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Paternal imprinting of mating preferences between natural populations of house mice (*Mus musculus domesticus*)

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Abstract

The evolutionary divergence of cues for mate recognition can contribute to early stages of population separation. We compare here two allopatric populations of house mice (*Mus musculus domesticus*) that have become separated about 3000 years ago. We have used paternity assignments in semi-natural environments to study the degree of mutual mate recognition according to population origin under conditions of free choice and overlapping generations. Our results provide insights into the divergence of mating cues, but also for the mating system of house mice. We find frequent multiple mating, occurrence of inbreeding and formation of extended family groups. In addition, many animals show strong mate fidelity, that is, frequent choice of the same mating partners in successive breeding cycles, indicating a role for familiarity in mating preference. With respect to population divergence, we find evidence for assortative mating, but only under conditions where the animals had time to familiarize themselves with mating partners from their own population. Most interestingly, the first-generation offspring born in the enclosure showed a specific mating pattern. Although matings between animals of hybrid population origin with animals of pure population origin should have occurred with equal frequency with respect to matching the paternal or maternal origin, paternal matching with mates from their own populations occurred much more often. Our findings suggest that paternally imprinted cues play a role in mate recognition between mice and that the cues evolve fast, such that animals of populations that are separated since not more than 3000 years can differentially recognize them.

Keywords: familiarity, imprinting, inbreeding, mate choice, social organization

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Introduction

Mating patterns are intrinsically linked to evolutionary processes. Assortative mating can lead to population splits (Dieckmann *et al.* 2004; The Marie Curie Speciation Network 2012), while disassortative mating can be required to avoid inbreeding depression (Pusey & Wolf 1996). Selective mate recognition is also thought to be beneficial for optimizing polymorphisms in immune defence genes (Penn & Potts 1999; Milinski 2006). House mice have long been a favourite subject for the analysis of mating patterns and the identification of associated genes. Mate recognition between individual mice is so far thought to be predominantly driven by scent communication (Hurst 2009). Products of highly polymorphic gene families have been implicated in this, including MHC peptides (Penn & Potts 1999; Milinski 2006) and the major urinary proteins (MUPs) (Thom *et al.* 2008; Roberts *et al.* 2010). But there is now also increasing evidence that acoustic communication in the ultrasonic range may be relevant for mate recognition (Hammerschmidt *et al.* 2009; Musolf *et al.* 2010) and signalling of individuality (Hoffmann *et al.* 2012) as well.

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Less attention has so far been paid to the question of how mating patterns diverge between populations and subspecies of house mice. A number of studies have addressed this indirectly by studying the ability of odour discrimination (Cox 1984, 1989) or the degree of gene flow between demes of natural populations (Baker 1981a,b; Singleton & Hay 1983). But these studies have mostly used very recently established populations in the United States or Australia where house mice have arrived only a few hundred years ago, that is, the respective populations are genetically still rather similar to each other. Other studies have investigated mutual recognition patterns between populations coming from different subspecies (*M. m. domesticus* and *M. m. musculus*) (Ganem & Searle 1996; Munclinger & Frynta 1997, 2000; Smadja & Ganem 2002, 2008; Ganem *et al.* 2008), but these are separated since such long times that postzygotic isolation mechanisms are already evident among them (Britton-Davidian *et al.* 2005).

We focus here on mice from two natural populations of *M. m. domesticus*: one from Germany (G) and one from France (F). These are derived from animals that have colonized Western Europe about 3000 years ago (Cucchi *et al.* 2005). They are molecularly well differentiated (Ihle *et al.* 2006; Teschke *et al.* 2008; Staubach *et al.* 2012), that is, have most likely split soon after the colonization. Hence, they are separated since up to 18 000 generations (assuming three generations per year and divergence in both lineages) and represent a typical case of initial population divergence under allopatry. We have used descendants of wild-caught mice from these populations in semi-natural environments to study the degree of mutual mate recognition according to population origin. Comparable enclosure studies have been previously used for studying various aspects of individual recognition, social organization and population dynamics in mice (Oakeshot 1974; Manning *et al.* 1992; Lenington *et al.* 1994; Meagher *et al.* 2000; Sherborne *et al.* 2007; Manser *et al.* 2011) but not in the context of population divergence questions.

In our study, mice were allowed to reproduce for up to three generations in the enclosures, nest occupation was monitored, and paternities were analysed by molecular typing approximately one thousand offspring. Successful matings were then inferred from the paternity analyses. We find complex mating patterns, including multiple mating, inbreeding in families and mate fidelity. Most interestingly, we find that hybrid animals between the populations mate preferentially with animals that have the same paternal population origin. Our results imply that a paternally provided component is part of the mate recognition system among mice.

Methods

Population origin of mice

The parental mice of the two populations chosen for our semi-natural enclosures were caught in the wild in Southern France (F) and Western Germany (G), whereby the sampling scheme took care to obtain a representative set of mice from the respective populations (Ihle *et al.* 2006). They were kept for 4–6 generations in the laboratory under a rotating outbreeding design (HAN rotation system – Rapp 1972) with 10 unrelated starting pairs. This design ensures a maximum degree of outbreeding (Nomura & Yonezawa 1996), that is, the mice put into the enclosures can be considered as mostly unrelated to each other. Both populations belong to the subspecies *Mus musculus domesticus*. Phylogeographic and fossil analysis suggest that they are derived from a colonization wave of Western Europe starting about 3000 years ago (Cucchi *et al.* 2005). The colonization was not associated with a major bottleneck. Systematic molecular screening for selective sweeps has shown that up to 1% of the genes may have been subject to differential selection since separation of these populations (Teschke *et al.* 2008; Staubach *et al.* 2012), indicating that they are at an early stage of genetic population divergence.

