Mate choice and reproductive strategies in recently diverged populations of the house mouse

(*Mus musculus domesticus*)

Inaugural – Dissertation

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List of Contents

List of Figures and Tables

Figures

Tables

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Zusammenfassung

Partnerwahl und Reproduktionsstrategien von differenzierten Populationen der Hausmaus (*Mus musculus domesticus*)

Populationsdivergenz ist ein wichtiger evolutionärer Prozess und kann schnell durch das Zusammenwirken von genetischer Drift, natürlicher und sexueller Selektion eintreten. Natürliche Selektion wirkt über Fitnessunterschiede durch unterschiedliche Anpassung an lokale Umweltbedingungen; sexuelle Selektion wirkt über Partnerwahl auf den Fortpflanzungserfolg von Individuen.

Für die vorliegende Studie habe ich die Partnerwahl in divergierenden Populationen der Westeuropäischen Hausmaus Mus musculus domesticus aus der Köln-Bonner Region (die „deutsche Population") und aus dem Zentralmassiv (die "französische Population") untersucht. Die Populationen sind seit höchstens 3.000 Jahren getrennt. Obwohl eine solche Zeitspanne evolutionär kurz ist, zeigt sich bereits genetische Differenzierung.

Ob eine Differenzierung der Populationen auch bei der Partnerwahl zu beobachten ist, habe ich in Langzeitexperimenten untersucht. Dazu habe ich individuell markierte Mäuse beider Populationen für 6 Monate in einem weitestgehend natürlichen Gehege gehalten. Für Kontrollexperimente habe ich ein Käfigsystem genutzt, bei dem Weibchen Kontakt zu Männchen beider Populationen hatten. Die Weibchen konnten über sechs Tage zwischen Männchen beider Populationen wählen; die Männchen hatten keinen Kontakt untereinander.

Die Vaterschaften aller Individuen in den Langzeitexperimenten wurden durch Mikrosatelliten-Typisierung als Maß für die Partnerwahl und den Fortpflanzungserfolg bestimmt. Die individuelle Überwachung der Tiere ermöglichte die Aufnahme ihres physischen Zustandes. Untersucht habe ich auch wie eine egoistische Genvariante, der t-Haplotyp auf die Partnerwahl in beiden Populationen wirkt. Schließlich habe ich geprüft, ob sich die Populationsdivergenz auch in relativen Häufigkeiten weiblicher Reproduktionsstrategien wie Polyandrie und gemeinsamer Jungenaufzucht widerspiegelt.

Die Gründerindividuen in den Langzeitexperimenten folgten keinem einheitlichen Muster bei der Partnerwahl. Mäuse die in den Gehegen geboren und aufgewachsen waren zeigten dagegen eine signifikante Präferenz für Partner, deren Väter aus derselben Population wie der eigene Vater stammte. Das Experiment im Käfigsystem lieferte keine

einheitlichen Präferenzen in Bezug auf Populationszugehörigkeit. Bemerkenswert jedoch ist, dass Schwestern, die gemeinsam in einem Käfig aufgewachsen waren Männchen aus derselben Population bevorzugten. Diese Ergebnisse werden im Kontext von ethologischer und genetischer Prägung diskutiert.

Einzelne Parameter zum Fortpflanzungserfolg (z.B. Anzahl der Nachkommen, Anzahl der erfolgreichen Verpaarungen, Nachkommen pro Verpaarung) unterschieden sich nicht signifikant im Vergleich von Tieren mit Eltern aus den verschieden Populationen („Hybride“) und Tieren mit Eltern aus der jeweils gleichen Population. Die Kombination der Parameter jedoch zeigte, dass in 5 von 6 Fällen die Nachkommen von Eltern aus der gleichen Population die Hybriden übertrafen. Dies weist auf eine leichte Abnahme der Hybrid-Fitness hin.

Ein unterschiedlicher Einfluss des t-Haplotypen auf Partnerwahl oder Verpaarungsverhalten von Weibchen wurde zwischen deutschen und französischen Mäusen und Hybriden nicht gefunden. Der einzig beobachtbare und statistisch signifikante Einfluss dieser egoistischen Genvariante besteht in einem leichten Rückgang der Nachkommenanzahl in erfolgreichen Verpaarungen zwischen Tieren die heterozygot für den t-Haplotypen waren.

Im Gegensatz zu theoretischen Annahmen und Experimenten anderer Wissenschaftler habe ich keine Hinweise auf erhöhte Polyandrie oder die Vermeidung von Partnern mit t/wt gefunden. Polyandrie und gemeinsame Weibchenaufzucht scheinen allgemeine Strategien von Weibchen zu sein. Beides trat vermehrt mit zunehmender Bevölkerungsdichte auf. Beide Strategien erhöhten leicht den individuellen Fortpflanzungserfolg im Langzeitexperiment: Weibchen, die Würfe von gleichzeitig mehreren Männchen hatten, zeigten einen höheren Reproduktionserfolg als Weibchen die nur Würfe hatten, die von jeweils einem Männchen gezeugt wurden. Ein höherer Fortpflanzungserfolg wurde auch bei Weibchen gefunden, die aus gemeinschaftlich aufgezogegnen Würfen stammten.

Zusammenfassend lässt sich feststellen, dass zwischen den untersuchten Popualtionen keine Unterschiede in Partnerwahl und Fortpflanzungsstrategien beobachtet wurden. Weibchen präferieren jedoch Männchen, deren Väter von der gleichen Population kommen wie ihr eigener Vater, ein Phänomen, das ich als „vaterbezogene assortative Präferenz“ bezeichne. Dies deutet auf die Existenz von Merkmalen hin, die ein Unterscheiden zwischen "eigener Population" und "anderer Population" möglich machen. Darüber hinaus gaben die Ergebnisse Einblicke in Vorteile durch kostspielige weibliche Reproduktionsstrategien.

Abstract

Population divergence is an important process in the evolution of lineages and can occur rapidly through the interaction of random genetic drift with natural and sexual selection. While natural selection operates on differences in fitness with respect to the local environment, sexual selection acts on the reproductive success of individuals through pre- and postcopulatory mate choice.

Recently separated populations of the Western European house mouse *Mus musculus domesticus* were investigated for mating preferences. The study system consisted of two populations, one sampled in the Cologne/Bonn region, referred to as the “German population” and one from the Massif Central, termed here the “French population”. These populations have been separated for at most 3,000 years. Although this time span is short in evolutionary terms, they already show genetic differentiation.

To test whether population divergence is reflected in mate choice, I carried out four replicates of a long-term experiment, in which individually tagged mice of both populations were held for 6 month in a semi-natural enclosure. As controls, I conducted cage experiments, where females could choose between males of both populations during a 6 day period.

Paternities in the enclosure populations were determined by microsatellite typing of all individuals and they were used as measures for mate choice and reproductive success. The frequent monitoring of the populations during which animals were examined individually allowed the assessment of their physical condition. Furthermore, I examined the influence of a selfish genetic element, the t haplotype, on pre- or postcopulatory mate choice for the different population backgrounds. Finally, I analyzed whether the population divergence is also reflected in relative frequencies of female strategies such as polyandry and communal breeding.

Founder animals of the long-term experiment did not follow a consistent mate choice pattern, while individuals born in the enclosures showed a significant preference for partners who had a father from the same population as themselves. In the controlled cage experiment, there was no consistent preference pattern regarding population background. However, female littermates that grew up in the same cage chose males

coming from one population, indicating an environmental influence. These findings are discussed in the context of behavioral and genomic imprinting.

German and French founder animals differed slightly in reproductive success. Among the F1 individuals, the comparison of reproductive success between individuals with a mixed population background (i.e. with parents from different populations) versus animals with a pure background (i.e. with parents from the same population) revealed no significant differences. Nevertheless, when looking at the combination of measures for reproductive success, such as offspring number, number of mating events, and offspring per mating, in 5 out of 6 parameters “pure” individuals outperformed the “mixed” individuals, which might indicate a slight decrease in hybrid fitness.

No different influences were detected between German, French and hybrid animals regarding the t haplotype or different frequencies of female multiple mating and communal breeding. Influences of the t haplotype were restricted to a slight decrease in offspring number in successful mating events between t/wt animals for all combinations of population backgrounds. Contrary to theoretical assumptions and other experiments, no evidence for an increased multiple mating frequency or avoidance of partners with t/wt was found. Polyandry and communal breeding seemed to be general strategies in females of pure as well as mixed population backgrounds, and both strategies increased in frequency with an increasing population density. Females displaying these strategies had a slightly higher reproductive success in semi-natural conditions: Mothers with litters sired by several males had a higher reproductive success than mothers with only single paternity litters. A higher reproductive success was also detected for females which grew up in communally reared litters.

Summarizing the outcome of the study, the recently diverged populations do not vary in partner choice: no differences in mate choice or reproductive strategies were observed. However, females preferred mates that had fathers from the same population as themselves, a pattern which I will call the “father related assortative mating pattern”. This suggests the presence of cues which enable the differentiation between “one’s own population” and “the other population”. In addition, the results gave insights into the benefits of costly female reproductive strategies.

Declaration

The project was designed together with my supervisors Dr. Meike Teschke and Prof. Dr. Diethard Tautz. Practical laboratory work was done by me with assistance of Heinke Buhtz and Cornelia Burghardt. The analysis and interpretation was done by me, with inputs from my supervisors.

1 General Introduction

1.1 Studying mate choice in the context of population divergence

Populations are the important units of evolution and will differentiate if they are subjected to different selective forces or drift (Ehrlich & Raven 1992). Considering this, population divergence is a decisive evolutionary process, since it captures the historic ecological differences in lineages. Following a population genetics model by Lande (1981), evolution can occur rapidly through the interaction of random genetic drift with natural and sexual selection. Similarly, Slatkin (1987) states that besides mutations and genetic drift due to finite population size, natural selection favors adaptations to local environmental conditions which lead to the genetic differentiation of local populations. This process is also described by Kimura & Weiss (1964), who pointed out that the genetic differentiation of geographical races may reflect local differences of selective patterns. Considering the above mentioned statements, these imply that the process of population differentiation is accelerated by adaptations to the local environmental conditions (Hartl & Clark 2007). According to the nearly neutral theory, adaptation may be due not to strong selection of rare variants with large effects, but to weak selection of common variants (Hurst 2009) and can act constantly on populations.

Important for the divergence of populations, however, is some sort of isolation which ensures the accumulation of genetic differences (Kimura & Weiss 1964). Mayr's species concept claims the importance of reproductive isolation (Mayr 1942, cited in De Queiroz 2005). One mechanism for such isolation could be assortative mating, biasing mate choice towards a partner from the same local population. Additionally, Ehrlich & Raven (1969) state that incompatibility can arise when two populations are subjected to differing selective regimes and selection operating against hybrids reinforces the divergence.

Genome wide studies have shown that genes involved in reproduction and immune defense are among the genes which evolve comparably fast (Waterston et al. 2002; Ellegren 2008; Swanson & Vacquier 2002). This might be reflected by a divergence in mate choice, since pre- and postcopulatory choice were shown to be influenced by for example, genes governing the immune defense (e.g., Milinski 2006) or genes coding for sperm and egg proteins involved in fertilization (Eady 2001). In addition to a consequence of genetic compatibility, divergence in partner preferences could

result from a situation where alleles, parameters or reproductive strategies favorable in one population context are different to alleles, parameters or strategies favorable in another context (Bussière et al. 2008),

To what extent parameters influencing mate choice evolve and diverge over time can be tested empirically by analyzing mate choice patterns between individuals of closely related populations. Even if such populations occur in allopatry, if they exhibit divergence in the respective parameters – driven either by drift or selection – some sort of assortative mating or an influence on reproductive success is expected.

Behavioral experiments and studies from the hybrid zone of two house mouse subspecies that have been separated for 800,000 years, showed assortative preferences as well as reduced fitness of hybrids (see below).

The system I studied is a very recent population divergence within the subspecies *M. m. domesticus*. The populations of French and German mice that I worked with have been separated for approximately 3,000 years.

1.2 The house mouse

1.2.1 The European Western house mouse as a model organism for evolutionary studies

The house mouse (*Mus musculus*) is an ideal model organism for studies on population divergence. Due to the fact that it is the ancestor of the lab mouse – an important model organism in medical research (Berry & Scriven 2005) – we know the complete genome sequence (Waterston et al. 2002). Moreover, specialized genetic tools such as genome wide screening platforms (microarrays or large scale SNP typing tools), that were originally developed for medical research can be applied to wild mice.

The house mouse has a relative short generation time (up to four generations per year, Geraldes et al. 2008). The ecology of the small rodent is well studied; it lives in feral populations or commensally with humans in fairly high population densities (Bronson 1979). The commensal lifestyle of the house mouse facilitates the study of its colonization history, and thus the study of population divergence.

Berry & Scriven (2005) pointed out that, although the house mouse has been used for many years as a model for evolutionary studies, most laboratory strains have

been inbred in captivity for many generations and have lost natural genetic variation, which is expected to bias results dramatically (e.g., McCarthy & Vom Saal 1986; Miller et al. 2000). Additionally, the complex social system of this rodent can't be appropriately mimicked in cage systems of animal houses because the artificial conditions bias the behavior (Latham & Mason 2004; Wolff 2003).

1.2.2 Mouse phylogeny

It is assumed that the species *Mus musculus* originated in India, from where it spread over the whole world, radiating into different subspecies around 0.5 Mio years ago (Boursot et al. 1993). *M. m. castaneus* expanded its range towards the East, *M. m. musculus* spread over Central and Eastern Europe and *M. m. domesticus* colonized Western Europe and subsequently the rest of the world (Figure 1.1).

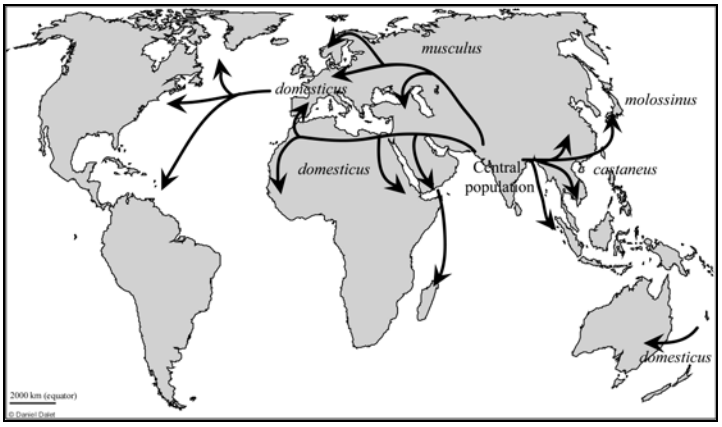


Figure 1.1: Colonization history of *Mus musculus domesticus* (after Morse 2007).

At the borders of their distribution, the subspecies form hybrid zones (zones of secondary contact), which are a major subject for speciation research. Especially the divergence between the two subspecies *M. m. musculus* and *M. m. domesticus* is intensively studied (Good et al. 2008) by crosses of both subspecies in the laboratory or the investigation of animals from the hybrid zone. These studies have found evidence for reduced fitness in hybrids in terms of higher parasite load (Sage et al. 1986), male sterility and reduced testis size as well as reduced female fertility in some crosses (Britton-Davidian et al. 2005). Additionally, different mate choice experiments between individuals of the two subspecies have been conducted to investigate whether the divergence is reinforced through sexual selection mechanisms. Applying two-way choice tests, Smadja and colleagues showed subspecies recognition mediated by urinary signals (Smadja & Ganem 2002) and

partner preferences for *M. m. musculus* males and females for individuals of the opposite sex from their own subspecies (Smadja et al. 2004).

I used two populations of mice that have only recently diverged; one population from the Massif Central region in France and the second from the Cologne Bonn region in Germany. From palaeontological studies it is known that the Western European house mouse reached Western Europe via the Mediterranean Sea at about 3,000 years ago (Cucchi et al. 2005). By this, the maximum divergence time of the two populations is not more than 3,000 years, which makes it an interesting system to investigate whether a divergence is observed in mate choice.

1.2.3 Life history of the house mouse

Life history of mice was extensively investigated in field studies and in captivity under semi-natural conditions (e.g., Crowcroft & Rowe 1961; Reimer & Petras 1967; Lidicker 1976; Singleton & Hay 1983; important reviews: Berry 1969; Bronson 1979; Berry & Bronson 1992; Berry & Scriven 2005). The following section summarizes facts that were important for the experimental design and the analysis of results in the present study.

Mice live in socially substructured populations composed of dominant and subdominant individuals where dominant males form breeding subpopulations (demes) and defend their territory by fighting with intruders and frequent urinary marking. Geneflow between demes is very rare (Selander 1970; Singleton & Hay 1983), but dispersal of young mice is frequent and an important mechanism for population expansion and colonization of new habitats (Bronson 1979; Berry & Bronson 1992).

It is estimated that commensal populations have up to three generations per year (Karn et al. 2002). Ovulation of females is every 4 days, and gestation lasts between 19-20 days. Normal litter size is between 5 – 8 pups and under normal conditions, equal numbers of males and females are born. Communal nests with several breeding females are common. Weaning takes place between 14 to 15 days. Many pups (up to 50%, Berry & Jakobson 1971) do not reach the adult age.

Polyandry and polygyny (mating with several partners) is widespread in house mouse reproduction. It was shown that both sexes benefit from mating with a

preferred partner (Drickamer et al. 2003; Gowaty et al. 2003) and sperm competition (Dean et al. 2006) seems to be common.

1.3 Aim of the study

The present study investigates partner preferences of diverged populations of the house mouse. The main question is, whether the observed genetic divergence is already reflected in mate choice. This could be indicated through assortative mating between individuals of the two populations and, possibly connected with this, an impact on hybrid fitness. On the other hand, a population divergence could also imply variation in reproductive strategies or a different influence of mate choice parameters.

The mate choice experiments were set up to observe any assortative mating and impacts on hybrid fitness (chapter 2). In chapter 3, I screen for a divergence in the role of mate choice parameters or reproductive strategies such as polyandry and communal breeding between the populations.

2 Mate choice between individuals of two separated house mouse populations (*M. m. domesticus*)

2.1 Introduction

For closely related sympatric or adjacent taxa it is known that assortative mating acts towards a stronger divergence by increasing reproductive isolation (Kirkpatrick 2000). The underlying mechanisms leading towards reproductive isolation were studied extensively in the case of the two house mouse subspecies *M. m. musculus* and *M. m. domesticus*, which came in secondary contact after 800,000 years of divergence. However, it is not known after which divergence time such reinforcing mechanisms evolve.

The here used *M. m. domesticus* populations from Germany and France are separated since approximately 3,000 years, which, considering generation times of house mice, means at most 18,000 generations. Figure 2.1 shows the migration route of the Western European house mouse: it made its way via the Mediterranean See and spread from there quickly over Western Europe (Cucchi et al. 2005).

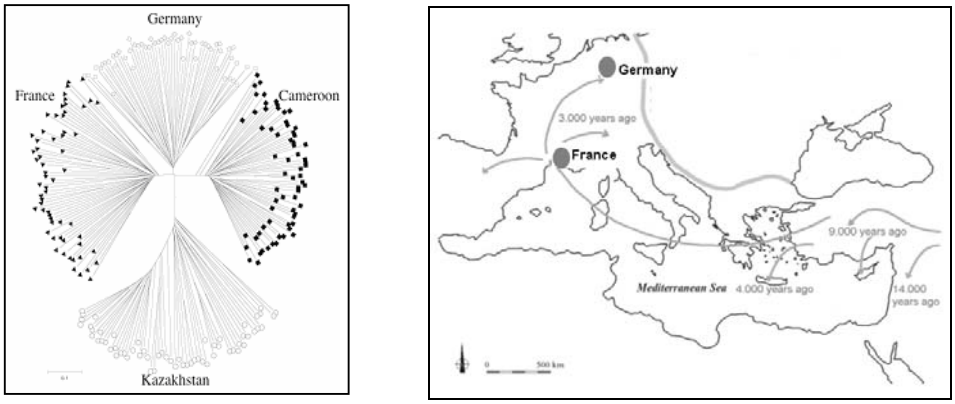


Figure 2.1: Left: Allele sharing tree based on 81 nuclear microsatellite loci, indicating recent genetic divergence between the populations. (Figure taken from Ihle et al. 2006). Right: Map showing the migration route from East to West of the house mouse *Mus musculus domesticus*. The spots mark the location of the sampling site of the Cologne Bonn ("Germany") and Massif Central ("France") population. Figure taken from (Thomas 2006).

Previous studies have shown that the populations are closely related but genetically distinct: D-loop sequences of the populations cluster together, while nuclear microsatellite loci show a clear divergence and indicate that no significant geneflow takes place between the populations (Figure 2.1, Ihle et al. 2006). Additionally, expression data also indicate divergence between the populations (Bryk et al. in prep). Considering this recent divergence and the observed genetic differentiation it

is interesting to investigate whether a divergence in mate choice can also be observed.

Long-term experiments in semi-natural enclosures are a useful setup to investigate behavior, population structure or mate choice in house mice. Several such experiments have been conducted previously (Crowcroft & Rowe 1963; Reimer & Petras 1967; Selander 1970; Lidicker 1976; Manning et al. 1995; Drickamer et al. 2003; Carroll et al. 2004; Ilmonen et al. 2008), as observations in the field are not so efficient (especially when individual observations are desired) and time consuming. Additionally, for house mice it is relatively easy to reconstruct their natural environments: since they live commensally in barns or stables, they are used to live in an indoor environment with times of artificial light and only slight temperature variation. The aforementioned long-term experiments were all long enough to allow the emergence of at least one new generation, lasting from several months to years.

Although long-term experiments are suitable setups for mate choice tests, many researchers opt for more controlled experiments in cages. The design of such experiments differs widely, lasting from few minutes to several days. Mice are exposed to different individuals (or olfactory stimuli) of the opposite sex, either allowing the free movements and enabling also mating (e.g., Rolland et al. 2003) or allowing only olfactory contact (Smadja & Ganem 2008; Drickamer et al. 2003; Lenington et al. 1994; Ramm et al. 2008). Such cage experiments have advantages, e.g. they are generally less cost intensive, they can be conducted under standardized conditions, individuals can be hindered to interact and compete with each other and outside influences can be controlled more easily. However, the mate choice behavior is not observed in a social context, which can be important depending on the research question.

To investigate the mate choice between French and German mice, I have chosen both experimental setups, which I describe in the following. In this chapter I focus on the results of assortative mating and compare the reproductive success of progeny from mate partners of the same population (“pure offspring”) and progeny from mate partners of the German and the French populations (“mixed offspring”). Figure 2.2 shows schematically the different possibilities of mate pairs and introduces the abbreviation system I used in the course of the thesis.

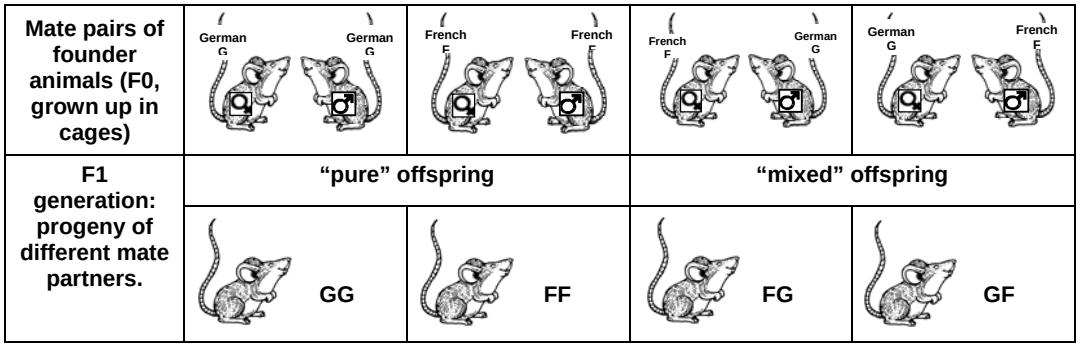


Figure 2.2: Schematic figure visualizing the animals in the F0 and F1 generations. Although not shown, also F2 and backcrosses were present in the long-term experiment. "G" stands for German population background, "F" for French background. In the F1, the population background of the mother stands on the left-hand side, of the father on the right-hand side.

