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The city-fox phenomenon: genetic consequences of a recent colonization of urban habitat

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Abstract

The red fox (*Vulpes vulpes*) is one of the best-documented examples of a species that has successfully occupied cities and their suburbs during the last century. The city of Zurich (Switzerland) was colonized by red foxes 15 years ago and the number of recorded individuals has increased steadily since then. Here, we assessed the hypothesis that the fox population within the city of Zurich is isolated from adjacent rural fox populations against the alternative hypothesis that urban habitat acts as a constant sink for rural dispersers. We examined 11 microsatellite loci in 128 foxes from two urban areas, separated by the main river crossing the city, and three adjacent rural areas from the region of Zurich. Mean observed heterozygosity across individuals and the number of detected alleles were lower for foxes collected within the city as compared with their rural conspecifics. Genetic differentiation was significantly lower between rural than between rural and urban populations, and highest value of pairwise F_{ST} was recorded between the two urban areas. Our results indicate that the two urban areas were independently founded by a small number of individuals from adjacent rural areas resulting in genetic drift and genetic differentiation between rural and urban fox populations. Population admixture and immigration analysis revealed that urban–rural gene flow was higher than expected from F_{ST} statistics. In the five to seven generations since colonization, fox density has dramatically increased. Currently observed levels of migration between urban and rural populations will probably erode genetic differentiation over time.

Keywords: *Canidae*, founder event, genetic differentiation, microsatellites, migration, urban habitat

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Introduction

With the expansion of cities during the 20th century, the occurrence of wild animals in suburban and urban habitats has been recorded all over the world and a wide range of different species have adapted to this man-made environment. Red foxes (*Vulpes vulpes*) are one of the most widely distributed mammals and are ecologically extremely flexible, utilizing a variety of habitats ranging from deserts to tundras (Voigt & Macdonald 1984). In urban habitat, foxes have been recorded in British cities since the 1930s (Teagle 1967) where they reached higher densities than had ever been observed (Harris 1981). Therefore, urban foxes were first thought to be a strictly British phenomenon (Harris 1977). However, during the past 20 years they have invaded cities or their suburbs in continental European (e.g. Møller Nielsen 1990), North America (e.g. Adkins & Stott 1998) and Australia (e.g. Marks & Bloomfield 1998). The colonization of Swiss urban and suburban environment by foxes is most apparent in the conurbations of Geneva and Zurich. In Zurich, fox density started to increase in 1985 and the current number of individuals living permanently in the built-up area within the boundaries of the municipality is estimated to be at least 500 adult animals (Gloor 2002).

As a vector for and carrier of rabies, the tapeworm *Alveolar echinococcoses* and sarcoptic mange, the red fox is a concern for public as well as domestic and wild animal health, especially in areas with high human density (e.g.

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Trewhella & Harris 1988; Hofer *et al*. 2000). Several studies have described fox density and social organization in urban habitats, and models of fox contact rate and its implication for rabies control were developed based on these findings (e.g. Trewhella & Harris 1988; White *et al*. 1995; Tischendorf *et al*. 1998). In Switzerland, the colonization of cities coincided with the successful eradication of the rabies epidemic, which lasted from 1967 to 1996.

In order to minimize the zoonotic risk posed by rabies, game wardens carried out prophylactic culling of foxes within and outside Zurich as intensively as possible between 1965 and 1995 (Gloor *et al*. 2001). Before 1985, the majority of culled foxes originated from rural areas around Zurich and only occasionally at the border of the city. No more than two foxes were recorded from the inner part of the residential area in 1964 and 1967, respectively (Gloor *et al*. 2001). Only in 1985, two years after the last rabies cases were recorded in the residential area, did the fox population start to increase, both in the urban and in the adjacent rural parts of the city. This increase was documented by both the number of foxes culled and the number of foxes found that had died of other causes such as road traffic accidents. The increasing population density of foxes following the successful eradication of rabies (Breitenmoser *et al*. 2000) may produce a rising number of dispersing foxes which in turn move into urban habitats not utilized previously.

After 1985, the number of foxes found dead (i.e. foxes which died of other causes than culling) increased significantly in urban compared to rural parts of Zurich (Gloor *et al*. 2001), indicating a higher mortality in urban environments. Doncaster & Macdonald (1991) observed high mortality and population turnover rates in urban habitats of Oxford, resulting in shifting territoriality, whereas morality rates were lower in suburban Oxford and Bristol (Doncaster & Macdonald 1996; White *et al*. 1996). Consequently, the urban population in Zurich may depend on immigrating foxes from surrounding areas.

Gloor *et al*.'s (2001) population pressure hypothesis (PPH) assumes that urban areas are suboptimal habitats for foxes and that they act as a population sink for dispersers from surrounding areas that lack territorial vacancies. A dispersal sink is defined as a habitat in which local births do not compensate for local deaths and where only immigration prevents decline to extinction (Andrewartha & Birch 1954). Even habitats which support high birth rates may act as dispersal sinks if mortality rate exceeds recruitment. Only a very small number of studies have convincing demographic data to demonstrate the existence of a dispersal sink (Watkinson & Sutherland 1995). Rousset's (1999) theoretical work indicates that source and sink habitats cannot be distinguished by comparison of gene diversity within sink and source populations because gene diversity in each habitat depends also on migration between habitats. Furthermore, Rousset's model shows that between-habitat differentiation is generally intermediate between within-habitat differentiations and that genetic differentiation is higher in the habitat with the lower gene diversity. Therefore, source and sink cannot be distinguished by analysis of population structure using *F*-statistics even if populations are partially isolated. Consequently, no genetic differentiation between fox populations within the city of Zurich and the surrounding rural population would be expected under the PPH.

