Heredity (2002) 89, 99–106
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www.nature.com/hdy

Geographical and altitudinal population genetic structure of two dung fly species with contrasting mobility and temperature preference

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Local adaptation of populations requires some degree of spatio-temporal isolation. Previous studies of the two dung fly species *Scathophaga stercoraria* and *Sepsis cynipsea* have revealed low levels of geographic and altitudinal genetic differentiation in quantitative life history and morphological traits, but instead high degrees of phenotypic plasticity. These patterns suggest that gene flow is extensive despite considerable geographic barriers and large spatio-temporal variation in selection on body size and related traits. In this study we addressed this hypothesis by investigating genetic differentiation of dung fly populations throughout Switzerland based on the same 10 electrophoretic loci in each species. Overall, we found no significant geographic differentiation of populations for either species. This is inconsistent with the higher rates of gene flow expected due to better flying capacity of the larger *S. stercoraria*. However, heterozygote deficiencies within populations indicated structuring on a finer scale, seen for several loci in *S. cynipsea*, and for the locus PGM (Phosphoglucomutase) in *S. stercoraria*. Additionally, *S. cynipsea* showed a tendency towards a greater gene diversity at higher altitudes, mediated primarily by the locus MDH (malate dehydrogenase), at which a second allele was only present in populations above 1000 m. This may be caused by increased environmental stress at higher altitudes in this warm-adapted species. MDH might thus be a candidate locus subject to thermal selection in this species, but this remains to be corroborated by direct evidence. In *S. stercoraria*, no altitudinal variation was found.
Heredity (2002) **89,** 99–106. doi:10.1038/sj.hdy.6800097

Keywords: allozymes; altitude; electrophoresis; genetic differentiation; *Scathophaga stercoraria*; *Sepsis cynipsea*

Introduction

Local adaptation of populations requires some degree of spatial or temporal isolation, that is, limited gene flow. Migration rates of individuals can be assessed via mark-recapture studies (Manly, 1985), but this direct approach is cumbersome and unfeasible with small organisms such as insects. One alternative is a population genetic approach, by which rates of gene flow between populations can be estimated indirectly from the degree of population differentiation at putatively neutral genetic markers (Wright, 1943, 1951). The study of protein variation using electrophoresis is the classic method (Berg, 1997; Eanes, 1999).

The yellow dung fly *Scathophaga stercoraria* and *Sepsis cynipsea* are the most common and abundant dung flies in Europe, at least in part because both are specialized on cow dung, which abounds in agricultural landscapes (Gorodkov, 1984; Zuska and Pont, 1984). In Switzerland, both species occupy environmentally heterogeneous habitats, ranging from the lowlands to the mountaintops close to the timberline (Blanckenhorn, 1997a). Previous studies of both species have demonstrated equally low levels of geographic and altitudinal genetic differentiation in quantitative life history and morphological traits such as fecundity, development time, growth rate, diapause and adult emergence, and revealed high degrees of phenotypic plasticity in response to key environmental variables such as temperature, photoperiod or resource availability (Blanckenhorn, 1997a,b, 1998a,b; Blanckenhorn *et al*, 2001). This suggests that gene flow preventing local adaptation is extensive despite considerable geographic barriers (ie, high mountains) and spatio-temporal variation in selection on these traits in local populations (Blanckenhorn, 1998a; Blanckenhorn *et al*, 1999; Jann *et al*, 2000; Kraushaar and Blanckenhorn, 2002). Here we address this hypothesis by investigating genetic differentiation of dung fly populations throughout Switzerland based on the same 10 electrophoretic loci in each species.

Differences in the degree of genetic differentiation between the two species can be expected, based on differences in their morphology and geographic distribution. First, unpublished field and laboratory observations including tethered-flight experiments indicate that the much larger, house-fly-sized yellow dung fly flies much faster and longer than the small, ant-sized *S. cynipsea*.