Assortative mating could be based on different parameters, e.g. Smith (1966) states that genes acting as "assortative mating genes" could be genes affecting signals or behavior used in courtship. However, to disentangle on which parameters assortative mating is based on, is a second step, considered in the next chapter of the thesis, where the influence of different mate choice parameters is analyzed. In the present chapter I concentrate on the observation of assortative mating.

2.2 Methods

Four replicates of a long-term mate choice experiment with individuals of both populations were conducted in a semi-natural enclosure. The experiments started with a mouse density of 1.5 mice/m^2 with equal numbers of German and French mice in an equal sex ratio. Two experiments were carried out in parallel, and the two sets varied only in duration of the experiment and in the starting condition (Table 2.2). The present chapter focuses on paternity data which serve as measures for mate choice and are analyzed considering population background. Chapter 3 will describe results on the role of other parameters than population background for mate choice. In addition to the long-term experiment, a controlled cage experiment was carried out where females could chose between two partners without interference through male contest.

2.2.1 Long-term Experiment

Experimental mice

All mice used for the long-term experiment originated from wild mice caught in 2004 and 2005 (F2-F4) in the Cologne/Bonn area (German mice) or the Massif Central

(French mice). Populations were kept using an outbreeding scheme. From the age of weaning (21 to 28 days) mice were housed in unisexual groups in standard macrolon cages (Type III, Techniplast) at light-dark cycles of 12:12 hours. At the age of 40 days they were housed solitarily in Type II L cages.

Conditions during the long-term experiment

Of the four replicates, experiments I & III were carried out in enclosures of 24 m^2, while experiments II & IV were carried out in enclosures of 18 m^2. At the age of 20 - 52 weeks, 10 females and 10 males (experiments I & III) or 7 males and 7 females (experiments II & IV) were tagged with passive glass transponders (Datamars and AEG) and then released in the enclosures. The initial mice density was approximately 1.5 mice/m^2 in all four experiments. For experiments I & II, some of these "founder mice" were siblings, while in experiments III & IV all founder mice were non-siblings (Table 2.2).

Mice were held in the enclosures (Figure 2.3) for 5 to 6.5 months. Water and food (Altromin 1324) were supplied ad libitum. The light : dark cycle was 12:12 hours, the ambient temperature 20 – 23 °C, and relative humidity 50 – 65 %. Enclosures were equipped with bedding, straw, and housings. Structural variation was provided by wooden walls (40 cm high) and plastic tubes. A "dispersal tube" with several entrances allowed mice to escape from the population enclosure in a connected cage system via an aquarium as designed by Gerlach (1996).

At the end of the experiments, all animals were euthanized individually with a CO_2/O_2 mixture, followed by cervical dislocation. Dead animals were weighed and tail and body length were measured. Liver, spleen, and testis were dissected; spleen and liver served as tissue samples and were stored in HOM Buffer (80mM EDTA, 100mM Tris, 0.5% SDS) at -20°C while the testes were weighed, shock frozen in liquid nitrogen and stored at -80°C to allow future gene expression/transcriptome analysis. In case of pregnant females, embryos were taken out of the uterus separately, shock frozen in liquid nitrogen, and stored in 70% ethanol for subsequent paternity analysis. Cadavers of all dissected mice were stored in 70% ethanol and kept at 4°C.

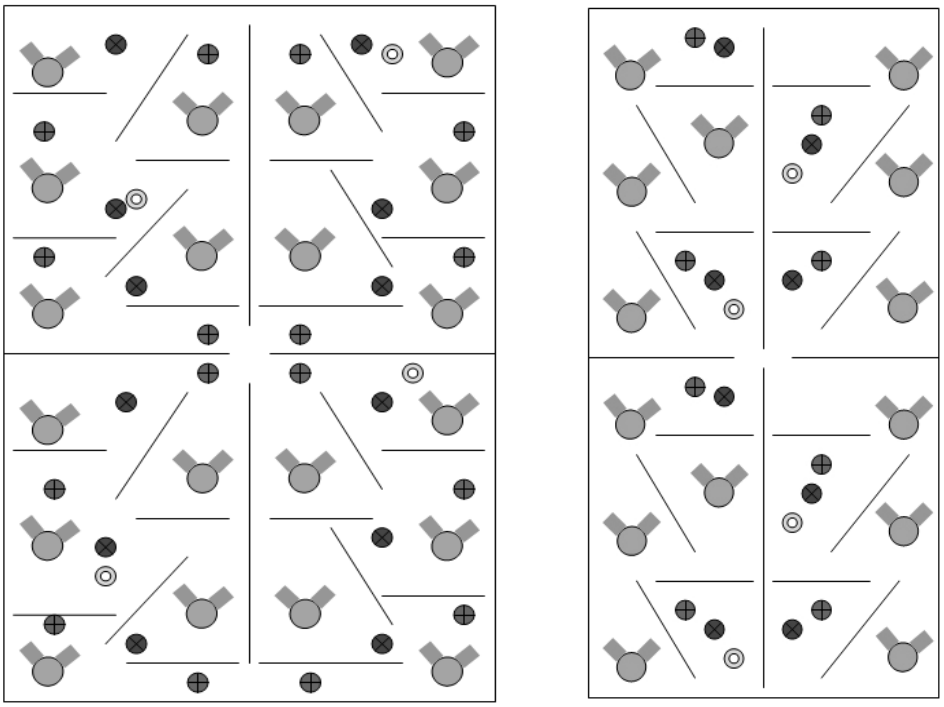


Figure 2.3: Enclosures used for experiment I and III (left) and II and IV (right). The biggest symbols show the localization of housings (bars demonstrate the two entrances). Lines represent wooden walls, lighter grey circles water bottles and darker grey circles feeding stations. The entrances to the dispersal tubes are shown as open circles.

Spatial association data

Animals born in the enclosures were tagged with an individual glass transponder at the age of 8 weeks (at a bodyweight of around 17 g). The identity of each mouse was assigned at the end of the experiment (see below).

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.

Monitoring of population development and individual condition

Every three to four weeks during the experiment, all mice were caught with live traps or by closing the houses and were checked for individual condition (check for bite marks, pregnancy), weight, and the existence of pups. During this monitoring activities, tissue samples of pups were also taken. Severely injured mice were taken out of the experiment and euthanized using a CO_2/O_2 gas mixture.

Genotyping for identity and paternity assignment

To obtain tissue samples of the founder mice, ears were clipped before the experiments, whereas offspring earclips were sampled at the age of 10-20 days during the experiment. Tissue samples from liver, spleen or tail were taken at the end of the experiment or at death in case animals died during the experiment. In this way, ideally two tissue samples from each animal were obtained which served for identity matching. DNA was extracted by salt extraction or with DNeasy 96 Blood & Tissue Kit (QIAGEN) following the "Purification of Total DNA from Animal Tissues" protocol with extended centrifugation times.

For each DNA sample, up to 14 microsatellite loci (Table 2.1) were typed by using the standard protocols of the QIAGEN Multiplex PCR Kit**.** Alleles were analyzed using Genemapper 4.0 (Applied Biosystems). Null allele frequency was estimated for each locus using the program CERVUS 3.0. None of the loci showed a null allele frequency higher than 0.05 and could be therefore used for identity matching and parentage analysis using the program CERVUS 3.0 (Kalinowski et al. 2007).

Table 2.1: Selected polymorphic microsatellites (described in Teschke et al. (2008)).

Locus	Number of alleles	Number of individuals typed	Observed heterozygosity	Expected heterozygosity	Estimated null allele frequencies
6G7	15	116	0.85	0.88	0.01
9C8	13	133	0.83	0.86	0.01
9F12	11	136	0.82	0.79	-0.02
3J6	16	135	0.87	0.90	0.02
8G7	17	130	0.85	0.90	0.02
6A4	14	136	0.84	0.88	0.03
7F9	10	134	0.87	0.84	-0.02
5H11	11	68	0.87	0.88	0.0001
9H5	14	133	0.93	0.87	-0.04
4C11	10	133	0.92	0.87	-0.03
7J6	11	133	0.85	0.85	-0.002
6G3	13	135	0.90	0.90	-0.0007
10C6	13	136	0.87	0.89	0.01
8H7	10	136	0.89	0.83	-0.03

Parentage analysis and identity matching

CERVUS 3.0 works with codominant, autosomal, unlinked genetic markers and assigns the most likely parent pair from a set of possible parents to the offspring.

Allele frequencies of the 14 loci were determined for all four experiments separately (including all animals in these analyses) and simulations for parentage assignment were run for 10.000 offspring previously to all parentage analyses, assuming 90% of possible parents sampled and typed with a minimum of 7 loci.

Prior to paternity assignment, birth dates of animals were determined by identity matching of individual genotypes with samples taken from 14 - 21 day old pups during the experiment (matching performed with CERVUS 3.0, min. number of typed loci: 7, max. number of allowed mismatches: 2). Following this analysis, animals could also be assigned to the litters from which they came and received, together with littermates, a special “Litter-ID”.

According to birth and death dates, animals were assigned as possible parents or offspring of defined experimental phases. Offspring of the first phase were born during the first three month of the experiment, while their possible parents were the founder animals. Possible parents of the second phase included offspring born in the first phase and founder animals (if still alive) and the offspring under consideration were born during the fourth and fifth month. Finally, offspring born until the end of the experiment were tested against possible parents born until the fifth month. Animals with uncertain birth dates were tested against all possible parents.

For paternity assignment, a maximum of 2 mismatches was accepted (following criteria from Araki & Blouin 2005, who allowed 2 mismatches for 8 microsatellite loci). The reliability of paternity assignment was verified manually for all individuals. Control factors taken into account were frequency of litters for individual females, age of assigned parents and paternity patterns in litters of females (e.g. a very high number of fathers in one litter is questionable).

Data Management

Data were managed using a self-constructed database in Microsoft Access 2002 (see Supplement). 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 experiment.

Statistical analysis

Statistical analysis was performed using SPSS (12.0) and Microsoft Excel (2002). For some analysis, p-values were calculated after obtaining chi-square values via Excel on the web site http://graphpad.com/quickcalcs. Two tailed t-tests for the paired comparisons and ANOVA for comparisons between more than two groups were used. The level of significance was set to 0.05.

The graphical presentation of data is mainly done with histograms or boxplots. For the latter case each box shows the median, quartiles and extreme values (outliers: cases with values between 1.5 and 3 box lengths from the upper or lower edge of the box, depicted as an open circle; extreme cases: values more than 3 box lengths from the upper or lower edge of the box, depicted as a star).

Measuring mate choice

Successful mating events (detected through paternity analysis) were taken as measures for mate choice. The use of “matings” and “mating events” in the text refer to successful matings (since matings were not observed directly).

2.2.2 Controlled female choice in a cage system

Experimental mice

Mice used in the cage experiments originated from the same wild mouse colonies as described above. Female and male siblings were separated after weaning and housed in different rooms to allow females to enter in anoestrus (Lee-Boot effect, Ma et al. 1998). Females were housed with two or three other females (sisters or non sisters), while the males were kept solitarily. Females were tagged with glass transponders. Both males and females were tested at an age of 30 – 60 weeks. At the beginning of the experiments mice were sexually inexperienced and female contact with male urine was avoided. The males were selected randomly (regarding size and weight). Females were used once (only two French females were tested repeatedly), while males were used in up to 4 experiments.

Cage system to test for female preference

The cage system consisted of three connected cages (Figure 2.4) which were all supplied with bedding, paper, wood wool, and egg carton for shelter. The female was

placed in the middle cage (macrolon, Type IV, Technoplast). To the left and right of this cage, two tubes connected the side cages (macrolon, Type III), which were subdivided into two parts by a perforated metal board. The cages were positioned allowing maximum distance between the males. The female could only enter one part of the side cages via the tube, while the male was placed in the other (closed) part. The location of the transponder-tagged female was registered by two antennas in each tube. The time at which a female passed the antennae and thus the time it spent close to each male was recorded. After each experiment, cages and tubes were thoroughly cleaned with water and ethanol. I used two such devices in parallel.

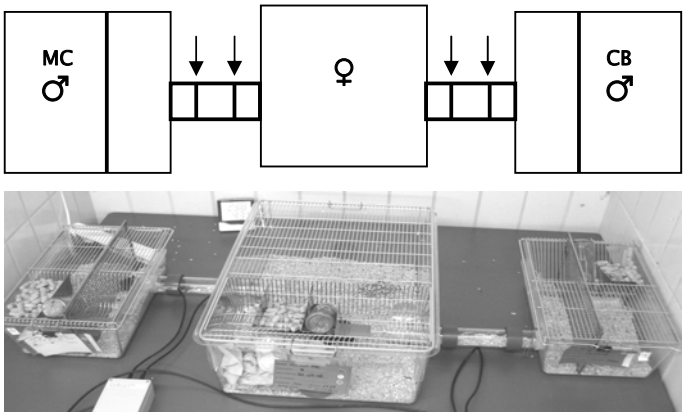


Figure 2.4: Experimental setup of the controlled cage experiment. **Above**: Diagram of the experimental device: the female was placed in the middle cage. Males of both populations were put into the left and right cages. The arrows indicate the positions of the inner and outer antennae to record the time a female spent at which side of the apparatus. **Below**: Photograph of the device.

Testing for side preference with test females

Before testing female preference for males in the system, several females were used to check general side attractiveness of the cage systems (e.g. through lower interferences) by recording the time these females spent in the side cages without the male stimulus. No general side preferences were observed: 4 females were tested in both used systems; comparing the means of relative time in each side cage did not show differences (cage device 1: p-value 0.55, cage device 2: p-value 0.91 paired t- test).

Procedures before, during and after the experiment

Right before an experiment started, females were weighed and placed in the middle cage of the choice apparatus. Males were also weighed and placed each one in the outer cages, selecting randomly the site for the different populations. Animals were left for 6 days in the apparatus, during which time movements of the female and the time it stayed in the outer cages was recorded. Well-being of mice was checked carefully without disturbing the animals. Food and water was supplied ad libitum.

2.3 Results from the Long-term Experiment

2.3.1 Population development

Mice of the two populations were left in the enclosures for 5 months (experiments I and II) and 6 ½ months (experiments III and IV). The experiment duration was extended in the second trials (experiments III and IV) in order to get more mice in advanced generations (after analysis of the first trials showed relatively few F2 animals).

During all four experiments, population densities increased considerably. Starting with 1.5 mice/m^2 population densities increased to 2.5 – 12.9 adult animals /m^2. The differences in population densities of experiments I and II in comparison with experiments III and IV were due to the longer experimental duration of the latter experiments. Surprisingly, despite a considerable increase in population densities, only 3 males escaped from the enclosures via the dispersal tube, all of which were juveniles at an age of approximately 30 days.

Table 2.2: Summary of population parameters for the four enclosure experiments.

	Exp I	**Exp II**	**Exp III**	**Exp IV**
Duration of experiment	147 days from 2nd of April to 27th of August 2008		196 days from 8th of October 2008 to 22nd of April 2009	
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	1.5 mice/m^2	1.5 mice/m^2	1.5 mice/m^2	1.5 mice/m^2
Initial spatial separation	F and G animals were initially separated for 7 days by dividing the enclosure in two parts		No separation of the two populations during the first week (both populations were immediately together)	
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
First litter born	5/12/2008	5/12/2008	11/1/2008	11/7/2008
Total number of animals recorded including embryos, dead pups, and newborns	193 mice	133 mice	647 mice	386 mice

In 3 out of 4 replicates, no significant deviations from equal sex ratios were detected when considering all animals (with the exception of embryos, animals which were newborn at the end of the experiment, and dead pups). Exp IV showed a significant male overrepresentation (chi-square test, p-value: 0.04, Figure 2.5, left side). The operational sex ratio (measured as the number of adult animals (>13 g) at the end of the experiment) gave no significant sex ratio distortion.

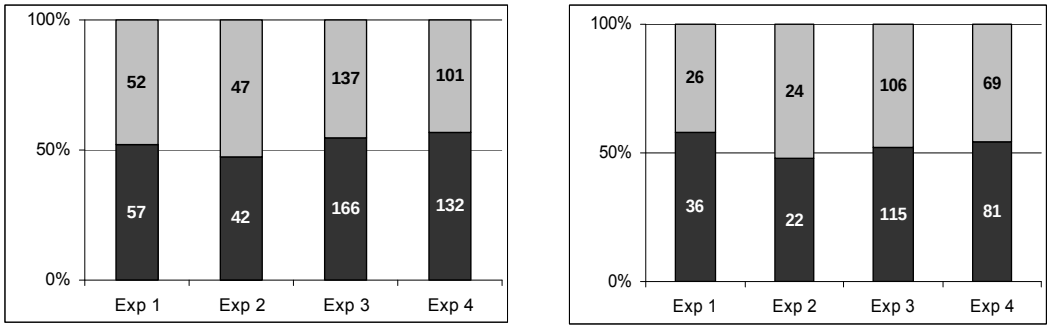


Figure 2.5: Sex ratio in the four replicates: **Left side**: sex ratio considering all animals monitored during the experiments. **Right side**: operational sex ratio (adults > 13 g) at the end of the experiments. Lighter grey: females, darker grey: males. Males were slightly overrepresented; however, in most cases the deviation from equal sex ratios was not significant. Only replicate IV showed for the overall sex ratio a significant male overrepresentation (chi-square test, p-value: 0.04).

The sex ratio of pups (recorded during tissue sampling approximately at the age of 14 days, data only available for Exp III and IV) showed a stronger sex ratio deviation (Figure 2.6). Binomial testing indicates that males in Exp III were significantly more times overrepresented when only pups are analyzed (binomial testing, p-value: 0.04). Exp IV didn't show such a strong skew towards males, but at the two last sampling dates, males were significantly overrepresented. These results coincide with an elevated population density towards the end of the experiments.

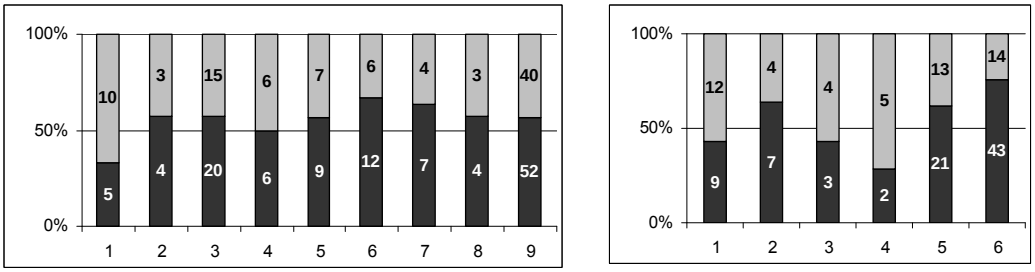


Figure 2.6: Sex ratio of offspring sampled at different dates. **Left side**: Exp III, **right side**: Exp IV. Lighter grey: females, darker grey: males. The absolute numbers of animals are indicated in the bars. Binomial testing showed that males in Exp III were significantly more often overrepresented when only pups were analyzed (p-value: 0.04). Experiment IV didn't show such a skew towards males, but at the last sampling date, males were significantly overrepresented.

2.3.2 Paternity assignment

A total of 1,220 offspring were analyzed for paternity. Paternity assignment was reliable, as determined in case of the embryos, where the mother was known. Nevertheless, in 53 cases (4.3%), paternity had to be changed manually, and in 96 cases (8%), paternity problems could not be solved and offspring remained "unassigned". One reason for these problems was the high degree of inbreeding, especially at the end of the experiments. The unassigned offspring were excluded from the analysis. A total of 1,124 offspring (92%) were assigned successfully.

After paternity analysis, the origin of 1,103 offspring was determined. In some cases, although the overall paternity analysis was successful, the origin of the pups could not be determined, resulting from unsolved paternity of one parent. Figure 2.7 gives an overview of the distribution of offspring within and between populations.

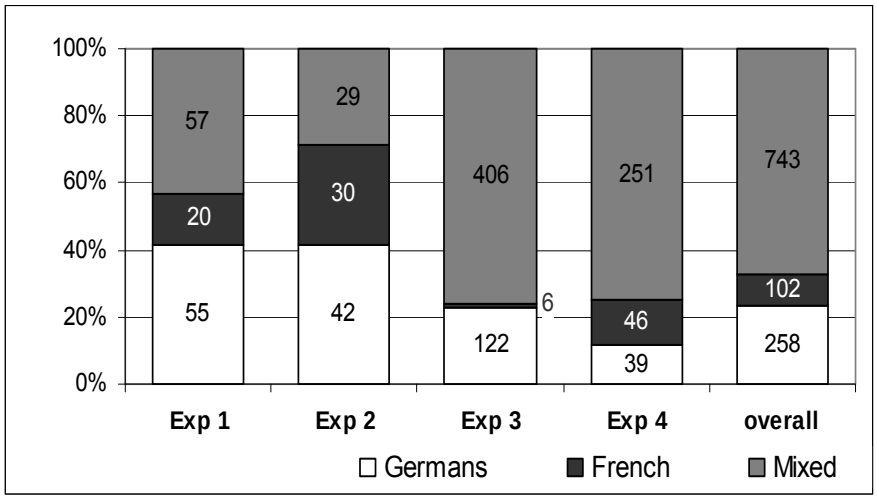


Figure 2.7: Relative proportion of "pure" German and French animals, as well as "mixed" animals. Absolute numbers of animals are indicated in the bars. Shown are all offspring of which population background could be assigned.

Animals descendent from individuals of the Cologne/Bonn population will be referred to as "German" or "pure German" individuals, and in figures and tables, the letter G is used for these. Animals originating from individuals from the Massif Central region will be labeled "French" or "pure French" individuals (F), and in figures and tables the letter F stands for these. Offspring originating from individuals of both populations are termed "mixed animals" and they are abbreviated with the letters GF or FG. Later on, the distinction between the origins of the mother and the father will be important. Here, the abbreviation for population background starts with the origins of the

mothers on the left-hand side and the origin of the fathers on the right-hand side; thus, a progeny of a German mother and a French father will be abbreviated GF, while an individual with both parents from the French population is abbreviated FF, etc. (see also introduction, **Figure 2.2**).

Assigning individuals to generations revealed the distribution of F1, F2 and "backcrosses" as shown in Table 2.3. As backcrosses, I refer to animals which result from mating events between animals of different generations.

Table 2.3: Distribution of animals (assigned to parents) over the generations in all the different replicates and the entire experiment.

Generation	Exp 1	Exp 2	Exp 3	Exp 4	Overall
F0	40	28	40	28	136
F1	113	71	133	91	408
F2	4	6	241	178	429
F3	0	0	1	0	1
Backcross F0/F1	15	23	108	58	204
Backcross F1/F2	0	0	45	5	50
Others or not clear	0	1	27	4	32
not assigned to parents	21	4	52	22	96
Overall	193	130	647	358	1328

2.3.3 Assortative mate choice

Mate choice

The fundamental data for the mate choice analysis are the successful mating events. These data were obtained through paternity analysis, i.e. only mating events which resulted in offspring could be considered. Mating events which did not result in fertilizations or where embryos died before birth could not be taken into account.

378 mating events were recorded in the four experiments. Four events could not be analyzed for population assortative mating, because the origin of one or both partners could not be determined. A total of 374 mating events remained for analysis.

Mating events between founder animals (125 events), between F1 animals (156 events) and backcross events were analyzed separately because founder animals had a different life history than the animals born in the enclosures which is expected to effect their mating behavior. The remaining events present pairings between F0 and F1 animals ("backcrosses") (76) and very few mating events between individuals with backcrosses. Mating events between founder animals are shown in Figure 2.8.