Gloor *et al*.'s (2001) alternative urban island hypothesis (UIH) postulates that foxes have adapted to specific urban conditions and resources. Divergent selective regimes and adaptation result in reproductive isolation (Thompson 1998). Therefore, between-habitat differentiation will be higher than the average of within-habitat differentiation (Rousset 1999). Alternatively, genetic differentiation between urban populations and the surrounding rural populations may be the result of founder events and random genetic drift alone. Using 11 polymorphic microsatellite markers, we describe the genetic structure the red fox population within the city of Zurich and the surrounding countryside. We quantify allelic diversity and genetic differentiation between the populations in order to test the prediction of no genetic differentiation under the source-sink concept.

Materials and methods

Study site and sample collection

Within the municipality of Zurich (92 km^2, 361 000 inhabitants) we established two sampling areas in urban environments, one in the eastern (U_{east}) and one in the western (U_{west}) part of the city (Fig. 1). These areas are separated by Lake Zurich, the river Limmat and the highly built-up city centre, which is less suitable for foxes (Gloor 2002). From spring 1996 to autumn 1998, 477 foxes were sampled in the municipality of Zurich. These animals were either killed by cars, shot by game wardens or originate from foxes that were captured during an ongoing research programme (Gloor 2002). From the total sample, we chose two subsamples representing the resident fox population of the two urban sampling areas. First, only foxes with known sampling locations were used ($n = 417$). Second, only foxes from within the built-up area of Zurich were selected ($n = 158$; Fig. 1). Third, we excluded all juveniles collected in October or later (Zimen 1984; Harris & Trewhella 1988). White *et al*. (1996) emphasize that a sampling regime, which does not account for the territorial status or origin of foxes, tends to over-sample dispersing and nonterritorial foxes. Therefore, our sampling regime avoids the inclusion of dispersers which may originate from rural areas but which do not settle in urban habitats (Funk 1994). However, dispersers which settled successfully in any of the study sites were included in our

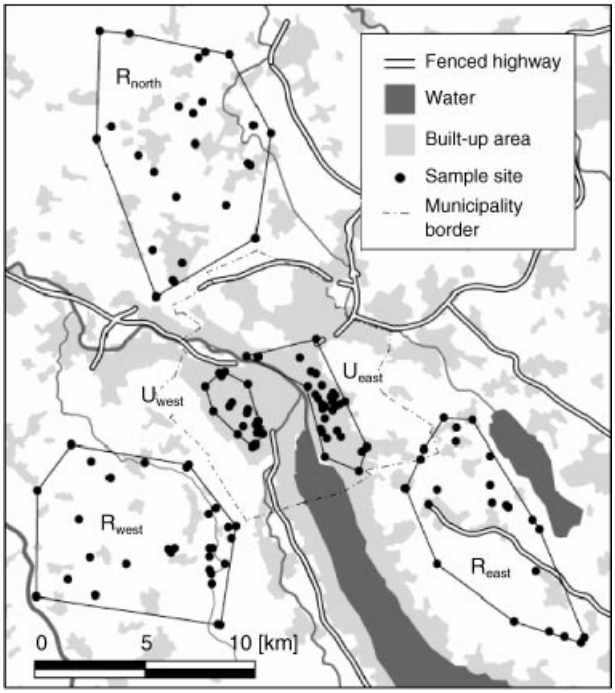

Fig. 1 Map of the study site: city of Zurich (U_{west} and U_{east}) and rural surrounding (R_{west}, R_{north} and R_{east}) sampling areas with the origin of foxes analysed (geographic base data: Vector200 © Federal Office of Topography, Switzerland).

sample, because we collected all adults after 4 March; we did not consider adult males during the mating season (December–February) because they may have been sampled during extra-territorial excursions (White & Harris 1994). Fourth, from the remaining samples, we chose only one individual randomly from the same collection site, defined by a radius of 50 m, in order to avoid related individuals such as cubs from the same den. In total, 50 foxes from the two urban areas were used for analysis.

Three rural sampling areas (R_{east}, R_{west} and R_{north}) were selected within a radius of 20 km around the city to represent the adjoining surrounding rural fox populations. These three rural areas are separated from each other by natural (Lake Zurich, river Limmat) or man–made barriers (fenced highways, built-up areas; Fig. 1). Using the same sampling criteria as in urban habitats a priori, 78 foxes were collected by game wardens and hunters between July and mid-October 1998.

Laboratory methods

Samples used in the study include muscle, tongue or lymph node samples from dead individuals (n = 118) and skin biopsies (n = 6) or hair (n = 4 samples; each sample at least 10 plugged hairs) from captured foxes. All fox carcasses were stored at –20 °C and dissected later at the Swiss Rabies Centre, University of Berne. Measurement of the relative width of the pulp cavity of a canine tooth by X-rays (Kappeler 1985) allowed discrimination between juvenile (< 12 months) and adult individuals.

