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ORIGINAL ARTICLE

Fine-scale population genetic structure and sex-biased dispersal in the smooth snake (*Coronella austriaca*) in southern England

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Human-induced alteration of natural habitats has the potential to impact on the genetic structuring of remnant populations at multiple spatial scales. Species from higher trophic levels, such as snakes, are expected to be particularly susceptible to land-use changes. We examined fine-scale population structure and looked for evidence of sex-biased dispersal in smooth snakes (*Coronella austriaca*), sampled from 10 heathland localities situated within a managed coniferous forest in Dorset, United Kingdom. Despite the limited distances between heathland areas (maximum <6 km), there was a small but significant structuring of populations based on eight microsatellite loci. This followed an isolation-by-distance model using both straight line and 'biological' distances between sampling sites, suggesting *C. austriaca's* low vagility as the causal factor, rather than closed canopy conifer forest exerting an effect as a barrier to dispersal. Within population comparisons of male and female snakes showed evidence for sex-biased dispersal, with three of four analyses finding significantly higher dispersal in males than in females. We suggest that the fine-scale spatial genetic structuring and sex-biased dispersal have important implications for the conservation of *C. austriaca*, and highlight the value of heathland areas within commercial conifer plantations with regards to their future management.
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Introduction

Anthropogenic pressures, through processes such as habitat alteration, have been attributed to the decline and disappearance of many populations (Pimm and Raven, 2000). However, such processes also have significant impacts on the genetic diversity, and ultimately, evolutionary potential of those populations that remain (Templeton *et al*., 2001). For a given species, the genetic structuring of populations reflects the range within that individuals are more closely related to one another than to those randomly selected from the general population (Repaci *et al*., 2007). Genetic structuring in contiguous habitats is typically expected to follow an isolation-by-distance model, whereby the geographical distance between two populations is the single biggest determinant of their genetic differentiation (Slatkin, 1993). In contrast, the population genetic structure of organisms inhabiting habitat mosaics can be facilitated or restricted by the specific habitat features an organism encounters (Adriaensen *et al*., 2003).

Limits to gene flow between populations are typically the result of a species reproductive mode and vagility (Lowe *et al*., 2005). However, habitat change can result in the creation of dispersal barriers (Hitchings and Beebee, 1998), which can alter patterns of gene flow and lead to the isolation of remnant populations (Gerlach and Musolf, 2000). Such small and isolated populations may continue to lose genetic variation as a consequence of genetic drift (Reed and Frankham, 2003), and become inbred (Madsen *et al*., 1996). Those populations that are able to persist and even expand in size following such perturbations are likely to be less genetically diverse because of bottleneck effects, which may result in reduced population fitness (Hoelzel *et al*., 2002).

Species at higher trophic levels have been shown both theoretically (Holt *et al*., 1999) and empirically (Komonen *et al*., 2000) to be particularly sensitive to habitat alteration. As snakes typically occur in the middle to higher levels of food webs, habitat changes are likely to have strong effects on their population dynamics (Lind *et al*., 2005) and have been linked to widespread population declines (Reading *et al*., 2010). Changes in the population status of snakes could also result in cascade effects, as has been seen with garter snakes (*Thamnophis* spp.) and their amphibian prey (Matthews *et al*., 2002). In addition to their high trophic level, snakes are typically limited in their dispersal ability, even in continuous habitats (Luiselli and Capizzi, 1997), which could have profound effects on their population genetics (Keogh *et al*., 2007). As a result, genetic structuring is likely to increase in populations that are subjected to habitat fragmentation.

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Although theoretical evidence supports the idea that dispersal ability is a critical factor in determining the persistence of species in a fragmented habitat (Hanski and Thomas, 1994), it remains one of the least understood factors in conservation biology (Macdonald and Johnson, 2001). This is largely a result of difficulties associated with its direct study (Templeton *et al*., 1990; Prugnolle and de Meeus, 2002), particularly for inconspicuous species such as snakes (Parker and Plummer, 1987; Gibbs and Weatherhead, 2001). In this respect genotypic data have become an invaluable tool to indirectly measure species dispersal capabilities, while simultaneously estimating differentiation between sampled populations (DeYoung and Honeycutt, 2005).

