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Restriction fragment length polymorphisms of different DNA regions as genetic markers in the hoverfly *Episyrphus balteatus* (Diptera: Syrphidae)

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Abstract

A polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) analysis using mitochondrial (A + T-rich region; mtDNA) and genomic (*zen*-region; nDNA) DNA was performed on 182 female individuals of *Episyrphus balteatus* (DeGeer), a widespread aphidophagous hoverfly with supposed migratory behaviour. Specimens originated from 13 sampling sites in six European countries. The analyses revealed 12 and 18 haplotypes, respectively, for the two DNA types, several of them with a wide distribution, although seven and eight haplotypes, respectively, occurred only in one location. In contrast to other studies on mobile insects, the genetic diversity was relatively high. However, lack of population subdivision, low genetic distances between populations, the very high gene flow rates, and the complete lack of isolation by distance suggest that *E. balteatus* populations are largely connected and that there is an absence of large-scale geographic structuring. These results support the hypothesis that *E. balteatus* is a migratory hoverfly species, capable of moving over large distances. These findings related to the seasonal migrations of this species are discussed.

Keywords: *Episyrphus balteatus*, Diptera, Syrphidae, migration, genetic diversity, Europe, overwintering, population structure

Introduction

Episyrphus balteatus (DeGeer) (Diptera: Syrphidae) is a common and widespread species of hoverfly in many parts of the world and is only absent in the Americas (Peck, 1988). Because of its periodically high abundance, the voracious feeding habits of the larvae, the efficient prey-finding abilities and high mobility of the adults, *E. balteatus* is considered in Europe to be one of the most important natural enemies of economically important aphids (e.g. Aubert *et al*., 1976; Ankersmit *et al*., 1986; Tenhumberg & Poehling, 1995; Bargen *et al*., 1998). Despite this importance for biological control, little is known on the population genetics and overwintering biology of *E. balteatus* and with regard to the latter, two hypotheses exist which have been the subject of substantial debate in the literature (e.g. Krause & Poehling, 1996; Salveter, 1996; Hart & Bale, 1997). First, *E. balteatus* is considered to be a migratory species, regularly moving between overwintering sites in the Mediterranean to its main breeding sites in central and northern Europe. This hypothesis is mainly based on occasional flight observations, e.g. on mountains, along coasts, and on ships (see Gatter & Schmid, 1990, and references therein), and two long-term studies where large numbers of southward moving individuals were caught in late summer and autumn by interception traps in the Alps (Aubert *et al*., 1976) and southwestern Germany (Gatter & Schmid, 1990). The second hypothesis postulates local overwintering of adult *E. balteatus* in central Europe. It is derived from flight observations, e.g. in northern Germany (Krause & Poehling, 1996) or Switzerland (Salveter, 1996) in late autumn to early

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spring, which were explained as indicating local hibernation by non-migrating individuals. Such residents go through a facultative diapause in which they attain relatively high levels of cold resistance (P. Hondelmann, unpublished data) as an adaptation to hibernation (Tauber *et al*., 1986).

Possibly both migrants and locally hibernating individuals contribute to the establishment of the *E. balteatus* summer populations in central Europe. However, even under such an assumption, various questions remain open: are populations or sub-populations connected? To what extent are populations subdivided and distinguishable? What is the genetic variability and diversity of populations and/or sub-populations? What are the sources for the high abundance of *E. balteatus* during the summer months in central Europe? What distances can migrants travel?

Hence the purpose of this study: to investigate if, and to what extent, European populations of *E. balteatus* are connected (in this paper individuals from one sample site are referred to as a population, even though these samples may be part of a larger population). As direct methods such as mark and recapture techniques or migration observations turned out not to be useful or feasible for hoverflies (Salveter & Nentwig, 1993; Nathan *et al*., 2003) indirect methods such as molecular markers were chosen, since they are reliable and can have a sufficiently high resolution, allowing detection of even small genetic differences within and between populations (Avise, 1994; Loxdale & Lushai, 1998).

The genetic structure of populations is determined by the balance between genetic drift, mutation, natural selection and gene flow (Roderick, 1996). Gene flow tends to homogenize the genetic structure of populations (e.g. Bohonak, 1999; Mun *et al*., 1999), although the effects of two-way migration are still little known (Freeland *et al*., 2003). Nevertheless, gene flow can also be restricted in highly mobile species like *E. balteatus*, e.g. by physical barriers (mountains, seas), isolation by distance or habitat patchiness (e.g. Loxdale & Lushai, 2001). In addition, founder effects may also influence population structure (Hedrick, 2000).