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Received 28 September 2001; accepted 10 March 2002

This would facilitate migration and thus gene exchange between populations. One might therefore expect a lower degree of geographic genetic differentiation among *S. stercoraria* populations than among *S. cynipsea* populations. Second, the distribution of yellow dung flies, as well as other species of this genus, up to Iceland reveals a preference for colder temperatures (Gorodkov, 1984). Both larvae and adults are very sensitive to heat (Ward and Simmons, 1990; Blanckenhorn, 1998a), and in the warmer regions of their distribution yellow dung flies typically disappear from pastures to spend the summer in the cooler forests in a physiologically quiescent state (Hammer, 1941; Parker, 1970; Gibbons, 1987; Blanckenhorn *et al*, 2001). *Sepsis cynipsea*, in contrast, like it hot: they are abundant only in summer (Blanckenhorn, 1997a; Blanckenhorn *et al*, 1999), and their range extends south to northern Africa (Zuska and Pont, 1984; Meier, 1996). From this one might expect that, for *S. stercoraria*, the cooler (but harsher) high altitude habitats would be more suitable and less stressful, whereas, for *S. cynipsea*, high altitude habitats would be less preferable and more stressful. Environmental stress can be expected to increase mutation rates (Hoffmann and Parsons, 1991). At the same time, particular enzymes typically used in electrophoresis may not be neutral but may have functional optima at different temperatures and are thus subject to thermal selection (Watt, 1977, 1983; Watt *et al*, 1985; reviewed in Huey and Kingsolver, 1989; Eanes, 1999). Indeed, this appears to be the case for the phosphoglucomutase (PGM) locus in *S. stercoraria* (Jann, 1997; Ward, 1998, 2000). One can therefore expect more rare and potentially thermally selected alleles in the more stressful environment for each species, ie at low altitudes for *S. stercoraria* and at high altitudes in *S. cynipsea*, or, at least, to find altitudinal clines in allele frequencies. In this study, we test these two expectations against the null-hypotheses of no geographic or altitudinal variation in the allozyme distribution of the two species.

Methods

Study sites

The samples were collected across Switzerland between spring 1995 and late autumn 1996. The sites range from 300 m to 2400 m above sea level. We generally considered pastures as populations. The entire sample included 34 sites for *S. stercoraria* and 29 sites for *S. cynipsea*. Due to differences in the phenology of the two species, the flies were sampled at different times throughout the season (April to October).

Field and laboratory procedures

We analysed the offspring of field-collected individuals reared in the laboratory at standard conditions. In the field we randomly collected approximately 30 pairs of the yellow dung fly and about 50 pairs of *S. cynipsea*. We transported the live animals to the laboratory as fast as possible. There we transferred pairs into glass vials and added a smear of dung on a filter paper as well as sugar and water *ad libitum*. The predatory *S. stercoraria* were additionally provided with *Drosophila melanogastor* as prey. Females immediately started to lay eggs into the dung. We transferred 10 to 15 eggs from each female to a plastic bottle containing a superabundant amount of ca. 40 g of cow dung (Amano, 1983), and allowed the offspring generation to develop in a climate chamber at 18ºC, 60% relative humidity and a photoperiod of 12 h (L:D 12h:12h). At emergence we randomly chose eight individuals of either sex from different clutches from each population, and stored them at –80ºC for later electrophoresis. Due to this limited number of individuals, rare alleles present in any particular population were less likely to be detected.

Electrophoresis

Anesthetized *S. stercoraria* individuals were crushed in 150 μl and those of *S. cynipsea* in 50 μl of a Mercapto-ethanol-buffer. Samples were centrifuged at 11000 rpm for 30 min and stored afterwards at –80ºC. Procedures for horizontal starch-gel electrophoresis followed those given by Murphy (1990). We assayed the same 10 allozymes in both species, permitting direct comparisons: mannose-6-phosphate isomerase (MPI) EC 5.3.1.8, glycerol-3-phosphate dehydrogenase (G3PDH) EC 1.1.1.8, glucose-6-phosphate isomerase (GPI) EC 5.3.1.9, diaphorase (DIA), phosphoglucomutase (PGM) EC 5.4.2.2, 3-hydroxybutyrate dehydrogenase (HBDH) EC 1.1.1.30, hexokinase (HK) EC 2.7.1.1, an undefined variant staining with HK (HKXY), phosphogluconate dehydrogenase (6PGDH) EC 1.1.1.44 and malate dehydrogenase (MDH) EC 1.1.1.37. GPI, G3PDH, DIA, HK, HKXY and MPI were run on a Tris-citrate electrode buffer of pH 8, while PGM, HBDH, 6PGDH and MDG were run on a JRP electrode buffer of pH 6.3. Only six loci were polymorphic in *S. stercoraria* (MPI, G3PDH, GPI, DIA, PGM and HBDH), while all loci were polymorphic in *S. cynipsea*. The enzyme abbreviations are those of Shaklee *et al* (1990). Multiple alleles encoding the same enzyme (allozymes) were designated by consecutive numbers, with '1' denoting the slowest migrating allozyme in the anodal direction.

