Molecular analysis of dispersal in giant pandas

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Abstract

Although dispersal in the giant panda (*Ailuropoda melanoleuca*) is a demographic mechanism which can potentially counteract the negative effect of habitat fragmentation, little is known about dispersal in this species because of difficulties in observing individuals. Using data from faecal microsatellite genotyping, we compared the spatial distribution of giant pandas in two populations and the proximity of relatives in one key population to infer their dispersal pattern. We conclude that giant pandas exhibit female-biased dispersal because: (i) vAIc (variance of assignment index) for females was significantly larger than for males, suggesting that females comprise both 'local' and 'foreign' genotypes; (ii) the average spatial distance of related female dyads was significantly larger than that of males; (iii) larger *r* (relatedness), F_{ST} (genetic variance among populations) and *mAIc* (mean of assignment index) values were found in males using the software FSTAT, although the differences were not significant; (iv) males set up territories neighbouring to their birth place; (v) significant population structure using microsatellites with a concomitant lack of mitochondrial structure was found in a previous study, possibly indicating more extensive female dispersal; and (vi) female-biased dispersal was strongly supported by evidence from concomitant ecological studies. Considering previous ecological data and life-history characteristics of the giant panda, female-biased dispersal is most likely to be due to competition for birth dens among females, inbreeding avoidance and enhancing inclusive fitness among related males.

Keywords: birth den, giant panda, inbreeding avoidance, inclusive fitness, noninvasive genetics sampling, sex-biased dispersal

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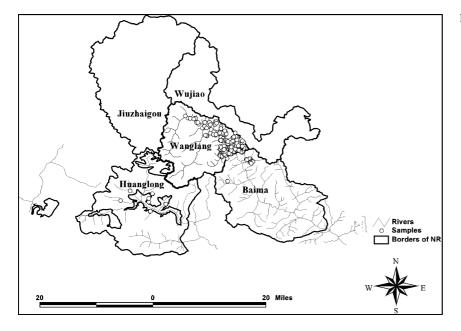
Introduction

Dispersal, the movement of an individual from its natal site to its reproductive site, is one of the key demographic forces shaping natural populations (Howard 1960; Proctor *et al.* 2004) and has become a focus for demographic and ecological research (Nathan 2001; Kokko & López-Sepulcre 2006; Nathan 2006). It has been reported that in contrast to birds, for example, mammals have predominantly male-biased dispersal patterns (e.g. Greenwood 1980; Wolff 1994; Clarke *et al.* 1997). However, there are many exceptions to this general observation (Clutton-Brock 1989;

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Waser 1996; Wolff & Plissner 1998) and dispersal patterns have been found to be contingent on breeding system, taxon and habitat type and fragmentation among other factors (Sinclair 1992; Swenson *et al.* 1998; Proctor *et al.* 2004; Goossens *et al.* 2006).

The giant panda (*Ailuropoda melanoleuca*) has attracted much attention in recent years and habitat fragmentation has been identified as one of the most serious issues surrounding its precarious conservation status (Hu *et al.* 1985; Wei *et al.* 2000; Liu *et al.* 2004). Since dispersal can promote gene flow (Slatkin 1987), facilitate range expansion (Lubina & Levin 1988; Swenson *et al.* 1998; Kokko & López-Sepulcre 2006) and resist range contraction (Channell & Lomolino 2000), it can potentially play a central role in maintaining population viability for giant pandas and could be a crucial

Fig. 1 Sampling locations in studied areas.

parameter for panda conservation management. However, to date, direct dispersal data in giant pandas are confined to just three observations: (i) Hu *et al*. (1985) found that a male juvenile named 'Longlong' underwent a so-called 'excursion' in Wolong Nature Reserve (NR), Sichuan Province; (ii) Pan *et al*. (2001) tracked two female juveniles, 'Shuilan' and 'Boshi', using radiotelemetry and found they dispersed a considerable distance (34 km and 24 km, respectively) in Qinling, Shaanxi Province; and (iii) a female juvenile ('Shenglin No. 1') dispersed into Dujiangyan, a populated city in Sichuan Province in 2005, leading to a highly publicized rescue and release operation. In general, however, difficulties in observing individuals have strongly hampered efforts towards studying general dispersal patterns in this species. Fortunately, the recent possibility of using indirect methods to infer dispersal processes from the spatial distribution of molecular genotypes represents a promising alternative (Austin *et al*. 2003; Palo *et al*. 2004; Goossens *et al*. 2006; Hammond *et al*. 2006). It is now clear that population genetic approaches based on molecular data can potentially accurately reveal spatial patterns of dispersal and differences in dispersal patterns and rates between the sexes (Goudet *et al*. 2002; Hammond *et al*. 2006).