Coronella austriaca is an ovoviviparous, colubrid species that primarily feeds on lizards and small mammals using ambush tactics (Spellerberg and Phelps, 1977). Radio-tracking studies suggest *C. austriaca* to be sedentary in nature (Gent and Spellerberg, 1993), with adults becoming sexually mature at 4 years of age and potentially living in excess of 17 years (Reading, 2004b). Although widely distributed throughout continental Europe and areas of western Asia, *C. austriaca* populations in the United Kingdom are limited to lowland heathland habitats in Dorset, Hampshire and Surrey (Braithwaite *et al*., 1989). A plagioclimax vegetation community dominated by ericaceous dwarf shrubs (Chapman *et al*., 1989), lowland heathland has undergone significant loss and fragmentation as a result of agricultural intensification, shrub encroachment, urbanisation and commercial afforestation (Bakker and Berendse, 1999; Rose *et al*., 2000). This has been implicated as the determining factor in historical population declines of *C. austriaca* in the United Kingdom (Spellerberg and Phelps, 1977). At present some of the largest patches of heathland that remain are contained within the boundaries of commercial forests. Silvicultural practices within forest sites have resulted in a heterogeneous landscape, comprising stands of *Pinus* spp. of varying ages interspersed with patches of heathland. These heathland patches represent important remnant habitats for *C. austriaca* populations (Reading, 2004a, b); whereas forest stands have been suggested as 'effective barriers' to dispersal between populations (Phelps, 1978). This assumption has remained unstudied to date.

Given our limited knowledge of snake populations in altered landscapes and particularly the impacts of heathland alteration on United Kingdom populations of *C. austriaca*, this study was designed to examine (a) whether fine-scale population genetic structuring occurs within Wareham Forest (maximum distance between sampling sites <6 km), and to determine whether the observed structure was a result of isolation-by-distance effects or the limits to dispersal ability conferred by the presence of coniferous forest stands, and (b) to determine whether dispersal in this species is sex-biased.

Methods

Field surveys and sampling

A total of 10 hexagonal arrays of 37 artificial *refugia* (*sensu* Reading, 1997) were located within the boundaries of Wareham Forest, Dorset (Figure 1). Arrays were located on homogeneous areas of lowland heathland between

