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POPULATION GENETIC DIFFERENTIATION IN THREE SYMPATRIC DAMSELFLY SPECIES IN A HIGHLY FRAGMENTED URBAN LANDSCAPE (ZYGOPTERA: COENAGRIONIDAE)

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The AFLP (Amplified Fragment Length Polymorphism) technique was used to compare the levels of genetic diversity and differentiation among *Paracercion calamorum*, *Ischnura senegalensis* and *I. asiatica* and to compare the genetic structure of populations found in highly fragmented urban habitats to populations in relatively continuous rural habitats. For all 3 spp., high genetic diversity was found in both areas. However, population genetic differentiation among urban populations was approximately twice that of rural populations, indicating that movements between habitat patches are more restricted in urban areas, probably due to human disturbances that may function as barriers. Inter-specific differences regarding genetic diversity and differentiation are further discussed in terms of habitat specificity.

INTRODUCTION

Habitat destruction and fragmentation due to human activity are a common phenomenon occurring throughout the world and is arguably the greatest threat to biodiversity (PRIMACK, 1998; WILCOVE et al., 1998). Thus roads, buildings and towns often provide barriers that interfere with an organisms' movement between the remaining habitat patches, causing population fragmentation (PRIMACK, 1998).

Fragmented populations directly impact gene flow within a species, leading to a

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loss of genetic diversity within each population and an increase in genetic differentiation between populations (FRANKHAM, 2006). This genetic force, along with demographic and environmental forces, could drive fragmented populations to extinction (GILPIN & SOULE, 1986; CROOKS & SANJAYAN, 2006). However, despite a growing concern over the influence of habitat fragmentation on genetic structure, few population genetic studies have been conducted in urban landscapes, where habitats are expected to be more fragmented and individual movements between fragmented patches may be more restricted than those in rural landscapes.

Ideally, population genetic structure presumably resulting from habitat fragmentation should be compared to that of a control site (i.e., unfragmented habitat) (VAN DONGEN et al., 1998) with the same spatial scale at the intra-specific level. However, the use of such a control site is often ignored (WILLIAMS et al., 2003; SOFIA et al., 2006). Furthermore, both sites should be located in relatively close proximity in order to eliminate any possible geographic effects on dispersal such as climatic conditions and altitude. Moreover, in order to detect low genetic differentiations resulting from a small spatial scale, molecular markers with high-resolution power should be employed (OUBORG et al., 1999). Therefore, finding a species inhabiting both fragmented and unfragmented habitats over a small geographical range and using a molecular marker with high-resolution power are important issues for examining the effects of habitat fragmentation on population genetic structure.

Amplified Fragment Length Polymorphism (AFLP) (VOS et al., 1995) is a molecular marker which has been used in the field of molecular ecology and evolution since 1995. Although the method has been widely employed in the study of plants, fungi, and bacteria, the advantages of the method (short start-up time, no prior sequence knowledge and good repeatability) should make it a valuable tool to investigate the population genetics of wild animal species (BENSCH & ÅKESSON, 2005). A major concern about the technique is that markers are dominant, and it is impossible to distinguish homozygous (1/1) from heterozygous (1/0) genotypes (BENSCH & ÅKESSON, 2005). This requires previous knowledge of the inbreeding coefficient (HOLSINGER et al., 2002) and, therefore, the Hardy-Weinberg equilibrium is sometimes assumed to estimate allele frequencies. However, the large number of loci counterbalances this drawback of dominant markers (PARSONS & SHAW, 2001) as it can reveal subtle but significant population structures (BENSCH & ÅKESSON, 2005).

