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Polyandry by wood mice in natural populations

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Abstract

Multiple paternity was investigated for the first time in natural populations of the wood mouse *Apodemus sylvaticus*. Thirteen females and their respective litters sampled within distinct habitats, seasons and years were screened for eight microsatellite loci. Allelic variation was compared with a dataset comprising 307 adult mice collected from the same source populations as pregnant females. Multiple paternity was unambiguously identified in seven litters (53.8%). In each case, a minimum of two or three male parents were involved. Populations of *A. sylvaticus* inhabiting the northern latitudes of the species range are characterized by annual cycles of abundance during which numbers can fluctuate by several orders of magnitude. Hence, the discovery of multiple paternity within litters sampled between May and July (high and low densities, respectively) in all years suggests that polyandry maximizes genetic diversity of the litter and, hence, survival of some of the offspring through such cycles. The results indicate that polyandry is a common mode of reproduction within wild populations of *A. sylvaticus*.

Introduction

Once considered rare, polyandry (female mating with multiple males) has now been reported in many species including birds (Primmer, Møller & Ellegren, 1995; Whittingham, Dunn & Magrath, 1997), reptiles (Fitzsimmons, 1998), invertebrates (Gosselin, Sainte-Marie & Bernatchez, 2005) and mammals (Baker, Makova & Chesser, 1999; Bartmann & Gerlach, 2001). The importance of this mating strategy has been recognized through the impact that it may have on effective population size, genetic diversity and levels of inbreeding (Sugg & Chesser, 1994). Relative to single paternity, litters composed of half-siblings resulting from multiple paternity may exhibit increased genetic diversity (Williams, 1975). Multiple paternity may also prevent inbreeding (Stockley *et al.*, 1993) and lead to increased interaction among offspring (Ridley, 1993). It is clear, therefore, that in order to address fundamental questions regarding effective population size, genetic diversity and social structure, precise quantitative measurements of the mating strategies must be carried out.

The social and reproductive behaviour of the wood mouse *Apodemus sylvaticus* has received considerable attention (Garson, 1975; Randolph, 1977; Montgomery & Gurnell, 1985; Wolton & Flowerdew, 2006; Jonsson & Silverin, 1997; Baker *et al.*, 1999; Bartmann & Gerlach, 2001) and a number of hypotheses regarding this species mating strategies have been proposed. Both Garson (1975) and Randolph (1977) observed the formation of bisexual pair bonds shortly after the beginning of the breeding season, supporting a monogamous mating system, at least during the initial part of the breeding season. However, among mammals, monogamy is considered rare (Ribble, 1991; Hohoff *et al.*, 2002). Two relatively recent studies based on molecular data have suggested the occurrence of polyandry and, subsequently, promiscuity in *A. sylvaticus* (Baker *et al.*, 1999; Bartmann & Gerlach, 2001). Baker *et al.* (1999) reported polyandry in the litters of three female *A. sylvaticus* out of six sampled from an area inadvertently exposed to radiation, an enclosure and a control area in the Chernobyl region of the Ukraine. A further study by Bartmann & Gerlach (2001) indicated multiple paternity occurring in 85% (29 out of 34) of litters produced by laboratory-bred females housed in an experimental outdoor cage under high density (experimental groups of 4:4 in a cage with a floor area of 4.25 m^2). Both investigations, however, were based on populations that could be considered unnatural.

The aim of the present study was to further elucidate the mating system of the European wood mouse *A. sylvaticus* living under natural conditions in an effort to estimate both the occurrence and frequency with which multiple paternity occurs.