Statistical analysis

Genotypic and allelic compositions were estimated with the program FSTAT, Version 2.9 (Goudet, 1995). For each locus, and over all loci, deviations from random mating were assessed by means of *F*-statistics. To test for departure from panmixia we used permutational analysis of the null distribution of each data set. Under the null hypothesis of random mating within populations, the pairing of alleles within individuals is random. We thus allocated alleles at random, without replacement, to a randomly chosen individual within each population. F_{IS} was estimated from each of 5000 random re-allocations of alleles to individuals within populations using FSTAT. We could therefore test for the significance of F_{IS}: if the observed value belonged to the highest 5% of the null distribution, it was deemed significant. We employed similar procedures for F_{IT}: under the null hypothesis of random mating all over Switzerland, we permuted alleles among individuals over the whole data set. Finally, for population differentiation quantified by F_{ST}, we permuted individuals among populations. These randomisation schemes were used to test whether populations were differentiated using the *G*-statistic, shown by

Goudet *et al* (1996) to be more powerful than F_{ST} to test for population differentiation. Standard errors for *F*-statistics were obtained by jackknifing among populations for per-locus estimates and among loci for overall estimates.

To test for geographic variation, we examined the correlations between matrices of pairwise genetic (F_{ST}) and pairwise geographic distances (Slatkin, 1993) using Mantel tests (Mantel, 1967). These were also used to test for significant correlations between genetic and geographic distances (ie, for isolation by distance). To test for altitudinal variation, we performed regressions of the total number of alleles and average gene diversity over all loci, as well as the principal component of these two variables (which are interdependent), on altitude (cf. Tables 1 and 3), separately for the species. Lastly, we used a Wilcoxon matched-pairs test to directly compare the population genetic structure (ie, F_{ST} and F_{IS}) of both species.

Results

Of the six polymorphic loci in *S. stercoraria*, G3PDH proved to have the lowest variability (Table 1). *F*-statistics revealed no overall structure whatsoever. F_{ST} was close to zero for all loci and overall (Table 2). Because of this lack of geographic population structure, further investigation by means of Mantel tests were not carried out. F_{IS} did not differ from zero for any locus in any individual population (not shown), nor over all loci. However, after Bonferroni corrections for multiple testing, F_{IS} for PGM deviated significantly from Hardy-Weinberg equilibrium within populations over all samples (Table 2), indicating heterozygote deficiency and some further structuring within populations.

In *S. cynipsea*, five of the 10 loci examined proved to be highly variable (MPI, DIA, HKXY, HBDH and 6PGDH), three showed some variability (PGM, GPI and MDH), and two were of rather low variability (HK and G3PDH; Table 3). We found considerable deviations from Hardy-Weinberg equilibrium (ie, significant F_{IS}) within several of our alleged populations for MPI, DIA, HKXY and 6PGDH (not shown). F_{IS} was also significant over all populations for MPI, DIA, PGM, HKXY, MDH and all loci combined (Table 2). F_{ST} was significant for MPI and MDH as well as for all loci combined, indicating genetic differentiation among populations, however almost entirely mediated by MDH (Table 2). A Mantel test yielded no isolation by distance (results not shown).

No difference in genetic structure was found between the two species ($P > 0.2$) when directly comparing the F_{ST}-values obtained (Table 2) using a Wilcoxon matched-pairs test, contrary to expectation. However, the same test was significant for F_{IS} ($P < 0.02$), the values of which were consistently greater in *S. cynipsea* than *S. stercoraria* (Table 2). These results were qualitatively the same irrespective of whether we included all 10 loci (assuming values of zero for the four loci monomorphic in *S. stercoraria*), or merely the six loci polymorphic in both species. Whereas no altitudinal variation was apparent in *S. stercoraria* ($r = 0.04$, $P > 0.5$), mean genetic diversity ($r = 0.44$, $P = 0.017$) and the total number of alleles over all loci (non-significant: $r = 0.27$, $P = 0.121$) increased with altitude in *S. cynipsea* (Figure 1); the principal component combining the two (interdependent) variables was also significant ($r = 0.38$, $P = 0.043$). This increase is primarily mediated by the locus MDH and, to a lesser extent, by DIA and MPI. We plot the individual variables rather than the principal component in Figure 1 because these numbers are more informative. Except for PGM, allelic diversity was generally greater in *S. cynipsea* (Tables 1 to 3; Figure 1).