We used the three Zygoptera species, *Paracercion calamorum*, *Ischnura senegalensis*, and *I. asiatica* to compare levels of genetic diversity and differentiation among populations found in highly urbanized fragmented habitat relative to populations in continuous rural habitat and thereby determine any effect of habitat fragmentation on their genetic structure. Adult odonates are known to be sensitive to the destruction and modification of their aquatic habitats (e.g. CARLE,

1979; BROWN, 1991; CLARK & SAMWAYS, 1996; SUHLING et al., 2006; SATO & RIDDIFORD, 2008), and this, along with their ease of capture and wide-spread distribution make these three species ideal for such a study. All three damselfly species occur sympatrically in the Kanto region in Japan and use lentic habitats, mainly occurring in agricultural ponds often connected by ditches and rice paddies in rural areas, and in distantly spaced city park ponds in urban areas. However, habitat specificity seems to differ among them. *P. calamorum* has a narrower selection of habitat with respect to the relative structure and arrangement of vegetation at the waterside compared to the other two species, and of the two congeneric species, *I. senegalensis* selects a more confined range of habitat types compared to *I. asiatica*. The sampling design allowed us to conduct both intra-specific comparison between two contrasting areas and inter-specific comparison among the three species since differences in habitat specificity could have profound influences on the genetic structure.

MATERIAL AND METHODS

STUDY SITES AND SAMPLE COLLECTION – We collected adult *P. calamorum*, *I. senegalensis*, and *I. asiatica* from a total of 23 different sites (11, 10, and 13 sites for each, respectively) in two contrasting areas on the Kanto Plain in Japan (Fig. 1). Since the main aim of our study was to examine the effects of habitat fragmentation on damselfly dispersal, we chose the most intensively urbanized area in Japan. 11 of the study sites were ponds in city parks in the Tokyo metropolitan area, and except for site U11, they were within the 23 wards of Tokyo, occupying much of the center of the Tokyo metropolitan area. This area is very built-up with business, commercial, and high-rise apartment buildings, which are potential physical barriers for dispersal and hence result in habitat fragmentation. The other 12 sites were selected in the surrounding countryside as a control against the urban data; 10 of these were located in Ibaraki prefecture, the other two on the border of Ibaraki and Chiba prefectures. These study sites were either ponds (natural, agricultural or park ponds) or rice paddies and were within an area approximately 50 to 60 km NE of Tokyo and mainly surrounded by relatively continuous agricultural land. Although there is some urbanization in this area, much of the area has still been used for rice paddies, lotus-cultivation, and arable lands due to the close proximity of Lake Kasumigaura, which is the second largest lake in Japan. Irrigated rice paddies, with irrigation ditches and shallow holding ponds, sustain many species of aquatic organisms and are considered to be artificial shallow wetlands in this respect (NAKAMURA & SHORT, 2001). Landcover types within approximately 1500 km^2 of each rural and urban area covering all study sites are summarized in Table I. The spatial scales of the two study areas are comparable. The minimum and maximum linear geographical distances between study sites were 4.5 km and 25.7 km in the urban area compared to 4.6 km and 27.4 km in the rural area, respectively, for *P. calamorum*, 8.6 km and 27.0 km in the urban area compared to 7.0 km and 25.1 km in the rural area, respectively, for *I. senegalensis* and 5.3 km and 38.9 km in the urban area compared to 4.5 km and 38.2 km in the rural area, respectively, for *I. asiatica*. Sampling was conducted in July and August 2004. Adult damselflies were collected with nets and immediately stored in 99 % ethanol in the field. An average of 15 individuals for each site for each species were caught and used for the analyses.

DNA EXTRACTION AND AFLP PROCEDURE – The head and thorax were ground in liquid nitrogen as a base material from which to extract total DNA following the slightly modified cetyltrimethylammonium bromide (CTAB) method suggested specifically for AFLP analysis for insect material (REINEKE et al., 1998). DNA was extracted with chloroform:isoamylalcohol (24:1),

and the DNA pellets were ethanol-precipitated and then suspended in TE buffer. DNA extracts were further purified using a QIAquick PCR Purification Kit (QIAGEN K.K., Tokyo) according to the manufacturer's protocols.

