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Population genetics of Daubenton's bat (*Myotis daubentonii*) in the Archipelago Sea, SW Finland

Veronika N. Laine*, Thomas M. Lilley, Kai Norrdahl & Craig R. Primmer

*Department of Biology, FI-20014 University of Turku, Finland (*corresponding author's e-mail: veronika.laine@utu.fi)*

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Due to their reclusive nature, information on the population structure of many bat species is lacking or scarce. The pattern of small scale population genetic structure could reveal the degree of gene flow among colonies, and the evolutionary consequences of short-distance dispersal. In this study, we used nine microsatellite loci to assess the small-scale genetic population structure of Daubenton's bats in the Archipelago Sea comparing it to samples from sites elsewhere in Finland and Europe. The Archipelago Sea is a highly variable environment with possible dispersal barriers. Our results indicate a low level of population genetic structuring among the populations sampled. We found significant isolation by distance in both sexes, indicating a gradual increase of population differentiation across a large geographic scale. In Finland alone, isolation-by-distance was also found, with high levels of gene flow among local populations. Isolation-by-distance was stronger in females, suggesting that males disperse longer distances.

Introduction

From an ecological perspective, a single population can be defined as a set of individuals separated in time and space from other individuals in regards to mating and dispersal tactics (Rockwood 2006). However, exact population definitions vary between species and studies (Waples & Gaggiotti 2006). In recent decades, molecular genetic methods have added an additional dimension to the identification of distinct population units, which can have important management and conservation implications (Moritz 1994). Applications of these methods have revealed that genetic discontinuities can occur within species at a range of geographical scales (Taberlet *et al*. 1998, Avise & Walker 1999, Waples & Gaggiotti 2006, Vähä *et al*. 2007). Such results have led to the conclusion that many factors such as behavior, physical barriers to gene flow, historical colonization patterns and environmental changes such as toxicants can influence the definition of population boundaries.

It is likely that population structuring in bat species is relatively low because of their ability to fly. Flying promotes long-distance dispersal, and the ability to disperse more efficiently almost always results in decreased population differentiation (Bohonak 1999, Bullock, Kenward, & Hails 2002). It is also possible that bat populations are panmictic across their range (Burland & Wilmer 2001). However, Castella

et al. (2000) reported that the Gibraltar Strait, which separates the Iberian Peninsula from the Maghreb in Marocco by a minimum gap of 14 km of the open sea, represents a barrier for gene flow for the greater mouse-eared bat (*Myotis myotis*). A similar, strong population structure is also evident in the Bechstein's bat (Kerth & Van Schaik 2012).

The Archipelago Sea, in southwestern Finland, is a highly variable environment; it is feasible that individuals living on islands can be genetically isolated. However, for some species, a chain of islands represents a possible path for gene flow. For example Seppä and Laurila (1999) showed that in the archipelago, overall differentiation was weak between islands for the common frog (*Rana temporaria*) and the common toad (*Bufo bufo*), although amphibians are considered poor dispersers and highly philopatric (Blaustein *et al*. 1994). Péténian and Néve (2003) found that in the Archipelago Sea, the populations of the butterfly silver-studded blue (*Plebejus argus*), were generally genetically uniform, but there was one island population of butterflies, where the sea had created a gene flow barrier between other island populations.

Features that define the bats, such as nocturnality and flight, make bats difficult to study using traditional ecological methods. In addition, hibernation and migration in some species make research even more difficult. Therefore, molecular genetic techniques provide an invaluable resource for bat research. In bat studies, molecular markers including microsatellites and also allozymes have been used in defining the population genetic structure and how it is affected by seasonal migration (Petit & Mayer 1999) and geographical barriers (Castella *et al*. 2000).

Daubenton's bat (*Myotis daubentonii*) is a small, widespread and common Eurasian bat, which in Finland is at the northernmost border of its distribution. Outside Finland, its range encompasses almost entire Europe and northern Asia (Dietz *et al*. 2009), although the phylogeny of the *daubentonii* group is unclear to the east of the Ural mountains (Kruskop 2004, Matveev *et al*. 2005). Daubenton's bats show strong roost fidelity and sexual segregation during the breeding season (Encarnação *et al*. 2005, Encarnação 2012a). As with many other species in the *Myotis* genus, Daubenton's bats swarm in early autumn. During swarming, bats from a wide area gather at a specific site, often outside their hibernation site, to mate (Parsons & Jones 2003). In addition, random mating occurs during summer as well as during hibernation, when males wake sporadically during winter (Dietz *et al*. 2009, Encarnação 2012b). After hibernation, Daubenton's bats typically migrate to their foraging areas and summer roost in their vicinity, which in Finland are often situated not far from the hibernation site (Nyholm 1965).

