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The Effect of Metapopulation Processes on the Spatial Scale of Adaptation Across an Environmental Gradient

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Running header: The spatial scale of adaptation

ABSTRACT: We show that the butterfly *Aricia agestis* (Lycaenidae) is adapted to its thermal environment in via integer changes in the numbers of generations per year (voltinism): it has two generations per year in warm habitats and one generation per year in cool habitats in north Wales (UK). Voltinism is an "adaptive peak" since individuals having an intermediate number of generations per year would fail to survive the winter, and indeed no populations showed both voltinism types in nature. In spite of this general pattern, 11% of populations apparently possess the "wrong" voltinism for their local environment, and population densities were lower in thermally intermediate habitat patches. Population dynamic data and patterns of genetic differentiation suggest that adaptation occurs at the metapopulation level, with local populations possessing the voltinism type appropriate for the commonest habitat type within each population network. When populations and groups of populations go extinct, they tend to be replaced by colonists from the commonest thermal environment nearby, even if this is the locally incorrect adaptation. Our results illustrate how stochastic population turnover can impose a limit on local adaptation over distances many times larger than predicted on the basis of normal dispersal movements.

Introduction

A critical question in evolutionary biology and ecology is "what is the spatial scale of adaptation?" In evolutionary terms, the answer determines the conditions under which populations are able to diverge (Lenormand 2002), and hence relates to questions about speciation and adaptation, and the ability of range boundaries to expand through successive local adaptations (e.g. Holt 1996; Kirkpatrick and Barton 1997). In an ecological context, local adaptation affects distributions, abundance, population dynamics and interactions between species (e.g. Singer & Thomas 1996; Tuda and Iwasa 1998; Hanski & Singer 2001).

Understanding adaptation requires both ecological and evolutionary approaches because it results from an interaction between local selection, migration (gene flow), colonization (founder events) and extinction. Within metapopulations such as those considered here, extinction rates are usually high in small and low-quality habitat patches, whereas migration rates of individuals between patches and the colonization rates of empty habitats are usually determined by patch isolation (e.g. Kindvall & Ahlen 1992; Thomas & Jones 1993; Hanski et al. 1994; Hanski 1999; J.A. Thomas et al. 2001). These ecological dynamics of local extinction and colonization are superimposed on a heterogeneous environment, where different selection pressures occur in different patches of habitat. This heterogeneity potentially generates an adaptive landscape with multiple peaks. The ability of a population to achieve a local adaptive peak depends on the balance between selection for the local environment and the effective rate of

immigration from populations occupying alternative environments that, for the focal population, carry genes that may be non-adaptive. Effective gene flow can be in the form of ongoing dispersal between extant populations or (re)colonization of empty patches of habitat. The geographical scale over which ecologically relevant traits are differentiated depends on the ratio of the scale of effective dispersal to the scale of the ecological or selective processes involved (Endler 1992; Mallet 2001; Lenormand 2002).

When local populations are subject to frequent extinction within metapopulations (Hanski & Gilpin, 1997), local adaptation is lost and the patch may subsequently be recolonized by migrants from nearby populations. Thus, in metapopulations with high turnover, adaptations to individual patches will be rare, and the traits that predominate are likely to be those associated either with the commonest type of habitat in a network, or the habitat present in the largest patches if these are the least extinction prone and generate most successful colonists (Hanski 1999; Wilson et al. 2002).

In this paper we examine the influence of population structure on the distribution and maintenance of allozyme markers and adaptive traits in a butterfly metapopulation distributed across a temperature gradient. At a coarse scale, the temperature changes are very gradual, but at a finer scale temperature is extremely heterogeneous, producing hotter and cooler habitat patches depending on the elevation and aspect of individual sites. In the temperate zone, adaptation to these environmental gradients often involves changes in the number of generations per year (voltinisms) for any species that can overwinter in only one stage of the life cycle (e.g., most insects). One and a bit generations per year would be fatal, so species of this type must translate a

gradual environmental change into an integer number of generations. The organism may be required to alter both its life history and responses to environmental cues (photoperiod and temperature sensitivity). Just on the "warm side" of the transition, development must be as fast as possible to ensure two full generations are achieved; just on the "cool side", development must be delayed to ensure exactly one generation. Such differences will be achieved either through local adaptation (populations have one or two generations) or through adaptive polyphenism (developmental response to an environmental trigger).

In this paper we evaluate the spatial scale of voltinism across a heterogeneous temperature gradient, and investigate whether adaptation to local environments occurs locally or is affected by population turnover in metapopulations.

Study System

In Britain, brown argus butterflies, *Aricia* Reichenbach (Lycaenidae) occur in populations that have either one (univoltine) or two (bivoltine) generations a year. There is a corresponding difference in adult flight period between these forms: the peak emergence of the univoltine populations falls between the two emergence peaks of the bivoltine populations (Figure 1). Breeding experiments in which univoltine and bivoltine broods are reared under the same conditions show that voltinism is determined by the response of larvae to photoperiod and is under genetic control (Jarvis 1966; A.S. Burke et al. unpublished). Voltinism variation in *Aricia* appears to be a

consequence of adaptation to climatic conditions, as in other butterflies such as *Pieris brassicae* (L.) (Held & Spieth 1999).

Univoltine and bivoltine *A. agestis* (see taxonomic status, below) populations occur in close proximity in North Wales (Figure 1; Wilson *et al*. 2002). Here, the habitat of the butterfly (unimproved limestone grassland supporting the larval food plant, *Helianthemum nummularium* (L.)) is patchily distributed across a thermal gradient: warmer in western, low-elevation coastal areas, and cooler in the east at higher elevation inland sites. Superimposed on this gradient is considerable local variation in thermal microclimate, determined chiefly by aspect, slope and altitude of individual hillsides. These environmental changes occur within very narrow latitudinal limits, so all populations experience approximately the same photoperiods. The fitness of each voltinism will therefore depend on the local thermal environment, and thermally intermediate patches might be expected to support populations with a mixture of voltinisms. But in nature, populations never showed polymorphisms in voltinism (Wilson et al. 2002). Furthermore, colonization and extinction of populations were observed, and small and/or isolated habitat patches were unlikely to be occupied, suggesting that population turnover may be common enough to affect thermal adaptation by *A. agestis*.

Taxonomic status of north Wales Aricia

Univoltine populations of *Aricia* in northern Europe have often been regarded as belonging to *A. artaxerxes* (Fabricius) and bivoltine populations to *A. agestis* (Denis &

Schiffermüller). These species can be mated in captivity and will produce fertile viable offspring (Jarvis, 1966), but the degree to which this occurs in the wild and its success is unknown. In a phylogeographic study of *Aricia* butterflies in north-western Europe, Aagaard et al. (2002) found two major clades of the mitochondrial gene cytochrome b (*cyt b*) separated by 3.3-4.6% sequence divergence. These correspond to the taxa *A. artaxerxes* and *A. agestis* from Scotland/northern Scandinavia and southern England/southern Scandinavia respectively. However, the univoltine and bivoltine forms in north Wales both appear to belong to a single mitochondrial clade corresponding to *A. agestis*, based on six bivoltine (two each from MD, MHW and GT; see Figure 2 for population codes) and six univoltine (two each from GF, LX and PG) individuals (A.S. Burke & I.R. Wynne, *unpublished*). Three mtDNA haplotypes were found among 12 individuals from north Wales sequenced for cytochrome b. When compared with the known haplotypes within *A. agestis* and *A. artaxerxes*, all individuals could be clearly assigned to *A. agestis* haplotypes of Aagaard *et al.* (2002: GenBank accession numbers AF408186 to AF408192): Haplotype 1 (MD, GF, LX, PG), Haplotype 2 (MHW) and Haplotype 3 (GT, LX, PG).

Materials and Methods

Distribution Mapping

The distribution of *Helianthemum nummularium* was mapped in north Wales during May – September 1996-1999 (Wilson et al. 2002). Habitat patches were considered distinct if separated by 50 m or more of habitat with no *H. nummularium*, or 25 m or

more if a scrub or woodland barrier was present. With these patch definitions, the closest neighboring patches will typically exchange ~20% of dispersing individuals, but most patches were further apart and should exchange far fewer (Wilson & Thomas 2002). Habitat patch area, aspect (degrees difference from south), slope and altitude were recorded, as well as shelter, *H. nummularium* cover, bare ground cover and turf height (see Wilson 1999).

The survey was also used to confirm the phenology of *A. agestis* previously monitored at many of the sites by regular visits and transects (R.W. Whitehead, unpublished data), at a comprehensive list of its populations in north Wales. On the Creuddyn Peninsula (Fig 1), the distribution of *A. agestis* was mapped, and the date, location and number of *A. agestis* seen anywhere from 1996-1998 were recorded (Wilson 1999). Numbers of *A. agestis* were monitored at 24 sites, from May 1996 to July 1998 on standardized weekly transects (Pollard & Yates 1993; Wilson 1999). During summer 1999, weekly transects were walked at a further six populations across mainland north Wales, from the Dulas Valley in the north-west to the Clwydian Hills in the south-east (Figure 1). The weekly transects were used to establish the phenology of bivoltine and univoltine *A. agestis* (Figure 1b). To determine the voltinism type of all other populations in north Wales, every habitat patch was searched in summer 1999 for adults, eggs or larvae of *A. agestis*. Patches were searched twice if no individuals were detected on the first visit, and at least one population per 2 km Ordnance Survey grid square was visited twice, once during the peak flight period of univoltine populations, and once during either the first or second peak flight period of bivoltine populations.