DNA was extracted either using a standard phenol/chloroform extraction procedure (Bruford *et al*. 1992) for tissue samples, a QIAamp™ tissue extraction kit (Qiagen) for biopsies and a Chelex-protocol for hair samples (Goossens *et al*. 1998).

Eleven canine microsatellites were selected on the basis of extensive tests for cross-species amplification in red foxes (Funk *et al*. unpublished). These included four dinucleotides (AHT-130, Holmes *et al*. 1995; CXX250, CXX466 and CXX 642, Ostrander *et al*. 1993, 1995) and seven tetranucleotides (c2001, c2010, c2017, c2054, c2079, c2088 and c2096, Francisco *et al*. 1996). Poolymerase chain reaction (PCR) was carried out in a 10-μL reaction volume containing 2 nmol of each dNTP, 5 pmol for dinucleotide primers and 1 pmol for tetranucleotides, 0.25 units of *Taq* polymerase (Gibco), 1 × PARR© PCR buffer (Cambio, Cambridge) and approximately 30 ng template DNA. A PCR amplification of 36 cycles was carried out (initial denaturation 94 °C for 4 min, 94 °C for 30 s, annealing temperature between 54 °C and 62 °C for 30 s, 72 °C for 30 s, followed by a final extension at 72 °C for 10 min) using Perkin Elmer GeneAmp PCR System 9700. All PCR products were separated electrophoretically using an ABI PRISM™ 377 DNA sequencer (Perkin Elmer). Allele sizes were scored against the size standard GS350 Tamra (Perkin Elmer) using GeneScan™ Analysis 2.1 and Genotyper™ 2.1 software. All homozygotes, rare genotypes and samples with poor DNA quality and quantity were reamplified independently at least once.

Statistical analyses

Genetic diversity was estimated within each sampling area as observed heterozygosity (H_O) and expected heterozygosity under Hardy–Weinberg equilibrium (H_E; Nei 1987) for each locus using FSTAT version 1.2 (Goudet 1995). With the same software package, we calculated single-locus F_{IS} values according to Weir & Cockerham (1984). Multilocus means and standard deviations for H_E, H_O, F_{IS} and the number of detected alleles (A) were described for each sampling area. Standard deviations were calculated by jackknifing over loci. In order to compare number of alleles between populations with differing sample sizes, we computed a saturation curve over the number of sampled individuals (Roy *et al*. 1994). Within each of the five sampling areas we selected individuals at random without replacement and calculated the cumulative number of alleles. Mean and standard deviation as a function of sample size were computed by

1000 iterations. We estimated mean individual observed heterozygosity within each sampling area and tested the variance for the effects of sampling area and habitat type in a nested ANOVA (mixed-effects nested design model) using the statistics package SPSS (SPSS Inc.).

Genotypic linkage disequilibrium between all pairs of loci (Garnier-Gere & Dillmann 1992) was evaluated using program GENEPOP 3.1b (Raymond & Rousset 1995). Deviations from Hardy–Weinberg equilibrium were tested for all locus–population combinations. We used either the complete enumeration method for loci with less than five alleles (Louis & Dempster 1987) or the Markov chain method for loci with more than four alleles (Guo & Thompson 1992), as implemented in the program GENEPOP. Critical significance levels were adjusted using the sequential Bonferroni method (Dunn–Sidák method) taking into account multiple tests on the same data. Fisher's exact test was used to test for significant deviations from Hardy–Weinberg equilibrium within populations across loci. One locus (CXX642) displayed significant deviation from Hardy–Weinberg equilibrium in more than one population. Therefore subsequent analysis, which was based on estimated allele frequencies (F_{ST}, assignment test, population admixture analysis), was carried out including and excluding this locus and results were compared using Spearman's rank correlation coefficient.

Genotypic differentiation (Goudet *et al*. 1996) between sampling populations was tested using GENEPOP. This software generates single-locus contingency tables of alleles obtained by permuting genotypes among population samples, classifies them by a log-likelihood (G)-based exact test and can be generalized to multilocus in a global test (Fisher's method). Because this approach is based on the hypotheses of independent sampling of genotypes, it does not require random mating within populations. Pairwise single-locus F_{ST} values (Weir & Cockerham 1984) between sampling areas were calculated and multilocus means and standard deviations computed by jackknifing over loci were obtained using FSTAT. In order to test for a sex-specific influence on genotypic differentiation between populations, *F*-values were calculated comparing differentiation between males and females within populations and tested for significance using Hudson *et al*.'s (1992) χ^2 test.

