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Three ways of assessing metapopulation structure in the butterfly *Plebejus argus*

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Abstract. 1. Three independent methods were used to investigate population structure in the butterfly *Plebejus argus*. First, migration and dispersal ability were measured by mark–release–recapture in seven adjacent habitat patches, and by release of butterflies in unoccupied habitat. Secondly, colonization of newly created habitat was observed over 7 years. Finally, genetic differentiation of local populations within a metapopulation was investigated. Sampled local populations included parts of the mark–release–recapture study area.

2. *Plebejus argus* is relatively sedentary: the maximum movement detected was 395 m, and only 2% of individuals moved further than 100 m between recaptures on different days. None the less, adjacent local populations in the mark–release–recapture study area were linked by occasional migration, with $\top$ 1.4% of individuals moving between patches separated by 13–200 m.

3. Despite low mobility, observed colonizations occurred rapidly over distances of $\leqslant$ 1 km. Because *P. argus* occurs at high population densities, 1.4% migration can generate enough migrants to colonize newly suitable habitat quickly at this spatial scale.

4. Mark–release–recapture data were used to predict that there would be limited genetic differentiation through drift between local populations at this spatial scale. The prediction was supported by allele frequency data for the same local populations.

5. Genetic differentiation often indicates higher levels of migration than are revealed by the movements of marked individuals. This study shows that when experimental releases and extensive marking are undertaken in areas that are large relative to most movements, indirect measures of gene flow and direct measures of dispersal can concur.

6. Evidence from the three different approaches was complementary, indicating that *P. argus* occurs as metapopulations within the study area.

Key words. Allozymes, conservation, dispersal, genetic differentiation, mark–release–recapture, migration, population structure.

Introduction

Metapopulations are characterized by occasional migration among extinction-prone local populations (Levins, 1970; Gilpin & Hanski, 1991; Hanski & Gilpin, 1997). Many species living in patchy or fragmented habitats (e.g. at least 50% of British butterfly species: Hanski & Thomas, 1994) are thought to have some attributes of a metapopulation structure. This has led to increasing interest in the properties of metapopulations, such as the minimum number of habitat patches needed to ensure persistence, and the probability of colonization of particular habitat patches as they are created or restored by conservation management (Hanski *et al*., 1996; Hanski & Gilpin, 1997). However, there is debate over the number of metapopulations, if any, that fit the classical Levins (1970) structure. Harrison (1994), Harrison & Taylor (1997) and others have invited critical assessment of the assumptions and predictions of metapopulation theory if the concept is to be applied to conservation problems.

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There are three general approaches to assessing how insect populations are structured: analysis of spatial pattern, direct measurement of the frequency of migration among local populations, and analysis of patterns of genetic differentiation.

Analysis of spatial pattern

Much of the existing evidence for butterfly population structure is based on observation of spatial patterns of occupied and unoccupied habitat, and frequencies of extinction and colonization events (Hanski & Gilpin, 1997; Thomas & Hanski, 1997). However, relying solely on analysis of spatial patterns may be misleading. For example, aggregations of individuals may occur in mobile species ('patchy populations': Harrison & Taylor, 1997), giving the false impression of local populations. The appearance and disappearance of all individuals from such transient aggregations can be misinterpreted as repeated demographic colonizations and extinctions. Conversely, aggregations may be entirely separate populations of sedentary species that do not exchange individuals at all ('relictual' or 'non-equilibrium' populations: Harrison & Taylor, 1997).

Direct measures of migration

Measurement of migration through the marking and recapture of individuals provides the most direct indication of the frequency of movements among patches (e.g. Hanski *et al*., 1994; Hill *et al*., 1996). However, long-distance migrants may emigrate undetected, and such movements are likely to be underestimated (Koenig *et al*., 1996). Because of the practical difficulties of documenting long-distance movements empirically, most studies have to make assumptions about migration rates, permitting different researchers to reach widely different conclusions from the same data.

Genetic differentiation

Measures of genetic differentiation integrate levels of gene flow, and hence migration, over many generations. This method has the advantage that even very rare long-distance dispersal events are recorded (Slatkin, 1985), and it usually gives increased estimates of migration (Slatkin, 1985; Koenig *et al*., 1996). In metapopulations, genetic differentiation of neutral markers through drift may typically be slight: if migration among habitat patches is frequent enough to ensure metapopulation persistence, gene flow is likely to be high enough to counteract drift in neutral markers (Lande, 1988). However, deductions about migration rates depend on assumptions about equilibrium population dynamics and rates of colonization and extinction. For example, repeated extinction and colonization might be expected to increase differentiation if the recolonizing individuals are few in number and genetically homogeneous (Whitlock, 1992; Hastings & Harrison, 1994).

Given the potential drawbacks of each approach, evidence from at least two different sources should be considered together to provide the best indication of how populations are structured (Slatkin, 1994). In this paper, data are presented on spatial patterns, migration and genetic differentiation in the silver-studded blue butterfly, *Plebejus argus* L. (Lepidoptera, Lycaenidae). Do the three sources of evidence lead to similar conclusions about population structure in this species?

Methods

Study species

Plebejus argus is a small (25–32 mm wingspan) lycaenid butterfly. It is a patchily distributed and declining species in the U.K., having almost disappeared from ⊤ 80% of its former range (Thomas, 1994). It occurs mainly on lowland heaths, where breeding is concentrated in early successional vegetation. Repeated disturbance, followed by succession, creates a 'shifting mosaic' of suitable habitat, which *P. argus* populations track (Thomas, 1985a,b; Ravenscroft, 1990; Thomas & Harrison, 1992). In North Wales, *P. argus* also occurs on limestone grasslands where there is a sparse, grazed sward (Thomas, 1985a,b).