In this paper the genetic characterization of a migratory hoverfly species collected over a broad geographical range is presented for the first time. The study involved a polymerase chain reaction–restriction fragment length polymorphism analysis (PCR–RFLP) of two DNA regions: the A + T-rich region (control region), and a non-coding region of mitochondrial DNA (mtDNA) near the origin of replication of the molecule. Because of the comparatively rapid evolution and lack of recombination, mtDNA has been used extensively in population genetic studies (Avise, 1994; Loxdale & Lushai, 1998). The A + T-rich region is one of the most variable parts of mtDNA (Simon *et al*., 1994; Ballard & Whitlock, 2004) and has been useful for resolving several population genetic questions (e.g. Brower & Boyce, 1991; Parker *et al*., 1998; Schultheis *et al*., 2002). To overcome certain disadvantages of mtDNA, e.g. only maternal gene flow, only one non-recombinant locus, and lower effective population size, another DNA region was also selected, namely a nuclear DNA region representing a derivative of the Hox genes. Hox genes are a set of related genes encoding homeodomain transcription factors, necessary for developmental regulation (Hughes & Kaufman, 2002). In this study a part of a Hox3 gene homolog called '*zerknüllt*' (*zen*), required for establishment of extraembryonic tissue (Stauber *et al*., 2002), was used. Using these methodological approaches, it was intended to identify patterns of population differentiation and to estimate genetic variability between European populations of *E. balteatus*, to analyse the genetic diversity within and among populations, to reveal the degree of connection of populations by gene flow, and to study the origin of the high numbers of individuals frequently observed in the summer months in central Europe.

Material and methods

Samples

Only adult female flies were used in this study since they are primarily the ones that migrate and overwinter (Aubert *et al*., 1976; Gatter & Schmid, 1990; P. Hondelmann, unpublished data). Samples from several European countries were collected by hand-net and immediately transferred to ethanol (70–98%). Malaise-trap samples from the island of Helgoland and Berlin (both Germany) (trapping fluid ethylene glycol) were transferred to ethanol (70–98%) after emptying the trap. After shipping, all material was stored in absolute ethanol and frozen at −20°C until further use. More detailed information on locations and collection dates are summarized in table 1.

DNA extraction, cloning and PCR-RFLP-analysis

Six to 25 individuals were analysed from each locality. DNA was extracted using a modification of the method originally developed by Aljanabi & Martinez (1997). For this, the thorax from an individual was homogenized in 400 µl extraction buffer (0.4 M NaCl, 10 mM Tris-HCl, pH 8.0, and 2 mM EDTA, pH 8.0), 40 µl 20% SDS, and 8 µl proteinase K (20 mg ml^{-1}) and then well mixed. After incubation at 65°C for 60 min, 300 µl 6 M NaCl was added and vortexed for 30 s. The tubes were centrifuged for 30 min at 10,000 *g* and thereafter the supernatant was transferred to a new tube and centrifuged for a further 10 min at 10,000 *g*. The resultant supernatant was then transferred to a new tube, mixed with an equal volume of −20°C cold isopropanol, and centrifuged for a final 20 min at 4°C and 10,000 *g*. Subsequently the pellet was washed twice with 70% ethanol, dried and finally re-suspended in RNase (0.01 mg ml^{-1}) in TE buffer (10 mM Tris, 0.1 mM EDTA, pH 7.6) and aliquots were frozen at −20°C.

The A + T-rich region (i.e. control region) of mtDNA was first amplified in PCR reactions with low specificity (annealing temperature for the first 4 cycles 45°C, then 33 cycles at 51°C). A primer pair located in the flanking regions of the published A + T-rich region sequence of *Lucilia eximia* (Wiedemann) (Diptera: Calliphoridae) (Lessinger & Azeredo-Espin, 2000) was designed. After cloning of amplified DNA fragments of suitable size in vectors (pGEM-T Easy, Promega; pbluescript II Sk$^-$, Stratagene) following procedures from Sambrook *et al*. (1989) and manufacturers protocols, respectively, these were sequenced (contract sequencing by MWG Biotech).

By means of the sequence, a specific primer pair was designed: ATsps: 5′-CATCGTCGCGCTGTAGTT-3′ and ATspas: 5′-CACTGTTAAAACGAGGACACCTTACA-3′. All amplifications were performed in a Biometra T3 thermocycler. For each amplification, 3–5 µl DNA was added to a 50 µl reaction mixture containing 1 × PCR buffer (Qiagen), 2.5 mM $MgCl_2$, 250 µM each of dATP, dTTP, dCTP and dGTP,

Table 1. Locality, sample numbers and dates of all *Episyrphus balteatus* samples.

Locality (abbreviation)	Longitude	Latitude	Individual number	Date of sampling
Germany				
Helgoland (Hel)	07°50′E	54°10′N	10	VII.02*
Harz-Braunlage (Hrz1)	10°40′E	51°40′N	21	27.VII.02
Harz-Kamschlacken (Hrz2)	10°30′E	51°50′N	15	27.VII.02
Schleswig-Holstein (S-H)	10°20′E	53°40′N	7	05.VIII.02
Elm (Elm)	10°50′E	52°10′N	18	28.VII.02
Münster (Mue)	06°40′E	51°50′N	8	15.VII.02
Berlin (Ber)	13°20′E	52°20′N	6	VII/VIII.00*
Italy				
Verona (Ital1)	10°60′E	45°30′N	11	25.VIII.02
Mantova (Ital2)	10°50′E	45°10′N	16	13.VIII.02
Spain (Esp)	00°40′E	41°40′N	18	VI.02
Norway (Nor)	05°40′E	58°50′N	24	24.VIII.02
United Kingdom (UK)	00°40′W	51°10′N	23	VI/VIII.00* 22. VII.02
France (Fra)	01°30′E	43°30′N	5	19.VI.02
Total			182	

* Malaise trap catches.