AFLP reactions were conducted using the AFLP Plant Mapping Kit (Applied Biosystems, Foster City, CA) for a regular genome following the procedures outlined in the manufacturer's instructions with minor modifications. Ten to 150 ng of initial DNA per individual were digested with restriction enzymes (MseI and EcoRI; New England Biolabs, Beverly, MA) before ligation of restriction site-

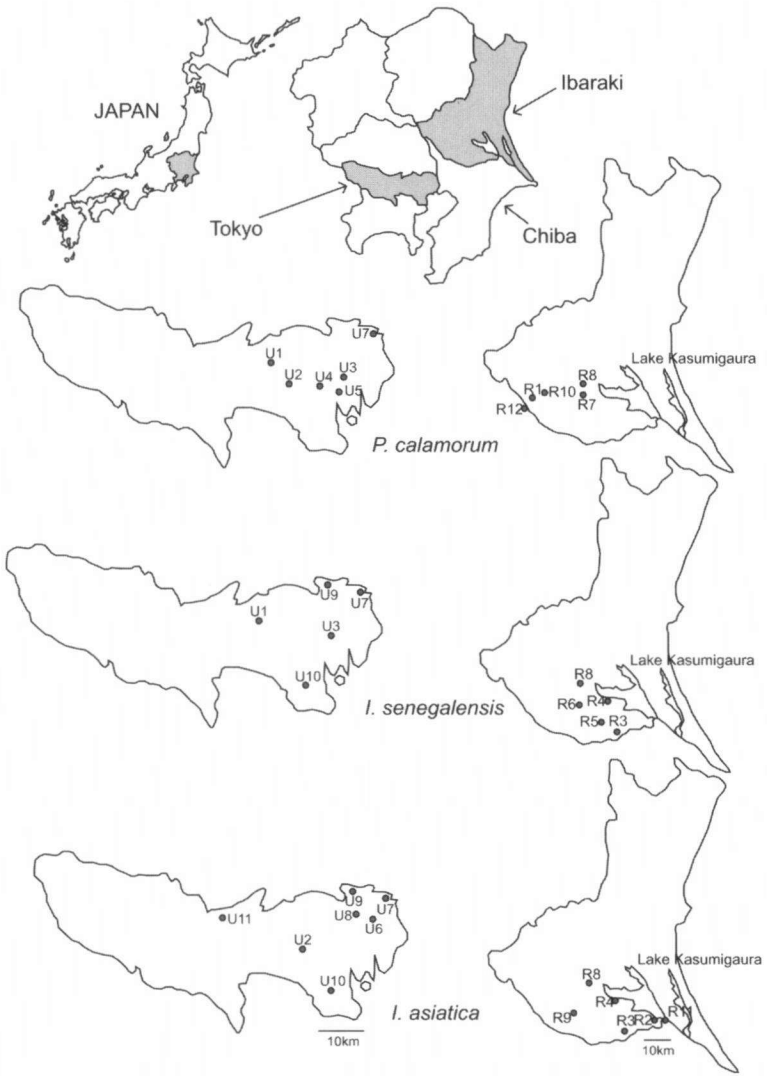

Fig. 1. Location of the sampling sites in urban (Tokyo: U1-U11) and rural (Ibaraki and Chiba: R1-R12) areas.

specific adaptors. The pre-selective and selective amplifications were carried out in a Perkin Elmer Gene Amp PCR System 9600 thermal cycler (Applied Biosystems). Modifications included reduction of the total volume of the solution to a half of the volume suggested in the manufacturer's instructions, which was 5.5μL for restriction and adaptor-ligation and 10μL for pre-selective and selective amplifications. In order to remove the inhibitory substances for amplifications, 1.6 μL of Ampdirect (Shimadzu Corporation, Kyoto, Japan) was added to the pre-selective amplification reaction mixture. Two primer combinations (EcoRI-ACA / MseI-CTG, EcoRI-ACT / MseI-CAC) that amplified a reasonable number of clear bands were used.

The fluorescence-labeled selective amplified products were resolved with a Gene-Scan 500 ROX internal size standard (Applied Biosystems), using an ABI 3100 automated sequencer (Applied Biosystems). The electrophoresis images were obtained from each run, and peaks were assigned base-pair sizes using the GENESCAN program version 3.1 (Applied Biosystems). Only DNA fragments ranging in size from 50 to 500 base pairs were discerned and manually scored as 1 (presence) or 0 (absence) to assemble a binary data matrix consisting of a total of 189, 215, and 203 bands for each of the *P. calamorum*, *I. senegalensis*, and *I. asiatica* individuals sampled, respectively. Ten DNA samples from each species were amplified at least twice to verify the reproducibility of the AFLP technique, and only intense and unambiguous bands were scored.