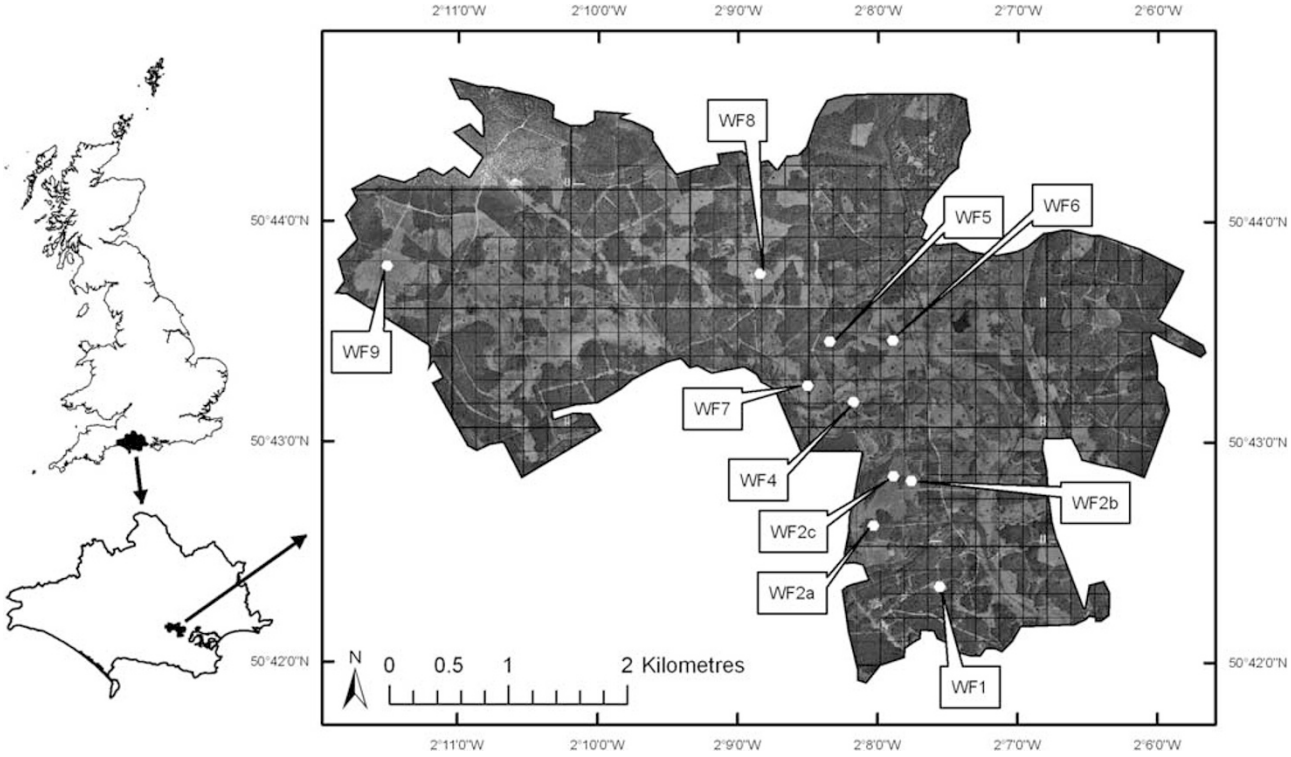

Figure 1 Localities of the 10 arrays of artificial *refugia* (white hexagons) within Wareham Forest, Dorset, from which individual *Coronella austriaca* were sampled. Darker coloured areas indicate stands of closed canopy *Pinus* spp., whereas lighter coloured areas include heathland, clearfelled stands and young *Pinus* spp. plantations.

Table 1 Matrix table of pairwise distances (m) between 10 arrays of artificial *refugia* used to collect DNA samples from populations of *Coronella austriaca* within Wareham Forest, Dorset

	WF1	*WF2a*	*WF2b*	*WF2c*	*WF4*	*WF5*	*WF6*	*WF7*	*WF8*	*WF9*
WF1	—	844.7	926.7	975.6	1720.4	2223.9	2295.6	2292.7	3092.1	5452.2
WF2a	1025.0	—	217.9	128.8	878.8	1385.6	1496.9	1450.2	2251.8	4724.0
WF2b	1124.6	206.7	—	211.0	831.2	1308.4	1368.6	1141.2	2204.6	4789.7
WF2c	1128.4	110.3	149.8	—	741.0	1255.3	1369.4	1318.3	2114.1	4614.3
WF4	1963.5	965.9	853.0	822.6	—	523.3	733.5	625.0	1376.1	4056.3
WF5	2468.1	1444.8	1338.9	1295.7	498.6	—	382.9	437.2	918.4	3806.0
WF6	2620.6	1635.9	1585.0	1541.7	883.8	435.3	—	822.4	1081.3	4093.9
WF7	2612.0	1595.2	1474.0	1460.6	633.9	494.9	873.5	—	832.4	3451.0
WF8	3502.5	2481.0	2375.7	2338.0	1524.9	1079.1	1297.2	885.9	—	3062.3
WF9	6046.4	5100.0	4940.8	4946.2	4103.4	3947.9	4355.4	3567.3	3299.4	—

Values above the dashed lines are the straight-line distances between the central *refugia* in each array. Values below the dashed lines are the 'biological' pairwise distances between the nearest edges of the two heathland samplings sites, containing arrays based on the assumption that smooth snakes only move through habitats with open canopies.

closed-canopy stands of Corsican pine (*Pinus nigra var. maritima*) and Scots pine (*Pinus sylvestris*). Straight-line distances (m) between the central *refugia* of each array at sampling sites were calculated in ArcGIS 9.1 using ortho-rectified aerial photographs. Habitat structure, in addition to geographical distance, is known to be a significant factor in determining population genetic structure for a number of species (Manel *et al*., 2003), and so a 'biological distance' between sampling sites within Wareham Forest was also measured from ortho-rectified aerial photographs. The 'biological distance' (m) was based on the assumption that snakes moving between pairs of heathland patches in Wareham Forest would preferentially use habitats that were primarily open canopied (for example, forest rides, clear-felled stands, young *Pinus* spp. stands, heath and moorland). *C. austriaca* is a lowland heathland specialist and, as such, is typically found in areas with few or no trees present (Spellerberg and Phelps, 1977). As a result the distance between the nearest edges of pairs of sampling sites, rather than the central *refugia* within arrays at sampling sites, was measured from the same aerial photographs, but based on a nonlinear path that avoided stands of trees that formed a closed canopy (Table 1).

Surveys for *C. austriaca* were completed between April and October of 2006 through 2008 (Pernetta, 2009). All snakes were captured by hand and sexed using tail length and shape (van Gelder *et al*., 1998; Reading, 2004a, b). Individuals were permanently marked using Passive Integrated Transponders (UKID Systems Model: UKID 122 IJ, 12-mm long, 2.12-mm wide), and DNA samples were collected upon first capture to prevent sampling individuals more than once.

