phenotypically recognised, apart from some island forms (e.g. *A. urticae ishnusa* of Corsica and Sardinia). This contrast of migratory habits corresponds to the ecological classification of butterflies into erratic or migrant species, and sedentary species. The latter have local populations, which may persist year after year. By contrast, migratory species usually occur in a wide range of habitat, but their presence at any given locality is more difficult to predict. Thomas (1984) stated that about 85% of British Butterfly species have “closed” populations, i.e. have viable colonies in distinct habitat patches, whereas the remaining 15 % have open or migratory populations. Long-term studies on the distribution of these butterflies have shown that species which have “closed” populations may disperse out of their habitat patches, as in the case of *Hesperia comma*, which recolonized many habitat patches from remnant populations (Davies et al. 2005). This questions the relationship between ecological data, either from population survival data or from MRR and the genetic make-up of population in a spatial context. By essence, dispersal events are rare and difficult to

record; this is a major drawback to comparative data among species (Bennetts et al. 2001). The tools of population genetics may be used in this context to assess the levels of gene flow among populations.

From a genetics point of view, a population is a group of individuals which share a common gene pool (Dobzhansky, 1950) and populations will be different if they do not share a common gene pool. Such differences may be quantified using genetical and statistical techniques. From a statistical point of view, two populations are different from each other if their allele frequencies, as estimated from the samples, are statistically different.

Without the exchange of individuals populations may become differentiated. In cases of complete isolation, each population has its own history of genetic drift and/or selection and over time the populations become more and more differentiated. (Box 1). Movements among populations do not need to be abundant to counteract the effect of genetic drift; an exchange of only one individual per generation is sufficient to avoid population differentiation (Hartl & Clark 1989). Obviously, such a low movement rate is difficult to detect in the field by MRR studies. Furthermore, it is not possible to infer the probabilities of long distance dispersal from the analysis of within-patch short-distance dispersal, as such movement follow different ecological clues. Usually an individual engaged in dispersal behaviour outside its preferred habitat flies higher and quicker (Baguette et al. 1998). Such movements are hard to detect in the field by direct observation. Two kinds of data may be useful in this respect. Firstly, ecological data on colonisation gives evidence that a movement from an occupied to an unoccupied patch has occurred. Colonisation of empty patches is a key component of the metapopulation dynamics of many butterfly species (see Chapter C1). Secondly, genetic data may tell how different populations are from each other. Generally, the more the population are differentiated, the less individuals they have exchange, directly or indirectly.

Genetic indices of population differentiation may be used to infer the level of migration of individuals between populations (Box 1). As such, the relationships between the genetic differentiation of populations and their geographical distances may be compared between areas within a species, or between species. Using this approach, Britten et al. (1995) showed that the isolation by distance in *Euphydryas editha* populations was much stronger in the Rocky Mountains than in the Great Basin, resulting from stronger barriers to dispersal in mountain areas compared to the plains.

Isolation by distance

In a particular species, isolation by distance (IBD) may be observed or not at the same scale, depending on geographic area. In *Parnassius apollo*, Descimon et al. (2001) showed that IBD was highly significant in the high Alps, but that populations from the southern Alps do not present such a pattern. This is because at some point in a recent past, the populations from the southern Alps were linked with each other in a single neighbourhood, and that recent barriers between these populations have not yet lead to isolation by distance. This is due to the large size of populations, and occasional migrations between them. By contrast, populations from the high Alps are more differentiated, due to the individual history of each of these populations, and

post glacial colonization occurring in a stepping-stone fashion, leading to a greater IBD (Box 2).

The spatial structure of butterfly populations depends on where individuals of each sex have come from when they mate, and where females lay eggs. As the adult stage is usually the only one when long distance dispersal is possible, population structure is strongly related to dispersal in the adult stage. In a habitat patch network where individuals all have the same movement potential, if there is a negative relationship between the distances from emergence to reproduction sites and their frequencies, a pattern of isolation by distance emerges (Wright 1943, Epperson, 2003). When a large number of populations have been sampled, it is possible to infer the spatial structure of the population from the genetic make-up of the individual populations and their geographical location (Box 2). Spatial statistics give information on how populations are alike to each other depending on their location. Because most adult butterflies move more or less freely within their natal habitat patch, population structure is generally studied at the between-population level. The isolation by distance model is only applicable if movements between neighbouring patches are more frequent than between patches further apart, as would be expected in sedentary species. Indeed, many studies were started suspecting that movement out of the natal patch would be rare, as very few individuals were ever sighted outside the preferred habitat (e.g. *Proclossiana eunomia, Boloria aquilonaris, Plebejus argus*). At the broader scale, butterflies which are known to move a lot raise interesting questions. *Aglais urticae* has a migratory habit (*e.g*. Roer 1968), so to what extent do individuals actually move? This question was recently answered using a combination of techniques on a series of samples coming from the whole of Eurasia. In the species with such wide distribution, the population structure would be expected to occur only at very large scale. A study of 9 populations from the Netherlands, Belgium and south France showed that these populations were hardly differentiated from each other (G_{ST} =0.03) and had a high heterozygosity (mean expected heterozygosity=0.248, Vandewoestijne et al. 1999), without any isolation by distance effect. As local density of this species is usually low, a high heterozygosity can only be maintained if individuals disperse over large distances. A phylogeography study, based on COI gene and the control region of the mitochondrial DNA of this species, showed that from Europe to Japan, there is a high genetic diversity with wide distribution of both common and rare haplotypes (Vandewoestijne et al. 2004). This corroborates high gene flow, and hence the strong dispersal power of this species. In *Maniola jurtina*, local densities are generally high, and the global fixation indexes (F_{ST}) are in the range of 0.015 to 0.065, without any isolation by distance effect either a the local (Birmingham, Islaes of Scilly) or at the continental scales (Europe) (Table 1) ; this implies frequent individual migration between populations of this species.

Porter and Geiger (1995) used the genetic approach to assess movements in the erratic species *Pieris napi*. In their study of 38 populations distributed throughout Europe, the isolation by distance model followed the relationship F_{ST} =0.03-0.45/(4x+1), which for F_{ST} =0 gives a estimated value of x=3.5 km, which is thus the estimated radius of the neighbourhood area for this species, and the estimated F_{ST} for the whole continent was 0.0887 (SE=0.0076), giving estimated numbers of migrants between populations at 2.6 (CI : 1.6 – 5.5). Such values suggest that there is a significant gene flow across the whole continent, which is not surprising, given the erratic behaviour

of individuals and the wide distribution of their reproductive habitats. However, the populations of the Nordic montane subspecies *Pieris napi adalwinda* and the lowland subspecies (*P. napi napi*) are ecologically and genetically separated (Espeland et al. 2007).

The situation for many species of European butterflies is very different from this. Most occur in patchily distributed habitats, from which dispersal is a rare event. If a species is distributed in a series of discrete patches with metapopulation dynamics (see chapter C1), the total effective size of its population will be much smaller than if each population were long lived. Even a low extinction probability will have a dramatic effect on the total effective population size (Whitlock, 2003).

Selection

Population differentiation may be caused by substantially different selection pressures occurring in different habitat patches. The identification of the cause of the selection pressure is always difficult (Manly 1985, Endler 1986). In butterflies the identified causes of selection concern primarily temperature, host plant availability and there is evidence that habitat structure may also be a selective factor. E.B. Ford studied *Maniola jurtina* in different habitat structures in the Isles of Scilly. Populations from small islands (<16 ha) were either unimodal with 0 or 2 wing spots, or bimodal at 0 and 2 spots, whereas the populations from the bigger islands were more evenly distributed. Evidence of constancy of spot pattern distribution in individual populations, even after a bottleneck, strongly suggested that spot pattern was under selection pressure rather than the result of random genetic drift in small island populations. Spot pattern frequencies changed after habitat changes, such as the removing of cattle grazing, rather than with population bottlenecks (studies summarized in Ford 1975, Brakefield 1984 & 1990). More recently, other evidence was found for selection in this species. Different PGM alleles were favoured in different areas of its English distribution (Goulson 1993). Indirectly, this explains why the relationship between genetic similarity and geographic distance between populations is steeper in Britain than for the whole of Europe (Thomson 1987). As several of the loci studied by the latter author are probably under selection, similarity between regions under equivalent ecological conditions is expected to occur, thus counterbalancing the general isolation by distance effect, which has not been found for this species in more recent studies at the regional or continent scales (Goulson 1993; Schmitt et al. 2005b; Grill 2007).

In the American species *Euphydryas editha*, the natural host plant at Schneider's Meadow (Nevada, USA) used to be the native plant *Collinsia parviflora*. Over a decade, the European plant *Plantago lanceolata* spread through the habitat. Singer et al. (1993) showed conclusively that the host plant choice switched from the native species to the introduced one. Associated with this host plant switch will be changes of selection regimes related to host plant quality and phenology.

For several American *Colias* species, temperature is a major factor affecting the polymorphism of the enzyme phosphoglucoisomerase (PGI). Watt et al. (1983) showed that the alleles present in different individuals were related to the temperature at which they fly, according to the optimal temperature of the PGI enzyme, as checked *in vitro*. At a broad scale, populations of the Alpine *Colias meadii* have

different PGI polymorphisms depending on the habitat they occupy (above tree line tundra *vs.* below tree line steppe), irrespective of the distance between these populations (Watt et al. 2003). The populations of these different habitats may be either isolated or exchange many individuals each year, including between the two habitat types. Nevertheless there is consistent 10 to 20 % difference in PGI frequencies between the two habitat types; such a pattern may only be explained through continuous strong selection at the PGI locus. This key enzyme of glucose metabolism affects flight capacity. In *Melitaea cinxia*, the dispersal ability of the individual bearing the different PGI alleles were significantly different, and that this in turn affected population growth and dispersal pattern (Haag et al. 2005; Hanski & Saccheri 2006). Using single nucleotide polymorphisms, Saastamoinen and Hanski (2008) showed that the two most common alleles for PGI in the Åland islands (Finland) populations of *M. cinxia* were linked with different temperature preferenda; the individuals with the PGI-f genotypes flew at lower temperature and laid 32 % larger clutch size than PGI-non-f females because they tend to initiate oviposition during the warmest time of the day when clutched tend to be larger.. As this leads to a strong selection against PGI-non-*f* alleles, the question then remains as to what favours the PGI- non-*f* alleles in the population system.