Where adults were found, sex and condition (from 4 - mint condition to 1 - completely worn) were recorded, and a transect was walked to give an estimate of population density (see Thomas, 1983a). Populations were assigned to a voltinism type (bivoltine or univoltine) according to the dates and life stages when *A. agestis* was observed; i.e., the condition and sex ratio of adult butterflies, or the stage of larval development, with reference to the populations where regular transects were carried out. Although there was a small amount of overlap between the end of the first bivoltine adult period and the beginning of the univoltine flight period, and between the end of the univoltine and the beginning of the second generation of the bivoltine flight period (Fig 1b), in practice it was easy to distinguish forms on the basis of adult condition (very old bivoltine and absolutely fresh univoltines in the first overlap period and the reverse in the second overlap period), even on the basis of a single visit.

Thermal Model

From May 1996 to April 1998, temperature was monitored at thirty locations, stratified by aspect and altitude, on the Creuddyn Peninsula (16 on Great Orme's Head (GO), 14 at Bryn Euryn (BE); Figure 2). Tinytalk dataloggers were placed beneath 5-7 cm tall turf on limestone grassland where *H. nummularium* grew. Temperature was recorded to the nearest 0.1°C, every 30 minutes from May to October, and every hour from November to April.

Experimental rearing of univoltine and bivoltine forms showed that larval development only occurred at temperatures greater than 10°C (A.S. Burke et al.,

unpublished data). Therefore, for each month at each Tinytalk location, we calculated the average number of day degrees > 10°C from the two years' data (Higley et al. 1986). A monthly thermal model was calculated using the linear regression of cumulative day degrees >10°C against aspect and altitude for all Tinytalk locations which had two years' data for a particular month (this did not include all locations for all months, because of loss or temporary damage, and because fewer dataloggers were used during winter). For this model, aspect was converted to a linear term by calculating the difference of site aspect from true south (~185° in north Wales in 1997). As a simple estimate of the length of the *Aricia* growing season, we used the monthly thermal models to estimate the annual number of day degrees > 10°C at each site, based on its aspect and altitude. This gives a measure of the thermal "development time" available to *A. agestis* within each habitat patch.

We used logistic regression (Norusis 1993) to test whether the thermal environment differed significantly between univoltine and bivoltine populations, and to calculate the probability of a population being bivoltine (rather than univoltine).

Connectivity

We estimated likely relative levels of immigration to each habitat patch by calculating connectivity (S_i) (Hanski 1994, 1999; Moilanen & Nieminen 2002). Connectivity of focal patch *i* depends on its distance from all other (source) patches (*j*), the area of each source patch, and the dispersal rate of the species in question. Connectivity for patch *i* (S_i) is defined as:

$$S_i = \sum_j e^{\alpha d_{ij}} A_j^b$$

where α describes how rates of dispersal decline with increasing distance (based on a Cauchy distribution with $\alpha = 3$; see Shaw 1995; Wilson *et al*. 2002); d_{ij} is the distance to patch i from each source patch j (where $i \neq j$); and A_j is the area of each patch j. Source population size scales with patch area, and emigration rate scales with patch area to the power b (set to 0.5, as an approximate description of how butterfly per capita emigration rate declines with increasing patch area; Thomas & Hanski 1997; Moilanen & Nieminen 2002). For each patch, we calculated S_i to all *A. agestis* populations; i.e., the potential rate of immigration from all other populations. However, we are primarily interested in the *relative* rates of gene flow (and colonization potential) from bivoltine and univoltine source populations, rather than the overall immigration rate. To estimate this, we calculated the difference between S_i calculated i) to all bivoltine populations, and ii) to all univoltine populations that existed during the survey period (connectivity difference). In a network, some habitat patches could be empty in a particular survey period, but might have been occupied in a previous year, at which time populations in them could have contributed to gene flow. Therefore, we also calculated a second term for thermal connectivity difference, based on the thermal characteristics of each habitat patch in the landscape (regardless of whether the habitat was occupied during the survey period). For this second measure, we first calculated whether each patch would be expected to favor bivoltine ("warm patches") or univoltine ("cool patches") populations using our logistic regression model that related

voltinism type to habitat thermal characteristics (see results). For each patch, we then subtracted its connectivity to all "cool patches" from its connectivity to all "warm patches."

Metapopulation modeling

The spatially realistic "incidence function" metapopulation model (IFM; Hanski 1994, 1999) was used to estimate relative persistence times of metapopulations of *A. agestis* in north Wales. IFM parameters relating extinction rate to patch area, and colonization rate to patch connectivity, were estimated using standard techniques (Moilanen 1999; see appendix material). Fourteen networks containing one or more patches of suitable habitat were defined (Fig 1a), each separated by more than 3 km of unsuitable habitat (Wilson et al. 2002). To estimate relative metapopulation persistence times, 100 IFM simulations of up to 200 generations were iterated for each habitat network. Patch occupancy was set to 100% in the first year of each simulation, in order to compare persistence times of networks that were occupied and unoccupied by *A. agestis* during the survey.

Population Genetic Structure

Butterfly samples were collected from 26 localities across the entire suitable area in North Wales in 1999 and 2000 (Figure 2). Localities with bivoltine populations were (codes in parentheses): Bwrdd Arthur (BA), Mariandyrys (MD), Penmon Quarry (PQ), Great Orme (GO), Mynydd Penygareg (MP), Pen-y-bont (PYB), Llangwstenin (LN), Bryn Euryn (BE), Marle Hall wood (MHW), Terfyn (TF), Pen-y-Corddyn Bach (PCB),

Mynydd Marian (MM), Bryn Meiriadog (BM), Bryn Cefn (BC), and Graig Tremeirchion (GT). Localities with univoltine populations were: Graig Fawr (GF), Ochr-y-Foel (OF), Gop Hill (GH), Lixwm (LX), Loggerheads (LOG), Cefn Mawr (CF), Aberduna (AB), Burley Hill Quarry (BHQ), Pistyll Gwyn (PG), Eryrys (EYS), Perthichweru (PW), and Castle Woods (CW). Sample sizes of at least thirty individuals were sought. However, in a few instances this proved difficult or, in the case of very small populations, was deemed undesirable. Samples were heavily male biased to minimize any potential impact on the populations.

Butterflies were snap frozen in liquid nitrogen in the field, and then stored at –80^{o}C. Butterflies were homogenized and prepared for electrophoresis by the methods described by Wynne and Brookes (1992) using half the thorax and abdomen in 250µl of extraction buffer.

Allozyme variation was assessed using cellulose acetate electrophoresis (Helena Laboratories - see Wynne *et al.*, 1992). A total of 24 enzymes (representing approximately 31 putative loci) were screened (at least 10 individuals per locus) for polymorphism. These were: adenylate kinase (AK; 2.7.4.3), aconitate hydratase (ACON; EC 4.2.1.3), alanine aminotransferase (GPT; EC 2.6.1.2), alcohol dehydrogenase (ADH; EC 1.1.1.1), diaphorase (DIA; EC 1.6), fumarate hydratase (FUM; EC 4.2.1.2), glucose dehydrogenase (GLDH; EC 1.1.1.47), glutamate-oxaloacetate transaminase (GOT; EC 2.6.1.1), glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49), glycerol-3-phosphate dehydrogenase (αGPD; EC 1.1.1.8), hexokinase (HK; EC 2.7.1.1), -

hydroxybutarate dehydrogenase (HBDH; EC 1.1.1.30), isocitrate dehydrogenase (IDH; EC 1.1.1.42), lactate dehydrogenase (LDH; EC 1.1.1.27), malate dehydrogenase (MDH; EC 1.1.1.37), malic enzyme (ME; EC 1.1.1.40), mannose-phosphate isomerase (MPI; EC 5.3.1.8), peptidases (PEP-A and PEP-D, using substrates leucyl-glycine and phenyl-alanine respectively) (PEP; EC 3.4.11), phosphoglucose isomerase (PGI; EC 5.3.1.9), 3-phosphoglycerate dehydrogenase (3PGD: EC ? See Mallet et al. 1993, for details), 6-phosphogluconate dehydrogenase (6PGD; EC 1.1.1.44), phosphoglucomutase (PGM; EC 2.7.5.1), and sorbitol dehydrogenase (SORDH; 1.1.1.14). The running buffers used were 50mM Tris-citrate, pH 7.8 (for ACON, ADH, DIA, αGPD, IDH, 3PGD, 6PGD and SORDH), 100mM Tris-citrate, pH 8.2 (for AK, GPT, FUM, GOT, G6PDH, HK, HBDH, MDH, and ME) and 25mM Tris-glycine, pH 8.5 (for MPI, PEP-A, PEP-D, PGI and PGM). For most enzymes the duration of the run was 30 minutes at a constant voltage of 200V. The exceptions were PGI and PGM, which were run for 40 minutes (visualized together as a double stain). Staining recipes were used directly or modified from Richardson et al. (1986) and Mallet et al. (1993). Of the loci run, nine were monomorphic (*Ak-1, Ak-2, Fum, Gpt, 6pgd, Dia-1, Ldh-2, Mdh-1,* and *PepA*) and only seven gave scorable polymorphisms (*Pgi, Pgm, Got-2, Mdh-2, Me, G6pd* and *αGpd*).