1.25–2.0 units of HotStar *Taq* polymerase (Qiagen) and 0.3 μM of each primer. The amplification profile consisted of one cycle of 15 min at 95°C (activation of HotStar *Taq* polymerase), 37 cycles of 30 s at 94°C, 30 s at 64°C, 90 s at 72°C and one cycle of 10 min at 72°C. PCR products were then stored at 4°C.

The second nuclear DNA sequence is a derivative of the Hox genes. The fragment used was sequenced by the Max-Planck-Institut für Biophysikalische Chemie in Göttingen, Germany, and was provided by Dr U. Schmidt-Ott. The specific primer pair used for this region was Spez1s: 5′-GTCCTCCGACTATCTTTCTAA-3′ and Spez1as: 5′-TACAATTCTAACAATCGGTGA-3′. For this primer pair the same PCR protocol as for the A + T-rich region was used, except for the cycling parameters which were one cycle of 15 min at 95°C, 37 cycles of 30 s at 94°C, 30 s at 52.5°C, 60 s at 72°C and one cycle of 10 min at 72°C. PCR products were then stored at 4°C.

After amplification, aliquots of the products were cleaved according to the manufacturers' protocols with the following restriction enzymes: for the *zen*-region: BsrI, HinfI, PaeR7I and PstI; for the A + T-rich region: AccI, BsaHI, HhaI and TaqαI (MBI Fermentas and NEB).

PCR products were separated by electrophoresis on 1% agarose gels and restriction fragments on 2–2.5% agarose gels, both in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.4) and stained with ethidium bromide. A standard DNA marker (100 bp DNA ladder, MBI Fermentas) was included on each gel.

Data analysis

For all banding patterns of the different restriction enzymes of both DNA regions tested, a 0/1 character code, i.e. the haplotype, was formulated for each individual. Haplotype diversity (h) and nucleotide diversity within populations were calculated using REAP 4.0 (McElroy *et al*., 1992). Haplotype diversity is equivalent to gene diversity and describes the average proportion of heterozygotes per locus in a randomly mating population. Nucleotide diversity is the average number of nucleotide substitutions within a population (Nei & Tajima, 1981; Nei, 1987). The genetic structure of the populations was analysed by analysis of molecular variance (AMOVA) using the Arlequin 2.001 software (Schneider *et al*., 2001). AMOVA estimates the amount of genetic variation attributable to genetic differentiation among self-defined groups (Φ_{CT}), among populations within groups (Φ_{SC}), and among populations relative to the total sample (Φ_{ST}). These Φ values, whose significance was tested using 16,002 non-parametric permutations, are analogous to conventional *F*-statistics (Excoffier *et al*., 1992). Slatkin's linearized F_{ST}s (Slatkin, 1995) between pairs of populations were used as input variables for a principal coordinates analysis (Gower, 1966) using the DistPCoA program (Legendre & Anderson, 1998). Correction for negative eigenvalues was performed using the Lingoes method (Lingoes, 1971). Effective migration rates (Nm) were calculated from F_{ST} (Φ_{ST}) values generated with Arlequin 2.001. *Nm* provides an estimate of the number of migrants per generation. It assumes the infinite-island model of population structure and gene flow (Wright, 1951). Although most populations probably do not conform to this assumption, it provides a useful estimation of the relative extent of gene flow. Isolation by distance was analysed by a regression of pairwise $\Phi_{ST}/1-\Phi_{ST}$ against natural logarithm (ln) of geographical distances in kilometres. A modified Mantel test over 10,000 permutations as implemented in the ISOLDE program in Genepop 3.4 (Raymond & Rousset, 2003) was used to test the null hypothesis that the two variables were independent. For both DNA regions, the relationships between haplotypes were evaluated using Nei & Li's (1979) distance between pairs of haplotypes and the Neighbor and Consensus routine in the software package Phylip 3.57c (Felsenstein, 1993).

Table 2. A + T-rich region haplotype distribution and number in all populations, total number of haplotypes and unique haplotypes per population of *Episyrphus balteatus*.

Haplotype/ sites*	Hel	Hrz1	Hrz2	S-H	Elm	Mue	Ber	Ital1	Ital2	Esp	Nor	UK	Fra
1	5	18	14	5	18	8	6	10	15	17	23	22	5
2												1	
3	2												
4		1											
5										1			
6	1		1										
7		1											
8		1											
9											1		
10	1			1									
11	1			1									
12								1	1				
Haplotype numbers	5	4	2	3	1	1	1	2	2	2	2	2	1
Unique haplotypes	1	3	0	0	0	0	0	0	0	1	1	1	0

* For abbreviations see table 1.

Table 3. *zen*-region haplotype distribution and number in all populations, total number of haplotypes and unique haplotypes per population of *Episyrphus balteatus*.