Mark–release–recapture (Clwyd)

To measure the frequency of migration among local populations, mark–release–recapture was carried out in the Dulas Valley (Clwyd, North Wales, SH9176). Here, *P. argus* occupies scattered habitat patches within an area of limestone grassland, and local populations are subject to periodic extinction and colonization (Thomas & Harrison, 1992). *Plebejus argus* was historically absent from this area, but was introduced experimentally to a single patch in 1942 (Marchant, 1956). By 1994, *P. argus* had spread to occupy nineteen distinct habitat patches up to 2.6 km from the release point (Fig. 1). The spread of *P. argus* through the system indicates that occupied patches are connected by dispersal, at least on a timescale of decades. The longest single colonization 'jump' between habitat patches was 600 m, while apparently suitable habitat isolated by more than 1–3 km remains unoccupied (Thomas & Harrison, 1992). For mark–release–recapture, a relatively isolated cluster of seven occupied patches at the south-east edge of the system (patches A–G; Fig. 1) was chosen. On the basis of a brief, small-scale mark–release–recapture study carried out previously, this was considered to be a large area relative to the likely movements of *P. argus*. Patches were defined as separate if they were isolated by at least 20 m from other suitable habitat (or 10 m if a scrub barrier was present) (Thomas & Harrison, 1992). Patches varied in size from 0.07 to 3.02 ha, and were 13–200 m from the nearest neighbouring patch (Table 1).

Mark–release–recapture fieldwork took place on 20 days between 15 June and 7 July 1994, corresponding to the early and peak flight period. Adult daily survival has been estimated at 0.8 (C. D. Thomas, 1983); assuming constant daily

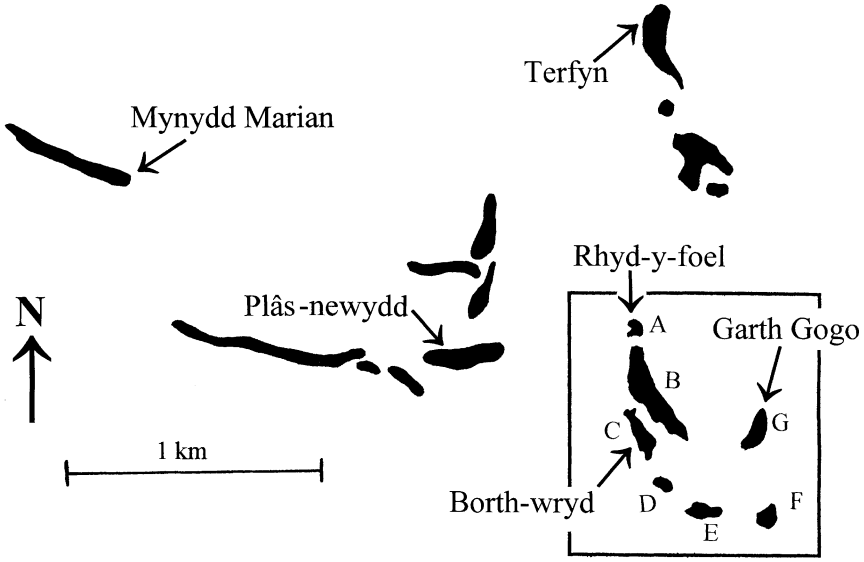

Fig. 1. The Dulas Valley metapopulation, updated from Thomas & Harrison (1992). Patches occupied in 1994 are shown as black areas (see text for definition of discrete patches). The patches in the box, labelled A to G, were included in the mark–release–recapture study. Allozyme frequencies were investigated at the six named patches. Patch A is the site of the original release in 1942.

Table 1. Summary information on habitat patches occupied by *Plebejus argus* within the Dulas Valley mark–release–recapture study area. Patch locations are shown in Fig. 1. Population estimates are approximate (see text) and total population sizes are likely to be approximately three times the value given (J. A. Thomas 1983). Edge is the minimum patch edge to patch edge distance. Centre is the distance between the centres of patch pairs.

	Patch		Distance to nearest neighbouring patch (m)	
Name	Area (ha)	Estimated peak population size	Edge	Centre
A	0.22	144	30	280
B	3.02	1975	13	158
C	1.02	667	13	158
D	0.07	46	35	100
E	0.38	248	35	100
F	0.18	118	188	288
G	0.47	307	200	315

probability of survival, estimated longevity is 3–4 days, with ⊤ 10% surviving 10 days, and 1% surviving 20 days. Thus the duration of mark–release–recapture fieldwork was sufficient to gain a reasonable estimate of per-generation migration. There was a single daily marking period of up to 4 person hours (scaled by patch area) in each habitat patch. *Plebejus argus* were netted and each individual was given a unique mark on the ventral wing surface, using Staedtler Lumocolour® permanent marker pens, as well as a site-specific mark using acrylic paint. Double marking reduced the possibility of erroneous records of movements between patches. No attempt was made to mark butterflies in areas between habitat patches, where *P. argus* was hardly ever seen. Each butterfly was released at the point of capture immediately after marking. The location at which each butterfly was marked and the location of all subsequent recaptures were recorded on 1 : 1000 maps of each patch.