Materials and methods

Sample collection

Thirteen pregnant female *A. sylvaticus* were available for this investigation, collected across two main habitat types between the months of April and August from studies carried out between 1990 and 2002. Eleven were collected from forests with a further two from a hedgerow system located on agricultural farm land, situated in County Down,

Table 1 *Apodemus sylvaticus* mother–litter groups analysed in this study

Female ID number	Number of offspring	Minimal number of sires	Paternal contribution ratio	Habitat type	Collecting date
1	4	1	/	Forest	April 1990
2	4	2	2:2	Forest	May 1990
3	5	2	2:3	Forest	May 1990
4	6	2	3:3	Forest	May 1990
5	4	1	/	Forest	May 1990
6	5	2	2:3	Forest	June 1990
7	6	3	2:2:2	Forest	July 1990
8[a]	5	2	4:1	Forest	June 2001
9	6	1	/	Forest	July 2001
10	4	2	2:2	Forest	July 2001
11	5	1	/	Hedgerow	July 2001
12	5	1	/	Hedgerow	August 2001
13	5	3	2:2:1	Forest	May 2002

[a]Not included in multiple-paternity litters as supported only by a single additional paternal allele at one locus.

Northern Ireland. Individual habitat type of origin and associated collection dates are given in Table 1. Mice were caught using Longworth live traps, each containing dry straw bedding in the nest box and baited with barley. Prebaiting is not considered necessary for the trapping of *A. sylvaticus* (Gurnell & Flowerdew, 2006). Each location within the chosen sampling sites/regions was trapped for three consecutive nights, with traps checked daily between 7:00 and 9:00 AM throughout the trapping period. Individuals collected in 1990 were returned to the laboratory and allowed to give birth before being euthanized, whereas females collected during 2001 and 2002 were immediately euthanized and the embryos were dissected out in the laboratory. The latter practice was used as a precautionary measure to prevent the possibility of embryo loss, which occurs in 2–5% of litters in *A. sylvaticus* (Pelikan, 1964). This practice also prevented the possibility of cannibalization of the young before tissue sampling could be performed, which could result in the underestimation of the frequency of multiple paternity. Biopsy tissue samples comprised either 1-cm tail clips removed from each female or tissue salvaged from whole embryos upon dissection. Tissues were placed in individually labelled vials containing 99% reagent-grade ethanol and were stored at 4 °C until DNA extraction. In order to obtain relevant population genetic data, tail clips were also taken from all males and non-gravid females within each study site. These individuals were released immediately after biopsy sampling at the exact location of capture. Microsatellite genotypic data were collected for 307 specimens representing the populations of origin of the 13 pregnant females surveyed in this study.

DNA extraction and microsatellite amplification

Total genomic DNA was extracted from tissues following the methodology of Taggart *et al.* (1992) with minor modifications, and samples were subsequently standardized to a final concentration of 50 ng μL. Females and their offspring were screened for eight polymorphic microsatellite loci: GACAB3A and GCATD7S (Makova *et al.*, 1998), As-7, As-11, As-12, As-34 (Harr, Musolf & Gerlach, 2000), WM2 (Barker, 2002) and WM4-6 (Booth, 2005). Polymerase chain reaction (PCR) settings for these microsatellites generally followed those described by their respective authors with minor modifications. Radioactively end-labelled (α^{32}P-dATP) microsatellite primers were used for manual screening, whereas fluorescently labelled IRD microsatellite primers were used for automated genotyping using a LiCor (Lincoln, NB, USA) (dual laser) system. PCR reactions were carried out in 12 μL volumes, each containing 1 × Promega Taq buffer, 1.5 mM $MgCl_2$, 100 mM dNTPs, 100 ng DNA template, 1 U *Taq* and ddH_2O to 12 μL. Primer concentration and annealing temperature varied depending on the screening method and between-individual loci (Table 2). Amplified PCR products were loaded onto 6% (1 × TBE) polyacrylamide gels containing 5.6 M Urea. Size standards (MicroStep-13b, 20a and 28a from Microzone™ (Haywards Heath, UK) were run every 15 samples to assist the sizing of allelic fragments. In all instances, maternal samples were run adjacent to their respective offspring, with at least one control sample (i.e. a sample of known genotype) run per gel to ensure accuracy and consistency of typing among different gels. The GeneProfiler (v3.46) software (Scanalytics Inc., Fairfax, VA, USA) was used to analyse genotypic data. Population samples were treated in a similar manner.

