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Multiple paternity does not depend on male genetic diversity

Kerstin E. Thonhauser^{a,*}, Shirley Raveh^{a,b}, Dustin J. Penn^a^a Konrad Lorenz Institute of Ethology, Department of Integrative Biology and Evolution, University of Veterinary Medicine, Vienna, Austria^b Department of Environmental Sciences, Zoology and Evolution, University of Basel, Basel, Switzerland

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Polyandry is common in many species and it has been suggested that females engage in multiple mating to increase the genetic diversity of their offspring (genetic diversity hypothesis). Multiple paternity occurs in 30% of litters in wild populations of house mice, *Mus musculus musculus*, and multiple-sired litters are genetically more diverse than single-sired ones. Here, we aimed to test whether female house mice produce multiple-sired litters when they have the opportunity to produce genetically diverse litters. We assessed the rates of multiple paternity when females could choose to mate with two males that were genetically dissimilar to each other (i.e. nonsiblings and MHC dissimilar) compared with when females could choose to mate with two males that were genetically similar to each other (i.e. siblings and shared MHC alleles). Multiple mating may depend upon a female's own condition, and, therefore, we also tested whether inbred (from full-sibling matings) females were more likely to produce multiple-sired progeny than outbred controls. Overall we found that 29% of litters had multiple sires, but we found no evidence that females were more likely to produce multiple-sired litters when they had the opportunity to mate with genetically dissimilar males compared with controls, regardless of whether females were inbred or outbred. Thus, our findings do not support the idea that female mice increase multiple paternity when they have the opportunity to increase the genetic diversity of their offspring, as expected from the genetic diversity hypothesis.

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Polyandry or multiple mating is common in many species and females can increase the number or quality of offspring produced when mating with multiple males (Firman and Simmons, 2008b; Fisher, Double, Blomberg, Jennions, & Cockburn, 2006; García-González & Simmons, 2005; Hoogland, 1998; Madsen, Shine, Loman, & Håkansson, 1992; Tregenza & Wedell, 1998). Many hypotheses have been proposed to explain how females gain benefits from multiple mating, which include both direct and indirect, genetic benefits (Hosken & Stockley, 2003; Jennions & Petrie, 2000; Simmons, 2005). For example, polyandry could provide females with genetic benefits by increasing offspring genetic diversity, as this can have positive effects on offspring performance and survival (Yasui, 1998). Therefore, multiple mating may depend upon the genetic similarity of potential mates and whether polyandry will increase the diversity of their litters. Increasing evidence also indicates that females' mate choice can be dependent on their own quality or condition, but almost nothing is known about whether polyandry is a facultative behaviour that depends on these factors.

Our goals were to investigate these hypotheses in an experiment with wild-derived house mice, *Mus musculus musculus*, in which we allowed females to select their mates and we measured the rates of multiple paternity.

In house mice, *Mus musculus domesticus*, genetic paternity analyses reveal that multiple paternity is common in enclosure populations (Lindholm, Musolf, Weidt, & König, 2013; Montero, Teschke, & Tautz, 2013; Potts, Manning, & Wakeland, 1991; Stockley et al., 2013). In feral populations, the rate of multiple-sired litters is 25% on average but it is unclear why there is so much variation among populations (6–43%, Dean, Ardlie, & Nachman, 2006; Firman and Simmons, 2008a). One study found that the rate of multiple-sired litters increases with population density (Dean et al., 2006; but see Firman and Simmons, 2008a), which suggests that polyandry is a facultative behaviour depending upon the number of available males or the risk of infanticide. Females actively engage in multiple mating (Rolland, MacDonald, de Fraipont, & Berdoy, 2003) and when females can choose to mate with either one or two males, 46% of their litters have multiple sires (Thonhauser, Raveh, Hettyey, Beissmann, & Penn, 2013a). Females are not consistent in producing multiple-sired litters when the same female is tested twice with different males, suggesting that females change their behaviour depending upon

* Correspondence: K. E. Thonhauser, Konrad Lorenz Institute of Ethology, Street: Savoyenstrasse 1a, 1160 Vienna, Austria.

E-mail address: kerstin.thonhauser@gmx.at (K. E. Thonhauser).

their own age, condition or perhaps differences in the available mates.

