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Low genetic variability, female-biased dispersal and high movement rates in an urban population of Eurasian badgers *Meles meles*

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Summary

1. Urban and rural populations of animals can differ in their behaviour, both in order to meet their ecological requirements and due to the constraints imposed by different environments. The study of urban populations can therefore offer useful insights into the behavioural flexibility of a species as a whole, as well as indicating how the species in question adapts to a specifically urban environment.
2. The genetic structure of a population can provide information about social structure and movement patterns that is difficult to obtain by other means. Using non-invasively collected hair samples, we estimated the population size of Eurasian badgers *Meles meles* in the city of Brighton, England, and calculated population-specific parameters of genetic variability and sex-specific rates of outbreeding and dispersal.
3. Population density was high in the context of badger densities reported throughout their range. This was due to a high density of social groups rather than large numbers of individuals per group.
4. The allelic richness of the population was low compared with other British populations. However, the rate of extra-group paternity and the relatively frequent (mainly temporary) intergroup movements suggest that, on a local scale, the population was outbred. Although members of both sexes visited other groups, there was a trend for more females to make intergroup movements.
5. The results reveal that urban badgers can achieve high densities and suggest that while some population parameters are similar between urban and rural populations, the frequency of intergroup movements is higher among urban badgers. In a wider context, these results demonstrate the ability of non-invasive genetic sampling to provide information about the population density, social structure and behaviour of urban wildlife.

Key-words: group size, outbreeding, population density, sex-biased dispersal, spatial genetic structure, sex typing.

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Introduction

Eurasian badgers (*Meles meles* L. 1758) have been known for some time to inhabit urban environments (e.g. Harris 1982; Cheeseman *et al*. 1988), where they can achieve burrow ('sett') densities comparable to those of most rural UK populations (Huck, Davison & Roper, in press). When urban and rural populations of the same species are compared, various animal taxa have been found to differ behaviourally in various respects (Ditchkoff, Saalfeld & Gibson 2006): for example, urban and rural Cooper's hawks (*Accipiter cooperii* Bonaparte 1828; Estes & Mannan 2003) differed in prey delivery rates, while urban red foxes (*Vulpes vulpes* L. 1758) have less stable territories than is typical of rural populations (Doncaster & Macdonald 1991). The same applies to badgers, in so far as urban badgers show less intense territorial behaviour than rural populations (Cheeseman *et al*. 1988), have smaller home ranges and differ in their pattern of sett use (Davison 2007). The study of urban populations can therefore offer useful insights into the behavioural flexibility of a species as a whole, as well as indicating how the species in question adapts to a specifically urban environment.

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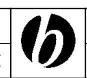
	J	A	E	1	4	1	5	Operator: LinPing		Dispatch: 30.05.08	PE: Penny Baker
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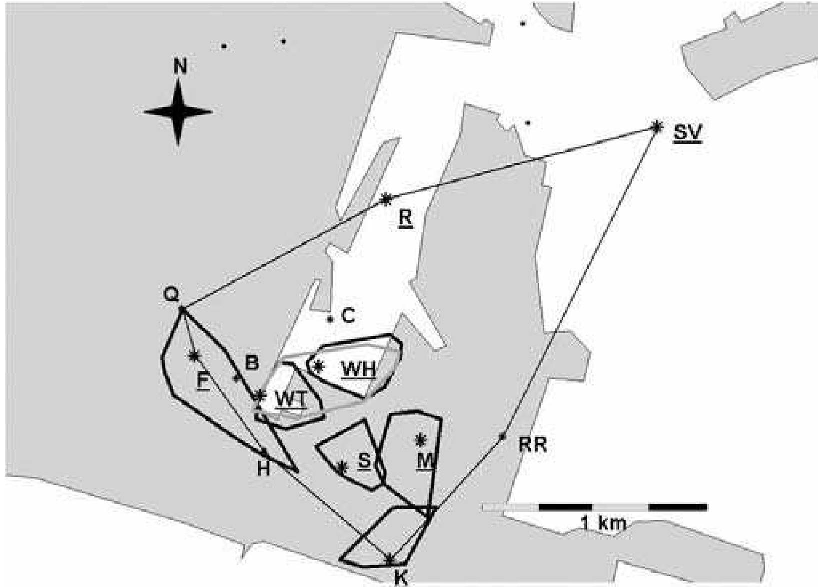

Fig. 1. Badger setts in Brighton where hair samples were collected. Large asterisks and underlined letters denote main setts, small asterisks setts of uncertain status. Dots show all known occupied badger setts in the vicinity of the study area that were not studied. The study area is bordered by the thin-lined polygon (195·6 ha). The dark background shows urbanized habitats (136·9 ha within study area). Bold-lined polygons signify group home ranges (Davison *et al*., submitted), with the exception of group WT that shows only the combined range of two females, while the range of a male is shown in grey. Outlier setts where no sampling took place are not depicted.

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Mating systems may also vary between different habitats (e.g. Langbein & Thirgood 1989), leading to variation in the genetic structure of the populations in question.

The genetic structure of a population can in turn provide information about social structure and movement patterns – and thus indirectly about some behaviours that might be difficult to obtain by other means such as radio-tracking or direct observation (e.g. Favre *et al*. 1997; Huck, Roos & Heymann 2007). In the case of badgers, microsatellite analysis based on blood samples has recently provided detailed information about the mating system and genetic structure of two rural populations (Carpenter *et al*. 2005; Dugdale *et al*. 2007, 2008), while DNA from non-invasively collected hair samples has been used to estimate badger population sizes and to track movements of badgers between social groups (Frantz *et al*. 2004; Scheppers *et al*. 2007). However, genetic information about urban badgers is completely lacking and there has been only one previous attempt to estimate population density in urban badgers (Harris & Cresswell 1987).

Our study used non-invasively collected genomic DNA (extracted from hair samples) in order to: (1) estimate the population density of badgers in a restricted urban area within the city of Brighton, England; and (2) determine population-specific parameters of genetic variability, and sex-specific estimates of outbreeding and dispersal. These results were compared to those of previous studies of rural badger populations, in order to determine whether urban and rural populations differ with respect to these parameters.