Semi-natural environment

The semi-natural enclosure set-up was aimed to provide the mice an opportunity for establishing territories and building sheltered nests, while still having access to all animals in the room. It consisted of two parallel rooms (room A with 24 m^2 and room B with 18 m^2) with appropriate nesting boxes and other enrichment (Fig. 1). The larger room included 20 nest boxes and the smaller 14. Water and food (Altromin 1324) were provided *ad libitum*. The light/dark cycle was 12:12 hours, the ambient temperature 20–23°C, and the relative humidity 50–65% with onefold air-exchange per hour. Structural variation was provided by wooden walls (40 cm high), and plastic tubes and a dispersal tube with several entrances allowed mice to escape from the population enclosure into a connected cage system via a water-filled aquarium, according to the design suggested by Gerlach (1996). Both rooms were stocked with sexually mature animals (age between 20 and 52 weeks) from both populations at equal sex ratio and at an initial density of 1.6–1.7 mice/m^2. The founder animals were raised in cages without contact to the other sex before they were released to the rooms. They were individually tagged with passive glass transponders (Datamars and AEG). As we used offspring of wild-caught

Fig. 1 View into a room with the semi-natural environment. The red plastic cylinders served as nests; each has two exits (grey tubes). They are covered with a tile that can be easily removed for checking the nest. There are additional tubes for hiding, scattered throughout the room, as well as several feeding plates and water bottles. Wooden walls provide additional structure. In set-up I, the horizontal middle wall was closed for the first week and the released populations were on either side of the wall.

animals for stocking the rooms, with minimal prior interference on their genetic constitution (see above), we had also some t-haplotype alleles segregating in the rooms, which could potentially interfere with mating patterns (Lyon 2003; Carroll *et al.* 2004). We have typed all animals for t-haplotypes and assessed all statistical parameters also by omitting animals which carried t-haplotypes. This did not change the overall results, if anything, some patterns, in particular paternal matching, became even more significant (Montero 2010). Hence, to avoid biasing the analysis in an unknown direction, we decided to retain all animals in the analysis, irrespective of their t-haplotype status.

In the first round (set-up I – run for 5 months simultaneously in both rooms), we kept animals of the same population initially separated in two subsections in each room, allowing them to become acquainted with the new situation and their own population background. The sections were opened after 1 week, and mice could intermix freely. This initial phase was omitted in the second round (set-up II – run for 6.5 months simultaneously in both rooms), that is, animals from both populations had immediate contact to each other.

Animals born in the enclosures were also tagged with individual glass transponders, at the age of 8 weeks and a bodyweight of around 17 g. During the experiment, every second to third day around noon, the positions of mice were recorded with a handheld transponder reader (Datamars). During this procedure, all houses and tubes were checked for the presence of a transponder-tagged mouse.

Genotyping and paternity assignment

Tissue samples were taken from all animals, mostly either when they died during the experiment or at the end of the experiment when they were all killed. DNA was extracted by standard methods. For each DNA sample, up to 14 microsatellite loci (Table S1) were typed using the standard protocols of the QIAGEN Multiplex PCR Kit. Alleles were analysed using GeneMapper 4.0 (Applied Biosystems). Paternity assignments were performed using the program CERVUS 3.0 (Kalinowski *et al.* 2007). None of the loci showed a null allele frequency higher than 0.05 and could therefore be used for identity matching and parentage analysis. Allele frequencies of the 14 loci were determined for all four enclosures separately (including all animals in these analyses), and simulations for parentage assignment were run for 10 000 offspring previous to all parentage analyses, assuming 90% of possible parents sampled and typed with a minimum of seven loci. Prior to paternity assignment, birth dates of animals were determined by identity matching of individual genotypes with ear punch samples taken from 14- to 21-day-old pups during the experiment. Following this analysis, animals could also be assigned to the litters from which they came and received, together with littermates, a unique 'Litter-ID'. According to birth and death dates, animals were assigned as possible parents or offspring within the respective time period. Animals with uncertain birth dates were tested against all possible parents. All assignments were repeatedly checked for consistency between the genetic analysis and birth dates. Only animals with unequivocal assignment were used for the statistical analysis.

Overall mating pattern statistics

We devised a test to assess whether mating patterns differ significantly from completely random choices across all phases of the two set-ups. To obtain a null distribution for random mating patterns, we divided the total time that the animals spent in the set-ups into phases of 20 days (about one gestation period) and counted the presence of every sexually mature animal during the respective phase in the respective room. The null expectation was then derived by calculating the proportions of matings in the tested category that would have been created by random matching. The differences of expected to observed values were then tested in a one-sample *t*-test with a total of 34 phases considered (33df) across all phases and across the rooms in both set-ups. This allows taking care of the inevitable variances between the phases and the rooms and is therefore the most conservative test possible.

Data management

Data were managed using a self-constructed database in Microsoft Access 2002. This database includes all information about the individual mice: sex, birth and death dates, transponder numbers, physical conditions and weight taken during the monitoring procedure, the spatial data obtained during locality check with the transponder reader, all genotype and origin information, the outcome of the parentage analysis, their assignment to a certain litter, as well as information on sample storage after the end of the study. An excerpt of this database containing all relevant information for the points discussed here is provided in Data S1. Statistical analysis was performed using SPSS (12.0 and 20.0) and Microsoft Excel.