As a result, there are now a considerable number of cases in which the dispersal of animals, including bears, has been characterized using molecular data, especially multilocus microsatellite analysis (Stow *et al*. 2001; Goudet *et al*. 2002; Proctor *et al*. 2004). We recently demonstrated that microsatellite markers could be successfully amplified from faecal samples, the commonest DNA source for pandas in the wild and could be used to accurately identify individuals and to census populations (Zhan *et al*. 2006). Here, we examine molecular data from the same key

populations of giant pandas to (i) explore the general dispersal pattern among the sexes based on molecular and ecological evidence, and (ii) study the spatial distribution of related individuals and its biological significance.

Materials and methods

Study site and sampling

The study site is located in the northern part of the Minshan Mountains, the largest giant panda habitat region in China and comprises Wanglang NR, Huanglong NR and Baima Tibetan Community (Fig. 1). In these areas, giant pandas in Wanglang NR and Baima Community were presumed to comprise a single connected population and Huanglong to comprise another because of high mountains separating them. The two populations are circled by the Jiuhuan highway, which probably now isolates them from populations in other parts of Min Mountains.

The sampling strategy was as described in Zhan *et al*. (2006). In total, we obtained 254 faecal samples in Wanglang, 27 in Baima, and 13 in Huanglong from February to August of 2004. Global Positioning System (GPS) coordinates were recorded for all samples and mapped into the study areas using ARCVIEW 3.2a (Fig. 1).

Molecular analysis

DNA was extracted from faeces according to Zhang *et al*. (2006) using standard controls. We screened each sample using mitochondrial DNA to ensure extracts were from giant panda as opposed to other species using primers and conditions as in Zhang *et al*. (2002).

Nine microsatellite primers, Ame-*μ5*, *μ10*, *μ26*, *μ15*, *μ19*, *μ22*, *μ27*, *μ13*, *μ24* (Lu *et al.* 2001; Zhan *et al.* 2006), were used to amplify DNA extracts. To obtain reliable genotypes, a multitubes approach (Taberlet *et al.* 1996) was used as follows: (i) we amplified faecal DNA twice in 20 μL containing 2 μL DNA, 10 μL Premix *Taq* (TaKaRa, DRR003A), 0.4 μM forward (labelled 5′-FAM, HEX or TET) and reverse primers and 1 μg/μL BSA (Sigma). Polymerase chain reactions (PCR) were performed in a Thermo MBS cycler, starting with 94 °C for 3 min, followed by a touchdown PCR (a total of 35–39 cycles of 94 °C/15 s, T_{anneal}/30 s, 72 °C/45 s) and a final step of 60 °C for 30 min. T_{anneal} was decreased by 2 °C every second cycle from 60 °C to a touchdown temperature (48–50 °C), which was used for following 25 cycles. Products were resolved using an ABI PRISM 377 automated sequencer, and analysed using GENESCAN version 3.1.2 and GENOTYPER 2.5 (Applied Biosystems). (ii) Loci that gave rise to the same heterozygous genotype twice were accepted. Otherwise, a third repeat was conducted. Some genotypes could be accepted from three positive PCRs. If not, four further repeats were conducted.

A species-specific sexing primer pair ZX1 (Zhan *et al.* 2006) was designed to amplify a 210-bp region of the Y chromosome of the giant panda. DNA from two male and two female pandas in captivity in Wolong NR were used as positive and negative controls. PCR and cycling conditions were similar to that for microsatellite amplifications with a touchdown temperature of 51 °C. Each sample was amplified with ZX1 three times and products were electrophoresed on a 3.0% agarose gel. A sample was identified as male if at least two experiments showed the 210-bp *SRY* band, and as female if no bands were produced.

We used MSTOOLS (Park 2001) in Microsoft Excel to find matching genotypes. Genotypes from different samples were considered to represent the same individual when all alleles at nine loci were identical or if there was only a single allelic mismatch (Bellemain *et al.* 2005; Solberg *et al.* 2006). The probability of full-sib or unrelated pairs of pandas, $P_{(ID)}$, bearing an identical multilocus genotype, was estimated using the software GIMLET (Valière 2002) to explore the discrimination power of the microsatellite locus combination. To further assess the reliability of individual identification, we designed three new primers, AY79, AY87, and AY95, and used them to amplify 30 samples (10.2% of the total).