Most studies on multiple mating have focused on the good genes and compatible genes hypotheses, whereas the genetic diversity hypothesis has received relatively little attention (but see [Cohas, Yoccoz, & Allainé, 2007](#); [McLeod & Marshall, 2009](#); [Schmoll, Schurr, Winkel, Epplen, & Lubjuhn, 2007](#)). Increased offspring genetic diversity (among offspring within litters) may increase litter survival if it reduces the risk of infectious diseases spreading between siblings. Studies on other species (social insects and birds) provide observational evidence that females gain fitness benefits by producing genetically diverse litters ([Dunn, Lifjeld, & Whittingham, 2009](#); [Liersch & Schmid-Hempel, 1998](#); [Seeley & Tarpay, 2007](#)). A study in bumblebees, *Bombus terrestris*, for example, showed that high-diversity colonies had fewer parasites and increased reproductive success compared with low-diversity colonies ([Baer & Schmid-Hempel, 1999](#)). In tree swallows, *Tachycineta bicolor*, the immune responses of nestlings from multiple-sired clutches were stronger compared with single-sired clutches ([Dunn et al., 2009](#)). Comparative studies on birds revealed that the degree of extrapair paternity increases with the species' genetic diversity ([Gohli et al., 2013](#); [Petrie, Doums, & Møller, 1998](#)). We recently found higher levels of genetic diversity within multiple- versus single-sired litters in wild house mice, *M. m. musculus* ([Thonhauser, Thoß, Musolf, Klaus, & Penn, 2013](#)); however, to our knowledge, it has never been experimentally tested whether females engage in multiple mating and increase the rate of multiple-sired litters when they have the opportunity to increase the genetic diversity of their offspring. In fact, no study on polyandry to our knowledge has ever manipulated the genetic differences of potential mates.

Similarly, multiple mating has been suggested to function as a mechanism to increase offspring diversity at the genes of the major histocompatibility complex (MHC; [Bollmer, Dunn, Freeman-Gallant, & Whittingham, 2012](#); [Evans, Dionne, Miller, & Bernatchez, 2012](#)). For example, in the Seychelles warbler, *Acrocephalus sechellensis*, females were more likely to seek extrapair copulations when their social mate had low individual MHC diversity ([Richardson, Komdeur, Burke, & von Schantz, 2005](#)). Similarly, a study with house mice suggested that females seek extrapair matings with males that are more disparate at the MHC than their social mate ([Potts et al., 1991](#)). Therefore, we additionally tested whether the frequency of multiple paternity is higher when females have the opportunity to increase the MHC diversity of their offspring. MHC genes are good candidates to assess the genetic benefits of mate choice, as they are highly polymorphic, they control immune resistance to infectious diseases and they influence disassortative mating preferences in mice ([Penn & Potts, 1999](#); [Penn, 2002](#)). As MHC genes control resistance to pathogens and parasites ([Apanius, Penn, Slev, Ruff, & Potts, 1997](#)), increasing offspring MHC diversity is potentially advantageous for the survival of litters, as it might allow a broader range of pathogens to be detected and combated. It has also been suggested that promiscuity drives increased MHC diversity ([MacManes & Lacey, 2012](#)). However, it has never been experimentally tested whether increased MHC diversity of potential mates elevates rates of multiple paternity.

Multiple mating may also depend upon the females' own condition, such as their age, body mass or genetic quality (e.g. inbreeding status). Increasing evidence indicates that female mate sampling and mate preferences are dependent on the females' condition ([Burley & Foster, 2006](#); [Cotton, Small, & Pomiankowski, 2006](#); [Hunt, Brooks, & Jennions, 2005](#)), including their infection ([Buchholz, 2004](#)) and inbreeding status ([Mazzi, Künzler, Largiadèr, & Bakker, 2004](#); [Michalczyk et al., 2011](#)). For example, house mice females prefer the odour of outbred versus inbred males and this

preference is more pronounced in inbred versus outbred females ([Ilmonen, Stundner, Thoß, & Penn, 2009](#)). Moreover, a study in the red flour beetle, *Tribolium castaneum*, showed that females with an inbreeding history had higher rates of polyandry than outbred controls and that polyandry effectively doubled previously inbred females' reproductive success ([Michalczyk et al., 2011](#)). Therefore, our goal was to test whether females' inbreeding status affects the rate of multiple paternity, as this hypothesis has never been tested in a vertebrate species to our knowledge.