Results

The semi-natural enclosure set-ups consisted of two rooms each, which were run in parallel (see Fig. 1 and Methods). Two consecutive set-ups were used, which differed with respect to the initial conditions in the first week of contact. In set-up I, animals were allowed to familiarize with members from their own population in separate halves of the rooms for 7 days before the separation was lifted. Set-up II was also run for a longer time to obtain offspring from the next generations. The details of the set-ups and the population development are listed in Table 1. In set-up I, the first litter was born 40 days after stocking of the rooms, that is, the first successful matings had occurred only after the separation was lifted (the gestation period is about 21 days) where animals had potentially free choice of partners.

Our analyses are focussed on mating outcome, that is, they are based on reconstructing successful matings from the paternity information, irrespective of the number of offspring that originated from a particular mating event. A total of 1083 offspring could be successfully assigned to fathers, and a total of 341 matings could be inferred from this (Table 1). Although the main motivation of our study was to assess divergence between populations, we paid also attention to other known parameters of mating patterns, because they are important for the whole context of the results. In the following, we discuss these parameters first, before coming to the question of population divergence.

Individual mating success

The animals of different population background showed no overall differences in numbers of successful matings. Across both set-ups, we found that 115 of 230 (50%) adult females and 93 of 273 (34%) adult males had offspring. There were no significant differences in relative mating success between the populations. For the founder generation, we found that 25 G vs. 21 F females and 21 G vs. 18 F males were successful

Table 1 Summary of room descriptions and population parameters for the two set-ups

	Set-up I		Set-up II	
	Room A 24 m^2	Room B 18 m^2	Room A 24 m^2	Room B 18 m^2
Duration of experiment	147 days, first litter born 40 days after start		196 days, first litter born 52 days after start	
Initial animal numbers	40 (10 G ♀, 10 F ♀, 10 F ♂, 10 G ♂)	28 (7 G ♀, 7 F ♀, 7 F ♂, 7 G ♂)	40 (10 G ♀, 10 F ♀, 10 F ♂, 10 G ♂)	28 (7 G ♀, 7 F ♀, 7 F ♂, 7 G ♂)
Initial population densities	1.7 mice/m^2	1.6 mice/m^2	1.7 mice/m^2	1.6 mice/m^2
Initial spatial separation	F and G animals were initially separated for 7 days by dividing the enclosure in two parts		No initial separation of the two populations	
Population densities at the end of the experiment	4.25 mice/m^2	2.5 mice/m^2	12.9 mice/m^2	11.2 mice/m^2
Operational sex ratio (adults >13 g) at the end of the experiments (♀:♂)	26:36	24:22	106:115	69:81
Total number of animals recorded including embryos, dead pups, and newborns	193	133	647	386
Total number of animals with successful paternity assignments	132	92	524	335
Total number of matings inferred from paternities	39	31	162	109

($P = 1.0$, Fisher's exact test). The numbers of successful matings among offspring between 'pure animals' and 'mixed animals' (with respect to their population origin) were not significantly different (females pure: 32/63; females mixed: 37/99, $P = 0.31$; males pure: 25/84, males mixed: 29/121, $P = 0.26$, Fisher's exact test). There were also no significant differences between matings among different genotypic classes, as tested by one-way ANOVA.

Hybrid fertility

To assess whether there was any indication for beginning postzygotic reproductive isolation between populations, we calculated the total number of offspring for each animal, both from pure and mixed population backgrounds. We found no significant differences between the populations (not shown) nor between 'pure' and 'mixed' males and females (Fig. 2, top row). Based on the paternity information, we compared also the number of successful matings and found also no significant differences (Fig. 2, bottom row).

Inbreeding and multiple paternities

The family inbreeding status was assessed for all litters that involved at least one offspring born in the enclosure. 73 of 341 (21%) successful matings were among relatives, 46 of them were among full-sibs (20 of them litter mates), 14 father–daughter and 13 mother–son matings. Multiple paternities were found for 78 of 250 litters (31%). The majority of multiple paternity litters were from two sires (82%): 12 litters (15%) had three sires and two litters (2.6%) had four sires. 58% (23 of 40) multiple paternity litters involving relatives had at least one nonrelative involved. This includes all mother–son litters and most father–daughter litters. Population background had no influence on the ratio of multiple versus single paternity (G 31%, F 33%, mixed 31%).