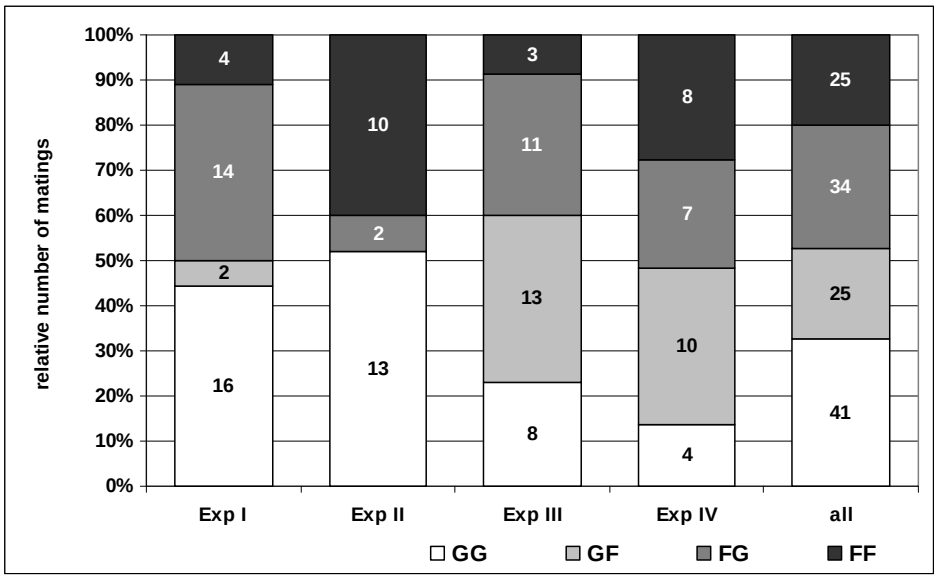


Figure 2.8: Relative proportions of mating events between individuals of F0 of both populations for all 4 replicates separately and together. Mating events between partners from the German population are shown in white, mating events of partners from the French population in black. Lighter grey are mating events where the female was G, and the male F, darker grey vice versa. The numbers in the bars refer to the total number of mating events observed for the different combinations.

The analysis for assortative mating showed no consistent pattern between the different experiments. Examining the different experiments showed similar results for experiments I and IV (Exp I: chi-square=0.8, df=1, p-value: 0.4; Exp IV: chi-square=1.01, df=1, p-value: 0.3), indicating random mating in regards to population background. Experiment II showed a very strong indication for assortative mating (chi-square=18.06, df=1, p-value < 0.0001) whereas experiment III pointed towards disassortative mating (chi-square=5.55, df=1, p-value: 0.02). This heterogeneity in the chi-square values showed that for the analysis of mating preferences in the founder generation the four experimental replicates could not be pooled. Leaving animals heterozygous for the t haplotype (a selfish genetic element, see chapter 3) out of the analysis (Figure 2.9) also did not allow the analysis of pooled data (chi-square values: Exp I: 1.1, Exp II: 17.06, Exp III: 0.28 and Exp IV: 1.8, chi-square for heterogeneity: 16.19, df=3, p-value: 0.01).

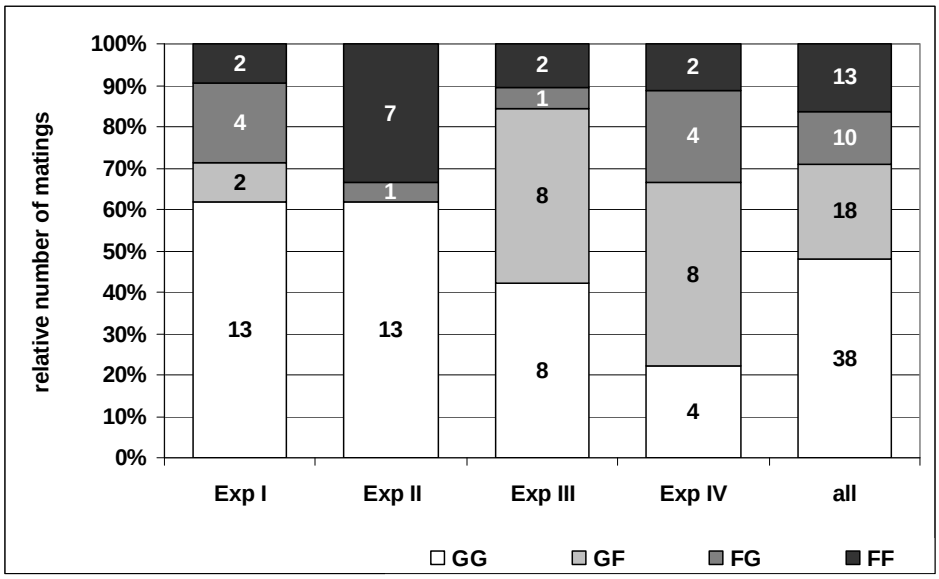


Figure 2.9: Matings between F0 animals, excluding t haplotype animals from the analyses. The heterogeneity test rejected homogeneity of the results from the four experiments (p-value: 0.01). Chi-square values of the different experiments are documented in the text.

Analyzing the mating events between animals of the F1 generation (156 successful mating events), no clear patterns for assortative or disassortative mating were observed (Figure 2.10). A contingency test showed a high chi-square value (89.5), which results in a p-value < 0.0001 (df=9), indicating unequal distribution of matings between individuals with different population background.

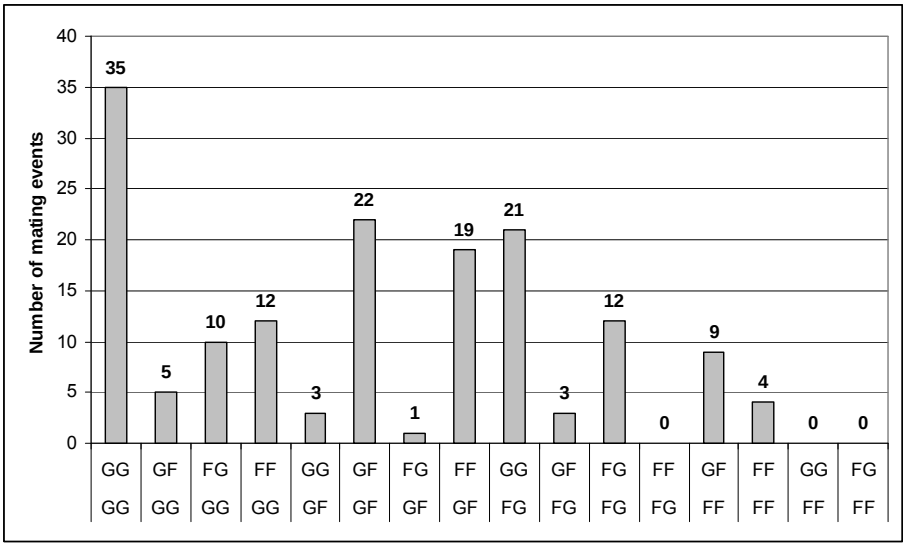


Figure 2.10: Number of successful mating events between F1 animals. X axis displays pairs. Upper part: female. Lower part: male

As depicted in Figure 2.11, a significant pattern indicating an assortative mating with regard to the father of the mate partner was observed. This means, if an individual descendent of, for example a French male, it mated preferentially with a partner also descendent of a French male. This pattern appears also in other parts of the thesis, and will be referred to as the *father related assortative mating pattern*. It is, taking all experiments together, highly significant (2X2 contingency table: chi-square = 77.22, p-value < 0.0001). For experiment I, only one F1-F1 mating was reported and for experiment II, only four F1-F1 matings were reported; these follow in all cases the above described pattern. F1-F1 matings in experiment III match significantly the pattern (chi-square: 76.67, p-value < 0.0001). For experiment IV, the analysis suggested no significance for the pattern (chi-square: 1.52, p-value: 0.2). However, when animals heterozygous for the t haplotype were taken out of the analysis, the *father related assortative mating pattern* was observed to be significant even for experiment 4 (Fishers Exact Test, p-values: all experiments: < 0.0001, experiment 3: < 0.0001, experiment 4: 0.001).

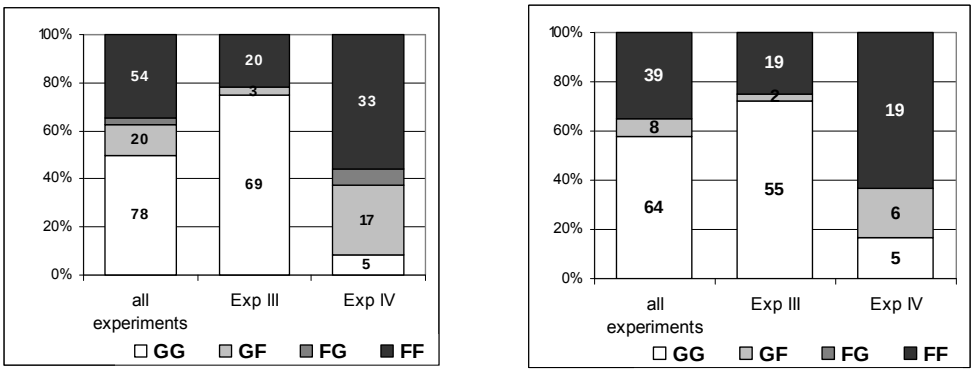


Figure 2.11: Mating events between F1. White and dark: father of mate partners from same population, grey: fathers from different populations. **Left**: Matings of all F1 animals included. **Right**: Only wildtype animals considered (t haplotype animals excluded).

This procedure was justified by a heterogeneity test, which was conducted to assess whether replicates III and IV can be pooled. The results showed that, without excluding the t haplotype animals, the replicates can not be pooled (chi-square: 5.13, 1 df, rejecting hypothesis of homogeneity of results with p < 0.02). When excluding the animals heterozygote for the t haplotype from the analysis, the homogeneity can not be rejected (chi-square: 0.55, df=1, p-value: 0.46).

For the analysis of the backcrosses, I distinguished between

a) matings where the female came from the F1 and the male from the F0 generation
b) matings where the female came from the F0 and the male from the F1 generation.

For all analyses, I pooled the results from all experiments, since it was not possible to statistically analyze them separately for the different experiments as numbers were too small to perform tests.

When considering all matings for the case a) (Figure 2.12), no deviation from random mating (in regards to population background) was found (chi-square: 2.59, df=3, p-value: 0.46). Excluding animals heterozygous for the t haplotype did not change the picture (chi-square: 2.98, df=3, p-value: 0.4).

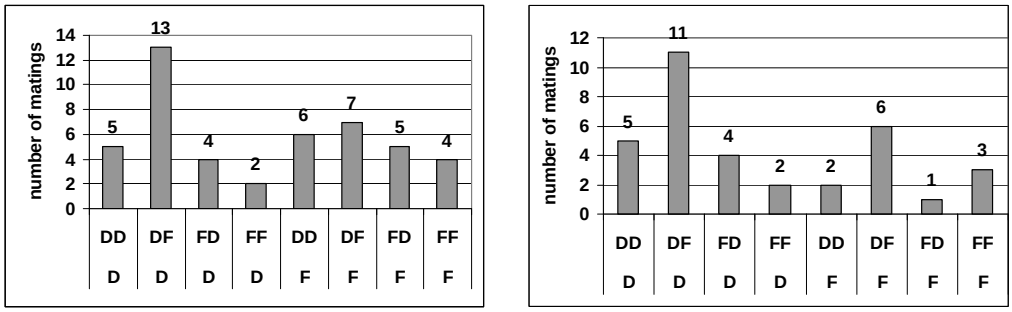


Figure 2.12: Number of successful mating events between a female from the F0 generation. **Left**: all animals, **right**: t haplotype animals were excluded. Matings were statistically equally distributed.

Testing for the father related assortative mating pattern was not significant (chi-spare: 0.73, df=1, p-value: 0.4.)

When considering all matings for the case b) (Figure 2.13), testing with a contingency table chi-square showed a statistically significant deviation of the distribution of matings from equality (p-value: 0.0008). This was also observed when testing only wildtype animals.

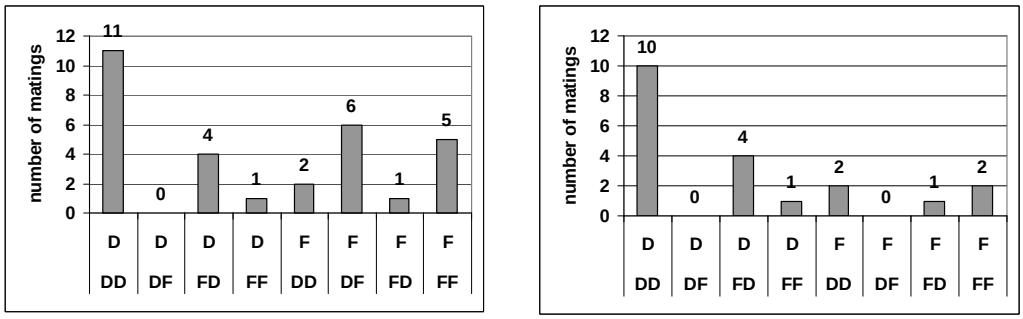


Figure 2.13: Number of successful mating events between a female from the F1 generation and the male from the F0 generation. **Left**: all animals, **right**: t haplotype animals were excluded. A deviation from equal distribution was found to be statistically significant (chi-square: 16.64, df=3, p-value: 0.0008). Without t haplotype animals, the deviation was still statistically significant (chi-square: 9.16, df=3, p-value: 0.03).

Testing for the *father related assortative mating pattern* was significant when considering all animals (chi-spare: 16.27, df=1, p-value < 0.0001). When considering only wildtype animals, the pattern was not quite statistically significant (p-value: 0.07) (Figure 2.14).

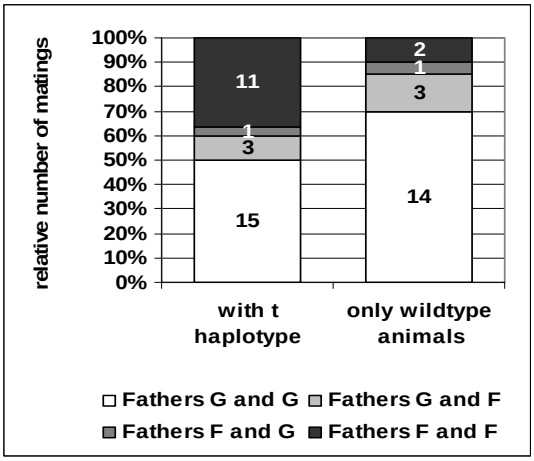


Figure 2.14: Mating events between F1 females and F0 males analyzed for the father related assortative mating pattern. White and dark: father of mate partner came from the same population, grey: fathers from different populations. Left bar: all animals analysed, right bar: animals with t haplotype excluded

A second way to test the validity of this *father related assortative mating pattern* was to analyze the observed pairs (in contrast to the successful mating events, repeated mating in different reproductive cycles between the same two animals was only counted once), which was done for the F1-F1 matings (Figure 2.15). By performing the heterogeneity test including all animals, homogeneity of the results from experiment III and IV had to be rejected (heterogeneity test, chi-square: 10.23, df=1, p-value: 0.001). When excluding the t haplotype animals, results could be pooled (heterogeneity test, chi-square: 1.97, df=1, p-value: 0.16).

The data clearly indicated the father related assortative mating pattern for pairs excluding t haplotype animals (experiment III: chi-square: 42.11, df=1, p-value: < 0.0001; experiment IV: chi-square: 6.3, df=1, p-value: 0.01).

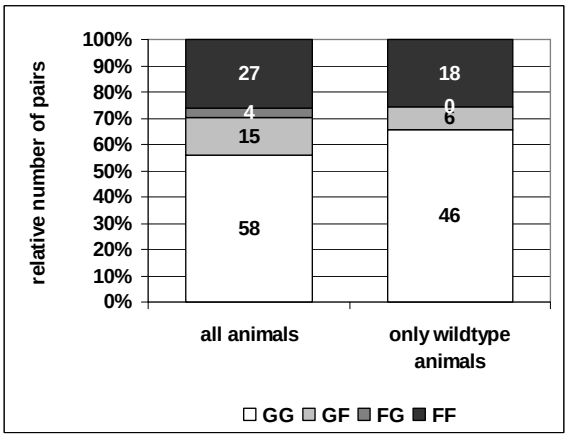


Figure 2.15: Pairs with mates of father with same (black and white) versus different population background.

2.3.4 Reproductive success

Successful individuals

Animals were considered “adult” at a minimum weight of 13 gram. Within all experiments, 123 out of 305 (40.3%) adult females and 97 out of 278 (34.9%) males had offspring. The ratio of successful males and females was analyzed according to their population background.

Considering the founder generation, there were no significant differences between the populations (Fisher’s Exact Test for F0 females: 26 G vs. 21 F, p-value: 0.29; F0 males: 21 G vs. 19 F, p-value: 0.80).

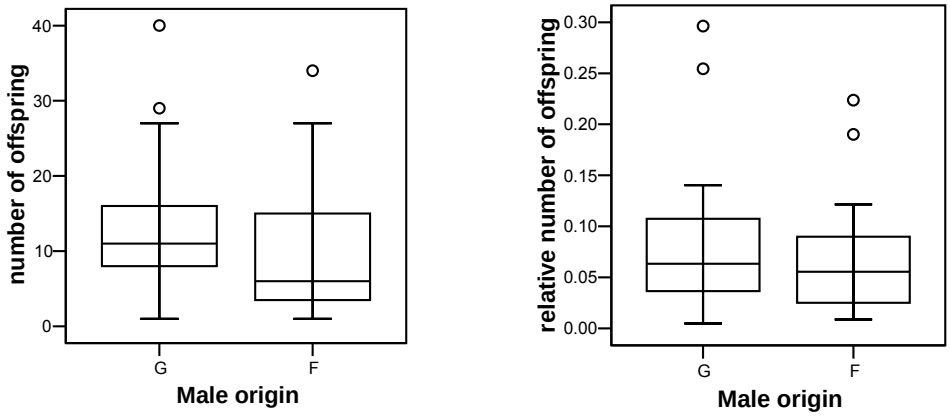
Among the F1, significantly more GG females were successful than FF females (Fisher’s Exact Test, p-value: 0.02; GG females: 25/42, FF: 7/24 successful). There were no significant differences between the “mixed” females (GF: 15/29 vs. FG: 17/29, p-value: 0.79). The numbers of successful females between “pure animals” and “mixed animals” were not significantly different (pure: 33/78; mixed: 40/119, p-value: 0.23). For males born in the enclosures, the results were similar: the analysis of “pure” vs. “mixed” animals (without F0) gave a p-value of 0.2 (Fisher’s Exact Test, “pure” males: 28/78, “mixed” males: 30/124 successful). F1 males were equally successful among the different population backgrounds.

Individual reproductive success

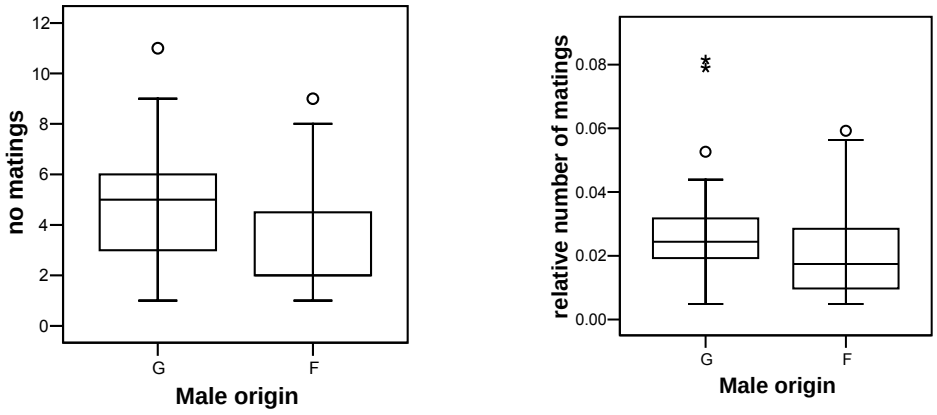
Individual reproductive success was analyzed as the number of offspring, the number of mating events and for females the number of offspring per litter and for males the number of offspring per mating event.

Again, the analysis was separated between founder animals and animals born during the experiment. Additionally, absolute numbers of offspring (all offspring an individual had during the experiment) were contrasted with relative numbers of offspring. The latter value is the number of offspring divided by the days an individual had been in the enclosure (until its death or the end of the experiment); a value only available for animals from which the exact dates of birth and death were known (with an accuracy of around 7 days). The “relative offspring number” takes into account that individuals born towards the end of the experiment had less opportunity to mate. The same logic was applied when analyzing the number of mating events per individual.

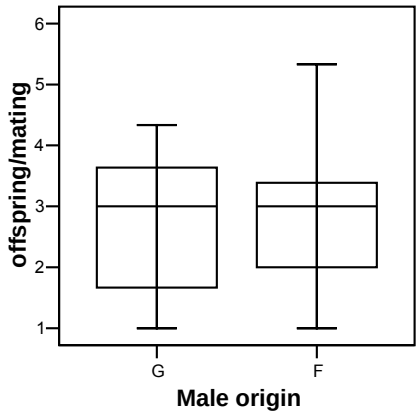
Founder animals (F0) were analyzed for reproductive and mating success and parameters did not differ significantly between populations (Figure 2.16).



A: Absolute (**left**) and relative (**right**) offspring numbers for F0 males. Mean number of offspring: F: 10.47, G: 13.19, t-test, p-value: 0.38 (absolute values) and p-value: 0.526 (relative values).



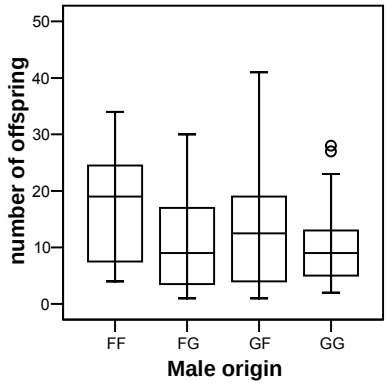
B: Numbers of mating events for F0 males. **Left**: Absolute numbers (mean number of matings: F: 3.42, G: 4.75. t-test, p-value: 0.17). **Right**: Relative mating numbers (p-value: 0.28).

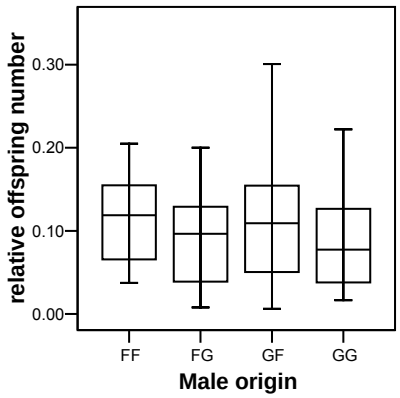


C: Average offspring number per mating analyzed for G and F males of founder generation (t-test, p-value: 0.62).

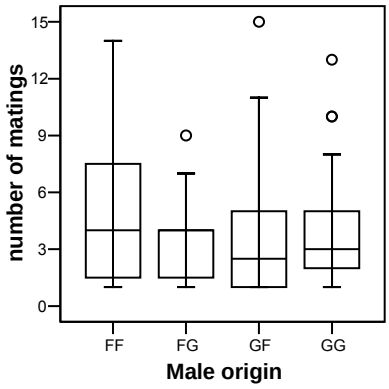
Figure 2.16: Reproductive success of F0 males (N_G: 21, N_F: 19). No significant differences were detected between G and F males.

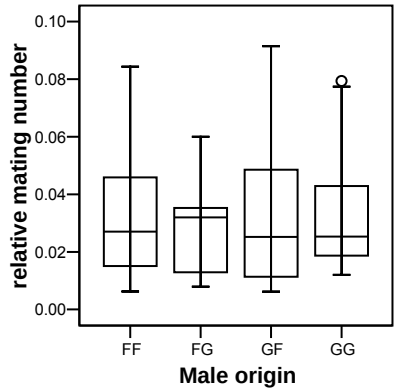
For the F1 generation, nearly all parameters for reproductive success were similar for the different population backgrounds. The only significant difference was the number of offspring per mating between GG and FF males indicating a higher offspring per mating number for FF males (Figure 2.17).