Methods

STUDY AREA

Our core study area comprised the areas of Kemptown and Whitehawk within the city of Brighton, England, where badgers had been subject to a radio-tracking study since September 2004 (Davison 2007). The area in question (minimum convex polygon around all sampled setts) covered 195·6 ha, including 136·9 ha of urban habitat consisting of private gardens, small patches of scrub unused by humans, allotments, public parks and areas of mown grass on playing fields and around housing estates. The area contained six main setts (urban setts F, K, M, S, WT, WH), several small setts that were known to be outliers of these main setts, and five setts whose status was unclear (B, H, Q, C, RR; see Fig. 1). These latter setts were usually separated by a larger distance from the nearest main sett than known outliers or were never visited by radio-collared individuals from adjoining main setts. In addition, data were collected from an adjacent suburban sett (R) and from the nearest rural sett (SV).

SAMPLE COLLECTION AND DNA EXTRACTION

The collection of hair samples followed the method described by Scheppers *et al*. (2007). Hair traps consisted of a strand of barbed wire supported by two metal stakes, placed approximately 30 cm apart and with the highest point of the wire about 22 cm above ground. Traps were placed across well-used badger paths ('runs'), where possible well hidden in vegetation such as brambles, or beneath fences, and usually in close proximity to a sett. Sett S was located on private school ground, and therefore was not accessible during the first period of hair collection, so that runs at some distance from the sett had to be used. For the second time-period, however, it was possible to collect samples from the runs around this sett.

We collected guard hair samples during two periods in 2006: from 20 March to 24 April and from 9 October to 16 November, except for sett S where samples were collected from 10 to 25 August. These periods were chosen because they coincide with peaks of reproductive activity in British badgers (Cresswell *et al*. 1992), thus enhancing the possibility of detecting the intergroup movements which were occurring for mating purposes. In addition, we wanted to calculate population densities before (spring) and after (autumn) the emergence of cubs from the dens. The sampling period of 4 weeks was based on previous studies (Frantz *et al*. 2004; Scheppers *et al*. 2007), but was prolonged in some cases where trapping proved difficult because of insufficiently dense vegetation or because traps were vandalized.

Hairs were collected daily using forceps. After each collection we flamed both the forceps and the barbed wire in order to avoid sample cross-contamination. Hairs collected from the same barb were considered to constitute one sample. Hairs from different barbs of the same trap were classed initially as separate samples, but after further analysis were considered separate only if shown to be genetically different. We also collected hair samples from eight cubs (caught during attempts to capture adults, Davison *et al*., submitted; see section 'Sex determination'). Samples were stored in separate paper envelopes at room temperature until DNA extraction. Genomic DNA was usually extracted on the day of collection and always within 2 days of collection.

Following the reasoning of Scheppers *et al*. (2007), we used only single hairs for both a main and a back-up extraction. Extractions took place in a laboratory where no previous work on badger DNA had been performed, using a Chelex protocol (Chelex-100; Bio-Rad, Hercules, CA, USA; Walsh, Metzger & Higuchi 1991) described by Frantz *et al*. (2004).

POLYMERASE CHAIN REACTION (PCR) AND GENOTYPING

DNA amplification and genotyping took place after each period of sample collection at the University of Sheffield. We tested a total of 32 badger microsatellite loci for their variability in our study population. The loci were developed originally by Bijlsma *et al*. (2000), Domingo-Roura *et al*. (2003), Carpenter *et al*. (2003) and D.A. Dawson (unpublished data). Six loci were not variable in a subset of samples, four appeared unreliable for scoring (where PCR products regularly included more than two electropherogram peaks), and two were discarded at a later stage of analysis (see below and Table 1), leaving 20 microsatellite loci available for the final analyses.

PCRs were set up and conducted in a separate room where no work with concentrated badger DNA had been performed previously, under an ultraviolet hood. The hood was cleaned thouroughly with bleach after setting up each PCR and was switched on daily after work for 20 min to remove potential for cross-contamination. For samples from the first sampling period, PCRs were performed as single or double-plex reactions using the conditions described in Carpenter *et al*. (2005) and Pope *et al*. (2006). Loci were amplified using the touchdown-profile described by Frantz *et al*. (2003). The total reaction volume was initially 25 µL, including 5 µL of DNA extract, but for some loci (Mel15, Mel101 and Mel105) this volume was reduced to 10 µL using the same concentrations of constituents, and with 1–5 µL of extracted genomic DNA extract.

DNA from the samples collected during the second sampling period was amplified in multiplex reactions, using the Qiagen Multiplex Kit (Qiagen, Hilden, Germany). Each multiplex reaction contained 1 × Qiagen Multiplex Master Mix, 0·2 µM of each primer and 0·5 × Q-solution. After drying 1 µL of DNA (*c*. 1–10 ng mL^{-1}) (or 5 µL in the case of some DNA extractions of poor yield or quality) for *c*. 15 min at 37 °C in a 384-well PCR plate (Greiner Bio-One, Stonehouse, UK), multiplex reactions were performed in a total volume of 2 µL. A touch-down profile was used, starting with 15 min denaturation at 95 °C, followed by denaturation at 94 °C for 30 s, annealing at initially 61 °C for 90 s and extension at 72 °C for 1min. The annealing temperature was then reduced by 1 °C per cycle for five cycles, then kept at 55 °C for the remaining 29 cycles. Final incubation was at 60 °C for 30min. A negative control, using double-distilled water instead of badger DNA, was included in each set of PCRs. Reactions were performed using a DNA Engine Tetrad thermocycler (MJ Research). PCR products were separated using an ABI 3730 automated DNA sequencer (Applied Biosystems, Warrington, UK) with the ABgene dye set DS-30, filter set D and ROX 500 size standard®, and the data were analysed using GeneMapper version 3·7 (Applied Biosystems). When fewer than four loci remained unscored in a sample, we reverted to single or duplex PCR as used for the initial genotyping performed, but reduced the total reaction volume to 10 µL. To ensure that allele size names were consistent using both amplification methods we genotyped at least three samples per locus using both methods (i.e. 'normal' PCR and Qiagen Multiplex Kit®).

SEX DETERMINATION

The only sex-typing marker currently available for badgers is based on the *SRY* gene (Griffiths & Tiwari 1993), which therefore amplifies in males (XY) but not in females (XX). An autosomal marker (microsatellite locus Mel7 or Mel109) was included in each sex-typing PCR. Samples that amplified the positive control without amplifying the *SRY* fragment were scored as females, while those that amplified both fragments were scored as males. This control is particularly important when working on non-invasive genomic DNA, which are potentially of low quality and quantity and therefore more liable to amplification failure. For sex-specific analyses, individuals were classified as females only if three repeat PCRs did not amplify a *SRY* fragment.