Immigrants in each sampling population were identified using Rannala & Mountain's (1997) test, which uses a Bayesian approach to derive the probability of allele frequencies in populations. First, the program assigns an individual of unknown origin to the population in which its genotype is most likely to occur. Then, the test calculates a probability for immigration for those individual that have not been assigned to their source population. Test power was calculated for each individual between its source population and the most likely assigned population using Monte Carlo simulations (Rannala & Mountain 1997). Mean power over all individuals was calculated for both 11 and 10 loci (after exclusion of locus CXX642). We used 10 000 simulations in order to determine critical values for the test statistics and applied a critical test value of 0.01. We estimated population admixture using Pritchard *et al*.'s (2000) model-based clustering method that infers population structure on the basis of unlinked genetic markers. Analyses were computed using the software STRUCTURE (Pritchard *et al*. 2000). Based on Bayesian statistics and a Markov chain Monte Carlo method (MCMC), individuals are assigned to population clusters in such a way that each population cluster is in Hardy–Weinberg equilibrium. Subsequently, we estimated the proportion of membership of each predefined (sampling) population in each of the population clusters. Geographic origin of individuals was used as prior population information. The approach requires specifying the immigration rate v, the probability that an individual is an immigrant or has an immigrant ancestor, as a prior. Because v is unknown, we applied three prior migration rates (0.1, 0.25 and 0.5, respectively) to examine the robustness of the model to migration patterns. STRUCTURE was also used to identify immigrants applying migration priors v of 0.1, 0.25 and 0.5, respectively, and a critical value for the test statistics of 0.01. We applied STRUCTURE by using 1 000 000 MCMC simulations, 100 000 burn-in runs and two replicates.

Results

Sampling, microsatellite typing and genetic diversity within sampling areas

We analysed a total number of 78 animals from rural and 50 animals from urban habitat (see for sampling areas Table 1). Numbers of juvenile foxes (< 12 months of age), adult males and adult females were 14, 8, 8 in R_{west}; 10, 7, 7 in R_{east}; 12, 6, 7 in R_{north}; 6, 13, 12 in U_{east}; and 8, 5, 6 in U_{west}, respectively. Mean successful amplification rate for all 11 microsatellites was 92.5%, with a range from 81% for locus c2096 up to 98.5% for locus CXX466. No significant linkage disequilibrium was found in any pair of loci in the five sampling populations after correction for multiple testing ($P < 0.05$, $k = 275$).

The number of successfully amplified microsatellites did not differ between foxes collected in urban and rural habitat (means of 10.28 and 10.08, respectively; $\chi^2_4 = 6.81$, $P > 0.15$; χ^2-test of independence). A total number of 93 alleles across loci and areas were detected and the number of alleles per locus varied between four at locus c2010 and 16 at locus c2054. The simulation results of selecting individuals at random within each sample cumulative number of alleles are shown in Fig. 2.

Over a large range of equal sample sizes across the five sampling areas, the two urban samples showed a consistently

Table 1 Measures of genetic diversity of foxes in two urban and three rural sampling areas. Shown are observed heterozygosity (H_O), expected heterozygosity (H_E), and number of alleles (A) at each locus and their means ± SD across loci. Mean F_{IS} ± SD for all sampling areas was estimated according to Weir & Cockerham (1984)

	Sampling areas (sample size)														
	R_{west} (n = 30)			R_{east} (n = 23)			R_{north} (n = 25)			U_{east} (n = 31)			U_{west} (n = 19)		
Locus	H_O	H_E	A	H_O	H_E	A	H_O	H_E	A	H_O	H_E	A	H_O	H_E	A
AHT-130	0.78	0.77	5	0.83	0.74	5	0.64	0.76	5	0.55	0.75	5	0.82	0.73	5
CXX250	0.72	0.79	7	0.74	0.81	7	0.63	0.81	6	0.70	0.69	6	0.68	0.57	4
CXX466	0.72	0.77	5	0.65	0.71	6	0.76	0.80	6	0.65	0.63	5	0.67	0.64	4
CXX642	0.86	0.90	12	0.68	0.86*	11	0.86	0.87	11	0.64	0.82	10	0.46	0.83*	8
c2001	0.55	0.64	5	0.45	0.74	7	0.57	0.76	9	0.76	0.82	7	0.58	0.72	5
c2010	0.47	0.54	4	0.50	0.44	3	0.55	0.44	4	0.26	0.27	4	0.32	0.40	4
c2017	0.79	0.81	9	0.77	0.76	8	0.82	0.83	8	0.77	0.73	8	0.53	0.80*	8
c2054	0.97	0.88	12	0.90	0.92	12	0.91	0.90	12	0.83	0.85	11	0.74	0.86	8
c2079	0.60	0.75	6	0.74	0.78	6	0.78	0.71	5	0.60	0.78	5	0.31	0.69	4
c2088	0.60	0.78	8	0.68	0.82	7	0.67	0.78	8	0.60	0.74	6	0.42	0.65	7
c2096	0.73	0.64	4	0.50	0.67	4	0.86	0.65	5	0.56	0.58	6	0.43	0.44	3
Mean	0.71	0.75	7.00	0.68	0.75*	6.91	0.73	0.76	7.18	0.63	0.70	6.64	0.54	0.67*	5.45
± SD	0.15	0.11	2.99	0.15	0.13	2.78	0.13	0.14	2.69	0.16	0.18	2.31	0.17	0.15	1.93
P (Fisher's exact test)	0.14			0.004			0.07			0.07			< 0.001		
F_{IS} (mean ± SD)	0.062 ± 0.119			0.089 ± 0.159			0.019 ± 0.188			0.087 ± 0.118			0.175 ± 0.249		

*Significant deficiency of heterozygotes after correction for multiple testing.