Allele frequencies, deviations from Hardy-Weinberg equilibrium expectation and tests for genetic differentiation were calculated using GENEPOP 3.2a (Raymond & Rousset 1995). F-statistics (Wright, 1978) were also calculated using this program using Weir & Cockerham's (1984) method. Pair wise F_{ST} comparisons were transformed to an estimate of *Nm* and used as a measure of genetic similarity to assess isolation by

distance (Slatkin 1993). Slatkin (1993) showed that F_{ST}, transformed into Nm ($\hat{M}$ in Slatkin's terminology), has a relatively simple relationship with geographical distance under a variety of assumptions about gene flow and population history. A few pairwise F_{ST} values between particularly close and/or well-connected *Aricia* populations in north Wales were slightly negative. To avoid losing these data points, and thus biasing the data set, a small quantity of F_{ST} (0.01) was added to all pairwise values before transformation of F_{ST} to Nm.

Results

Distribution

In mainland north Wales, bivoltine populations were found in 87 habitat patches, and univoltine populations in 68 habitat patches in the mainland study region (Figure 1). Population genetic samples were taken from a further three bivoltine populations on the island of Anglesey (Figure 1). The first flight period of bivoltine *Aricia* stretched from late April to early June, peaking at the end of May; the second flight period extended from mid July to early September, peaking in mid August. The flight period of univoltine *Aricia* extended from early June to mid August, peaking at the end of June (Figure 1b).

Thermal Model

Monthly day degrees > 10°C were always negatively related to aspect (degrees difference from south) and altitude (Table 1). The regression coefficients for aspect and

altitude were significant ($P < 0.05$) for eight and five months respectively. They were rarely significant during the winter months, when fewer dataloggers were used and the number of day degrees > 10°C were much smaller. All monthly equations were used to estimate the annual thermal environment at all habitat patches across north Wales: the non-significant, winter equations have a relatively minor effect on overall estimates, as the constants and coefficients for these months are small (Table 1).

The modeled thermal environment was cooler in sites where univoltine populations occurred than where bivoltine populations occurred. Logistic regression using the thermal model differentiated significantly between bivoltine and univoltine populations (Table 2). Using the thermal model, patches receiving more than 782 day degrees in excess of 10°C per year were expected to support bivoltine populations, and cooler patches were expected to support univoltine populations. Modeled thermal environment misclassified voltinism at 17 (11%) populations (out of 155). Misclassified populations were located in networks 1, 2, 6, 9 and 10 (Figure 1), so it is unlikely that latitude or proximity to the coast affected results. In particular, the thermal model misclassified five univoltine populations in network 6, and four univoltine populations in network 10: these two habitat networks were at much lower altitudes than other univoltine populations, and had a number of steep, south-facing slopes.

Density within populations was also related to thermal environment. A generalized linear model (GLM) of $\log_e$ (population density) against thermal environment and number of generations per year ($R^2 = 0.17$, $F_{3,40} = 2.66$, $P = 0.06$) found significant effects

of thermal environment ($F_{1,40} = 6.62$, $P = 0.01$), voltinism ($F_{1,40} = 5.04$, $P = 0.03$), and of their interaction ($F_{1,40} = 7.20$, $P = 0.01$). The interaction term is significant because population density in bivoltine populations decreased at cooler temperatures, whereas the density in univoltine populations increased in cooler environments (Figure 3). Furthermore, density in univoltine populations was positively related to vegetation height (using Spearman's rank correlation because vegetation height was recorded on a categorical scale; $n = 20$, $r_s = 0.62$, $P = 0.03$), again suggesting higher population density in cooler environments (taller vegetation is likely to result in lower day degree accumulation; Thomas 1983b).

Alternative models using either population connectivity difference or thermal connectivity difference in the logistic regression model explained much more of the observed voltinism pattern than did modeled thermal environment alone (Table 2). Population connectivity difference, calculated to estimate the potential for gene flow from bivoltine and univoltine forms, discriminated between all univoltine and bivoltine populations (Figure 4), such that thermal environment and thermal connectivity difference ceased to be significant in a stepwise model. Thus, some patches that appeared to favor one voltinism form on the basis of the local thermal environment were occupied by the other, probably because of immigration by the unfavored form. Based on the thermal model, we calculated the area in each habitat network that was suitable for each form (<782 day degrees >10°C for univoltine, >782 for bivoltine) (Table 3). No network was occupied by the wrong form, but several networks contained relatively large areas of habitat suitable for the other form. For example, network 6

contains the third largest quantity of habitat thermally suitable for the bivoltine form, but is occupied only by the univoltine form.

Metapopulation simulations

Incidence function modeling predicted that metapopulations in only three habitat networks would persist indefinitely (Table 3, Figure 5). Two of the persistent networks supported bivoltine butterflies, and one persistent network supported univoltine butterflies (1, 2 and 9 respectively; Fig 1). All other networks occupied by *A. agestis* had median estimated times to extinction of between 2 and 84 years, shorter than the modeled time to extinction (median 110 generations) of the most isolated unoccupied network (Table 3). Metapopulation simulations appear to support the conclusion of Wilson et al. (2002), based on habitat network size and configuration, that some small but surviving *A. agestis* metapopulations may have periodically become extinct, only to be recolonized from the large, persistent metapopulations.

Genetic Variation

Allele frequencies for the seven polymorphic loci are given in the Appendix. Tests for deviations from Hardy-Weinberg equilibrium revealed only 8 significant deviations ($P<.05$) out of a total of 150 locus x population tests performed. This is similar to the number of significant results expected by chance alone (7.5 expected) and the significant tests were not associated with any particular locus or population (see appendix material). Overall, the results do not indicate deviation from random mating within populations.

Population Genetic Structure in North Wales

No fixed differences were found between univoltine and bivoltine populations at any of the seven polymorphic loci in north Wales. However there was significant variation of allele frequency among all populations in north Wales (F_{ST} = 0.093, P<< 0.001, Table 4). Genetic differentiation within voltinism types was also significant (F_{ST} = 0.088, P<< 0.001 and F_{ST} = 0.064, P<< 0.001 amongst bivoltine and univoltine populations respectively, Table 4). Pairwise tests between populations revealed that this differentiation was widespread; only 16 out of 325 comparisons were not significant (at P< 0.05).

Genetic distance between pairs of populations (14 univoltine, 12 bivoltine) declined with increasing geographic distance (Figure 6). In a three-way Mantel test with 20000 randomized matrices, genetic similarity [log (Nm +1)] was significantly, and negatively, related to geographic distance [log (km+1)] even after controlling for voltinism (correlation coefficient g = -0.29, P < 0.001), but genetic similarity was unrelated to voltinism after controlling for geographic distance (g = -0.09, P=0.25). Geographic distance is therefore the most important determinant of genetic structure within voltinism types, and has a similar effect in each, but it does not control genetic differentiation between voltinism types (the slope is weakly in the opposite direction; Figure 6), suggesting that gene flow between voltinism types may be very low.

Average heterozygosity, over all polymorphic loci, varied between 0.245 and 0.354 for bivoltine and between 0.266 and 0.354 for univoltine populations. There was no

detectable relationship between within-population heterozygosity and the degree to which populations were connected (S_i) to the population network of the same voltinism type (Spearman's Rank $r_s = 0.509$, $P > 0.05$ and $r_s = 0.490$, $P > 0.05$ for bivoltine and univoltine populations respectively).

Genetic differentiation of each population from the within-voltinism network mean allele frequency was again calculated using pairwise *Nm* derived from F_{ST}. This measure was significantly correlated with connectivity for bivoltine populations (Spearman's Rank $r_s = 0.827$, $P < 0.01$) and the non-significant trend for univoltine populations is in the same direction ($r_s = 0.469$, $P > 0.05$) (Figure 7). Thus, it would appear that drift during colonization events or in small established populations, whilst insufficient to generate detectable losses of heterozygosity, is sufficient to disturb allele frequencies in isolated populations.

Genetic differentiation within the three networks considered persistent by metapopulation modeling was low but significant: Creuddyn peninsula (network 1; GO, MP, MHW, PYB, LN and BE), $F_{ST} = .0240$ ($P << 0.0001$); Dulas Valley (network 2; MM, TF and PCB), $F_{ST} = .043$ ($P = 0.0446$); the Clwydian Hills (network 9; LOG, CF, AB, PG, BHQ, EYS and PW), $F_{ST} = .0504$ ($P << 0.0001$). Thus population substructure is evident even well-connected networks of local populations. We also sampled three populations (GF, OF and GH) from network 6, which had a predicted metapopulation survival of 43% after 100 generations (Figure 5): differentiation among these populations was again low but significant ($F_{ST} = .0296$, $P << 0.0001$).

Discussion

The spatial scale of adaptation

Our results indicate that the spatial population dynamics of patchily-distributed species can play a major role in determining patterns of both adaptive and "neutral" genetic variation, at landscape scales that are much larger than expected from dispersal distances achieved by most individuals. Despite the relatively short distances of most individual movements, rare long distance dispersal events have the capacity to dominate patterns of genetic variation at broad scales, given sufficient turnover of populations and metapopulations.

Aricia agestis appears to show "correct" adaptation to variation in the environment in 89% of the habitat patches in the landscape studied: it converts a smooth, but heterogeneous, thermal gradient into a binary response of either one or two generations per year. In other words, it achieves alternative adaptive peaks. Yet it is not 100% successful in achieving the "correct" local adaptation in every habitat, and most "local" adaptations almost certainly arise not because of *in situ* evolution within each patch but because the patch of habitat was colonized by a phenotype that already had appropriate adaptations.