Behaviour may also be subject to selection: individuals from isolated patches of habitat from which emigration would be extremely unlikely to be successful may be selected against. In the UK, severe isolation of the last remnant populations of both *Maculinea arion* and *Papilio machaon* resulted in decrease of thorax size in recent museum specimens compared to older ones (Dempster 1991). A more thorough study on the effect of isolation on flight ability was conducted in UK populations of the silver-spotter skipper (*Hesperia comma*), which was once widespread in southern and eastern England. It declined to its smallest range in the 1970s and 1980s, because a decrease of grazing rabbit populations, due to myxomatosis, led to a loss of habitat areas. With the recovery of rabbit populations from the beginning of the 1980, the species has recolonised some areas (Thomas & Jones, 1993). Hill et al. (1999) related morphology to colonization and demonstrated that thorax size was bigger in the area where recolonisation had been the quickest (East Sussex), than where it was slower (Surrey). They suggested that selection had operated more strongly against large thorax size and mobility in Surrey where the species had persisted in small (<1ha) isolated refuges, compared to East Sussex where the population had persisted in a large (18 ha) refuge. The stronger flight ability of East Sussex populations resulted in a higher colonisation rate and gene flow, whereas the lower flight ability of Surrey populations resulted in a higher isolation by distance effect.

The identification of selection pressure implicitly asks the question of what populations are. The East Sussex and Surrey populations of *Hesperia comma* had suffered different selection pressures according to the characteristics of the two regions. However, such clear-cut situations are infrequent, as most butterflies exhibit gene flow between habitat patches. The question of the identification of what constitutes a population is central to many problems in ecology, and – as seen above - the genetic makeup in a population is under selection from its environmental conditions. The scale at which selection will affect butterfly populations will depend on gene flow among these populations. *H. comma* displays discrete populations which suffered a bottleneck in the 1970s and 1980s (Hill et al. 1999), and a differential effect of selection could be detected between Surrey and East Sussex. In widely distributed

species with strong flight abilities, such as *Pieris napi*, such a phenomenon does not occur. The case of *Colias meadii* where there is a significant difference of PGI allele frequencies according to altitude (Watt et al. 2003) may be due to individual actively seeking a habitat according to their individual temperature requirements. The question remains open as to how often this may occur in other species. The number of generations per year for *Aglais urticae* may also be under a similar selection pressure, although at a larger scale, as *A. urticae* is trivoltine in central France, mostly bivoltine in England and univoltine in northern Scotland, with local variation according to altitude (Brakefield & Shreeve, 1992b).

Techniques in population genetics

Wing pattern

The first techniques used in population genetics concerned phenotypic variations, using these as surrogates for genetic information. The number and size of spots of several species of Satyrinae often display variable numbers of spots on their wings. The number and size of spots in *Coenonympha tullia* vary with sex (females have more spots than males) and with locality (Turner 1963, Dennis et al. 1984). Spot pattern also varies among localities in *Maniola jurtina* and it was used to study population differentiation (Dowdeswell & Ford 1953; Dowdeswell 1981). It was assumed that the wing spotting was heritable, on the basis that the pattern was consistent among years. However, heritability of this character was only formally demonstrated later, and was found to be sex linked: it was first tentatively estimated at 0.14 in males and 0.63 in females (McWhirter 1969), and later at 0.66 in males and 0.89 in females (Brakefield & van Noordwijk 1985).

Protein electrophoresis

Upon the general availability of protein electrophoresis from the 1960s (Johnson 1971), this technique has been widely used for population genetic studies of butterflies, from the pioneering studies of Handford (1973a & b) to date. Nowadays this technique still remains the most widely used in population genetics studies of butterflies. The main reason for this choice is a combination of relative ease of scoring, and a fairly low price (Wynne et al. 1992). The scoring of the resulting zymograms is usually straightforward (Richardson et al. 1986), and the Mendelian basis of the observed polymorphism may be checked using the known quaternary structure of the given protein, by experimental crosses or by Hardy-Weinberg equilibrium expectations. This technique proved powerful as many species exhibit a high degree of polymorphism. Studies usually focus on 3 to 25 polymorphic loci. Most of the studies on protein electrophoresis assume that allele variation is neutral, or at least that no selection could be detected (Besold et al. 2008). As the proteins of interest have all definite functions, this is unlikely to be true (van Oosterhout et al. 2004) but population differentiation based on protein electrophoresis has been, and still is, widely studied.

Some species (e.g. *Lycaena helle*) or life-stages (e.g. caterpillars) have sometimes proved difficult to be studied by protein electrophoresis, due to toxic compounds (e.g. oxalic acid, phenols) which interfere with enzyme activity. In this case the homogenization procedure should extract or neutralize these compounds. Polyvinylpolypyrrolidone (PVPP) and a few grains of instant coffee have been

mentioned as compounds which may improve enzyme stability by removing phenolic compounds during the grinding and homogenization procedures (Hebert & Beaton 1993). For adult butterflies, I have used the following homogenizing solution : 50 mM Tris-HCl, 0.5 % (v/v) triton X-100 (optional), 15 % (w/v) sucrose, adjusted to pH 7.1 with HCl (Wynne & Brookes 1992). For 4th and 5th instar *Melitaea cinxia* larvae, the following homogenizing solution has been used : 100 ml distilled water, 10 mg NADP, 100 µl β-mercaptoethanol (Saccheri pers. com. ; solution from Richardson et al. 1986).

Molecular techniques

Protein electrophoresis has the major drawback that a common protein migration rate may result from two different alleles, hiding heterogeneity (Johnson, 1977). Recent genetic studies on butterflies often rely on DNA-based molecular techniques. These are rapidly improving tools, and the choice of a method depends primarily on the questions asked, the scale of the study and on the chosen organism. Several good reviews of methods are currently available (*e.g.* Parker et al. 1998, Avise 2004, Behura 2006).

Mitochondrial DNA sequencing

Variation of mt DNA is studied by sequencing one or several genes of the short strand of mt DNA. The most studied parts are the control region (CR), cytochrome oxydase I or II (COI and COII). The region of interest is amplified by PCR and then sequenced for each individual (Avise 2004). Sequences of mitochondrial DNA are usually scored for phylogeography studies aiming at an understanding the pattern of colonization at the continental scale (e.g. Vandewoestijne et al. 2004). Variation in mt DNA sequence has also been used to assess levels of genetic variation in cases where the conservation of frozen specimens would have been difficult. Diversity of populations of *Mycalesis orseis* within forest fragments in Malayan Borneo, assessed by the number of mt haplotypes, was negatively affected by isolation of their habitat patch, but not by population size of patch size (Benedick et al. 2007). The low level of mutation within the mitochondrial DNA allow the study of long term processes: in the North American *Parnassius smintheus*, the local variation of mt DNA haplotypes could be linked with the range expansion and retraction during glacial-interglacial cycles. During warm periods, populations persisted at mountain tops, whereas they expanded during cold spells. As a result, populations from an area within a mountain range have a series of possible refugias during warm periods, and end up being more diverse than those from an area with fewer refugias (DeChaine & Martin 2004).

Randomly Amplified Polymorphic DNA (RAPD)

This technique uses short PCR primers (ca. 10 bp) to amplify DNA fragments. This primer length is short enough to find several annealing sites in the genome by chance alone, but long enough as not to amplify too many fragments. Usually several possible primers are tested, and the ones yielding recognisable and repeatable banding patterns are then selected for the study. The major drawback of this method is that it is not possible to identify from which genome region each band is amplified. Furthermore, the banding pattern is very sensitive to laboratory conditions. Due to these drawbacks,

RAPD results are difficult to replicate. Zakharov et al. (2000) managed to amplify DNA from museum specimens of *Atrophaneura alcinus* and four *Parnassius* species, but these authors did not publish further studies based on RAPD. Vandewoestijne & Baguette (2002) showed that RAPD on 18 polymorphic loci in *Boloria aquilonaris* yielded significant IBD, while isozymes (4 polymorphic loci) on the same populations did not. This draws attention to the fact that the lack of genetic differentiation found with one marker does not necessarily mean that the populations are not differentiated. From a statistical point of view, it is simply that the hypothesis that the populations are not differentiated may not be rejected (Bossart & Prowell 1998).

Amplified fragment length polymorphism (AFLP)

To avoid the drawbacks of RAPD, primer pairs are used to amplify known regions of the nuclear genome and then digested by restriction enzymes. These generate a series of bands, according to the length of the amplified regions. Three primer pairs used on 190 specimens from across the North American range of *Lycaedes melissa* yielded a total of 143 bands ranging in size from 71 to 481 bp (Gompert et al. 2006).

Microsatellites

Given the highly functional nature of enzymes studied by allozyme electrophoresis, and the proven selection which may occur on these loci, a neutral marker is desirable for population studies. Microsatellites are tandem repeats of 1 to 6 bp motifs such as ACACACAC. This marker seemed to be the “Holy Grail” for population geneticists, as microsatellites are non-coding lengths of repetitive DNA. It was thought that these would be neutral and, by providing high levels of polymorphism, could end up as excellent tools for population genetics. Although it has been shown that they may be linked by hitch-hiking to a gene under selection pressure or that they may even be under selection themselves (Estoup & Cornuet 1999), they are still regarded as the first choice of neutral markers (Golstein & Schlötterer 1999). Compared with allozymes, which require fresh or frozen material, microsatellite analysis can be conducted with dry material. This facilitates the study of museum material (Meglécz et al. 1998b, Harper et al. 2006) or the use of non-lethal sampling (Lushai et al. 2000, Keyghobadi et al, 2005). From a practical point of view, each microsatellite locus is specifically amplified by PCR using locus specific primer pairs which recognise the flanking region of each side of the studied loci. The identification of these microsatellite loci with the design of primers is the most time consuming task for the set-up for microsatellite based studies. This has to be done for each new species studied, but workable pairs of loci in one species are typically tested in other congeneric species, with various success. From a series of 17 loci identified for *Papilio zelicaon*, between 5 and 14 loci could be amplified in other *Papilio* species but their polymorphism in these other species still remains to be tested (Zakharov & Hellmann 2007). In the 1990s, many butterfly biologists tried to develop microsatellite methods for butterfly population biology studies. By the end of the decade, it became apparent that the recurrent problems faced by the butterfly geneticists might be linked to the structure of the Lepidoptera genome rather than to the expertise of the involved laboratories (Meglécz & Solignac 1998, Nève & Meglécz 2000, Sunnucks 2000a & b). Many researchers tried to apply microsatellite techniques to butterflies, but gave up because of the low number of usable microsatellite loci. An analysis of the flanking regions of the microsatellites of

Euphydryas aurinia and *Parnassius apollo* showed that many microsatellites loci could be grouped by similar flanking regions. Thus the numbers of microsatellites with unique flanking regions were drastically reduced (Meglécz et al. 2004). Subsequently this was found to be the case for many other Lepidoptera species, and also other insects (Meglécz et al. 2007).