Statistical analyses

Summary population sample statistics (i.e. allelic diversity and heterozygosity) were estimated using the GENEPOP 3.1 software (Raymond & Rousset, 1995). Exclusion probabilities for the markers used in this study were calculated following the method described by Dodds *et al.* (1996), as implemented in the GERUD v1.0. The incidence of multiple paternity was assumed when, after subtracting maternal alleles, more than two paternal alleles were observed in at least two loci within a litter. As the number of loci (within a litter) meeting this criterion increases, so does the robustness of the multiple-paternity inference. This was initially carried out by visual inspection and subsequently with the assistance of the GERUD v1.0 software (Jones, 2001). Where multiple paternity was clearly detected, the program GERUD v1.0 was also used to estimate the minimum number and ratio of paternal contribution of males involved.

Results

Microsatellite variation

Summary population sample statistics are presented in Table 3. Overall, allelic diversity was found to be 15.33 alleles/locus. The average observed heterozygosity across

Table 2 Microsatellite primer details indicating the source, annealing temperature, number of cycles, primer concentration and $MgCl_2$ concentration

		Automated screening – LiCor				Manual screening – ^{32}P isotope			
Locus	Source	Annealing temperature (°C)	Number of cycles	Primer concentration (pM)	$MgCl_2$ concentration (mM)	Annealing temperature (°C)	Number of cycles	Primer concentration (pM)	$MgCl_2$ concentration (mM)
GCATD7S (F)	Makova *et al.* (1998)	55	27	2.5	2	60	28	5	1.5
GACAB3A (R)	Makova *et al.* (1998)	N/A	N/A	N/A	N/A	55	28	5	1.5
As-7 (F)	Harr *et al.* (2000)	55	27	0.8	1.5	48	28	5	1.5
As-11 (F)	Harr *et al.* (2000)	57	26	1.2	1.5	55	28	5	1.5
As-12 (F)	Harr *et al.* (2000)	56	26	1.2	1.5	48	28	5	1.5
As-34 (F)	Harr *et al.* (2000)	55	27	1.25	1.5	55	28	5	1.5
WM2 (F)	Barker (2002)	55	30	0.85	2	53	28	5	1.5
WM4-6 (F)	Booth (2005)	45	30	2.5	2	45	30	5	2

The end-labelled primer indicated in parentheses after locus name (N/A–the GACAB3A locus was screened manually only.

the panmictic sample was 72.7%. Population samples were found to be in Hardy–Weinberg equilibrium (HWE). Population summary statistics were unavailable for two microsatellite loci (*As*-11 and *GACAB3A*) across all sampled locations due to difficulties encountered during optimization for use with the Li-Cor™ dual laser automated DNA analyser. Although problematic for population analyses, these loci were scored unambiguously for mating system determination following the manual radioisotope-based methodology. Table 3 summarizes the usefulness of the marker loci used for parentage analysis, as expressed by the exclusion probability. Although individual locus exclusion probabilities ranged from 16.4% (*WM*2–Hedgerow) to 82.4% (*As*-12–Hedgerow), when combined across all loci, the exclusion probability per sample was higher than 99% when one of the parents is known.

The number of embryos per female varied from four to six (Table 1). Genotypic data at eight microsatellite loci were collected for 13 female *A. sylvaticus* and their combined 64 offspring with two exceptions. Females ID-4 and ID-13 failed to amplify at the loci *WM*4-6 and *GACAB*3*A*, respectively. However, as more than two paternal alleles were detected at the additional loci screened for these females, these two loci were redundant. Genotypes of all young were consistent with the females being the mothers of their respective litters, that is, all embryos possessed a maternal allele at each locus.