Information on the genetic population structure of Daubenton's bat is scarce; only a few molecular studies were carried out on this species. These studies focused on molecular systematics (Mayer & von Helversen 2001, Ruedi & Mayer 2001), paternity (Senior *et al*. 2005, Encarnação 2012b) and dispersal related to European bat lyssavirus type 2 (Ngamprasertwong *et al*. 2008, Atterby *et al*. 2010), rather than on the population genetic structure of Daubenton's bat *per se*. Information on the population genetic structure of Daubenton's bat on a smaller scale would help to determine the degree of gene flow among colonies and reveal the evolutionary importance of short-distance dispersal in the species. Daubenton's bats are specialized in flying and foraging over water, therefore we predicted that stretches of water are unlikely to be genetic barriers in this species, at least in patchy archipelagoes. However, there are other possible barriers to gene flow besides the water barrier, such as social barriers, large industrial areas, which together with less conspicuous physical constraints, such as chemical toxicants, may form larger than expected uninhabitable areas or areas which become less attractive for a bat to cross (e.g. lighting, traffic), although in some cases they may fly far to forage (Encarnação *et al*. 2010). In this study, we used nine microsatellite loci collected from Daubenton's bats from the Archipelago Sea and other sites in Finland and Europe to (1) assess the small-scale genetic population structure and isolation-by-distance in bats in order to (2) assess whether these could be affected by their dispersal behavior or the patchy environment of the Archipelago Sea, and to (3) discuss the potential conservation needs in light of our results.

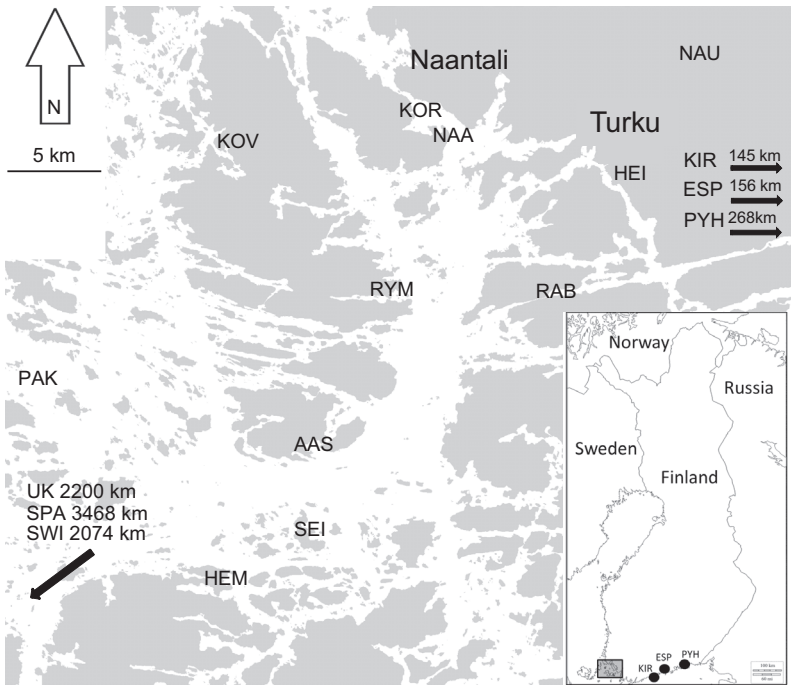

Fig. 1. Sampling locations in the Archipelago Sea, SW Finland. Grey shading indicates land and white water.

Material and methods

The study area and its characteristics

The sampling sites were situated on the mainland and islands in the northern part of the Archipelago Sea, situated in southwestern Finland (60°N, 22°E) (Fig. 1). The islands constitute ca. 2000 km^2 of the total area of 9436 km^2 (Helminen *et al*. 1998). The area has a large river input of fresh water and therefore the salinity is very low (typically less than 5 psu). This greatly affects the species composition of the area, including insects with aquatic lifestages (Canedo-Arguelles & Rieradevall 2009). The rivers running into the Archipelago Sea also carry large amounts of organic and clay-rich sediments, which accumulate on the sea floor, especially in the northern part. The Archipelago Sea is eutrophicated due to the nutrient runoff from rivers (Jumppanen & Mattila 1994). Because of heavy sea traffic caused by two major ports and a large repair shipyard, the northern part of the Archipelago Sea suffers from higher than average organic tin compounds in sediments, which affect the local distribution of e.g. chironomids (Lilley *et al*. 2012a, Lilley *et al*. 2012b). Most of the coastline in the northern part of the Archipelago Sea is covered by a wide (up to approx. 30 m) bed of common reed (*Phragmites australis*) (Pitkänen 2006), considerably increasing the insect biomass production of the area. Open-water areas in the vicinity of the reed beds are primary feeding areas for local Daubenton's bats (pers. obs.).