Single Nucleotide Polymorphism

Due to problems in the use of microsatellite loci in Lepidoptera, other markers useful for population genetics markers were desirable. Orsini et al. (2007; 2008) identified a series of single nucleotide polymorphisms (SNP) in *Melitaea cinxia*. Among these two could be identified with known variants of the Phosphoglucoisomerase (PGI) locus, a key enzyme in the glycolysis cycle, which was already known to affect dispersal rate in this species (Haag et al. 2005). As the use of SNP could be done without killing the individuals (a 2 mm diameter part of the hind wing was enough), the PGI genotypes could be studied on individuals which were later followed for their behaviour, with collected data on their flight body temperature, oviposition time and clutch size.

The new partial sequencing of the coding region of the *M. cinxia* genome gave sequence information on over half of the genes of this species (Vera et al. 2008; Ellegren 2008). As the method used a pool of *ca*. 80 individuals (caterpillars, pupae and adults from 8 families), this approach provided unprecedented access to *M. cinxia* genome polymorphism, leading to the identification of numerous SNP and to future detailed quantitative trait loci studies. No doubt the future of butterfly population genetics will increasingly use SNPs in their approach.

Differentiation among butterfly populations

In Europe, a total of 87 studies of spatial aspects of population genetics have been located (Table 1). Of these, the great majority involved allozyme electrophoreses (80 cases), 3 studies involved RAPD and 5 involved microsatellites. Generally the authors give a value of population differentiation, either F_{ST}, or one of its derived estimates (θ, G_{ST}). Each study species has its own ecological needs, and history of postglacial colonisation, from one or several refugia. These species may have widespread populations with frequent movements, as is the case of migratory species such as *Aglais urticae* (Vandewoestijne et al., 1999), whereas others are very sedentary, such as *Plebejus argus* (Brookes et al., 1997).

Most of the values of the global fixation index (F_{ST}) among populations of European butterflies show that generally populations show little genetic differenciation (*sensu* Wright 1978): the median F_{ST} value is 0.053. The lowest F_{ST} value (0.004) is found in *Polyommatus icarus*, a common and widely distributed butterfly which thus shows numerous movements among its populations. The highest value (0.291) is found among isolated populations of the mountain species *E. epiphron*, showing the ancient separation of its populations and the lack of movements between mountain massifs. The 86 studies of spatial population genetics on European butterflies show a general relationship between F_{ST} and the size (log scale) of the study area for all species combined, which is close to significance (Figure 3, t=1.94, 84 df, P=0.055). However, global F_{ST} tends to vary among the five butterfly (sub)families (excluding the four species of Hesperidae ; F=2.88, 4 and 79 df, P=0.03). Probably due to the narrow

habitat choice of many species, butterflies of the families Papilionidae and Nymphalinae tend to have higher F_{ST} than those of the families Lycaenidae, Pieridae and Satyrinae (Figure 8). This is also due to a bias in the studied species, as e.g. many Lycaenidae species have narrow ecological requirements, and probably a low colonization power. Within four of these families (Lycaenidae, Papilionidae, Pieridae, Nymphalinae), there is no trend between the size of a study area and the observed F_{ST} (P>0.3). For Satyrinae, however, the size of the study area, in logarithmic scale, is correlated with the observed F_{ST} (t=3.07, 22 df, P=0.006, Figure 3). Such a trend is probably the consequence of similar open structures of most studied Satyrinae populations, and therefore may be linked with a global isolation by distance process affecting Satyrinae species in a similar way, despite the absence of IBD in *Maniola jurtina*. The choice of study species may also have biased these results. It is noteworthy that only two genetic studies, involving four species, could be found on Hesperidae, despite numerous studies on their ecology and distribution. Within Papilionidae, only isolated populations or mountain species have been studied. The genetic structures of *e.g.* the widespread *Iphiclides podalirius* and *Papilio machaon* have not been worked out. The only study of *P. machaon* has been carried out in Britain where the species is localized and threatened. In Pieridae the bias is the other way, only the widespread species *Pieris napi* has been thoroughly investigated. The diverse Lycaenidae family has been studied both in localised threatened species, such as the *Maculinea* species and in widespread species such as *Polyommatus icarus* and *Aricia agestis*. With the exception of the widespread and migratory *Aglais urticae, studies* on the Nymphalinae have focussed on species with localised populations, often with vulnerable and decreasing distributions. The various studies on Satyrinae, like those of Lycaenidae, have involved both common species such as *Maniola jurtina* and *Coenonympha pamphilus*, and species with very restricted ranges such as *Erebia triaria* and *Coenonympha hero*.

Dispersal ability and population differentiation

Species with low dispersal abilities show larger F_{ST} values. *Parnassius apollo* is known to be very vagile and it is therefore not surprising that population from the same mountain massif are hardly different from one another, with a non-significant isolation-by-distance effect in the southern Alps. However, when all French populations are included, the slope of F_{ST} against distance is -0.54 indicating that areas between mountain ranges act as effective barriers to dispersal (Descimon et al. 2001), even if vagrants sometimes occur there (Lafranchis 2000). In the case of large scale disturbances (such as fires or drought) the local genetic diversity of a population will depend on the scale at which migration and colonization events take place. In the tropical species *Drupadia theda*, populations in areas near to undisturbed habitats tend to be more diverse then more isolated populations (Fauvelot et al. 2006).

When a range of species within a single habitat network are studied, their dispersal abilities may effectively be compared. With a MRR survey, Baguette et al. (2000) showed that *Cupido minimus* had much less dispersal ability than the sympatric *Melanargia galathaea and Aporia craetegi*. Both MRR and genetic approaches showed that *Euphydryas aurinia* is less prone to inter patch movement between patches than *Melitaea phoebe* (Wang et al. 2003 & 2004). Genetic studies of three *Thymelicus* species in Luxembourg and Germany showed that the three species have

very different genetic structures as a result of their different dispersal ranges and habitat requirements. *Thymelicus lineola* displays a high dispersal ability and has broad habitat requirements, resulting in a panmictic genetic structure at the regional scale; *Thimelicus sylvestris* displays a lower dispersal ability in the same habitat matrix, which results in isolation-by-distance effect. The third species, *Thymelicus acteon*, has narrow habitat requirements in combination with a low dispersal ability, resulting in populations being more isolated from each other, as reflected by this species having the highest F_{ST} value of the three species in the same habitat patch network, without any isolation by distance effect (Louy et al. 2007). *T. acteon* has declined in many European countries, and is of conservation concern, while the other two are stable (van Swaay and Warren 1999). Population in southern mountain areas tend to be more variable than low elevation ones. *Pieris bryonae* populations within the Swiss Alps are more isolated from each other than *Pieris napi* populations between south France, Germany and Hungary (Porter & Geiger 1995). Similarly, populations of *Proclossiana eunomia* from the Pyrenees display more differentiation than those from the Ardennes, as the slope of the IBD is -0.91 in the Pyrenees and -0.53 in Ardenne (Fig. 4).

Population differentiation generally occurs when population are isolated from each other in space. In some cases, however, there seem to be isolation through ecological preferences. In *Carterocephalus palaemon*, two morphotypes (*Carterocephalus palaemon palaemon* and *C.p. tolli*), probably originating from distinct glacial refugia, are found in Białowisża primeval forest (NE Poland). These are maintained because of their ecological differentiation both in habitat and phenology, resulting in assortative mating (Ratkiewicz & Jaroszewicz 2006). The two subspecies of *Proclossiana eunomia* at Białowisża (Krzywicki 1967) may show the same phenomenon.

Population isolation

For conservation biology, the consequences of population isolation set challenges. An isolated population may evolve locally according to local conditions, but it may also undergo genetic drift, leading to loss of genetic variability, or to stochastic demographic extinction. If populations are long lived, the total effective size of the population is higher if it is the sum of a series of isolated demes (Whitlock & Barton, 1997). However, in a metapopulation system (see Chapter F3), as local populations result from an equilibrium between extinction and colonisation, there is high gene flow between the populations, and little room for local adaptation to take place; selection will then operate more at the metapopulation scale. Genetic drift and selection are difficult to distinguish in population differentiation. In *Erebia triaria*, the isolated population of Xistral (NW Spain), named *Erebia triaria pargapondalense*, is as different from Cordillera Cantabrica populations (*ca*. 120 km apart) as from Pyrenean populations (720 km away), according to a study using four microsatellite loci and mitochondrial DNA. In this case, the genetic approach confirmed ecological and morphological data (Vila et al. 2005 & 2006).

Population size

Small and isolated populations undergo genetic drift,due to the low numbers of reproducing individuals, and the homozygosity of such populations tends to increase.

Isolated populations also suffer from several ecological effects, which affects their reproduction and hence their long term survival: demographic stochasticity, a low buffering effect due to the small habitat patch size and micro-habitat diversity, behaviour alterations due to low population density or close proximity to habitat boundaries, and often an overall lower habitat quality. These phenomena are often exarcerbated by the position on the edge of the relevant species' range (see Chapter E2). Such situation lead to increased homozygosity in individuals, as observed on westernmost populations of *Coenonympha hero* (Cassel & Tammaru 2003) or *Polyommatus bellargus* (Harper et al. 2007). By contrast phenotypic variation may increase, due to lower canalization in populations with low genetic variation (Debat & David 2001), as seen in peripheral populations of *Polyommatus icarus* (Artemyeva 2005).