Haplotype/ sites*	Hel	Hrz1	Hrz2	S-H	Elm	Mue	Ber	Ital1	Ital2	Esp	Nor	UK	Fra
1	2	5	9	3	8	3	1	6	6	7	4	16	2
2			2										
3		4	1	1	1				1	1	1	2	
4	1	2	1			1			1				
5	1		2	1	1	1						1	1
6										1		2	
7	5	2		1				5	2	6	8	1	
8	1	3			1	2	3		3	2	2		2
9		4			3		2		1	1	2		
10									2		3		
11					3								
12											1		
13											2		
14						1					1		
15				1									
16		1											
17												1	
18					1								
Haplotype number	5	7	5	5	7	5	3	2	7	6	9	6	3
Unique haplotypes	0	1	1	1	2	0	0	0	0	0	2	1	0

* For abbreviations see table 1.

Results

Distribution of haplotypes

PCR-amplifications of the A + T-rich region led to an approximately 1470 bp long fragment with no detectable size differences between the populations. Using this sequence, four restriction enzymes (BsaHI, AccI, TaqαI and HhaI) were selected to obtain RFLP markers. All enzymes produced banding patterns and all cutting sites polymorphisms. In total, 12 haplotypes were found (table 2), with approximately two haplotypes per population. Unique haplotypes (in total seven) were found in five populations, three of them from Harz-Braunlage (Germany) (table 2). Haplotype 1 was present in all populations, and haplotypes 6, 10, 11 and 12 in two populations. Haplotypes found in two locations were not spatially aggregated but distributed over the entire sampling region (table 2).

PCR-amplifications of the *zen*-region yielded an approx. 1170 bp long fragment, also with no detectable size differences between the populations. The following restriction enzymes were selected: HinfI, BsrI, PaeR7I and PstI. All enzymes cut at polymorphic sites; in total 18 haplotypes were found, with approximately five haplotypes per population (table 3). The highest numbers of unique haplotypes (in total eight) were recorded in the populations from Elm (Germany) and Norway; seven populations had no unique ones (table 3). Five haplotypes occurred once and three haplotypes more often, though only at one site. Haplotype 1

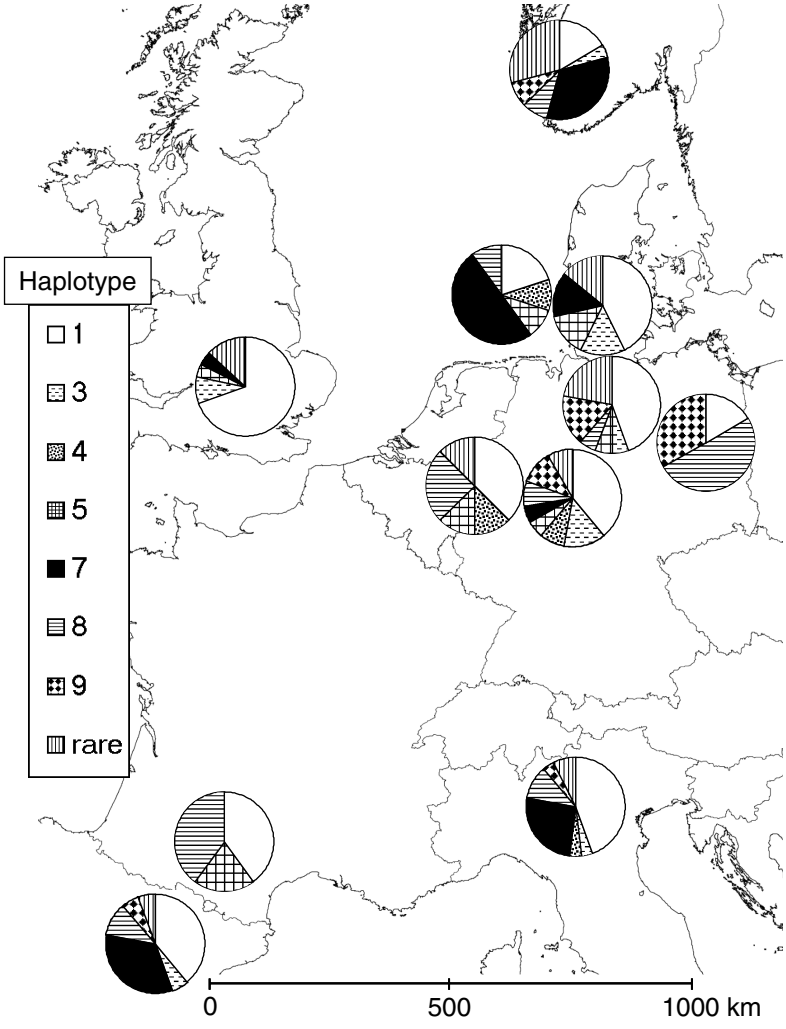

Fig. 1. Map showing distribution of *Episyrphus balteatus zen*-haplotypes in Europe. Data for both Harz and Italy were combined in one pie-chart. Data for rare haplotypes occurring in less than three populations were also pooled.

was present in all populations; haplotypes 3, 5, 7 and 8 were also rather common, and occurred in more than half of the populations sampled (fig. 1, table 3).

The Nei & Li's (1979) distance between pairs of haplotypes indicated no geographic patterns or genetic lineages visible in the resulting trees and, for most of the branches, the bootstrap support was comparatively weak (data not shown).