DATA ANALYSIS – We assumed the Hardy-Weinberg equilibrium to estimate genetic diversity and differentiation as no prior information on the mating system of the three damselflies was available. Allele frequencies were estimated according to a Bayesian method with non-uniform prior distribution of allele frequencies (ZHIVOTOVSKY, 1999), using the computer program AFLP-SURV 1.0 (VEKEMANS, 2002). Based on the estimates of allele frequencies, we determined genetic diversity as Nei's unbiased expected heterozygosity (NEI, 1973). We estimated the genetic differentiation among populations within each area using two different methods. We first calculated Wright's F_{ST}, the fixation index, which is a parameter indicating how populations are genetically differentiated according to the Lynch and Milligan method (LYNCH & MILLIGAN, 1994), using AFLP-SURV 1.0 (VEKEMANS, 2002). The method was specifically developed for calculating differentiation and suitable for use with dominant data types. The significance of F_{ST} was tested by comparing the observed F_{ST} with the distribution of the set of F_{ST} generated by 1000 random permutations from individuals. We also used the method that does not assume the Hardy-Weinberg equilibrium, but instead, uses the Euclidean distance to estimate the components of variance attributable to differences among populations and among individuals within populations (EXCOFFIER et al., 1992). The Analysis of MOlecular VAriance (AMOVA) (EXCOFFIER et al., 1992) was performed to calculate Φ_{ST} (F_{ST}

Table I

Percentage of major landcover types within about 1500 km² for each urban (Tokyo) and rural (Ibaraki and Chiba) area. Vegetation and land use data derived from 15 grid cells, each of which is approximately 10×10 km of the National Survey on the Natural Environment (Biodiversity Center of Japan, 1999), were re-categorized into four landcover types. Urban landscapes include residential areas, factories, and buildings. A G-test showed that the composition of landcover types in the urban area is significantly different from that of the rural area ($G = 921.5, p < 0.001$)

	Urban area		Rural area	
Land cover type	Area (km²)	% of total	Area (km²)	% of total
Paddy field / Wetland vegetation	50.5	3.4	433.2	32.1
Arable land / Meadow	217.1	14.6	413.9	30.6
Urban landscapes	1002.0	67.3	233.8	17.3
Others	218.9	14.7	267.4	19.8
Total	1488.6	100	1348.4	100

Table II

The number of rural and urban populations sampled (n), estimates of genetic diversity (Hw), and genetic differentiation (F_{ST}, Φ_{ST}) in *P. calamorum I. senegalensis*, and *I. asiatica* among populations in each urban and rural area. − indicate significant differences from 0 at the $p < 0.05$ level based on 1000 and 1023 random permutations for F_{ST} and Φ_{ST}, respectively

	Urban area				Rural area			
	n	H_W	F_{ST}	Φ_{ST}	n	H_W	F_{ST}	Φ_{ST}
P. calamorum	6	0.274	0.079*	0.145*	5	0.300	0.036*	0.076*
I. senegalensis	5	0.324	0.031*	0.065*	5	0.308	0.018*	0.036*
I. asiatica	7	0.304	0.006*	0.031*	6	0.313	0.003*	0.015

analogue), using the program Arlequin 3.1 (EXCOFFIER et al., 2005). This Φ_{ST} is similar to Weir and Cockerham's θ (WEIR & COCKERHAM, 1984), which is calculated within an analysis of variance framework and supposed to be a better estimate when sample sizes are fewer than 30 individuals (LOWE et al., 2004). Arlequin also calculated pairwise Φ_{ST} values for all pairs of populations within each area. The significance of Φ_{ST} and pairwise Φ_{ST} were tested using non-parametric permutation of the data set with 1023 permutations.

For all pairs of populations in each area, genetic distances were calculated (NEI, 1972) using AFLP-SURV 1.0 (VEKEMANS, 2002), and geographical distances were obtained as simple linear distances, using Kashmir 3D (SUGIMOTO, 2002). We then tested whether there was any correlation between the genetic and geographical distance for each species. Since the independence of pairwise elements of distance matrices violates the basic assumptions for using a simple correlation coefficient, we used the Mantel test (e.g. MANTEL, 1967; MANLY, 1986; SOKAL & ROHLF, 1995).