Samples comprised either blood spots collected on Whatman grade 2 filter paper strips (Whatman plc, Maidstone, UK), obtained by puncturing the caudal sinus below the cloaca, or shed skin samples in cases where animals were in the process of sloughing when captured. Both sample types were air dried at room temperature before being frozen (−20 °C) before analysis. All blood samples were collected under licence from the Home Office (No: PIL 80/9699) Natural England (No: 20070553).

Sample extraction and microsatellite analysis

DNA was extracted from the dried blood and/or skin samples using a modified version of Gemmell and Akiyama's (1996) method for vertebrate tissues. Samples were placed in 300 µl of digestion solution (100 mM TRIS, 1%sodium dodecyl sulfate, 100 mM NaCl) and 7 µl of protinase K (10 mg ml^{-1}), and mixed overnight (≈12 h) on a rotating wheel at 37 °C. Following digestion, 300 µl of 10 M LiCl and 600 µl of chloroform were added and the samples mixed on the rotating wheel for a further 30 min at room temperature. The samples were then centrifuged for 10 min at 13 000 rpm to separate the supernatant, which was transferred to a new eppendorf tube. DNA was then precipitated with ethanol, dried and dissolved in 50-µl sterile distiled water, and stored at −20 °C.

We used eight previously characterised microsatellite loci *Ca19*, *Ca20*, *Ca26*, *Ca27*, *Ca30*, *Ca40*, *Ca47* and *Ca66* (Bond *et al*, 2005), on the basis of their consistency in amplification and ease of scoring. Amplifications were carried out in 20 µl volumes containing 1 µl of the DNA extract. Reactions contained 10 mM Tris-HCl pH 8.3, 50 mM KCI, 1.5 mM $MgCl_2$, 0.2 µm of each oligonucleotide primer, 100 µm dGTP, dCTP and dTTP, 5-µm unlabelled dATP, 3.7 KBq [α-^{33}P]dATP (37 TBq mmol^{-1}) and 0.5–1.0 units *Taq* polymerase (New England Biolabs, Hitchin, Hertfordshire, UK). Conditions for each primer set used followed those of Beebee (2008) and comprised an initial 4-min denaturation period (94 °C), followed by 35 amplification cycles each with 1-min denaturation at 94 °C, 1-min annealing (*Ca20*, *Ca30*, *Ca40* = 51 °C, *Ca19*, *Ca26*, *Ca27*, *Ca47*, *Ca66* = 60 °C) and 1-min elongation at 72 °C. All reactions culminated with a 4-min elongation step at 72 °C. PCR products were electrophoresed alongside M13 sequence markers in standard sequencing gels (6% wv, polyacrylamide). Following electrophoresis, gels were visualised using autoradiography and genotypes scored from the X-ray film.

Statistical analyses

Loci were examined for null alleles and mis-scoring using MICRO-CHECKER (Van Oosterhout *et al*, 2004). All loci were tested, at 99.5% confidence intervals, for evidence of selection using LOSITAN (Antao *et al*, 2008) with 50 000 simulations based on a stepwise mutation model. All retained loci were also tested for the presence of linkage disequilibria between loci using GENEPOP v4.0 (Rousset, 2008), and for deviations from Hardy–Weinberg equilibrium (HWE) using GENALEX v6.2 (Peakall and Smouse, 2006). In both cases the critical

probability was adjusted for multiple comparisons using a sequential Bonferonni correction (Weir, 1990). Observed and expected heterozygosities (H_O, H_E) were calculated in GENALEX, whereas Allelic richness (A_R) and the inbreeding coefficient within populations (F_{IS}) were calculated using FSTAT v2.9.3.2 (Goudet, 1995). The significance of F_{IS} estimates was tested in FSTAT based on 1600 randomisations. Allelic richness and measures of genetic diversity were compared between populations using Kruskal–Wallis one-way analysis of variance tests implemented in Minitab v.15 (Minitab inc., Philadelphia, PA, USA).

Bayesian clustering methods of population assignment were implemented using STRUCTURE 2.3.3. as an initial attempt to determine whether the sampling regions represent genetically distinct populations (Pritchard *et al*., 2000). A correlated alleles model with admixture incorporating sampling locality information was run in triplicate for each potential real population size ($K=$ 1–10). An initial burn-in period of 30^5 Markov chain replicates, followed by 10^6 iterations was used for each run. The number of 'true' populations was determined by calculating ΔK using the method as described by Evanno *et al.* (2005).