It is apparent that variation in generation number (voltinism) in *A. agestis* butterflies is an adaptation to the thermal environment, as in many other northern temperate insects. The larvae of both voltinism types have similar minimum temperature requirements above which development is possible (A.S.Burke *et al*. unpublished), so

the 'best' voltinism strategy will depend on the 'growing season' available in a particular site (measured as day degrees available for development). A bivoltine (two generation per year) strategy will be more successful in warm, low-elevation and/or south-facing conditions and a univoltine (one generation per year) strategy more successful in cool, high-elevation and/or north facing slopes. The reduced density of bivoltine butterflies in relatively cool habitats, and the reduced density of univoltine butterflies in relatively warm habitats suggests that both voltinism types "struggle" in thermally marginal environments.

In heterogeneous conditions, a mixture of the two voltinisms might be expected if (a) life cycle timing was solely a phenotypic response to local environmental conditions or (b) local populations were independently adapted to each local habitat patch. The first of these explanations is not plausible: a variety of microclimates occur within every single habitat patch, and patches differ in thermal environment within each patch network, so we would expect a mixture of voltinism strategies to be observed within most patches and patch networks. The butterflies only ever achieved a single voltinism strategy within each patch and network. Laboratory rearing revealed genetically-based differences in photoperiod responses of univoltine and bivoltine caterpillars determining whether they enter diapause for the winter, or continue to develop directly to produce a second generation of adult butterflies later in the same summer (A.S.Burke *et al*., unpublished). We conclude that genetic differences among populations are responsible for the geographic pattern of generation numbers shown in Figure 1.

At a patch level, the "wrong" voltinism type in about 11% of local populations, as well as reduced population density in patches with intermediate thermal environments, suggests that local adaptation is incomplete. For example, the north-facing slope of GO (Fig 2) is predicted to be most suitable for a univoltine population on the basis of microclimate, whereas bivoltine butterflies actually occupy the site (at low density). Similarly, butterflies at GF are univoltine (at low density) when bivoltinism is expected. In contrast, all networks of patches contain the voltinism type that is adaptive, based on the amount of each thermal environment that is available across the whole network. The spatial scale and arrangement of habitat patches within the landscape are likely to be important determinants of the traits that predominate (Endler, 1992). Understanding the scale of adaptation requires consideration of the population dynamics as well as the dispersal behavior of *A. agestis*.

Metapopulation dynamics

Many species occur as metapopulations whereby entire population systems persist through a dynamic equilibrium between extinction and recolonization (Hanski, 1991; 1999; Thomas and Hanski, 1997). Patterns of patch and network occupancy, observed local and network-level extinctions (Wilson *et. al*., 2002) and metapopulation simulations suggest that turnover is common in *A. agestis* metapopulations in north Wales. Simulations and observations suggest that metapopulations in small patch networks are extinction-prone, whereas groups of large habitat patches in the Creuddyn peninsula, Dulas Valley and the Clwydian Hills (Figure 1) form extinction-resistant areas important for regional persistence.

The population genetic structure deduced from allozyme analysis is entirely consistent with this population dynamic interpretation. Populations peripheral to patch networks are genetically more differentiated from the rest of the network, presumably because of genetic drift and/or founder effects, than central and well-connected populations. Founder events are likely to contribute to the significant F_{ST} values found within each patch network (Taneyhill et al. 2003). However, isolated populations did not have significantly reduced heterozygosity, which initially seems surprising because many of the small and isolated populations in north Wales must have low effective population sizes ($N_e \sim < 50$). This is probably in part because heterozygosity is relatively insensitive to mild population bottlenecks (Brookes et al. 1997), but is also consistent with metapopulation interpretation: genetic variation within individual populations is not expected to decline over many generations because of the high population turnover rates (Whitlock, 1999). In a conservation context, it is useful to note that areas of high population persistence could be deduced from allozyme data via studies of F_{ST}, but not of heterozygosity.

Given the population dynamics of the system, it is not surprising, then, that adaptation appears to take place at the scale of patch networks, rather than individual patches. Local populations with the "wrong" voltinism apparently become extinct faster than they achieve a switch of adaptive peak to the locally "correct" voltinism type. Following local extinction, the population is likely to be replaced by colonists from the commoner habitat type within the landscape, even though they possess the locally incorrect adaptation. Because only one type of voltinism is present within each network, and

local populations are extinction-prone, the "correct adaptation" within each of the remaining 89% of patches also owes much more to prior colonization by an appropriate phenotype than it does to local adaptation once the population is founded (see Hanski & Singer 2001).

It is likely that a very small fraction of all *A. agestis* dispersal events is responsible for the network-level patterns of voltinism observed. Colonization of empty patches and networks, and movements between existing populations are distance-dependent. In a mark-release-recapture study, the maximum recorded movement was less than 1km (Wilson & Thomas 2002). Even correcting raw dispersal data because of the finite nature of the mark-release-recapture study area (Wilson & Thomas 2002) produces an estimate of only 0.0004% of butterflies moving the 3.6 km between the habitat networks nearest to one another in the current study. Thus, only tiny fractions of individuals probably move between habitat networks, but those that do appear to exert an important effect on the observed distribution of genotypic and phenotypic variation.

Reservoirs of neutral and adaptive variation

Network-level adaptation would appear to be sufficient to explain patterns of adaptation in this system. But, the butterfly's population dynamics suggest that long-term persistence of *A. agestis* in N. Wales depends on three key networks (1, 2, and 9, and perhaps a fourth in Anglesey, which was not modeled – see Figures 1, 5). We therefore suggest that adaptations are likely to be maintained at an even larger spatial scale, again because of the process of colonization and extinction. Metapopulations in

small networks have a short predicted times to extinction (Figure 5), and *A. agestis* has been observed to become extinct from two networks in recent years (networks 13, 14; Fig 1). Metapopulations in small but surviving networks probably owe their existence to proximity to the most persistent networks in the landscape, through very rare, long-distance dispersal events (Wilson *et al*., 2002). Of the persistent systems, the Creuddyn peninsula (network 1) and Dulas Valley (network 2) are clearly dominated by thermal environments suitable for bivoltine *A. agestis*, whereas the Clwydian Hills (network 9) contain predominantly much cooler habitat, suitable for univoltine forms. Ultimately, the distribution of the adaptive variation in north Wales seems to stem from the persistence of these key systems and the metapopulation-scale adaptations appropriate within them. Large, well-connected and persistent systems apparently also act as the long-term repository of supposedly neutral allozyme variation within the region, with smaller and more isolated metapopulations containing increasingly divergent allozyme frequencies. In the long run, any new variation that arises within a smaller metapopulation is likely to be lost, and the empty patch network will ultimately be recolonized from one of the persistent population systems.

In this patchy landscape, extinction-prone, small metapopulations may switch voltinism type from time to time, depending on the origin of recolonists. For example, network 6 is currently populated by univoltine forms, and cool habitats appropriate for univoltine forms are indeed commoner than warm habitats within the network. Nonetheless, network 6 contains more warm "bivoltine-type" habitat than any of the other networks except for 1 and 2. If the current metapopulation became extinct (which

models suggest might occur on the order of once every hundred years), the network could be recolonized by either voltinism type: the nearest potential colonists would be in networks 3 and 5, which are both currently bivoltine (Figure 1).

Our conclusions are analogous to source-sink and range boundary ideas about limits to adaptation (e.g., Holt 1996; Kirkpatrick and Barton 1997), but in a non-equilibrium system. In an equilibrium context, range and adaptive boundary models usually imply that asymmetric gene flow from better (core-type) to worse (marginal-type) habitats can prevent the establishment of adaptations to the marginal environment because of gene swamping. This is most likely when levels of movement are high; in contrast, per generation rates of *A. agestis* movement seem rather too low. But in metapopulation systems where entire patch networks become extinct and are recolonized from core areas, the effective rate of migration / gene flow may be orders of magnitude greater than expected from ongoing measures of "normal" dispersal. Because many other species also have landscape-scale population dynamics and patterns of persistence (Hanski 1999), key areas within distributions may often hold the key to the maintenance of both adaptive and neutral traits. This evolutionary / population dynamic model is similar to a scaled down (over 10s of kilometers and 100s of years; for these butterflies) version of the conceptual framework used to explain genetic patterns within species that experience major distribution shifts in response to glacial-interglacial cycles (over 1000s of kilometers, and 10000s of years). In such systems, it is commonplace to refer to "refugia" that retain and accumulate neutral and adaptive variation within persistent parts of the geographic distribution (e.g., Hewitt 1993, 1996, 1999); refugia are the

equivalent of the persistent metapopulations within north Wales. Areas of population persistence within generally unstable population systems may dominate patterns of adaptation over surprisingly large areas.

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Table 1. Linear regressions of monthly day degrees > 10°C against aspect and altitude.

Month	n	R^2	F	*a* (constant)	*b* (aspect$^+$)	*c* (altitude$^\wedge$)
Jan	10	0.57	4.70^{NS}	15.92 ^{NS}	- 0.043 ^{NS}	- 0.082 ^{NS}
Feb	10	0.63	6.03*	8.47 ^{NS}	- 0.020 ^{NS}	- 0.046 ^{NS}
Mar	8	0.94	38.18***	49.06**	- 0.110**	- 0.250*
Apr	7	0.70	4.66^{NS}	102.54 ^{NS}	- 0.247 ^{NS}	- 0.381 ^{NS}
May	19	0.52	8.69**	165.38***	- 0.281**	- 0.515*
Jun	19	0.42	5.88*	230.19***	- 0.246*	- 0.541*
Jul	19	0.57	10.72**	324.84***	- 0.429**	- 0.569*
Aug	19	0.55	12.14***	320.59***	- 0.437***	- 0.507*
Sep	16	0.62	10.44**	207.86***	- 0.502***	- 0.306 ^{NS}
Oct	15	0.79	22.18***	103.36***	- 0.304***	- 0.213 ^{NS}
Nov	14	0.76	17.30***	17.15***	- 0.072***	- 0.021 ^{NS}
Dec	9	0.48	2.72^{NS}	5.13 ^{NS}	- 0.017 ^{NS}	- 0.024 ^{NS}

Notes: n = number of dataloggers with two years' data for each month; $^+$degrees difference from 185; $^\wedge$metres above sea level; significance levels NS $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 2. Logistic regression models for the voltinism of *Aricia agestis* populations, based on 1. Modeled thermal environment, 2 Thermal connectivity difference (measured to warm and cool *habitats*), 3. Population connectivity difference (measured to bivoltine and univoltine butterfly *populations*).