We conducted an experiment with wild-derived house mice (F2 from wild-caught *M. m. musculus*), in which we allowed females to choose to mate between two males. All males were unrelated to the females, but the males were experimentally selected to be either genetically similar (brothers with identical MHC haplotype) or dissimilar (unrelated with different MHC haplotype) to each other. We chose to manipulate MHC sharing as MHC is the only locus to our knowledge that is highly polymorphic, influences individual odour and mate choice and simultaneously can confer potential fitness benefits to offspring (i.e. MHC controls immune resistance to pathogens and parasites). We expected that the rate of multiple paternity (using genetic paternity analyses) would be higher when potential mates are genetically dissimilar as females would have the opportunity to increase the genetic diversity among offspring as expected from the genetic diversity hypothesis. To test whether female inbreeding status affects the likelihood of producing multiple-sired litters, the females were experimentally inbred (parents were full siblings) or outbred (parents from different families). We had no prediction for how inbreeding might affect multiple paternity because poor condition may increase ([Michalczyk et al., 2011](#)) or decrease ([Huchard et al., 2012](#)) multiple mating.

METHODS

Experimental Animals

All experimental animals were second-generation descendants of wild-trapped house mice, *M. m. musculus*, in Vienna (48°12'38"N; 16°16'54"E). Progenitor mice were trapped at 14 different locations within a 500 m radius and crossed between trapping sites. Before we assigned the breeding pairs, we genotyped mice to exclude individuals carrying *t* alleles, since these alleles cause meiotic drive and may influence females' mating preferences ([Lenington, 1991](#)). F1 mice were arranged in two breeding lines to generate both inbred and outbred mice. Inbred mice resulted from one generation of brother–sister matings and outbred mice resulted from matings of nonsiblings. One generation of full-sib mating has been shown to cause significant inbreeding depression ([Meagher, Penn, & Potts, 2000](#)) and it also influences female odour preferences for males ([Ilmonen et al., 2009](#)). Experimental mice were weaned at the age of 21 ± 1 days and then housed individually in standard mouse cages (type II cages, 26.5 × 20.5 cm and 14 cm high) containing woodchip bedding (ABEDD), wood shavings and a nestbox. Food (Altromin rodent diet 1324) and water were provided ad libitum. At weaning all animals received an ear punch which was necessary for individual identification and tissues were stored at –20 °C for genetic analyses. A standard 12:12 h light cycle was maintained and the temperature was 22 ± 2 °C. All animals were sexually naïve and between 10 and 22 weeks of age when the experiment started.

Mate Choice Assay

Each female could choose to mate with one or two males and these males were either siblings (genetically similar) or unrelated (genetically dissimilar) to each other. Males were located in two

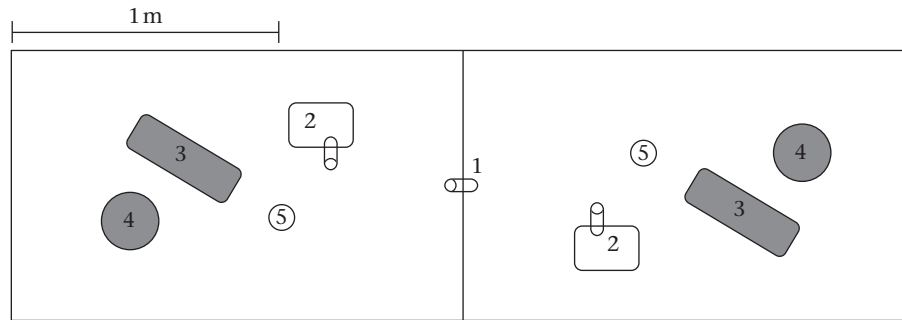


Figure 1. Experimental set-up of neighbouring males' enclosures. Females could move between male enclosures through a connection tube (1) and could escape male harassment in a separate shelter cage (2). Males could not pass through the tubes as they wore collars. Each enclosure contained a shelter box (3), a nestbox (4) and a water dispenser (5).