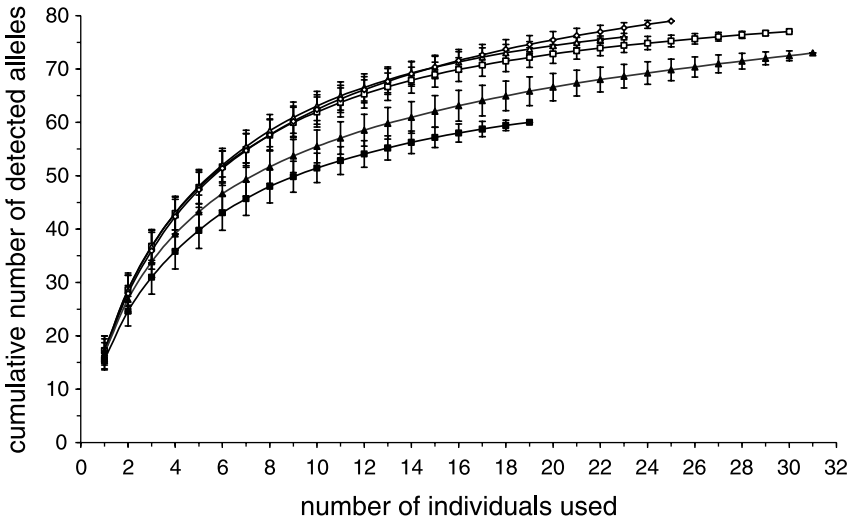

Fig. 2 Results of the simulation of selecting individuals within each of the five sampling areas at random without replacement, and counting the cumulative number of alleles. Shown are mean and SD for the urban areas U_{west} (■), U_{east} (▲) and the rural areas R_{west} (□), R_{east} (△), R_{north} (◇).

lower number of detected alleles indicating a lower number of alleles in the two urban areas in general. Mean ± SD individual observed heterozygosity across loci within each sampling area were 0.70 ± 0.14 in R_{west}, 0.68 ± 0.18 in R_{east}, 0.72 ± 0.18 in R_{north}, 0.63 ± 0.14 in U_{east} and 0.56 ± 0.18 in U_{west}. Tested ANOVA revealed a lower observed heterozygosity in urban habitat by computing a significant effect of the habitat factor ($F_{1,123}$ = 13.86, P = 0.031), but no significant effect of the individual sampling areas ($F_{3,123}$ = 0.98, P = 0.40). Pairwise F_{ST} values between sexes within sample populations ranged between 0.018 and 0.001 and none of the χ^2-tests indicated significant differentiation between females and males (range P = 0.14–0.76).

Significant deviations from Hardy–Weinberg equilibrium were observed in two areas, R_{east} and U_{west}, across all loci (Fisher's exact test; Table 1). In all samples, expected heterozygosity exceeded observed heterozygosity and all mean F_{IS} estimates were positive, indicating heterozygote deficiency at most loci in each of the areas. Three of 55 individual tests for deviations from Hardy–Weinberg equilibrium yielded a significant result after table-wide sequential Bonferroni correction for multiple tests (Table 1,

$\alpha < 0.05$). Two significant values were observed in U_{west} and one locus, CXX642, deviated significantly from Hardy–Weinberg equilibrium in R_{east} and U_{west}.

Genetic diversity between sampling areas

The global test for homogeneity of genotypic distribution detected significant genetic differentiation ($P < 0.001$) in all pairwise comparisons after correction for multiple testing ($k = 10$) except for the comparison between R_{east} and R_{north}. Therefore, for subsequent calculation we pooled these two rural sampling areas. Analysis of *F*-statistics among the areas and all 11 loci revealed a mean ± SD F_{ST} value of 0.035 ± 0.007. Pairwise F_{ST} values between areas varied from 0.009 ± 0.003–0.068 ± 0.020, indicating little to moderate genetic differentiation (Fig. 3a). The lowest level of differentiation was observed between the two rural populations $R_{east\ \&\ north}$ and R_{west} and greatest differentiation between the two urban populations U_{east} and U_{west}. All pairwise F_{ST} values between rural and urban samples were intermediate. With one exception the same pattern, but with opposite relative values, was found using the population admixture analysis irrespective of the values of the prior migration rates *v* (Fig. 3b). Smallest admixture coefficients were between the urban populations and between the urban and the rural populations on opposite river banks. Admixture coefficients between urban and rural populations on the east bank were, like F_{ST}, intermediate between the smaller admixture coefficients among urban populations and the larger coefficients among rural populations. Admixture coefficients between urban and rural populations on the west bank were, however, larger than expected on the basis of F_{ST} values with admixture coefficients being of the same magnitude as coefficients between the rural populations (Fig. 3a,b).