Study species

The IUCN Red List classifies Daubenton's bat of Least Concern due to its wide distribution and increasing population size (Stubbe *et al*. 2012). Outside Finland, in central Europe, movements up to 304 km between summer roosts and hibernation sites have been recorded (Steffens *et al*. 2004), although mean movements are under 100–150 km (Tress *et al*. 2004). Daubenton's bat prefers to roost in woodpecker cavities, but also use small-bird nest boxes, especially after they are vacated by breeding birds in July (Arnold *et al*. 1998). Female Daubenton's bats forage close to the roost, usually less than 1 km away, but males can forage considerably further, up to 6 km (Senior *et al*. 2005). The majority of the

bats hunt over water or in the vicinity of water, but bats may sometimes hunt in forests, especially in Scandinavia during brighter light conditions (Nyholm 1965); Daubenton's bats generally avoid light and illuminated areas. Daubenton's bats are partially opportunistic trawling bats; insects are usually caught directly from the water surface (Dietz *et al*. 2009). Their diet consists mostly of newly-hatched adult chironomids, but also other Diptera (crane-flies, mosquitoes), aphids, mayflies, lacewings, Hymenoptera, moths and caddis flies are seasonally captured (Vaughan 1997, Flavin *et al*. 2001; E. Vesterinen unpubl. data).

Because of its feeding habits, water bodies with extensive still and vegetation-free water surfaces and trees on both banks represent key foraging patches for this species, as they provide high insect densities and a suitable foraging habitat during the seasonal activity period (Taake 1992, Warren *et al*. 2000, Encarnação *et al*. 2004, 2010, Almenar *et al*. 2013) In Finland, due to the low salinity of the northern Baltic Sea, Daubenton's bats regularly forage over the sea surface.

Bat trapping and sample collection

Bats were caught with a combination of mist nets and harp trap at summer roosting and feeding sites between July–August 2008–2010. This multi-trap combination was placed across the flying corridor of bats for approx. 1.5 hours at civil dusk to catch individual bats commuting between roosts and foraging areas. The mist nets used for capturing bats were 6 meters in length and 3 meters high with a mesh size of 36 mm, positioned on each side of the harp trap as guides. A Sussex Autobat siren, which gives species specific ultrasound social calls at steady intervals, was placed in the center of the harp trap to attract the bats (Hill & Greenaway 2005). Bats were also caught emerging from known woodpecker cavities and bird boxes at summer breeding sites and a swarming site.

All tissue sampling was conducted in the field. Captured bats were measured (weight and forearm length), sex and reproductive status were checked and tissue samples were collected from the wing membrane between fourth and fifth finger with a 3-mm hole punch. The samples were subsequently placed in Eppendorf tubes, labelled by individual, filled with 70% ETOH, and stored at –20 °C. Sampling was conducted under the license ESLH-2009-04960/Ym-23. We also received DNA samples from Spain (Itxulegor/ Sierra de Entzia), United Kingdom (Sussex) and Switzerland (Cathy/Pleine Lune) to compare our population structuring to distant Daubenton's bat populations.

DNA extraction and microsatellite genotyping

DNA was extracted from a single biopsy punch by using the modified salt-extraction protocol (Aljanabi & Martinez 1997). The yield of genomic DNA varied from 1 to 160 ng μl^{-1} with an average final concentration of 5 ng μl^{-1}.

All samples were analysed at 9 microsatellite loci shown previously to be polymorphic in Daubenton's bats (Senior *et al*. 2005, Ngamprasertwong *et al*. 2008, Jan *et al*. 2012) (Table 1). The unlabelled primer included a 5´-GTTT-3´ tail to promote Taq DNA polymerase adenylation (Brownstein *et al*. 1996). The PCRs were carried out in a final volume of 10 μl in a single multiplex PCR reaction that included 1× Qiagen Multiplex PCR Master Mix, fluorescently labelled forward primers, reverse primers, approx. 15 ng of genomic DNA, and dH_2O to a final volume of 10 μl. Primer concentrations and labels are listed in Table 1. The thermal cycling program for the multiplex was: 95 °C for 15 min; 35 cycles of 94 °C for 30 s, 59 °C for 1 min 30 s, 72 °C for 1 min 30 sec, followed by 60 °C for 30 min. PCR products were subsequently diluted with water 1:100 and electrophoresed on an ABI3130xl Genetic Analyzer with GeneScan-600 LIZ size standard (Applied Biosystems, Foster City, CA). Genotypes were scored using GeneMapper 4.0 software (Applied Biosystems, Foster City, CA).