AMOVA and Φ-statistics

The AMOVA results for the A + T-rich and *zen*-region are summarized in tables 4 and 5, respectively. In both cases, the populations were partitioned into three groups, one comprising the northern populations (i.e. Norway, and the two German sites in Schleswig-Holstein and Helgoland), one comprising the southern populations (i.e. France, Italy, Spain), and the third the remaining populations. In the A + T-rich region, >99% of the genetic variation appeared as individual variation within populations, with almost no variation between populations or the three groups. Conversely, in the *zen*-region with approximately 12% of the genetic variation, considerably more variation was found between the populations, although with *c.* 88% of genetic variation most of the variation appeared within the populations. Other groupings showed the same patterns of variation (results not shown). The fixation indices Φ_{ST}, 0.00469 ($P = 0.40864$) and 0.121 ($P < 0.0001$) for the A + T-rich and *zen*-region, respectively, also indicated low genetic differentiation between populations sampled.

Diversity

Within populations, haplotype and nucleotide diversities of the A + T-rich region indicated distinct differences between such populations. Thus in the populations from Helgoland and Schleswig-Holstein (both Germany) diversity was high, whereas it was low or even equal to zero in all other populations (table 6).

In the *zen*-region, haplotype and nucleotide diversities within populations were substantially higher than in the A + T-rich region (table 6). The highest haplotype diversities

Table 4. Analysis of molecular variance (AMOVA) using A+T-rich region haplotypes of *Episyrphus balteatus*.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Φ-Statistics (fixation indices)
Among groups	2	0.693	0.0026	1.17	−0.0071[a]
Among populations within groups	10	2.026	−0.00158	−0.70	0.0047[b]
Within populations	169	37.76	0.2234	99.53	0.0117[c]
Total	181	40.48	0.2245		

Probability (16002 permutations): [a] 0.4908 ± 0.0040; [b] 0.4086 ± 0.0036; [c] 0.0596 ± 0.0019.

Table 5. Analysis of molecular variance (AMOVA) using *zen*-region haplotypes of *Episyrphus balteatus*.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Φ-Statistics (fixation indices)
Among groups	2	6.542	0.0014	0.12	0.1199[a]
Among populations within groups	10	27.44	0.1336	11.98	0.1210[b]
Within populations	169	165.66	0.9802	87.90	0.0012[c]
Total	181	199.64	0.2255		

Probability (16002 permutations): [a] 0.0000 ± 0.0000; [b] 0.0000 ± 0.0000; [c] 0.4010 ± 0.0035.

Table 6. Haplotype and nucleotide diversity of the A+T-rich region and *zen*-region within all tested populations of *Episyrphus balteatus*.

Location*	A+T-rich region		*zen*-region	
	Haplotype diversity ± SE	Nucleotide diversity	Haplotype diversity ± SE	Nucleotide diversity
Hel	0.756 ± 0.130	3.667	0.716 ± 0.087	2.620
Hrz1	0.271 ± 0.124	1.629	0.850 ± 0.021	2.331
Hrz2	0.133 ± 0.112	0.800	0.616 ± 0.089	1.807
S-H	0.524 ± 0.209	5.000	0.791 ± 0.086	2.181
Elm	0.000 ± 0.000	0.000	0.756 ± 0.057	4.006
Mue	0.000 ± 0.000	0.000	0.800 ± 0.066	2.711
Ber	0.000 ± 0.000	0.000	0.667 ± 0.091	2.897
Ital1	0.182 ± 0.144	2.182	0.520 ± 0.038	3.818
Ital2	0.125 ± 0.106	1.500	0.807 ± 0.048	3.816
Esp	0.111 ± 0.096	0.556	0.737 ± 0.047	3.542
Nor	0.083 ± 0.075	0.750	0.837 ± 0.035	2.430
UK	0.087 ± 0.078	0.174	0.506 ± 0.086	1.904
Fra	0.000 ± 0.000	0.000	0.711 ± 0.086	4.247
Mean	0.175	1.251	0.716	2.947

* For abbreviations see table 1.

were found in populations from Norway, Muenster and Harz-Braunlage (both Germany), and Verona (Italy), whereas populations from Elm (Germany) and France showed the highest nucleotide diversities.

Nm-estimates

Substantial levels of gene flow were found between all sampling sites, though *Nm*-estimates for the *zen*-region were smaller than those for the A+T-rich region (table 7). For the latter region, populations from the two German sites in Helgoland and Schleswig-Holstein, and for the *zen*-region, the Berlin (Germany) population, had lower gene flow estimates. Most of the *Nm*-estimates were >1 and many approached infinity, thus indicating panmixia.

Isolation by distance

Isolation by distance will generate positive correlations between pairwise geographic distances and estimates of $\Phi_{ST}/1-\Phi_{ST}$ (genetic distance). When plotting $\Phi_{ST}/1-\Phi_{ST}$ against geographical distance of all *E. balteatus* populations, no positive correlations were found (fig. 2). For both regions, $\Phi_{ST}/1-\Phi_{ST}$ were independent of geographical distances (A+T-rich region: Spearman's $P = 0.67820$; *zen*-region: Spearman's $P = 0.69060$; Mantel test with 10,000 permutations). Thus no evidence for an isolation by distance effect was found.