In the Åland islands (Finland), the main factors affecting *Melitaea cinxia* population survival are population size, density of neighbouring populations, patch size and cattle grazing (Hanski et al. 1995). A genetic study conducted on individuals caught in 1996 showed that heterozygosity had a significant extra effect on the extinction risk of the 42 genetically studied *M. cinxia* populations: the seven populations which went extinct between 1995 and 1996 had both ecological factors affecting their survival and a lower than average heterozygosity (Saccheri et al. 1998). A low heterozygosity was thus shown to be a significant extra factor affecting population survival (Fig. 5). Further evidence that population heterozygosity affects survival was given by an experiment in which the founder individuals of each populations were either full sibs, generating individuals with an inbreeding coefficient of 0.25, or outbred individuals from parents from two different populations, thus having a zero inbreeding coefficient. Three larval groups from either outbred or inbred individuals were introduced into one of 12 unoccupied habitat patches. Only two of the inbred populations attained adulthood and reproduction, and went extinct by the next year, while four of the six outbred populations survived until the next year (Nieminen et al. 2001). As inbreeding is deleterious, mate choice could avoid inbreeding, but experimental result showed that individual butterflies are unable to recognize sibs from non-sibs for mating (Haikola et al. 2004). As a result, inbreeding is more likely in small than in large populations, and this affects egg hatching rate, larval surviving rate (Haag & de Araú 1994, Haikola et al. 2001), and adult survival rate (Saccheri et al. 1998); furthermore, a second generation of inbreeding, by pairing full sibs, further decreased clutch size (Haikola 2003). A comparison of inbreeding effect conducted on French and Finnish individuals showed that a reduction of hatching rate from full sibs occurred in all cases, but was more pronounced in French individuals than in Finnish individuals. The genetic load thus seems to be less severe in Finnish populations than in French ones. This is probably due to the repeated bottlenecks through which Finnish populations have been through, which have purged them from a number of deleterious recessives (Haikola et al. 2001). Data on low reproductive output in isolated populations of *Parnassius apollo* also indicates that inbreeding was the main factor affecting small populations of this species as well (Witkowski et al. 1997) ; in some cases the reproductive power of an isolated population was enhanced by the introduction of individuals from nearby populations with which exchanges are now unlikely to occur (Nakonieczny et al. 2007).

Coenonympha hero is a species which has already vanished from most of its former European range (van Swaay & Warren 1999). In Sweden, some local populations are

extremely small, with an estimated size of 7 to 15 individuals during the flight period, while others are larger, with estimated population sizes from 51 to 128 individuals. Furthermore, large populations are connected to each other by suitable habitat corridor structures, such as grassy roadbanks. Cassel et al. (2001) collected eggs from the different populations, and placed them in semi-natural conditions on tussocks of *Festuca ovina*. The hatching rate of eggs from small populations was lower, and their death rate and proportion of unfertilized eggs larger, compared to eggs from larger populations (Fig. 6). This phenomenon is most likely to be related to the increased homozygosity of the small populations, due to local inbreeding. Furthermore females from small populations had a higher probability of not being mated, and thus to be effectively infertile. It is unlikely that small populations of this species will remain viable, both for the genetic reasons of increased homozygosity, and for ecological reasons such as a reduced microhabitat variability which will not effectively buffer against environmental variation. Furthermore, Swedish populations of *C. hero* already have a heterozygosity which is much lower (Hobs=0.017) that in the more central populations of Estonia of the Urals (Hobs=0.052; Cassel & Tammaru 2003).

The studies of inbreeding effects *on Melitaea cinxia* and *Coenonympha hero* indicate that inbreeding depression can have a significant effect on small and isolated populations. As reintroduction schemes are being considered for a number of species in parts of their range from which they have disappeared, there is therefore a requirement for genetic diversity to be considered to avoid inbreeding effects.

Host plant range

Populations which use a number of host plants tend to be more varied than ones using a single host plants. In this respect the case of *Euphydryas aurinia* in France is spectacular. In the Atlantic and continental part of the country it feeds on one or two Dipsacacae species: mainly *Succisa pratensis* and sometimes on some *Knautia* species. Sampled populations, up to 700 km apart, show a low F_{ST} value of 0.0648, whereas populations from South France (up to *ca*. 650 km apart) show a F_{ST} value of 0.112. Descimon et al. (2001) concluded that the high F_{ST} obtained from southern populations is due to the large number of food plants used in this part of the range: Dipsacacae (*Cephalaria, Knautia, Scabiosa, Succisa*), Caprifoliacae (*Lonicera*), Valerianacae (*Centrentus*) and Gentianacae (*Gentiana*). Each local population seems to use only one food plant in a given locality (Mazel 1986). In the South of France, a neighbour-joining dendrogram (Fig. 7) shows that populations mainly cluster according to their geographical origin, i.e. according to distance. However some populations show strong difference from this tendency. The population from Sommail is more similar to ones in southwest France, than to others in Languedoc, and this population is the only sampled Languedoc population which feeds on *Succisa pratensis*, like the ones in the southwest, and unlike the others in Languedoc, which feed on *Cephalaria*. Similarly, two populations from the Pyrenees, found feeding on *Succisa*, do not group with the other ones from the same area feeding on *Lonicera*, but with the ones from Languedoc, also feeding on *Succisa*. In a study of 11 populations of *E. aurinia* from south France and north Spain, scored by AFLP markers, larvae of *Euphydryas aurinia* found on *Succisa* or on *Lonicera* at the same site were shown to be as different as two allopatric populations feeding on different hosts are (Singer & Wee 2005). Differences between individuals at the same site, whether on the same or on different hosts, are generally smaller within than between populations. A high level

of differentiation between larvae found on different host plants will be more likely to occur in allopatry than in sympatry, but the latter may be relevant in some cases (Wee 2004, Singer unpubl.) This tends to confirm Descimon's interpretation that *E. aurinia* may be undergoing speciation in South France. However, such a differentiation pattern in not the rule. *Euphydryas editha* caterpillars at Sonora junction (California, USA), were not genetically different whether they came from eggs laid on *Penstemon* or *Castilleja*, thereby discarding any speciation event between the individuals on the two hosts. Furthermore, the selection pressure put forward by the different hosts may affect the variability of the population. Singer and Wee (2005) showed that larvae of *E. editha* developing on *Castilleja* (from eggs naturally laid on this host) had a higher heterozygosity (0.137, SE=0.007) than larvae from *Pedicularis* (0.119, SE=0.007) ($p<0.001$, Mann-Whitney U test) at the same site (T-junction, Ca, USA). As female *E. editha* from this site all accept both hosts to lay their eggs, a process of sympatric speciation may be ruled out. The only cause seems to be that the two hosts induce different mortality rates between egg hatch and time of sampling, thereby indicating different selection pressure on the populations.

Barriers to dispersal

In Germany, populations of *Chazara briseis* have declined. Present populations on igneous and calcarious hills are separated from each other by farmland and urban development. Most individuals of this species stay in their natal patch, with a mean distance between capture events of 80 m (n=191), while about 2% (n=5) of individuals moved more than 1000 m. The question then is: are these occasional long distance movements efficient in maintaining gene flow within the landscape? An analysis of 165 individuals from 9 populations up to ca. 10 km apart gave a mean F_{ST} of 0.022 for the 15 polymorphic loci. Johannesen et al. (1997) suggested that these populations show limited substructure and that the agricultural landscape does not constitute a barrier for this species. Dispersive individuals moving out of their preferred habitat tended to move to a neighbouring habitat patch, leading to an isolation by distance effect, which was indeed detected.

Post-glacial dispersal pattern

Since the last glacial maximum (18,000 yr BP), the climate of Europe has changed dramatically, and this has lead to the colonisation of northern areas from southern refugia (Huntley & Webb 1989). This change in distribution of organisms can have lasting consequences for the genetic make-up of populations. For species with a low dispersal ability, post-glacial colonisation event took place slowly, and usually by stepping stone patterns (see Chapter E2). The main consequence for the genetic diversity is that the centres of origin of this post-glacial northern migration are in southern Europe or Asia. Consequently there is generally a decrease of genetic diversity from the centre of origin to the edge of a species range (Hewitt 1996). In *Polyommatus bellargus*, for example, a study of the mitochondrial control region and of a section of the 12SrRNA gene (totalling 722 bp) showed a much lower variation within the UK (mean pairwise difference between two individuals: 0.000 in non-Dorset populations and 0.295 in Dorset) than in France (mean pairwise difference : 7.42). Such a small variation is unlikely to have remained over a long period. It is concluded that the British populations of *P. bellargus* probably originate from western

European stock during historic time, and that this colonization was subjected to a bottleneck (Harper et al. 2007).

Open questions and conclusion

Generally, populations of butterflies show differentiation according to the geographical distance separating them, but the pattern may differ widely between species within a given landscape. As expected, widely distributed and highly mobile species display less differentiation than more sedentary species. The high fragmentation of habitats decreases gene flow between populations. As most species of European butterflies occur in discrete populations, this effect is of major consequence, and may lead to the quick decline of populations once a critical threshold of habitat connectivity has been reached (With & Crist 1995). However, the type of habitat between patches also has crucial importance in the dispersal ability of individuals. For example, forest habitats are effective barriers to dispersal for open habitat species such as *Erebia medusa* (Schmitt et al. 2000) and *Parnassius smintheus* (Keyghobadi et al. 2005), while the reverse is true for several forest species, such as the moth *Operophtera brumata* (van Dongen 1994) or *Pararge aegeria*, as it seems that individuals from forest areas need to perceive the presence of a forest to move towards it (Merckx et al. 2003). How other habitat structures affect butterfly dispersal remains an open, but critical, question. The response of individuals will also depend on regional habitat structure, as dispersal behaviour is expected to be selected against in very fragmented habitats, while such behaviour will be more common in more continuous habitats (Baguette et al. 2003). As dispersal ability is heritable – it was recently shown to be heritable from mother to female offspring in *Melitaea cinxia* (Saastamoinen 2008) -, the selection pressure of landscape structure on individual dispersal abilities may occur, and result in differential dispersal abilities according to regional landscape structure (Schtickzelle et al. 2006, Van Dyck & Baguette 2005), as was first suggested by difference in thorax width among *Hesperia comma* populations (Hill et al. 1999).