GENELAND version 1.0.5 (Guillot *et al.* 2005), a computer package in R 2.3.1 (Ihaka & Gentleman 1996), was used to verify our definition of panda populations. The software makes use of georeferenced individual multilocus genotypes for inferring the most likely number of populations in a data set (e.g. Coulon *et al.* 2006). Five independent runs of the GENELAND model were performed with 1 000 000 iterations, of which only every hundredth one was saved,

treating the number of genetic clusters as unknown and using the spatial D-model as a prior for all allele frequencies. Since the sampling in this study was clustered in two sampling areas, we also used a clustering method that does not use a spatial prior as implemented in STRUCTURE 2.2 (Pritchard *et al.* 2000) to detect the most likely number of populations (*K*). Eight independent runs of *K* = 1–8 were performed with 1 000 000 Markov chain Monte Carlo (MCMC) repetitions after 50 000 burn-in period. The admixture ancestry model was chosen and allele frequencies were assumed to be correlated. *K* was identified using the maximal values of Ln $P_{(D)}$ (the posterior probability of the data for a given *K*) returned by structure and Δ*K* based on the rate of change in the log probability of data between successive *K* values (Evanno *et al.* 2005). Globally and for each population, estimates of genetic variation and tests for deviation from Hardy–Weinberg expectation (F_{IS}) were carried out using ARLEQUIN version 3 (Excoffier & Schneider 2005), FSTAT version 2.9.3.2 (Goudet 2001) and GENEPOP version 3.4 (Raymond & Rousset 2003).

Relatedness (*r*) among individuals at a locality is expected to be higher for the more philopatric sex than that of the more dispersing sex (Goudet *et al.* 2002). As reported in Zhan *et al.* (2006), we intensively sampled most panda individuals in the Wanglang/Baima population, which was used to examine relatedness structure. Therefore, we calculated average relatedness among males, females and across all individuals only in this population using RELATEDNESS 5.08 (Goodnight & Queller 1999). Standard errors were calculated by jackknifing over loci. We also explored potential differences in dispersal rates between females and males over both populations. FSTAT version 2.9.3.2 was used to quantify genetic variance among populations (F_{ST}) mean (*mAIc*) and variance (*vAIc*) of assignment index for both sexes using 10 000 permutations in the menu of biased dispersal. F_{ST} is a statistic expressing the proportion of the total genetic variance that resides among populations (Hartl & Clark 1997). Allelic frequencies for individuals of the sex dispersing most should be more homogeneous than those for individuals of the more philopatric sex. F_{ST} for the more philopatric sex, therefore, is expected to be higher than that of the more dispersing sex (Goudet *et al.* 2002). Assignment index (*AI*) refers to the probability of an individual occurring in a locality (Paetkau *et al.* 1995; Favre *et al.* 1997). Because populations can contain very different levels of gene diversity, the multilocus probabilities of individuals in different populations are not directly comparable. To remove this problem, a corrected assignment index (*AIc*) is used. In general, A positive value indicates a resident individual and a negative value indicates a potentially disperser. Because immigrants tend to have lower *AIc* values than residents, under sex-biased dispersal, *mAIc* for the more philopatric

sex should be higher than that of the more dispersing sex. In contrast, *vAIc* should be larger for the dispersing sex because members of this sex usually include both common genotypes from residents and rare ones from immigrants (Goudet *et al.* 2002).

Pairwise relatedness estimates use the population allele frequencies and the genotypes of the two individuals to calculate the likelihood that this genotype combination could have been produced by the relationship specified (Queller & Goodnight 1989). We used KINSHIP 1.2 to obtain a likelihood ratio for each pair of individuals and to calculate a significance level for this ratio by 10 000 simulations. Because of the large number of dyads analysed and to reduce the probability of Type I error (Radespiel *et al.* 2001), giant pandas were only classified as being related ($0.125 \leq r \leq 0.5$) or closely related ($r = 0.5$) if their respective likelihood ratios were at the 0.01 significance level. We used extensions of ARCVIEW 3.2a, XTOOLS and center of mass, to produce the centre of the movement range for every individual based on the 'territory' identified by the GPS coordinates generated during repeated sampling of an individual's genotype. However, a single male panda (WL107) moved very large distances within the reserve during the sampling period and the previous year. Since we wished to avoid any sampling bias introduced by attempting to determine the territory centre of this male and calculate the spatial distance between him and other related pandas, we excluded this aberrant individual from later geographical analyses.

Spatial distances between centres for related individuals were compared between different sex classes with an Independent Sample t-test in SPSS. If one sex tends to disperse more, we predict that the spatial distance between related individuals from the more dispersing sex should be significantly larger than that of the opposite sex. To explore biological meaning of gender difference in spatial distribution of related individuals, we examined the relationship between the geographical distance among related individuals and previous home-range estimates for giant pandas. There are two studies on the home range of the giant pandas (Hu *et al.* 1985 in Wolong NR and Pan *et al.* 2001 in Changqing NR), we use these two values and obtained average range radiuses assuming that the home range of giant pandas was approximately circular (e.g. brown bear *Ursus arctos*; Proctor *et al.* 2004). Then we took 1×, 2× and > 2× radius as scales to examine the number of related dyads at the corresponding home range multiplier. However, since the application of such home range multipliers derived from pandas in other places into our study may be subjective, we also plotted the number of related dyads ($0.125 \leq r \leq 0.5$) against five specific geographical distance classes (1000, 2000, 3000, 4000 and 12 000 m, which was the upper limit of interindividual distance in the present study).