Griffiths & Tiwari (1993) described primers for the amplification of a 216 base pairs (bp)-long fragment of the *SRY* gene. We used a shorter version of the forward primer RG4 in this study: 5′-GGTCAAGCGACCCATGAACG-3′. The sequences published in Griffiths & Tiwari (1993) were used to design a reverse primer (5′-AAGCATTTTCCACTGGCACCCCAA-3′) to amplify a shorter fragment (122 bp) that would be suitable for amplification in non-invasively collected DNA samples. Frantz *et al*. (2006) tested these sex marker primers on 12 individuals of known gender (six males, six females), which were all sexed correctly. For this study, we tested the sex marker with hair samples collected from a total of 23 adults (15 males, eight females) that were live-captured for purposes of radio-collaring (Davison *et al*., submitted), found dead in the study area or live-trapped at other locations in Britain, and whose sex was therefore known. Hair samples had been stored in an envelope at room temperature for up to 11 months, so we included approximately 10–20 hairs in each extraction. In 22 cases the results of the genetic sexing confirmed the previously known sex based on morphology. One individual that was caught as a subadult in October 2005 (estimated to have been born in 2004) was thought to be a female when caught, but the genetic results of several independent PCRs from different samples suggested it was a male. Testicles of badgers undergo significant weight changes throughout the year with lowest weights in autumn (Page, Ross & Langton 1994). Furthermore, about 4% of males have only one descended testicle (Page *et al*. 1994), which might result in misidentifying a male badger as a female. With the high proportion of correctly PCR-sexed individuals we are confident that the sex of this single badger was mistaken at the time of capture and that the *SRY* marker is a reliable indicator of sex for badgers.

COMPILING CONSENSUS GENOTYPES

When identifying individuals through genotyping, a trade-off exists between the number of loci needed to (a) ensure detection of all individuals, prevent 'shadow effect' individuals (Mills *et al*. 2000)

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Table 1. Summary statistics of the Eurasian badger (*Meles meles*) microsatellite loci tested. Loci that were discarded are shown in italic type

Locus	Refs.	Fluorolabel	'Pig-tail'*	*n*	No. of alleles	Exp. allele size range (bp)†	Obs. allele size range (bp)	H_O	H_E	HWE deviation	Est. null allele freq.	Parent pair exclusion probability	Locus used for study
Mel1	1	6FAM	Yes	73	5	262–274	276–286	0·26	0·27	NS	0·02	0·259	Yes
Mel4	*1*	*HEX*	*No*	*(67)*	*Unreliable*	*141–147*	*144–146*						*No*
Mel7	1	HEX	No	71	2	134–144	134–138	0·09	0·08	NS	–0·01	0·073	Yes
Mel10	*2*	*6FAM*	*Yes*	*(14)*	*1*	*154*	*160*						*No*
Mel12	2	NED	Yes	74	3	153	154–162	0·50	0·53	NS	0·03	0·352	Yes
Mel14	2	6FAM	Yes	74	4	188	186–196	0·66	0·556	NS	–0·09	0·392	Yes
Mel15	2	HEX	Yes	72	3	270	246–262	0·64	0·51	NS	–0·12	0·305	Yes
Mel18	*2*	*HEX*	*No*	*(8)*	*1*	*359*	*363*						*No*
Mel101	3	6FAM	Yes	74	4	120–136	116–136	0·61	0·60	NS	–0·01	0·474	Yes
Mel102	*3*	*6FAM*	*Yes*	*(14)*	*1*	*193–199*	*195*						*No*
Mel103	3	6FAM	Yes	74	3	255–263	255–261	0·61	0·55	NS	–0·07	0·474	Yes
Mel104	3	6FAM	Yes	73	4	315–331	315–327	0·57	0·57	NS	0·01	0·404	Yes
Mel105	3	HEX	Yes	73	3	136–150	136–142	0·74	0·60	NS	–0·12	0·471	Yes
Mel106	3	HEX	Yes	72	3	220–226	222–226	0·56	0·51	NS	–0·05	0·446	Yes
Mel107	3	HEX	Yes	74	2	284–288	286–288	0·37	0·32	NS	–0·07	0·327	Yes
Mel108	*3*	*HEX*	*Yes*	*(12)*	*1*	*322–326*	*322*						*No*
Mel109	3	NED	Yes	74	2	106–129	116–127	0·15	0·14	NS	–0·03	0·116	Yes
Mel110	3	NED	Yes	60	3	324–334	324–332	0·67	0·62	NS	–0·04	0·464	Yes
Mel111	3	6FAM	Yes	71	3	130–138	136–140	0·55	0·45	NS	–0·11	0·306	Yes
Mel112	*3*	*6FAM*	*Yes*	*(14)*	*1*	*418–430*	*418*						*No*
Mel113	3	HEX	Yes	66	3	120–130	120–130	0·59	0·66	NS	0·06	0·513	Yes
Mel114	*3*	*HEX*	*Yes*	*(11)*	*1*	*231–237*	*233*						*No*
Mel115	3	HEX	Yes	70	5	330–351	330–351	0·51	0·48	NS	–0·03	0·425	Yes
Mel116	*3*	*NED*	*Yes*	*(52)*	*Unreliable*	*113–135*	*113–135*						*No*
Mel117	*3*	*NED*	*Yes*	*73*	*2*	*174–193*	*174–193*	*NS*					*No*
Mel125b	4	6FAM	No	71	5	143	135–145	0·56	0·57	NS	0·01	0·469	Yes
Mel126	3	6FAM	No	74	3	158	161–165	0·49	0·40	NS	–0·10	0·270	Yes
Mel127	*3*	*HEX*	*No*	*(32)*	*Unreliable*	*184*	*191–216*						*No*
Mel128	3	HEX	No	73	6	206	197–207	0·64	0·52	NS	–0·11	0·373	Yes
Mel131	*3*	*HEX*	*No*	*74*	*2*	*116*	*124–126*	***					*No*
Mel135	3	6FAM	No	72	3	131‡	236–240	0·19	0·22	NS	0·06	0·197	Yes
Mel140	*4*	*6FAM*	*No*	*(42)*	*Unreliable*	*229*	*220–230*						*No*
Average				72	3·45			0·50	0·46		–0·04	First parent: 0·921 Combined: 0·999	