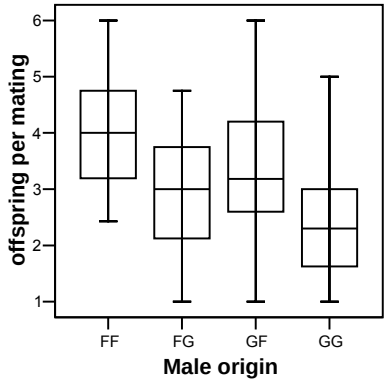


A: Number of offspring (absolute and relative) for F1 males. ANOVA testing indicated no significant difference for the different population backgrounds (p-value: 0.54).





B: Number of mating events of F1 males. Animals of different population background did not differ significantly in the absolute or relative number of mating events (ANOVA absolute: p-value: 0.841, relative: p-value: 0.846).

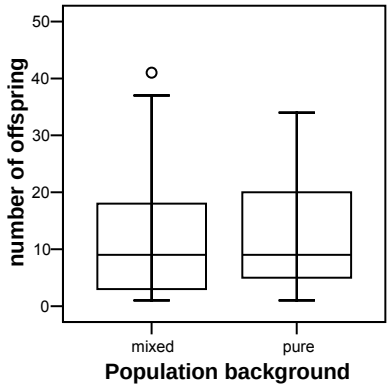


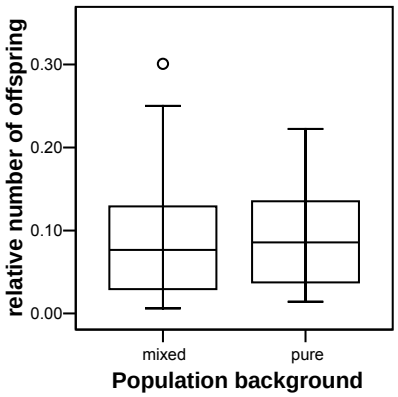
C: Average offspring number per mating. ANOVA p-value: 0.074.

Significant higher value for FF males in comparison with GG males (t-test p-value: 0.009). The average numbers of offspring per mating were: GG: 2.54, FF: 4.05. GF: 3.22, FG: 2.92.

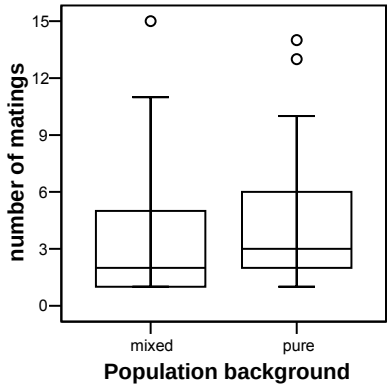
Figure 2.17: Reproductive success of F1 males of different population background (N_{GG}: 17, N_{FF}: 7, N_{GF}: 14, N_{FG}: 11).

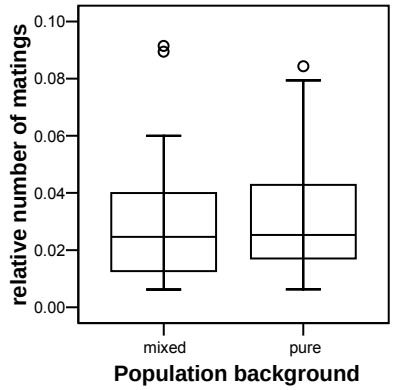
The reproductive success for all “pure” vs. “mixed” males born in the enclosure (Figure 2.18) was analyzed. No significant differences were observed.



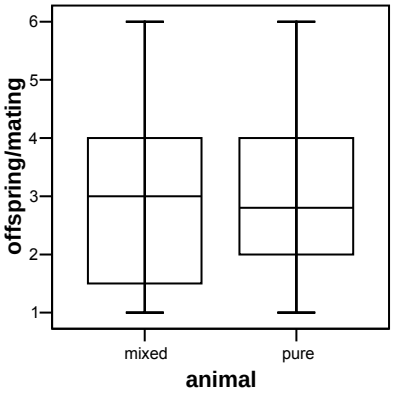


A: Offspring number (absolute and relative) for individuals born in the enclosure. p-values t-test: absolute values: 0.761, relative values: 0.896.





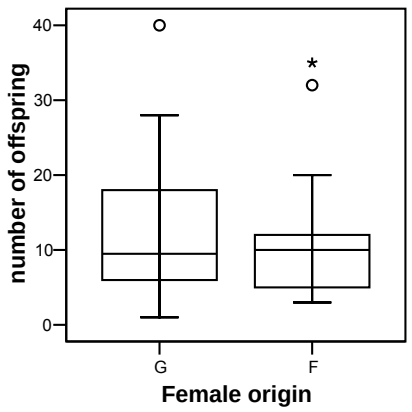
B: Number of mating events (absolute and relative values) for males born in the enclosure. No significant differences were observed (p-value t-tests: absolute values: 0.39, p-value relative values: 0.46).

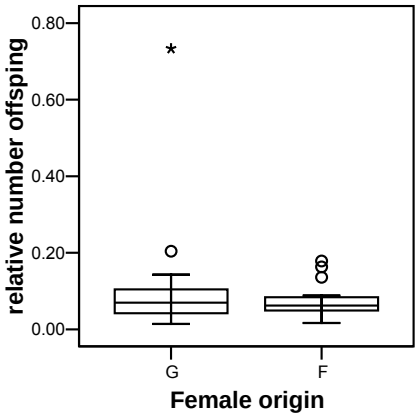


C: Average offspring number per mating. No significant difference was detected (p-value: 0.97).

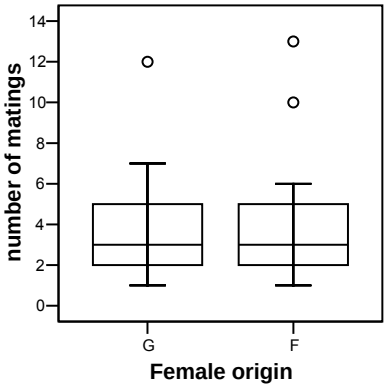
Figure 2.18: Reproductive success of males born in the enclosure, comparing “pure” and “mixed” population background, N_{pure}: 25, N_{mixed}: 29. No significant differences were observed.

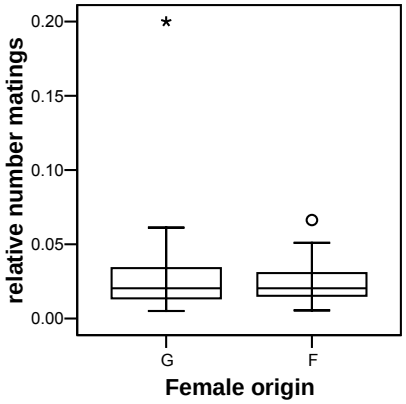
As previously shown for males, females were analyzed for their reproductive success. Results are shown for F0 (Figure 2.19), F1 (Figure 2.20) and all animals born in the enclosure (Figure 2.21).



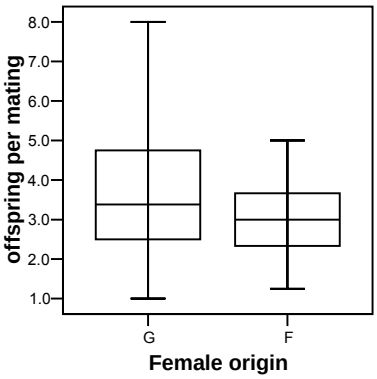


A: Number of offspring (absolute and relative values) for F0 females. No significant differences were detected (absolute values: t-test p-value: 0.62, mean for F: 10.95, mean for G: 12.23; relative values: p-value: 0.37).





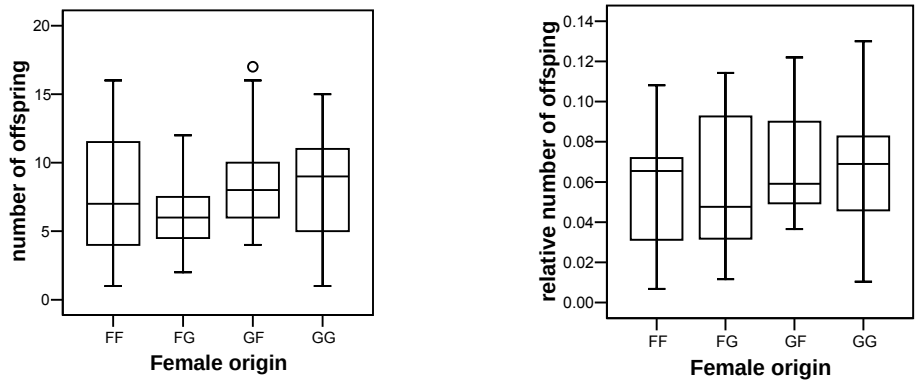
B: Number of mating events (absolute and relative values). Absolute values: p-value: 0.64, mean for F: 3.95, mean for G: 3.58. Relative values: p-value: 0.59.



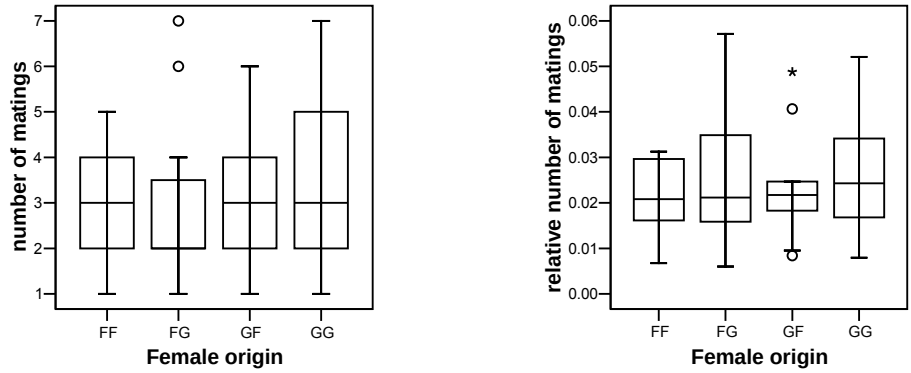
C: Average offspring number per mating for F1 females (t-test p-value: 0.18).

Figure 2.19: Reproductive success of F0 females (N_F: 21, $N_{G:}$ 26). No significant differences were found.

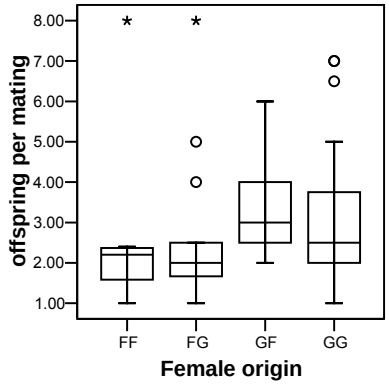
For F1 females, there were no significant differences, except for the absolute numbers of offspring in some pairwise comparisons (Figure 2.20).



A: Offspring numbers (absolute and relative values) for F1 females. No significant differences were found. Absolute values: ANOVA p-value: 0.11, means: GG: 8.36, FF: 7.86, GF: 9.24, FG: 5.93. Relative values: ANOVA p-value: 0.69.



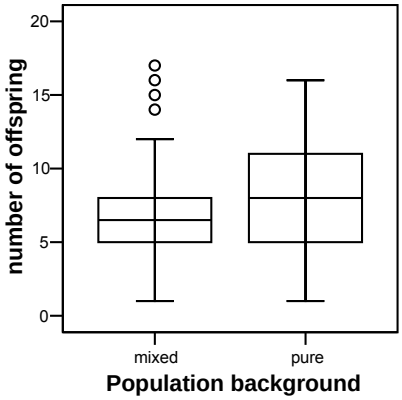
B: Mating events (absolute and relative values) for F1 females. Mean values: GG: 3.16, GF: 3.06, FF: 3.0, FG: 2.87. No significant differences were detected (ANOVA absolute values, p-value: 0.96, relative values, p-value: 0.78).

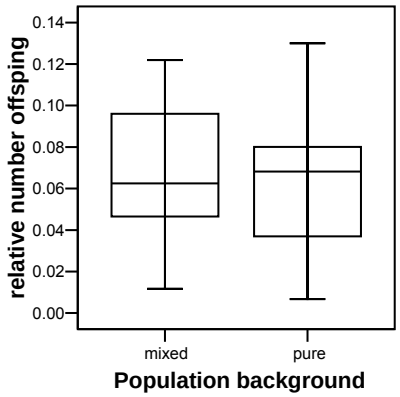


C: Average offspring number per mating for different population backgrounds. No significant differences were found (ANOVA: p-value: 0.62).

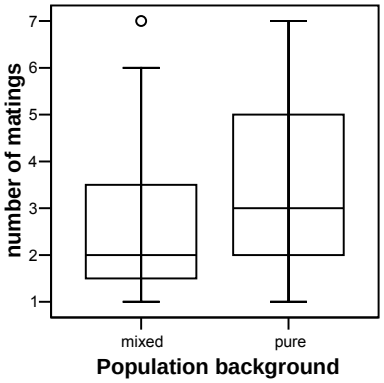
Figure 2.20: Reproductive success of F1 females (N_{FF}: 7, N_{GG}: 24, N_{GF}: 17, N_{FG}: 14). No significant differences were found, with the exception of the absolute numbers of offspring which differed significantly for two pairwise comparisons (GG-FG: p= 0.041, GF–FG: p=0.013). However, relative numbers did not differ significantly.

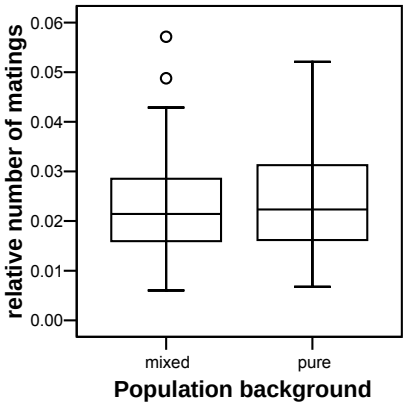
Females born in the enclosure with “pure” and “mixed” population background showed no significant differences (Figure 2.21).



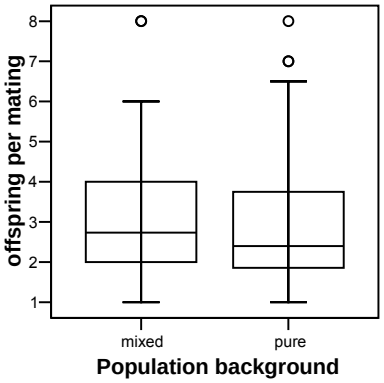


A: Offspring number (absolute and relative values) for females born in the enclosure. No significant differences were found (absolute values: t-test p-value: 0.349, relative values: p-value: 0.53).





B: Number of mating events (absolute and relative values). No significant differences were found (absolute values: t-test p-value: 0.30, mean values “pure” females: 3.06, “mixed” females: 2.68; p-value for relative values: 0.88).



C: Average offspring number per mating. P-value (t test): 0.76. Mean “pure” females: 3.07; mean “mixed” females: 3.2.

Figure 2.21: Reproductive success for females born in the enclosure. Compared were “pure” vs. “mixed” females (N_{pure}: 33, N_{mixed}: 40). No significant differences were found.

Combined analysis of reproductive success

Although the measures for reproductive success did in most cases not differ significantly between F and G (founder animals) and “pure” and “mixed” (others than founders) animals, a Sign test was conducted to evaluate whether the overall reproductive success depended (even slightly) on population background. Results are summarized in Table 2.4. Although differences are visible, they are not statistically significant.

Table 2.4: Table summarizing comparisons of measures for reproductive success between groups with different population background

	G versus F (founder animals)		“pure” vs “mixed” (F1)	
	Males	females	males	females
Proportion of successful individuals	+	+	+	+
absolute no of offspring	+	+	+	+
relative number of offspring	+	-	+	+
Absolute no of matings	+	+	+	+
Relative number of matings	+	+	+	+
Offspring per mating	=	+	-	-
Sum	**5:1**	**5:1**	**5:1**	**5:1**
P-value Sign test (one sided)	0.11	0.11	0.11	0.11

Relative fertilization success

The average fertilization success of individuals was calculated for males which sired offspring in multiple paternity litters. Single father litters were excluded, as it was not possible to distinguish between one father litters where only one male inseminated the female and those where only one male fertilized all ova, despite of inseminations of several males.

The number of offspring sired per litter was analyzed for each male; the average relative fertilization success was then calculated as the mean of the values of the different mating events. Figure 2.22 shows the data for F0 and F1 males. No significant effect of population background on the individual average fertilization success was found.

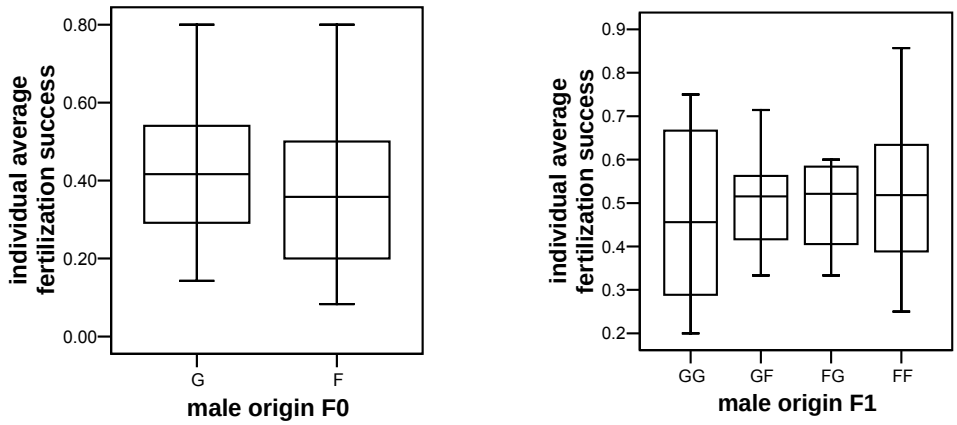


Figure 2.22: Individual average of fertilization success for F0 males and F1 males analyzed considering population background. **Left**: F0: p-value t-test: 0.35 (N_F: 14, N_G: 15). **Right**: F1: p-value ANOVA: 0.86 (N_{FF}:8: N_{FG}: 8, N_{GF}:11, N_{GG}:14).

Furthermore, it was investigated whether the relative fertilization success of a male depended on the population background of the female (relative fertilization success in intra-population matings versus inter-population matings). Thus the fertilization success of males in the different matings was calculated. Figure 2.23 shows that the relative fertilization success was significantly higher in mating events with a partner from the other population than with a partner from the same population.

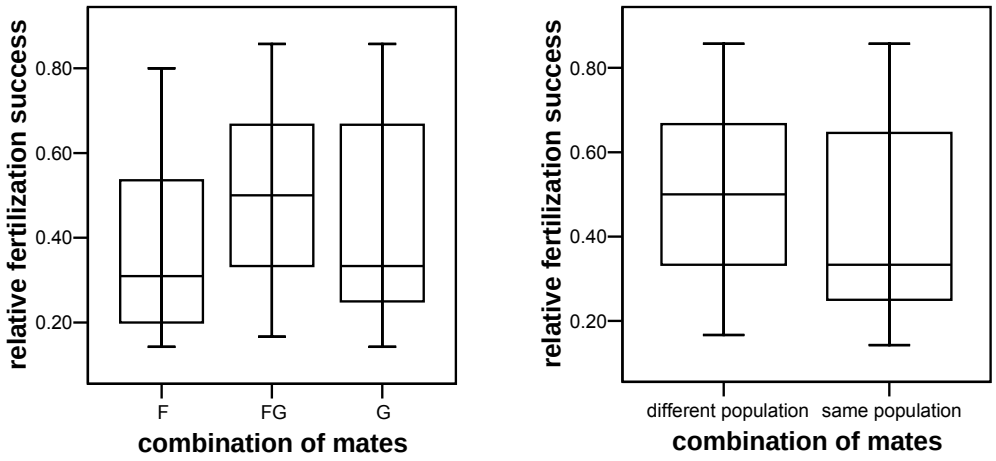


Figure 2.23: Relative fertilization success in intra- ("F" and "G") versus inter-population matings ("FG"). **Left**: Separating between F (N=20) and G (N=43) as intra-population mating and FG as inter-population mating (N=82). P-value (ANOVA): 0.047. **Right**: Intra-population matings F and G taken together. P-value (t test): 0.025.

Considering the *father assortative mating pattern* (see Figure 2.11 and Figure 2.14.), relative fertilization success in matings was analyzed for case A (mate partner with both fathers from the same population) versus case B (mate partner with fathers from different populations). No significant differences were found (Figure 2.24).

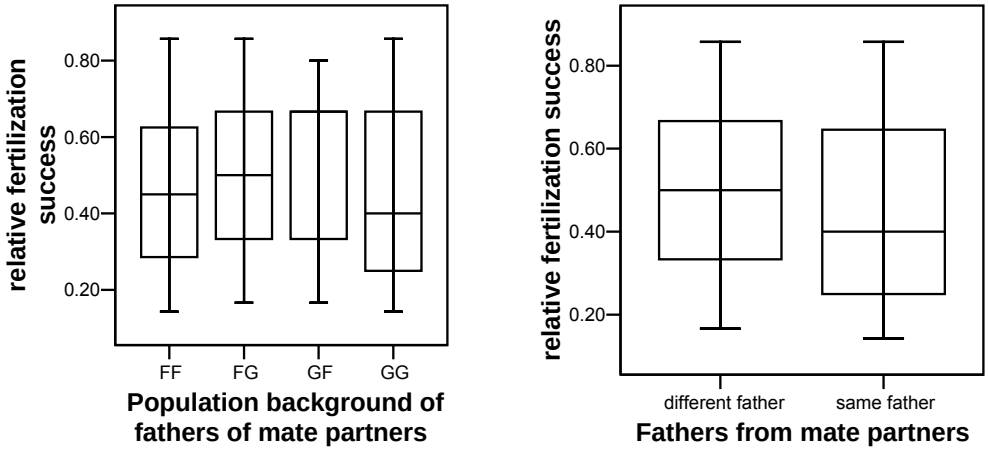
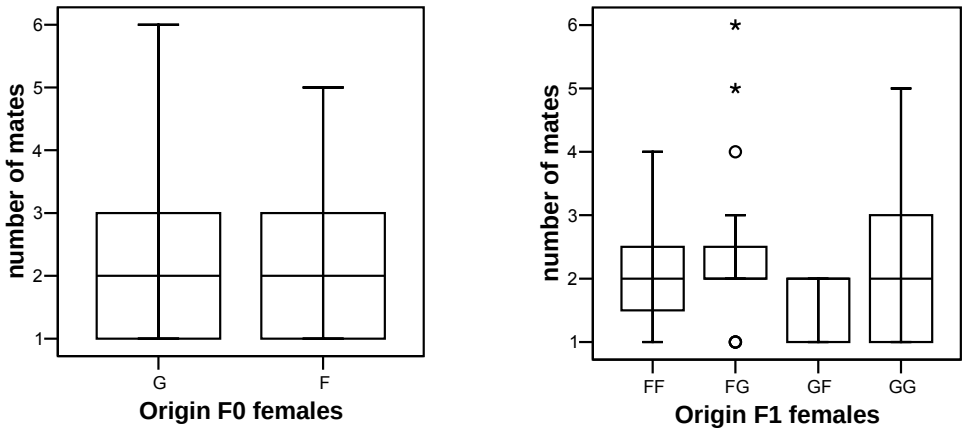


Figure 2.24: Relative fertilization success in matings between mates of same and different father. **Left**: FF: Mates with fathers from French population (N=42). GG: Mates with fathers from German population (N=61). FG: Mates where female's father was from French and male's father from German population (N=28). GF: Mates with female's father from German population and male's father from French population (N=14). No significant differences are found (ANOVA p-value: 0.59). **Right**: mates with fathers of the same population (N=103) and mates with fathers of a different population (N=43) were grouped. Differences were not statistically significant (t-test p-value: 0.19).