Discussion

From our data we conclude that populations of both dung fly species are not genetically differentiated in Switzerland. Formally, a significant overall F_{ST} for all loci combined suggests differentiation in *S. cynipsea*. However, this is almost entirely mediated by MDH (Table 2), and may be caused by selection as discussed below. We also found no isolation by distance. Moreover, when directly comparing the F_{ST}-values obtained for the same allozymes, no difference in genetic structure between the two species was found. Contrary to our expectation (see Introduction), gene flow due to dispersal is apparently equally effective in both species in preventing genetic isolation among populations, despite ubiquitous geographic barriers (ie, high mountains) and the better flying ability of the larger *S. stercoraria*. Significant gene flow preventing population differentiation by genetic drift in both species agrees well with previous findings of low degrees of geographic and altitudinal genetic differentiation in quantitative traits, potentially facilitating the evolution of phenotypic plasticity (Blanckenhorn, 1997a, 1998b).

Failure to find genetic differentiation may to some extent relate to our sampling scheme, which was conservative with regard to the null hypothesis. First, we considered pastures as populations. This is probably justified in the mountains, where pastures are large and isolated: highland populations are known to be local, as the flies typically arrive before the cows, ie, they do not migrate up with them (personal observation). In the lowlands, however, where in some regions many dairy farms are located next to each other, this may not hold. Second, because we tested only eight individuals per population, very rare alleles might not have been detected. Third, our set of allozymes was less variable overall in *S. stercoraria*. All this limits the statistical power to detect population differentiation (ie, significant F_{ST}), perhaps more so in one species than the other. However, greater allozyme variation did not result in a significant F_{ST} in *S. cynipsea*, while F_{IS} was often significant (discussed below). Furthermore, for our species comparison this is a minor problem because it is based on our overall mean F_{ST} and F_{IS} values, the estimates of which should not be affected by the different number of polymorphic loci. We therefore believe that our methods have not strongly affected our results, and that the low degree of population differentiation and the differences in allozyme variation between the species are real. It is likely that the use of finer scale, highly polymorphic, DNA markers would result in significant (albeit low) F_{ST}, but in some way this is trivial. Furthermore, a direct comparison using microsatellite markers would not be possible because the same markers are not shared by both species (TWJ Garner, unpublished data). Nevertheless, to investigate genetic variation at the broader geographic scale across Europe in *S. stercoraria*, we are currently conducting

Table 1 Geographic coordinates, altitude, number of alleles (top) and gene diversity (bottom) for each locus and *Scathophaga stercoraria* population