*Primer included the 'pigtail' sequence 'GTTTCTT' at the 5′ end of the unlabelled primer to prevent non-specific adenylation during polymerase chain reaction (following Brownstein, Carpten & Smith 1996), *n*: number of individuals genotyped; †expected allele size range based on sequenced allele or allele size range observed as cited in reference; HWE deviation: deviation from Hardy–Weinberg equilibrium (calculated using GENEPOP version 3·4, Raymond & Rousset 1995); est. null allele freq: estimated frequency of null alleles (calculated with CERVUS version 3·0, Marshall *et al*. 1998); NS: not significant; bp: base pairs. ***$P < 0{\cdot}001$. ‡Based on the size of the product observed compared to that expected, locus Mel135 may be amplifying a different locus to that from which the primers were designed. References for loci tested: 1: Bijlsma *et al*. (2000); 2: Domingo-Roura *et al*. (2003); 3: Carpenter *et al*. (2003); 4: D.A.D. unpublished data. New primer sets designed from badger microsatellite sequences isolated by Carpenter *et al*. (2003). Mel 125b: forward [6FAM] TGAGGGATAAGGCTGTCC and reverse: TTTCATTTCCTACCCGAGTG; Mel 140: forward: [6FAM] GGGAGATGTGAAAGAAATGA and reverse: ACAGTCAACTAAACAAAGAGGC.

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and perform paternity analyses, and (b) minimize the cumulative probability of genotyping errors, leading to 'false' genotypes. Earlier studies (e.g. Domingo-Roura *et al*. 2003; Pope *et al*. 2006) suggested that the available microsatellite loci were not very polymorphic and that a large number would be needed to conduct parentage analysis. While other studies (e.g. Frantz *et al*. 2003) found that seven loci were enough to differentiate even among siblings in badgers, we found that seven (10%) individuals in our population would have been not detected using only the seven most informative loci. Therefore, we typed individual samples at a minimum of nine loci or more until the probability among siblings (Pi_{sib}; Waits, Luikart & Teberlet 2001) was less than 0·001. (Note that because of the combination of primers in the multiplex sets, most samples were typed at a minimum of 15 loci.) PI_{sib} was calculated with the program GENCAP version 1·2 (Wilberg & Dreher 2004), which we also used to identify samples that were complete matches and those that differed only by one or two alleles. We repeated genotyping until the same alleles were observed at least twice in a heterozygous individual, or seven times in a homozygous individual (Taberlet & Luikart 1999a; Taberlet, Waits & Luikart 1999b). If after this process two genotypes differed at only one or two loci, with at least one of the samples being homozygous, and if – excluding the mismatching loci – $PI_{sib} < 0{\cdot}001$, we treated these samples as stemming from one individual in order to avoid overestimating the population size. We chose these thresholds because the comparison of some known siblings (five live-trapped cubs in group S, and three in group WT) gave a mean number of mismatches between the siblings of 5·2 in group S and 7·3 in group WT (minimum 3·0), and an average PI_{sib} in group S of 0·003 and in group WT of 0·009. Thus, it is unlikely that this compilation of genotypes resulted in an underestimate of population size.

The program DROPOUT version 2·0 (McKelvey & Schwartz 2004, 2005) indicates how many loci should be typed to avoid the 'shadow effect' (Mills *et al*. 2000; see above). The indicated threshold was 16 loci for a PI_{sib} value of less than 0·001. Individual samples that could be typed at only 15 or fewer loci and that did not match with any other sample were therefore not included in further analyses, as they were difficult to type, and so even the remaining loci might have been unreliable.

DATA CHECKING

Because of the large number of loci typed, the probability of genotyping error was relatively high. We calculated the initial (i.e. before data checking and compiling of genotypes) error rate manually by dividing the number of incorrectly genotyped PCR samples by the total number of genotyped PCR samples, averaged over all loci. The initial error rate was 0·08, with allelic dropout accounting for 0·04. After applying a multiple-tubes approach (Taberlet & Luikart 1999a; Taberlet *et al*. 1999b) and compiling genotypes as described above we used two further approaches to check our final data set for errors. The software MICRO-CHECKER version 2·2·3 (van Oosterhout *et al*. 2004) tests the data for the presence of errors due to null alleles, allelic dropout of larger alleles, and stuttering because of errors during the PCR. This program indicated that none of these posed a problem in our data. Progam DROPOUT version 2·0 (McKelvey & Schwartz 2004; McKelvey & Schwartz 2005) identifies samples and loci that are likely to contain errors that would affect the estimation of population size by conducting two tests. The 'bimodal test' calculates the number of loci that are different between each pair of samples. A bimodal distribution would indicate an excess of incorrect genotypes. The 'difference in capture history test' determines those loci that produce most errors. Briefly, the test first identifies how many and which loci are needed to obtain a sufficiently low PI and PI_{sib} to guarantee that individuals should be identified correctly. The program then calculates the number of unique individuals obtained with this combination of loci (the tag) and compares it to the number of unique individuals generated through adding additional loci and changing the composition of the tag. The addition of error-free loci will not result in more individuals being inferred. By rotating the order of loci the program evaluates the errors generated by each locus. If new individuals are produced when a particular locus is added then this locus is considered problematic. This test indicated that the genotypes at locus Mel117 were not reliable, so we discarded this locus from further analyses. The bimodal test, when used with the data set including genotypes that differed at only one or two loci, but with PI_{sib} of less than 0·001 (see above), showed two peaks, but not if these ambiguous cases were considered to belong to the same individual. Together, these results show that our methods to minimize errors were successful.

We checked whether any locus deviated from Hardy–Weinberg and linkage equilibrium using the program GENEPOP version 3·4 (Raymond & Rousset 1995) using 1000 dememorizations, 100 batches and 1000 iterations, with a false discovery rate control for multiple testing (Miller *et al*. 2001) that assumed the tests to be dependent. Only locus Mel131 was not in Hardy–Weinberg equilibrium, so genotypes obtained for this locus were not used in further analyses. Four pairs of loci showed significant linkage disequilibria (Mel12 and Mel14, Mel110 and Mel111, Mel12 and Mel109, Mel109 and Mel125). Given the high proportion of individuals sampled over a relatively small area, we expect a high proportion of relatives to be present in the data which may lead to the artefact of many loci appearing to be linked. As no consistent linkage disequilibria were observed among these loci in other populations (e.g. Frantz *et al*. 2003; Pope *et al*. 2006; Dugdale *et al*. 2007) suggesting that these loci were not physically linked. Therefore, we retained these loci in the analysis.