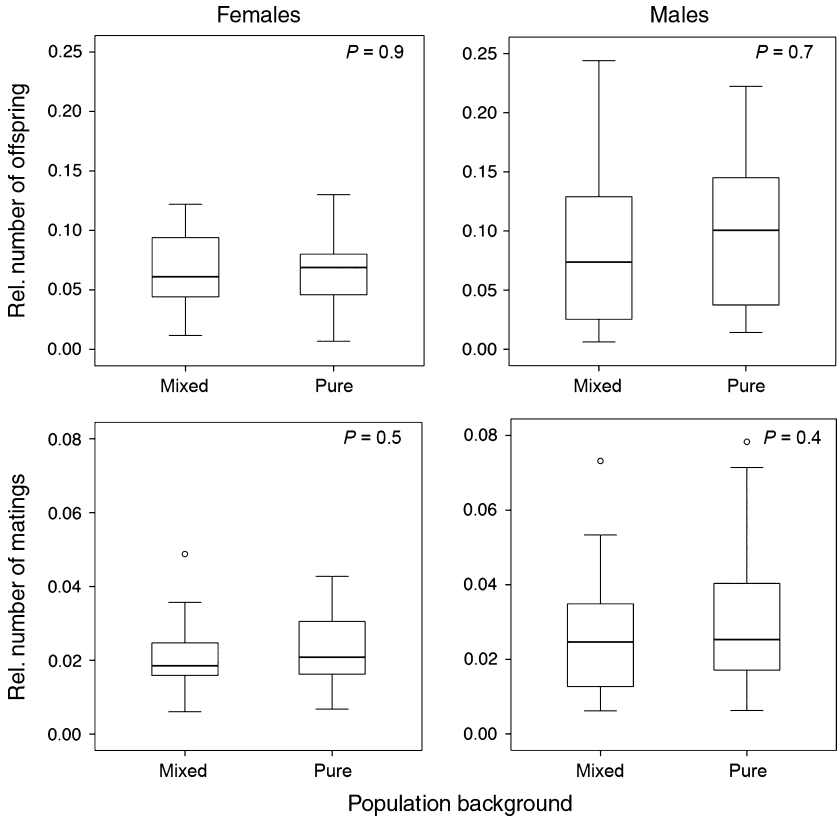

Fig. 2 Comparison of fertility parameters for pure and mixed females (left) and males (right). 'Mixed' population background refers to matings (as inferred from offspring litters) between animals that had a combination of G and F genotypes, 'pure' population background refers to animals from either G or F. The numbers are relative numbers, that is, corrected for time of presence in the enclosure. This correction was performed because younger animals born in the enclosure had of course a lower total number of offspring, because they had fewer possibilities for repeated breeding. But the conclusion would not be different if total numbers were compared (Montero 2010). The boxplots show the median, quartiles and extreme values.

Mate fidelity

Although the large frequency of multiple matings would suggest a promiscuous mating system, we noticed that there was often a high fidelity towards remating with mates that had already been chosen before. We have quantitatively analysed this for the animals of the founder generation, because they had the longest time in the set-ups, giving them the most opportunity for remating or finding new mates. For females that had remated at least once, we found that 61% (43 of 70) of matings occurred with a male that was chosen at least once before. For males, the corresponding frequency was 58% (44 of 76). Although the founder animals had the opportunity to mate with offspring as these had grown up, most of their matings were still with other founder animals (females 113/157 = 72%; males 113/139 = 81%). Among the matings between founder animals and offspring, many were parent–offspring matings (females 11/44 = 25%, males 12/26 = 46%).

Patterns of house occupation, as determined by frequent monitoring (see Methods), showed no consistent patterns that could easily explain the mate fidelity patterns. While some animals showed specific strategies, such as either stable associations with houses or continuous free ranging, others changed these patterns during their lifetime. A rough classification for the monitored reproductively active males in set-up II showed approximately a third each with (i) stable association to a given house (but usually not the one in which they were born), (ii) association with a certain quarter of the room (but partly across shielding walls) and (iii) no stable associations.

Patterns of remating, parent–offspring mating and changes in house occupation are particularly evident in the genealogies of large families – one example is shown in Fig. 3. These observations suggest that familiarity with a previously encountered mate is important for remating, that is, the second mating is not independent of the first one.

Assortative mating

To obtain an overall view of assortative mating patterns, we developed a statistical approach to compare all matings, but taking into account that available mates changed during the course of the experiment. We counted for consecutive phases all available adult mates and calculated expected mating frequencies under a random mating assumption, which were then compared with the observed numbers (see Methods). In this overall analysis, we found that assortative mating was significantly more likely (null hypothesis of random mating rejected with $P = 0.033$, one-sample t-test, 33df).

However, we have to take into account that matings could have been influenced by the two factors described above: family inbreeding and mate fidelity. To remove the overlap with these factors, we devised a second approach, by counting only successful matings between nonsiblings and only the first mating with a new partner. In set-up I (with initial separation), we found that 28 of 36 such mating events occurred assortatively according to population background, which is significantly different from random mating ($P = 0.001$, binomial test, two tailed). However, most of the nests, in which the respective offspring were raised, were located within the sections of the rooms where the mice were originally released, before the separation was lifted. Hence, it is possible that the observed pattern reflects a spatial effect in the establishment of home ranges within the initial phase of the experiment. But, as noted above, many animals tended to shift their home ranges during later phases.

In set-up II (without initial separation), there was no possibility for an initial establishment of population-specific home ranges for the colonizing mice. Among them, we found only 12 of 36 first successful matings had occurred assortatively, which is not significantly different from random mating ($P = 0.07$, binomial test, two tailed). Hence, although we have an overall effect of assortative mating, some form of learning or acquaintance within the enclosure appears to be required (see Discussion).

Paternal matching

Set-up II was run for a longer time to see how animals that were born in the enclosure mate among each other. This resulted in many different possible combinations of matings, including matings between the generations. However, for the purpose of the present study (i.e. influence of population differences), we focus the analysis on the matings between animals of the first offspring generation, that is, those that were born in the enclosure and had chosen mates of their own generation. We use the following annotation for these cases: each animal is represented by two letters: the first representing the population origin of the mother and the second one of the father (e.g. GG when both are German, GF when the mother was German and the father was French). In matings, we write the female first, for example GG × FF, when a pure German female mated with a pure French one.