Population	*Location Latitude/Longitude*	*Altitude (m)*	*Loci*						
			MPI	*G3PDH*	*GPI*	*DIA*	*PGM*	*HBDH*	*Total*
Aecherlipass	46°52′N/8°21′E	1400	2	1	1	2	1	2	9
			0.333	0	0	0.464	0	0.125	0.154
Arisdorf	47°31′N/7°46′E	350	2	1	1	2	2	1	9
			0.200	0	0	0.167	0.143	0	0.085
Boudevilliers	47°01′N/6°55′E	754	3	1	1	3	2	2	12
			0.446	0	0	0.339	0.125	0.125	0.173
Brüschwil	47°33′N/9°19′E	453	2	1	2	2	3	2	12
			0.500	0	0.125	0.232	0.241	0.125	0.204
Bulle	46°37′N/7°04′E	770	2	1	1	2	2	1	9
			0.357	0	0	0.524	0.143	0	0.171
Cadagno	46°45′N/8°44′E	1950	2	1	1	2	1	1	8
			0.321	0	0	0.417	0	0	0.123
Chur	46°52′N/9°32′E	600	2	2	1	3	2	2	12
			0.464	0.125	0	0.589	0.125	0.125	0.238
Col de la Croix	46°19′N/7°04′E	1750	1	1	1	3	2	1	9
			0	0	0	0.274	0.232	0	0.084
Engelberg	46°49′N/8°25′E	1003	3	1	2	2	1	1	10
			0.482	0	0.125	0.321	0	0	0.155
Fehraltorf	47°23′N/8°45′E	536	3	1	1	2	3	1	11
			0.527	0	0	0.482	0.241	0	0.208
Flüelapass	46°46′N/9°57′E	1900	2	1	1	4	3	2	13
			0.250	0	0	0.518	0.274	0.125	0.195
Furkapass	46°34′N/8°25′E	2430	2	1	1	2	2	1	9
			0.464	0	0	0.500	0.125	0	0.182
Gilbach	46°29′N/7°34′E	1439	2	1	1	3	2	1	10
			0.350	0	0	0.241	0.125	0	0.119
Hoher Kasten	47°17′N/9°26′E	1700	2	1	1	2	3	1	10
			0.262	0	0	0.143	0.357	0	0.127
Jaunpass	46°36′N/7°21′E	1400	2	1	1	2	3	1	10
			0.300	0	0	0.500	0.488	0	0.215
Julierpass	46°30′N/9°45′E	2100	2	1	1	2	3	2	11
			0.125	0	0	0.500	0.286	0.143	0.176
Klausenpass	46°52′N/8°52′E	1750	2	1	1	2	4	1	11
			0.411	0	0	0.464	0.438	0	0.219
Netstal	47°05′N/9°04′E	461	2	1	1	2	3	1	10
			0.321	0	0	0.250	0.241	0	0.135
Nussbaumen	47°28′N/8°19′E	450	1	1	2	2	3	1	10
			0	0	0.125	0.429	0.518	0	0.179
Oberalppass	46°39′N/8°35′E	1850	4	1	1	2	3	1	12
			0.667	0	0	0.464	0.348	0	0.247
Parpan	46°47′N/9°34′E	1490	2	1	1	3	2	1	10
			0.533	0	0	0.517	0.125	0	0.196
Pragelpass	46°59′N/8°53′E	1560	2	1	1	3	2	4	13
			0.232	0	0	0.545	0.250	0.348	0.229
Prês d'Orvin	47°10′N/7°13′E	1100	3	1	2	3	2	1	12
			0.524	0	0.232	0.491	0.125	0	0.229
Reichenbach	46°38′N/7°42′E	706	2	1	1	2	2	1	9
			0.381	0	0	0.232	0.125	0	0.123
Rigi	47°03′N/8°33′E	1604	2	1	1	3	3	1	11
			0.411	0	0	0.274	0.348	0	0.172
Roggen	47°18′N/7°43′E	810	2	1	1	2	3	2	11
			0.300	0	0	0.500	0.405	0.143	0.225
Schwarzenburg	46°49′N/7°21′E	792	2	1	2	2	2	2	11
			0.500	0	0.143	0.262	0.143	0.143	0.199
Sils Maria	46°27′N/9°46′E	1817	3	2	1	3	2	1	12
			0.571	0.143	0	0.536	0.125	0	0.229
Tann	47°13′N/8°12′E	666	2	1	1	2	2	2	10
			0.411	0	0	0.464	0.125	0.143	0.191
Truttikon I	47°38′N/8°45′E	472	2	1	1	2	1	1	8
			0.350	0	0	0.500	0	0	0.142
Truttikon II	47°38′N/8°45′E	472	2	1	1	2	4	1	11
			0.143	0	0	0.357	0.393	0	0.149
Urnerboden	46°53′N/8°55′E	1389	2	1	1	2	1	1	8
			0.321	0	0	0.339	0	0	0.110

Continued

Table 1 *Continued*

Population	*Location Latitude/Longitude*	*Altitude (m)*	*Loci*						
			MPI	*G3PDH*	*GPI*	*DIA*	*PGM*	*HBDH*	*Total*
Wangenried	47°14′N/7°40′E	473	2	1	1	2	3	2	11
			0.393	0	0	0.518	0.339	0.125	0.229
Wasserauen	47°17′N/9°26′E	872	2	1	1	2	2	1	9
			0.452	0	0	0.357	0.125	0	0.156
Average	–	–	2.15	1.06	1.15	2.32	2.32	1.38	10.38
			0.362	0.008	0.022	0.403	0.208	0.049	

Table 2 *F*-statistics (mean ± SE) for each locus and for all loci combined for *Scathophaga stercoraria* and *Sepsis cynipsea*