Model	-2LL #	R^2	Model Chi^2	DF	Significance
1. Thermal environment+	98.76	0.70	113.78	1	<0.001
2. Thermal connectivity difference^	29.53	0.93	183.01	1	<0.001
3. Population connectivity difference*	0	1	212.54	1	<0.001

Notes: n = 68 univoltine populations, 87 bivoltine populations; # -2LL represents model $-2 \log_e$ likelihood; R^2 calculated as in Nagelkerke (1991); +Thermal environment is modeled annual day degrees above 10°C; ^ = (connectivity to patches predicted by model 1 to be bivoltine) – (connectivity to patches predicted to be univoltine); * = (connectivity to bivoltine populations) – (connectivity to univoltine populations).

Table 3. Occupancy status, thermal environment and modeled time to metapopulation extinction of *A. agestis* networks in north Wales.

Network	Status	Area "warm" (ha) #	Area "cool" (ha) #	Median generations to extinction+
1	Bivoltine	201.4	5.3	>200
2	Bivoltine	92.6	1.9	>200
3	Bivoltine	1.3	0	5
4	Unoccupied	0.2	0	2
5	Bivoltine	5.8	0	12.5
6	Univoltine	9.0	35.5	83.5
7	Unoccupied	0.03	2.4	7
8	Univoltine	0.03	11.7	33.5
9	Univoltine	0.1	132.6	>200
10	Univoltine	0.5	0.7	4
11	Unoccupied	0.02	0.1	1
12	Univoltine	0	0.7	4
13	Unoccupied	0.01	2.0	7
14	Unoccupied	4.1	4.6	110

Notes: #Area "warm" has modeled thermal environment > 782 annual day degrees > 10°C , area "cool" has < 782 day degrees > 10°C. +From 100 Incidence Function model simulations starting with all patches occupied.

Table 4. Standardized gene frequency variance F_{ST} among univoltine, bivoltine and all populations of *Aricia* in North Wales for seven polymorphic loci.

Locus	Bivoltine	Univoltine	All
Pgi	0.0870	0.0709	0.0971
Pgm	0.0913	0.0825	0.0926
Me	0.0301	0.0498	0.0693
Got-f	0.1887	0.0436	0.1226
G6pd	0.0889	---	0.1163
αGpd	0.0272	0.0130	0.0373
Mdh-f	0.0772	0.0697	0.0854
All	0.0881	0.0643	0.0934

Figure legends

Figure 1. (a) The distribution of bivoltine (black) and univoltine (gray) populations of *A. agestis* in north Wales, and of suitable but unoccupied habitat patches (white). Symbol sizes greatly exaggerate patch size, and are proportional to log patch area. The line shows the north coast of Wales. Place names and numbers are as referred to in the text. (b) Adult emergence patterns for univoltine (n = 4) and bivoltine (n = 24) populations.

Figure 2. Sample localities for univoltine (open triangles) and bivoltine (closed circles) *A. agestis* populations in North Wales. Gray areas show the distribution of limestone. For a key to sample codes see Materials and Methods.

Figure 3. Peak population density at univoltine (open triangles) and bivoltine (solid circles) populations, plotted against modeled thermal environment (annual modeled day degrees > 10°C).

Figure 4. Modeled thermal environment (annual modeled day degrees > 10°C) against connectivity difference for univoltine (open triangles) and bivoltine (solid circles) populations. Populations with positive connectivity difference were better connected to bivoltine populations than to univoltine populations, and vice-versa. Dashed lines show: horizontal – the modeled thermal environment (782° day degrees > 10°C) at which logistic regression predicts a 50% probability of either voltinism type; vertical – zero connectivity difference (i.e. equally well connected to univoltine and bivoltine populations).

Figure 5. Survival over time of Incidence Function metapopulation simulations for each habitat network. a) networks containing bivoltine *A. agestis*; b) networks containing univoltine *A. agestis* ; c) networks unoccupied by *A. agestis* during the study. Network numbers correspond to those on Figure 1a.

Figure 6. Relationship between genetic similarity (*Nm* +1) and distance (km+1) in *A. agestis* populations in northWales: bivoltine x bivoltine (solid circles), univoltine x univoltine (solid triangles) and bivoltine x univoltine (open circles) pair wise comparisons. Note the use of Log_{10} scales.

Figure 7. Relationship between genetic similarity, *Nm*+1 (of individual populations to within voltinism mean frequency), and connectivity (of patches to within voltinism type patch network) for univoltine (open triangles) and bivoltine (closed circles) populations.

Figure 1.

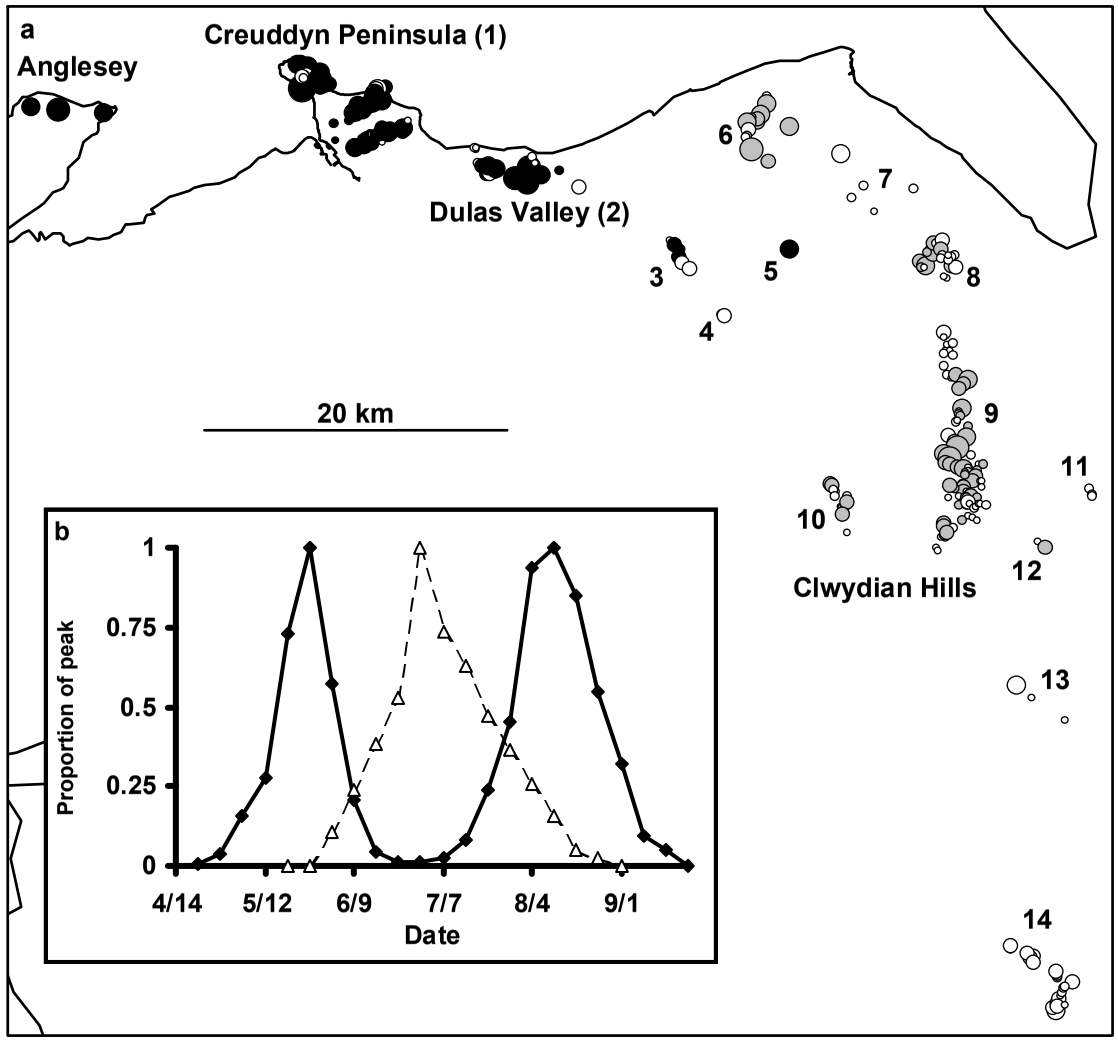
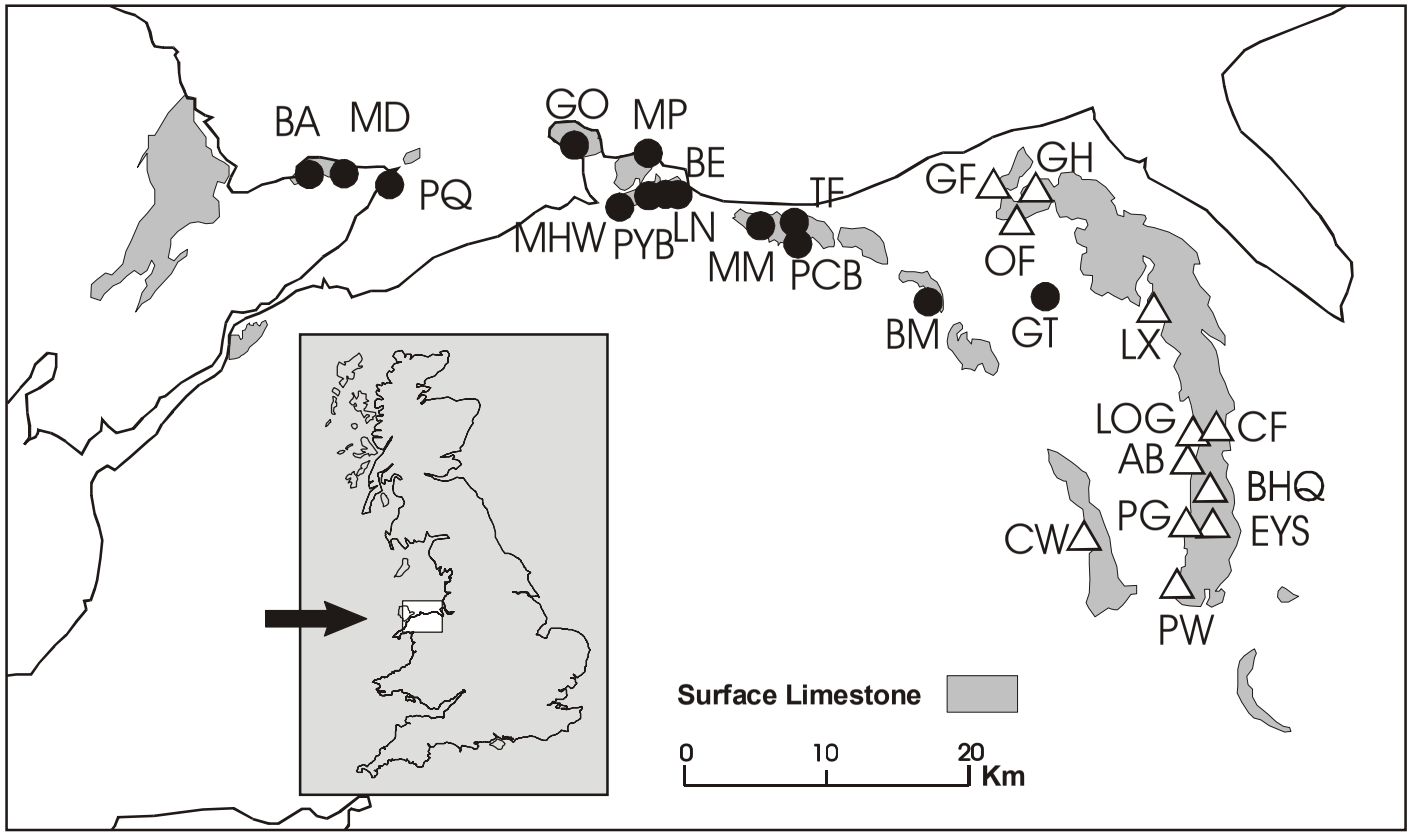