The two tests for immigration that were applied identified differing numbers of immigrants, but showed similar trends for migration between populations (Fig. 4). Using Rannala & Mountain's (1997) assignment test, 30 individuals (23.4%) were not assigned to their sampling area ($P < 0.01$; Fig. 4a). Mean power of the test (min–max) over all individuals was 0.948 (0.636–1.0) for 11 and 0.935 (0.636–0.999) for 10 loci. Using Pritchard *et al.*'s (2000) admixture-based approach, seven (5.5%), 14 (10.9%) and 23 (18.0%) immigrants were identified for the migration priors (*v*) of 0.10, 0.25 and 0.50, respectively ($P < 0.01$; Fig. 4b). Spearman's rank correlation coefficients were 0.15, 0.17 and 0.49 for the comparisons between assignment test and the admixture-based approach for migration priors of 0.10, 0.25 and 0.50, respectively, and 0.60 between the results for migration priors of 0.10 and 0.50. Among the nonassigned individuals were cubs and juveniles, which were too young to have dispersed. This apparently incorrect misassignment is more frequent for the assignment test (17.6% of 51 cubs and juveniles) compared to the admixture-based approach (2.0% and 7.8% and 11.8% for migration priors of 0.10, 0.25 and 0.50, respectively). Even the immigration rate estimated for the very high migration prior of 0.50 is 11.8%, noticeably lower than the 17.6% estimated with the assignment test. The higher misassignment in Rannala & Mountain's (1997) test stems from its low power to distinguish between immigrants and nonimmigrants with a recent immigration history such as first-generation hybrids. Despite the four tests for

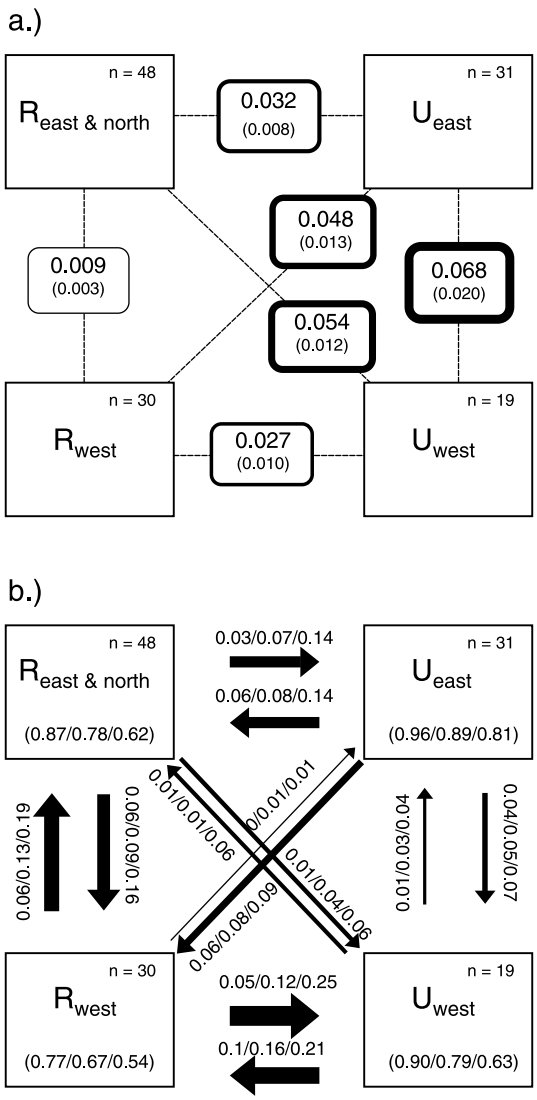

Fig. 3 Genetic differentiation (a) and population admixture (b) for sampling areas within and around the city of Zurich. Rounded boxes in (a) show pairwise F_{ST} values (Weir & Cockerham 1984; SD values in parentheses) and thickness of boxes is proportional to pairwise F_{ST} values. For the population admixture approach (Pritchard *et al.* 2000), admixture proportions are shown for migration priors $v = 0.10$, $v = 0.25$ and $v = 0.50$, respectively. Thickness of connecting arrows is proportional to admixture proportions at $v = 0.50$.