In detecting significant barriers to dispersal, Bayesian methods may prove to be very useful. The emerging field of landscape genetics uses techniques of Bayesian clustering to identify clusters of individuals, and hence the barriers between clusters (Software GENELAND, Guillot et al. 2005, or EASYPOP, Balloux 2001). Bayesian methods may also help to delineate populations in very mobile species such as *Aglais urticae* or various *Colias* species. Graphical methods using multivariate analyses offer some grouping, but do not allow confidence to be made about the barriers. In contrast, Bayesian methods may prove useful in this respect, as these may combine the information from the genotypes of the sampled individuals and tests which of several barriers may be the most likely (Corander et al. 2004). Unfortunately, such important studies involving population genetics and landscape ecology need high research investment, and may be carried out only in few, carefully chosen study systems.

The inbreeding consequences shown in Finnish *Melitaea cinxia* populations most probably occur generally. As more and more populations become isolated, they end up occurring in non-equilibrium metapopulations, where extinctions are more frequent than colonisation, eventually leading to the total extinction, due to both local inbreeding and demographic disequilibrium. In extreme cases where active management aims at rescuing a declining population, on top of habitat restoration,

"genetic restoration" should also take place by pairing individuals which come from populations which used to be part of the same metapopulation, even if populations in between have now become extinct.

The distribution of many European butterflies is likely to change dramatically over the coming decades as a result of global warming. Several species have already started a northward shift (Parmesan et al. 1999). The future of this pattern is likely to have far reaching consequences, as populations of the mountains of southern Europe will move upwards, which will result in their increased isolation, and in many cases eventual extinction (Wilson et al. 2005). Furthermore, species should not be viewed generally as adapted to one particular environment; they generally include populations genetically adapted to a range of environmental conditions (see Chapters B5 and D1). Populations from the middle of the range, will also be affected, as these will have to adapt to changing conditions, in a typical "Red Queen" fashion: they will have to adapt to new environmental conditions to stay in the same place (Lythgoe & Read 1998), through selection of genotypes adapted to warmer temperatures, as has been shown for numerous other species (Parmesan 2006). In erratic species, alleles adapted to warm conditions presently present in the south of the range will migrate north, through a change in the selective gradient due to climatic conditions getting warmer. For more sedentary species, this process may be more difficult, as most species occur in patchy habitats, which are now isolated from each other (Bridle and Vines 2007). In such species, the genetic variation on the edges of the distribution is generally lower than at its center, as shown in *Erynnis propertius* and *Papilio zelicaon* (Zakharov & Hellmann 2008). This in turn may affect the future shift in distribution, as the species with less gene flow (*E. propertius*) will be more affected than the more vagile species (*P. zelicaon*).

For species responding to photoperiod to time specific life-stages, this adaptation is under a strong selection pressure. With climate change the environmental conditions associated with specific photoperiods will alter, imposing new selection pressures and requirements for changes of responses to specific photoperiods. Populations of butterflies will then be affected in numerous ways, involving a large set of ecological and physiological characters, ultimately depending on their genetic make-up governing their response to photoperiod, dispersal behaviour and temperature range for adult and larval activities. How these strong selection pressures will globally affect European butterflies is largely unknown, as such diverse and large-scale environmental change has not been observed before.

Acknowledgements

This chapter benefited from the constructive comments of Emese Meglécz, Sören Nylin, Thomas Schmitt and the editors.

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Box 1

Genetics drift and population differentiation

The population genetics of population differentiation are based on the simple principle that two opposite forces act on this phenomenon: genetic drift tends to differentiate populations, whereas gene flow tends to homogenize populations. If two small populations are isolated from each other, they tend to diverge in allele frequencies more quickly than do large populations (Fig. 1). This principle is used backward to evaluate how population are different from each other, from their allele frequency differences using different methods of calculating a genetic distance between populations (Hartl & Clarck 1989, Hedrick, 2000). Within populations, individuals may mate at random, in which case the number of heterozygotes in the population will be dependant solely on allele frequency. For an allele frequency of *p*, the frequency of heterozygotes is *2p(1-p)*. If populations do not show heterozygote deficiency (in which cases the individual samples may actually result from a local deviance of random mating), the degree of difference among populations may be measured by means of Wright's F statistics, now easily computed using programs such as Hierfstat (Goudet 2005) or Genepop (Rousset 2008). When a large number of populations are studied, F statistics may be ranked to study hierarchical clustering of populations, to study differentiation within a cluster of habitat patches, or among them, which may be river systems, mountain tops or otherwise discrete habitats.

In particular, patterns of allozyme genotype frequencies allow the use of Sewall Wright's F_{ST} index, which gives a concise way of expressing the degree of population differentiation between populations (Wallis 1994). In a group of populations, the population differentiation is measured by the fixation index (symbolized F_{ST}), which is estimated as $F_{ST} = (H_T - \overline{H}_s)/H_T$, with $\overline{H}_s$ the mean expected heterozygosity of an individual in an equivalent population mating randomly, and H_T the expected heterozygosity of an individual in a total population mating randomly. Wright (1978) suggested some guidelines to interpret the resulting values. A value of F_{ST} smaller than 0.05 may be considered as indicating little genetic differentiation, the range 0.05 to 0.15 indicates moderate genetic differentiation, 0.15 to 0.25 great genetic differentiation and over 0.25 very great genetic differentiation. The higher the value of F_{ST} is, the lower the number of migrant among populations per generation would be.

In an island model, where individuals moving out of a population may move with an equal probability to any other population, not just to the ones nearby, the migration rate among populations may be expressed as

$$Nm \approx \frac{1}{4}\left(\frac{1}{F_{ST}} - 1\right) \qquad \text{(Slatkin 1993)}$$

with *m* the migration rate and *N* the effective population size.

This equation allows an estimate of the number of migrating individuals from the F_{ST} differentiation statistics. The given value always has to be taken with caution. The island model assumptions are rarely valid with field data; the model assumes that (1) there is no selection, (2) there is no mutation, (3) all populations host the same number of individuals and contribute equally to the migration pool, (4) migration is random (i.e. irrespective of the distance between the populations), (5) the system is at equilibrium (Whitlock & McCauley 1999). In practice most of these assumptions are

violated with field studies, but comparative analyses using F_{ST} nevertheless yield valuable information on among-population migration (Bohonak 1999). Furthermore, if the number of populations from which F_{ST} is estimated is small, the variance of F_{ST} is large (Douwes & Stille 1988), rendering the estimate of the number of migrants impossible to infer. However, as F_{ST} increases or decreases monotonously with the number of migrants, F_{ST} values or the estimate number of variants may still be used to compare different population system, either within a species or among species (Wang & Whitlock 2003).Other indexes of population differentiation have been implemented since the description of the F_{ST} index. G_{ST} is a generalisation of F_{ST} for the study of different loci simultaneously (Hartl & Clark 1989). These indices both study the population differentiation, irrespective of the relative spatial positions of the relevant populations.
[Figure 1]

Box 2
Spatial aspect of population genetics
The two main parameters of spatial population genetics are the genetic neighbourhood and the isolation by distance. The isolation by distance approach compares populations two at a time, and assess whether there is a correlation between geographic distance and genetic differentiation of populations. The slope of the curve is then an indication of the level of gene flow between the populations, and this parameter varies both among species and among region within species, according to the distribution of habitat patches (Nève et al. 2008).
If migration occurs more frequently between neighbouring populations than between distant ones, one would expect the allele frequencies to be more similar between neighbouring populations then between more distant ones. The genetic neighbourhood is the area within which the individuals mate at random (Wright 1978). Isolation by distance occurs if the genetic relatedness between populations decreases as the geographic distance between them increases. This may be estimated using Moran's I (Legendre & Legendre 1998), which computes the correlation between the allelic frequencies in pairs of populations according to the geographical distance between them. In cases of isolation by distance, the correlation decreases continuously with distance, going from a strong positive correlation for populations close to each other to a nil or negative correlation for populations the furthest away (Fig. 2a). The pattern showing a decrease of autocorrelation with an asymptotic approach to zero suggests that populations from a given area are similar to each other, but with no correlation at a wider scale (Fig. 2c).

The spatial pattern of differentiation may also reveal the different scales at which the different kinds of movements occur: in sedentary species most individuals reproduce within a small genetical neighbourhood, whereas the dispersal of a few individuals out of this area, and the distance to which they will eventually move determines the level of isolation by distance between populations. The local effective population size, and hence the neighbourhood size, may be estimated by using the y-intercept of the relationship between Nm and geographic distance, using logarithmic scales on both axes (Slatkin 1993). For sedentary species, this would be equivalent to their natal habitat patch. The slope of the isolation-by-distance, on the other hand, reveals the extent to which individuals move away to other patches. In migratory or erratic species, both values are of high interest. As it would be expected that populations

which are closer to each other would be more similar than more distant ones, there is a need to address the relationship between the degree of population differentiation and the distance between populations. The first approach on this topic is to assess the isolation by distance, by a study of the relationship between the geographical distance between populations and their index of differentiation. This is usually done by using the estimated number of migrants per generation (Nm) between any two populations, using the relationship G_{ST} =1/4(Nm+1) and the geographic distance between these two populations, usually both log-transformed (Slatkin 1993). The estimated number of migrants should not be taken literally; Whitlock and McCauley (1999) stressed that the relationship between Nm and G_{ST} or F_{ST} is based on a number of assumptions, most of which are unrealistic in natural populations. The most important concern here is that this relationship is based on an island model, where the number of migrants between populations is not correlated with the distance between populations. This is obviously not true in most butterfly populations. In consequence, the estimated number of migrants between two populations should be taken as an index of their differentiation, but not at face value (Neigel 2002, Whitlock & McCauley 1999).

This approach is usually applied on each studied allele separately (e.g. Porter & Geiger 1995, Nève et al. 2000, Descimon et al. 2001), but may also be applied using the information from the different loci simultaneously (Smouse & Peakall 1999), as performed by Harper et al. (2003) on English populations of *Polyommatus bellargus*.