Results

Individual identification, population definition and genetic variability

Genotyping errors have been frequently reported in noninvasive genetics using faecal samples (e.g. Taberlet *et al.* 1996; Pompanon *et al.* 2005) and some preselection of samples and rigorous laboratory procedures always need to be taken to produce accurate genotypes. As part of this process, we conducted mitochondrial DNA analysis for species verification and our microsatellite genotyping protocol followed the criteria of Taberlet *et al.* (1996), which produced reliable genotypes at a confidence level of 99%. Nine microsatellite loci could be amplified from 290 samples that were collected in the study area (eight loci with approx. 90% success, one with approx. 50% success). Analysis using GIMLET showed that these loci combined would only produce an identical genotype by chance in the case of full sibs with a probability of 0.165%. There was no increase in discriminatory power with results based on nine or 12 loci (three additional loci were screened in 10% of samples, data not shown). However, if the three most informative loci could not be scored, the probability of sib false identity was 2.55%, so we excluded four samples where less than six loci could be reliably amplified.

Through analysing georeferenced multilocus genotypes using GENELAND, we found two distinct populations in our study areas: the Wanglang/Baima population and samples from Huanglong (Fig. 2). The same population definitions were produced using STRUCTURE and assuming no prior geographical partition, because both the maximal Ln P(D) (–1706.1125) and ΔK (20.5983) were maximal when the population number *K* was equal to 2. From the faecal samples, we identified 72 individuals: 36 males and 36 females, in the Wanglang/Baima population and 10 (3 males, 7 females) in Huanglong. Overall expected heterozygosity was 0.609 for Wanglang and Baima and 0.67 for Huanglong. All the populations were in Hardy–Weinberg equilibrium. Mean F_{IS} values were –0.033 for Wanglang/Baima and –0.023 for Huanglong, and none were significantly different from zero.

Gender differences in genetic indices

The average relatedness, *r*, among males in the Wanglang/Baima population was 0.114 ± 0.067, significant greater than zero, while the average *r* was 0.085 ± 0.080 for females, not significantly different from zero. The difference between the average *r* values of the two sexes was not significant. Additionally, both F_{ST} and *mAIc* were larger for males than females, but in neither case were they significantly so (Table 1). However, *vAIc* for females, at 10.28, was significantly greater than that for males (4.33). In an
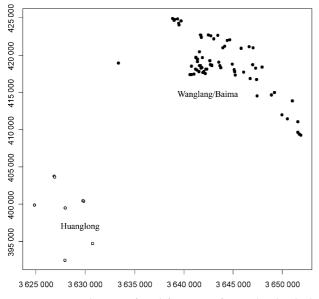
Fig. 2 Two populations inferred from georeferenced individual multilocus genotypes using GENELAND. Faecal samples collected in Wanglang NR and Baima Tibetan Community were clustered into one population (solid circles) and samples from Huanglong were clustered into another population (empty circles) in the analysis. Each sample was mapped using its horizontal UTM Northing coordinate and vertical Easting coordinate.

Table 1 Mean F_{ST}, mean (*mAIc*) and variance (*vAIc*) of assignment index of giant pandas in Wanglang/Baima and Huanglong populations calculated using FSTAT 2.9.3

	Sample size	F_{ST}	*mAIc*	*vAIc**
male	39	0.011	0.25	4.33
female	43	0.062	−0.22	10.28

*Significant difference.

assessment of dispersal indices from genetic data, Goudet *et al.* (2002) concluded that a pronounced difference in *vAIc* between the sexes provides the strongest genetic evidence for sex-biased dispersal when the dispersal rate is below 10%.

Spatial distance between related individuals

The average spatial distance at 5339.9 m (*n* = 125, SE = 328.7) between related female dyads was larger than the 4088.2 m (*n* = 97, SE = 271.3) for related male dyads and this difference is highly significant (d.f. = 222, *P* < 0.01) in an Independent Sample t-test in SPSS. As shown in Table 2, we found there were more closely related male dyads (*r* = 0.5, *P* < 0.01) within 1× and 2× home ranges but more females beyond 2× home ranges using either Hu *et al.*'s criterion (1985) or Pan *et al.*'s (2001). Moreover, the number of related male dyads was more than that of females within 1× and 2× home ranges according to the home range estimated by Pan *et al.* (2001). If we divided the geographical scale into five specific classes defined as above, there were 13 related male dyads (three closely related) in contrast with nine related female dyads (no closely related) in the first class, 1000 m. In the geographical class of 2000 m, the number of related male dyads was only one more than that of females. But there were more related female dyads than related males beyond 2000 m.