RESULTS

No statistical differences were found in the genetic diversity, as measured by the mean expected heterozygosity, between urban and rural areas for *P. calamorum, I. senegalensis* and *I. asiatica* (Unpaired t-test; $p = 0.130$, $p = 0.120$, $p = 0.201$, respectively) (Tab. II). However, in comparisons between species, a significant difference was found in the total mean expected heterozygosity (combined urban and rural populations) (ANOVA; $F = 6.781$, $p = 0.003$). A *post hoc* test revealed that *P. calamorum* had significantly less genetic diversity than that of *I. senegalensis* and *I. asiatica* (LSD test; $p = 0.001$ and $p = 0.009$, respectively), but no difference was found between *I. senegalensis* and *I. asiatica* (LSD test; $p = 0.357$).

Significant population differentiations with an F_{ST} and Φ_{ST} were found for all three species except a Φ_{ST} value of *I. asiatica* among rural populations that was not significantly different from zero (Tab. II). There was a consistent trend for all species with F_{ST} and Φ_{ST} values about twice as high in urban compared to rural populations (Tab. II), demonstrating that populations in the urban area were more differentiated than in the rural ones. The pairwise Φ_{ST} values revealed that significant genetic differentiation was more common among urban compared to rural populations and the pattern was consistent for all three species (Tab. III). For *P. calamorum*, of the 15 pairwise comparisons within the urban area, all

pairwise Φ_{ST} values (100 %) were significant, compared to seven (70 %) out of 10 pairwise comparisons within the rural area. For *I. senegalensis*, 10 (100 %) out of 10 pairwise Φ_{ST} values within the urban area were significant, and seven (70 %) out of 10 within the rural area. For *I. asiatica*, of the 21 pairwise Φ_{ST} values

Table III

Pairwise estimates of Φ_{ST} in (a) *P. calamorum*, (b) *I. senegalensis*, and (c) *I. asiatica* for urban and rural populations. – indicate significant differences from 0 at the $p < 0.05$ level

(a) *P. calamorum*

	U1	U2	U3	U4	U5	R1	R7	R8	R10
U2	0.060*								
U3	0.189*	0.200*							
U4	0.088*	0.096*	0.250*						
U5	0.096*	0.069*	0.192*	0.096*					
U7	0.101*	0.175*	0.320*	0.147*	0.171*				
R7						0.094*			
R8						0.088*	0.072*		
R10						0.060*	0.080*	0.065*	
R12						0.117*	0.053	0.070	0.050

(b) *I. senegalensis*

	U1	U3	U7	U9	R3	R4	R5	R6
U3	0.137*							
U7	0.043*	0.113*						
U9	0.052*	0.082*	0.026*					
U10	0.051*	0.104*	0.032*	0.021*				
R4					0.078*			
R5					0.041*	0.041*		
R6					0.044*	0.043*	0.011	
R8					0.028*	0.045*	0.017	0.010

(c) *I. asiatica*

	U2	U6	U7	U8	U9	U10	R2	R3	R4	R8	R9
U6	0.013										
U7	0.083*	0.053*									
U8	0.029	0.107*	0.040								
U9	0.000	0.006	0.027	0.044							
U10	0.000	0.013	0.083*	0.085	0.000						
U11	0.010	0.022	0.050*	0.060*	0.007	0.000					
R3							0.000				
R4							0.000	0.000			
R8							0.017	0.000	0.052*		
R9							0.006	0.000	0.026	0.025	
R11							0.024	0.014	0.059*	0.085*	0.039

within the urban area, six (29 %) were significant, and of 15 pairwise Φ_{ST} values within the rural area, three (20 %) were significant. There was also a trend that, in both areas, *P. calamorum* showed the highest F_{ST} and Φ_{ST} values among the three species, and the F_{ST} and Φ_{ST} values of *I. senegalensis* were higher than those of *I. asiatica* (Tab. II). The Mantel test showed no significant relationship between genetic distance and linear geographical distance in each area for each of the three species ($p > 0.1$).