Table 1

Example of MHC genotypes (class II $A\alpha$ and $E\beta$ locus) in genetically similar versus dissimilar males in relation to the tested females

Treatment	δ Relatedness	δ MHC genotype	OB δ MHC genotype	IB δ MHC genotype	No. of shared alleles	No. of new alleles			
Genetically similar	Brothers	ab	cd	ag	ch	aa	cc	2	2
		ab	cd						
Genetically dissimilar	Unrelated	ab	cd	ag/be	ch/df	aa	cc	2	2–4
		ae	cf						

Regardless of female inbreeding status, females always shared the same number of alleles with potential mating partners, both when males were genetically similar and when they were dissimilar to each other. However, in the genetically dissimilar treatment, the number of potential new alleles females could gain for their litters from multiple mating was at maximum doubled. OB = outbred; IB = inbred.

neighbouring enclosures (each measuring 1×1.7 m and 0.8 m high) separated by an opaque plastic divider (Fig. 1). Each enclosure contained one nestbox and one shelter both equipped with bedding and nesting material, one water dispenser and randomly distributed food (Altromin rodent diet 1324). Males were introduced into their enclosures 1 day before females to enable them to establish a territory. Simultaneously to male introduction, 20 μ l of female urine (pool of seven females collected on 5 consecutive days) were deposited between the nestbox and the shelter to sexually stimulate males. Females could move freely between the males' enclosures through a small passage tube at the base of the divider (3 cm diameter). Males were prevented from entering the passage by small collars to ensure that both established their own territory and to avoid injuries from fighting. At the base of the divider, four mesh-sealed holes (4 cm diameter) allowed visual and olfactory contact between males. To prevent male harassment, we provided a cage within each male's enclosure (including separate water and food), which was accessible only to females through another passage tube.

Genetic Similarity

Experimental males were selected in matched pairs according to their degree of relatedness and MHC genotypes (see *MHC Genotyping*). 'Genetically similar' males were full siblings that shared identical MHC genotypes, whereas genetically dissimilar pairs were unrelated males that only shared one allele at each MHC locus (see Table 1). In total we had 24 pairs of males, 12 genetically similar and 12 genetically dissimilar. We tested a total of 48 females (thus male pairs were used twice) 24 of which were inbred; all other experimental animals were outbred. The genetic background of male pairs (similar or dissimilar) was balanced for female inbreeding status (inbred or outbred). Inbred and outbred females always shared the same number of alleles with both males they were tested with independent of whether the males were genetically similar or dissimilar. However, the number of new alleles females could potentially obtain for their litter by mating with two

genetically dissimilar males is at maximum twice what they gain from mating with two genetically similar males (see Table 1). Individual MHC genotypes of females and their potential mates in the different treatment groups are listed in Tables 2–5.

Experimental Procedure

Females were sexually naïve and always unrelated and unfamiliar with the males with which they were tested. Males were also

Table 2

Individual MHC genotypes of outbred females that could choose between genetically dissimilar males

δ Genotypes				δ Genotypes				Reproductive outcome
$A\alpha$ locus		$E\beta$ locus		$A\alpha$ locus		$E\beta$ locus		
430	453	328	340	445	453	319	328	No reproduction
				430	445	319	340	
430	453	328	340	445	453	319	328	No reproduction
				430	445	319	340	
430	445	319	340	434	445	319	338	Single sire
				445	453	319	328	
430	445	319	340	445	453	319	328	Single sire
				430	453	328	340	
430	445	319	340	430	453	328	340	Single sire
				445	453	319	328	
430	445	319	340	445	453	319	328	Single sire
				430	453	328	340	
430	453	328	340	445	453	319	328	Single sire
				430	445	319	340	
440	445	319	332	430	445	319	340	Single sire
				445	453	319	328	
445	453	319	328	430	445	319	340	Single sire
				430	453	328	340	
445	453	319	328	430	445	319	340	Single sire
				430	453	328	340	
430	445	319	340	430	453	328	340	Multiple sires
				445	453	319	328	
430	453	328	340	445	453	319	328	Multiple sires
				430	445	319	340	

Table 3
Individual MHC genotypes of inbred females that could choose between genetically dissimilar males