a
Anglesey
Creuddyn Peninsula (1)
Dulas Valley (2)
3
4
5
6
7
8
9
10
11
12
13
14
20 km
Clwydian Hills
b
Proportion of peak
1
0.75
0.5
0.25
0
4/14
5/12
6/9
7/7
8/4
9/1
Date

Figure 2.


BA
MD
PQ
GO
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LN
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PG
EYS
CW
PW
Surface Limestone
0
10
20
Km

Figure 3.

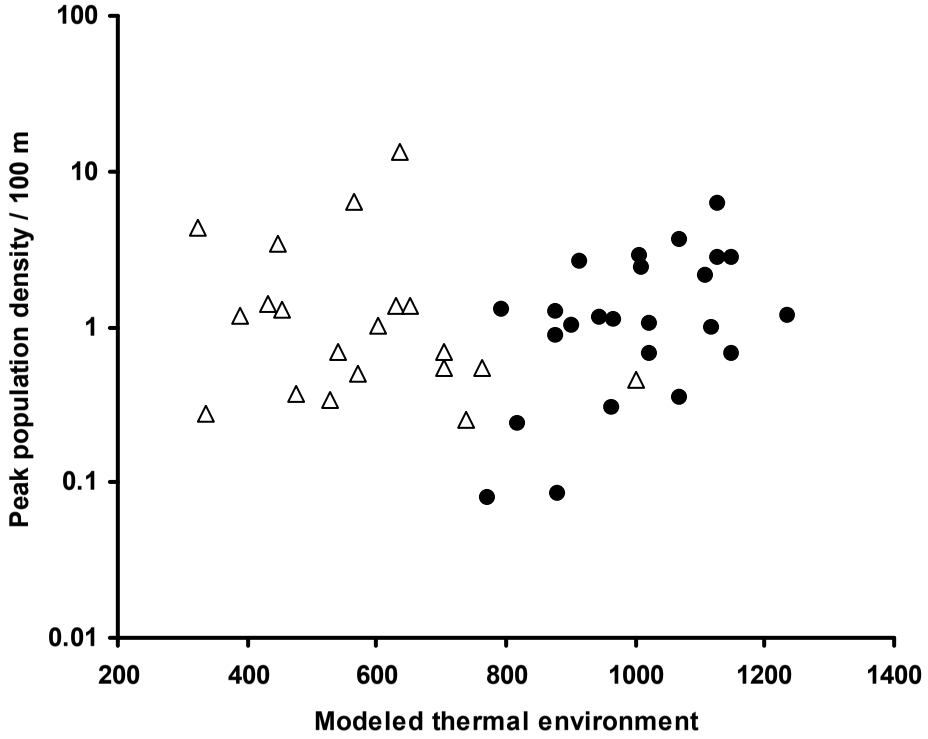

100
10
1
0.1
0.01
Peak population density / 100 m
200
400
600
800
1000
1200
1400
Modeled thermal environment

Figure 4.

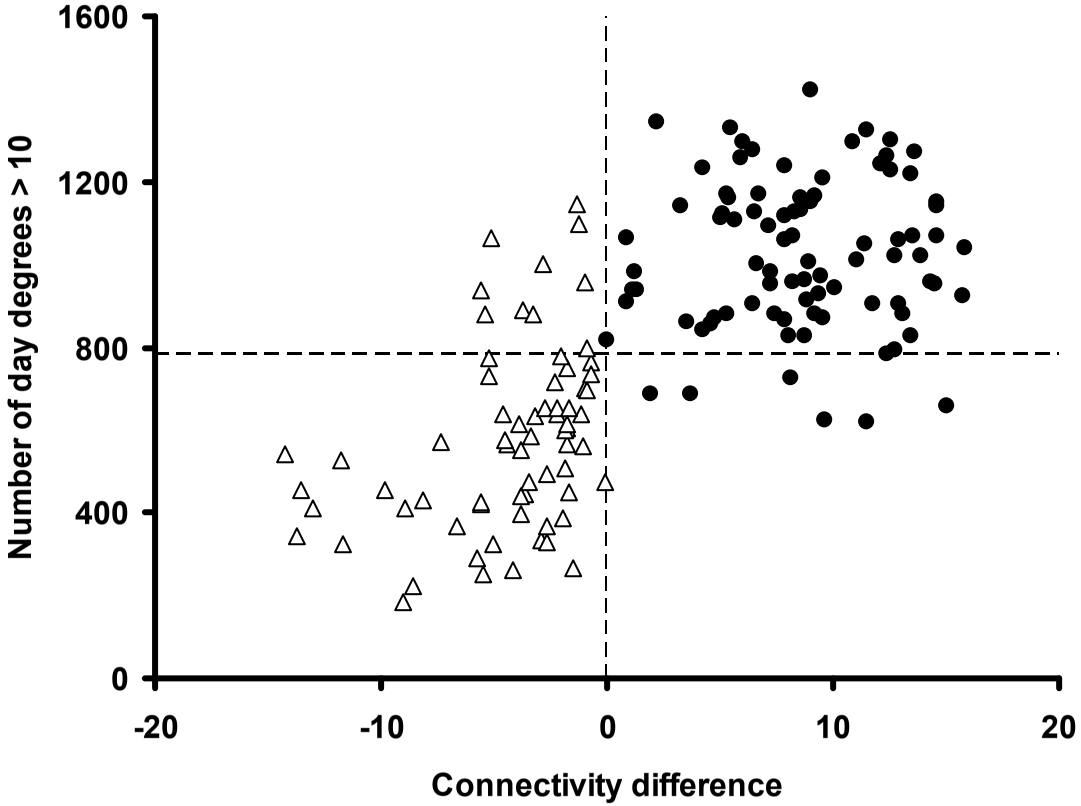

1600
1200
800
400
0
Number of day degrees > 10
-20
-10
0
10
20
Connectivity difference

Figure 5.