Genetic differentiation was assessed using F_{ST} analysis, which generally performs well when divergence among samples is expected to be low (Balloux and Goudet, 2002). Total genetic differentiation between populations was assessed using an analysis of molecular variance (AMOVA), in addition to pairwise estimates of F_{ST} between populations using GENALEX. The significance of these estimators was assessed using a non-parametric permutation approach (9999 permutations) with a subsequent Bonferonni correction applied to significance values.

Isolation by distance between populations was assessed using Mantel tests conducted in GENALEX (9999 permutations). Both linear and 'biological' distances (Ln transformed) between pairs of populations were compared with the genetic difference between population pairs ($F_{ST}/(1-F_{ST})$; a useful transformation for testing isolation-by-distance effects (Rousset, 1997)). Four tests for evidence of sex-biased dispersal were conducted using FSTAT, for the samples collected within Wareham Forest, based on 10 000 permutations. The first test compared relatedness (r) between sexes, with the assumption that the dispersing sex should exhibit lower levels of within population relatedness than the philopatric sex. The second test compared among population F_{ST} values between sexes, based on the principle that allelic frequencies of the dispersing sex should exhibit higher levels of homogeneity across the populations than the philopatric sex. The third and fourth tests are based on the use of a corrected assignment index to determine the probability of a genotype originating from the population in which the individual was collected (Favre *et al*, 1997). The first of these two tests compared the mean of the corrected assignment index (*mAIc*). In this instance, immigrants to a population are expected to have lower *AIc* values than residents. Therefore, the dispersing sex is likely to have a significantly lower *mAIc* value. The second of these tests compared the difference in the variance of *AIc* (*vAIc*). In this instance, the dispersing sex is likely to have a larger *vAIc* value than the philopatric sex because of increased probability of both resident and immigrant individuals being present in the sampled populations (Dubey *et al*, 2008). Because of the inequality in the number of males and females sampled at each site, we used a random re-sampling procedure to create five new data sets ($n=110$) with an equal sex ratio (male = 55 females = 55) and calculated the mean statistic and probability values for each test (*sensu* Dubey and Shine, 2010)

Results

A total of 149 individual smooth snakes were captured within Wareham Forest. Samples from two individuals failed to amplify any of the eight loci and so were excluded from further analyses. Initial examination of the data with MICRO-CHECKER showed no evidence of null alleles, and LOSITAN analyses showed no evidence of any loci being under selection. There was also no evidence of linkage disequilibrium at the 5% level (Bonferonni adjustment $P<0.000625$). Tests for deviation from HWE found five significant results (Table 2). However, these were not limited to a single locus or sampling site and so all eight loci were retained for further analysis. The mean allelic richness, inbreeding coefficients, observed and expected heterozygosities are shown for each sample site in Table 2. Kruskall–Wallis one-way ANOVA tests showed no significant difference between sample sites in any of the calculated measures of genetic diversity (A_R, $H=0.960$, $P=0.384$; H_O,

Table 2 Total sample size (n), based on males (M) and females (F) sampled at each array, average number of individuals genotyped per locus (N), allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficients (F_{IS}) and loci showing significant deviations from Hardy–Weinberg equilibrium (HWE) for 10 smooth snake-sample sites within Wareham Forest, Dorset

Array	N	*M*	*F*	N	A_R	H_O	H_E	F_{IS}	*HWE*
WF1	15	6	9	13.5	2.113	0.455	0.568	0.236 (0.0013)	*Ca47*
WF2a	17	12	5	13.38	2.295	0.476	0.591	0.249 (0.0019)	
WF2b	24	11	13	18.63	2.236	0.393	0.526	0.280 (0.0006*)	*Ca19, Ca20, Ca40*
WF2c	8	3	5	7	2.492	0.414	0.555	0.336 (0.0056)	
WF4	21	11	10	18.25	2.603	0.507	0.582	0.156 (0.0025)	
WF5	10	6	4	7.75	2.536	0.388	0.507	0.305 (0.0013)	*Ca40*
WF6	14	7	7	9.88	2.705	0.448	0.518	0.189 (0.0063)	
WF7	12	11	1	9	3.013	0.448	0.601	0.314 (0.0006*)	
WF8	12	7	5	7.88	2.768	0.394	0.455	0.212 (0.0331)	
WF9	14	9	5	9.5	2.807	0.357	0.418	0.220 (0.0038)	

Probabilities of F_{IS} estimates are given in parentheses. *significant at adjusted nominal 5% significance level for multiple samples of 0.00063.