Multiple-paternity analyses

According to the chosen criteria based on the number of paternal alleles per locus as an indicator of the paternal contribution to each litter, multiple paternity was unambiguously detected in seven (53.8%) of 13 litters examined (Table 1). In these instances, more than two paternal alleles were evident at more than one locus (Table 4). In five cases (i.e. 38.5%), litters were sired by a minimum of two males, while for the remaining two cases (15.4%), litters were found to have been sired by a minimum of three males, which were inferred through the detection of five paternal alleles at two loci. The litter of female ID-8 exhibited an additional paternal allele at a single locus (*As*-12), but as this does not fit the criteria for the multiple paternity as described earlier, this litter was not considered a multi-sired litter. Multiple paternity was evident in litters of females collected in forest habitats sampled across all years. No incidence of multiple paternity was detected in the litters of females sampled in the hedgerow habitat. However, with a sample size of two, little can be deduced from this. Multiple paternity was observed during the months of May, June and July. Both multiple paternity and monogamous litters were observed within the same sampling location and sampling year (Table 1). With one exception (female ID-8), the males involved in multiple paternity were equally successful in siring the litter. Thus, there was no apparent skew in the reproductive success of the males involved (Table 1).

Table 3 Summary statistics for *Apodemus sylvaticus* population samples for which pregnant females were obtained and screened for eight microsatellite loci

Sample/microsatellite locus	*GCATD7S*	*WM2*	*As-7*	*As-11*	*As-12*	*As-34*	*GACAB3A*	*WM4-6*	Average
Panmictic population									
n	303	305	307	N/A	278	305	N/A	303	300.2
NA	18	6	16	N/A	25	18	N/A	9	15.33
H_o	0.763	0.376	0.882	N/A	0.921	0.733	N/A	0.688	0.727
H_e	0.775	0.381	0.825	N/A	0.905	0.789	N/A	0.837	0.752
ExPr	0.553	0.194	0.652	N/A	0.782	0.626	N/A	0.661	0.998[a]

[a]Total combined over loci/population sample).

n, number of individuals screened per sample; NA, number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity (Nei, 1987); ExPr, exclusion probability per locus (Dodds *et al.*, 1996).

N/A, population genetic data unavailable.

Table 4 Microsatellite genotypes in *Apodemus sylvaticus* for eight microsatellite loci screened for 13 families