There are four possible classes of mating under these conditions: class 1 – those that share the same paternal and maternal origin (GG × GG, FF × FF, GF × GF and

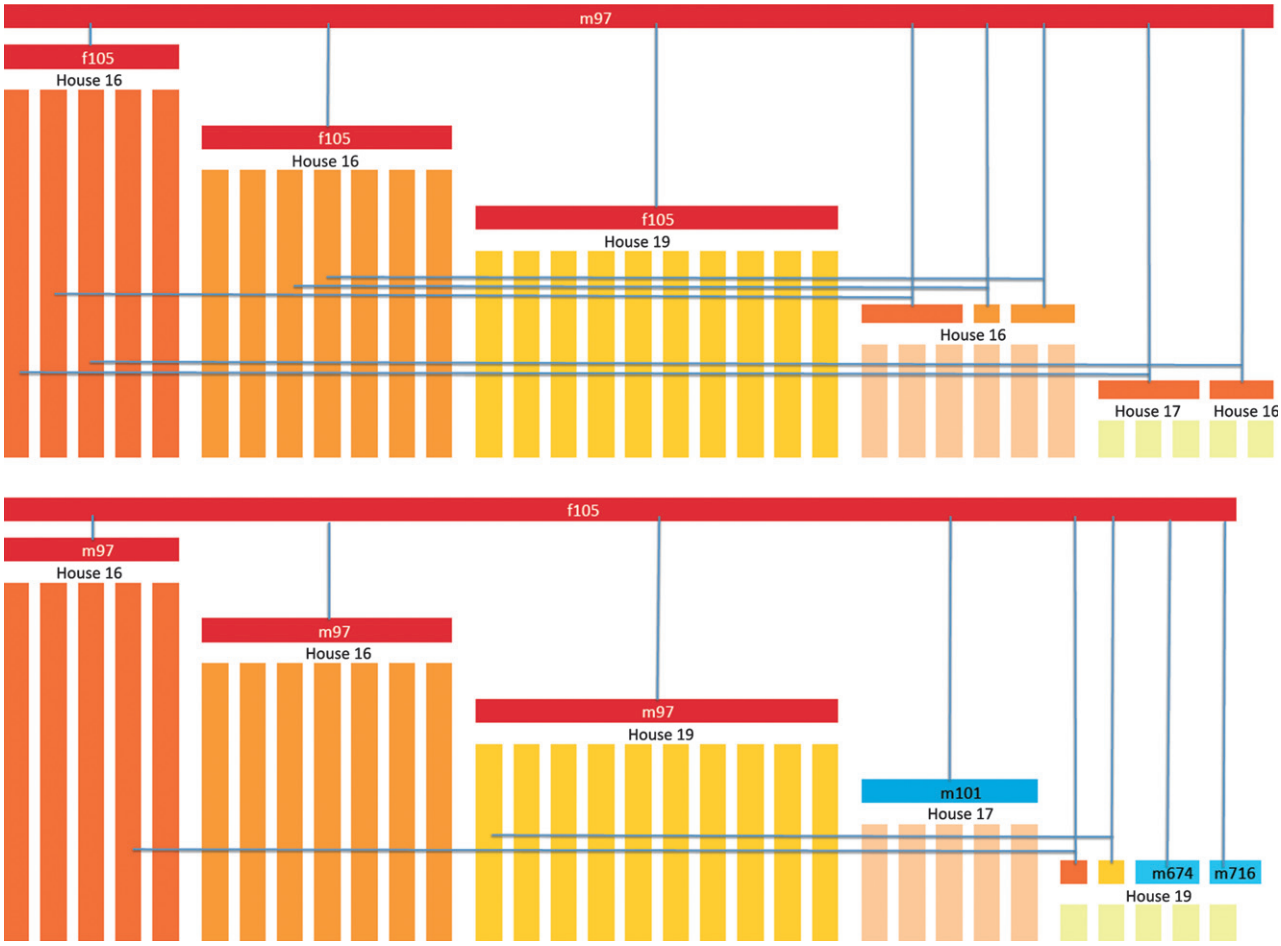

Fig. 3 Mating patterns within one extended family originating from the founder male 97 and founder female 105. Both were present during the whole duration of the experiment (symbolized by horizontal bars), and both were involved in five mating phases (differently coloured blocks). Each bar represents one offspring generated between the respective founder animal and the mate indicated in the horizontal bar on top. Red-yellow colours represent matings within the family; blue coloured animals are with unrelated animals. The first three mating events occurred only between the founder animals, the third one in a different house (i.e. a joint change of territory). The founders split up in the fourth mating period, with the male returning to its first house and mating with daughters of the first and second litter, while the female moved to a new house (house 17) and mated with an another male of the founder generation (m101). In the fifth mating period, female 105 moved back to house 19 and mated with four males: two of them sons of previous litters, one from house 19, and two unrelated males of later generation offspring (m674 and m716). Male 97 mated in house 16 and 17 with daughters of the first litter. At the end of the experiment, female 105 was pregnant with a litter fathered by male 716 (not shown). Comparable patterns were found for the other large families as well.