INTERGROUP MOVEMENTS

Genotypes that were represented by a single sample were assigned to the sett where this sample was collected. However, some genotypes were found at more than one sett. If the majority of samples (more than twice as many) came from one sett we assumed this to be the main sett of the corresponding badger, and that the animal had only 'visited' the other location. If a similar number of samples was collected at more than one sett (i.e. not more than twice as many) we did not assign a main sett to the badgers in question and excluded these individuals ('floaters') from group-related analyses. If several samples of one genotype were found only or mainly at one sett in one of the sample periods, and only or mainly at another sett in the second period, we assumed a change of main sett ('dispersal').

Five individuals (including two radio-tracked badgers: Davison *et al*. submitted) made visits or floated between the setts F, Q, B and H (Table 2). These four setts seemed therefore to be associated more closely than other setts. Setts Q, B and H were small (one to two entrance holes), although breeding took place in at least two of them (Q and B, and in other years in F). These setts might therefore be either one main sett with three outliers or might house a 'supergroup', i.e. an association of badger groups that use different setts for breeding but have overlapping ranges and that sleep frequently during the day in one another's setts (Evans *et al*. 1989; T. Scheppers, unpublished data). For spatial analyses, samples from these setts were treated as stemming from one group, FF. Although one male floated regularly between setts WT and WH, and one individual from WH visited

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Table 2. Individual badgers observed (by hair samples) to visit, emigrate to or 'float' between other groups. Events that were witnessed by radio-tracking only are written in italic type. Figures in brackets indicate the number of hair samples of the same genotype found at a particular sett, 'obs.' indicating that the event was witnessed by radio-tracking

Genotype	Sex	Main sett of resident group*	Visits/emigration to sett*	Distance (m)	Evidence for sett visits based on
Visits					
WT-1	F	WT(10)	F(1) & WH(1)[1]	340 & 285	Genotyping
WT-10	F	WT(6)	F(1)	340	Genotyping
WT-90	M	WT(4)	F(1)	340	Genotyping
F-1003	F	Q(7)	WT(1)	515	Genotyping
K-1007	*F*	K (19)	*M(2 obs.)*	*550*	*Radio-tracking*
M-3206	*F*	*M(obs.)*	*S(obs.)*[2]	*365*	*Radio-tracking*
WT-3217	*F*	*WT(obs.)*	*F(obs.)*	*340*	*Radio-tracking*
F-3226	*F*	*F(obs.)*	*B & Q(obs.)*	*210 & 215*	*Radio-tracking*
Emigration					
F-1003	F	F(8)	Q(7)[3]		Genotyping
M-1008	*M*	*M(obs.)*	*Found dead*	*3260*	*Radio-tracking*
Floaters					
B/H/F-71	F		B(5), H(13), F(9)[4]	208, 350 & 525	Genotyping
WT/H-119	F		H(1), WT(2)	255	Genotyping
M/WH-142	F		M(1), WH(1)	560	Genotyping
K/WT-199	F		K(1), WT(1)	930	Genotyping
M/Q-436	F		M(1), Q(2)	1210	Genotyping
B-1001	M		B(obs), H(2)[4]	350	Genotyping & radio-tracking
F-1005	M		*F & H(obs.)*	*525*	*Radio-tracking*
WT/WH-39	M	*WT(obs.)*	WT(1) & WH(1)[5]	285	Genotyping

*Setts are identified by the codes B, F, H, K, M, Q, S, WH and WT. [1]No sample in group WT in autumn, possibly emigration. [2]Possible emigration: the female was tracked regularly in group M but after moving to group S the signal stopped moving (i.e. female died or the collar was lost). [3]Only 'supergroup' dispersal. [4]Only 'supergroup' floater. [5]WT classified as main sett because radio-tracking data showed that this sett was used on 79% of days (Davison *et al*. in press).

group WT at least once, we treated these as separate social groups because four radio-tracked individuals from the two groups were clear residents of only one main sett (Davison 2007).

GROUP SIZE

Population and group sizes with 95% confidence intervals (CI) were estimated using the program CAPWIRE version 4/22/05 (Miller *et al*. 2005). This program has been recently developed to maximize the use of DNA-based mark-recapture data and performs well for smaller populations ($N = 100$) with substantial capture heterogeneity (Miller *et al*. 2005). The program has two models to estimate population size, based on the absence ('even capture probability model') or presence ('two innate rates model') of capture heterogeneity. Selection of the appropriate model can be defined by a likelihood ratio test or by the user.

Abundance estimates were generated for both sampling periods separately (i.e. pre- and post-breeding) and for the complete data set. Using the complete data set obviously violates the assumption of a closed population. However, sample sizes for the two sampling periods were low for some groups and the results appeared to be more robust and conservative using the whole data set. We used the 'two innate rates' model, as we expected heterogeneity in individual capture probabilities. For example, in other studies the trappability of badgers differs between study areas, seasons and years (Tuyttens *et al*. 1999; Scheppers *et al*. 2007). For these calculations we excluded samples of individuals that were known to be dead or that were only live-trapped, because they were captured with a different method and at a different time, as well as those that stemmed from a 'visit' or from 'floaters' (see above).

SPATIAL ANALYSIS

For spatial analysis the samples from setts F, B, H and Q were treated as from one group, FF, but spatial coordinates for the individual setts were retained. For 'floaters' we used the mean for the X and Y coordinates of all setts at which the genotype was found. For those analyses where the main group to which an individual belonged needed to be known, we excluded floaters other than floaters within group FF.

To assess the fine-scale genetic structure of adults of both sexes in the population, we performed individual-based statistical correlation analyses between a measure of genetic kinship and the (log-transformed, see Rousset 2000) pairwise spatial distances using SPAGeDi version 1·2. The slope of this relationship offers a convenient measure of the degree of spatial genetic structuring (Hardy & Vekemans 2002). As suggested by Vekemans & Hardy (2004), the kinship coefficient presented in Loiselle *et al*. (1995) was chosen as a pairwise estimator of genetic relatedness (Loiselle's R), as it is a relatively unbiased estimator with low sampling variance and performs well with markers that are not very polymorphic. We used the same six distance classes chosen automatically by the program for the data set of all adult badgers and the same allele frequencies (calculated from all adult badgers) when calculating values for females and males separately. The spatial genetic structure was tested by numerical resampling in which spatial locations were permuted,

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a procedure equivalent to a Mantel test (Hardy & Vekemans 2002). We compared the slopes (calculated from the mean values per distance class) for adult females and males using a *t*-test (program SsS version 1·0b, Engel 1998) after checking that the residuals were distributed normally.