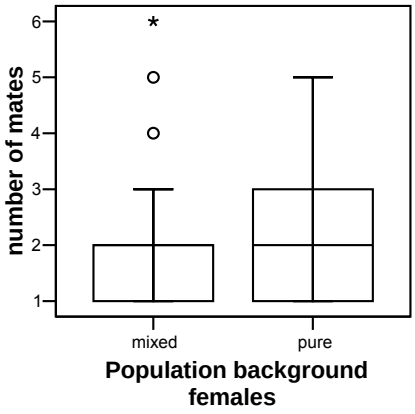
Individual attractiveness

The number of mates of males and females was recorded and analyzed regarding population background. Similarly to the analyses of reproductive success, founder animals and F1 animals were treated separately.

For the females, no significant difference was observed (Figure 2.25).



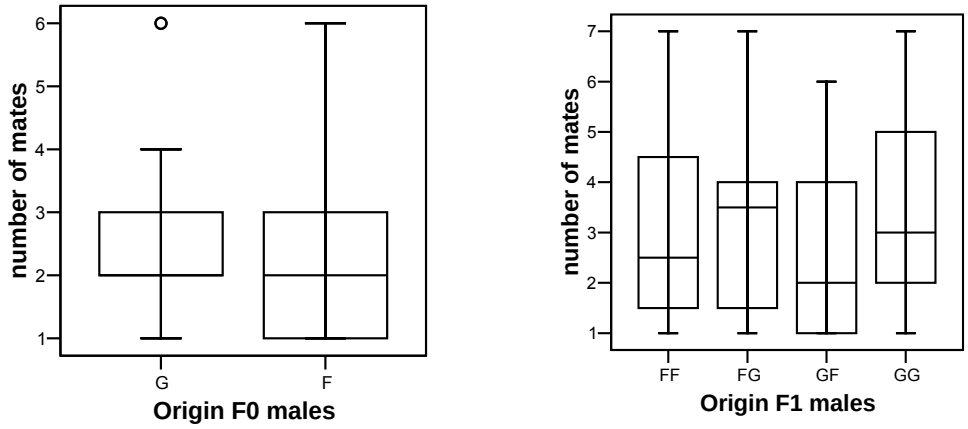
A: Number mate partners. **Left**: F0 females (p-value: 0.68, N_G26: N_F: 21). **Right**: F1 females. N_{FF}: 7, N_{FG}: 15, N_{GF}: 17, N_{GG}: 25. No significant differences were found.



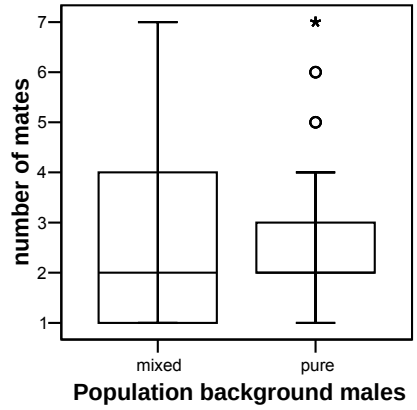
B: Number of mates of females from parents of the same population ("pure animals") or different populations ("mixed animals"). P-value (t test): 0.27.

Figure 2.25: Female attractiveness, measured as number of mate partners per female. No significant differences were found.

The analysis for male attractiveness (measured in numbers of females an individual male fertilized successfully) also showed no significant differences regarding population background. This hold for F0 males, F1 males and also for the comparison of “pure” vs. “mixed” animals (Figure 2.26). The mean number of females per male was in all cases approximately 2.7.



A: Attractiveness of males. **Left**: Males from the founder generation. P-value: 0.31. N_F: 19, N_G: 21. **Right**: Males from the F1 generation. None of the pairwise comparisons showed significant differences (N_{FF}: 8, N_{FG}: 12, N_{GF}: 14, N_{GG}: 16).



B: Comparison of number of females for “pure” and “mixed” males. No significant difference was detected (t test, p-value: 0.99. N_{pure}: 65, N_{mixed}: 30.)

Figure 2.26: Male attractiveness measured as the number of mates per male. No significant differences were found.

Relative testis weight

Relative testis weight (testis weight / bodyweight) was calculated for all males >13 g from individuals of experiments III and IV. There was no significant difference between the populations, or comparing "pure" with "mixed" animals (t test p-values: G vs. F: 0.18, G vs. mixed: 0.06, F vs. mixed: 0.98 and "pure" vs. mixed: 0.12 (Figure 2.27).

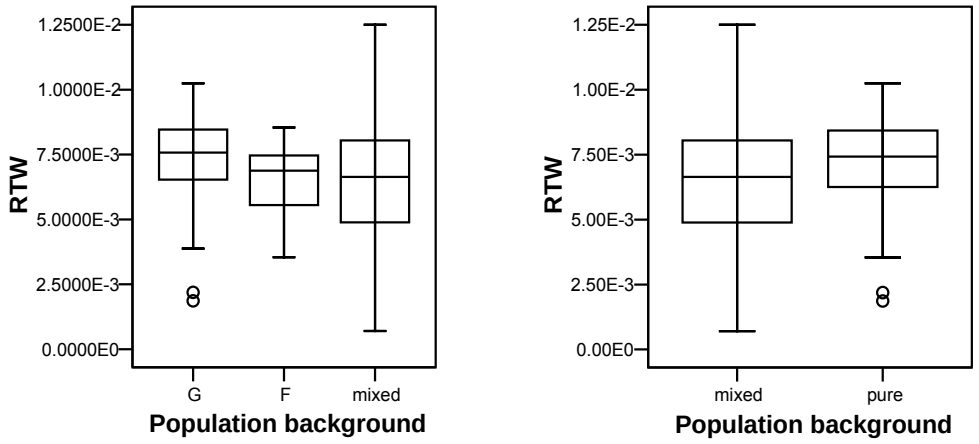


Figure 2.27: Relative Testis Weight compared between populations (**left side**) and between "pure" animals and "mixed" animals (**right side**) (N_G:36, N_F:14, N_{mixed}:134, N_{pure}:50). No significant differences were found.

2.3.5 Multiple mating frequencies

To determine whether females of the two populations followed different mating strategies, I looked at the frequencies of multiple paternities separately from litters of "pure" G and F females and from females with a "mixed" population background (Table 2.5).

Table 2.5: Number of multiple paternity litters vs. one father litters, considering population background. The frequencies were in all three cases around 30%.

Population background female	Number of all litters	Number of one father litters	Number of multiple paternity litters (MP)	Ratio MP/OFL (%)
G	121	88	33	27
F	61	42	19	31
mixed	70	47	23	33

From these data, I analyzed the percentage of females from both populations who at least once mated multiply (Table 2.6). Of the three population backgrounds (G, F, mixed), around 50% of the successful females had at least one multiple paternity litter. No significant differences were found (p-value Fisher's Exact Test: 0.60

comparing F and G, p-value 0.54 comparing G and F values vs. “mixed” population females).

Table 2.6: Number of successful females with at least one multiple paternity litter. No significant differences were found.

Population background female	Total no of successful females	Females with multiple paternity litters	Females without multiple paternity litters	% Females with multiple paternity litters
G	52	25	27	48
F	28	14	14	50
mixed	40	18	22	45

2.3.6 Social partner choice

Communal Breeding

Litters found together in the same nest with pups of nearly the same age, were considered as communal breeding litters. I obtained information about the rearing status (communal breeding/no communal breeding) from 166 litters. 85 (51%) were communally reared litters.

I compared the number of communal breeding litters with the number of litters found alone in a nest for F and G females. G females showed a higher frequency of communal breeding than F females (G females: 48.7%, N=78; F females: 31.1%, N=45). The difference, however, was not statistically significant (Fisher’s Exact Test, p-value: 0.06). Comparing between F1 females of pure population background and “mixed” background, no difference was observed (Fisher’s Exact Test, p-value: 0.160, pure: 39%, N= 41, mixed: 23.8%, N=42).

Preference for breeding partner

Out of 31 communal breeding events (in part with more than two breeding females, repeated pairs were counted as often as they bred together), only six were not sister-sister or mother-daughter pairs. Three of these were G-G pairs; the other three were “mixed” pairs.

2.3.7 Social status

Occupation of houses

I analyzed how many times an individual was found in a house compared with encounters outside houses. For this, the relative numbers of encounters in houses of the individuals were compared.

In the founder generation, German males significantly more often were encountered in houses than French males (t test, p-value: 0.007), while females did not differ significantly (p-value: 0.13). In the F1 generation, “pure” and “mixed” males did not differ significantly (p-value: 0.34), while “mixed” females were encountered significantly more often in houses than “pure” females (p-value: 0.04).

In addition, I measured how many times an animal was found in the same house (in relation to the total encounters). As a measure for philopatry (or spatial dominance) an average was calculated from the frequency of occupying houses. For example, if an animal was found 20 times in total, and among these encounters 10 times in house A, 4 times in house B and the remaining 6 times somewhere free in the enclosure, the average frequency in houses was calculated from 0.5 (house A) and 0.2 (house B) as to be 0.35. Animals met fewer than 5 times were excluded from the analysis and when animals were recorded only once in a certain house, these records were excluded from the analysis (but included in the total number of encounters).

Males of different background (F (N=23) versus G (N=29) and “pure” (N=20) versus “mixed” (N=35) animals) were similar in occupying certain houses (t-test p-value: 0.34 for F versus G and 0.54 for “mixed” versus pure). Likewise, for females no difference was detected (t-test females: p-value: 0.88 for F (N= 34) versus G (N= 34) and 0.81 for “mixed” (N=28) versus “pure” (N=15) animals).

Monitoring records

The information about the condition of animals obtained during monitoring was classified in five categories: *good condition*, *modest* (generally mice which had sparse fur or one bite mark), *bites* (mice with several bite marks), *severely bitten* (animals who had so many bite marks that they had to be considered as too weak to stay in the experiment) and *bad condition* (mice which had nearly no fur and showed no vital behavior, these were also taken out of the experiment).

I analyzed the overall number of monitoring records with regard to population background and sex, separating between records from founder animals and animals born in the enclosure (from the founder animals I had considerably more monitoring data, since F1 animals were transponder-tagged only at an age of 6 weeks or even later when they weighed 17 gram.

The sex specific analysis showed that females, especially those born in the enclosure, are mostly in good condition (Figure 2.28). For males, the occurrence of bites was much more frequent.

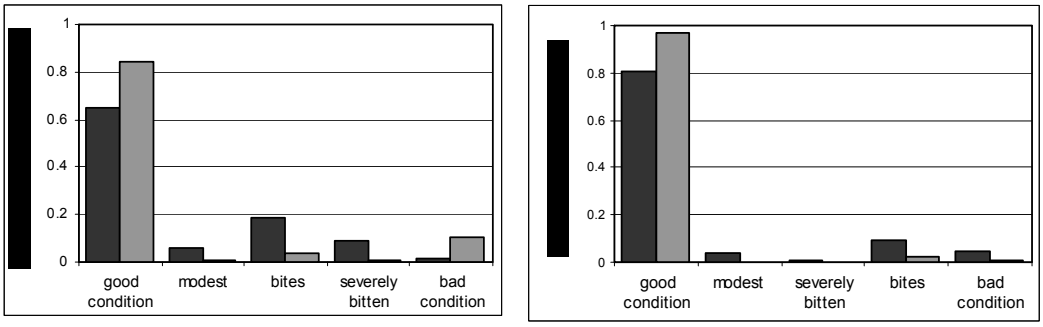


Figure 2.28: Monitoring records for individuals separated for sexes. **Left**: Founder generation, **right**: animals born in enclosure. Males are depicted in black, females in grey. Females were almost always in good condition, while males were more frequently found in bad condition and with bite marks.

The analysis of monitoring records with regard to population background is shown in Figure 2.29.

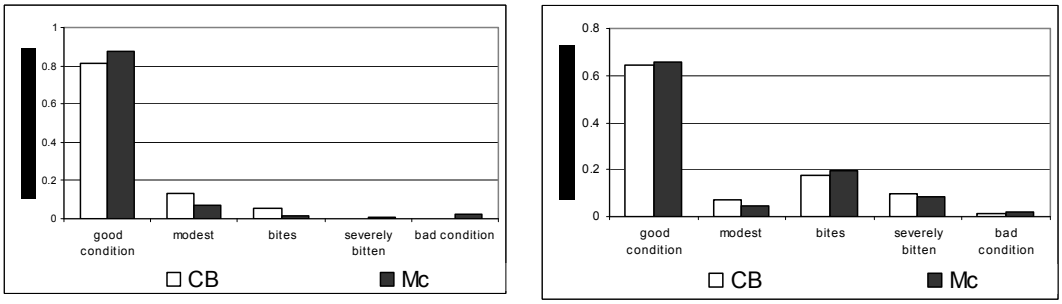


Figure 2.29: Monitoring records of F0 population analyzed according to population background. **Left**: females, **right** males. White: German individuals, black, French individuals.

For animals born in the enclosures, females were almost always in good condition (92 out of 95 records). For males, the condition was analyzed with regard to population background, but no significant differences were detected (chi-square: 4.47, df: 8, p-value: 0.81, Figure 2.30).

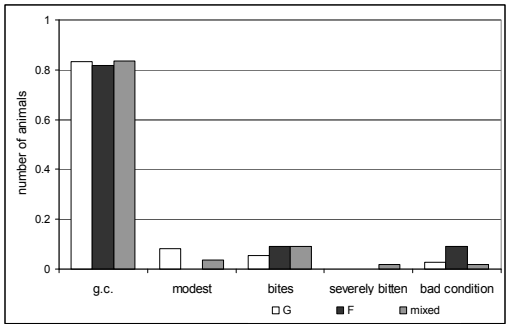


Figure 2.30: Monitoring records (relative) for males born in the enclosure, analyzed regarding population background. No significant differences were detected.

Individual condition

The analysis shown above was based on the overall number of records obtained during the monitoring. In the following, the results of individual conditions are described. All mice which were recorded at least 3 times during monitoring were analyzed. The ratio of good condition vs. other conditions was calculated and tested statistically for differences between animals of different population background.

When analyzing F0 animals, no significant differences were detected (t test, p-value: 0.7, Figure 2.31). Similarly, the condition of F1 individuals did not differ between "pure animals" and "mixed animals" (p-value: 0.2). This was also the case when testing only males (F0 generation, test between G and F, p-value: 0.8; F1 generation, test between "pure" and "mixed", p-value: 0.8, data not shown) and females (F0 generation, test between G and F, p-value: 0.4; F1 generation: all females recorded more than twice had 100% "good condition" records).

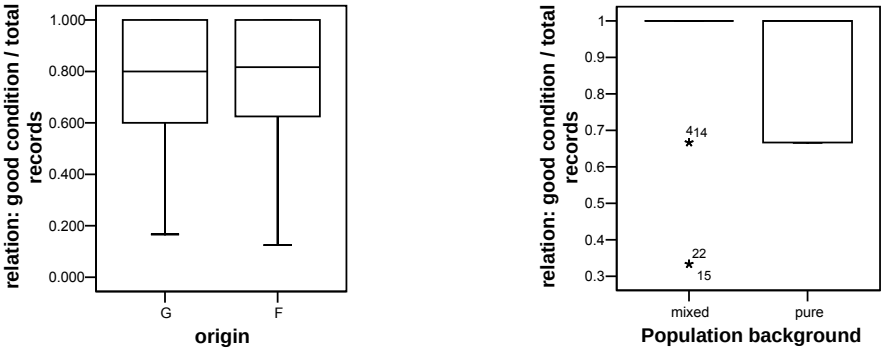


Figure 2.31: Individual condition recorded during monitoring. Plotted is the relative number of records individuals were found in "good condition". Left: F0 animals, only animals found at least 3 times were considered. N_G: 56, N_F: 54. Right: Individuals born in the enclosure and found during monitoring at least 2 times. N_{pure}: 16, N_{mixed}: 27.

In addition, I compared the number of French versus German founder animals which were reported as "severely bitten" or found in "bad condition". The numbers for F and G animals are 20 were 14, respectively (Fisher's Exact Test, p- value: 0.264).

2.4 Results from the Controlled Cage Experiment

In total 28 females (11 German and 17 French) were tested in the cage experiment for displaying a preference for German versus French males. The relative time a female spent close to one of the males and the numbers of visits were taken as measure for preference.

When looking at the relative time a female spent close to the males, 24 (85.71%) of the 28 females showed a significant preference. The signal for preferences was much lower for the relative number of visits a female made to the males cages: Only 13 (46.43%) of the females showed a significantly different number of visits to one of the two males. In both cases, no assortative preferences (regarding population background) were detected (Figure 2.32). Moreover, females of both populations displayed no significant difference in their preferences (relative time spent: p-value: 1; relative number of visits: p-value: 0.878, Exact Fishers Test).

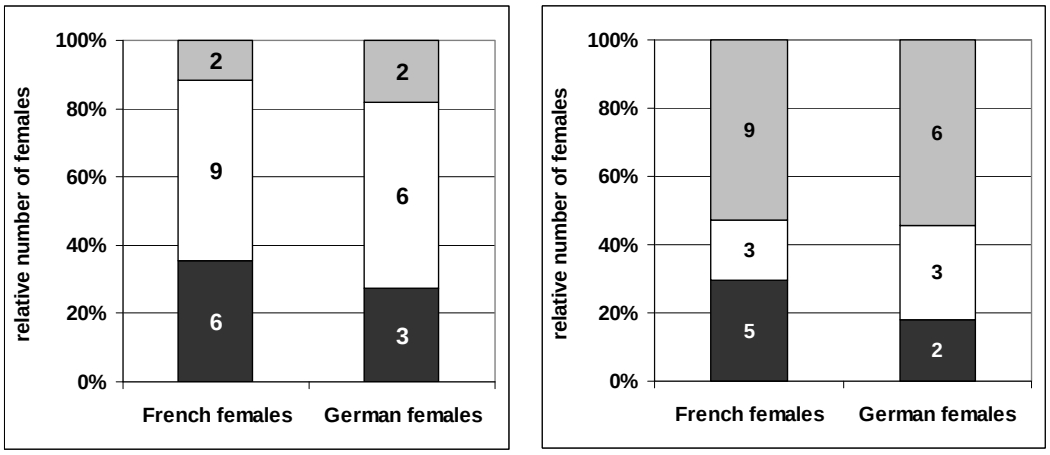


Figure 2.32: Female preferences. **Left**: Female preferences measured as the relative time a female spent close to the males. **Right**: Female preferences measured as the relative number of visits a female made to the males cage. Dark: preference for French males, white preference for German males, grey: no significant preference.

All but one female showed a consistent preference pattern comparing relative times spent and relative number of visits. For two females (both from the French population), the cage test was repeated. One female showed preferences for the male of the same population in both trials, while the other female showed different preferences in the two trials.

Among the females tested, 22 individuals were sister pairs which had been kept in the same cage before the experiment. Out of these 11 sister pairs, 10 pairs showed significant preferences for one of the males, and among these, only two sister pairs

differed in their preference, while 8 pairs preferred the male from the same population. This finding was statistically significant (chi-square: 7.2, p-value: 0.007).

Additionally to the overall time a female spent close to the males I analyzed whether the preference patterns differed between active daytime (empirically shown to be from 6 p.m. to 6 a.m.) and the rest of the day. 13 females (46%) showed no significant differences between the active daytime and the rest of the day. Only three females (11%) showed a significantly different preference. However, four females (14%) showed a significantly stronger preference during the active time and 8 females (29%) a significantly weaker preference.

2.5 Discussion

Results of the mate choice experiments indicated some kind of assortative mating between animals from the German and French populations as well as slightly reduced hybrid fitness: While social partner choice (choice of the nest mate in communal nests) was influenced mainly through kinship, a remarkable hint for sexual partner choice influenced through population background came from the analysis of mating events between F1 animals. Consistently, females born in the enclosures had offspring with a partner who had a father from the same population as themselves, a phenomenon called here the *father related assortative mating pattern*. The biological significance for this pattern is as yet unclear; several possible explanations are discussed below. Regarding fitness of hybrids, none of the parameters tested as measures for reproductive success showed significant reduction in the “mixed” versus “pure” animals. However, the measures for reproductive success are nearly always higher for “pure” animals compared to the “mixed” animals.

In the following, different topics of the results are discussed in more detail.

2.5.1 Paternity analysis

After Araki & Blouin (2005), incorrect paternity assignments may result from genotyping errors, finite number of loci, mutations, and null alleles. An important factor for errors in paternity assignment is not to have sampled all potential parents. In the case of this study, missing parents can nearly be excluded, as the enclosures presented a closed system and the probability of missing dead animals was low. Nevertheless some potential parents were not found. As Marshall et al. (1998) pointed out one can work around this problem by estimating accurately the number of

missing parents, which was done in my paternity analysis by setting the frequency of sampled parents to 0.9.

The quality of paternity assignment (92% of offspring assigned) is comparable to other studies. For example, Carroll et al. (2004) report 4% of unassigned offspring (out of 1,159). The aforesaid study constrained kinship among the founder animals, which lowered inbreeding rates and thus the problem of paternity assignment.

2.5.2 Population Development

The populations in the enclosures developed with rates that were similar to other studies (Lidicker 1976). The mouse densities (Table 2.2) were in the range of other reported densities (e.g. Bronson 1979: 10 mice/m^2) which could explain that only very few animals emigrated from the enclosure via the dispersal tube. The striking differences in population density between experiment I and II vs. III and IV can be explained with the longer experiment duration (5 vs. 6.5 months). The lower offspring numbers in experiments I and II could also result from a higher pup or embryo mortality caused by unidentified unfavorable conditions.

For the analysis of multiple mating frequencies, communal breeding, and reproductive success it is important to mention that litter size decreased towards the end of the experiment. Causes for this observation could not be identified, but probably one reason is the lower survival rate of newborn pups, as observed by Reimer & Petras (1967) and Lidicker (1976) in their enclosure experiments lasting for eight and twelve months, respectively. Lidicker (1976) reported that “nearly no young were surviving this critical period” and identified as one reason lactation failure. Additionally, he suggested cannibalism and abandonment as contributing causes, which could equally apply to my experiments.

The sex ratios in three of the four experiments were not significantly skewed. However, generally more males were recorded (except for experiment II). This pattern is also evident when looking at the sex ratio of pups (recorded at tissue sampling at an age of around 14 days), and the effect increased towards the end of the experiments (Figure 2.5, Figure 2.6). This could be due to elevated population densities. Some studies report a deviation of sex ratio at overcrowding, while other mouse researchers consider other parameters responsible for a sex ratio deviation (food availability and competition: Wright et al. 1988; Meikle & Thornton 1995). However, the results are in contrast to Trivers & Willard (1973), who showed

theoretically and experimentally that as maternal condition declines, the adult female tends to produce a lower ratio of males to females.

2.5.3 Assortative mating

The different results regarding assortative mating for founder animals and F1 animals are not surprising, as both generations were exposed to very different conditions during their development. Diverse impacts of animal house conditions on mouse behavior were reviewed in Latham & Mason (2004).

The founder animals showed no consistent pattern regarding assortative or disassortative mate choice (Figure 2.8). These animals were born and grew up in cages, and held solitarily for some weeks, which alters the life of the adult.

For this reason, the F1 generation is much more appropriate for such an analysis. Here I observed a consistent pattern, where animals mate with partners which had a father from the same population as their own father. This pattern was highly significant for all experiments when taking out animals heterozygous for the t haplotype (Figure 2.10 and Figure 2.11). Especially for experiment IV this should make sense, as this population differed regarding t haplotype frequencies (see chapter 3). For backcrosses, the pattern was consistent (and highly significant for all experiments) when looking at mating events where the female came from the F1 generation, and the male from the F0 (Figure 2.14). In addition, testing the parent pairs gave the same signal.