Figures

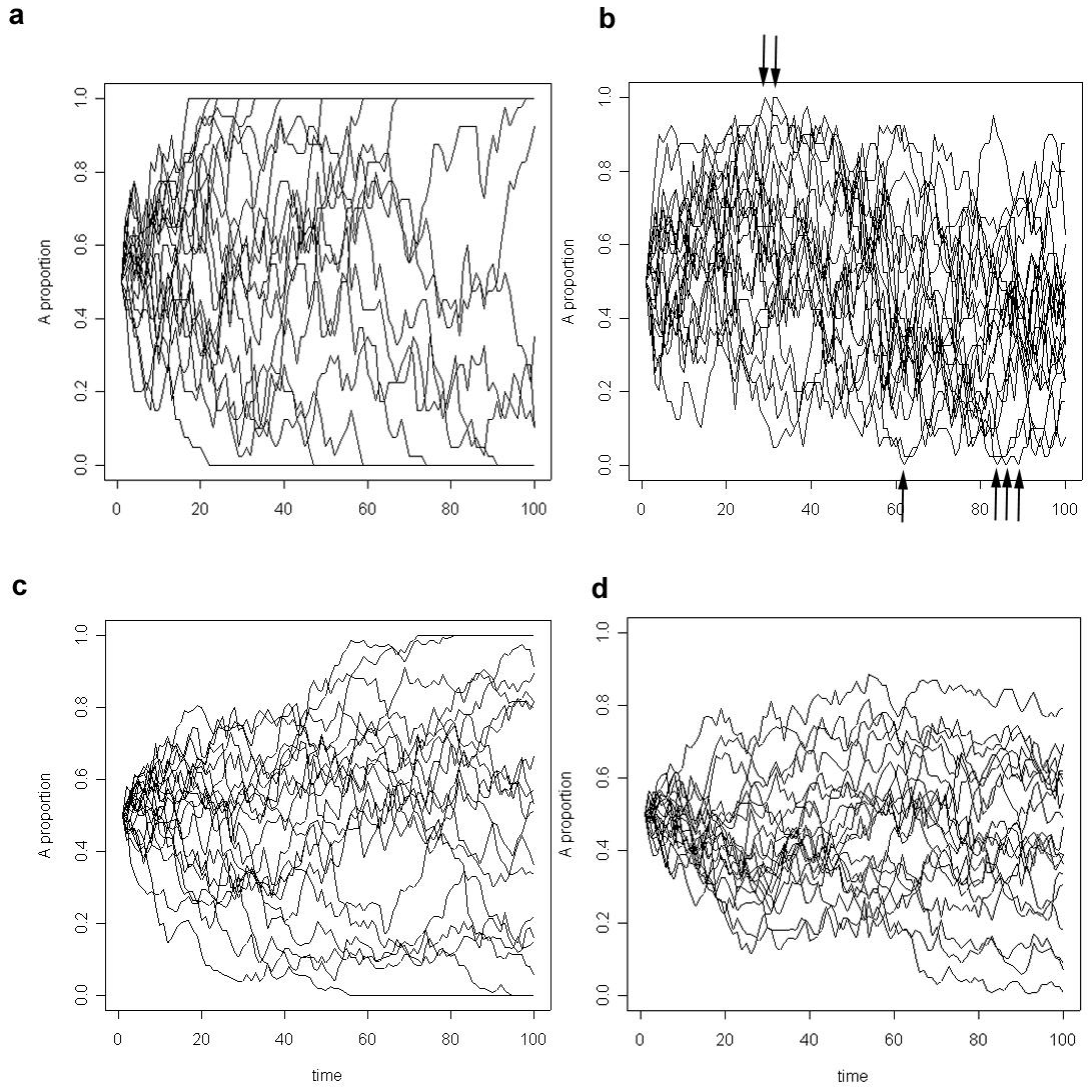

Figure 1.

Example of simulation of allele frequencies of 20 populations of 20 individuals each, with an initial allele frequency of 0.5.

a. With no migrant between populations, most populations loose one of the two alleles within 100 generations. In this run only 4 of the 20 populations retain both alleles, with a highly variable proportion.
b. With one migrant per population per generation, all populations retain their variability in the long run, as each event of allele fixation (arrows) is subsequently rescued through migration of an individual from another population.

Example of simulation of allele frequencies of 20 populations of 100 individuals each, with an initial allele frequency of 0.5.

c. With no migrant between populations, some populations loose one of the two alleles within 100 generations. In this run 16 of the 20 populations retain both alleles, with a highly variable proportion.
d. With one migrant per population per generation, all populations retain their variability in the long run, with allele frequencies closer to each other than if no migration occurred.

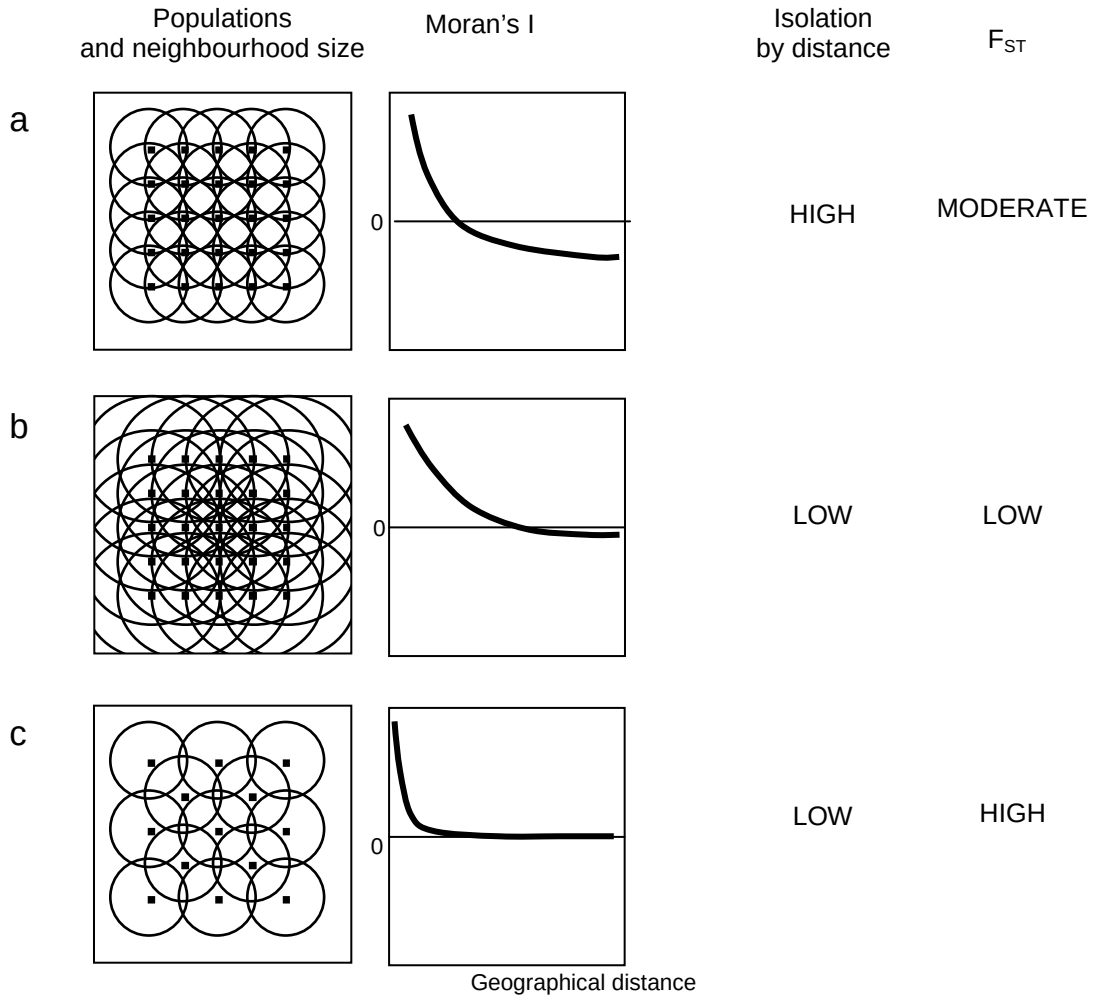

Figure 2. Spatial population structures. Black squares depict suitable habitat patches, and circles the neighbourhood sizes. (a) Each of the 25 populations exchanges individuals with its direct neighbours at each generation. (b) Each of the populations exchanges individuals with its direct and indirect neighbours, resulting in a much higher gene flow throughout the system. (c) The neighbourhood size is the same as in (a), but with a matrix of half of the habitat patches, each population ends up being isolated, and is subjected to genetic drift independently of its neighbours.

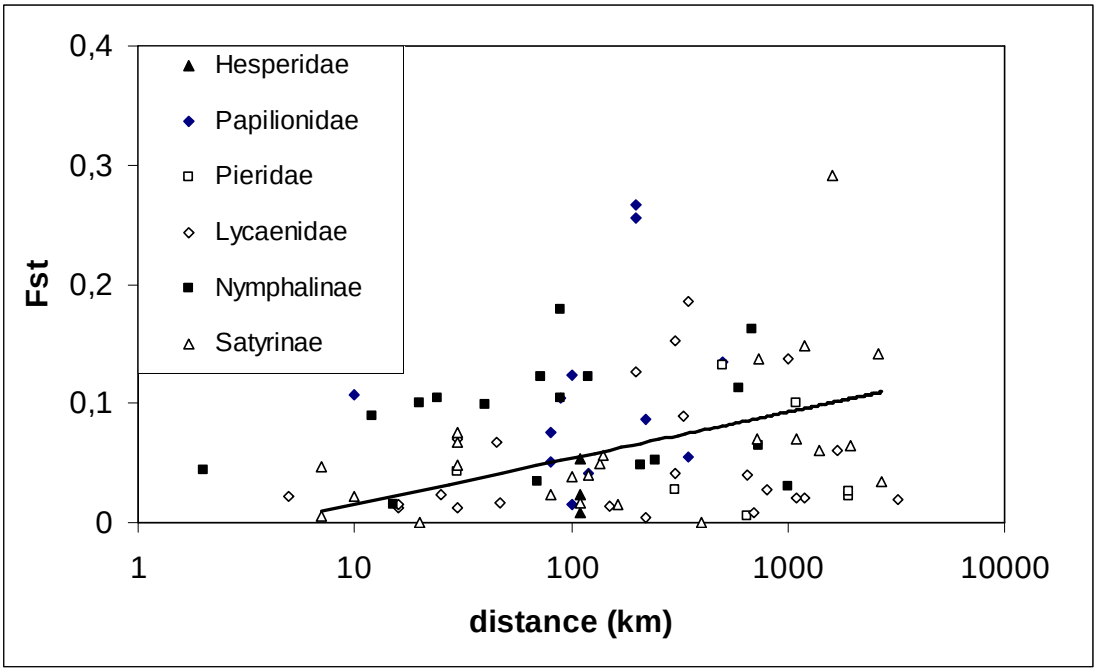

Figure 3. Fixation index (F_{ST}) as a function of the longest geographic distance involved in studies of European butterflies. There is a significant correlation only within the subfamily Satyrinae.