Species	*Locus*	F_{IT}	F_{ST}	F_{IS}
S. stercoraria	MPI	0.076 ± 0.059	-0.010 ± 0.018	0.086 ± 0.062
	G3PDH	–0.002 ± 0.001	–0.001 ± 0.007	–0.001 ± 0.007
	GPI	–0.008 ± 0.004	0.021 ± 0.024	–0.029 ± 0.025
	DIA	–0.035 ± 0.054	0.010 ± 0.014	–0.045 ± 0.060
	PGM	0.166 ± 0.063***	–0.008 ± 0.012	0.173 ± 0.064***
	HBDH	–0.016 ± 0.005	0.003 ± 0.015	–0.018 ± 0.016
	All loci	0.037 ± 0.055	0.000 ± 0.008	0.038 ± 0.062
S. cynipsea	MPI	0.142 ± 0.044***	0.029 ± 0.013**	0.126 ± 0.044***
	G3PDH	0.000 ± 0.000	–0.003 ± 0.002	0.003 ± 0.002
	GPI	–0.016 ± 0.005	0.004 ± 0.012	–0.020 ± 0.013
	DIA	0.129 ± 0.059*	0.003 ± 0.014	0.126 ± 0.062*
	PGM	0.544 ± 0.226***	0.010 ± 0.018	0.539 ± 0.231**
	HBDH	0.019 ± 0.058	0.008 ± 0.017	0.012 ± 0.065
	HK	–0.002 ± 0.002	–0.004 ± 0.002	0.001 ± 0.001
	HKXY	0.170 ± 0.059**	0.020 ± 0.025	0.154 ± 0.065*
	6PGDH	0.106 ± 0.074*	0.035 ± 0.024	0.073 ± 0.071
	MDH	0.383 ± 0.253***	0.252 ± 0.121***	0.242 ± 0.410*
	All loci	0.139 ± 0.020***	0.025 ± 0.010***	0.117 ± 0.018***

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

further studies at the allozyme and finer scale DNA levels.

Whereas F_{ST} was low and largely non-significant, we found considerable within-population heterozygote deficiency, ie, significant F_{IS}, for several loci and overall in *S. cynipsea*. In *S. stercoraria* this occurred only for PGM. Significant F_{IS} may be caused by null alleles that do not show up on the gel. Alternatively, substructuring within a pasture may result from inbreeding, non-random mating or reproductive failure of heterozygote individuals, but we have no direct evidence for any of these mechanisms from the field or the lab. In *S. stercoraria*, PGM is a highly polymorphic locus that has been implicated in cryptic female choice (Ward, 1998, 2000) and shows systematic seasonal and temperature variation (Kraushaar, 1994; Jann, 1997). It is thus possible that PGM may not be neutral but subject to (thermal) selection (eg, Watt, 1977, 1983; Watt *et al*, 1985; Eanes, 1999), which is another potential explanation for deviations from Hardy-Weinberg equilibrium. Selection may equally be invoked in explaining significant F_{IS} in *S. cynipsea*, but in this species we have no further evidence corroborating this supposition (except perhaps for MDH; see below), so we consider this unlikely.

We also investigated altitudinal genetic variation and obtained some evidence that gene diversity increases at higher altitudes in *S. cynipsea*. This effect was primarily mediated by MDH (in addition to MPI and DIA). MDH was completely monomorphic in *S. stercoraria*, whereas in *S. cynipsea* a second allele was present only in populations above 1000 m. The latter was also true for the slowest allele of the locus DIA. For the warm-adapted *S. cynipsea*, the colder and harsher high altitude pastures should be the more stressful habitats (see Introduction). For example, development time is much prolonged at cooler temperatures (Blanckenhorn, 1997a), increasing the time larvae are exposed to mortality factors. In contrast, gene diversity was not greater in lowland *S. stercoraria*, as we had expected based on the same reasoning. Hot weather results in high mortality in this species (Ward and Simmons, 1990; Blanckenhorn, 1998a), and lowland habitats are considerably hotter on average (Blanckenhorn, 1997a): *S. stercoraria* should therefore prefer the cooler, high altitude habitats (Gorodkov, 1984). However, (re)colonization of high altitude pastures is only possible via the lowlands, so recurrent migration should homogenize genetic variation with regard to altitude in this species. This cannot necessarily be expected in *S. cynipsea*, which should migrate only occasionally (perhaps by accident) from the preferred lowlands (the population sources) to the highlands (the population sinks). However, since we found no difference in the

Table 3 Geographic coordinates, altitude, number of alleles (top) and gene diversity (bottom) for each locus and *Sepsis cynipsea* population