Additionally, we compared mean relatedness values for individuals of the same sex living either in the same or in different groups by using matched-pair randomization (10 000 randomizations) or exact permutation tests calculated with the program SsS 1·0b (Engel 1998). Because Loiselle's *R*, although performing better in spatial analysis, has lower accuracy and precision, here we used Li's Relatedness value (Li's *R*: see Hardy & Vekemans 2002). For these calculations we had a value pair (i.e. one value for mean relatedness to individuals from the same group and one value for mean relatedness to individuals from a different group) for each individual, minimizing the degree of dependence of values. For comparison with other studies we calculated additionally the more commonly used Queller & Goodnight's *R* (Q&G's *R*: Queller & Goodnight 1989), calculated without bias-correction with SPAGeDi (Hardy *et al.* 2002). Li's *R* and Q&G's *R* were highly correlated (adjusted R^2 between Q&G's & Li's $R = 0{\cdot}78$, $n = 2701$, randomization test: $P < 0{\cdot}001$).

Parentage

We employed the program CERVUS version 3·0·3 (Kalinowski, Taper & Marshall 2007) to determine potential parent pairs. For the simulation determining confidence levels we used 10 000 'offspring'. The minimum number of loci typed was 16. We considered all individuals as adults, and thus as potential parents, except those that were live-trapped as cubs and individuals that were sampled only in autumn and for which parents were found in the parentage analysis. We calculated the proportion of sampled females and males by dividing the number of potential parents of each sex by the estimated population size (see above), plus the live-caught individuals, minus the number of cubs and minus the number of individuals of the opposite sex. This resulted in 87% sampled females and 88% sampled males. To include potentially related individuals we determined the number of adult same-sex pairs with Li's $R > 0{\cdot}25$, and calculated the mean of this value for each sex and the proportion of same-sex relatives in the whole population. Thirteen per cent of the females had Li's $R > 0{\cdot}25$, with a mean of 0·389, while 42% of males were thus related, with an average of Li's $R = 0{\cdot}422$. The error rate was set at 0·001, because the error-checking programs used (see above) indicated a low remaining error rate. As potential mothers we included only females from the same sett as the cub (or in the case of cubs from setts B, Q or F, females from setts B, Q, F or H), because it is unlikely that either mothers or cubs would migrate so soon after independence of the cubs (Cheeseman *et al.* 1988; da Silva, Macdonald & Evans 1994). All males were included as potential fathers.

Results

We obtained 395 reliable genotypes from 416 DNA samples (200 spring, 186 autumn) collected at hair traps or from live-trapped animals, stemming from a total of 74 badgers. Of these, 35 were male and 23 (scored at least three times) or 32 (scored at least once) female. For the remaining samples it was not possible to determine the sex due to poor sample quality. On average, 97% of the individuals were genotyped at any one locus. The initially high error rate of 0·08 shows that data checking procedures are essential to obtain reliable results, and that the ease with which badger hair DNA can be amplified from single hair samples may differ between studies (compare to Frantz *et al.* 2004; Scheppers *et al.* 2007). The 20 loci used had on average 3·45 alleles per locus (Table 1), giving an average allelic richness of 3·40.

Intergroup movements

Seven females (or six, if 'supergroup' visits are excluded) and one male visited other setts (Table 2). One male and possibly one female emigrated, and one female (F-1003) changed her main sett within the 'supergroup'. For five females and three males, or four females and two males if 'supergroup' floaters are excluded, it was not possible to determine the main sett ('floaters'). This leads to a total of 17 (14 excluding supergroup movements) of 74 individuals, or 23% (18·9%), that left the original group range at least once. More females than males moved, but this difference was not significant (*G*-test, $G = 2{\cdot}89$, d.f. = 1, $P = 0{\cdot}089$; excluding super-group movements: $G = 3{\cdot}29$, $P = 0{\cdot}069$). Assuming that cubs do not migrate before maturity, and calculating the value relative to the number of adult individuals ($n = 50$), the proportion is even higher (34%, or 28% excluding supergroup movements).

Group size and population density

If capture success was similar between the seasons (as is suggested by the fact that similar numbers of samples were collected in similar time spans) then the spring figures should reflect minimum group sizes (i.e. group sizes after emigrations and winter deaths but before new cubs are born). The relevant data suggest a population minimum of 33–37 badgers, i.e. 0·169–0·189 adults ha^{-1} (Table 3). In principle, the autumn values should reflect population peaks more accurately than the 'total' values (including individuals that were captured either only in spring, only in autumn, or in both seasons), because the latter will include individuals that have meanwhile died or emigrated. However, in some groups CAPWIRE estimates were lower for the complete data set than for the autumn samples alone (Table 3). This suggests that low seasonal capture frequencies of some individuals that were present in both periods, i.e. those which did not migrate, led the program to overestimate seasonal population sizes. Choosing the lowest and the highest estimates from the combined autumn and total data sets gives a population maximum (i.e. including cubs) of 56–69 badgers, corresponding to 0·286–0·353 badgers ha^{-1}. Densities excluding the suburban and rural setts would lead to even higher values, namely, 0·316–0·329 adults ha^{-1} (25–26 individuals) or 0·506–0·594 badgers ha^{-1} (40–47 individuals). The average minimum group size (based on the number of genotypes and live trapped individuals, and excluding the rural sett SV where only three samples were collected) was 6·4. The more conservative of the CAPWIRE estimates (i.e. the lower confidence interval) gave an average of 7·8 individuals, or 4·1 adults, per group (considering F, Q, H and B as one group). The sex ratio was about even in both sampling periods with, on average, 2·8 resident females and

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Table 3. Total number of individuals caught ('Trapped') in spring and autumn 2006 at 13 setts in Brighton, and the CAPWIRE estimate of group sizes. Figures (except CAPWIRE estimate) include radio-collared individuals that were known to be present at the time but were not caught in hair traps, as well as 'floaters' and visitors. CAPWIRE estimates give only estimates for known residents. Numbers in brackets after the CAPWIRE estimate give the 95% confidence intervals. If no values are given the confidence interval includes only one figure