One possible biological explanation for this observation could be paternal imprinting. Paternal imprinting on mate choice is known to occur in some animals (Tramm & Servedio 2008). However, it is probably not a sufficient explanation for the pattern observed here since it would raise the question of how the offspring would know its father, since male participation in parental care in house mice is described to be comparatively low (e.g., Patris & Baudoin 2000), which - considering the high frequency of multiple paternities observed - is not expected to differ in the populations of my study. Alternatively, nest mates or other environmental components could influence mating preferences. Some studies have shown familial imprinting on mate choice of house mice during early life. Through cross-fostering experiments, Penn & Potts (1998) showed that MHC disassortative mating was influenced by familial imprinting. One support for this is the observation from the controlled cage experiment, where sister pairs showed the same preference patterns.

Evidence for another form of imprinting – genomic imprinting – comes from recently published work. Gregg et al. (2010) showed a sex-specific parent-of-origin allelic expression in the mouse brain of offspring. Their data suggest a strong expression bias of paternal alleles in the hypothalamus of female offspring. Interestingly, the hypothalamus is known to influence mating behavior which could explain the *father related assortative mating pattern*.

2.5.4 Reproductive success

Differences between populations were for most of the tests statistically not significant. Nevertheless, some patterns were detected: Looking at the F0 generation, the reproductive success of French animals was almost always below values of German animals (e.g., Figure 2.16, Figure 2.19, and Table 2.4). In the F1 generation, animals with “pure” population background outperformed the animals with a “mixed” population background (Table 2.4, Figure 2.18, and Figure 2.21).

Considering the number of mates as a measure for individual attractiveness, no significant differences between the population backgrounds were observed (Figure 2.25 and Figure 2.26).

2.5.5 Relative fertilization success

The average relative fertilization success is interesting in the context of multiple mating and gives an idea about postcopulatory sexual selection, including sperm competition and cryptic female choice. Sperm competition is the competition between the sperm of different males to fertilize the ova of a female, while cryptic female choice is the ability of a female to bias the fertilization success of the males they mated with. It is assumed that both forms of sexual selection present important evolutionary forces (Birkhead & Pizzari 2002) and considering this, it is interesting to see whether mating events with animals of different population backgrounds show distinct effects.

The analysis of the average fertilization success between individual males with different population backgrounds gave no significant differences (Figure 2.22). This suggests that the fertility of males did not depend on population background. Examining the fertilization success depending on the mate partner combination (Figure 2.23) showed significantly higher fertilization success for males which had a different population background than the female they inseminated. Confirming this result would propose that disassortative postcopulatory mate choice or population

specific sperm competition influences the fertilization of ova. The *father assortative mating pattern* suggested an analysis of fertilization success regarding mate partners with fathers from the same versus a different population background. This analysis showed no significant differences in fertilization success (Figure 2.24) and indicated that the *father assortative mating pattern* did not hold for postcopulatory mechanisms.

2.5.6 Relative testis weight

Through the comparison of RTW no significant differences dependent on population background of the males were found. Nevertheless, G males in comparison to other males and “pure” vs. “mixed” population background males showed a higher RTW. This is consistent with the observed differences in reproductive success (Table 2.4). However, RTW and number of offspring did not correlate significantly (see chapter 3: Results 3.3.3 and discussion).

2.5.7 Multiple paternity

Multiple mating frequencies did not differ between population backgrounds (Table 2.5 and Table 2.6); the ratio of multiple paternity litters to all litters was in all cases around 30%. The important information here is that there was no increased frequency of multiple mating in mating events between individuals of different populations. An increased frequency can be interpreted as a strategy to counteract possible genetic incompatibilities between individuals of the separated populations. However, as already mentioned above, multiple paternity frequencies underestimate the frequency of multiple mating (see also chapter 3).

2.5.8 Communal breeding as a measure for social partner choice

Communal breeding was very common in the long-term experiment (50% of litters shared nests with other litters). There was no significant difference in the frequency of communal breeding between the different population backgrounds. A general preference for partners from the same population could not be shown, but remarkably nearly all communal breeding pairs were relatives: 25 out of 31 breeding pairs were sisters or daughter – mother pairings (for more detailed analysis see chapter 3). This finding is in line with a study of König (1994) who showed in a laboratory setting that females nursing communally with sisters had a higher reproductive success than females nursing with unrelated but familiar females.

2.5.9 Fitness

The occupancy of houses showed a significant difference in the founder generation: German males were significantly more often found in houses than French males, and for females, the higher frequency in houses is found for F1 animals of mixed population background, compared to pure F1. The analysis of all monitoring records (of all transponder-tagged mice) did not show any differences of animal conditions according to population background (Figure 2.29 and Figure 2.31). The observed difference between males and females was also reported by Reimer & Petras (1967) and can be explained by the occasionally very aggressive behavior of males towards competitors. Similarly, the individual condition analysis showed no difference for individuals of different populations.

2.6 Conclusion

Similar to studies reported from mice of the hybrid zone between the subspecies *M. m. musculus* and *M. m. domesticus,* I observed some kind of assortative mating and slightly reduced hybrid fitness. The comprehensive experimental design of the present work allowed me to uncover mating patterns at a fine scale, showing that females have significantly more offspring with males whose fathers came from the same population as themselves. In addition, the decrease in hybrid fitness is another indication for reproductive divergence between the two recently separated populations.

3 The role of different molecular parameters for mate choice and individual reproductive strategies

3.1 Introduction

3.1.1 Background and focus of the investigation

Molecular mate choice parameters and reproductive strategies related to sexual and social partner choice were investigated for the two recently separated house mouse populations from Germany and France. The study aims to examine their influence on mate choice and reproductive success, analyzing the results obtained from the long-term experiment described in chapter 2. Simultaneously, a comparison between both populations was conducted to determine whether they differ in mate choice and reproductive strategies such as communal breeding and multiple mating.

Several studies investigated the influence of molecular parameters on mate choice in house mice. These parameters include genes of the major histocompatibility complex (MHC) (e.g., Penn & Potts 1998), overall heterozygosity (Ilmonen et al. 2009), the current social status (Rolland et al. 2003) or health status (Ilmonen et al. 2008), mayor urinary proteins (Hurst 2009; Ramm et al. 2008; Sherborne et al. 2007) and the t haplotype (Lenington et al. 1994; Lenington et al. 1992). However, many of these studies were done with laboratory mice or under unnatural conditions, e.g. in cages or y-maze devices. Additionally, most studies focus only on one parameter rather than trying to consider the whole set of possibly interacting traits. Nevertheless, it is important to study mate choice on wild animals with natural genetic and phenotypic variation and within a social context. Moreover, the interaction of different parameters has to be considered, possibly disentangling their individual role for mate choice.

In the following, genetic parameters considered important for mate choice in house mice as well as widespread reproductive strategies such as polyandry (multiple mating in females) and communal breeding are described.

3.1.2 Mate choice and its benefits in house mice

The existence of mate choice is reported for a wide range of animals (Andersson 1994). Potential fitness gains are diverse and range from resource benefits to indirect benefits. Many studies address the identification and quantification of such benefits,

but empirical demonstration especially for indirect benefits remains challenging (Andersson & Simmons 2006; Kokko et al. 2003).

In house mice, both sexes show partner preferences. This was demonstrated amongst others by Drickamer and colleagues who reported fitness benefits for females and males which were bred to a preferred partner: their offspring had a higher fitness compared to individuals bred to a mate they did not prefer (Drickamer et al. 2003; Gowaty et al. 2003, Drickamer et al. 2000).

3.1.3 Strategies related to reproduction

Communal breeding

Besides mate choice, also social partner choice plays an important role in house mouse reproduction. An interesting behavior is communal breeding of females, which describes the peculiarity that females nest together and nurse pups of a similar age indiscriminately together. Manning et al. (1995) showed that females of such nursing pairs were not able to distinguish between their own pups and pups of the nest mate, and several studies tried to identify social mate preferences (Weidt et al. 2008; König 1994a) and fitness benefits for females leading to such costly behavior (König 1994b) (discussed benefits are e.g. enhanced immune system through antibodies of different females, which are transmitted during lactation). Following a theory of Roulin & Hager (2003), communal breeding could be influenced through male genomic imprinting.

Recent studies found evidences for enhanced postpartum maternal care in females rearing pups in communal nests (Curley et al. 2009). The same authors showed transgenerational effects on emotional (through reduced anxiety-like behavior) and reproductive (through higher levels of maternal care and larger litter size) behavior of the offspring and grand-offspring of mice grown up in communally reared litters. Similarly, another recent study reports changes in brain function and behavior in communally reared mice (Branchi 2009).

Multiple mating

Polyandry – females mating with several males during one reproductive cycle - is known in many species (Cornell & Tregenza 2007), including house mice (Bronson 1979). This female mating strategy has important implications for sexual selection and speciation; it induces sperm competition and cryptic female choice (Evans &

Simmons 2008), and varying levels of polyandry can lead to the evolution of complex male behaviors and alternative mating strategies (Bretman & Tregenza 2005).

In many species, females receive enough male gametes through copulating with one male and mating causes costs including time and energy during courtship and copulation, increased risk of predation while mating and risk of disease from parasite transmission and injury (Daly 1978; Keller & Reeve 1995). This contradicts the widespread existence of polyandry, and the potential benefits leading to this costly behavior are highly discussed (Jennions & Petrie 2000) and are in many cases not obvious (Tregenza & Wedell 2000).

The benefits of multiple mating can be direct, e.g. nuptial gifts, future parental care or reduced male harassment, and often the benefit for the female is evident and measurable. Contrary to this, indirect benefits are difficult to assess. These include potential benefits through genetic compatibility (by reducing the risk of fertilization by genetically incompatible males (Zeh & Zeh 1997; Zeh & Zeh 1996), genetic bet-hedging against various future unforeseeable incidents and increased genetic diversity in the offspring (Yasui 1998) or inbreeding avoidance (by reducing the degree of inbreeding in a female's grandchildren (Cornell & Tregenza 2007). Supporting theoretical assumptions about genetic benefits, there is evidence for female benefits through a higher offspring performance in different taxa (Gowaty et al. 2010, Klemme et al. 2008; Edvardsson et al. 2007; Fisher et al. 2006; Sakaluk et al. 2002).

Another aspect of polyandry is a possible sibling conflict, as the maternal genome in the offspring of promiscuous females combines with the genomes of several males (Birkhead & Pizzari 2002). Offspring produced by polyandrous females may be full or half siblings, which may have important consequences for social interactions among offspring (Evans & Kelley 2008).

Multiple mating in house mouse females is reported from several recent studies (e.g. Dean et al. 2006; Firman & Simmons 2008) and is assumed to be common. A behavioral experiment showed that nearly all tested females mated by choice with different males (Rolland et al. 2003). Nevertheless, the frequency of polyandry of wild house mice is difficult to assess in nature. To estimate the frequency of multiple mating in the wild, the occurrence of multiple paternity in litters is used as an indicator. This, however, underestimates the real numbers in cases where only sperm of one male win the competition to fertilize the ova of the female.

Recent studies showed that the frequency of multiple paternity varies between populations. In natural populations, Dean and colleagues detected an average of 23% litters with multiple paternity, while Firman and Simmons detected 6-43% multiple paternity litters (Dean et al. 2006; Firman & Simmons 2008). Ehman and Scott showed in an enclosure experiment with CD1 outbred mice a multiple paternity rate of 64% (Ehman & Scott 2004), while Carroll and colleagues measured a frequency of 19.3% in a study with wild mice (Carroll et al. 2004). These differences could indicate individual or population specific mating patterns or differences in postcopulatory sexual selection mechanisms, and could be explained with variances in possible benefits or population histories. Identifying a pattern on which parameters the frequency of multiple mating depends could help to identify mechanisms driving evolution and maintenance of this behavior.

3.1.4 Genetic parameters involved in mate choice

Several parameters are considered to play a role in mate choice. Up to date, I completed the analysis of the influence of the t haplotype, while other analyses are still under way. However, in the following I will describe briefly the different genetic parameters which are considered to play important roles in mate choice.

t Haplotype

It has been shown that the presence of selfish genetic elements with deleterious effects can promote polyandry (Price et al. 2008; Martin 2009). For house mice, this could hold for populations with the t haplotype, a selfish allele of the t complex, a region of 20 cM on chromosome 17. t alleles are responsible for a high degree of transmission ratio distortion in males carrying one t and one wild type allele: sperm carrying the t haplotype achieve 80 – 100% of fertilizations (Lyon 2003). The homozygous state or two t alleles in an individual leads to prenatal lethality or male sterility, hence it is predicted that heterozygous females would have a fitness benefit when mating with several males (Haig & Bergstrom 1995) or avoiding heterozygous males (Lenington et al. 1992). Lenington et al. (1992) showed this by demonstrating disassortative mating preferences between mice with different t haplotypes. Carroll et al. (2004) showed a fitness decline for males heterozygous for the t haplotype, which could influence reproductive success.

Major Urinary Proteins

Major Urinary Proteins (MUPs) are signaling molecules and serve additionally as transporters for olfactory information. Adult mice express a fixed individual pattern of different MUP isoforms in their urine determined by their *Mup* genotype. Hurst and colleagues found that MUPs mediate individual recognition (Hurst et al. 2001) and enable the direct assessment of a partners heterozygosity and competitive behavior, as well as the avoidance of inbreeding through kin recognition (Hurst 2009; Ramm et al. 2008; Thom et al. 2008; Sherborne et al. 2007; Cheetham et al. 2007).

Major Histocompatibility Complex

The major histocompatibility complex (MHC), a genomic region coding for proteins with key roles in the immune system of all jawed vertebrates, is known for the extraordinary polymorphism (Klein 1986) and heterozygosity (Duncan et al. 1979) of some of the genes. In the 1970th, when the importance of MHC genes and their medical implications became evident, the system was intensively studied on house mice (*Mus musculus domesticus*), where the complex is termed “H2” (Klein 1979).

Since Yamazaki and colleagues detected MHC related mating preferences in laboratory mice in 1976, more and more studies reported evidence for an influence of MHC loci on mate choice in nearly all classes of vertebrates (Ziegler et al. 2005; Milinski 2006). The findings mostly indicated MHC disassortative mating, which could be explained by enhanced immune competence for offspring or a means of inbreeding avoidance. However, recent studies on wild populations also found mate choice patterns suggesting avoidance of extremely MHC-dissimilar mates (Woelfing et al. 2009) and even MHC assortative mating (Bos et al. 2009).

Several studies have addressed the question of MHC influence on partner choice for house mice (reviewed in Penn & Potts 1999). Nevertheless, a clear picture of MHC influence on mate choice in natural populations can not be drawn (Penn 2002). This is in part due to the fact that the majority of the studies was carried out on laboratory mice missing a natural genetic background and under laboratory conditions which is expected to bias results by influencing behavior (Latham & Mason 2004; Wolff 2003). In addition, very few studies investigated the interaction of other mate choice parameters with MHC alleles.

Multiple parameters used in mate choice

The above mentioned parameters are considered to influence mate choice, beside other parameters, here not described. It is challenging to disentangle the different importance of these parameters, which is only addressed in very few studies. An exception is a study of Roberts & Gosling (2003) who showed in laboratory mice strains that MHC dissimilarity and a “good genes” indicator (investment in scent-marking) both had a role in determining female preference.

3.2 Methods

Data were obtained from the long-term experiments (chapter 2). The following measures were taken to determine the frequencies of communal breeding and multiple mating:

Identification of communally reared litters

Litters of pups with approximately the same age and which were shown to have different mothers (as a result from paternity testing) were defined as “communally reared”.

Determining the frequency of multiple mating

As a measure for multiple mating, I considered the presence of more than one sire in a litter, assessed through paternity testing. However, it has to be pointed out that this value underestimates the real frequency of multiple mating, as I did not observe mating directly. Mating events which did not result in offspring could thus not be considered.

Identification of t heterozygote animals

All experimental mice were genotyped at the t complex based on fragment length variation at the Hba-4ps and Tcp-1 loci (Schimenti & Hammer 1990; Morita el al. 1993). The following primers were used for amplifications via PCR: Hba4ps_F: 5'–gagtgacctgcatgcccacaagctgtg-3' and Hba4ps_R: 5'-gagctgtggagacaggaagggtcagtg-3' (sequences following Schimenti & Hammer 1990). Primer sequences for the amplification of the Tcp-1 locus were taken from Planchart et al. (2000): Tcp1_F: 5' -gacaatcatagccttgtctcag-3' and Tcp1_R: 5'-gcagtgttatctttcactgg-3'. Forward primers for both loci were HPLC purified and labeled with the fluorescent marker FAM at the 5' -

ends. Fragments were amplified in separated 5 µL reactions using 2-5 ng DNA template with a multiplex PCR kit (Quiagen) and a primer concentration of 100pg. A standard PCR reaction was carried out using ABI Fastcyclers applying the following cycling protocol: 95°C for 15 min, 28 cycles at 95°C for 30 s, annealing temperature (66°C for Hba-4ps and 58°C for Tcp-1) for 1.30 min and 72°C for 1.30 min, and a final extension step at 72°C for 10 min. PCR products were subsequently diluted with 100 µL HPLC water. 1 µL of this dilution was added to 10 µL HiDi formamide + 0.01 µL 500 ROX size standard (ABI) per single well. After a denaturation step, incubating the reaction for 2 min at 90°C and 5 min at 20°C, the fragments were run on an ABI Sequencer and subsequently analyzed with Genemapper 4.0 (Applied Biosystems).

Samples of mice heterozygous at the t complex (t/wt) show fragment sizes for Hba-4ps with 214bp/198bp and for TCP1 600bp/425bp, whereby the longer fragments in both cases originate from the t haplotype (**Figure 3.1**, **Figure 3.2**). Animals heterozygous for both loci have a complete t haplotype, while animals heterozygous at Tcp-1 but homozygous for the shorter fragment at Hba-4ps have a partial t haplotype.

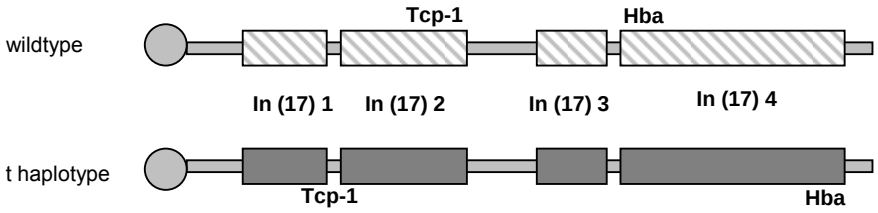


Figure 3.1: Genetic map of chromosome 17 of wild-type and t haplotype mice is shown. The four chromosomal inversions [In(17)1-4] are shown by the shaded or solid boxes. Tcp-1 and Hba represent t-complex polypeptide-1 and hemoglobin a pseudogene 4, respectively (modified after Morita et al. 1992)

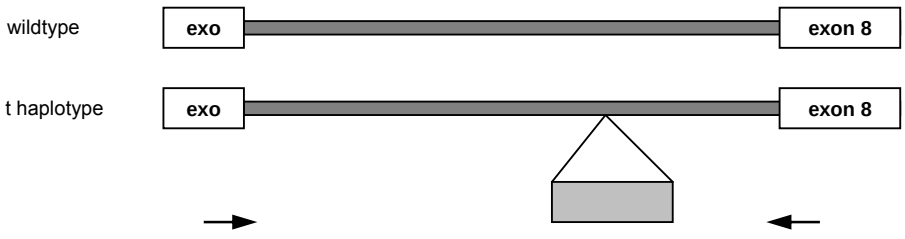


Figure 3.2: Partial structure of the wild-type and t haplotype Tcp-1 genes of mouse, showing the region used for PCR and fragment length analyses. Two exons (exon 7 and 8) are shown as open boxes. The t haplotype specific insertion is indicated by the grey box. The PCR primers, Tcp1_F and Tcp1_R are indicated by arrows (modified after (Morita et al. 1992).

Data Management and statistical analysis

Data obtained from the above described genotype analyses were imported to the same Access data base used for paternity analysis and monitoring records (see chapter 2). Statistical analysis was performed using SPSS 12.0 and Microsoft Excel 2002. To analyze reproductive success depending on different parameters as the t haplotype, multiple mating and communal breeding, I used two tailed t-statistics. Results were considered significant at a p-value < 0.05.

The graphical presentation of data was mainly done with histograms or boxplots. For the latter case each box shows the median, quartiles and extreme values (outliers: cases with values between 1.5 and 3 box lengths from the upper or lower edge of the box, depicted with an open circle; extreme cases: values more than 3 box lengths from the upper or lower edge of the box, depicted with a star).

3.3 Results

3.3.1 Communal breeding

Littersize in communal breeding litters

No statistically significant differences in litter size (per female) in communal nests and solitary nests were found (Figure 3.3).

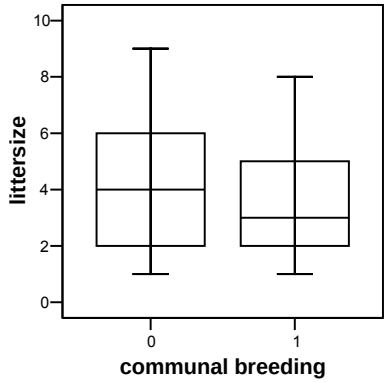
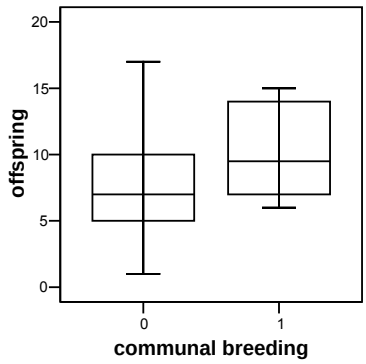


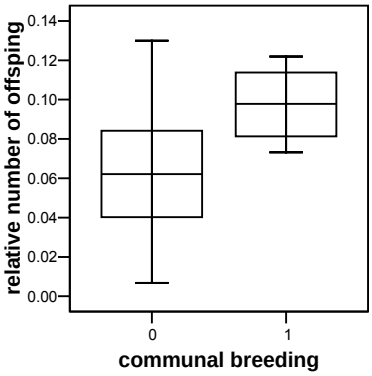
Figure 3.3: Litter size in communal nests vs. solitary nests (bred by one female). 1: communal breeding, 0: one female litter. N1=84. N0=81. Mean 1: 3.65, mean 0: 4.12, p-value (t test): 0.15.

Reproductive success of animals grown up in communal breeding litters

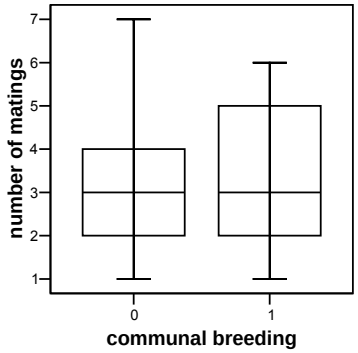
It was determined, whether animals grown up in communal litters had the same probability to reproduce as animals grown up in solitary nests. For this analysis, animals at a minimum weight of 13 gram were considered “adult”. Results showed that the proportion of successful females (>13 g) grown up in communally reared litters was significantly lower than the proportion of successful females grown up in solitary litters (11% versus 40%, p-value Fishers Exact Test < 0.0001). Contrary to this finding, successful females grown up in communally reared litters had significantly higher relative offspring numbers than females grown up in solitary nests (p-value: 0.006). Numbers of mating events and offspring per mating showed no significant differences, but were in all tests slightly higher for females born in communal breeding litters (Figure 3.4).

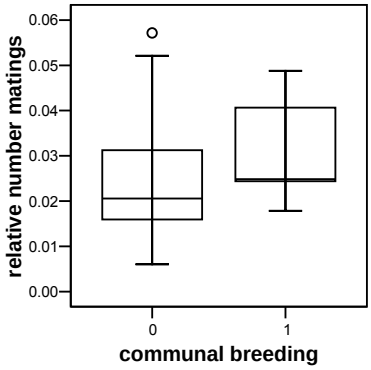
Males born in communal breeding litters did not show a higher reproductive success (analyzing only those who had reproductive success). The different measures for reproductive success did not differ significantly. Nevertheless, values for males born in solitary litters showed for all parameters higher values (Figure 3.5). As for females, I observed a significantly higher reproduction failure in communally reared males than in other males (13 % versus 37.8 %, p-value < 0.0001).