Discussion

Dispersal in natural populations of the giant panda

We can conclude that dispersal is a key process in giant panda populations based on previous macro-ecological evidence (Hu *et al.* 1985; Pan *et al.* 2001) and the genetic data in the present study. Based on spatial distances between related individuals (Table 2; Fig. 3), we find evidence for dispersal in both genders. However, there is also evidence

		The number of related dyads			
		Hu *et al.*'s criterion		Pan *et al.*'s criterion	
Scope	Gender	$0.125 \leq r \leq 0.5$	$r = 0.5$	$0.125 \leq r \leq 0.5$	$r = 0.5$
1× HR	male dyads	16	2	27	4
	female dyads	14	0	18	1
2× HR	male dyads	15	2	31	2
	female dyads	15	1	20	0
> 2× HR	male dyads	64	3	36	1
	female dyads	96	1	87	1

Table 2 The number of related dyads of both genders at different geographical distance classes defined according to the HR (home range of the giant panda) reported by Hu *et al.* (1985) and Pan *et al.* (2001), respectively
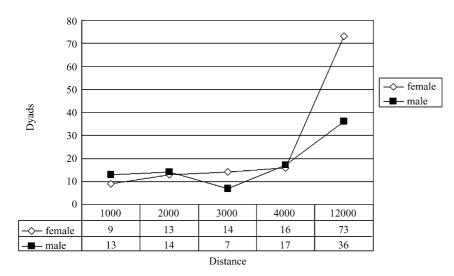
Fig. 3 The number of related dyads ($0.125 \leq r \leq 0.5$, calculated from KINSHIP software) between female–female, and male–male at five geographical distance classes, 1000, 2000, 3000, 4000 and 12 000 m.

	1000	2000	3000	4000	12000
female	9	13	14	16	73
male	13	14	7	17	36

Distance

Table 3 Recent confirmed dispersal events*

Individual	Sex	Age(year)	Discovery site	Presumed source	Date
1 (Shuilan)	♀	3.5	Yang County	—	1991
2 (Boshi)	♀	2.5	Yang County	—	1993
3 (Siguniang)	♀	~4	Xiaojin County	Wolong NR	2003
4 (Shenglin No. 1)	♀	2–4	Dujiangyan City	Zhaogongshan	2005
5	♀	~4	Jiangyou City	Beichuan County	2004
6	♀	2.0	Laoxiancheng NR	Foping NR	1992
7	♀	2.0	Laoxiancheng NR	Foping NR	1995

*Individual 1 & 2 from Pan *et al.* (2001); others collected by our team. Dispersal distances in all cases were more than 20 km.

that male pandas more often set up their territories adjacent to their birth place, while females disperse further away (Table 2; Fig. 3). Therefore, as spatial distance between related individuals increases we expect there to be fewer related male dyads with more related female dyads detected. This is the case not only because we found 16 related females dyads and only four related male dyads more than 10 000 m apart in our study, but also because it has been found that all reported long-dispersal observations involved females (Table 3).

Is dispersal sex biased?

There are currently two opposing ideas on sex-biased dispersal in giant pandas: some researchers have argued that giant pandas might have male-biased dispersal because of the observation in Wolong NR (Hu *et al.* 1985) and similar dispersal patterns in two related species, the brown bear (Swenson *et al.* 1998; Proctor *et al.* 2004) and the American black bear (*Ursus americanus*, Rogers 1987; White *et al.* 2000). Others have proposed that the giant panda has female-biased dispersal (e.g. Pan *et al.* 2001). The conclusion of Hu *et al.* (1985) was only based on the

observation of a single individual and Pan *et al.* (2001) inferred female dispersal from just two dispersing individuals. It seems unlikely that so few observations have any biological meaning at the population level. In contrast, our analysis of dispersal is based on microsatellite genotypes from nearly the whole population in Wanglang/Baima (Zhan *et al.* 2006), and may therefore reflect the dispersal pattern of the population more accurately.

Our molecular data suggest that female pandas disperse farther or longer than males because: (i) the *vAIc* for females is significantly larger than that of males, suggesting that females in the populations comprise both 'local' and 'foreign' genotypes; (ii) males commonly establish territories neighbouring to their birth place; (iii) the average dyadic spatial distance of related females is significantly larger than that of males, and (iv) larger r, F_{ST} and $mAIc$ values were found in males, although these differences were not statistically significant. Further evidence comes from a related study (Lu *et al.* 2001) which found evidence for significant population structure using microsatellites (biparentally inherited markers) in giant panda populations with a concomitant lack of mitochondrial (maternally inherited) structure, suggesting that the observed patterns

of population differentiation might be due to limited male dispersal with more extensive female dispersal (e.g. Lyrholm *et al*. 1999; Wright *et al*. 2005). However, such a conclusion would be strengthened by the use of paternally inherited Y-chromosome markers.

Our conclusion is also supported by recent evidence from macro-ecological studies. Pan *et al*. (2001) described female-biased dispersal in a family of giant pandas in the Qinling population: two juvenile males established home ranges largely overlapping with their mother and one of these males successfully mated with an unrelated female. In contrast, the daughter of the same female established her home range further away from her mother and success-fully reproduced. In recent years, pandas have been observed to disperse long distances to potential habitats or nondistribution areas, even into a crowded city (Table 3). Inspection of these data finds that all dispersing individuals were juvenile females. Therefore, based on our analysis and observed dispersal observations, it seems highly plausible that the giant panda has female-biased dispersal, different from many other mammals (e.g. Greenwood 1980; Wolff 1994; Clarke *et al*. 1997).