	Spring		Autumn		Complete data set	
Group	Trapped	CAPWIRE estimate	Trapped	CAPWIRE estimate	Trapped	CAPWIRE estimate
B	1	ND	1	C	2	2
F	4	2	4	3 (3–5)	8	5 (5–7)
Q	1	1	3	4 (4–6)	4	6 (6–8)
H	2	2	2	2	3	3 (3–5)
FF*	7	7 (6–9)	7	8 (8–9)	12	12 (11–15)
K	3	5 (3–7)	1	1	3	5 (3–7)
M	6	6 (6–6)	11	14 (12–19)	13	13
S	2	2	6	6	7	8 (8–9)
WT	6	6 (6–8)	9	9 (6–13)	13	10 (10–11)
WH	4	2 (2–2)	10	18 (9–30)	10	11 (9–16)
C	2	ND	2		3	3
RR	1	1	Not trapped	1	1	
R	4	6 (4–15)	11	26 (12–47)	13	24 (14–39)
SV†	1	ND	2	ND	3	ND
Total*	33	33 (30–37)	56	58 (52–68)	68	66 (63–69)

ND: not done; *the values do not necessarily add up because 'floaters' between different subsetts might appear at several setts; †only one sample was collected in spring and two samples in autumn at sett SV.

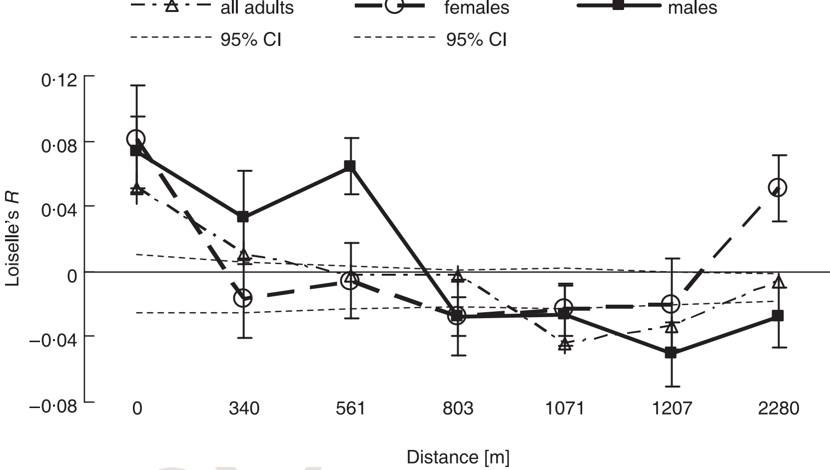

Fig. 2. Mean relatedness values (Loiselle's *R*) and standard deviation for females, males, and the entire population over different spatial distance categories. 95% confidence intervals (dotted lines) are calculated by permuting individual locations among all individuals under the null hypothesis that genotypes of all adults are distributed randomly. Note: statistical analyses (see text) were performed using logarithmic spatial distances.

3·3 resident males per group in each season (and 0·7 individuals of unknown sex).

SPATIAL ANALYSIS

Mean kinship coefficients (Loiselle's *R*) did not vary over different logarithmic spatial distance categories in females ($b = 0{\cdot}01$, $R^2 = 0{\cdot}005$, $P = 0{\cdot}43$), but the correlation was significant for males ($b = -0{\cdot}06$, $R^2 = 0{\cdot}084$, $P < 0{\cdot}001$) and for adults overall ($b = -0{\cdot}02$, $R^2 = 0{\cdot}01$, $P < 0{\cdot}001$; Fig. 2). The slopes calculated using the mean of each distance class (excluding distance class 0 m) differed between the sexes (*t*-test, $t_8 = 2{\cdot}603$, $P = 0{\cdot}031$).

RELATEDNESS AND SEX-BIASED DISPERSAL

Dyads of adult males living in the same group were related significantly more closely [average Li's $R = 0{\cdot}33$, standard deviation (SD) = 0·24] than dyads of males living in two different groups (average Li's $R = 0{\cdot}15$, SD = 0·18; exact permutation test, 18 pairs, $P = 0{\cdot}002$). The difference for females (average Li's *R* for females of the same group = 0·07, SD = 0·32, of different groups = −0·09, SD = 0·20) was also significant (exact permutation test, 12 pairs, $P = 0{\cdot}049$), but less pronounced. Female dyads living in the same group were related significantly less closely than male dyads (randomization test, 10 000 permutations, $P = 0{\cdot}009$). The average Li's

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relatedness (calculated as the mean of the means of all groups) of individuals within groups of the total data set was 0·24; when including only adults it was 0·19. The corresponding values for Q&G's *R* were 0·17 and 0·12, respectively.

PARENTAGE

When we treated all individuals as offspring we usually found several potential mothers (on average 3·6 from the same group as the offspring, maximum = 9), fathers (on average 7·2, maximum = 20) and parent pairs with no triplet mismatch (3·4 if the mother of the pair was from the same group as the offspring, maximum = 27). We found probable parent pairs for 23 likely (including eight known) cubs at seven different setts (six groups, if setts B and Q are considered to belong to one group, FF). For 12 of the 35 individuals which were caught only in autumn we did not find any probable parent, so these individuals were considered to be adults. Up to five probable cubs were born within the same sett (or at least three if considering only live-trapped cubs). In three of five setts with more than one cub (or four of six groups) at least two females were assigned maternity, while in two of five setts more than one male sired offspring (or three of six groups). In three of six groups fathers came from a different group (or four of seven setts). Li's Relatedness value of breeding pairs was on average 0·081 (for Q&G's *R*: 0·01, $n = 8$), not differing from random expaction (randomization test, $P > 0{\cdot}05$).

Discussion

GROUP SIZE AND POPULATION DENSITY

This is the first study to use remotely collected DNA to measure social group sizes and population density in an urban badger population. The results suggest that group sizes range up to 11 individuals, with an average of 4·1 adults per group. This is within the range observed in rural populations in Luxembourg (Schley, Schaul & Roper 2004; Scheppers *et al*. 2007) and Ireland (Smal 1995) and only slightly lower than has been reported for rural Britain as a whole (average: six adults per group, Clements, Neal & Yalden 1988). While the methods in our study and that of Scheppers *et al*. (2007) are strictly comparable, the sampling periods that we used were relatively long by comparison with most live-trapping capture–mark–recapture studies. It could be argued that, as a consequence, our method was more likely to record badgers that were temporarily visiting another sett and, thus, to overestimate group sizes. However, previous studies in Luxembourg, using the same method as was applied here, have shown a good correspondence between social group sizes estimated by hair trapping and those revealed by direct observation (Frantz *et al*. 2004; Scheppers *et al*. 2007).