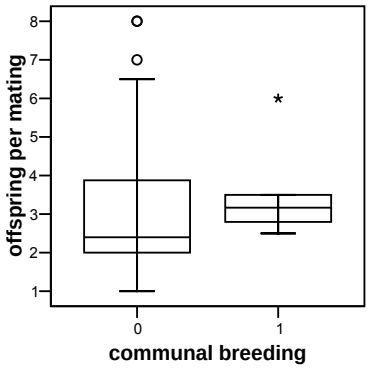


A: Absolute and relative offspring numbers for females born in communal breeding litters with 0: females from solitary litters, 1: females from communal breeding litters, 0: females from solitary litters. **Left**: Absolute offspring numbers: t-test result: p-value: 0.13. N_1: 6, mean 10.17, N_0: 60, mean 7.65. **Right**: relative offspring numbers. t-test, p-value: 0.006.



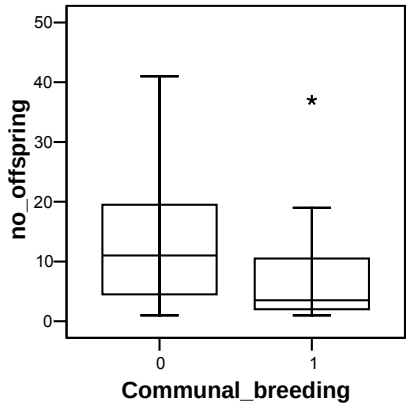


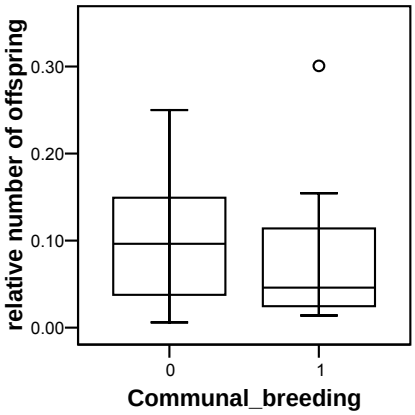
B: Absolute and relative mating numbers for females born in communal breeding litters, with 1: females from communal breeding litters, 0: females from solitary litters. **Left**: Absolute mating numbers: t-test result: p value = 0.59 (mean 1: 3.33; mean 0: 2.97). **Right**: relative mating numbers. t-test: p-value: 0.17.



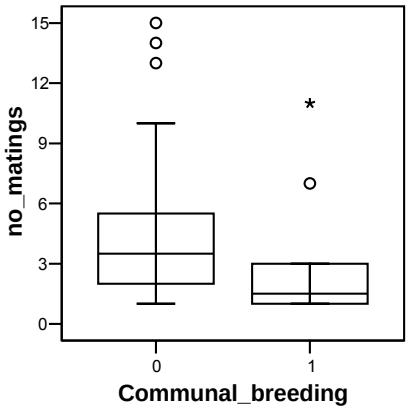
C: Offspring number per mating for females born in communal breeding litters, with 1: females from communal breeding litters, 0: females from solitary litters. t-test result: p-value: 0.43. Mean value 1: 3.52, mean value 0: 2.95.

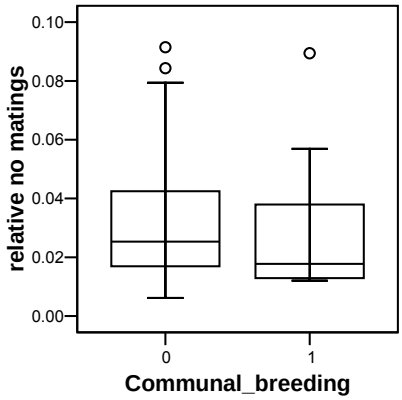
Figure 3.4: Reproductive success for females born in communal breeding litters vs. born in solitary litters. N of communally reared females: 6, N of females reared in solitary nests: 60.The relative offspring number was higher in females grown up in communal nests.



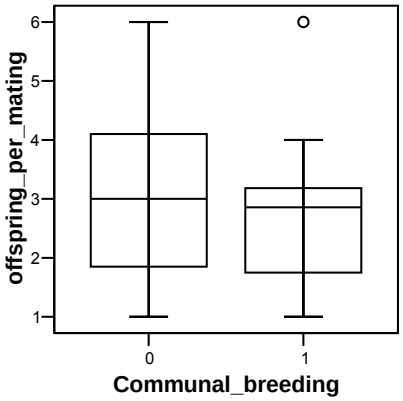


A: Absolute and relative offspring numbers for males born in communal breeding litters with 1: males from communal breeding litters, 0: males from solitary litters. **Left**: Absolute offspring numbers: t-test, p-value: 0.18. N_1: 12, mean 8.5, N_0: 40, mean 13.15. **Right**: relative offspring numbers. t-test result: p value =0.5.





B: Absolute and relative mating numbers for males born in communal breeding litters, with 1: males from communal breeding litters, 0: males from solitary litters. **Left**: Absolute mating numbers: t-test result: p-value: 0.16. **Right**: relative mating numbers. t-test: p-value: 0.61.



C: Offspring number per mating for males born in communal breeding litters, with 1: males from communal breeding litters, 0: males from solitary litters. t-test, p-value: 0.51. Mean value 1: 2.71, mean value 0: 3.01

Figure 3.5: Reproductive success for males which were born in communal breeding litters vs. males from solitary litters.

3.3.2 Multiple mating

Frequency of multiple paternity

252 litters, with a total of 1,072 offspring were analyzed for multiple (MP) or single paternity (SP). 44 animals without data about their birth date had to be excluded from the analysis, as it was not clear to which litter they belong. 179 litters (71%, with 715 offspring) showed single paternity, whereas 73 litters (29%, 357 offspring) had 2 to 4 sires. The majority of multiple paternity litters were from two sires (84.9%), 13.7% (10 litters) had three sires, and just 1 litter (1.4%) had four sires. Frequencies of multiple paternities were additionally analyzed separately for all 4 experiments. There was no significant difference (contingency table: chi-square: 7.33, df=3, p-value: 0.06 (Table 3.1).

Table 3.1: Frequencies of multiple paternities, calculated for all 4 experiments separately. The last row gives the result for all experiments together. SP: single paternity litters, MP: multiple paternity litters.

	All (litters)	SP (litters)	MP (litters)	frequency of MP
Exp I	29	23	6	0.21
Exp II	23	12	11	0.48
Exp III	124	94	30	0.24
Exp IV	76	50	26	0.34
all	252	179	73	0.29

Multiple mating and kinship

Compared to the frequency of multiple paternity in overall litters (179 SP vs. 73 MP) the frequency of multiple paternity in litters where mating events between relatives (siblings or parent – offspring matings) were involved is significantly higher (MP: 62 litters, SP: 58 litters). As expected frequencies, the frequencies in overall litters were used (chi-square, df=1, p-value < 0.0001).

Additionally, different pairings of relatives (daughter – father mating, half-sib mating, full-sib mating and son – mother mating) were tested, to estimate whether significantly more litters were multiply sired than sired by one male. For this purpose, the frequency of MP versus SP was compared and a deviation from equality was tested with a chi-square test. Only in the case of mother – son pairings, the frequency of multiple paternity litters was significantly higher than the frequency of single paternity litters (p-value: 0.013). Compared to this, the frequency of multiple

versus single paternity litters was significantly lower in no kinship matings (p-value: 0.016) (Figure 3.6).

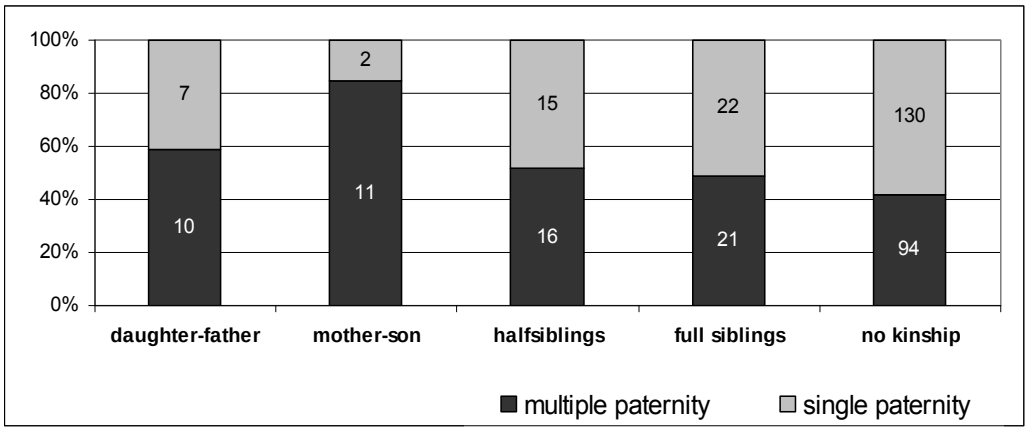


Figure 3.6: Relative occurrence of multiple paternity litters for related pairs. The last bar shows the frequency of multiple paternities in unrelated pairs (p-value: 0.02, indicating significantly more single paternities). The mother-son matings showed a significant higher frequency of multiple paternity litters than single paternities (p-value: 0.01). Numbers in the bars refer to observed numbers of mating events.

The frequencies of MP versus SP litters in the context of kinship mating where also analyzed considering different population backgrounds. No significant differences between "pure" or "mixed" couples were found (Table 3.2).

Table 3.2: Multiple paternity versus single paternity litters in mating events between relatives, considering population background. The lighter grey rows show the frequencies of MP versus SP in "mixed" and "pure" couples. The darker grey rows show the results between "pure French" and "pure" German pairings. Differences were not significant.

mating	daughter father mating			mother son mating			half sib mating			full sib mating			no kinship mating		
	MP	SP	p -value	MP	SP	p -value	MP	SP	p -value	MP	SP	p -value	MP	SP	p -value
mixed	4	2		6	2		15	15		3	8		62	90	
pure	6	5	1	5	0	0.487	1	0	NA	18	14	0.162	32	40	0.664
G	4	5		3	0		1	0		17	12		17	27	
F	2	0	0.454	2	0	NA	0	0	NA	1	2	0.568	15	13	0.234

Effects on reproductive success of multiple mating

Litter size and paternity

Litter size was significantly elevated in multiple paternity litters (t-test p-value: 0.02) (Figure 3.7).

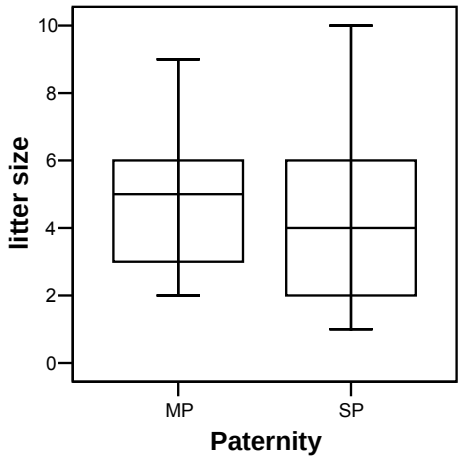


Figure 3.7: Litter size in litters with multiple paternity (MP) and single paternity (SP). Litters are significantly larger in litters sired by several males (p-value: 0.02).

Mean litter size for MP: 4.89 offspring, mean for SP: 3.95 offspring. N_{MP}: 73, N_{SP}: 181

Reproductive success and multiple mating

The reproductive success of females who had (at least one) multiple paternity litter was analyzed (Figure 3.8): Females with MP litters had a higher overall reproductive success (in terms of absolute and relative offspring number).

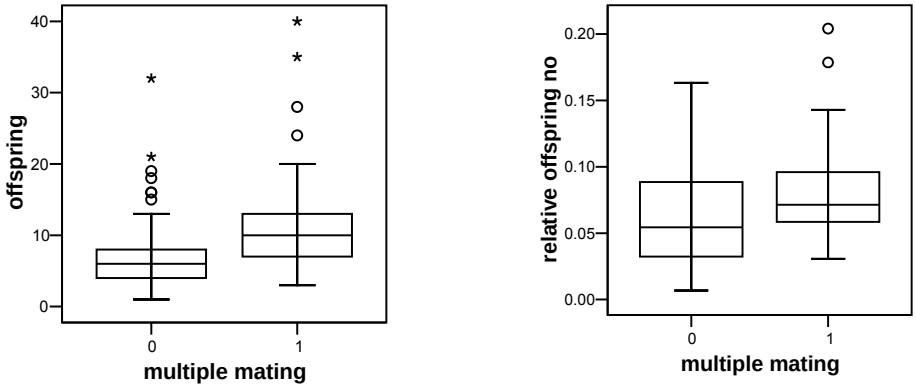


Figure 3.8: Reproductive success of females with and without MP litters (0: females without any MP litters, 1: females with at least once an MP litter). **Left**: Absolute number of offspring, p-value: 0.01. Means: females with MP litter: 11.18 offspring, females without MP litter: 7.38 offspring. N_1: 66, N_0: 57. **Right**: Relative number of offspring, related to the number of days in the enclosure. This value is also higher for females which had at least one multiple paternity litter. P-value: 0.06.

This analysis was also conducted separately for females with different population backgrounds (Table 3.3). The pattern was the same for the different population backgrounds: females which had at least one multiple paternity litter had a higher offspring number, but differences are not statistically significant.

Table 3.3: Reproductive success of females with and without any MP litters, analyzed for different population backgrounds (G: German, F: French).

Female's population background (in brackets number of females analyzed)	**Mean number of offspring for females with MP litters**	**Mean number of offspring for females with SP litters**	**P-value (MP versus SP, Fisher's Exact Test)**	**P-value comparing relative offspring numbers**, data not shown)
G (F0) (N_{MP}:12, N_{SP}:14)	16	9	0.046	0.075
F (F0) (N_{MP}:10, N_{SP}:11)	13.4	8.73	0.222	0.333
GG (F1) (N_{MP}:13, N_{SP}:12)	9.69	6.92	0.066	0.286
FF (F1) (N_{MP}:4, N_{SP}:3)	8.25	7.33	0.846	0.701
GF and FG (F1) (N_{MP}:14, N_{SP}:18)	9.07	6.61	0.073	0.045
F1 and Backcrosses "pure" background (N_{MP}:17, N_{SP}:16)	9.35	6.87	0.08	0.281
F1 and Backcrosses "mixed" background (N_{MP}:16, N_{SP}:20)	8.44	6.36	0.072	0.072

Multiple paternity and population densities

I tested whether the frequency of multiple paternity increased with population densities. Therefore, a comparison of frequencies of multiple paternity litters born in the first three months and the second three months of the experiment was conducted. In all experiments, the frequencies were higher in the second phase, but differences are only significant in the case of experiment III. However, when data of all experiments were taken together, the increase of multiple paternities in the second phases of the experiments became evident (p-value: 0.0005).

Table 3.4: Number of multiple paternity litters in the first and second phase of the experiments. The p-values indicate if there was a statistical significance for a deviation of multiple paternity frequencies in the first phase versus frequencies in the second phase. In all experiments, the frequencies were higher in the second phase, but differences were only significant in the case of experiment III.

Exp	**First phase**		**Second phase**		**Comparison of frequencies**	**p-value Fisher's Exact Test**
	MP	**SP**	**MP**	**SP**	**MP 1st phase / MP 2nd phase [%]**	
1	1	11	5	14	8.3 / 26.3	0.36
2	3	8	8	4	27.3 / 66.7	0.1
3	0	19	30	75	0 / 28.6	0.006
4	1	8	25	42	11 / 37.3	0.15
all	5	46	68	135	9.8 / 33.5	0.0005

The same analysis was conducted considering population background and it was shown that for both populations the observation was consistent with the overall

experiment (Table 3.5): When the frequency of multiple mating was analyzed for the experiments separately, no significant increase was detected, but in all but one case, frequencies for all tested sets increased. Looking at the data for all experiments together, the German population showed a significant increase in multiple paternity in the second phase of the experiment (p-value: 0.004), while this increase was just about not significant for the French population (p-value: 0.07).

Table 3.5: Frequency of multiple paternity litters in first and second phase of the experiment, considering population background. Analysis for all experiments separately, the last two rows of the table show the analysis when data of experiments were pooled

(*): test conducted to examine whether frequencies differed between populations.

litter		First phase		p-value Fisher's Exact (*)	Second phase		p-value Fisher's Exact (*)	Comparison of frequencies MP 1st phase / MP 2nd phase [%]	p-value frequency increase
		MP	SP		MP	SP			
Exp I	G	0	6	1	4	7	0.34	0 / 36.4	0.237
	F	1	5		1	7		16.6 / 12.5	1
Exp II	G	1	5	1	1	3	0.14	16.6 / 25	1
	F	1	3		4	0		25 / 100	0.14
Exp III	G	0	10	1	15	45	1	0 / 25	0.104
	F	0	5		3	12		0 / 20	0.539
Exp IV	G	0	4	1	11	13	0.77	0 / 45.8	0.1323
	F	0	3		9	14		0 / 39.1	0.529
All exp	G	1	25	0.558	31	68	0.852	3.8 / 31.3	0.004
	F	2	16		17	33		11.1 / 34	0.074

Multiple paternity and costs

To estimate a possible cost of multiple mating for females, the survivorship of MP (females with at least one multiple paternity litter) versus SP females (females with only single paternity litters) was compared. Days in the enclosure were taken as a measure for survivorship. No significant difference was found between the founder females (p-value: 0.11; Mann-Whitney-U-Test, N_{MP}: 22, N_{SP}: 22). Also, no significant differences were found for females which died before the end of the experiment (p-value: 0.79, N=11) or all females (p-value: 0.17, N_{MP}: 60, N_{SP}: 58).

Individual condition records were analyzed from information obtained during the monitoring (described in chapter 2). Numbers of bad condition versus good condition records did not differ significantly between females with multiple paternity litters and females without multiple paternity litters (Fisher's Exact Test, p-value: 0.80).

Heritability of multiple mating

Daughters of females which had multiple paternity litters showed no significantly higher probability to have multiple paternity litters than females whose mothers did not have multiple paternity litters (Fisher's Exact test: p-value: 0.06) (Table 3.6).

Table 3.6: Number of daughters with MP or SP litters, depending on the paternity in the litters of their mothers.

Mother	Daughter	
	Multiple paternity litter	**No multiple paternity litter**
Multiple paternity	24	18
No multiple paternity	11	22

Multiple mating and success of sons

The reproductive success of males was tested comparing sons of MP mothers and SP mothers. The number of mate partners and mating events (as a measure for attractiveness), the number of offspring and offspring per mating did not differ (Figure 3.9 and Figure 3.10).

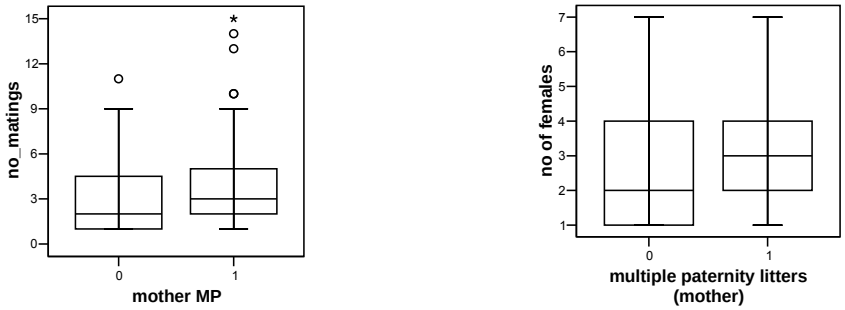


Figure 3.9: Males born from mothers with multiple paternity litters (1) had more mating events (**left**) and fertilized more females (**right**) than males born from mothers with exclusively single paternity litters (0). However, differences are not significant. Mating events: p-value (t test): 0.33, means: 0:3.37, 1: 4.35. Number females: p-value: 0.27. Means: 0: 2.58, 1: 3.16. N_1: 37, N_0:19.

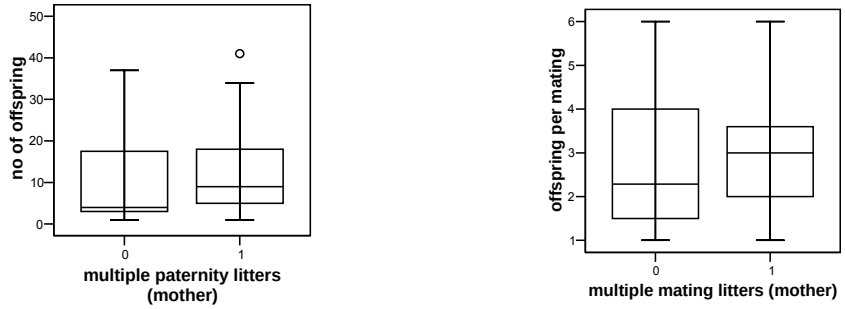


Figure 3.10: Reproductive success of males born from mothers with (1) and without (0) multiple paternity litters. **Left**: Number of offspring, p-value: 0.49. Means: 0: 10.21, 1: 12.22. **Right**: Offspring per mating. P-value: 0.79. Means: 0: 2.8, 1: 2.9.

It was not possible to test for individual success of males born in multiple paternity litters, as too few males born in multiple paternity litters had offspring (N=5). This is due to the fact that most multiple paternity litters were born towards the end of the experiment, and males were still too young to sire litters.

3.3.3 Effects of the t haplotype

t haplotype frequencies in the enclosures

Out of 1,230 animals, 18.6% (229 animals) were heterozygous for the complete t haplotype and 2.8 % (34 animals) for the partial t haplotype (21.4 % of t haplotype occurrence in total). No individual was found to be homozygous for the t haplotype. In the following analyses, partial and complete t haplotypes were treated together. The initial and final frequencies for the 4 experimental replicates are shown in Figure 3.11.

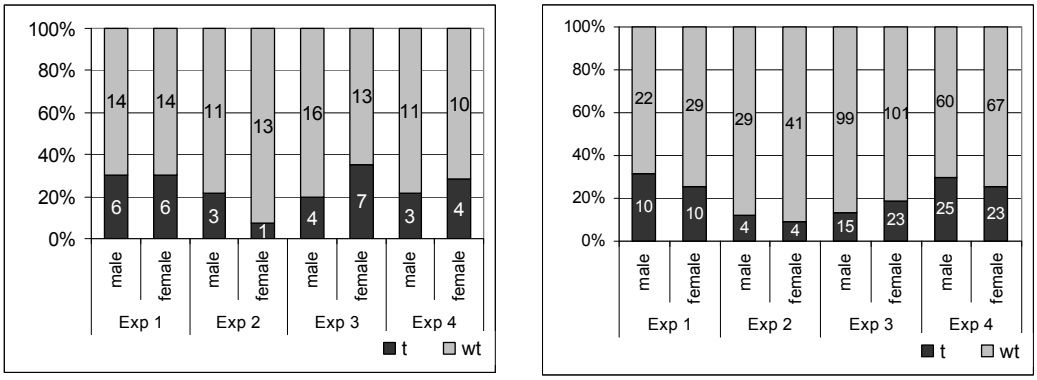


Figure 3.11: Frequencies of t/wt heterozygous animals. The numbers in the bars indicate the number of individuals. Dark bars: t/wt, light bars: wt/wt animals. **Left**: Initial frequencies (at the beginning of the experiments). **Right**: Frequencies of adult animals at the end of the experiments.

Comparing initial t frequencies with t frequencies over the whole experimental duration showed a significant decrease in t frequency for 2 of the 4 replicates (Exp II: p-value: 0.04, Exp III: p-value: 0.014). In Exp I a statistically not significant decrease was observed (p-value: 0.0636), while in Exp IV, a statistically significant increase in the t frequency was found (p value: 0.033) (Figure 3.12). For the statistical testing, chi-square was applied, using initial frequencies as expected frequencies.