However, the rate of dispersal in giant pandas in our study populations is nonetheless low overall because the *vAIc* value and its significance level indicated that the dispersal rate is lower than 10% (Goudet *et al*. 2002). The low dispersal rate of the giant panda is in agreement with the view that habitat specialists (bamboo for pandas) may view complex fragmented landscape as barriers to move-ment (Laurence 1985; Wolff 1999). We have also found that unlike other species with female-biased dispersal (e.g. Hammond *et al*. 2006), bias in the giant panda is relatively low because some indices, such as relatedness, F_{ST} and *mAIc*, showed trends but no statistical support. Another possibility, considering the giant panda's body size and mobility, is that our sampling areas may not have been large enough for exploring large-scale or long-distance dispersal.

Several mechanisms could play a role in forming female-biased dispersal or male philopatry in the giant panda. First, inbreeding avoidance is usually thought to be the primary cause of sex-biased dispersal in mammals (Greenwood 1980; Perrin & Mazalov 2000). While male offspring can potentially recognize their mothers because they are dependent upon them until 1.5 years old (Hu 2001), such recognition between fathers and daughters is less likely because males often mate with different females in a breeding season (Hu *et al*. 1985). Therefore, the dispersal of juvenile females could be an effective measure for minimizing inbreeding. Mating with relatives does not necessarily imply a cost for male pandas since they can mate with a number of different females over a mating season, however, females only reproduce once every 2.33 years on average, even though they can also mate with many males (Pan *et al*. 2001). This asymmetry in breeding cost between the sexes could also favours female-biased dispersal.

Second, male-male competition for the opportunity of consortships with females during the mating season is common in the giant panda (Hu *et al*. 1985; Pan *et al*. 2001). Male pandas, thus, may best enhance their own (inclusive) fitness by remaining near relatives and may form kin groups that facilitate cooperative mating efforts based on kin selection (Wrangham 1980; Ross 2001). In the field, researchers have found that dominant male pandas sometimes allow other males to mate with females (Hu *et al*. 1985), which may support this hypothesis.

Last but not least, competition for key resources is another possible factor promoting female-biased dispersal. Although food is not a limiting factor for female pandas in that bamboo is abundant in most areas most of the time, birth dens may be an important potential resource that triggers competition between female pandas, and to date this has not been taken to account in studies of dispersal. The giant panda is a typical *K*-strategist with slow repro-duction, a long generation time and small litter size (Hu *et al*. 1985; Li *et al*. 1997; Hu 2001). Moreover, newborn pandas are extremely poorly developed such that the ratio of weight between infant and mother is about 1:900, the largest recorded in a eutherian mammal (Hu *et al*. 1985). Suitable birth dens are therefore needed to guarantee safety and thermal protection for mothers and their infants during the breeding period. Female pandas are known to be extremely particular in selecting birth dens. In Sichuan, they usually utilize tree holes (Hu *et al*. 1985) while they generally select caves in over-exploited forest such as in Qinling, Shaanxi Province (Zhu *et al*. 2001). Further, it is not easy for a female to find a birth den because there are few old trees in the forest after years of human exploitation and only large trees, more than 200 years old, provide suitable dens (Hu *et al*. 1985). Therefore, female dispersal may reduce competition for a key resource in panda reproduction, which may eventually be competed for by mother and daughter.

Studying dispersal in giant pandas is problematic not only because it is very difficult to observe in the wild, but also because very few individuals have been studied using traditional radio-tracking. Our faecal DNA-based method of exploring dispersal did not require the capture/ observation of individuals and was therefore less intrusive than radio-tracking methods. Moreover, exhaustive faecal sampling allowed us to identify nearly all individuals in Wanglang NR using microsatellites (Zhan *et al*. 2006). Based on microsatellite analyses, dispersal is low in this population of giant pandas, but females disperse farther than males. Such dispersal patterns suggest connecting currently fragmented habitats could be a vital first step towards restoring population viability in the giant panda.

Acknowledgements

References

Austin JD, Dávila JS, Lougheed SC, Boag PT (2003) Genetic evidence for female biased dispersal in the bullfrog *Rana catesbeiana* (Ranidae). *Molecular Ecology*, **12**, 3165–3172.

Bellemain E, Swenson JE, Tallmon D, Brunberg S, Taberlet P (2005) Estimating population size of elusive animals with DNA from hunter-collected feces: four methods for brown bears. *Conservation Biology*, **19**, 150–161.

Channell R, Lomolino MV (2000) Dynamic biogeography and conservation of endangered species. *Nature*, **403**, 84–86.