Table 3 Pairwise estimates of F_{ST} among 10 smooth snake-sample sites within Wareham Forest

	WF1	*WF2a*	*WF2b*	*WF2c*	*WF4*	*WF5*	*WF6*	*WF7*	*WF8*	*WF9*
WF1	—									
WF2a	**0.060**	—								
WF2b	**0.072**	**0.113**	—							
WF2c	0.020	0.030	**0.067**	—						
WF4	**0.082**	**0.099**	**0.072**	**0.089**	—					
WF5	**0.074**	**0.098**	0.017	**0.094**	**0.067**	—				
WF6	**0.062**	**0.054**	0.031	0.067	**0.091**	0.013	—			
WF7	**0.093**	0.076	0.033	**0.089**	**0.054**	0.034	0.045	—		
WF8	**0.107**	**0.140**	0.034	**0.147**	**0.073**	0.041	0.039	0.043	—	
WF9	**0.137**	**0.165**	**0.091**	**0.174**	0.163	**0.103**	**0.063**	**0.086**	0.024	—

Pairwise F_{ST} values that are significantly different from zero following Bonferonni's correction are highlighted in bold.

$H = 2.54$, $P = 0.980$; H_E. $H = 15.21$, $P = 0.085$; F_{IS}, $H = 2.11$, $P = 0.990$). Mean inbreeding coefficients were high within all 10 sampling sites (overall mean = 0.250, s.e. ± 0.018) and strongly significant for two of them (Table 2).

Bayesian clustering analysis using STRUCTURE identified two 'true' populations, based on the samples obtained. However, the two identified 'populations' did not correspond to the geographical locality of samples, suggesting the relatively low levels of variability for each loci resulted in a failure to correctly resolve discrete population clusters. Despite the close proximity of sample sites within Wareham Forest, AMOVA showed a small, but highly significant genetic differentiation between populations ($F_{ST} = 0.078$, $P < 0.001$). In addition, a total of 29 of the 45 pairwise F_{ST} values were significantly different following Bonferonni's correction ($P < 0.0011$; Table 3). Although 'biological' distance tended to be longer than the Euclidean distance between sampling sites, there was a highly significant correlation between both measures (Pearson's $r = 0.998$, $P > 0.001$). Mantel tests showed significant effects of isolation by distance using both straight-line ($r_S = 0.511$, $P = 0.003$) and 'biological' distances between sampling sites ($r_S = 0.445$, $P = 0.005$; Figure 2).

Analysis of sampling sites for sex-biased dispersal showed significant evidence for males being the dispersing sex. Female smooth snakes had significantly higher levels of both within-population relatedness (Figure 3a, mean $P = 0.011$) and between population genetic differentiation (Figure 3b, mean $P = 0.0072$). In addition, females showed significantly higher *mAIc* values (Figure 3c, mean $P = 0.046$), as would be expected from the more philopatric sex. Finally, the difference in mean *vAIc* values was not statistically significant (mean $P = 0.134$), the higher mean value for females is not consistent with the other three results in indicating males to be the dispersing sex (Figure 3d).

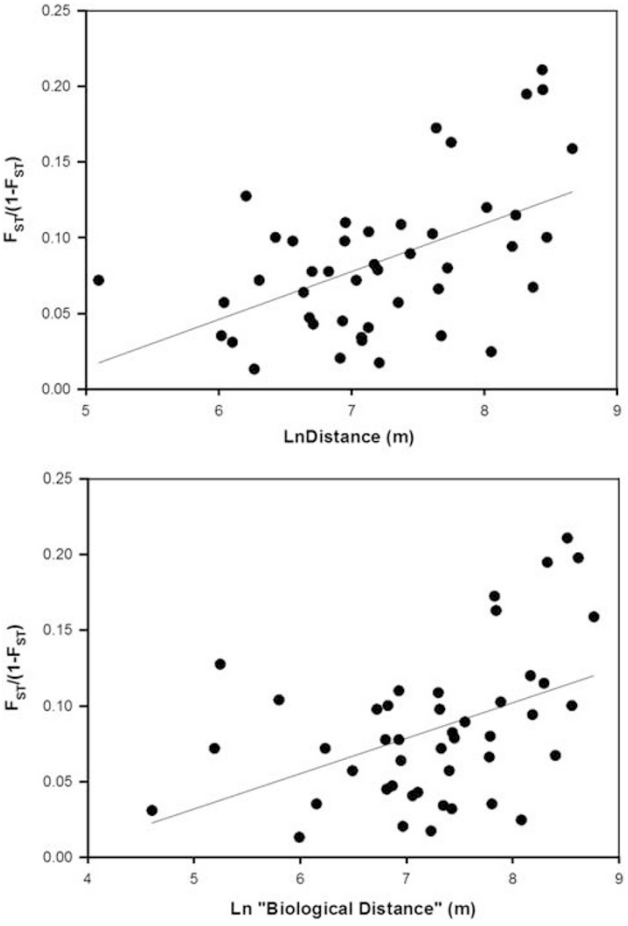

Figure 2 Relationship between pairwise F_{ST} values and (**a**) the straight-line distance ($r_S = 0.511$, $P = 0.003$), and (**b**) the 'biological' distance ($r_S = 0.445$, $P = 0.005$) between smooth snake-sampling sites in Wareham Forest.