Principal coordinates analysis

Figure 3 shows the principal coordinates analysis scattergrams derived from all *E. balteatus* populations. As shown, no clear patterns of relationship between populations were detected. In the A+T-rich region, only populations from the two German sites in Schleswig-Holstein and Helgoland were substantially outside the main cluster. In the *zen*-region, populations from the UK and Harz-Kamschlacken (Germany) formed a second cluster, and those from Verona (Italy), Berlin (Germany) and Norway were considerably outside the two clusters (fig. 3b).

Table 7. *Nm*-Estimates between all populations of *Episyrphus balteatus*. *zen*-region estimates (in the lower left segment), A + T-rich region estimates (in the upper right segment).

	Hel	Hrz1	Hrz2	S-H	Elm	Mue	Ber	Ital1	Ital2	Esp	Nor	UK	Fra
Hel	–	55.8	54.4	inf	5.03	inf	inf	16.6	7.00	6.98	5.60	7.87	inf
Hrz1	4.58	–	inf	inf	inf	inf	inf	inf	inf	inf	118	inf	inf
Hrz2	7.85	3.07	–	8.61	39.4	inf	inf	inf	217	inf	79.7	296	inf
S-H	35.0	inf	inf	–	2.86	24.0	inf	9.44	4.53	3.79	4.96	9.41	inf
Elm	5.93	inf	4.50	inf	–	inf	inf	10.1	65.5	inf	inf	inf	inf
Mue	3.96	inf	2.33	inf	18.0	–	inf	inf	inf	inf	inf	inf	inf
Ber	0.49	2.51	0.30	0.82	1.44	2.39	–	inf	inf	inf	inf	inf	inf
Ital1	inf	1.98	3.01	2.15	1.92	1.19	0.19	–	inf	52.7	inf	inf	inf
Ital2	7.77	inf	2.14	10.1	11.4	inf	2.07	2.17	–	1183	inf	inf	inf
Esp	inf	7.01	5.22	15.1	6.08	6.12	0.62	66.3	29.1	–	inf	inf	inf
Nor	7.35	6.05	1.46	3.77	4.80	7.64	1.80	2.15	inf	8.64	–	inf	inf
UK	6.35	2.78	9143	9.37	2.47	1.89	0.26	4.08	2.01	6.38	1.35	–	inf
Fra	4.07	inf	2.36	inf	inf	inf	3.97	0.94	inf	7.46	14.3	1.52	–

inf, infinite gene flow. For other abbreviations see table 1.

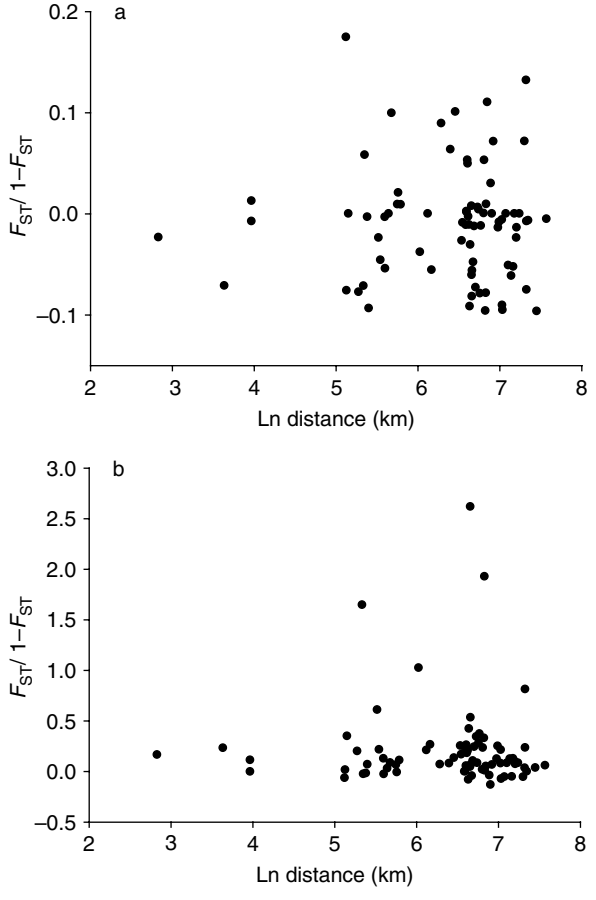

Fig. 2. Scatterplots of pairwise $F_{ST}/1\text{-}F_{ST}$ and ln of pairwise geographical distance between all populations of *Episyrphus balteatus* for (a) the A + T-rich region (a = −0.00787, b = −0.000357, r^2 = 0.000035), and (b) for the *zen*-region (a = 0.24840, b = −0.00564, r^2 = 0.00015).