Clarke AL, Saether BE, Roskaft E (1997) Sex biases in avian dispersal: a reappraisal. *Oikos*, **79**, 429–438.

Clutton-Brock TH (1989) Female transfer and inbreeding avoidance in social mammals. *Nature*, **337**, 70–72.

Coulon A, Guillot G, Cosson JF *et al.* (2006) Genetic structure is influenced by landscape features: empirical evidence from a roe deer population. *Molecular Ecology*, **15**, 1669–1679.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study.

Excoffier LG, Schneider S (2005) ARLEQUIN ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.

Favre L, Balloux F, Goudet J, Perrin N (1997) Female-biased dispersal in the monogamous mammal *Crocidura russula*: evidence from field data and microsatellite patterns. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **264**, 127–132.

Goodnight KF, Queller DC (1999) Computer software for performing likelihood tests of pedigree relationships using genetic markers. *Molecular Ecology*, **8**, 1231–1234.

Goossens B, Setchell JM, James SS *et al.* (2006) Philopatry and reproductive success in Bornean orang-utans. *Molecular Ecology*, **15**, 2577–2588.

Goudet J (2001) *FSTAT: A Program to Estimate and Test Gene Diversities and Fixation Indices*, Version 2.9.3. Lausanne University, Lausanne, Switzerland.

Goudet J, Perrin N, Waser P (2002) Tests for sex-biased dispersal using bi-parentally inherited genetic markers. *Molecular Ecology*, **11**, 1103–1114.

Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour*, **28**, 1140–1162.

Guillot G, Mortier F, Estoup A (2005) GENELAND: a computer package for landscape genetics. *Molecular Ecology Notes*, **5**, 712–715.

Hammond RL, Handley LJ, Winney BJ, Bruford MW, Perrin N (2006) Genetic evidence for female-biased dispersal and gene flow in a polygynous primate. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **273**, 479–484.

Hartl DL, Clark GC (1997) *Principles of Population Genetics*, 3rd edn. Sinauer & Associates, Sunderland, Massachusetts.

Howard WE (1960) Innate and environmental dispersal of individual vertebrates. *American Midland Naturalist*, **63**, 152–161.

Hu JC (2001) *Research on the Giant Panda*. Shanghai Publishing House of Science and Technology, Shanghai, China.

Hu JC, Schaller GB, Pan WS, Zhu J (1985) *The Giant Panda of Wolong*. Sichuan Publishing House of Science and Technology, Chengdu, China.

Ihaka R, Gentleman R (1996) R: a language for data analysis and graphics. *Journal of Computational and Graphical Statistics*, **5**, 299–314.

Kokko H, López-Sepulcre A (2006) From individual dispersal to species ranges: perspectives for a changing world. *Science*, **313**, 789–791.

Laurence WF (1985) Extinction and survival of rainforest mammals in a fragmented tropical landscape. In: *Landscape Approaches in Mammalian Ecology and Conservation* (ed. Lidicker WZ), pp. 46–63. University of Minnesota Press, Minneapolis, Minnesota.

Li XH, Li DM, Rong YG, Zhang J (1997) A preliminary analysis on population viability analysis for giant panda in Foping. *Acta Zoologica Sinica*, **43**, 285–293.

Liu XH, Bronsveld MC, Skidemore AK *et al.* (2004) Mapping habitat suitability for giant pandas in Foping Nature Reserve, China. In: *Giant Pandas: Biology and Conservation* (eds Lindburg D, Baragona K), pp. 176–186. University of California Press, Berkeley, California.

Lu Z, Johnson WE, Menotti-Raymond M *et al.* (2001) Patterns of genetic variation in remaining giant panda populations. *Conservation Biology*, **15**, 1596–1607.

Lubina JA, Levin SA (1988) The spread of a reinvading species: range expansion in the California sea otter. *American Naturalist*, **131**, 526–543.

Lyrholm T, Leimar O, Johanneson B, Gyllenstein U (1999) Sex-biased dispersal in sperm whales: contrasting mitochondrial and nuclear genetic structure of global populations. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **266**, 347–354.

Nathan R (2001) The challenges of studying dispersal. *Trends in Ecology & Evolution*, **16**, 481–483.

Nathan R (2006) Long-distance dispersal of plants. *Science*, **313**, 786–788.

Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, **4**, 347–354.

Palo JU, Lesbarrères D, Schmeller DS, Primmer CR, Merilä J (2004) Microsatellite marker data suggest sex-biased dispersal in the common frog *Rana temporaria*. *Molecular Ecology*, **13**, 2865–2869.

Pan WS, Lu Z, Zhu XJ *et al.* (2001) *A Chance for Lasting Survival*. Peking University Press, Beijing, China.

Park SDE (2001) Trypanotolerance in West African cattle and the population genetic effects of selection. PhD Thesis, University of Dublin, Dublin, Ireland.