♀ Genotypes				♂ Genotypes				Reproductive outcome
A α locus		E β locus		A α locus		E β locus		
430	430	340	340	430	445	319	340	No reproduction
				430	453	328	340	
445	445	319	319	430	445	319	340	No reproduction
				445	453	319	328	
430	430	340	340	430	445	319	340	Single sire
				430	453	328	340	
430	430	340	340	430	445	319	340	Single sire
				430	453	328	340	
430	430	340	340	430	445	319	340	Single sire
				430	453	328	340	
445	445	319	319	445	453	319	328	Single sire
				430	445	319	340	
445	445	319	319	434	445	319	338	Single sire
				445	453	319	328	
430	430	340	340	430	445	319	340	Multiple sires
				430	453	328	340	
445	445	319	319	430	445	319	340	Multiple sires
				445	453	319	328	
445	445	319	319	445	453	319	328	Multiple sires
				430	445	319	340	
445	445	319	319	445	453	319	328	Multiple sires
				430	445	319	340	

sexually inexperienced in their first trial. We measured individual body mass (g) the day we introduced the animals into the enclosures to assess whether female mate choice is related to their own or the male's body mass. The mice were allowed to interact in the experiment for 14 days before all animals were returned to the colony. Male collars were removed immediately and females were placed individually in type IIL mouse cages (36.5 × 20.5 cm and 14 cm high) to give birth under controlled conditions.

Table 4
Individual MHC genotypes of outbred females that could choose between genetically similar males

♀ Genotypes				♂ Genotypes				Reproductive outcome
A α locus		E β locus		A α locus		E β locus		
430	453	328	340	430	445	319	340	No reproduction
				430	445	319	340	
445	453	319	328	434	453	328	338	No reproduction
				434	453	328	338	
445	453	319	328	434	445	319	338	No reproduction
				434	445	319	338	
445	453	319	328	430	445	319	340	No reproduction
				430	445	319	340	
430	453	328	340	430	445	319	340	Single sire
				430	445	319	340	
440	445	319	332	430	445	319	340	Single sire
				430	445	319	340	
440	445	319	332	430	445	319	340	Single sire
				430	445	319	340	
445	453	319	328	430	445	319	340	Single sire
				430	445	319	340	
445	453	319	328	434	453	328	338	Single sire
				434	453	328	338	
430	445	319	340	434	445	319	338	Multiple sires
				434	445	319	338	
434	453	328	338	445	453	319	328	Multiple sires
				445	453	319	328	
440	445	319	332	430	445	319	340	Multiple sires
				430	445	319	340	

Table 5
Individual MHC genotypes of inbred females that could choose between genetically similar males

♀ Genotypes				♂ Genotypes				Reproductive outcome
A α locus		E β locus		A α locus		E β locus		
430	430	340	340	430	445	319	340	No reproduction
				430	445	319	340	
445	445	319	319	440	445	319	332	No reproduction
				440	445	319	332	
430	430	340	340	430	445	319	340	Single sire
				430	445	319	340	
445	445	319	319	430	445	319	340	Single sire
				430	445	319	340	
445	445	319	319	430	445	319	340	Single sire
				430	445	319	340	
445	445	319	319	445	453	319	328	Single sire
				445	453	319	328	
445	445	319	319	430	445	319	340	Single sire
				430	445	319	340	
445	445	319	319	430	445	319	340	Single sire
				430	445	319	340	
445	445	319	319	430	445	319	340	Single sire
				430	445	319	340	
445	445	319	319	430	445	319	340	Single sire
				430	445	319	340	
445	445	319	319	430	445	319	340	Single sire
				430	445	319	340	
445	445	319	319	434	445	319	338	Multiple sires
				434	445	319	338	
445	445	319	319	434	445	319	338	Multiple sires
				434	445	319	338	