Population	*Location Latitude/Longitude*	*Altitude (m)*	*Loci*										
			MPI	*G3PDH*	*GPI*	*DIA*	*PGM*	*HBDH*	*HK*	*HKXY*	*6PGDH*	*MDH*	*Total*
Aecherlipass	46°52′N/8°21′E	1400	5	1	2	2	1	2	1	2	3	1	20
			0.770	0	0.232	0.393	0	0.400	0	0.536	0.607	0	0.294
Arolla	46°02′N/7°29′E	2000	5	1	1	4	2	2	1	2	2	1	21
			0.845	0	0	0.643	0.125	0.500	0	0.429	0.500	0	0.304
Beromünster	47°13′N/8°12′E	666	6	1	1	2	2	2	1	2	2	1	20
			0.848	0	0	0.500	0.125	0.262	0	0.357	0.518	0	0.261
Brülisau	47°17′N/9°27′E	790	4	1	2	2	1	3	1	2	2	1	19
			0.759	0	0.125	0.393	0	0.339	0	0.518	0.411	0	0.255
Bulle	46°37′N/7°04′E	770	5	1	1	3	1	2	1	2	3	1	20
			0.726	0	0	0.524	0	0.143	0	0.500	0.583	0	0.248
Chur	46°52′N/9°32′E	600	6	1	1	2	1	2	1	3	2	1	20
			0.804	0	0	0.393	0	0.400	0	0.545	0.500	0	0.264
Col de la Croix	46°19′N/7°04′E	1750	7	1	1	3	1	2	1	3	2	2	23
			0.902	0	0	0.688	0	0.232	0	0.625	0.533	0.250	0.323
Engelberg	46°49′N/8°25′E	1003	6	1	2	2	1	1	1	2	3	2	21
			0.821	0	0.125	0.536	0	0	0	0.500	0.616	0.250	0.285
Flüelapass	46°46′N/9°57′E	1900	6	1	1	2	1	3	1	2	2	1	20
			0.839	0	0	0.500	0	0.420	0	0.571	0.518	0	0.285
Flumserberge	47°09′N/9°15′E	1230	5	1	1	2	1	2	1	3	2	2	20
			0.777	0	0	0.411	0	0.350	0	0.554	0.300	0.500	0.289
Hoher Kasten	47°17′N/9°26′E	1700	5	1	1	3	1	3	1	2	2	1	20
			0.804	0	0	0.598	0	0.241	0	0.232	0.518	0	0.239
Netstal	47°05′N/9°04′E	461	4	1	1	3	1	3	1	3	3	1	21
			0.738	0	0	0.482	0	0.241	0	0.607	0.482	0	0.255
Novazzaro	46°01′N/8°57′E	300	5	1	2	3	1	2	1	2	3	1	21
			0.804	0	0.125	0.661	0	0.232	0	0.393	0.563	0	0.278
Nussbaumen	47°28′N8°19′E	450	5	1	2	3	1	4	1	3	2	1	23
			0.833	0	0.125	0.545	0	0.348	0	0.571	0.429	0	0.285
Oberalppass	46°39′N/8°35′E	1850	5	1	1	3	1	2	1	3	3	2	22
			0.762	0	0	0.583	0	0.125	0	0.625	0.550	0.125	0.277
Parpan	46°47′N/9°34′E	1490	5	1	2	4	2	2	1	3	2	1	23
			0.821	0	0.125	0.759	0.250	0.232	0	0.661	0.464	0	0.331
Pragelpass	46°59′N/8°53′E	1560	5	1	1	3	1	2	1	3	2	2	21
			0.817	0	0	0.571	0	0.167	0	0.589	0.500	0.500	0.314
Prês d'Orvin	47°10′N/7°13′E	1100	7	1	1	3	1	1	1	2	2	1	20
			0.830	0	0	0.438	0	0	0	0.518	0.536	0	0.232
Quartino	46°01′N/8°57′E	250	5	1	1	2	1	3	1	2	3	1	20
			0.795	0	0	0.536	0	0.241	0	0.476	0.536	0	0.258
Reichenbach	46°38′N/7°42′E	706	5	1	2	2	1	3	2	2	3	1	21
			0.786	0	0.125	0.554	0	0.286	0.125	0.536	0.598	0	0.301
Roggen	47°18′N/7°43′E	800	5	1	1	2	3	3	1	2	4	1	24
			0.839	0	0	0.500	0.357	0.339	0	0.464	0.652	0	0.315
Romanshorn	47°34′N/9°23′E	399	4	1	1	3	1	2	1	3	3	1	20
			0.741	0	0	0.580	0	0.125	0	0.598	0.560	0	0.260
Samedan	46°33′N/9°53′E	1721	5	1	1	3	1	3	1	2	2	1	20
			0.795	0	0	0.598	0	0.429	0	0.536	0.339	0	0.270
Schwarzenburg	46°49′N/7°21′E	792	6	1	1	2	1	2	1	2	2	1	19
			0.830	0	0	0.518	0	0.125	0	0.567	0.518	0	0.256
St. Gotthard	46°15′N/8°02′E	1760	4	1	1	3	2	2	1	3	2	2	21
			0.800	0	0	0.375	0.200	0.200	0	0.600	0.500	0.400	0.308
Truttikon	47°38′N/8°45′E	472	3	1	3	3	1	2	1	2	2	1	19
			0.667	0	0.241	0.500	0	0.339	0	0.500	0.554	0	0.280
Urnerboden	46°53′N/8°55′E	1389	5	1	2	3	1	3	2	2	3	2	24
			0.750	0	0.125	0.643	0	0.429	0.125	0.536	0.571	0.250	0.343
Vue des Alpes	47°01′N/6°55′E	1283	6	2	1	3	1	3	1	2	2	2	23
			0.848	0.125	0	0.518	0	0.554	0	0.554	0.232	0.250	0.308
Wangenried	47°14′N/7°40′E	473	5	1	1	2	1	2	1	2	2	1	18
			0.786	0	0	0.482	0	0.125	0	0.464	0.500	0	0.236
Average	–	–	5.18	1.03	1.34	2.66	1.21	2.34	1.07	2.34	2.41	1.28	20.86
			0.799	0.004	0.046	0.532	0.036	0.270	0.009	0.523	0.506	0.087	