Experimental release (Powys)

To measure within-habitat, per-generation dispersal, an artificial release of *P. argus* was carried out in unoccupied limestone grassland, where the habitat fell within the criteria used to define suitable breeding habitat (Thomas, 1985b). Two hundred and fifty-nine (146 male and 113 female) adult *P. argus* were released on 15 June 1993. To minimize the effects of handling, butterflies were captured and transported while they were inactive, and released in the evening at a central roost site. On limestone, *P. argus* often roost in dense aggregations (C. D. Thomas, 1983), so the release density was not unnaturally high. Tendency to disperse may change during an adult's lifetime (Johnson, 1969; Mallet, 1986), so only recently emerged butterflies (as indicated by lack of wing wear) were released. On each of the 8 days following the release, and after 10, 14 and 23 days, the area around the release site was

searched thoroughly. Small flags at 10-m intervals were used to mark out a grid so that the position of individuals could be plotted accurately. The habitat was searched systematically to reduce the likelihood of recording an individual more than once on any one day. However, because rates of movement are so low (see below), such double recording is unlikely. All suitable habitat up to 200 m from the release point was searched with equal recording effort per unit area. Suitable habitat between 200 m and 1 km away was searched with ⊤ 50% recording effort. Habitat beyond 1 km was not searched, because 1 km was further than the longest known colonization on limestone in North Wales (Thomas & Harrison, 1992).

Artificial releases overcome two major drawbacks of mark–release–recapture methods for measuring dispersal: the potential problems of marking and handling butterflies (e.g. Morton, 1984), and the difficulty of detecting small numbers of marked individuals among a large number of unmarked conspecifics. Releases should make it easier to detect long distance movements, which are expected to be relatively rare but are of great importance in the context of gene flow and colonization. The site was a linear strip of habitat, 30–50 m wide and over 2 km in length, making it unlikely that dispersal in the longest dimension would be truncated by the size of available habitat (see Results).

Habitat tracking (Sussex)

Spatial patterns of *P. argus* colonization were investigated at Iping and Stedham Commons (West Sussex, SU 856219). Iping and Stedham form a continuous heathland area of ⊤ 125 ha. The site has historically supported a *P. argus* population, but by 1989 had become dominated by dense stands of uniformly aged heather, *Calluna vulgaris* (L.) Hull, that had grown up following an extensive fire in 1976. These stands were unsuitable as breeding habitat for *P. argus*, and the butterfly had become restricted to a few small patches where early successional vegetation persisted along paths and firebreaks (T. P. R. Crane, unpublished data). In an attempt to conserve *P. argus*, a programme of heather mowing (with subsequent removal of brushwood), scrub clearance and herbicide spraying of invading bracken, *Pteridium aquilinum* (L.) Kuhn, was introduced in 1990. Additional areas were burned in 1989. Annual changes in the distribution of *P. argus* resulting from this management are described. The whole heathland area was searched each year between 1988 and 1995, in good weather during the flight period. The position and extent of occupied habitat was plotted on a 1 : 10 000 Ordnance Survey map.

Genetic differentiation (Clwyd)

The degree of genetic differentiation of local populations was measured at six patches of habitat within the Dulas Valley system (Rhyd-y-foel, Borth-wryd, Garth Gogo, Terfyn, Mynydd Marian and Plâs-newydd; Fig. 1). Three of these patches (Rhyd-y-foel, Borth-wryd and Garth Gogo) were included in the mark–release–recapture study as patches A, C and G, respectively. Male *P. argus* were frozen in the field in liquid nitrogen and stored at –80 °C. Alleles were separated using standard cellulose acetate plates using methods given by Emelianov *et al*. (1995). Eleven allozyme loci were polymorphic and scorable (Table 2). Samples from the six patches were scored for allele frequencies. Allele frequencies for these populations are given by Brookes *et al*. (1997). Differences in gene frequencies were measured using estimates of F_{ST} (Wright, 1969). The value of F_{ST} varies between 0 (equal gene frequencies in different local populations) and 1 (completely fixed differences between local populations). The method of Weir & Cockerham (1984) was used to calculate θ as an estimate of F_{ST} using the programme FSTAT version 1.2 (Goudet, 1995). Ninety-five per cent confidence limits on these estimates were obtained by bootstrapping over loci (Weir, 1990).

Data analysis

In the mark–release–recapture experiment, recaptures of individuals marked in the same patch on previous days (‘residents’), and recaptures of butterflies marked in different patches (‘transfers’) were recorded. Recaptures of individuals on the day of marking were not included, to avoid underestimating dispersal as a result of the temporary effects of marking and handling (Gall, 1984). Single and multiple recaptures are distinguished by recognizing ‘individuals recaptured’ and ‘recapture events’, respectively. If n individuals are recaptured at least once in site X, but p of these are recaptured on 2 days and q on 3 days, then there are $(n + p + q)$ recapture events. Similarly, ‘individuals transferring’ and ‘transfer events’ are distinguished: if a butterfly moves from site X to site Y, but is recaptured in site Y on a different days, there are a transfer events. The distinction may be of biological significance: 50% migration could be 50% of individuals migrating early in adult life, or migration of every individual after 50% of its life.