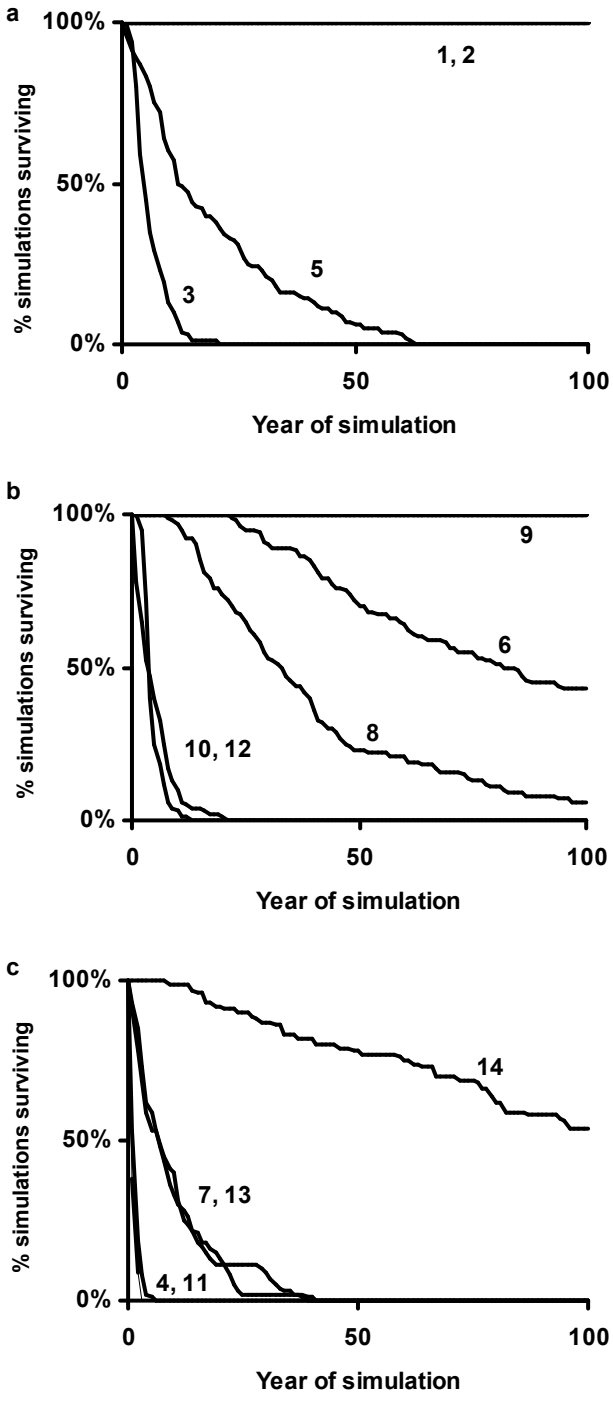

a
100%
50%
0%
% simulations surviving
1, 2
5
3
0
50
100
Year of simulation
b
100%
50%
0%
% simulations surviving
9
6
8
10, 12
0
50
100
Year of simulation
c
100%
50%
0%
% simulations surviving
14
7, 13
4, 11
0
50
100
Year of simulation

Figure 6

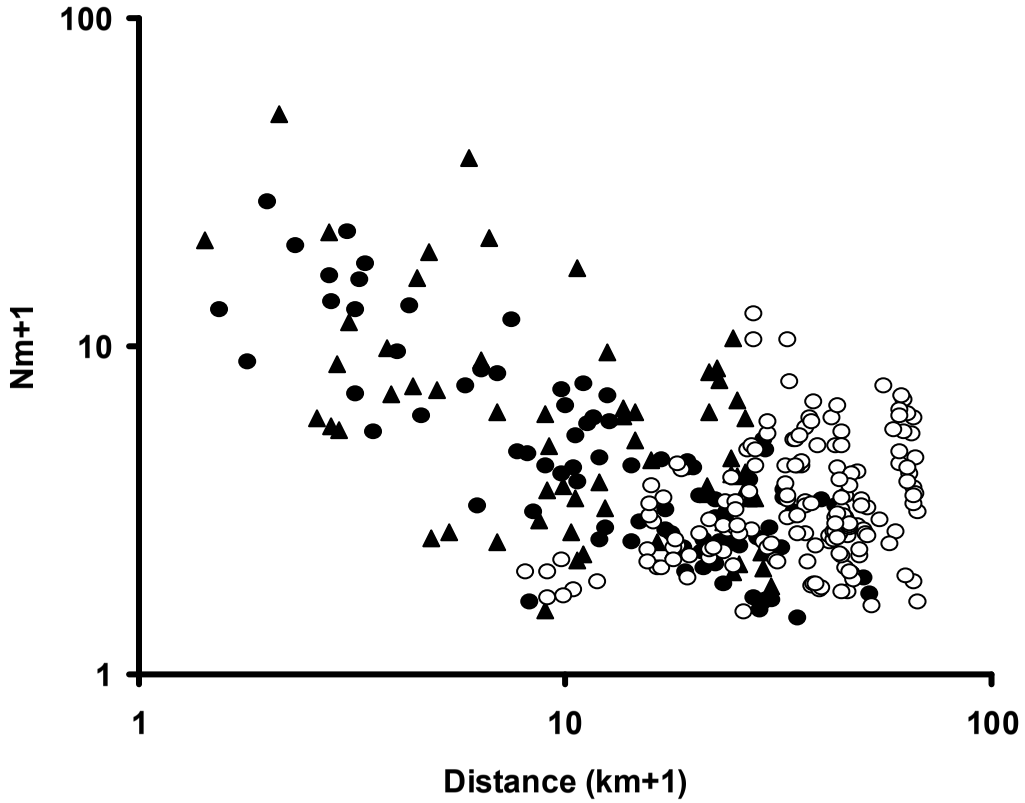
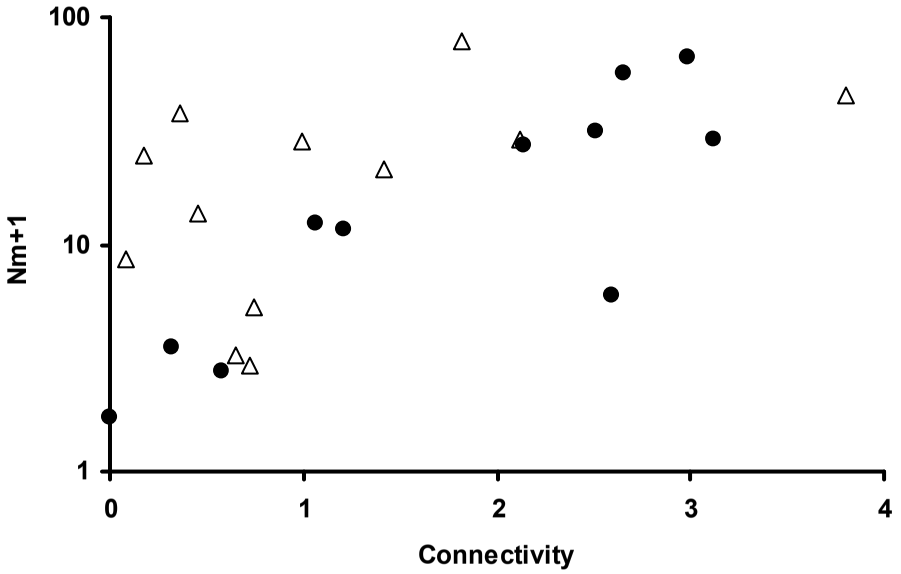


Figure 7



Electronic enhancements.
Appendix material: Allozyme allele frequencies

Allele frequencies for eight polymorphic loci in (A) bivoltine and (B) univoltine populations of *Aricia*. Also provided are the number of individuals sampled (N), observed (Obs.), Expected (Exp.) and mean Heterozygosity (* indicates a significant deviation from Hardy-Weinberg expectations, $P < 0.05$) .

A

	MD	BA	PQ	GO	MP	MHW	PYB	LN	BE	MM	TF	PCB	BM	GT
Pgi														
(N)	50	30	30	52	50	50	59	50	49	25	50	42	51	21
A	.300	.267	.350	.519	.530	.390	.398	.560	.408	.180	.240	.119	.245	.048
B	.240	.183	.350	.240	.150	.140	.314	.200	.214	.200	.220	.286	.010	.310
C	.010	.050	.067	.077	.110	.310	.127	.070	.214	.500	.350	.369	.206	.548
D	.450	.500	.233	.163	.210	.160	.161	.170	.163	.120	.190	.226	.539	.095
E														
Het:														
Obs.	.540	.600	.633	.750	.440*	.620	.831	.680	.735	.680	.780	.762	.647	.571
Exp.	.640	.643	.696	.640	.640	.707	.701	.613	.715	.663	.735	.717	.607	.593
Pgm														
(N)	50	30	30	52	50	50	59	50	49	25	50	42	51	21
A	.200	.267	.233		.020		.017		.010	.080	.090	.083		
B	.650	.683	.667	.769	.530	.410	.542	.580	.439	.520	.580	.488	.912	.405
C	.150	.050	.100	.173	.320	.510	.347	.270	.398	.400	.320	.369		.595
D				.058	.130	.080	.093	.150	.153		.010	.060	.088	
Het:														
Obs.	.380*	.567	.467	.385	.580	.480	.712*	.600	.571	.600	.560	.524*	.176	.429
Exp.	.515	.459	.491	.375	.599	.565	.576	.568	.626	.563	.553	.615	.161	.482
Me														
(N)	50	30	30	51	50	49	51	50	43	25	50	42	51	21
A	.460	.317	.533	.676	.670	.531	.657	.590	.628	.700	.580*	.560	.667	.452
B	.540	.683	.467	.324	.330	.469	.343	.410	.372	.300	.420	.440	.333	.548
Het:														
Obs.	.480	.433	.600	.569	.580	.612	.451	.460	.512	.280	.680	.643	.510	.524
Exp.	.497	.433	.498	.438	.442	.498	.451	.484	.467	.420	.487	.493	.444	.495