Deviations from the Hardy-Weinberg equilibrium (HWE) within all populations (within loci) and across all loci (within populations) were calculated using the population genetics program GENEPOP 4.0 (Rousset 2008) using Guo and Thompson's (1992) exact test. The GENEPOP

Table 1. Primer sequences, labels and primer specific concentrations and information for the microsatellite loci used in the study. Underlined sequence is the GTTT tail. F is for forward and R is for reverse primer.

Locus	Sequences (5´–3´)	Array	Fluorescent tag	No. of alleles	Allele size range (bp)	Marker concentration (both F & R, μM)
A24-Mluc[a]	F: GTGGTATGAAATAACCAGTTCACTTTG R: GTTTCAGACTGCATTACTGAAGAAATTATGG	$(AC)_n$	FAM	11	336–356	0.2
C113[b]	F:GTTTACCTCCCTGCCCTGCAC R:GCAATGCTTCCTCCAAGTCC	$(AAC)_n$	FAM	4	94–103	0.1
Clone A2-Mluc[a]	F:TGGCCCATGCTCATCATC R:GTTTCTGGTCTCAACTGGGTGCTC	$(CA)_n$	VIC	14	93–127	0.05
D9[b]	F:GTTTCTTTCCTCCCCTGTGCTC R:TCTGGACCCAAAATGCAGG	$(CT)_n$	NED	26	111–175	0.2
E24[b]	F:GTTTGCAGGTTCAATCCCTGACC R:AAAGCCAGACTCCAAATTCTG	$(TC)_n$	FAM	23	216–262	0.2
ES43-MLuc[a]	F:GTTTAAGGGGGAGAGGAGTGG R:GCTGCGTGTCCAGAGG	$(AC)_n$	FAM	16	368–398	0.2
H29[b]	F:GTTTCAGGTGAGGATTGAAAACAC R:GCTTTATTTAGCATTGGAGAGC	$(CA)_n$	FAM	13	162–214	0.4
MM1[c]	F:GTTTGTTAATGACTGTAGC R:TTGCCCTTCCTGTCCTTTAA	$(GT)_n$	VIC	15	157–207	1.2
Paur6[d]	F:GATCAGATTTCCAAACAGAG R:GTTTAGGTTCTTTCTTCAGCTATG	$(AC)_n(AG)_n$	PET	16	145–179	0.2

[a] Jan *et al.* 2012, [b] Castella & Ruedi 2001, [c] Petri *et al.* 1997, [d] Burland *et al.* 1998.

4.0 program was also used for assessing genotypic linkage equilibrium among every locus pair. The Markov chain method was used to estimate the p values for linkage among each pair of loci in every population individually. Fisher's method was used to calculate the overall significance values for each locus pair across all populations. False discovery rate (FDR) procedure using the program QVALUE (Storey & Tibshirani 2003) with an FDR threshold of 0.05 was used to detect false significant results. This program calculates q values, which are extensions of FDR describing the proportion of false positives incurred within a set of significant features (Storey & Tibshirani 2003). Null alleles from every marker were examined with the program MICRO-CHECKER 2.2.3 (Van Oosterhout *et al*. 2004).

For every sample site, the genetic diversity indices, observed number of alleles (A), expected gene diversity (H_e), and the observed proportion of heterozygotes (H_o) was calculated by using MICROSATELLITE TOOLKIT add-in for EXCEL (Park 2001). Allelic richness (R_t) averaged across loci was calculated with FSTAT 2.9.3.2 (Goudet 1995). This method corrects allele number for sample size using the rarefaction method summarized in El Mousadik and Petit (1996), making the calculated values comparable across varying sample sizes.

Population differentiation was estimated by the GENEPOP 4.0 program. Fisher's exact test as described in GENEPOP 4.0 and in Raymond and Rousset (1995a, 1995b) estimated the p values for genetic differentiation between each population pair at every locus and across all loci. The pairwise F_{ST} values over all loci for each population pair were also calculated with GENEPOP 4.0. An F_{ST} value measures the extent of variance in allele frequencies among each population pair (Weir & Cockerham 1984). Its theoretical minimum is 0 (no genetic divergence) and a theoretical maximum is 1 (fixation of alternative alleles in different populations). However, found maximum is usually much smaller than 1. Wright (1978) proposed the following guidelines for interpretation of F_{ST}: the values 0–0.05, 0.05–0.15, 0.15–0.25, and > 0.25 indicate little, moderate, great and very great genetic differentiation, respectively.