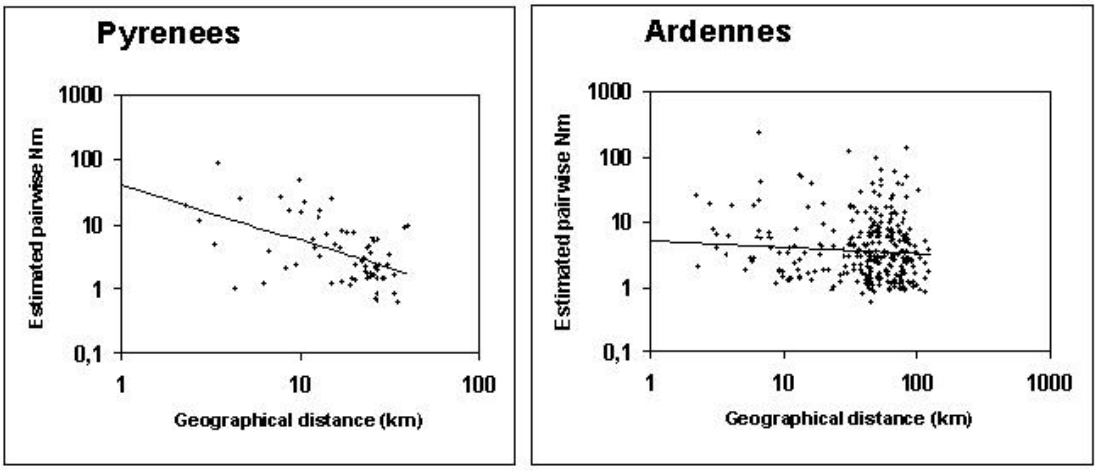

Figure 4. The estimated pairwise number of migrants Nm, as a function of the geographical distances in *Proclossiana eunomia* populations in Ardennes and Pyrenees. The slopes indicate the levels of isolation by distance, while the intercept indicate the neighbourhood size (from Nève et al. 2008).

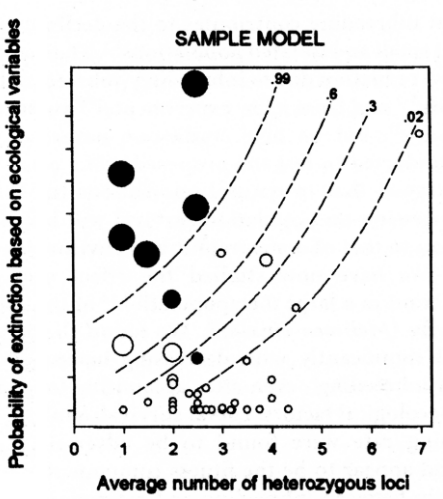

Figure 5. The probability of extinction in *Melitaea cinxia* populations in the Åland islands (Finland) depends on both ecological and genetical factors. The vertical axis gives the probability of extinction predicted by a model including several ecological factors. The horizontal axes gives the average number of heterozygous loci per individual (from 8 enzyme and microsatellite loci). The size of the symbol indicates the extinction probability. Black dots indicate populations which went extinct in one year (from Saccheri et al. 1998).

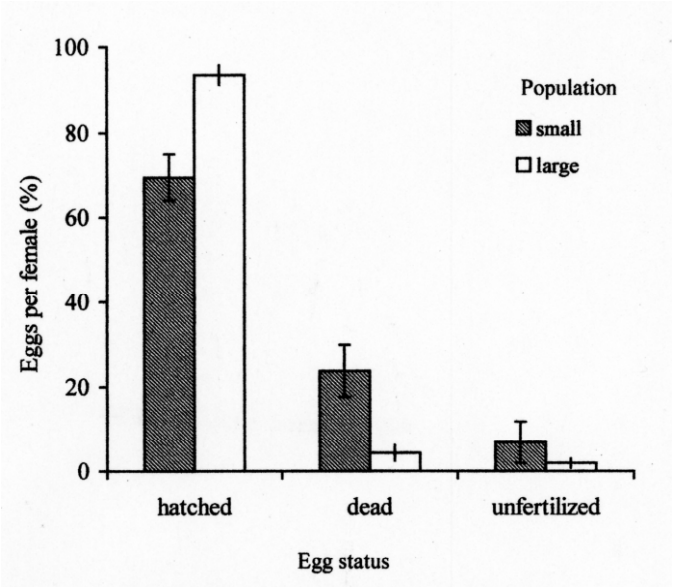

Figure 6. Percentages of total number of eggs per female *Coenonympha hero* females (± 1 SE) that hatched, died as zygotes, or remained unfertilized according on whether they came fro large or small Swedish populations (from Cassel et al., 2001)

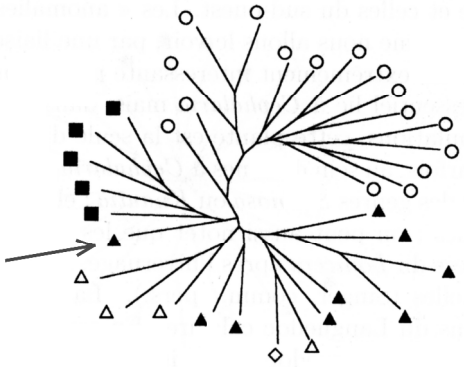

Figure 7. Neighbour-joining dendrogram of French *Euphydryas aurinia* populations, based on genetic distances between populations calculated for 10 enzyme loci. Circles : Provence, black triangles : Languedoc, open triangles : Pyrenees, squares :SW France. The populations from Sommail (Languedoc) is indicated by an arrow. (from Descimon et al. 2001, fig 4c).

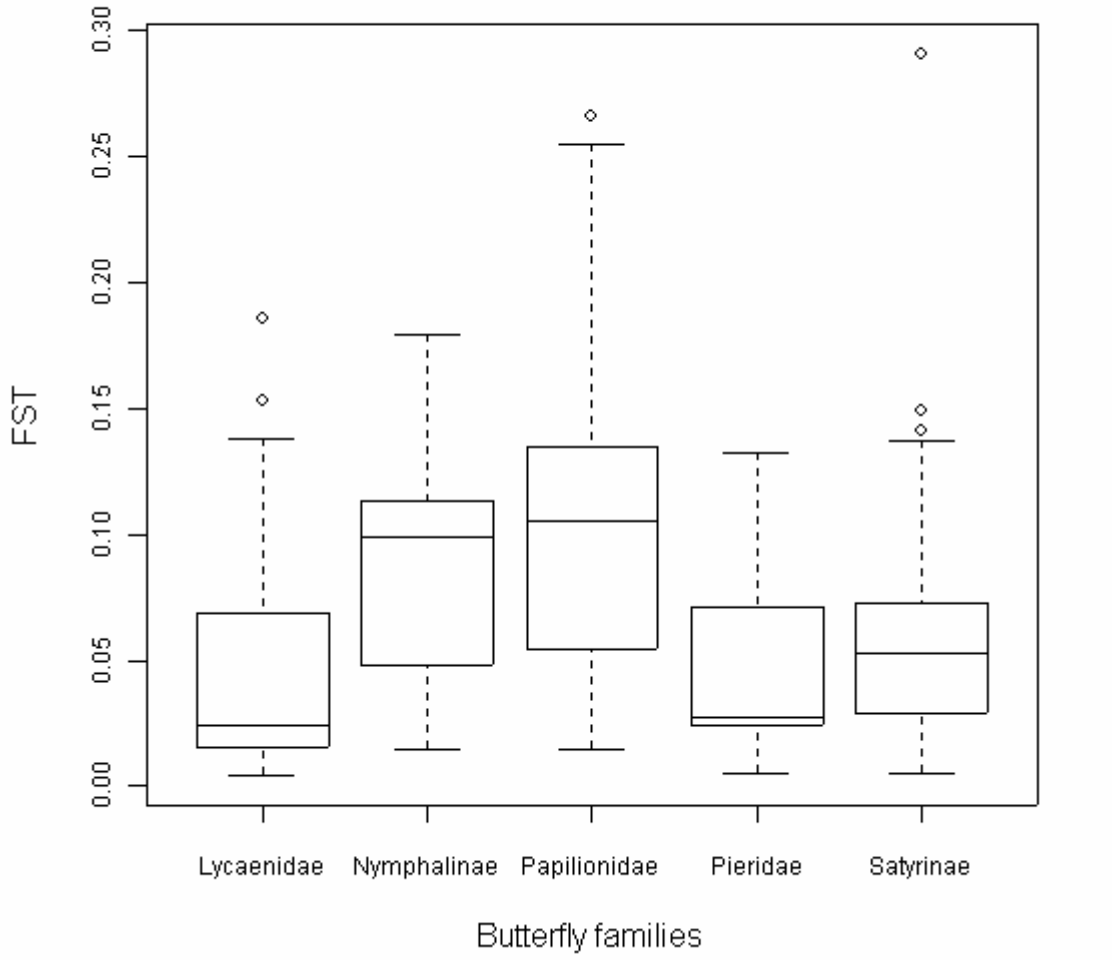

Figure 8. Box plots of the global fixation index (F_{ST}) for all studied species (Table 1). The horizontal bars indicate the median values, the boxes includes 50 % of the values and the vertical bars indicate the range of the values. Outliers are indicated by circles. Studies on Nymphalinae and Papilionidae clearly indicate higher values of than those of Lycaenidae, Papilionidae and Satyrinae.

Table 1. Population genetics studies of butterflies conducted in Europe.