Got-f														
(N)	50	30	30	52	50	50	59	50	49	25	50	42	51	21
A														
B	.650	.717	.683	.933	.890	1.000	.958	.920	.959	.740	.850	.810	.618	.238
C	.350	.283	.317	.067	.110		.042	.080	.041	.260	.150	.190	.382	.762
Het:														
Obs.	.540	.300	.367	.135	.180	.000	.085	.160	.082	.360	.260	.238	.490	.381
Exp.	.455	.406	.433	.126	.196	.000	.081	.147	.078	.385	.255	.308	.472	.363
G6pd														
(N)	50	30	30	52	50	50	59	50	49	25	50	42	51	21
A	.260	.167	.083		.100	.020	.068		.133	.040	.010	.060		
B	.740	.833	.917	1.000	.900	.980	.932	1.000	.867	.960	.990	.940	1.000	1.000
C														
Het:														
Obs.	.440	.333	.167	.000	.200	.040	.136	.000	.265	.080	.020	.071	.000	.000
Exp.	.385	.278	.153	.000	.180	.039	.126	.000	.230	.077	.020	.112	.000	.000
αGPD														
(N)	47	30	30	50	50	49	51	50	47	23	50	41	50	21
A	1.000	.883	.900	.940	.980	.990	.961	.980	1.000	.913	.990	.988	.950	1.000
B		.117	.100	.060	.020	.010	.039	.020		.087	.010	.012	.050	
Het:														
Obs.	.000	.167	.200	.120	.040	.020	.078	.040	.000	.174	.020	.024	.100	.000
Exp.	.000	.206	.180	.113	.039	.020	.075	.039	.000	.159	.020	.024	.095	.000
Mdh-f														
(N)	50	30	30	48	48	49	51	50	45	25	50	42	51	20
A														
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.912	1.000
C													.088	
D														
Het:														
Obs.	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.176	.000
Exp.	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.161	.000
Mean Exp.	.313	.303	.306	.211	.262	.229	.251	.231	.265	.283	.259	.284	.243	.242

B

	GF	OF	GH	LX	LOG	CF	AB	PG	BHQ	EYS	CW	PW
Pgi												
(N)	41	54	50	30	20	50	50	60	50	50	18	50
A	.573	.241	.420	.317	.150	.180	.220	.183	.180	.170	.194	.050
B	.220	.370	.280	.183	.550	.370	.350	.550	.410	.580	.389	.570
C	.024		.030	.167	.050		.110	.083	.080	.130	.417	.130
D	.183	.389	.270	.333	.250	.450	.320	.183	.330	.120		.250
E												
Het:												
Obs.	.634	.630	.620	.733	.750	.620	.700	.633	.700	.640	.611	.660
Exp.	.589	.654	.671	.727	.610	.628	.715	.623	.684	.603	.637	.593
Pgm												
(N)	42	54	50	30	20	50	50	60	50	50	18	50
A	.012	.074	.140	.033	.175	.160		.075	.030	.070	.111	.020
B	.440	.491	.410	.583	.525	.580	.470	.575	.600	.570	.806	.150
C	.440	.435	.440	.367	.300	.230	.440	.300	.300	.320	.083	.830
D	.107		.010	.017		.030	.090	.050	.070	.040		
Het:												
Obs.	.548	.407	.620	.500	.600	.580	.600	.567	.380*	.540	.333	.300
Exp.	.600	.564	.619	.524	.604	.584	.577	.571	.544	.566	.332	.288
Me												
(N)	42	54	49	29	19	50	50	59	50	50	18	50
A	.250	.269	.367	.517	.421	.380	.490	.424	.400	.400	.167	.650
B	.750	.731	.633	.483	.579	.620	.510	.576	.600	.600	.833	.350
Het:												
Obs.	.500*	.389	.571	.483	.526	.480	.540	.508	.520	.520	.333	.380
Exp.	.375	.393	.465	.499	.488	.471	.500	.488	.480	.480	.278	.455
Got-f												
(N)	42	53	50	30	20	50	49	60	49	50	18	50
A												
B	.810	.717	.910	.733	.625	.610	.816	.650	.612	.710	.583	.580
C	.190	.283	.090	.267	.375	.390	.184	.350	.388	.290	.417	.420
Het:												
Obs.	.333	.377	.180	.400	.450	.460	.204*	.467	.531	.500	.389	.520
Exp.	.308	.406	.164	.391	.469	.476	.300	.455	.475	.412	.486	.487

G6pd												
(N)	42	54	50	30	20	50	50	60	50	50	18	50
A												
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
C												
Het:												
Obs.	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
Exp.	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
αGPD												
(N)	42	52	50	30	20	50	49	60	49	50	18	49
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.975	1.000	1.000	1.000	1.000
B								.025				
Het:												
Obs.	.000	.000	.000	.000	.000	.000	.000	.050	.000	.000	.000	.000
Exp.	.000	.000	.000	.000	.000	.000	.000	.049	.000	.000	.000	.000
Mdh-f												
(N)	42	53	48	29	19	47	48	60	47	50	18	49
A												
B	1.000	1.000	1.000	1.000	.789	.862	.969	.933	.957	.920	.778	.969
C					.211	.138	.031	.067	.043	.080	.222	.031
D												
Het:												
Obs.	.000	.000	.000	.000	.211	.277	.063	.133	.085	.160	.444	.061
Exp.	.000	.000	.000	.000	.332	.238	.061	.124	.081	.147	.346	.059
Mean Exp.	.234	.252	.240	.268	.313	.300	.269	.289	.283	.276	.260	.235

Appendix material: Metapopulation modeling methods

Incidence function model (IFM)

The Incidence Function Model (Hanski 1994, 1999) is a spatially realistic metapopulation model, based on the assumptions that population extinction rate is negatively related to habitat patch area, and that patch colonization rate is positively related to patch connectivity.

Connectivity for patch i (S_i) is defined as $S_i = \sum \exp(-\alpha d_{ij}) A_j^b$ where α is the slope of the dispersal kernel (the cumulative proportion of per generation dispersal over distance d km or greater corresponds to $\exp^{-\alpha d}$ for a negative exponential dispersal kernel); d_{ij} is the distance to patch i from each occupied source patch j (where $i \neq j$); and A_j is the area (ha) of each patch j. Source patch emigration rate scales with patch area to the power b.

IFM is based on the equation (Hanski 1994, 1999):

$$\ln\left(\frac{J_i}{1-J_i}\right) = -\ln(ey) + x\ln A_i + 2\ln S_i$$

where J_i is the incidence or long-term probability of occupancy of patch i, A_i and S_i are respectively the area and connectivity of patch i, and e, y and x are parameters of extinction and colonization. The annual colonization probability of patch i is $C_i = S_i^2 / (S_i^2 + y^2)$; and annual extinction probability, $E_i = (e / A_i^x)(1-C_i)$. In the model, patches with high connectivity (S_i) are more likely to be colonized than patches with low connectivity, and patches with

large area (A_i) are less likely to go extinct than small patches. Patches with high annual colonization probability are more likely than isolated patches to be "rescued" from extinction, and extinction rate is multiplied by 1-C_i to take account of the rescue effect. The risk of extinction is unity where minimum patch area $A_0 = e^{1/x}$. Thus, A_0, e and x scale the relationship between patch area and extinction, and y determines the relationship between connectivity and colonization probability.

We estimated e, y, and x using 1997 occupancy data for the Creuddyn Peninsula, where we were confident that most if not all habitat patches had been identified (n=89), and where the distribution of limestone grassland has been relatively stable for the last 100 years (9% decline, Cowley et al. 1999). We used the method described in software available from the website at http://www.helsinki.fi/science/metapop/ (see Hanski 1994, 1999, Moilanen 1999). For *A. agestis* we used a negative exponential dispersal kernel of α=2, as has been used for species with similar dispersal rates determined by mark-release-recapture studies (e.g., Hanski 1994, Wahlberg et al. 1996, Wilson and Thomas 2002). Per capita emigration rate tends to decline with increasing patch area (Thomas and Hanski 1997, Hanski et al. 2000), and parameter b was set to 0.5 (A. Moilanen personal communication). Minimum patch area (A_0) was estimated from field observations to be 0.05 ha. The probability of colonization from outside the network was set to zero, because the nearest habitat patches were 5 km away, well beyond the empirically observed maximum dispersal distances of *A. agestis* (Wilson and Thomas 2002).

Regional stochasticity was set to zero, based on data which showed asynchronous dynamics of *A. agestis* in patches less than 1 km apart (Wilson 1999). The Monte Carlo Markov Chain (MCMC) method was used for the final parameter estimation, with 1000 function evaluations in initiation and 4000 function evaluations in estimation (Moilanen 1999).

Incidence function parameters estimated from the distribution of occupied and vacant habitat patches in the Creuddyn Peninsula were: x = 0.604 (95% C.I.s 0.306-1.314), $ey^2 = 0.329$ (95% C.I.s 0.107-0.466), e = 0.164, y = 1.418. To estimate metapopulation persistence, we ran 100 IFM simulations of up to 200 generations for each habitat network, using estimated values of *e*, *y*, and *x*, and the above values for α, *b* and A_0. We set 100% patch occupancy in the first year of each simulation, and zero regional stochasticity, so estimated extinction rates should be conservative.