DISCUSSION

In our study, we found relatively high genetic diversity, but the patterns of increased genetic structuring (as estimated by F_{ST} / Φ_{ST}), among populations from urban habitats compared to the populations from rural habitats, were consistent with the predicted effects of habitat fragmentation on genetic structure for all three species (Tab. II).

HITCHINGS & BEEBEE (1997), CAIZERGUES et al. (2003), and WILLIAMS (2003) all found decreased genetic diversity and increased genetic differentiation in either naturally or anthropogenically fragmented landscapes when compared to those in control sites. Interestingly, however, no decreased genetic diversity was detected in our study and those by GIBBS (1998), KNUTSEN et al. (2000) and ARNOUD et al. (2003) although increased genetic differentiation was found. We propose the following two non-exclusive explanations for the high genetic diversity found in the present study. Habitat fragmentation may result in a reduced genetic diversity through random genetic drift (BAHL et al., 1996; ARNAUD et al., 2003). As random genetic drift is known to depend on effective population size (BEEBEE & ROWE, 2004; LOWE et al., 2004), that of each population in the urban area is large enough to maintain high genetic diversity. An alternative explanation is that there is sufficient gene flow among urban populations to maintain high genetic diversity.

We did not find any correlation between the genetic distance and the linear geographical distance either in the urban or in the rural areas. Whenever such a correlation is found, the factor limiting dispersal among populations is considered to be geographical distance, and this is the basic premise of the theory of 'isolation-by-distance' (WRIGHT, 1946). The absence of such an association in the present study may simply reflect insufficient time for an equilibrium between gene flow and drift to be reached and hence the expected genetic patterns of isolation--by-distance to build up (HUTCHISON & TEMPLETON, 1999; KNUTSEN et al., 2000). However, pairwise Φ_{ST} values between site U3 and other sites were consistently higher for both *P. calamorum* (range 0.189-0.320) and *I. senegalensis* (range 0.104-0.137) compared to the pairwise Φ_{ST} values between sites isolated by equal or greater distances. Considering that site U3 is located in the centre of the Tokyo metropolitan area, we suspect that human disturbances such as buildings

and roads could be limiting gene flow and have thus created the observed genetic differentiation which cannot be explained by geographical distance.

Although all three species showed the same pattern, with urban populations being more genetically differentiated than rural populations, the F_{ST} / Φ_{ST} values of *P. calamorum* were twice as high as those of *I. senegalensis*, which were also more than twice as high as those of *I. asiatica*. In addition, the genetic diversity of *P. calamorum* was significantly lower than that of *I. senegalensis* and *I. asiatica*. To our knowledge, this is one of only a few studies that have demonstrated how the effects of habitat fragmentation on population genetics differed among closely-related, sympatric species. Habitat specificity could be one explanation for the observed differences in genetic diversity and differentiation among the three species.

P. calamorum has specific habitat requirements, generally inhabiting ponds with submerged or floating vegetation but with relatively little emergent aquatic vegetation (UÉDA, 1985). They also require trees in close proximity to the water as male roosting sites (UÉDA, 1976). On the other hand, both *I. senegalensis* and *I. asiatica* use a wide range of water types including ponds, small channels, sewage drains estuaries with emergent aquatic vegetation and rice paddies (INOUE & TANI, 1999; WATANABE & MATSU'URA, 2006). Due to the higher habitat specificity of *P. calamorum* compared to that of *I. senegalensis* and *I. asiatica*, *P. calamorum* utilized a smaller range of habitats, which may result in restricted gene flow between available patches, especially in the highly fragmented urban landscape.

When comparing the two *Ischnura* species, the wider range of habitat requirements for *I. asiatica* might also explain the observed differences in F_{ST} / Φ_{ST}. In addition to the habitat types used by both species described above, *I. asiatica* is also seen around ponds with grassy types of vegetation (e.g. Poasceae, Cyperaceae) that grow in wet areas even though the pond itself does not have any aquatic vegetation (pers. obs.). These eurytopic characteristics of *I. asiatica* may enable them to find available patches of habitat and colonize successfully in other patches, even in fragmented areas.