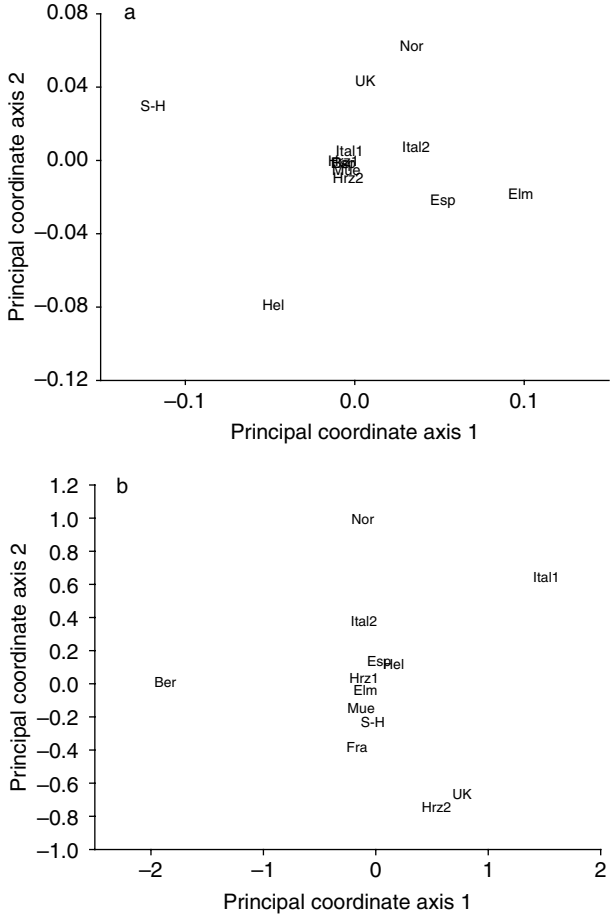

Fig. 3. Principal coordinates analysis of Slatkin's F_{ST} distances of (a) the A + T-rich region for all analysed populations of *Episyrphus balteatus* (the dot accumulation at zero coordinates contains Harz-1, Harz-2, France, Muenster and Berlin), and (b) the *zen*-region for all analysed populations. For abbreviations see table 1.

Discussion

Results of this study strongly suggest that European populations of *E. balteatus* are connected to a large extent and form one large panmictic population. These findings support the hypothesis of seasonal migrations in *E. balteatus* as suggested by several authors (e.g. Aubert *et al.*, 1976; Verlinden & DeCleer, 1987; Gatter & Schmid, 1990; Torp,

1994; Sullivan & Sutherland, 2000). This hypothesis was until now primarily based on two long-term studies of mass movements of *E. balteatus* and other hoverfly species along passes in the Schwäbische Alp (south-western Germany) and the Swiss Alps and numerous occasional flight observations across Europe (Aubert *et al*., 1976; Gatter & Schmid, 1990 and references therein). These observations were interpreted as southbound migrations heading to Mediterranean regions for hibernation. Moreover, physiological experiments, revealing low levels of cold hardiness in *E. balteatus* (Hart & Bale, 1997), suggested an overwintering in warmer climates.

However, to date, northbound migration of *E. balteatus* in spring from the Mediterranean to central and northern Europe has never been observed, although essential for this hypothesis. Unfavourable climatic conditions and the periodic depletion of food sources for adults and larvae of *E. balteatus* during the hot summer months in the Mediterranean are quoted as reasons for such a migratory pattern (Gatter & Schmid, 1990). The results of this study suggest that in the framework of the seasonal migration hypothesis, northbound migrations are very likely to occur, because the genetic diversity of the sampled populations of *E. balteatus* turned out to be high. Assuming that the high densities of *E. balteatus* recorded frequently during the summer months in central and northern Europe derive only from overwintering individuals in these areas, and considering the extremely low numbers of hibernating residents (findings of residents in *E. balteatus* are extremely rare) this would consequently result in bottleneck effects with largely reduced genetic diversity (Nei *et al*., 1975; Hedrick, 2000).

Even so, it is still uncertain how regular these migrations are. For example, Salveter (1996) stressed that during the period of southward migrations of hoverflies, the vegetation in the Mediterranean is still very dry, thus providing insufficient food for the adults and the aphidophagous larvae of *E. balteatus*. Hence, a more facultative or irregular migration pattern might occur (e.g. in the sense of Svensson & Janzon, 1984), only triggered by abiotic (e.g. weather) or biotic conditions (e.g. food resources). This would not exclude the probability that *E. balteatus* migrates over long distances, leading to the observed highly connected populations, with low genetic distances, high gene flow and high genetic diversity.

Physical barriers to migration such as mountains or seas and isolation by distance effects can strongly influence gene flow even in mobile insects (e.g. Gimnig *et al*., 1999). However, in European populations of *E. balteatus*, no such effects were recorded as shown by the lack of population subdivision, the low genetic distances between sampled populations, the very high gene flow rates and the complete lack of isolation by distance even between populations separated by mountains (Swiss Alps, Pyrenees) and the sea (North Sea).

At present, studies on the population genetics of mobile hoverflies covering broad geographical regions are lacking. So far, work has focused on genera with patchy distributions and non-migratory behaviour such as *Portevinia* or *Cheilosia* spp. (Diptera: Syrphidae) (Wynne, 2001; Milankov *et al*., 2002; Ludoski *et al*., 2003). In *E. balteatus*, Sullivan & Sutherland (2000) studied variation in morphological patterns (i.e. asymmetry measures) between specimens collected from nine European sites. They found considerable homogeneity in most morphological measurements, except for total body size, which led to the assumption that only one population, from the island of Madeira (> 450 km from the African continent), might be considered isolated. Despite numerous observations of incoming migrants along coastlines (e.g. Gatter & Schmid, 1990; Svensson & Janzon, 1984), it is presently unclear to what extent *E. balteatus* is capable of migrating offshore, as it is not known if or how often it needs refuelling and resting periods during migration. In the present study, *E. balteatus* populations from islands (UK and Helgoland, Germany), showed a slightly increased similarity and a higher genetic distance compared to populations from other sites.