Locus	F1 (*n*=4)	F2 (*n*=4)	F3 (*n*=5)	F4 (*n*=6)	F5 (*n*=4)	F6 (*n*=5)	F7 (*n*=6)	F8 (*n*=5)	F9 (*n*=6)	F10 (*n*=4)	F11 (*n*=5)	F12 (*n*=5)	F13 (*n*=5)
As-7	**113123**	**113113**	**113113**	**113129**	**113125**	**107113**	**109127**	**121127**	**113123**	**111121**	**113113**	**113121**	**113119**
	113123	**113**113	**113**113	**113**121	**113**113	*107113*	**109**113	113**127**	**113**113	113**121**	**113**125	**121**129	115**119**
	123123	**113**115	**113**113	121**129**	**113**113	**113**113	113**127**	119**127**	*113123*	**111**135	**113**125	117**121**	**119**125
	113125	**113**119	107**113**	*113129*	**113**113	*107113*	107**109**	119**127**	*113123*	**121**123	**113**125	**121**129	**119**121
	113125	**113**113	**113**113	**113**113	**113**119	*107113*	**109**117	113**127**	*113123*	**113**113	**113**117	117**121**	**113**113
			107**113**	119**129**		**113**113	113**127**	113**121**	111**123**		**113**125	**113**129	**119**121
				113113			**109**113		111**123**				
NPA	2	3	2	3	2	2	3	2	2	3	2	2	4
As-11	**220246**	**250252**	**246250**	**246256**	**240252**	**220246**	**220240**	**218248**	**242242**	**218244**	**248252**	**242252**	**234246**
	240**246**	240**250**	240**250**	238**246**	**240**256	**220**240	**220**244	232**248**	**242**242	*218244*	246**252**	*242252*	**234**234
	240**246**	246**252**	**246**248	**256**256	**240**246	**246**246	**240**246	**218**252	**242**242	**218**248	246**252**	*242252*	244**246**
	220240	**252**256	*246250*	238**246**	**240**246	**246**252	**220**220	232**248**	**242**248	*218244*	242**248**	**252**252	**234**250
	220246	*250252*	238**246**	238**256**	**252**256	**246**246	**240**250	**218**232	**242**248	242**244**	246**248**	**242**242	**234**236
			246248	238**256**		**220**240	*220240*	232**248**	**242**248		242**248**	*242252*	**234**250
				256**256**			**220**242		**242**248				
NPA	2	4	4	2	2	2	5	2	2	3	2	2	4
As-12	**258258**	**248264**	**254260**	**268274**	**244254**	**260268**	**258270**	**232234**	**232232**	**228232**	**232258**	**232258**	**234254**
	258270	**228**248	**254**254	228**274**	**244**260	244**268**	**258**264	**232**254	**232**258	**232**248	**258**266	**232**254	**254**254
	258258	**248**258	228**260**	268274	NA	244**260**	264**270**	*232234*	**232**258	**228**264	**232**266	**258**264	**234**238
	258270	**248**254	244**260**	228**268**	**244**260	260268	254**258**	**234**268	**232**258	**232**248	248**258**	**232**254	**254**254
	258258	254**264**	228**260**	268274	228**254**	244**268**	258**258**	**234**268	**232**258	**232**264	**232**248	**232**254	**234**263
			244**254**	NA		244**260**	258**258**	**234**254	**232**258		248**258**	**232**254	**254**254
				228**268**			236**270**		**232**258				
NPA	2	3	3	2	2	2	4	3	1	2	2	2	4
As-34	**130130**	**130136**	**144144**	**130136**	**130154**	**130136**	**136150**	**136136**	**130130**	**154154**	**154162**	**138150**	**130136**
	130130	**130**154	NA	*130136*	**130**130	**130**130	**136**154	**136**154	**130**130	130**154**	158**162**	**150**150	**136**162
	130130	**130**154	130**144**	**136**144	**130**130	**130**130	144**150**	**136**136	**130**130	130**154**	146**162**	**150**150	**136**166
	130130	**130**144	**144**144	**136**144	**130**130	**136**144	130**136**	**136**154	**130**130	130**154**	146**162**	**150**160	**136**164
	130130	**130**130	**144**144	*130136*	**130**130	*130136*	**144**150	**136**154	**130**130	130**154**	146**154**	*138150*	**136**166
			144144	**136**156		*130136*	**130**136	**136**154	**130**130		**154**158	*138150*	*130136*
				136156			**130**136		**130**130				
NPA	1	3	2	3	1	2	3	2	1	1	2	2	3
GCATD7S	**241241**	**259259**	**201257**	**195245**	**201259**	**195201**	**201259**	**205205**	**203245**	**201245**	**245245**	**205245**	**201247**
	241255	245**259**	245**257**	*195245*	**201**245	197**201**	199**259**	**205**245	201**245**	203**245**	**245**259	201**205**	203**247**
	241241	199**259**	*201257*	*195245*	**245**259	*195201*	195**201**	201**205**	201**245**	**201**247	**245**259	201**245**	**201**245
	241255	**259**259	*201257*	**195**257	**201**245	**195**245	199**259**	201**205**	**203**203	**245**245	**245**245	201**205**	**201**259
	241241	245**259**	*201257*	**245**257	257**259**	**195**199	259**259**	201**205**	201**245**	**201**203	**245**245	201**245**	245**247**
			201201	**195**257		**195**199	195**259**	**205**245	**203**203		**245**245	201**245**	**201**203
				245257			*201259*		201**203**				