FG × FG), class 2 – those that share the same maternal origin (GF × GG, FG × FF, GG × GF and FF × FG), class 3 – those that share the same paternal origin (GF × FF, GG × FG, FF × GF and FG × GG) and class 4 – those that share neither (FG × GF, GG × FF, FF × GG and GF × FG). Class 1 is equivalent to assortative mating, whereas class 4 is equivalent to disassortative mating. Classes 2 and 3 imply some population recognition, if they are more frequent than would be expected by chance. In addition, they can reveal a possible paternal or maternal bias with respect to mate choice. To be conservative, we exclude matings among siblings from the analysis, because these could simply reflect familiarity. For the same reason, we focus also only on first matings of a given pair, that is, we do not count repeated matings between the same partners. Of a total of 142 matings among first-generation animals, 46 occurred among siblings, and among the remaining ones, 25 were repeated matings with the same partner. This leaves 71 matings for the following analysis.

Table 2 lists all possible combinations of matings and classes and their respective occurrence. As not all possible genotypes are equally represented, we included also the expected numbers if random mating had occurred

Table 2 Mating events among first-generation offspring animals (without sib-matings and only first choices), sorted according to genotype combinations. The first value represents the observed numbers, the second the expected (calculated as if all animals in the analysis would have mated freely and randomly according to their respective frequencies)

Female	Male	(1) Mat/pat matched (assortative)	(2) Maternally matched	(3) Paternally matched	(4) Unmatched (disassortative)
Matings among pure × pure					
GG	GG	3/5.9			
GG	FF				7/5.9
FF	FF	1/0.9			
FF	GG				0/1.1
Matings pure × mixed					
GG	GF		5/5.3		
GG	FG			7/5.9	
FF	GF			3/1.0	
FF	FG		0/1.1		
Matings mixed × pure					
GF	GG		3/4.8		
FG	GG			13/7.2	
GF	FF			8/4.1	
FG	FF		0/6.1		
Matings mixed × mixed					
GF	GF	6/4.3			
FG	FG	11/7.2			
GF	FG				1/4.8
FG	GF				3/6.5
Overall*		21‡/18.3	8†/17.3	31†/18.2	11‡/17.4

*Overall significance across the four classes: $P = 0.0008$, chi-square goodness-of-fit test, comparing observed with expected values, df = 3.
†Paternal versus maternal matching: $P = 0.0004$, binomial test, two tailed.
‡Assortative versus nonassortative matching: $P = 0.11$, binomial test, two-tailed.

among them. Overall, we find a highly significant deviation from random mating ($P = 0.0008$, chi-square goodness-of-fit test). This is mostly due to a highly significant difference between paternally and maternally matched matings (31 vs. 8; Table 2). There is also an excess of assortative versus nonassortative matings (21 vs. 11) as discussed above, but this is marginally not significant in this reduced data set (Table 2).

We applied also the overall analysis across consecutive mating phases (see Methods) to the question of paternal versus maternal matching and found that paternal matching was significantly more frequent than expected ($P = 0.019$), while maternal matching was not significantly different from random expectation ($P = 0.264$) across the whole data set of all matings.

Repeatability of mate choice

Many animals mated more than once with a new mate that they had not mated with before, and it is therefore of interest to assess whether they retained the population preference that they showed in the first mating. In Table 3, we have compiled all these animals (a subset of those analysed above) and listed their mates in temporal succession (consecutive phases in the experiment). For males, 12 of 18 mated with exactly the same genotypic combination for two or more times and for females 11 of 19. However, one has to take into account that a combination of class 1 and 2 as well as class 1 and 3 matings is not really different with respect to mate choice preferences, because class 1 implies both, maternal and paternal matching. Hence, animals that, for example, prefer paternally matched mates would find them in both class 1 and class 3. When accounting for this, the fidelity becomes higher: 14 of 18 for males ($P = 0.03$, binomial test, two tailed) and 15 of 19 for females ($P = 0.02$, binomial test, two tailed). Interestingly, there are also three cases (one male, two females) where a disassortative mate choice (class 4) was repeated, which suggests that animals tended to repeat their first mating preference decision.

Discussion

The goal of our study was to assess the extent to which individuals of the French and the German population

Table 3 Mating events among F1 animals, which mated with more than one partner (without sib-matings and only first choices), listed according to phases and mating classes. Each line represents one animal and entries separated by commas represent more than one mate in the respective phase (implying multiple paternities in case of females). The phases represent approximately gestation periods (see Methods – note that no F1 matings occurred during phases 1–4), the genotypic symbols are defined in the text

	Phase 5	Phase 6	Phase 7	Phase 8	Phase 9	Phase 10	Mating class*
Males	Genotype combinations of female mating partners						
GG						GG, GF, GF	1, 2
GG						GG, GF	1, 2
GG		GG, GG, GG	GG				1
GG	FG, FG	FG, FG					3
GG			FG	FG			3
GF				FG	GG, GG		4, 2
GF		FF, FF, FF		FG			3, 4
GF		GF	GF				1
GF				GG		GG	2
FG	FG		FG	FG			1
FG						FG, FG	1
FG	FG, FG	FG					1
FG						GG, GG, GG	3
FG		GG, GG, GG	GG				3
FF		FF		GG, GG			1, 4
FF	GF	GG, GF, GF, GF, GF					3, 4
FF		GF, GF		GF			3
FF		GG	GG				4
Females	Genotype combinations of male mating partners						
GG				FF	GF		4, 2
GG		FF, FF			GF		4,2
GG						FG, GG	1, 3
GG			FF, FF				4
GG		FF				GG	4, 1
GF			GF	GF			1
GF		FF, FF					3
GF	FF			FF			3
GF		FF, FF					3
GF						GG, GG	2
FG			GG			FG, GF	3, 1, 4
FG	FG, FG, GG		FG				1, 3
FG	FG, FG						1
FG		FG		GG			1, 3
FG		GG		FG		GG	3, 1
FG		GG	GG				3
FG						FG, FG	1
FG		GG, GG					3
FG				GF, GF			4