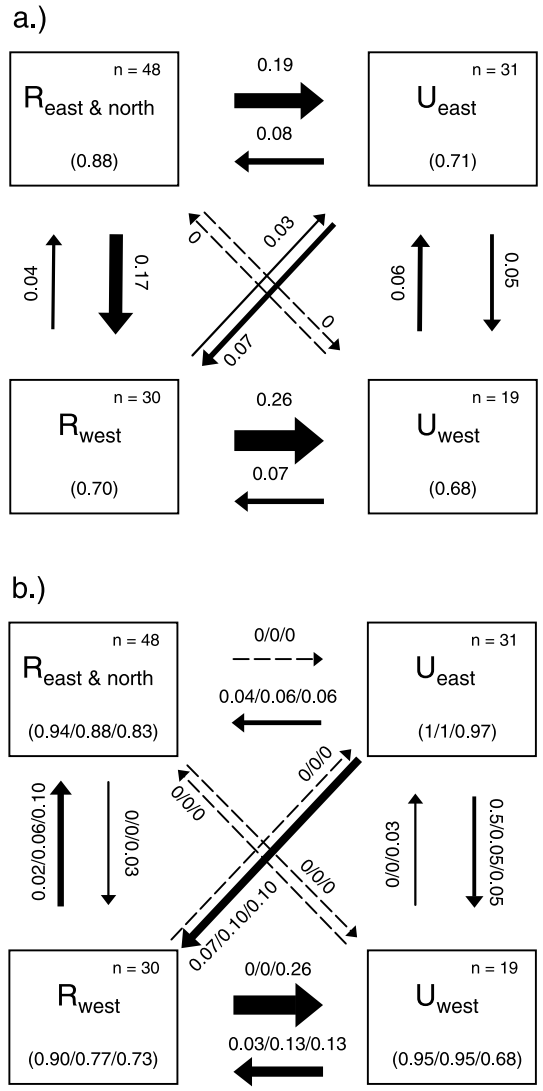

Fig. 4 Migration patterns between sampling areas according to Rannala & Mountain's (1997) assignment test (a) and Pritchard *et al*.'s (1999) population admixture analysis for migration priors $v = 0.10$, $v = 0.25$ and $v = 0.50$, respectively (b). For both approaches, percentages of individuals with significant immigration history ($P < 0.01$) are shown alongside arrows. Relative thickness of arrows represent the proportion of immigrants for the assignment test or the proportion of immigrants based on $v = 0.50$ for the admixture approach. Total proportion of nonimmigrants are shown in parentheses.

immigration differing in absolute numbers of immigrants, all tests showed the same trend with the lowest exchange rates found between the two urban populations, low immigration from rural population into the urban population on the opposite riverside and highest exchange rates between rural populations as well as rural and urban populations from the same river side (Fig. 4).

Due to the significant heterozygosity deficiency at locus CXX642 in the samples R_{east} and U_{west}, we reanalysed pairwise F_{ST}-values and carried out the assignment and admixture analysis with this locus excluded. Pairwise F_{ST} values ($r^S = 0.98$), assignment proportions ($r^S = 0.92$) and population admixture ($r^S = 0.90$, $r^S = 0.85$ and $r^S = 0.88$ for the migration priors $v = 0.10$, $v = 0.25$ and $v = 0.5$, respectively) correlated strongly between the analyses including and excluding locus CXX642 ($n = 16$ in each of the analyses).

Discussion

The urban environment provides suitable habitat for a wide range of wildlife, including red foxes, coyotes *Canis latrans* (Grinder & Krausman 1999) and raccoons *Procyon lotor* (Riley *et al*. 1998) among carnivora. Highest densities were observed in red foxes and racoons within urban and suburban habitats (Riley *et al*. 1998; Baker *et al*. 2000). It has to be differentiated whether cities were colonized actively by new species, such as foxes (Møller Nielsen 1990; Adkins & Stott 1998; Marks & Bloomfield 1998; this study), or whether they were trapped in habitat fragments due to urbanization, such as badgers (Harris 1984). Boal & Mannan (1999) compared the breeding ecology in Cooper's hawks *Accipiter cooperii* between urban and exurban environment. Failure rate was greater among urban nests than among exurban nests due a parasite (*Trichomoniasis*) rendering an 'ecological trap' for hawks in these types of habitat (Boal & Mannan 1999). High densities and stable social organization in red fox were described in suburban areas of Bristol and Oxford, whereas in more urban areas of Oxford high population turnover, based on a higher mortality rate, was found (Doncaster & Macdonald 1996; White *et al*. 1996). Today, fox density in the city of Zurich exceeds densities recorded from most rural areas greatly but did not reach the exceptionally high densities of some areas in Bristol (Gloor 2002). At present, there are no sufficient data available on mortality and natality within the city of Zurich and therefore prediction on the demographic status of the two urban fox populations are difficult to make. However, colonization history suggests a sink–source situation between urban and rural habitat in Zurich.

The prediction that urban areas act as dispersal sinks, which are maintained by constant immigration from rural population, is not consistent with our results. There was significant genetic differentiation between rural and urban fox populations, and the differentiation was relatively high (2.7–5.4%). The observed differentiation cannot be explained by the geographical arrangement of the samples. Genetic differentiation between the two rural populations was much lower (0.9%) than between urban and rural areas, although the geographical distance is greater and migration barriers appear stronger between the rural sites than between urban and rural sites (Fig. 1).

The observed significant genetic differentiation between the urban and rural fox populations could be caused by different mechanisms. First, reproductive isolation may evolve rapidly due to divergent selection regimes and adaptation (Thompson 1998). However, little is known about how quickly reproductive isolation may evolve in newly colonized habitats. The fastest evolution of reproductive isolation in the wild reported so far — and controversially discussed — may have occurred after 13 generations in introduced salmon, leading to the conclusion that the observed differentiation is a consequence of adaptation in divergent habitats (Hendry *et al*. 2000; Gustafson *et al*. 2001). Although we cannot reject the hypothesis that the significant differentiation between urban and rural populations is a consequence of selection and adaptation, available information on general fox ecology, colonization patterns in Zurich and a parsimonious alternative hypothesis indicate that selection and adaptation are unlikely to have caused the differentiation. We observed significant differentiation between urban and rural populations within only 15 years, which is equivalent to approximately five to seven generations, assuming a generation time for foxes of between two and three years within Zurich (mean age of 285 adult animals collected in the municipality of Zurich was 2.40 years; unpublished data). Second, red foxes exhibit an unusual behavioural plasticity and can utilize a large range of different habitat types and food resources (Voigt & Macdonald 1984). This indicates that the inherent plasticity allows the species to utilize urban environments and that a specific adaptation is not a prerequisite for successful colonization of cities. Second, the observed genetic differentiation between urban and rural foxes and between the two urban populations is consistent with recent founder events and random genetic drift.