MHC Genotyping

For MHC genotyping two class II MHC loci A α and E β on mouse chromosome 17 were screened with single strand conformation polymorphism (SSCP). Therefore, genomic DNA was extracted from frozen ear punch samples using a proteinase K/isopropanol protocol (Sambrook, Fritsch, & Maniatis, 1989). A two-step PCR (Bio-metra-T1 thermocycler) was used to amplify the products. The first denaturation step started at 94 °C for 2 min followed by 10 cycles of denaturation at 94 °C for 30 s, annealing at 59 °C for 30 s and extension at 72 °C for 1 min. The second step was followed by 25 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s, extension at 72 °C for 1 min and a final extension at 72 °C for 10 min. The PCR for E β differed in the two annealing temperatures which were at 53 °C and 48 °C. The PCR reaction contained 1 μ l DNA (100 ng/ μ l), 1 μ l 10 \times B-buffer, 1 μ l dNTPs (2 mM), 1.5 μ l MgCl₂ (25 mM), 0.2 μ l Taq polymerase (1 U/ μ l), 0.3 μ l of both A α forward and reverse primer and 0.5 μ l of both E β forward and reverse primer (modified after [Schad, Sommer, & Ganzhorn, 2004](#)). Nucleotide sequences of primers for the two loci were: A α -Forward: 5'-ACCATTGGTAGCTGGGGTG-3'; A α -Reverse: 5'-CTAAATC-CATCAGCCGACC-3'; E β -Forward: 5'-GAGTGTCAITTTCTACAACGGGACG-3'; E β -Reverse: 5'-GATCTCATAGTTGTGTCTGCA-3'. Reaction volume was 10 μ l and ddH₂O was added to reach the desired volume.

For the CE-SSCP analyses 1 μ l of the diluted (1:60) PCR product was added to 9 μ l of loading dye mix (8.5 μ l Hi-DITM formamide, 0.5 μ l GeneScan ROX 350 standard, Applied Biosystems, Foster City, CA, U.S.A.). The reaction was denatured at 95 °C for 5 min and immediately chilled on ice before being analysed by capillary electrophoresis on an ABI PRISM 3130xl automated DNA Sequencer (Applied Biosystems). The CE-SSCP polymer consisted of 5% conformational analysis polymer (CAP) which is made of 9% CAP, 10 \times genetic analyze buffer, 100% glycerol and HPLC-water and a 1 \times ABI running buffer was used. The separation of the allelic variants was achieved by using the following running conditions: injection voltage at 1.2 kV, injection time of 18 s, run voltage at 12 kV for

40 min, run temperature at 22 °C. The retention times of the allelic variants were identified relative to the ROX 350 standard. GeneMapper software package 4.05 from Applied Biosystems was used to analyse the SSCP data.

Genetic Paternity Analyses

For genetic paternity analyses DNA was extracted from frozen ear punch samples using a proteinase K/isopropanol protocol (Sambrook et al., 1989). Individuals were genotyped at a minimum of six and a maximum of 24 microsatellite loci (D9Mit34, D9Mit135, D10Mit20, D11Mit150, D17Saha, D17Mit28, D17Mit21, D1Mit404, D1Mit456, D2Mit252, D2Mit380, D5Mit25, D6Mit138, D7Mit227, D15Mit16, D19Mit39, D4Mit 17, D4Mit 164, D4Mit 139, D4Mit 243, D4Mit 288, D4Mit 217, D4Mit 241, D4Nds6, see Mouse Microsatellite Data Base of Japan, <http://www.shigen.nig.ac.jp/mouse/mmdbj/top.jsp>) which were arranged in multiplex PCRs using a Multiplex-PCR MasterMix (Qiagen Multiplex PCR kit). Amplification mixes were subjected to a denaturation step at 94 °C for 15 min followed by 30 cycles at 94 °C for 30 s, 55 °C for 90 s and 72 °C for 60 s, followed by an elongation step at 72 °C for 10 min. Amplification products were analysed using an automated sequencer (Beckman Coulter CEQ 8000). Allele scoring was done with Beckman Coulter CEQ 8000 System software, and allele sizes were determined with SLS+400 as size standard. Paternity assignment was made by complete exclusion. Paternity results were confirmed using CER-VUS 3.0.3 (Kalinowski, Taper, & Marschall, 2007; Marshall, Slate, Kruuk, & Pemberton, 1998). The program assigned paternity with a 95–99% confidence (given dam–sire–offspring relationship) based on male allele differences.