*Class 1, maternal and paternal matching; class 2, maternal matching; class 3, paternal matching; class 4, no matching.

have already diverged with respect towards differentially recognizing each other. The results showed that not only cues of population origin, but also additional factors influence mating patterns in mouse populations. Most notably, we found a component of familiarity among animals as a factor for successful mating, which includes inbreeding among family members. This suggests that acquaintance of the animals with each other is important determinant of mating success. Accordingly, we found more evidence for assortative mating among the founder animals in the first set-up where they had the chance to get familiar with mates from their own population before they came in contact with the animals from the other population. However, animals born in the enclosure had contact to possible mates from both populations, and their tendency to choose partners of the same paternal origin allows two important conclusions: (i) paternal imprinting plays some role in mate choice and (ii) the cues that convey the paternally imprinted mate choice have diverged

between the populations, because they would otherwise not be able to differentially recognize each other.

In the following, we discuss this in detail, including discussion of general aspects of the mating system as revealed in our study.

Semi-natural conditions

Our set-up of semi-natural conditions mimicked that of previous studies (Oakeshot 1974; Manning *et al.* 1992; Lenington *et al.* 1994; Meagher *et al.* 2000; Carroll *et al.* 2004; Sherborne *et al.* 2007; Manser *et al.* 2011). The rooms provided spatial structure for territory establishment, as well as shelter for nesting. We had also installed a dispersal tube that allowed mice to escape from the population enclosure in a connected cage system (see Methods). However, only few animals made use of this, suggesting that the population densities did not become too high. In the rooms, mice could interact completely freely, particularly in the first half of the experiment, where unoccupied houses were continuously available. The set-ups provided both populations equal opportunities for mating, and they showed no relative differences in mating success. We found also no fertility differences for hybrids between the populations, indicating that their divergence has not led to noticeable postzygotic incompatibilities.

Multiple mating and inbreeding

Dean *et al.* (2006) found for a wild population of mice that 20% of litters analysed had multiple paternities. They suggested that the true frequency might even be higher, because it was not possible to assign specific sires to individual embryos due to extensive allele sharing. This could imply that related males were the respective fathers, as it was also often the case in our study. A study on island mice (Firman & Simmons 2008) found 6–43% multiple paternity litters. These numbers correspond well with our average estimate of about 31% multiple paternities. Many possible reasons have been offered to explain strategies of multiple matings in females, including inbreeding avoidance or acquisition of better genes (Zeh & Zeh 2001; Colegrave *et al.* 2002; Tregenza & Wedell 2002; Wolff & Macdonald 2004). We found indeed that females mating with their sons or their fathers did always mate in addition with an unrelated male, which would support these hypothesis.

It is generally thought that animals avoid inbreeding because this could lead to genetic problems due to homozygosity of recessive lethal or sublethal alleles (Pusey & Wolf 1996). Long-term studies in semi-natural enclosures have suggested that inbred animals have indeed a lower fitness (Meagher *et al.* 2000). Our set-ups did not run for long enough to assess this, that is, we cannot exclude that this would also have been the case in our study.

In mice, urinary odour cues based on different alleles of MUP have been suggested as a signal to avoid inbreeding (Isles *et al.* 2002; Sherborne *et al.* 2007). However, a certain degree of inbreeding has in fact been observed in these studies as well (Sherborne *et al.* 2007), because family members can carry different alleles at the MUP loci in a polymorphic population. But even naturally caught mice tend to show an overall deficit of heterozygosity (Dean *et al.* 2006; Ihle *et al.* 2006; Hardouin *et al.* 2010), implying that local inbreeding within families is part of the natural breeding system. Alternatively, the inbreeding seen for wild-caught mice could also be a simple consequence of the deme structure, due to patchy habitats and small dispersal ranges (Berry & Bronson 1992), within which the availability of potential mates might well be restricted to relatives. On the other hand, even in our semi-natural enclosures, that is, under conditions where unrelated mates were available within the activity range, family structures were formed and inbreeding occurred frequently. However, we do not consider the observed level of inbreeding as a reflection of a specific strategy, but would rather interpret it as a reflection of the general familiarity component of mate choice.

Familiarity and pair bonding

Several studies have shown that mice discriminate mates on the basis of familiarity and other social cues due to previous experience (Barnard & Fitzsimons 1988; Drickamer *et al.* 2000; Gowaty *et al.* 2003; Rolland *et al.* 2003). However, the issue of long-time pair bonding has not been assessed so far. Our approach allowed quantifying the incidence of repeated mating in separate reproduction cycles. We find that the frequency of remating with a known mate is high, both for females and males, and appears to decline only when the density of available mates increases. Even multiple matings can repeatedly occur with the same group of mates. Hence, pair bonding is an important component of the mating system of mice. This may partly be connected to joint territory defence, but most pair-bonded mice had offspring in different houses (compare also Fig. 3). Generally, we observed that whole nests including full litters were easily moved during the course of the experiment, in line with the general observation that most spatial territories were not very stable over time.