Table 4 Continued

NPA	2	3	2	2	2	4	3	2	2	3	2	1	4
GACAB3A	**379383**	**381391**	**379387**	**383395**	**383391**	**383387**	**379387**	**281291**	**383391**	**377385**	**381385**	**391391**	/
	375**383**	383**391**	*379387*	387**395**	*383391*	361**383**	371**379**	*381391*	*383391*	*377385*	**381**381	385**391**	/
	379383	**381**387	383**387**	**383**383	*383391*	*383387*	**379**379	**381**381	**391**397	**385**391	**381**381	371**391**	/
	375**379**	383**391**	*379387*	387**395**	**391**391	381**383**	**387**387	379**381**	*383391*	379**385**	**385**385	371**391**	/
	375**379**	375**381**	**379**379	**383**383	*383391*	*383387*	371**387**	379**391**	*383391*	*377385*	385385	371**391**	/
			379379	*383395*		*383387*	*379387*	*381391*	*383391*		**385**385	NA	/
				383395			383**387**		*383391*				

NPA	2	3	2	2	2	3	4	2	2	3	2	2	/
WM2	**192206**	**192192**	**192192**	**192192**	**192192**	**192202**	**192202**	**192192**	**192194**	**192192**	**192202**	**192192**	**192192**
	192192	**192**192	**192**192	**192**192	**192**192	*192202*	*192202*	**192**192	*192194*	**192**194	**192**192	**192**192	**192**192
	206206	**192**192	**192**192	**192**192	**192**192	**192**192	**192**206	**192**194	*192194*	**192**192	*192202*	**192**192	**192**192
	192192	**192**192	**192**192	**192**200	**192**206	**202**202	*192202*	**192**192	**192**196	**192**194	194**202**	**192**192	**192**206
	NA	**192**192	**192**192	**192**200	**192**192	**202**202	*192202*	**192**192	*192194*	**192**192	**192**194	**192**192	**192**192
			192192	NA		**192**192	*192202*	**192**192	*192194*		*192202*	**192**192	**192**192
				192192			**192**206		**192**192				

NPA	2	1	1	2	2	2	2	2	2	2	2	1	2
WM4-6	**157173**	**161165**	**169173**	**NA**	**161161**	**157169**	**161169**	**161173**	**169169**	**165173**	**161169**	**173181**	**153165**
	157165	**165**173	**169**181	157169	**161**185	**157**165	**161**165	**173**181	161**169**	*165173*	**161**165	153**181**	**153**157
	165**173**	**161**169	161**169**	169173	**161**169	*157169*	157**169**	**173**173	**169**169	157**165**	**161**173	169**173**	157**165**
	161**173**	**161**169	**173**185	161173	NA	**157**185	*161169*	**173**173	**169**169	*165173*	165**169**	169**181**	NA
	161**173**	157**165**	**173**185	169173	**161**169	**157**157	**161**181	NA	161**169**	**165**165	**169**173	169**181**	157**165**
			169185	157169		**157**165	**161**165	**173**173	161**169**		**161**173	169**173**	157**165**
				157169			**161**177		**169**169				

NPA	2	3	3	/	2	3	5	2	2	2	2	2	1

Maternal genotype and inherited alleles are given in bold. Paternally inherited alleles are given in regular font. Where determination of the paternal allele was impossible due to the mother and offspring sharing complete genotypes is given in italics.
NPA, number of paternal alleles; *n*, number of offspring; NA, non-amplification.

Discussion

The results suggest that polyandry is common in female *A. sylvaticus*, with the multi-sired litters examined often characterized by roughly equal success of sires. We report, for the first time, the occurrence of multiple paternity in litters sampled throughout the breeding season and in different sampling years within natural populations of this species. Our findings, therefore, remove doubt over the applicability of the results presented in the previous studies (i.e. Baker *et al.*, 1999; Bartmann & Gerlach, 2001) to natural populations of this species. Owing to the inherent statistical improbability of collecting more than one litter sired by the same male in a sample of 13 litters, we were unable to document polygyny. Nevertheless, field observations of territory size and overlap (Brown, 1969; Wolton & Flowerdew, 2006) strongly suggest that males are polygynous. Thus, if the documentation of polygyny by Bartmann & Gerlach (2001) is considered in combination with field behavioural observations, it is likely that the mating system of *A. sylvaticus* is predominantly promiscuous.