Although group sizes were similar to those reported for other populations, badger density in our study area was, at 0·32–0·33 adults ha^{-1}, considerably higher than in the only other urban population for which data are available (0·04 adults ha^{-1}: Harris & Cresswell 1987) and higher than in almost all rural locations (for review see Kowalczyk, Bunevich & Jêdrzejewska 2000). Indeed, the population density in Brighton approaches that of Wytham Woods, Oxfordshire (0·44 individuals ha^{-1}: Macdonald & Newman 2002), which is generally believed to have the densest population of badgers in the world (Kowalczyk *et al*. 2000). The explanation for this high population density, despite average group sizes, is that group ranges in our study area, as revealed by a radio-tracking study, were extremely small (range of minimum convex polygons: 5·2–14·0 ha, mean 9·0 ha, Davison 2007). By contrast, the high population density in Wytham Woods results from unusually large social groups occupying moderately sized ranges (Macdonald & Newman 2002).

GENETIC VARIABILITY AND INTERGROUP MOVEMENTS

Analysis of nucleotide diversity in the mtDNA control region suggests that the Eurasian badger, as a species, is not genetically depauperate (Marmi *et al*. 2006). However, most studies investigating populations within restricted geographical areas have reported low levels of genetic diversity, based on microsatellites (Domingo-Roura *et al*. 2003; Pope *et al*. 2006), minisatellites (Pertoldi *et al*. 2001), allozymes (Evans *et al*. 1989) or the mtDNA cytochrome b sequence (Kurose *et al*. 2001, as cited in Pope *et al*. 2006). We found a mean allelic richness value of 3·4, which is at the lower end of what has been found in other British and Irish badger populations (mean = 4·0, range 3·2–4·5: see Pope *et al*. 2006), although higher than in some European populations (range: 2·0–5·7, Pope *et al*. 2006). Microsatellite studies on other mammals often report higher average numbers of alleles (e.g. 11·8 in brushtail possums, *Trichosurus vulpecula* Kerr 1792, Taylor *et al*. 2000; 7·4 in moustached tamarins, *Saguinus mystax* Spix 1823, Huck *et al*. 2005; 6·6 in cheetahs, *Acinoyx jubatus* Schreber 1775, Gottelli *et al*. 2007).

On the other hand, the results provide some indicators that badgers avoid incestuous matings within groups. First, we found a high rate of extra-group paternity, comparable to that reported in other studies (Carpenter *et al*. 2005; Dugdale *et al*. 2007) for rural populations. Secondly, a high proportion of individuals (at least 28% of the adult population) visited the setts of other social groups at least occasionally. Although some of these excursions might constitute exploratory forays to assess dispersal opportunities (Roper, Ostler & Conradt 2003), the most likely explanation is that they occurred for purposes of extra-group mating (Evans *et al*. 1989; Woodroffe & Macdonald 1993a; Christian 1994).

FEMALE-BIASED DISPERSAL

The overall relatedness value (Q&G's *R*) for badgers living in the same group was 0·17, or 0·12 for adults only. This is similar to the results from a long-term study of a rural population of badgers in Woodchester Park, Gloucestershire, where within-group relatedness had an overall mean of 0·15 and varied from 0·12 to 0·19 between years (Carpenter 2002). The Q&G's

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R-values in this latter study, as well as our own, were calculated without using the bias correction recommended by Queller & Goodnight (1989). Without a bias correction, relatedness values will be underestimated. A more recent study on the Wytham Woods population that used the bias correction reported slightly higher values (average within-group relatedness: 0·2, Dugdale *et al*. 2008). The within-group relatedness for females was significantly lower than for males (Li's $R = 0{\cdot}07$ vs. 0·33, Q&G's $R = 0{\cdot}07$ vs. 0·24). Furthermore, for female dyads Loiselle's *R* dropped steeply even when females lived relatively close together, while for males these values remained higher for distances up to about 560 m (Fig. 2). This suggests that males have more relatives in neighbouring setts than females. Together with the lower within-group relatedness of females this indicates that females are the main dispersers, at least on a relatively small scale as studied here. In contrast, Dugdale *et al*. (2008) found significantly higher values for females (Q&G's *R* with bias-correction = 0·25 vs. 0·16 for adult females and males, respectively).

Our findings suggesting female-biased dispersal are consistent with some previous studies (da Silva 1989, as cited in Woodroffe & Macdonald 1993a; Woodroffe, Macdonald & da Silva 1993b; Christian 1994; Tuyttens *et al*. 2000), although other studies have indicated male-biased dispersal in badgers (Kruuk & Parish 1987; Cheeseman *et al*. 1988; Roper *et al*. 2003). Whether the female bias in dispersal in our study reflects only the current population structure (e.g. current effective sex ratios) in our population, or is typical of urban badgers, remains to be determined.

OVERALL CONCLUSION

Two major conclusions can be drawn from this study. First, badgers can attain locally very high population densities in an urban environment, showing that they can adapt as successfully to urban as to rural habitats. However, in our study area, unlike in rural habitats, high population density has resulted from small group range sizes rather than large social groups (Davison 2007). Secondly, the results indicate a combination of relatively low genetic variability (by comparison with other mammals) together with outbreeding at a local scale (i.e. frequent matings between groups rather than within groups). Possibly, our population is subject to a relatively high influence of genetic drift by comparison with mutation or long-distance dispersal. Drift might keep genetic variability low if long-distance dispersal is rare, even if short-distance dispersal and breeding between groups are common.

Most of the characteristics of our population fell within the range of those exhibited by rural badger populations, the most notable exception being the high rate of intergroup movements. In the only previous study of urban badgers, the rate of intergroup movements was similarly high, at 30·8% (calculated from Cheeseman *et al*. 1988). By contrast, a previous study of rural badgers in Luxembourg, using the same methodology as ours, reported that 13% of genotype profiles were found at more than one sett (Scheppers *et al*. 2007), while data from a capture–mark–recapture study of rural badgers in Britain showed an intergroup movement rate of 12·2% (Cheeseman *et al*. 1988). Thus, the available data suggest that movements between groups are more frequent in urban than in rural populations. The proximate and ultimate reasons for this difference require further investigation.

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