Paternal matching

The most unexpected result of our study is a strong paternally conveyed component in mate choice. For

hybrid animals mating with an animal of pure population origin, it should have been equally likely to match paternally or maternally, but matching occurred mostly paternally. This effect is very strong, even if one excludes all possible confounding effects associated with inbreeding and familiarity. Hence, there must be population-specific cues that allow the animals to find the paternally matching partners. As these cues were apparently not used by the animals that were directly released into the enclosures from cages in set-up II, we conclude that a learning component is involved. A role for learning in population and species discrimination has long been hypothesized (Immelmann 1975; Irwin & Price 1999). There is also increasing experimental evidence that learning plays a role in population divergence in various species, such as sticklebacks (Kozak & Boughman 2009) or damselflies (ss *et al.* 2010), but it has best been studied in birds, where population-specific songs are part of the cues that convey the discrimination (Zeigler & Marler 2004). Many bird species copy the songs of the adults in their surroundings and produce songs that are similar to those of these adults. This is not a purely cultural inheritance, because most species have also a genetic predisposition for learning their population-specific songs (Williams 2004). We propose that such a combination of a learned cue together with a genetically imprinted component (see below) may also explain the results of our study. Once learned, the cues are stably used, as it is evident from the high fidelity of repeated choices made by the animals (Table 3).

The similarities to bird song might even go further. Mice are also known to 'sing', but only in the ultrasonic range (Portfors 2007). It has been shown that these songs are used for mate recognition (Hammerschmidt *et al.* 2009; Musolf *et al.* 2010) and that males' vocalizations contain specific signatures of individuality and kinship (Hoffmann *et al.* 2012). Furthermore, there seems to be both a genetic (Hammerschmidt *et al.* 2012; Kikusui *et al.* 2012) and a learning component in shaping the songs (Arriaga *et al.* 2012). Thus, it seems well possible that pups or growing infants learn paternal patterns of songs. Similar as in birds, these would have to include population-specific genetic components to explain our results for mating according to population origin. Learning of paternal songs requires that males of the respective paternal population background stay at the nests where the pubs were born and raised. Indeed, males were frequently found in nests together with pups during our monitoring surveys, and all F1 animals that are included in the analysis in Tables 2 and 3 came from single-father litters.

However, although ultrasonic vocalization may play a larger role in mate choice than previously assumed, the influence of olfactory cues cannot be ruled out either. In humans, paternally inherited HLA alleles have been suggested to influence preferences for male odours (Jacob *et al.* 2002). But it is difficult to explain a population-specific discrimination on this basis. Although olfactory cues based on urinary proteins (MUP) or MHC peptides have been implicated in individual recognition in mice (Hurst 2009), they are not likely to play a direct role for paternal matching. As these genes are not known to be genetically imprinted, they would be codominantly expressed in hybrid animals. Also, both systems are highly polymorphic and one can expect that the two populations share a set of alleles at these loci (already confirmed for the MHC alleles: Montero and Teschke, in preparation), which would make a population-specific discrimination very difficult. However, one of the MUPs was shown to convey general receptivity to females (Roberts *et al.* 2010). This could be a possible candidate pheromone for discrimination between populations, but we found that the two populations show no coding position difference for this gene (data not shown). However, it might act via differences in expression or secondary modification, which needs to be further explored.

Irrespective of whether vocalization or odour is used as mating cue, a genetic component for the observed paternal matching would imply that genetic imprinting of at least some of the genes involved in such behavioural traits is involved. Paternally expressed alleles of genes could influence either the population-specific cues or the recognition of cues, or both, and they could have different functions in males and females. A complex pattern of imprinted gene expression has been described for the mouse brain, with both maternal and paternal biases (Gregg *et al.* 2010), although there is some dispute on how many genes are actually affected (DeVeale *et al.* 2012). Interestingly, a stronger paternal bias of gene expression was found in the adult cortex and the hypothalamus (Gregg *et al.* 2010), that is, brain regions that are particularly relevant for behaviour, vocalization and mating. One imprinted gene, Grb10, was shown to directly convey specific behavioural responses through paternal imprinting of a brain-specific transcript in mice (Garfield *et al.* 2011). Imprinting of genes was also implied in inbreeding avoidance and dispersal behaviour (Isles *et al.* 2002) as well as the possibility of selective abortion (Wolf & Hager 2009). Evolution of imprinting phenomena is generally considered to be linked to social system evolution, like sex-biased dispersal, variance in reproductive success and mate choice (Haig 2000; Isles *et al.* 2006; Tramm & Servedio 2008; Brandvain *et al.* 2011). Modelling of mate choice imprinting has indeed suggested that paternal

imprinting is more likely to evolve than maternal imprinting (Tramm & Servedio 2008).

Population divergence

Our results imply that the signals and/or the neuronal recognition system that is required for mate recognition in mice are subject to particularly fast divergence, such that the two mouse populations, which have diverged for only 3000 years, can discriminate each other. Animals of these populations are not in regular contact with each other, that is, reinforcement mechanisms are not expected to play a role. Rather, they represent a case for divergence in allopatry. The divergence process must therefore be based on mechanisms that happen within populations, such as sexual selection. Sexual selection is known to depend on maternal and paternal influences (Qvarnström & Price 2001). Hence, it will be of interest to revisit the population divergence and speciation models that take mate recognition preferences into account to further assess the effect on the evolutionary dynamics of population subdivision (Tramm & Servedio 2008; Brandvain *et al.* 2011).

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Data accessibility

Lists of all animals in the experiment, including all relevant data for the analysis are available at Dryad doi:10.5061/dryad.53356.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Microsatellite loci used for paternity analysis.

Data S1 Data matrix for all matings, all F1 matings and only first F1 matings without sib-matings.