As first described by Wright (1943), isolation-by-distance (IBD) is defined as a decrease in the genetic similarity between populations as the geographic distance between them increases. F_{ST} estimates from GENEPOP were used to test for IBD using the Mantel test in GENALEX 6.4 (Peakall & Smouse 2006). This test assessed whether the direct distance (km) between sample-site pairs correlated with their genetic distances (pairwise F_{ST}). Statistical significance of the correlation estimates was attained by performing 9999 permutations. According the recommendations of Rousset (1997), the final correlation between $F_{ST}/(1 - F_{ST})$ and the logarithm of Euclidean geographical distance between populations in kilometres was examined. IBD was determined using all individuals and also for males and females from each sampling site separately. To detect sex-biased dispersal assignment tests implemented within GENALEX were used.

Results

Seven sampling sites deviated significantly from the HWE expectations (p value for deviations > 0.05), and after a Bonferroni correction five populations remained statistically significant. All but two of the sample sites showed significant inbreeding coefficient (F_{IS}) estimates at a maximum of two loci. Because there is no information about population structuring, it is not possible to exclude the possibility that low sample sizes might have played a role in these deviations. Linkage disequilibrium was tested for each pair of loci in every population individually and across all populations, and after Bonferroni correction significant linkage disequilibrium was found between three pairs of loci: C113 and D9, D9 and E24, A24 and H29. Of the 540 Fisher exact probability tests undertaken, only 4 gave significant results after the false discovery rate procedure. Null alleles were found from one or two different markers across populations. However, these infrequent deviations are unlikely to affect the results.

The heterozygosity values for each populations varied between 0.82 to 0.54, and the mean number of alleles and allelic richness ranged from 6.56 to 11.11 and from 3.61 to 4.17, respectively (Table 2).

Considerable variation in the levels of population differentiation was apparent (Table 3). The F_{ST} values ranged from –0.0099 to 0.0637, with 57% (78 of 136) of the values being significant (Fisher's exact test: $p < 0.05$). The global F_{ST} value over all populations, including also non-Finnish samples, was 0.012. The global F_{ST} for females and males separately was 0.023 and 0.007, respectively. Without foreign samples the global F_{ST} across all samples was 0.005, 0.011 for females, and 0.001 for males. A significant difference in dispersal between sexes was found: the mean corrected assignment index (mAIc) obtained for females (mAIc = 0.245) was significantly higher than in males (mAIc = –0.136) ($p = 0.045$ for mAIc; 1000 randomizations) indicating male-biased dispersal.

A positive correlation between $F_{ST}/(1 - F_{ST})$ and log[Euclidean geographical distance] was found for all populations (Fig. 2a; Mantel test: $r = 0.71$, $p < 0.001$). When the two sexes were considered separately, correlations between genetic and geographical distances were also found (Mantel test: $r = 0.55$, $p = 0.001$ for males [Fig. 2b]; $r = 0.75$, $p = 0.002$ for females [Fig. 2c]), but females showed a higher degree of isolation by distance than males.

When non-Finnish samples were excluded, isolation by distance remained significant but weak for both sexes combined and for females, but not for male bats only (Mantel test: $r = 0.46$, $p = 0.005$ for all [Fig. 3a]; $r = 0.10$, $p = 0.277$ for males [Fig. 3b]; and $r = 0.21$, $p = 0.021$ for females [Fig. 3c]).

Discussion

Our results indicate a generally low level of population structuring in Daubenton's bats, as heterozygosity was high and F_{ST} values low, even with all the European samples included. These diversity levels were within the range normally found for microsatellite loci in Daubenton's bats (Ngamprasertwong *et al*. 2008, Atterby *et al*. 2010). Our results thus indicate that there are no evident barriers to gene flow in the Archipelago Sea, or the rest of Finland and Europe.

Although distinct barriers were not evident, we found significant isolation by distance (IBD)

Table 2. Genetic diversity indices for each of the population averaged across microsatellite loci and the *p* values for the HWE exact tests; *n* = sample size.