Multiple paternity may occur frequently in *A. sylvaticus* because males are unable to guard females successfully after mating due to long and unpredictable oestrus cycles, which can range from 1.3 to 15.8 days (Jonsson & Silverin, 1997). Remote mate guarding using a copulatory plug is thought to be a strategy utilized by many mammals (especially rodents) for paternity assurance. However, the observed frequent multiple paternity in *A. sylvaticus* argues that copulatory plugs prove to be ineffective or inefficient barriers to insemination by other males, especially as it appears to be characterized by roughly equal fertilization success between sires. Spines on the glans penis of muroid rodents, which includes the genus *Apodemus*, may have evolved to aid in plug removal and would help to explain their apparent inefficiency for chastity enforcement (Milligan, 1979; Dewsbury, 1984).

As sperm plugs seem to be ineffective in preventing further copulations, it seems likely that other post-copulatory mechanisms may exist to maximize the success of sperm by each copulating male. The relative testis to body-mass ratio of around 5% in male *A. sylvaticus* is higher than for almost all other rodent species and suggests that sperm competition is a prominent feature of the reproductive behaviour of this species (Gage & Freckleton, 2002; Moore *et al.*, 2002). In addition, the spermatozoa of *A. sylvaticus* display a unique postcopulatory morphological transformation, which results in cooperation among sperm to form aggregations or 'trains' composed of hundreds or even thousands of cells within 1–5 min of ejaculation (Moore

et al., 2002). 'Trains' were found to increase the sperm motility significantly. Thus, it may be hypothesized that 'trains' form to ensure that sperm progress rapidly through the reproductive tract of the female, ensuring that the unfertilized eggs are reached before the sperm of rival males. If these trains function to confer increased motility in response to sperm competition, it would imply that the period between copulation of a single female by multiple males must often be very short. Moreover, this function would imply that sperm trains should only form between sperm from the same male.

With the possible exception of obtaining nutrition from multiple sperm plugs, it is unlikely that females benefit directly (e.g. through additional paternal care of offspring) from copulating with several males. In addition, as the paternity of litters examined in this study appears not to be biased towards one outright, highly competitive male, the advantage of multiple paternity in terms of female fitness is not obvious. However, genetic benefits may play a significant role in the evolution of promiscuous reproductive behaviour and multiple paternity in *A. sylvaticus*. Polyandry resulting in increased within-litter offspring diversity may enhance the fitness of the mother by decreasing sibling competition. Furthermore, this may enhance the survival probability of the offspring across variable habitat types and environmental conditions, effectively serving as an insurance against environmental uncertainty (Loman, Madsen & Håkansson, 1988; Foerster *et al*., 2003; Fisher *et al*., 2006). This may be particularly important in *A. sylvaticus* occupying the northern latitudes of the species range, where populations are characterized by annual cycles of abundance during which numbers may fluctuate by several orders of magnitude. Hence, polyandry during periods of low numbers may maximize the genetic variation within litters, and thus maximize the chance that some offspring survive.

This study demonstrates that the production of single litters sired by multiple males, as a result of polyandry, is common in females in natural populations of *A. sylvaticus*. This polyandrous mating system is further supported by relatively large testes size and the formation of post-copulatory sperm trains, suggesting that male *A. sylvaticus* are adapted for sperm competition. The outcome of this mating system in *A. sylvaticus* appears to be the production of litters with increased allelic diversity, with a nearly equal genetic representation of successful males. If this increased allelic diversity can indeed be linked to enhanced likelihood of survival and offspring of a higher reproductive value, as suggested by Foerster *et al*. (2003), the evolution of polyandry, and more than likely promiscuity, may play a fundamental role in the maintenance of genetic diversity within *A. sylvaticus* populations. A fundamental question that remains to be addressed is related to the possible variation of mating strategy within this species as proposed by Montgomery & Gurnell (1985). The authors have suggested that a shift from monogamy to polygyny may occur between the commencement of the breeding season and the end in *A. sylvaticus*. This may be associated with the annual fluctuations in abundance common to members of this species occupying the northern latitudes of its range. This relevant question could be addressed with a more comprehensive sampling regime using the same approach used in this study.

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