Statistical Analyses

We applied a *t* test to test for differences in litter size between inbred and outbred females and a chi-square test to assess any difference in the likelihood of reproduction between inbred and outbred females. To test for the effect of male genetic similarity and female inbreeding status on multiple paternity, we applied a generalized linear mixed-effects model (GLMM) with a binomial distribution and a logit link function. Paternity (single or multiple) was included as the dependent variable, male genetic similarity (similar or dissimilar) and female inbreeding status (inbred or outbred) as fixed factors and female body mass and the two males' body mass difference as a covariate. As males were used twice in the experiment, we included male pair nested in trial as a random

factor to control for nonindependence of male pairs and increased experience over trials. We verified that model assumptions were fulfilled. We applied a backward stepwise removal procedure (Grafen & Hails, 2002) to avoid problems from including nonsignificant terms (Engqvist, 2005) and the removed variables were re-entered one by one to the final model to obtain relevant statistics. Statistical analyses were performed using 'R' version 2.14.1 (R Development Core Team, 2011). We implemented the generalized mixed-effects model using the 'lmer' function in the 'lme4' package.

Ethical Note

This study was discussed and approved by the institutional ethics committee of the University of Veterinary Medicine, Vienna, in accordance with Good Scientific Practice guidelines and national legislation. Trapping of the founder individuals was additionally approved by the MA 22 (Municipality for Environment and Conservation of Vienna). For detailed information on animal trapping, tissue sampling and male collaring see Thonhauser, Raveh, Hettyey, Beissmann, and Penn (2013b). Collars were used to ensure that males kept to their home range, thereby avoiding aggressive encounters between males and to protect females from male harassment and coercion by blocking males' entrance into females' cages (see Mate Choice Assay). After the experiment, all individuals were reintegrated and kept in the colony. Experimental offspring were weaned at the age of 21 ± 1 days before they were also integrated into the colony.

RESULTS

In total 78% (38/48) of all females successfully gave birth to an average litter size of 6.5 ± 2 SD. There was no difference in litter size ($t_{35} = 0.319$, $P = 0.751$) or the likelihood of reproduction between inbred and outbred females (outbred = 18 litters, inbred = 20 litters; $\chi^2 = 0.105$, $P = 0.746$). Overall, the rate of multiple-sired litters was 29% (11/38); however, multiple paternity was not influenced by male genetic similarity (GLMM: $z = -0.120$, $\beta = -0.091$, $SE = 0.762$, $N = 38$, $P = 0.899$; Fig. 2a) or female inbreeding status (GLMM: $z = 0.099$, $\beta = 0.074$, $SE = 0.749$, $N = 38$, $P = 0.921$; Fig. 2b). Also, none of the covariates in our model explained multiple paternity (female body mass GLMM: $z = -0.958$, $\beta = -0.183$, $SE = 0.191$, $N = 38$, $P = 0.338$; body mass differences between males GLMM: $z = 0.925$, $\beta = 0.164$, $SE = 0.177$, $N = 38$, $P = 0.355$).

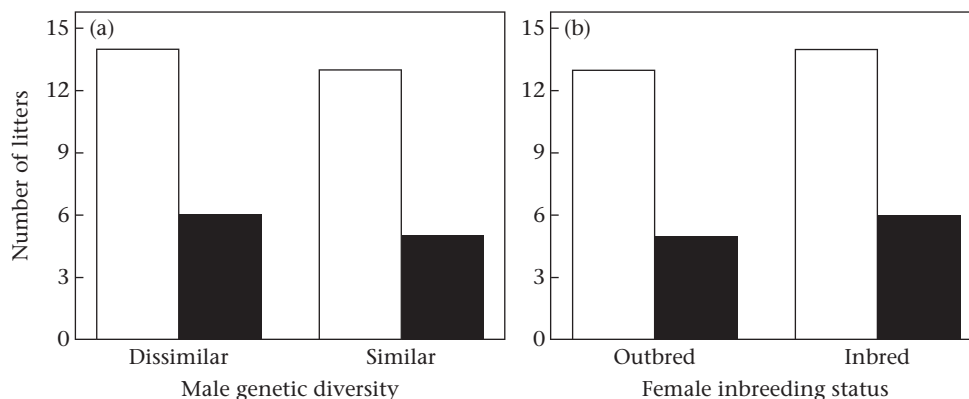


Figure 2. (a) Multiple paternity in relation to male genetic diversity. Number of litters with single (white) and multiple (black) sires when potential mating partners were genetically similar or dissimilar to each other. (b) Multiple paternity in relation to female inbreeding status. Number of litters with single (white) and multiple (black) sires in inbred and outbred females.