The presence of genetic drift is confirmed by the significantly lower heterozygosity found in urban foxes, which is consistent with founder effects. Further, the significant genetic differentiation of 6.8% between foxes in the eastern and western parts of the city indicate two independent colonizations. Subsequently, two populations were established that remained isolated by the river Limmat. When comparing urban with rural populations, genetic differentiation was always smallest between immediate neighbouring areas, indicating that the urban populations were founded by dispersers from adjacent rural areas rather than by long-distance dispersal. Similar reduced levels of variation within recently founded populations and increased differentiation between recently colonized *vs*. established populations has been reported in several species, including red foxes on Phillip Island (Lade *et al*. 1996) and urban plant populations (Hollingsworth & Dickson 1997).

The disappearance of rabies may have promoted the red fox colonization in Zurich in two ways. First, the Swiss fox density has increased steadily from 1986 onwards (Breitenmoser *et al*. 2000), thus providing an increasing number of dispersers that may settle in urban habitats. Second, the eradication of rabies — due to the successful oral vaccination campaign starting in the early 1980s (Wandeler 1991) — may have led to a more tolerant attitude in humans towards foxes as the threat of rabies to human life has disappeared. During the rabies epidemic in continental Europe, individual foxes entering any human settlements have been subject to intensive efforts to be removed (Labhardt 1990). In the municipality of Zurich, intense prophylactic culling was carried out during the rabies epidemic (Gloor *et al*. 2001). However, colonization of urban areas on the European continent may not be linked directly to rabies, but may have been caused by changes in the urban environment, thus providing suitable surroundings for foxes. Behavioural observations in central Europe on dispersing foxes indicate that dispersers avoid urban environments (Ziemen 1984; Labhardt 1990; Funk 1994) although the successful eradication of rabies and the subsequent increasing fox density is likely to have increased the number of foxes entering cities.

Once the initial immigrant foxes have established themselves successfully, the general high abundance of food resources in urban habitats may have led to a rapid increase in fox density (Doncaster *et al*. 1990; Baker *et al*. 1998). The highest fox densities are recorded in cities, which are reflected by small and vastly overlapping home ranges (Harris 1981; Baker *et al*. 1998; Baker *et al*. 2000). The rapid increase in fox density could explain why the genetic signature of a founder event was detectable, despite the presence of immigrants during our study. Fox populations with high density are characterized by reduction of reproducing females and the formation of groups larger than the breeding pair (review in Cavallini 1996; Baker *et al*. 1998). This complex social organization is the probable cause of the deviation from Hardy–Weinberg equilibrium in two of our study populations.

The urban populations show a lower number of alleles and in heterozygosity than the rural populations. Nevertheless, they are relatively variable (Fig. 2), suggesting that either multiple founders or multiple rural to urban migration events have occurred. The largest genetic differentiation was observed between the two urban populations, thus indicating that separate founder events have occurred in different parts of the city. Using different analytical approaches to estimate dispersal between the habitat types, Rannala & Mountain 1997) assignment test and the immigration test based on Pritchard *et al*.'s (2000) population admixture analysis, we detected significant numbers of dispersers in both directions. The precise amount of dispersal and directionality could not be sufficiently well resolved and depends on the prior assumptions used for the analyses (e.g. migration priors in the admixture analysis). Both approaches recorded lower immigration

rates of urban foxes into the rural surroundings than vice versa. While we sampled across the whole of the urban habitat, the rural study sites covered only small proportions of areas suitable for dispersers to settle. Therefore, total dispersal from the urban to the rural habitat may be higher than our results indicate and may be similar or even exceed the proportion of rural to urban habitat dispersal. The latter is supported by the admixture analysis, where the urban populations are generally less admixed than the rural populations and urban to rural vs. rural to urban admixture is more balanced than in the immigration analysis. From radio-tracking and tagging studies, little is known about migration of foxes between rural and urban habitat except that urban to rural dispersal occurs at low frequency (Harris & Smith 1987; Gloor 2002).

Population admixture and migration rates between urban and rural populations were generally of the same magnitude as the rural–rural comparisons, despite more pronounced rural–urban compared to rural–rural genetic differentiation. This discrepancy between genetic differentiation, pointing to isolation, and immigration and admixture analysis, pointing to gene flow, indicates that observed genetic differentiation, which we quantified approximately 15 years after successful colonization of the city of Zurich, is a legacy of the initial colonization by founder events. Whether genetic differentiation will erode over time depends on the amount of gene flow between the habitat types. Despite the discrepancies of detail, the two statistical approaches for testing immigration and the population admixture analysis support the hypothesis that urban–rural migration is low but not rare. Therefore, we expect that continuous dispersal on similar levels as currently estimated will reduce genetic differentiation in the future. The time-scale to complete removal of any differentiation will depend on reproductive success of immigrants, which in turn will depend on the relative fox density and the social organization within the urban habitat.

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