In the experimental release, displacements from the release point were pooled for all days of recording. Although the data between days are not independent, the resulting distribution of observations (combining data for all days) gives a measure of the net distribution of individuals over the whole adult lifetime. From both a demographic and a genetic point of view, this is of more interest than the distances moved by a sample of individuals at any one time (when one observation would equal one individual), because it gives a better indication of the proportion of an average individual’s lifetime reproductive effort likely to be invested at different distances from its point of emergence. The distribution of distances moved in one direction (the less constrained direction, along the hillside) was used to calculate the dispersal parameter σ (the standard deviation of per-generation dispersal in one dimension). Use of σ to infer genetic population structure may often assume that the distribution of distances moved is a bivariate normal, which is rarely the case (Wright, 1969; Crawford, 1984). Dispersal usually has a leptokurtic distribution, and σ is a poor

Table 2. Estimates of F_{st} (θ) for six populations in the Dulas Valley, and for the three local populations (Rhyd-y-foel, Borth-wryd, Garth Gogo) in which mark–release–recapture was carried out. Full names for isoenzyme loci are: *Pgm*, phosphoglucomutase; *Gpi*, glucose-6-phosphate isomerase; *Mpi*, mannose-6-phosphate isomerase; *Me*, malic enzyme; *Sdh*, sorbitol dehydrogenase; *Idh*, isocitrate dehydrogenase; *Fum*, fumarate hydratase; *Pp*, peptidase (phe-pro); *Lgg-f*, *Lgg-s*, peptidase (leu-gly gly); *Ak-f*, adenyl kinase. Sample sizes (individuals analysed per locus) are: Rhyd-y-foel, $n \geqslant 31$; Borth-wryd, $n \geqslant 35$; Garth Gogo, $n \geqslant 25$; Terfyn, $n \geqslant 74$; Mynydd Marian, $n \geqslant 72$; Plâs-newydd, $n \geqslant 67$.

	θ in six Dulas Valley local populations	θ in three local populations within the mark–release–recapture study area
Total individuals tested	396	138
Locus		
Pgm	0.011	0.007
Gpi	0.012	0.008
Mpi	0.017	–0.006
Me	0.027	0.088
Sdh	0.004	0.002
Idh	0.000	–0.001
Fum	0.096	0.134
Pp	0.005	–0.003
Lgg-f	0.015	0.024
Lgg-s	–0.003	0.001
Ak-f	0.001	–0.014
Over all loci	0.028	0.039
Lower 95% confidence limits	(0.009)	(–0.002)
Upper 95% confidence limits	(0.056)	(0.084)
All loci excluding *Fum*	0.014	0.019
Lower 95% confidence limits	(0.006)	(–0.005)
Upper 95% confidence limits	(0.020)	(0.053)

estimator of colonization ability. However, σ is useful in assessing the consequences of dispersal for the variation of neutral markers provided that the leptokurtosis is not extreme (Wright, 1969).

Results

Numbers marked and recaptured within local populations (Clwyd)

In total, 3924 butterflies (2348 males and 1576 females) were marked. One-thousand, two-hundred and six (31%) individuals were recaptured at least once in the habitat patch in which they were marked, and there were 1713 recapture events (Table 3). The maximum number of times an individual was recaptured was six (a male) and the same individual had the longest time between its first and last captures (17 days). Males are behaviourally and physically more conspicuous, and were more likely to be recaptured than females in terms of both individuals ($\chi^2 = 80.1$, d.f. = 1, $P < 0.001$) and recapture events ($\chi^2 = 178.2$, d.f. = 1, $P < 0.001$).

Daily recapture frequencies were too low in most habitat patches to allow calculation of reliable estimates of local population size. However, for one of the largest local populations, C, daily population sizes could be estimated using the Jolly–Seber method and a mean daily population estimate for the 6 consecutive days of peak abundance was calculated. Population densities in all patches studied were high, and are uncorrelated with patch areas (Hanski & Thomas, 1994), so peak population sizes for the remaining sites were estimated by assuming similar peak population densities (654 butterflies ha^{-1}) in all patches. The resulting estimates (Table 1) are approximate, as the mark–release–recapture investigation was designed to investigate migration rather than to calculate population sizes. However, even approximate estimates of population size are often of value (Watt *et al*., 1977); in the current context they allow estimation of the potential for genetic drift and translation of migration rates into approximate numbers of migrants. As a rule of thumb, total adult population sizes are roughly three times the population size at the peak of the flight period (C. D. Thomas, 1983; J. A. Thomas, 1983), so ⊤ 10 000 adult *P. argus* may have been present in the mark–release–recapture study area in 1994.

Migration among local populations (Clwyd)

Sixteen individuals (1.3%) transferred between local populations, with one individual transferring twice to give a total of seventeen (1.4%) transfers. In terms of transfer events, twenty-six recapture events were of individuals that had transferred between patches and 1713 were of residents, giving an estimated migration rate of 1.5% (Table 3; Fig. 2). The slightly different ways of calculating the frequency of migration gave very similar results, although this may not always be the

Table 3. Summary of the results of the Dulas Valley mark–release–recapture. Recapture figures refer to recaptures on days subsequent to marking or previous capture.

			Recaptured			
			Residents		Transfers	
Patch	Individuals marked		Individuals	Events	Individuals	Events
A	Males	246	68	91	0	0
	Females	202	33	36	0	0
	Total	448	101	127	0	0
B	Males	1226	384	484	6	6
	Females	760	117	125	0	0
	Total	1986	501	609	6	6
C	Males	471	208	316	3	3
	Females	304	61	74	3	3
	Total	775	269	390	6	6
D	Males	18	11	17	1	1
	Females	10	2	2	0	0
	Total	28	13	19	1	1
E	Males	125	53	108	4	13
	Females	83	25	33	0	0
	Total	208	78	141	4	13
F	Males	37	25	51	0	0
	Females	14	9	19	0	0
	Total	51	34	70	0	0
G	Males	225	144	273	0	0
	Females	203	66	84	0	0
	Total	428	210	357	0	0
All	Males	2348	893	1340	14	23
patches	Females	1576	313	373	3	3
	Total	3924	1206	1713	17	26

case. Of the sixteen transfers, three involved females; given the higher probability of capturing and recapturing males within habitat patches (see above), males and females did not differ in their probability of transferring between habitat patches ($\chi^2 = 0.60$, d.f. = 1, NS). Distances moved by individuals transferring between habitat patches ranged from 37 to 395 m, although movements of up to 1.2 km could have been detected had these occurred. Immigrants or emigrants were detected for all sites except F.