Discussion

As far as we are aware, the results presented in this study provide the first detailed microsatellite-based analysis of the population genetic structure of *C. austriaca* at any spatial scale. Importantly, these results have shown that there is significant genetic structuring of populations at a fine-scale, within Wareham Forest. Comparable results have been found in North American species, notably eastern massasauga rattlesnakes (*Sistrurus catenatus catenatus*; Gibbs *et al*., 1997), black rat snakes (*Elaphe obsoleta obsoleta*; Prior *et al*., 1997), timber rattlesnakes (*Crotalus horridus*; Clark *et al*., 2008) and eastern foxsnakes (*Mintonius gloydi*; DiLeo *et al*., 2010). Typically, these patterns result from the presence of anthropogenic barriers or a lack of permeable habitats between subpopulations. North American snakes associated with water bodies also have significant population genetic structuring at fine geographical scales (*Nerodia sipedon*, Prosser *et al*., 1999; *Thamnophis sirtalis* and *Thamnophis elegans*, Manier and Arnold, 2005), likely a result of their

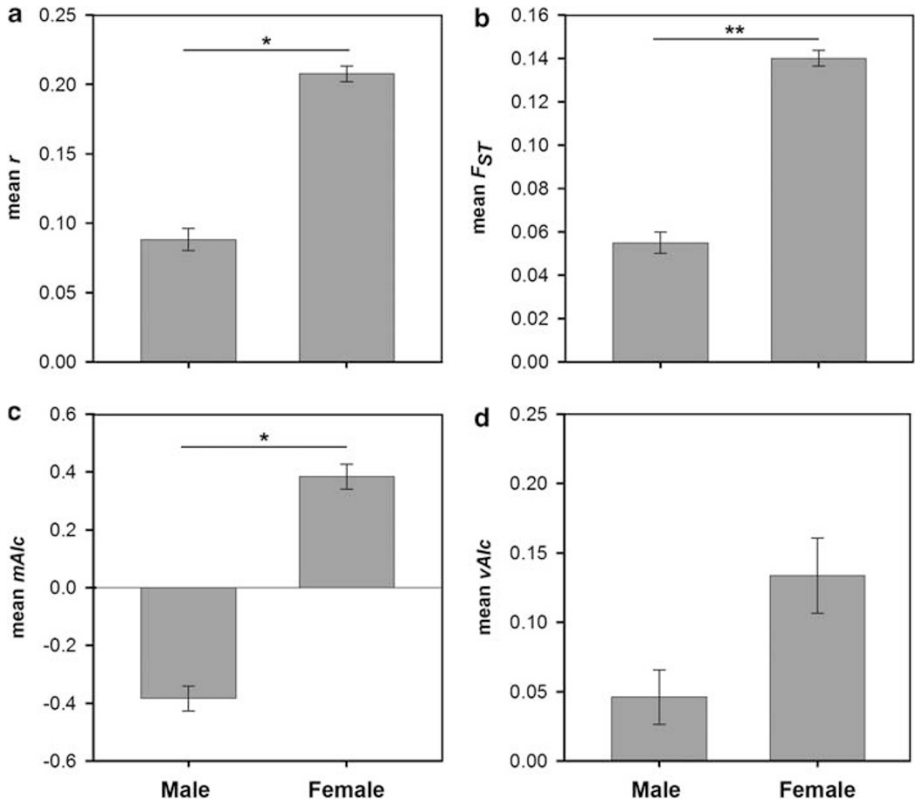

Figure 3 Mean ± s.e. (**a**) relatedness, (**b**) F_{ST}, (**c**) *mAIc* and (**d**) *vAIc* values for male and female smooth snakes from Wareham Forest, Dorset. Significant differences between the sexes are indicated by asterisks (*$P<0.05$, ** $P<0.01$).

habitat specificity (Manier and Arnold, 2005). Genetic differentiation of snake populations has also been linked to body size (King and Lawson, 2001), which may be a reflection of absolute dispersal capacity (DiLeo *et al*., 2010).