Species and study area	Methods	N populations	F_{ST}	Number of polymorphic loci	Approximate longest distance	IBD	Reference
Hesperidae							
Carterocephalus palaemon							
Białowisża forest, Poland	Allozymes	2	0.191	16	(sympatry)	NA	Ratkiewicz & Jaroszewicz 2006
Thymelicus acteon							
GD Luxemburg + neighbouring areas	Allozymes	12	0.053	18	110 km	NA	Louy et al. 2007
Thymelicus sylvestris							
GD Luxemburg + neighbouring areas	Allozymes	12	0.023	18	110 km	*	Louy et al. 2007
Thymelicus lineola							
GD Luxemburg + neighbouring areas	Allozymes	12	0.008 NS	18	110 km	NA	Louy et al. 2007
Papilionidae							
Parnassius mnemosyne							
Hungary	Allozymes	8	0.075	3	80 km	*	Meglécz et al. 1998a
Hungary	Microsatellites	8	0.051	3	80 km	NA	Meglécz et al. 1998b
Alps+ Pyrenees	Allozymes	24	0.135	9	500 km	NA	Napolitano & Descimon 1994
S Alps (France)	Allozymes	4	0.035 – 0.175	9	90 km	NA	Napolitano et al. 1988
Parnassius apollo							
French Alps + Jura	Allozymes	17	0.0548	14	350 km	**	Descimon et al. 2001
French southern Alps	Allozymes	10	0.041	14	120 km	NS	Descimon et al. 2001
Jura	Allozymes	2	0.124	12	100 km	NA	Descimon et al. 2001
Pyrenees	Allozymes	7	0.087	14	220 km	NA	Descimon et al. 2001
Massif central	Allozymes	3	0.266	10	200 km	NA	Descimon et al. 2001
Spain	Allozymes	2	0.152	12	NA	NA	Descimon et al. 2001
Parnassius phoebus							
French Alps	Allozymes	12	0.255	11	200 km	NA	Descimon 1995
Papilio hospiton							
Corsica	Allozymes	6	0.015 NS	8	100 km	NS	Aubert et al. 1997

Papilio machaon							
England	Allozymes	3	0.107	7	10 km	NS	Hoole et al. 1999
England	RAPD	3	NA	109	10 km	NS	Hoole et al. 1999
Pieridae							
Pieris napi napi							
Europe	Allozymes	16	0.0226	9	1900 km	*	Porter & Geiger 1995
Pieris napi bryoniae							
Alps	Allozymes	8	0.0277	9	300 km	*	Porter & Geiger 1995
Pieris napi meridionalis							
South Europe	Allozymes	7	0.0052	9	650 km	NS	Porter & Geiger 1995
Pieris napi britannica							
Britain	Allozymes	6	0.1322	9	500 km	*	Porter & Geiger 1995
Birmingham	Allozymes	8	0.042 (NS)	5	30 km	NS	Angold et al. 2006
Pieris napi adalwinda							
Scandinavia	Allozymes	2	0.1010	9	1100 km	*	Porter & Geiger 1995
Pieris napi napi+bryoniae+meridionalis							
Europe	Allozymes	31	0.0258	9	1900 km	*	Porter & Geiger 1995
Lycaenidae							
Maculinea teleius							
Poland	Allozymes	3	0.041	3	300 km	**	Figurny-puchaska et al. 2000
Hungary, Rumania, Slovenia	Allozymes	13	0.008	14	700 km	NA	Pecsenye et al. 2007b
Maculinea nausithous							
Poland	Allozymes	4	0.153	5	300 km	**	Figurny-puchaska et al. 2000
Hungary	Allozymes	3	0.013	14	16 km	NA	Pecsenye et al. 2007b
SW Germany	microsatellites	14	0.068	7	45 km	***	Anton et al. 2007
Maculinea alcon							
Denmark	Allozymes	13	0.09	5	330 km	NA	Gadeberg & Boomsma 1997
Hungary, Slovenia, Rumania	Allozymes	13	0.138	14	1000 km	NA	Pecsenye et al. 2007b
Maculinea arion							
Hungary, Rumania	Allozymes	3	0.040	5	650 km	NA	Pecsenye et al. 2007b
Plebejus argus							
Britain	Allozymes	9	0.07	12	30 km	NA	Brookes et al. 1997
South Spain	Allozymes	5	0.016	7	47 km	NS	Péténian & Nève 2003

Finland	Allozymes	9	0.015	10	16 km	NS	Péténian & Nève 2003
Polyommatus bellargus							
Britain	microsatellites	26	0.127	4	200 km	***	Harper et al. 2003
Polyommatus icarus							
Portugal to Germany	Allozymes	29	0.0187	19	3200 km	*	Schmitt et al. 2003
W Germany	Allozymes	15	0.0041	19	220 km	NS	Schmitt et al. 2003
Polyommatus coridon							
W Europe	Allozymes	18	0.021	17	1200 km	NA	Schmitt & Seitz 2001a
E Europe	Allozymes	18	0.028	17	800 km	NA	Schmitt & Seitz 2001a
Europe	Allozymes	36	0.060	17	1700km	NA	Schmitt & Seitz 2001a
W Germany	Allozymes	22	0.014	20	150 km	NS	Schmitt & Seitz 2002c
Lower Saxony (Germany)	Allozymes	17	0.013	19	30 km	NS	Krauss et al. 2004
France, Italy, Germany	Allozymes	39	0.021	20	1100 km	NA	Schmitt et al. 2002
Aricia artaxerxes issekutzi							
Bükk (Hungary)	Allozymes	2	0.022	13	5 km	NA	Pecsenye et al 2007a
Aggtelek (Hungary)	Allozymes	6	0.024	13	25 km	NA	Pecsenye et al 2007a
Aricia agestis							
Britain	Allozymes	6	0.1857	12	350 km	NA	Lai & Pullin 2005
Nymphalinae							
Proclossiana eunomia							
Belgium	Allozymes	26	0.123	3	120 km	*	Nève et al. 2000
Belgium	RAPD	4	0.0887	24	12 km	NS	Vandewoestijne & Baguette 2004
Pyrenees (France)	Allozymes	12	0.099	3	40 km	***	Descimon et al. 2001
Asturias (Spain)	Allozymes	4	0.123	10	73 km	*	Nève et al. 2008
Morvan (France)	Allozymes	11	0.105	3	24 km	NS	Barascud et al. 1999
Czech R.	Allozymes	11	0.035	3	70 km	***	Nève et al. 2009
Boloria aquilonaris							
Belgium	RAPD	8	0.179	18	90 km	**	Vandewoestijne & Baguette 2002
Belgium	Allozymes	8	0.105	4	90 km	NS	Vandewoestijne & Baguette 2002
Aglais urticae							
Europe	Allozymes	9	0.030	5	1000 km	NS	Vandewoestijne et al. 1999
Euphydryas aurinia							
Britain	Allozymes	17	0.1621	6	690 km	NA	Joyce & Pullin 2003

South Britain	Allozymes	8	0.0522	6	244 km		Joyce & Pullin 2003
S France	Allozymes	35	0.113	10	600 km	***	Descimon et al. 2001
N France	Allozymes	26	0.065	10	730 km	**	Descimon et al. 2001
Melitaea cinxia							
Åland Is	Allozymes + Microsatellites	369	0.1	6 + 2	20 km	« strong »	Saccheri et al. 2004
Melitaea didyma							
C. Germany	Allozymes	21	0.006 – 0.090	25	210 km	NS	Johannesen et al. 1996
Hammelburg (Germany)	Allozymes	14	0.015	25	15 km	NS	Johannesen et al. 1996
Mozel (Germany)	Allozymes	4	0.044	25	2 km	NA	Johannesen et al. 1996
Satyrinae							
Coenonympha pamphilus							
Birmingham	Allozymes	10	0.075	10	30 km	NS	Angold et al. 2006
Monte Baldo (Italy)	Allozymes	4	0.005	17	20 km	NS	Besold et al. 2008
Coenonympha hero							
Sweden, Estonia,Russia	Allozymes	13	0.141	5	2600 km	*	Cassel & Tammaru 2003
Maniola jurtina							
S England	Allozymes	15	0.049	2	135 km	NS	Handford 1973a§
Isles of Scilly	Allozymes	13	0.047	2	7 km	NS	Handford 1973b§
SE England	Allozymes	14	0.015 (NS)	12	165 km	NS	Goulson 1993
Europe	Allozymes	48	0.034	20	2700 km	NS	Schmitt et al. 2005b
Birmingham	Allozymes	10	0.048 (NS)	10	30 km	NS	Angold et al. 2006
Sardinia	Allozymes	5	0.057(NS)	15	140 km	NS	Grill et al. 2007
Europe	Allozymes	12	0.065	15	1930 km	NS	Grill et al. 2007
Maniola nurag							
Sardinia	Allozymes	6	0.04(NS)	15	120 km	NS	Grill et al. 2007
Pyronia tithonus							
Birmingham	Allozymes	11	0.068	6	30 km	NS	Angold et al. 2006
Erebia epiphron							
Europe	Allozymes	16	0.291	18	1600	NA	Schmitt et al. 2005a
Erebia embla							
Sweden	Allozymes	4	0.024	5	80 km	NA	Douwes & Stille 1988

Erebia medusa							
Germany, Hungary	Allozymes	53	0.149	19	1200 km	NA	Schmitt & Seitz 2001b
NE Hungary	Allozymes	6	0.005	20	7 km	NS	Schmitt et al. 2000
Balkan peninsula	Allozymes	28	0.137	17	730 km	NA	Schmitt et al. 2007
Erebia triaria							
Spain	Microsatellites	6	0.07	7	720 km	NS	Vila et al. 2006
Melanargia galathea							
Belgium	Allozymes	7	0.016	6	110 km	*	Vandewoestijne et al. 2004
South France	Allozymes	7	0.038	4	100 km	NS	Nève (unpubl)
C Europe	Allozymes	11	0.061	11	1400 km	NA	Habel et al. 2005
South east Europe	Allozymes	16	0.070	11	1100 km	NA	Schmitt et al. 2006
Morocco	Allozymes	4	0.088	11	400 km	NA	Habel et al. 2008
Chazara briseis							
Germany	Allozymes	9	0.022	15	10 km	**	Johannesen et al. 1997

NA : not available, NS : not significant, * : P<0.05, ** : P<0.01, *** : P<0.001, § F_{ST} and isolation by distance computed by present author