DISCUSSION

We found no evidence that genetic similarity of females' potential mates (MHC-identical siblings versus MHC-dissimilar non-siblings) influenced the rate of multiple paternity. We also found no evidence that female inbreeding status affected the rate of multiple paternity. We analysed 38 litters, which is not a large sample size, but we detected no trends in our results to suggest that a larger sample size might reveal differences (see Fig. 2a, b). Thus, we can rule out the possibility that these factors have strong effects on multiple paternity, and if there are small effects, then a much larger sample size would be needed to detect a difference. We found that 29% of the litters had multiple sires, which is identical to wild populations of *M. m. musculus* (Thonhauser et al., 2013) and similar to feral *M. m. domesticus* populations in the U.S.A. and Australia (Dean et al., 2006; Firman and Simmons, 2008a). This finding indicates that our experiment did not artificially alter the rate of multiple paternity, and, moreover, it confirms that females show multiple mating when they can select their mates rather than because of sexual coercion.

Our negative results could be caused by an inability of females to discriminate male genetic similarity; however, this explanation is unlikely since there is considerable evidence that relatedness and genetic similarity influence variation in individual odour in mice (see the appendix in Thom & Hurst, 2004). Moreover, in our study we manipulated males' MHC similarity, and several studies have shown that MHC influences odour in mice and rats (Penn & Potts, 1998; Yamazaki et al., 1979) and other species. It has been suggested that MHC effects on odour may not be as salient in wild, heterozygous mice as found in congenic strains of laboratory mice (in which background genes are controlled; Penn & Potts, 1998). Some studies have suggested that major urinary proteins (MUPs), which have been shown to control kin recognition and inbreeding avoidance in house mice (Cheetham et al., 2007), have an even stronger influence on odour variation than MHC genes. If so, we would have more reason to expect that females are able to detect differences in male genetic similarity and assuming that brothers are more likely to share MUPs than nonsiblings, variation in MUPs cannot explain our negative results. The level of genetic differences in MHC, as well as in MUPs, in our experiment may not have been pronounced enough to affect females' mating behaviour; however, males' genetic diversity in our study was comparable to that in natural conditions (where polyandry increases the genetic diversity of litters; Thonhauser et al., 2013). Thus, our findings suggest that although female mice can assess the genetic similarity of males by their scent, they do not increase offspring diversity when provided with the opportunity to mate multiply, at least under our experimental conditions. Therefore, our results raise the question of whether increasing genetic diversity of offspring increases litter survival or provides other fitness benefits, as predicted by the genetic diversity hypothesis (Yasui, 1998).

Multiple mating may depend upon females' condition, but we found no evidence that female inbreeding status influenced that rate of multiple paternity, regardless of the males' genetic similarity. A previous study with flour beetles found that inbred females (generated from experimentally bottlenecked populations) were more likely to engage in multiple mating than outbred controls, which enabled them to reduce the negative fitness consequences of inbreeding (Michalczyk et al., 2011). Since the inbred females in the study with flour beetles were generated by eight generations of full-sib matings, it is possible that one generation of inbreeding is not sufficient to increase female promiscuity. However, if eight generations of close inbreeding are necessary to increase female promiscuity, then the relevance of this study is rather limited. Another study on grey mouse lemurs, *Microcebus murinus*, found

that heavier females were more likely to mate with multiple males than lighter females, suggesting that females in poor condition cannot afford the costs associated with multiple mating (Huchard et al., 2012). However, the rate of multiple-sired litters was not related to female body mass in our study.

Finally, female multiple mating may not be a facultative behaviour, as we assumed, and the variation in multiple mating might be due to genetic polymorphism. However, it is unlikely that multiple mating is due to a genetic difference since we previously found no consistency in the rate of multiple paternity within individual females when repeatedly tested (Thonhauser et al., 2013a).

In summary, we did not find support for the hypothesis that females are more likely to mate with multiple males when they have the opportunity to increase the genetic diversity of their progeny. Although previous work shows higher levels of genetic diversity within multiple- than in single-sired litters (Thonhauser et al., 2013), female mice did not have more multiple-sired litters when they had the opportunity to increase the genetic diversity of their progeny. Moreover, we found no evidence that inbred females were more likely to give birth to multiple-sired litters than outbred females, contrary to experimental findings with beetles (Michalczyk et al., 2011), regardless of the genetic diversity of the available males.

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