Experimental release

After an initial 2-day period of dull weather, during which recording did not take place (since individuals remained at the release point), 637 observations of *P. argus* were made. Observations of males (463) were more frequent than observations of females (174; $\chi^2 = 22.5$, d.f. = 1, $P < 0.001$). Males moved significantly further than females (Fig. 3; median displacement from release site for males = 28 m, for females = 8 m; Mann–Whitney $W = 160412$, $n_1 = 463$, $n_2 = 174$; $P < 0.001$). The maximum recorded displacement from the release point was 265 m (a male, 7 days after the release). σ (the standard deviation of dispersal in one dimension) was calculated as 42 m for males and 29 m for females. An unbiased overall value for σ was calculated as

$$\sigma_{P.\ argus} = \sqrt{[(\sigma^2_{\text{male}} + \sigma^2_{\text{female}})/2]} = 36 \text{ m}.$$

Habitat tracking (Sussex)

Following habitat improvement at Iping and Stedham Commons, and using the same criteria as before to define patches, *P. argus* had spread from just twelve 'refuge' patches in 1989 to occupy twenty-two patches by 1995 (Fig. 4). The total area occupied increased by 360% over this period. Time to colonization was longer for habitat subject to burning (median time to colonization = 4 years) than for habitat that was mowed (median time to colonization = 1.75 years; Mann–Whitney, $W = 39$, $n_1 = 9$, $n_2 = 8$, $P = 0.002$). The maximum colonization distance was 865 m, assuming colonization from

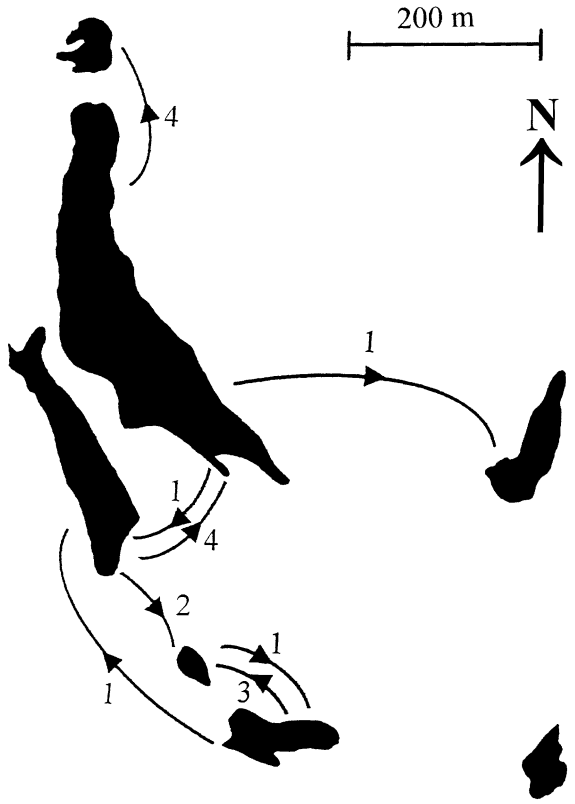

Fig. 2. Observed movements of individuals between local populations in the Dulas Valley mark–release–recapture study area. The numbers next to arrows indicate the number of individuals transferring in each direction between patch pairs. One-thousand two-hundred and six individuals were recaptured in the habitat patch in which they were marked.

the nearest occupied patch. This is close to the maximum distance possible within the study area.

Genetic differentiation (Clwyd)

Allozyme data (Table 2) reveal low levels of genetic variability among subpopulations, giving F_{ST} estimates of $\theta = 0.039$ (bootstrap 99% confidence limits –0.002, 0.084) for the three patches sampled within the mark–release–recapture study area, and $\theta = 0.028$ (bootstrap 99% confidence limits 0.009, 0.056) for the six patches sampled throughout the Dulas Valley system. Drift should affect each locus similarly, but much of the differentiation is the consequence of variation in allele frequencies at a single locus, fumarase (Table 2). Excluding fumarase from the analyses resulted in estimates for F_{ST} of $\theta = 0.019$ (bootstrap 99% confidence limits –0.005, 0.053) for the three patches within the mark–release–recapture study area and $\theta = 0.014$ (bootstrap 99% confidence limits 0.006, 0.020) for the six sampled patches in the Dulas Valley. Whether or not fumarase is included, the confidence limits for Rhyd-y-foel, Borth-wryd and Garth Gogo include zero, so there is little evidence of genetic differentiation of local populations within

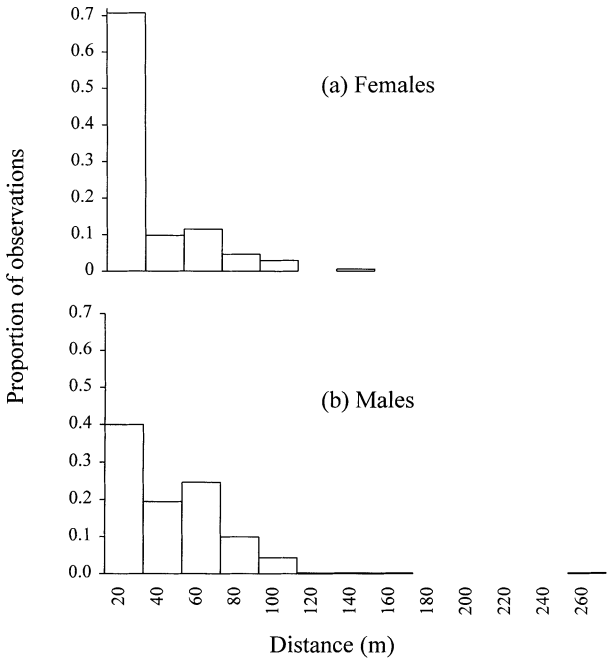

Fig. 3. The distributions of within-habitat dispersal distances from the release experiment for (a) females and (b) males.

the mark–release–recapture study area. When all six locations are included there is weak but significant genetic differentiation, with or without fumarase.