Perrin N, Mazalov V (2000) Local competition, inbreeding, and the evolution of sex-biased dispersal. *American Naturalist*, **155**, 116–127.

Pompanon F, Bonin A, Bellemain E, Taberlet P (2005) Genotyping errors: causes, consequences and solutions. *Nature Reviews Genetics*, **6**, 847–859.

Pritchard JK, Stephens P, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Proctor MF, Mclellan BN, Strobeck C, Barclay RM (2004) Gender-specific dispersal distance of grizzly bears estimated by genetic analysis. *Canadian Journal of Zoology*, **82**, 1108–1118.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.

Radespiel U, Sarikaya Z, Zimmermann E, Bruford MW (2001) Socio-genetic structure in a free-living nocturnal primate population: sex-specific differences in the grey mouse lemur (*Microcebus murinus*). *Behavioral Ecology and Sociobiology*, **50**, 493–502.

Raymond M, Rousset F (2003) GENEPOP version 3.4. Updated from Raymond M, Rousset, F. (1995). GENEPOP version 1.2, population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **8**, 248–249.

Rogers LL (1987) Effects of food supply and kinship on social behavior, movements, and population growth of black bears in northeastern Minnesota. *Wildlife Monograph*, **97**, 72.

Ross KG (2001) Molecular ecology of social behaviour: analyses of breeding systems and genetic structure. *Molecular Ecology*, **10**, 265–284.

Sinclair ARE (1992) Do large mammals disperse like small mammals? In: *Animal Dispersal, Small Mammals as a Model* (eds Stenseth NC, Lidicker WZ), pp. 229–242. Chapman & Hall, London.

Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science*, **236**, 787–792.

Solberg KH, Bellemain E, Drageset O, Taberlet P, Swenson E (2006) An evaluation of field and non-invasive genetic methods to estimate brown bear (*Ursus arctos*) population size. *Biological Conservation*, **128**, 158–168.

Stow AJ, Sunnucks P, Briscoe DA, Gardner MG (2001) The impact of habitat fragmentation on dispersal of Cunningham's skink (*Egernia cunninghami*): evidence from allelic and genotypic analyses of microsatellite. *Molecular Ecology*, **10**, 867–878.

Swenson JE, Sandegren F, Söderberg A (1998) Geographic expansion of an increasing brown bear population: evidence for presaturation dispersal. *Journal of Animal Ecology*, **67**, 819–826.

Taberlet P, Griffin S *et al.* (1996) Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research*, **26**, 3189–3194.

Valière N (2002) GIMLET: a computer program for analyzing genetic individual identification data. *Molecular Ecology Notes*, **2**, 377–379.

Waser PM (1996) Patterns and consequences of dispersal in gregarious carnivores. In: *Carnivore Behavior, Ecology and Evolution* (eds Gittleman JL), pp. 267–295. Cornell University Press, Ithaca, New York.

Wei FW, Feng ZJ, Wang ZW, Hu JC (2000) Habitat use and separation between the giant panda and the red panda. *Journal of Mammalogy*, **81**, 448–455.

White TH, Bowman JL, Leopold BD *et al.* (2000) Influence of Mississippi alluvial valley rivers on black bear movements and dispersal: implications for Louisiana black bear recovery. *Biological Conservation*, **95**, 323–331.

Wolff JO (1994) More on juvenile dispersal in mammals. *Oikos*, **71**, 349–352.

Wolff JO (1999) Behavioral model systems. In: *Landscape Ecology of Small Mammals* (eds Barrett GW, Peles JD), pp. 11–40. Springer, New York.

Wolff JO, Plissner JH (1998) Sex biases in avian natal dispersal: an extension of the mammalian model. *Oikos*, **83**, 327–330.

Wrangham RW (1980) An ecological model of female-bonded primate groups. *Behaviour*, **75**, 262–300.

Wright TF, Rodriguez AM, Fleischer RC (2005) Vocal dialects, sex-biased dispersal, and microsatellite population structure in the parrot *Amazona auropalliata*. *Molecular Ecology*, **14**, 1197–1205.

Zhan XJ, Li M, Zhang ZJ *et al.* (2006) Molecular censusing doubles giant panda population estimate in a key nature reserve. *Current Biology*, **16**, R451–R452.

Zhang BW, Li M, Ma LC, Wei FW (2006) A widely applicable protocol for DNA isolation from fecal samples. *Biochemical Genetics*, **44**, 494–503.

Zhang YP, Wang XX *et al.* (2002) Genetic diversity and conservation of endangered animal species. *Pure and Applied Chemistry*, **74**, 575–584.

Zhu XJ, Lindburg DG, Pan WS, Karin AF, Wang DJ (2001) The reproductive strategy of giant pandas (*Ailuropoda melanoleuca*): infant growth and development and mother-infant relationships. *Journal of Zoology*, *London*, **253**, 141–155.