Similar or lower levels of genetic differentiation have been found over wide spatial scales in other mobile insect species such as the monarch butterfly, *Danaus plexippus* (Linnaeus) (Lepidoptera: Nymphalidae) (Brower & Boyce, 1991), stable fly, *Stomoxys calcitrans* (Linnaeus) (Diptera: Muscidae) (Szalanski, 1995), the planthoppers *Nilaparvata lugens* (Stål) and *Sogatella furcifera* Horvath (both Hemiptera: Delphacidae) (Mun *et al*., 1999), the dragonfly, *Anax junius* (Drury) (Odonata: Aeshnidae) (Freeland *et al*., 2003) and the aphid, *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae) (Llewellyn *et al*., 2003). Hence, this seems to be a relatively typical phenomenon, at least if migration and gene flow are linked, which is not always so (Slatkin, 1985). In *E. balteatus* populations, reproduction is proven in northern and southern areas of Europe (e.g. Tizado-Morales *et al*., 1991; Steenis *et al*., 2001), but it was previously not known if, and to what extent, migrants contribute to the local gene pool. Our findings that a number of common haplotypes are distributed over the entire study area in Europe and the strong genetic similarity between these populations imply that long distance migration determines the genetic structure and that local gene pools cannot be separated.

The observed high genetic diversity within the *zen*-region in most *E. balteatus* populations is particularly remarkable. Often low genetic diversity is connected with lack of genetic structuring and high diversity with high levels of structuring (e.g. Brower & Boyce, 1991; Szalanski, 1995; Gimnig *et al*., 1999), whereas high diversity and low geographic structuring is rarely found (e.g. Mun *et al*., 1999; Freeland *et al*., 2003). A general explanation for the high genetic diversity and the widespread distribution of common haplotypes in most populations may be an adaptation of *E. balteatus* to a broad range of environmental conditions (e.g. temperature, humidity, photoperiod) and biotic factors (e.g. prey species and distribution, food plants, natural enemies). All developmental stages of a species have to be adapted to this array of selection pressures, and a high genetic diversity can increase fitness and the ability to cope with these challenges (Hansson & Westerberg, 2002), although direct correlations are often weak (David, 1998).

In this study the *zen*-region showed larger genetic diversity and revealed more population subdivision compared with the A + T-rich region. Yet, the latter is one of the most variable mtDNA regions and evolves much faster than coding nDNA regions (Simon *et al*., 1994; Parker *et al*., 1998; Ballard & Whitlock, 2004). However, variability can vary to a large extent between species, and species with low A + T-rich region variability are known (e.g. Taylor *et al*., 1993; Dueñas *et al*., 2002). There are three possible

explanations for the observed lower genetic variability in the A+T-rich region:

1. Based on haploid structure and maternal inheritance, the mtDNA effective population size is only a fourth of that of nuclear DNA (Zhang & Hewitt, 2003), which increases the power of genetic drift, leading to a decline in within-population heterozygosity. Consequently, migration influences genetic structure more strongly by easier fixation of haplotype lineages over all populations. The effective population size of *E. balteatus* is likely to be considerably smaller than one might expect based on the high numbers frequently found during the summer, since the lowest population density during the autumn and winter months largely determines the overall effective population size (Hedrick, 2000).
2. In *Drosophila* spp. (Diptera: Drosophilidae), the A+T-rich region consists of three domains with different variability (Brehm *et al*., 2001), making it possible that a more conserved domain of this region was sequenced.
3. The evolutionary rate and variability of the *zen*-region is unknown; thus its variability possibly exceeds that of the mtDNA region. The fundamental functional changes of this region during its evolution might be indicative of this (Hughes & Kaufman, 2002; Stauber *et al*., 2002).

For pest management purposes, it would be disadvantageous if the large summer populations of *E. balteatus* mainly develop from immigrants. This is because in northern Europe, these individuals often arrive too late for sufficient control of for example, cereal aphids. This lack of synchronization between hoverflies and cereal aphids is believed to be one of the main reasons for frequent pest outbreaks in northern Germany (Tenhumberg & Poehling, 1995), whereas in southern parts of Germany, aphids are most often under the control of natural enemies owing to the earlier arrival of hoverflies and other beneficial insects.

The findings of this study strongly suggest a northbound migration of European populations of *E. balteatus* during the spring. However, the results do not exclude the possibility of local overwintering in central and northern regions of Europe. Since both hibernation strategies entail high risks of perishing, adult survival and in consequence population build-up in the summer probably depends primarily on weather conditions, as the severity of winter, wind direction and rainfall, affect both migrants and residents alike. Thus, a double strategy of local overwintering and large-scale migration is a possibility as it would spread the risks and possibly increase the chances of survival. Hence, in future studies, attempts should be made to quantify the relative contribution of such migrants and residents to the summer populations of *E. balteatus* from central and northern Europe.

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