Population	Abbreviation	Sampling site type	*n*	Sex distribution (male/female)	Observed heterozygosity	Expected heterozygosity	Number of alleles	Allelic richness	HWE *p*
Aasla	AAS	Foraging	32	21/11	0.75	0.79	10.00	3.93	0.008
Espoo	ESP	Foraging	10	5/5	0.82	0.81	7.22	4.00	0.875
Heikkilä	HEI	Swarming	61	40/21	0.75	0.79	11.11	3.96	0.0025
Hemsundet	HEM	Foraging/mix roost	19	14/5	0.74	0.78	9.33	3.94	0.4231
Kirkkonummi	KIR	Foraging/mix roost	21	9/12	0.77	0.78	8.33	3.86	0.3962
Korjaustelakka	KOR	Foraging/maternity	45	18/27	0.75	0.76	9.89	3.76	0.1274
Koverinlahti	KOV	Foraging	17	8/9	0.77	0.76	8.22	3.76	0.4749
Naantali	NAA	Foraging	64	59/5	0.73	0.76	10.56	3.84	0.0851
Nautelankoski	NAU	Mix roost	27	21/6	0.77	0.77	9.22	3.87	0.1527
Pakinainen	PAK	Maternity	40	12/28	0.72	0.76	10.22	3.85	0.0985
Pyhtää	PYH	Foraging	11	6/5	0.67	0.73	6.56	3.61	0.3635
Turku islands	TUR	Foraging	60	42/18	0.77	0.78	10.67	3.89	0.0347
Rymättylä	RYM	Foraging	22	16/6	0.73	0.80	9.33	3.95	< 0.001
Seili	SEI	Foraging	12	4/8	0.75	0.80	7.44	3.97	0.5808
Spain	SPA		20	19/1	0.54	0.80	8.00	3.91	< 0.001
United Kingdom	UK		13	13/0	0.79	0.81	9.44	4.17	< 0.001
Switzerland	SWI		17	17/0	0.57	0.78	8.44	3.90	< 0.001

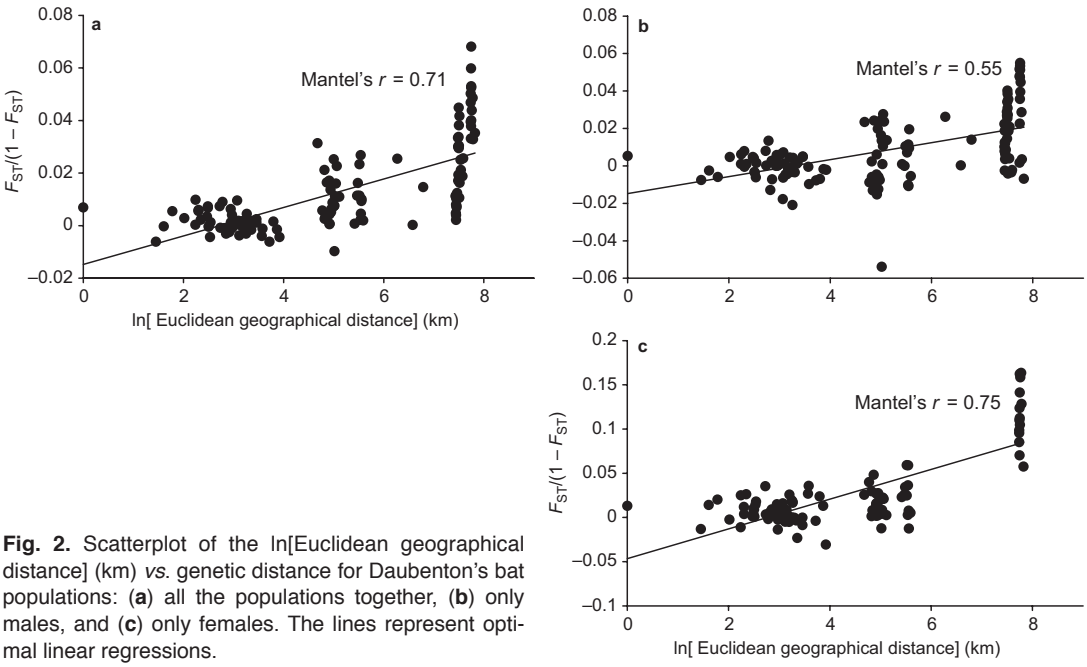

Fig. 2. Scatterplot of the ln[Euclidean geographical distance] (km) *vs.* genetic distance for Daubenton's bat populations: (**a**) all the populations together, (**b**) only males, and (**c**) only females. The lines represent optimal linear regressions.

in both sexes, indicating some population differentiation on a large scale. Isolation-by-distance was found within Finnish populations, indicating that there are higher levels of gene flow among local populations and less among more distant ones. Significant IBD was found only in females, indicating males disperse longer distances, as found in Daubenton's bats and other species of *Myotis* elsewhere (Kerth *et al*. 2002, Ngamprasertwong *et al*. 2008).

Table 3. The pairwise F_{ST} values and geographical distances for all seventeen sample sites. Geographical distances (km) are above the diagonal and pairwise F_{ST} values are below the diagonal. The significant values are underlined ($p < 0.01$) and in boldface ($p < 0.05$).