Calculation of expected genetic differentiation of local populations

Data on population sizes from mark–release–recapture, and on per-generation dispersal from the experimental release, allow calculation of whether differentiation between local populations in the mark–release–recapture system is expected through genetic drift. Wright's (1943) neighbourhood size, Nb, is defined as the population occupying an area from which the parents of individuals born near the centre can be treated as if drawn at random. For a two-dimensional habitat, Nb is calculated as:

$$Nb = 4\pi\sigma^2 d$$

where σ is the standard deviation of per-generation dispersal in one dimension, and d is the genetically effective population density. Neighbourhood size is similar to Hanski & Gilpin's (1991) 'local population' (Mallet, 1996), since, if dispersal has a bivariate normal distribution, 86.5% of the progeny of a parent giving birth at the centre will be found within a neighbourhood with radius 2σ. The calculated population density in the Dulas Valley was 1962 ha^{-1}, and from the release experiment, $\sigma = 36$ m. By expressing σ in the appropriate units, Nb can be estimated as $Nb = (4 \times \pi \times 0.36^2 \times 1962) = 3195$ individuals. Such a population would occupy an area of $\top$ 3195/1962 = 1.6 ha. In the study system, only habitat patch B is larger than 1.6 ha, so each patch, except patch B, can be

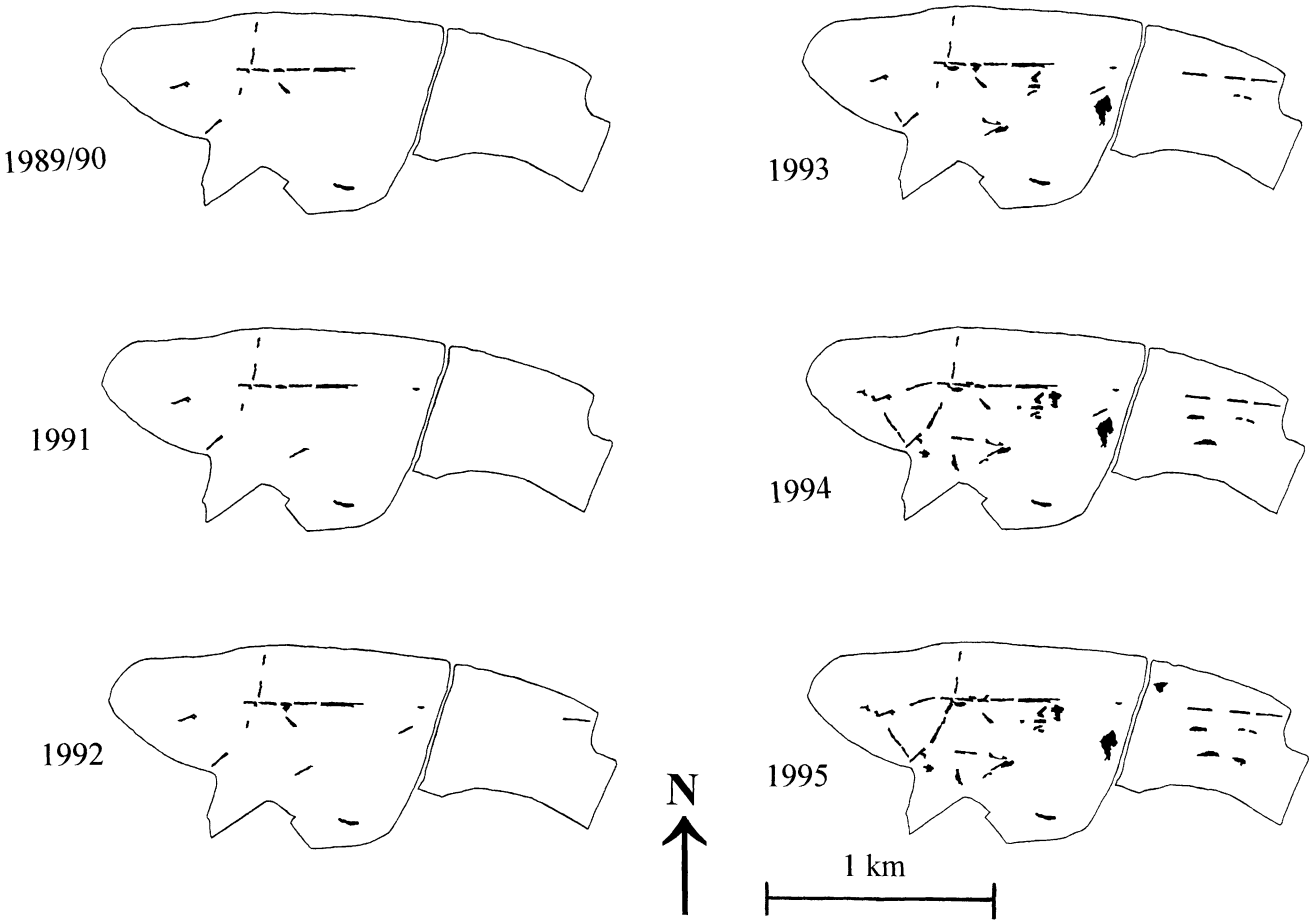

Fig. 4. Changes in the distribution of *Plebejus argus* on Iping and Stedham Commons between 1989 and 1995. Black areas represent occupied habitat, and the boundary line marks the limits of heathland vegetation. There was no change in the distribution of *P. argus* between 1989 and 1990.