Unlike these previous studies (but see Clark *et al*., 2008), the fine-scale population genetic structuring observed in this study is consistent with an isolation-by-distance model. This result suggests that the observed differentiation may be because of the previously observed low vagility of the species (Gent and Spellerberg, 1993). Given the highly significant correlation relating straight-line and biological distances between sampling sites, it remains difficult to explicitly state that stands of mature *Pinus* spp. do not represent an 'effective barrier' to *C. austriaca* dispersal (*sensu* Phelps, 1978). However, it does seem that in this particular site smooth snakes are either capable of using these stands, or other non-heathland habitats within Wareham forest, such as rides bordering such stands, as routes for dispersal between populations.

In contrast to Gent and Spellerberg (1993), whose telemetry-based study found no evidence of sex-biased dispersal, the microsatellite analyses showed clear evidence for male-biased dispersal in smooth snakes. More recently, long-term mark-recapture studies on the ranging behaviour of smooth snakes at a site in Wareham Forest have shown that, although the mean range of male and female smooth snakes is similar (≈0.5–0.6 ha), males typically exhibit increased ranging movements once sexual maturity is reached (Reading, 2005). The biased dispersal observed in this study may therefore be a result of sexually mature males dispersing to find suitable mates, which can be an adaptive trait to reduce inbreeding (Dubey *et al*., 2008). *Coronella austriaca* effectively discriminates kin in a foraging context (Pernetta *et al*., 2009), although further work is required to understand whether this has any functional role in mate selection and male dispersal. The second possible explanation for the observed sex-biased dispersal could be a result differences in dispersal between sexes following parturition. Male neonatal slatey-grey snakes (*Stegonotus cucullatus*) typically disperse further from natal sites than female litter mates (Dubey *et al*., 2008). However, it is unknown whether this also occurs in *C. austriaca* because of the very-low incidence of neonate snake captures (Pernetta, 2009). Positive F_{IS} values were recorded from all of the sampling localities within Wareham Forest, suggesting that relatively high levels of inbreeding may still be occurring. Although positive values of F_{IS} may also be indicative of the Wahlund effect (the sampling of an assumed single population that is actually composed of two or more populations; Sinnock, 1975), in this instance this seems extremely unlikely, because of the restricted area covered by each array of artificial *refugia* (≈0.284 ha).

To date only four published studies have used genetic data to investigate sex-biased dispersal in snakes, all of which have found a male-bias (Rivera *et al*., 2006; Keogh *et al*., 2007; Clark *et al*., 2008; Dubey *et al*., 2008). Male-biased dispersal in mammalian species has been attributed to a female-defence polygyny mating system (Greenwood, 1980), and while polygyny has been regarded as typical for snakes (Duvall *et al*., 1993), more

recent research suggests that this may not always be the case (Rivas and Burghardt, 2005). As a result, further work is required to determine the reproductive system of *C. austriaca*, through paternity analysis of litters, thereby establishing whether the observed bias in dispersal can be explained by their reproductive strategy.

From a conservation perspective our data emphasise the sedentary nature of *C. austriaca* on United Kingdom lowland heathland sites within commercial coniferous forest plantations; a land-use change that has been suggested as the cause of significant declines in heathland reptiles (Spellerberg, 1988). However, this study has shown that *C. austriaca* is able to use areas of open heath and areas containing young stands of *Pinus spp.* before complete canopy closure. In addition, although areas containing mature stands of trees may not represent suitable permanent habitats, the presence of isolation-by-distance effects in population genetic structuring suggests that such stands, under current management regimes, do not represent significant barriers to dispersal. Wareham forest represents a heterogeneous landscape, despite the presence of large stands of *Pinus* spp., as it also contains areas of forest rides, clearfelled trees and moor land, which could exert an effect as corridors for *C. austriaca*. As a result, movements of individuals between heathland patches are more likely to be limited by their dispersal capabilities. Inevitably, the commercial nature of such sites results in conflict between maximising profits from forestry and the conservation benefits of land for biodiversity. However, this research suggests that maintaining a mosaic of habitats within managed commercial forests could benefit smooth snake populations. Although plantation forests are not able to support the biodiversity of primary habitats, they can have a role in complementary conservation services (Barlow *et al*., 2007). Further research concentrating on determining the precise stage at which young forestry stands may become unsuitable habitat for smooth snakes should be seen as a priority for forest management practices.

Conflict of interest

The authors declare no conflict of interest.

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