Pop.	KOR	NAA	RYM	KOV	TUR	HEI	AAS	PAK	SEI	NAU	HEM	KIR	ESP	PYH	UK	SWI	SPA
KOR		1	9	10	12	12	19	22	23	26	28	136	149	249	1726	1814	2322
NAA	**0.007**		10	10	12	12	20	22	24	26	29	136	149	249	1727	1814	2322
RYM	**0.010**	**0.006**		5	15	19	13	13	17	35	21	142	155	257	1717	1804	2312
KOV	0.006	0.002	0.000		19	22	17	12	21	36	25	145	159	259	1717	1806	2314
TUR	**0.007**	0.001	**0.007**	0.001		8	15	27	19	26	24	127	140	242	1729	1812	2321
HEI	**0.007**	**0.003**	0.000	0.002	**0.003**		23	32	26	19	32	124	137	238	1735	1819	2328
AAS	**0.006**	0.001	0.004	0.003	0.001	0.004		17	4	41	9	137	151	255	1714	1797	2305
PAK	**0.009**	0.002	0.001	0.000	0.001	0.002	0.001		20	48	20	153	167	269	1705	1794	2301
SEI	0.002	0.000	0.001	0.000	0.003	0.003	0.006	0.004		45	6	137	151	255	1712	1793	2302
NAU	**0.004**	0.003	0.004	0.001	0.001	0.000	0.006	0.002	0.002		51	119	130	226	1752	1838	2347
HEM	**0.002**	0.002	0.001	0.002	0.000	0.001	0.000	0.000	0.005	0.004		141	156	260	1706	1787	2296
KIR	**0.017**	**0.004**	**0.006**	**0.009**	**0.004**	**0.003**	0.001	**0.011**	0.001	**0.006**	**0.005**		16	124	1829	1873	2389
ESP	**0.025**	**0.007**	**0.011**	**0.022**	**0.013**	**0.004**	**0.008**	**0.011**	0.010	**0.016**	**0.016**	**0.009**		108	1845	1888	2405
PYH	**0.023**	0.011	0.005	0.009	0.016	**0.011**	0.002	0.002	**0.026**	0.001	0.010	**0.021**	**0.030**		1953	1983	2502
UK	**0.010**	**0.011**	**0.004**	0.008	**0.007**	**0.007**	0.002	**0.011**	**0.005**	**0.012**	**0.011**	**0.010**	**0.016**	0.018		722	890
SWI	**0.043**	**0.037**	**0.029**	**0.033**	**0.029**	**0.030**	**0.019**	**0.029**	**0.017**	**0.040**	**0.032**	**0.024**	**0.021**	**0.025**	0.000		530
SPA	**0.064**	**0.050**	**0.036**	**0.056**	**0.050**	**0.039**	**0.032**	**0.048**	**0.038**	**0.042**	**0.045**	**0.046**	**0.032**	**0.034**	**0.014**	**0.025**	

The very low pairwise F_{ST} values (Table 1) and high gene diversity indices (Table 2) indicate very high levels of gene flow among local Daubenton's bat colonies. Additional cluster analysis with the software STRUCTURE (Pritchard *et al*. 2000) did not show any cryptic population structuring (data not shown). Possible explanations for the lack of population structuring are mating behavior or individual dispersal among local colonies. Only one swarming site has so far been found from the study area (site HEI), which also showed high diversity indices. A paucity of swarming sites should aggregate individuals from large areas together, promoting mixing of different colonies. Swarming behavior is regarded as an important method of avoiding inbreeding and also an important reproductive strategy especially for young and sub-dominant males at the end of the summer when they are reproductively active (Parsons & Jones 2003, Encarnação *et al*. 2004), although, mating may also occur in breeding colonies (Senior *et al*. 2005).

The findings of this study are in agreement with those of Ngamprasertwong *et al*. (2008), who reported low global F_{ST} values over all Daubenton's bat populations in Scotland, and an AMOVA analysis for microsatellite-derived genetic divergence indicating low genetic differentiation among populations. They also noticed that no significant F_{ST} value was found among populations located less than 25 km apart. In our study, a significant differentiation value was obtained from some sample sites located as little as 1 km apart. However, the differentiation value was not high and was between a male-dominated foraging area (sample site NAA) and a female maternity roost/foraging area (sample site KOR), which can be explained by sexual segregation and dispersal patterns in Daubenton's bats (Angell *et al*. 2013). In addition, the landscape of the study in Scotland maybe different to the Archipelago Sea, therefore flying distances for bats can be longer if they avoid certain areas, such as long stretches of open water or illuminated urban areas.