treated as occupied by a single, panmictic local population. F_{ST} is a measure of the genetic differentiation of such local populations, defined as the correlation between random gametes within local populations relative to the total population (Wright, 1969). In an 'island model' of population structure, a balance between local drift and gene flow will give, at equilibrium:

$F_{ST} \top 1/(1 + 4N_e m_)$

where N_e is the genetically effective local population size and m is the proportion of individuals migrating between populations per generation (Wright, 1969). Although this result was obtained for an unrealistic 'island' population structure, it holds approximately true in more general situations such as 'stepping stone' and continuous population models (Slatkin & Barton, 1989). Numbers of *P. argus* immigrants captured during mark–release–recapture are too low to allow calculation of accurate immigration rates separately for different patches, so m is assumed to be similar in all local populations (there were in fact no significant differences in numbers or proportions of recorded migrants for different patches). The observed value of m for the system as a whole was 0.014, and the average local population size, N, is $\top$ 10515/7 = 1502. N_e is likely to be smaller than the census population size, N, because of greater than Poisson variations in reproductive success among individuals (Nunney, 1993), and has been estimated as $0.5N$ in *P. argus* (Brookes *et al*., 1997). Thus on average,

$F_{ST} \top 1/\{1 + (4 \times 751 \times 0.014)\} \top 0.024$

This 'direct' (Slatkin, 1985) estimate, based on data on migration rates and within-habitat dispersal, is close to the 'indirect' value estimated independently from allozyme data for the same set of local populations.

Discussion

Direct measurements of dispersal

Practical constraints mean that direct evidence on the frequency of migration between patches of habitat (of the kind gathered for *P. argus*) has been quantified for relatively few butterfly species (e.g. Ehrlich, 1961; Harrison, 1989; Baguette & Nève, 1994; Hanski *et al*., 1994; Hill *et al*., 1996). Results presented here confirm that *P. argus* is relatively sedentary,

and support the statement, based on analysis of spatial patterns, that 'migration over distances greater than 1 km is very rare' for limestone populations in the U.K. (Thomas & Harrison, 1992). Even with the detailed data collected on migration, it is impossible to quantify how rare such long-distance movements are, because this would require an extrapolation well beyond the range of the data. Indirect sources of evidence (spatial patterns of occupancy, colonization and genetic differentiation) may provide better ways of detecting long distance movements.

Despite such limited dispersal, local migration was detected for six of the seven local populations in the mark–release–recapture study system. The only habitat patch into which no immigration was detected was the most isolated one (F), and the only patches from which emigrants were not detected were the two most isolated patches (F and G). Of these, F had a small population size and would not be expected to act as a source of many transfers. Although the percentage of individuals migrating between habitat patches was low (1.4%), the total population size of *P. argus* in the system was so large ($\top$ 10 000) that some individuals ($\top$ 100–200) will migrate between patches during their lifetime. It seems likely that even the most isolated local populations within the mark–release–recapture study area receive immigrants regularly, probably every generation, although in years when weather during the flight period is poor, or population sizes are low, numbers of migrants may be much reduced (e.g. Watt *et al*., 1977).

Direct measures of dispersal ability have their disadvantages. Marking individuals may affect their mobility, and movements early in adult life may be missed. In this study the concordance of dispersal distances of marked (mark–release–recapture) and unmarked (experimental release) individuals suggests that dispersal was not affected seriously by marking. Perhaps a more serious problem is that marked individuals moving long distances have a lower probability of recapture and may leave the study area entirely, leading to underestimates of dispersal ability (Slatkin, 1985; Koenig *et al*., 1996). In both the mark–release–recapture and the experimental release, the dimensions of the study areas were so large relative to typical movements that this is unlikely to have been a serious problem. The longest movements detected were 395 m (mark–release–recapture) and 265 m (experimental release), even though movements of up to 1 km could have been recorded in both cases. Similarly, the estimate of migration rates is likely to be unbiased, because mark–release–recapture was carried out in *all* local populations in a relatively isolated subset of habitat patches. If mark–release–recapture was carried out in a subset of patches within a larger system with high levels of linkage, migration rates might be underestimated because of migration of marked individuals to patches outside the study area, and of unmarked individuals into the study area from elsewhere in the system. In the case of the present study area, mark–release–recapture results suggest that no more than one or two individuals per generation would be expected to transfer successfully between the mark–release–recapture study area and the rest of the Dulas Valley system.