degree of geographic differentiation between the two species, and thus presumably in the extent of gene flow among populations, (stronger) selection on particular rare alleles may instead better explain the altitudinal increase in allozyme variation in *S. cynipsea*. Here we might have found a candidate for a temperature-dependent enzyme perhaps allowing its bearer to function better at lower temperatures. MDH is a major enzyme of the citric acid cycle and therefore influences energy provision and thus major life functions of an individual. This

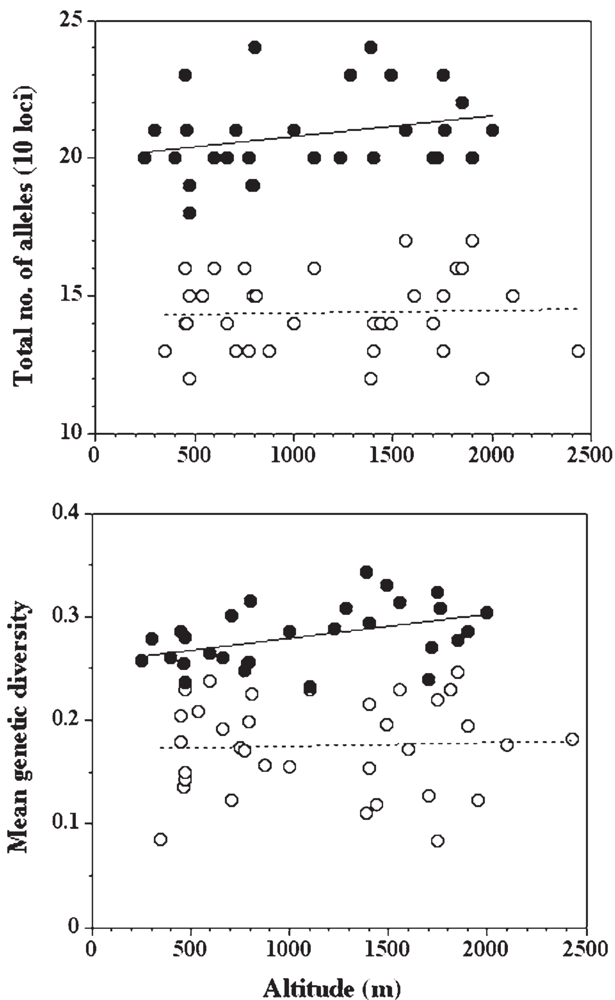

Figure 1 The total number of alleles at the same 10 loci (top) and the mean genetic diversity of six and 10 (respectively) polymorphic loci (bottom) for 34 and 29 Swiss populations of the dung flies *Scathophaga stercoraria* (open circles and dashed lines) and *Sepsis cynipsea* (filled circles and solid lines) in relation to altitude.

would be consistent with the expectation that harsher, more stressful, environments can increase mutation rates (Hoffmann and Parsons, 1991) and may lead to particular alleles being favoured by selection because they have the corresponding temperature optima (cf. Watt, 1977, 1983; Watt *et al*,1985; Eanes, 1999). However, so far we have no direct evidence for these mechanisms, so they need to be investigated further.

Acknowledgements

We thank C Mühlhäuser for help with collecting flies, Jeannette Fanti for help in the laboratory, Paul Ward, Nicolas Perrin, Bob Martin and Trent Garner for comments, and the Swiss National Fund for funding this project.

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