Most species of mammals show male-biased dispersal (Greenwood 1980) whilst females may benefit from being philopatric due to the strong selection they are under for their ability to exploit local resources in order to provide parental care for immobile offspring (Johnson & Gaines 1990, Wolff 1993). Also, if inbreeding is to be avoided and therefore is a selective factor driving dispersal, then only one sex should disperse, the other sex being more philopatric (Perrin & Mazalov 1999). In concurrence with this, most temperate-zone bats are no exception to the male-biased mammalian dispersal pattern: male bats disperse and are responsible for gene-flow whereas, despite their relatively high dispersal abilities as compared with that of other mammals, the maternity colony-forming females remain philopatric (Burland & Worthington Wilmer 2001). This is also supported

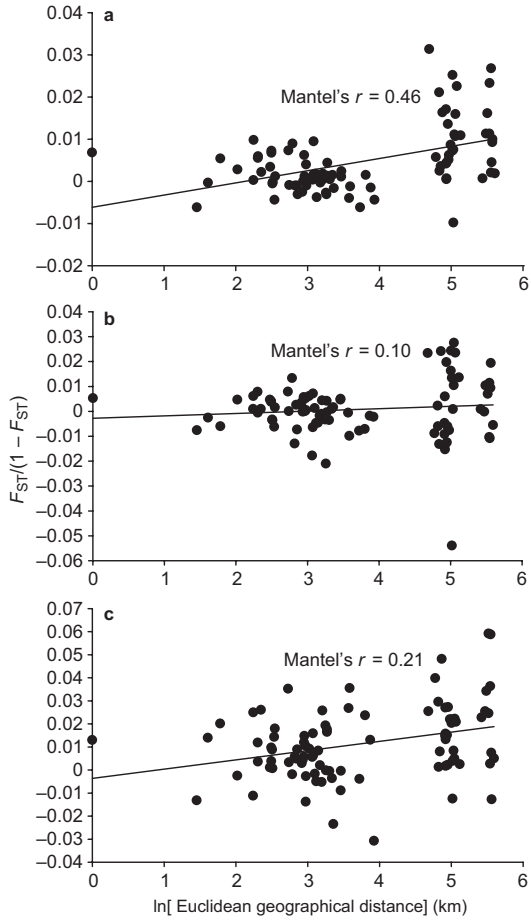

Fig. 3. Scatterplot of the ln[Euclidean geographical distance] (km) *vs.* genetic distance for Finnish Daubenton's bat populations: (**a**) the Finnish populations together, (**b**) only males, and (**c**) only females. The lines represent optimal linear regressions.

by the results of the present study with stronger IBD in Finnish female bats as compared with that in male bats. However, when including all the sample sites, the significant IBD is more likely to be due to distant populations rather than strong philopatry. In fact, many molecular analyses show that female natal philopatry is often very pronounced (Petit and Mayer 1999, Burland and Wilmer 2001, Castella *et al*. 2001, Kerth *et al*. 2002). However, a recent study by Encarnação (2012a) reports average home ranges of 47.3 km^2 for Daubenton's bat males, whereas female ranges were much smaller (9.7 km^2). This may also partially account for the differences in sexes in IBD.

Many bat species have suffered population declines in past decades mainly due to human activities such as destruction of natural habitats and foraging areas (Hutson *et al*. 2001, Rebelo & Rainho 2008), pesticide poisoning (Geluso *et al*. 1976) and chemical pollution (Walker *et al*. 2007). In the study area, high local concentrations of sediment bound tributyltin (TBT) has been a conservation concern, as TBT accumulates in the main prey of Daubenton's bat, chironomids (Lilley *et al*. 2012b; E. Vesterinen unpubl. data). However, the apparent wide-scale mobility of Daubenton's bats suggests that possible negative effects of small-scale pollution are diluted as the bats hunt also in less polluted areas. This indicates that these populations may not be chronically exposed and affected by local pollutants as also suggested by Lilley *et al*. (2013).

Conclusions

Due to their high mobility and complex breeding systems, it is difficult to interpret population boundaries in bats even with molecular methods used here. Instead of populations, one could consider grouping bat individuals in terms of colonies, feeding areas or swarming areas. In our study, genetic diversity indices were high across the range. This can be due to high gene flow during swarming behavior or frequent long-distance movements by males (dispersal). The observed sex-biased dispersal warrants more detailed investigation.

Our results show that genetic isolation is not a conservation concern in Daubenton's bat as long as the mechanisms that maintain high genetic diversity are preserved. As the ecology of Daubenton's bats is not exceptional among temperate bats, our results can probably be generalized to other bat populations with winter hibernation and swarming behavior. Swarming and wintering sites are likely to play an important role as they ensure the genetic mixing of local populations. Our results highlight the need to preserve the swarming and wintering sites of bats to ensure the genetic diversity in local populations.

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