Although the three damselfly species investigated in this study are neither threatened nor endangered, the fact that the population genetic differentiation differed among closely-related, sympatric species has important management implications. Habitat specificity is a well-known ecological attribute of species that increases vulnerability to extinction (WALDRON et al., 2000). Thus, if habitat fragmentation has a more severe impact on habitat specialists, as this study indicates, this may be one of the mechanisms explaining why habitat specialists are more vulnerable to extinction.

It is worth noting that *I. senegalensis* has a larger body size than *I. asiatica* (adult body length (mm): *I. senegalens* = 23-25; *I. asiatica* = 20-25, SUGIMURA et al., 2001). Contrary to our intuition that larger species are better dispers-

ers, the smaller body size of *I. asiatica* showed a lower genetic differentiation. CORBET (1999) considered these two *Ischnura* species as migrants based on the reports that adults of *I. asiatica* were caught more than occasionally over water far from land (ASAHINA & TURUOKA, 1970), and larvae of *I. senegalensis* inhabited temporary pools in seasonal rainfall areas (WEIR, 1968; WATSON, 1980 as cited by CORBET, 1999; DUMONT, 1981 as cited by CORBET 1999). If being small is a prerequisite for migrant Zygoptera to move a long distance by exposing themselves to air currents as CORBET (1999) inferred, *I. asiatica*, which has a smaller body than *I. senegalensis*, should have an advantage in dispersing.

An F_{ST} value of 1 indicates perfect genetic differentiation and 0 indicates no differentiation, with moderate differentiation at values between 0.05 and 0.15 (WRIGHT, 1978). The magnitude of the F_{ST} / Φ_{ST} values found for both urban and rural populations suggests a relatively high dispersal capability of all three damselfly species. This finding is not in accordance with the generalization that damselflies are weak fliers that can disperse only between water bodies less than 1 km apart (CONRAD et al., 2002). Since moderate genetic differentiation was detected for *P. calamorum* in the urban area, the resolution power of the genetic marker used in this study was considered to be high enough to detect difference in dispersal ability. One should bear in mind that dispersal ability measured by direct observations (i.e. Mark-Release-Recapture) and indirect measurements (i.e. F_{ST} / Φ_{ST} values) have substantially different meanings. The former is a snapshot of a one-time dispersal event. Not all the marked individuals are located and long-distance and rare movements tend to be particularly difficult to detect because of the spatio-temporal constraints of the method (e.g. RIECKEN & RATHS, 1996; NATHAN, 2001; OSBORNE et al., 2002). On the other hand, dispersal measured as F_{ST} / Φ_{ST} values is a cumulative effect of gene flow. Individuals might successfully disperse to new habitat patches, either by flying directly or in a stepping-stone way, or even by being passively dispersed by air currents. This colonization event might happen within a generation in some cases and over several generations in other cases, and may result in gene flow. Therefore, whilst the results of this study suggest that urban populations are experiencing the effects of habitat fragmentation, relatively high dispersal events are expected to be occurring among the populations. Caution should be taken as this cumulative effect of gene flow expressed as F_{ST} / Φ_{ST} may not be reflecting the current status of dispersal where habitat destruction and modification are still likely to be occurring.

In conclusion, human disturbances in urban areas are likely to restrict the dispersal of individuals, and the extent of habitat fragmentation effects on the population genetic differentiation differed among the three damselfly species probably because they have different habitat specificity. However, this study is only the first step towards determining how we should conserve Odonata species and maintain

biodiversity in an urban landscape, as we do not know specifically what caused or created the population genetic structures obtained. *P. calamorum* are apparently heavily dependent on city park ponds in urban areas, which are more or less spatially separated. Our findings indicate that even for *P. calamorum*, there seems to be sufficient dispersal among urban fragmented habitats to maintain high genetic diversity. In other words, connectivity, the pattern of interconnectedness that determines how individuals move among habitat patches, should be sufficient for the persistence of metapopulations (WIENS, 1996; WITH, 2004). Therefore, further study of landscape effects which limit or facilitate dispersal in urban areas should be particularly important for understanding and predicting animal dispersal and increasing connectivity in highly urbanized landscapes.

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