Analyses of spatial pattern

Despite low mobility, *P. argus* was able to track changes in heathland habitat quite rapidly over the spatial scale investigated at Iping and Stedham Commons (Fig. 4). Species favouring temporary habitats are expected to have relatively high mobility (Southwood, 1962), but *P. argus* appears to track suitable habitat through a combination of rather low mobility and very high population densities. Patch isolation was not a major barrier to the colonization of new habitat, and hence 'stepping-stone' colonization did not appear to be an important factor at the spatial scale investigated: most colonizations represented consolidation of the existing distribution (Fig. 4). On heathlands much larger than the Iping/Stedham system, considering the steepness with which the distribution of dispersal falls away with distance (Fig. 3), relatively short-distance colonizations may bring whole new arrays of previously isolated empty habitat patches within colonization range with each generation. However, dispersal between habitats separated by more than $\top$ 5 km is unlikely, and the modern fragmented distribution of heathlands and limestone vegetation across much of the U.K. (Ratcliffe, 1984; Webb, 1986) may already have restricted *P. argus* metapopulations to patch networks within vegetation fragments, as at Iping and Stedham Commons. Provided that habitat management continues to be favourable, these metapopulations may persist indefinitely even in the absence of immigration. However, natural recolonization is unlikely if entire metapopulations go extinct.

Genetic differentiation of local populations

There is much current interest in the genetic and evolutionary implications of metapopulation structure (Hastings & Harrison, 1994; Harrison & Hastings, 1996; Hanski & Gilpin, 1997). Although allozyme frequencies have been investigated in several butterfly species (e.g. McKechnie *et al*., 1975; Descimon & Napolitano, 1993; Britten *et al*., 1994; Nève *et al*., 1997), the results have rarely been interpreted in terms of genetic differentiation of local populations within metapopulations (cf. Debinski, 1994; Nève *et al*., 1997), despite the fact that butterflies have been among the most popular organisms for metapopulation research (Thomas & Hanski, 1997). In *P. argus*, it seems that high local population densities are sufficient to prevent much genetic differentiation of local populations through drift, in spite of the low levels of migration. The low levels of genetic differentiation found for *P. argus* in the Dulas Valley are consistent with the population density and dispersal data observed directly. This contrasts with studies of *Drosophila pseudoobscura* (Coyne *et al*., 1982) and the butterfly *Euphydryas editha* (Slatkin, 1985), where levels of genetic differentiation were much lower than indicated by observed dispersal.

However, some caution in the interpretation of the allozyme data is required. Assessment of the genetic differentiation of local populations using F_{ST} requires two main assumptions (Hastings & Harrison, 1994). The first is that populations are at equilibrium. The Dulas Valley system may not be at

equilibrium (Brookes *et al*., 1997), because it is the result of an artificial introduction of *P. argus* in 1942, into a single patch (Fig. 1), and all other populations in the system are the result of colonization from that patch. Stepping stone colonization, or extinction followed by recolonization by a small number of individuals, may lead to differentiation through the founder effect (Whitlock, 1992), suggesting a lower frequency of migration among local populations than actually occurs (Slatkin, 1985). However, given the low levels of differentiation actually recorded (Table 2; Brookes *et al*., 1997), this potential bias is unlikely to be important.

The second assumption is of neutrality – that gene frequencies are not influenced by differential selection in patches. In the study system, it is possible that this assumption is violated at one locus, fumarase, which shows higher levels of variability among local populations than other loci. However, reanalysing the data excluding this locus did not alter the conclusions substantially.

Comparison of methods

The methods used to assess how populations are structured each have their advantages and disadvantages (Slatkin, 1985, 1994). Where possible, it seems sensible to use several independent sources of evidence. If the results are consistent, there can be increased confidence in the impression of how a particular population is structured. If the results are not consistent, the nature of the inconsistency may provide additional or unpredicted information about biases in some types of data, about population structure, or about historical or stochastic events within the system (Slatkin, 1985, 1994). In the context of metapopulations and other spatially structured systems, evidence from a variety of sources will provide the most accurate picture of the relative importance of within-population and among-population processes (Nève *et al.*, 1997). Sound empirical evidence of this kind will be essential if the metapopulation concept is to be applied to conservation problems (Harrison, 1994; Harrison & Taylor, 1997).

The three methods of assessing population structure do not give identical results for *P. argus*, but the results are complementary: none of the approaches on its own would have provided such a clear picture. The occurrence of colonizations and extinctions (Thomas & Harrison, 1992), direct evidence of dispersal among habitat patches (Fig. 2), observations of the rate of colonization of newly suitable habitat (Fig. 4), and indirect evidence of gene flow from allozyme data (Table 2) all suggest that *P. argus* fits the metapopulation concept reasonably well. Within *P. argus* habitat patches, local population dynamic processes must be the major determinants of population size, but local populations are linked by low levels of migration. The Dulas Valley *P. argus* system may come closer to the idealized metapopulation than other systems involving more mobile species, where higher frequencies of transfers between habitat patches have been detected (Baguette & Nève, 1994; Hanski *et al*., 1994; Hill *et al.*, 1996; Sutcliffe *et al.*, 1997). These other systems appear to lie further along the spectrum towards 'patchy populations,' with demographically less distinct local populations.

However, movement by *P. argus* within patches (Fig. 3) is so low that the largest continuously occupied areas of habitat stretch Hanski & Gilpin,'s (1991) definition of a local population as 'a set of individuals which all interact with each other with a high probability.' The largest occupied limestone habitat patches in North Wales have an area > 10 ha (Thomas & Harrison, 1992). The estimated neighbourhood area of $\top 1.6$ ha suggests that the probability of any interaction between butterflies emerging at the opposite margins of such sites will be very low, probably as low as the likelihood of interaction between butterflies emerging in immediately adjacent but distinct habitat patches. If so, 'local populations' in 'habitat patches' may be more descriptions for human convenience than real entities. In real systems, rapid mixing within local populations may occur only when exchange rates among patches are high, or where the permeability of patch boundaries is low.

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