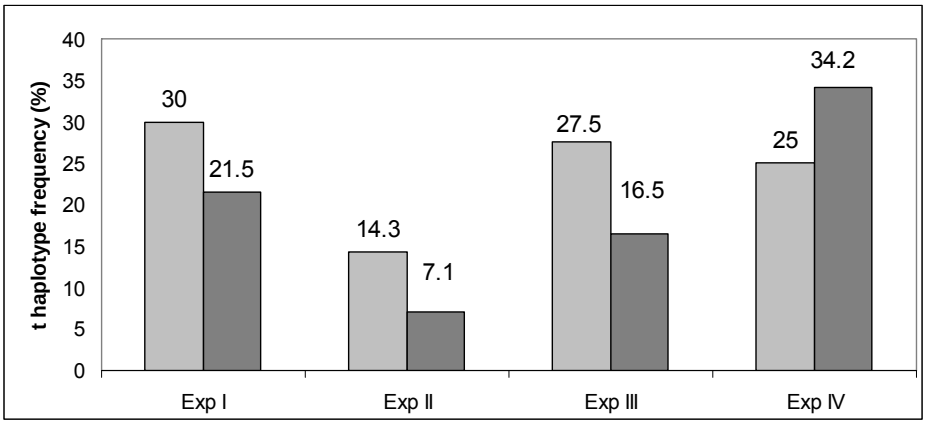


Figure 3.12: Initial (light grey) and overall t haplotype frequencies (darker grey) for the different replicates.

Transmission ratio distortion

The observed transmission ratio distortion (TRD) in litters of t/wt males with wt/wt females was 0.69 (excluding litters where a single t male only sired one progeny: TRD 0.67, mating events: 26). This value was calculated from the ratio of t/wt to wt/wt offspring resulting from matings between a wt/wt female and a t/wt male (total number of these mating events was 31). It has to be pointed out that some of these litters are multiple paternity litters; to calculate the TRD, only offspring of the t male were considered. The transmission ratio for t of females, calculated from litters of t/wt females which mated with wt/wt males, was 0.45 (45 litters) (TRD 0.46 when excluding 3 litters with one offspring). Binomial testing (number litters with TRD > 0.5 against number of litter with TRD < 0.5, litters with TRD = 0.5 excluded) showed for males a significant TRD (p-value: 0.016; 22 litters TRD > 0.5, 8 litters < 0.5, 2 excluded) and for females no TRD (p-value: 0.64; 18 litters TRD > 0.5, 22 litters < 0.5, 7 excluded).

t Haplotype and multiple paternity

The influence of the t haplotype on multiple paternity frequencies of females was analyzed, comparing the ratio of females who had at least one multiple paternity litter with females who never had multiple paternity litters. This analysis showed that frequencies for wt/wt females (48.4%, 46 out of 95 females) were slightly higher than for t/wt females (39.3%, 11 out of 28 females).

t haplotype and mate choice

Through analysis of 252 pairs (from which I had full individual information about the t haplotype) no significant preferences were found (Figure 3.13, Fisher's Exact test for all experiments: p-value: 0.34, exp. I: p-value: 0.63, exp. II: not applicable (because of "null" values), exp. III and IV: p-values: 1).

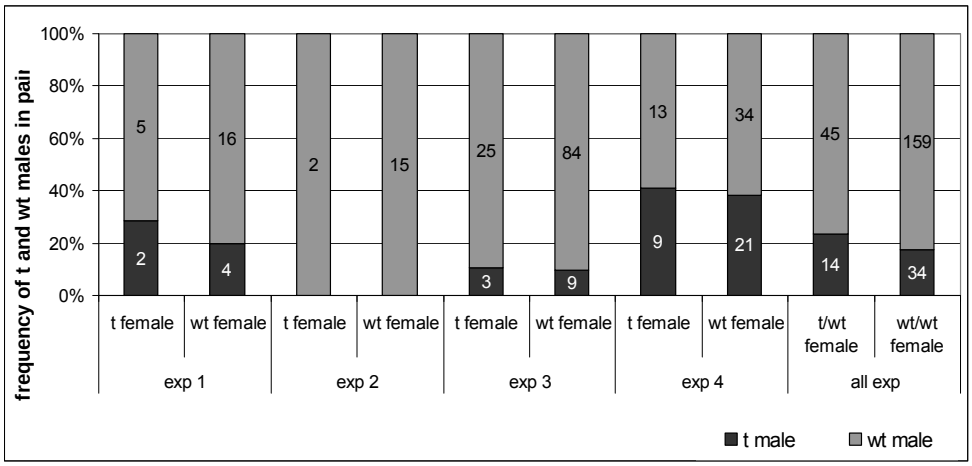


Figure 3.13: Frequency of different pairs for all experiments separately and for all experiments together. Numbers in the bars represent numbers of pairs observed.

Considering not only pairs, but also the frequency of mating events between the different pairings gave the same pattern (Figure 3.14, Fishers Exact Test: all experiments: 0.202, exp. I: p-value: 1, exp. II: not applicable, exp. III: p-value: 1, exp. IV: p-value: 0.29): no significant preferences were observed.

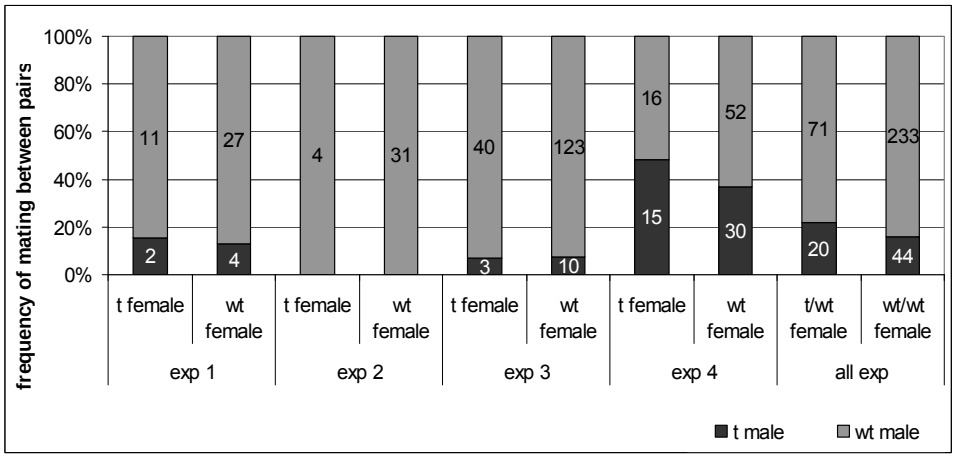


Figure 3.14: Frequency of mating events between wt/wt and t/wt individuals. Numbers in the bars represent numbers of mating events.

t haplotype and reproductive success

Ratio of successful males and females

The proportion of adult males and females which reproduced was, over all replicates, not significantly different between t/wt and wt/wt males (Figure 3.15). Nevertheless, experiment IV was outstanding: significantly more males and females heterozygous for the t haplotype were successful than wt/wt individuals (p=0.023 comparing males, p=0.01 comparing females).

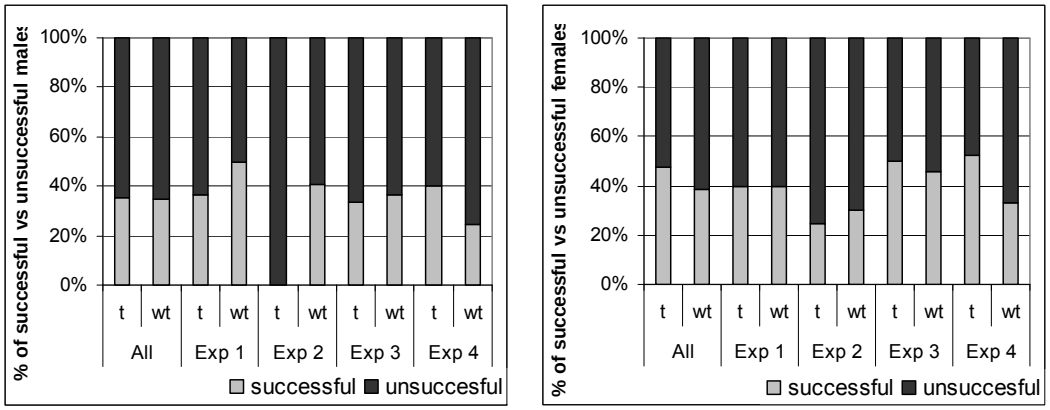


Figure 3.15: Ratio between successful and unsuccessful males (**left**) and females (**right**) in comparison between t/wt and wt/wt individuals.

Individual attractiveness

The number of mates an individual had was analyzed separately for sexes regarding t haplotype. For both sexes, no significant differences were shown (Figure 3.16).

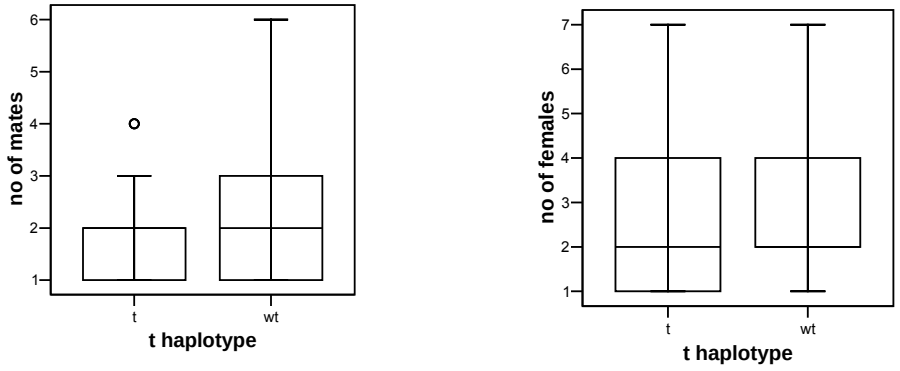


Figure 3.16: Number of mates for t vs. wt animals. No significant difference was shown. **Left**: females, N_t: 29, N_{wt}: 92. P-value t-test: 0.55. **Right**: males, N_t: 18, N_{wt}: 72. P-value (t test): 0.81.

Offspring number per mating

A significant reduction in offspring number for mating events between t/wt females and t/wt males (unpaired t-test: p-value: 0.038, average offspring number t/t: 2.1 vs. t/wt: 3.18) was found. In general however, t/wt females did not show a significantly reduced offspring number per mating (p-value: 0.96, average offspring number 2.95 for t females vs. 2.96 for wt females). Also for males, no effect of the t haplotype on offspring number per mating (p-value: 0.69, average offspring number 2.86 for t males vs. 2.97 for wt males) was detected. For t males, there was no significant difference when mating with t or wt females (p-value: 0.063, average offspring number: 3.2 with wt females vs. 2.1 when mating with t females).

Litter size

The analysis of litter size of t females which mated with at least one t male against the litter size of t females which did not mate with a t male showed no significant difference (unpaired t-test: p=0.42, average litter size of t females mated with a t male: 3.57, vs. t females mated with wt males: 4.13).

Number of offspring

The absolute number of offspring in males did not differ between wt and t males. The mean for t type males (N=18) was 10.17, while the mean for wt males (N=75) was 12.32 (two tailed t-test: p=0.42). This was also observed when controlling for the number of days an individual spent in the enclosure, and the same was found for females.

Fertilization bias

The individual average of relative fertilization success of males was calculated, to examine whether it correlated with presence or absence of the t haplotype. For this analysis, only multiple paternity litters were considered, and the relative number of offspring sired by t males versus wt males was determined. Subsequently, the average was calculated for the individual males. The results showed that there was no difference (Figure 3.17) (t test: p-value: 0.39, average relative fertilization success: t males: 0.595, wt males: 0.644).

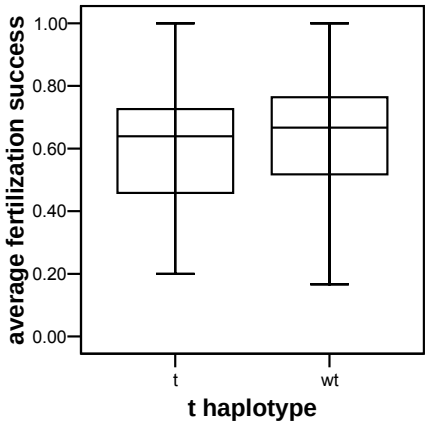


Figure 3.17: Average fertilization success of t/wt males in comparison with wt/wt males.

Individual fitness

The number of animals in bad condition or severely bitten (see definitions chapter 2) did not differ significantly between wildtype animals (N=29) and t type animals (N=11), considering the frequency of heterozygous animals (Fisher's Exact Test, p-value: 0.34). When analyzing the percentage of good condition in relation to the number of individual records (only animals with a minimum of three monitoring records were considered), wild type animals and t type animals did not differ significantly (p- value=0.62) (Figure 3.18).

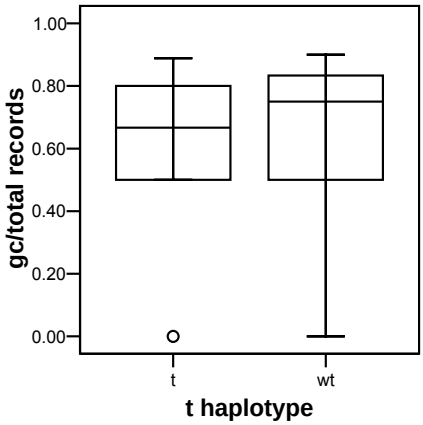


Figure 3.18: Individual condition, measured as the relation of good condition records to total number of records. N_{wt}: 75, N_t:21.

t haplotype influence on different population background

With respect to population background, no effects of the t haplotype on the number of successful females or males were detected. Table 3.7 summarizes the outcome. Only F0 and F1 animals were tested, as the numbers of the other animals were too low for statistical analysis.

Table 3.7: Number of successful versus unsuccessful t/wt individuals. Light grey: F0 generation. Darker grey: F1 generation.

	Population background	successful	unsuccessful	Fishers Exact Test
Females	G	2	1	p-value: 1
	F	11	4	
	GG	1	0	p value: 0.45, "pure" vs. mixed: p-value: 0.47
	FF	10	4	
	GF	9	6	
	FG	10	4	
Males	G	1	2	p-value: 1
	F	5	8	
	GG	0	0	p-value: 0.67, "pure" vs. mixed: p-value: 0.44
	FF	10	3	
	GF	5	4	
	FG	5	3	

Likewise, the reproductive success of t/wt animals showed no difference for the different population backgrounds (Table 3.8).

Table 3.8: Summary of reproductive success of animals heterozygous for the t haplotype in consideration of population background. The only significant difference was the number of offspring per mating in F1 males. Light grey: F0 generation. Darker grey: F1 generation.

	Population background	N	No of offspring (mean)	Statistical analysis	Mating events (mean)	Statistical analysis	Offspring/ mating (mean)	Statistical analysis
MALES	G	1	6.00	t-test: p-value: 0.66	6.00	t-test: p-value: 0.2	1.00	t-test: p-value: 0.13
	F	5	8.40		2.80		3.10	
	GG	0	0	ANOVA: p-value: 0.61	0	ANOVA: p-value: 0.77	0	ANOVA: p-value: 0.03
	FF	3	15.00		3.67		4.722	
	GF	4	16.50		5.75		2.958	
	FG	3	6.67		3.33		1.595	
FEMALES	G	2	17.50	t-test: p-value: 0.40	3.50	t-test: p-value: 0.88	4.550	t-test: p-value: 0.11
	F	11	11.27		3.82		3.130	
	GG	0	0	ANOVA: p-value: 0.92	0	ANOVA: p-value = 0.29	0	ANOVA: p-value: 0. 13
	FF	4	7.25		3.50		1.82	
	GF	6	6.50		2.17		3.44	
	FG	4	7.25		3.00		2.50	

3.3.4 Parameters correlating with male reproductive success

Age and mating events

The number of offspring correlated with the number of mating events (regression analysis: R^2= 0.83, p-value < 0.0001, Figure 3.19).

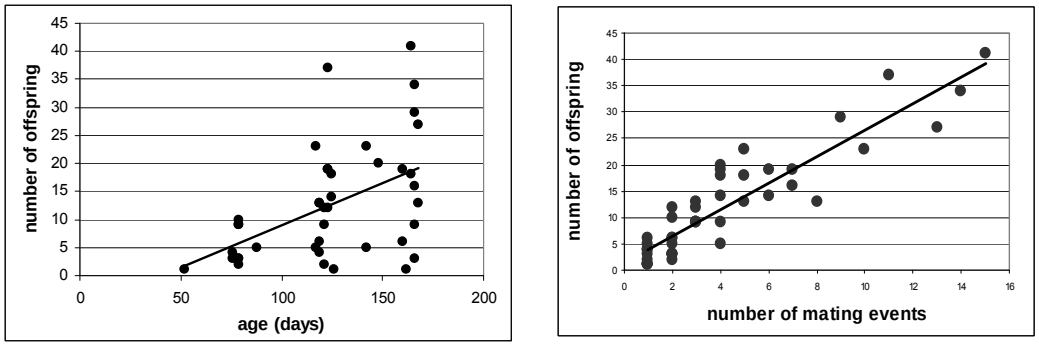


Figure 3.19: Reproductive success dependent on age (**left**) or number of mating events (**right**). The correlation between age and number of offspring was not very strong (R^2 = 0.2405), whereas a correlation between number of mating events and offspring number was clearly shown (R^2= 0.83).

Relative testis weight

RTW had no influence on the reproductive success: the analysis of mating numbers with RTW showed no correlation (R^2 = 0.02, data not shown). Likewise, no correlation was observed between RTW and absolute and relative offspring number (absolute: R^2 = 0.04, relative: R^2= 0.04). This was also observed when considering all males (including those which had no offspring) (Figure 3.20).

Nevertheless, a comparison of RTW of unsuccessful versus successful males showed a significantly higher RTW for successful males (p-value: 0.02, N unsuccessful =108, N successful= 45).

The result of the analysis whether RTW depended on the age of the animals is shown in Figure 3.21: there is no correlation detected (R^2 = 0.02). In addition, the number of successfully fertilized females (as a measure for male attractiveness) did not correlate with RTW (R^2= 0.08).

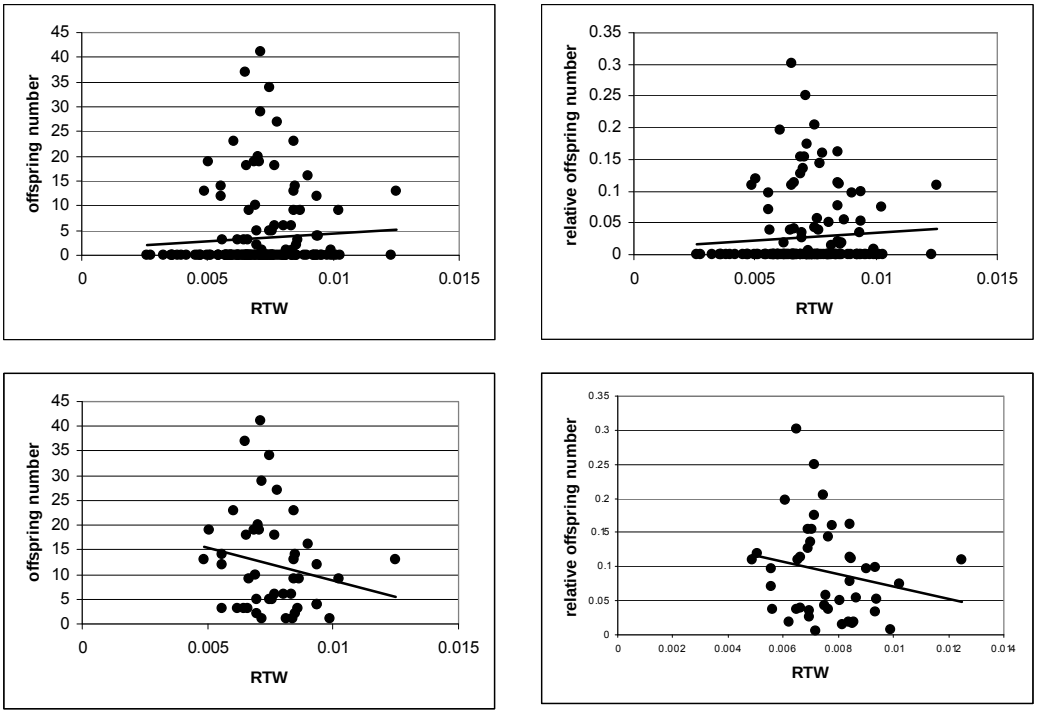


Figure 3.20 : Relative Testis Weight (RTW) and reproductive success: no correlations were observed when testing all males including those which had no offspring (plots above) or when including only successful males. **Left** plots: absolute offspring number. **Right** plots: relative offspring number.

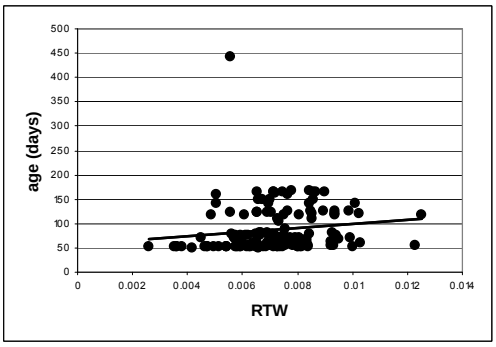


Figure 3.21: RTW and age of the males: No correlation was observed (R^2 = 0.02).

3.4 Discussion

This part of the study aims to determine whether partner preferences differ between individuals of two recently separated house mouse populations and whether this is reflected in their reproductive strategies. A possible explanation would be that individuals base their partner preferences on traits which are favorable for one situation, but not necessarily for the other, as a consequence of differences in the environment in which their offspring develop (Bussière et al. 2008). Also historically differences could differently shape mate choice behavior and reproductive strategies in diverged populations.

Reproductive success is determined by an integrated set of traits whose relationships need to be quantified and interpreted within a life history context (Cornwallis & Uller 2010). The long-term mate choice experiment analyzed here presented an ideal situation to study the role of different parameters for mate choice and mating strategies, as important behavioral traits influencing reproductive success.

Overall, it can be stated that no differences regarding effects of the t haplotype and the analyzed strategies between the populations were detected.

Through the careful analysis of multiple mating frequencies and benefits as well as the implications of communal breeding, it became evident that communal breeding and multiple mating slightly increased reproductive success under semi-natural conditions. The balance between benefits and costs seems to vary, as otherwise these strategies would become fixed in populations.

Observed effects of the t haplotype were restricted to a slight decrease in offspring number in mating events between t/wt animals. I found no evidences for an increased multiple mating frequency or the avoidance of partners with t/wt.

In the following, the results are discussed in detail.

3.4.1 Communal breeding

Overall it can be stated that communal breeding was frequently observed in the experiment and found for both populations (shown in chapter 2), and that, rather than population background, kinship was important for social partner choice.

As described by Manning et al. (1995), female house mice often nest communally, and within these communal nests appear to indiscriminately nurse all pups. This costly behavior of house mouse females is expected to have benefits.

Direct benefits for females in form of higher reproductive success could not be observed: the results presented here showed that females nursing in communal breeding litters had a smaller litter size than females nursing alone (Figure 3.3). However, the difference was not statistically significant, and it is important to mention that towards the end of the experiment the communal breeding frequency increased, probably as a consequence of limited nest sites. At the same time, littersize per female decreased, most likely due to resource competition and stress. Another possible benefit for females could be indirectly through enhanced fitness for their offspring. Therefore, the reproductive success of animals grown up in communal breeding litters was compared with the success of animals born in “normal” litters. The analysis showed that females grown up in communal breeding litters showed a significantly higher offspring number than other females (Figure 3.4), which can not be explained through an increased mating success. This effect was not seen for males (Figure 3.5). These findings are interesting in the light of two recently published studies: Curley et al. (2009) showed in an experiment with Balb-C mice, that communal breeding had transgenerational positive effects on the offspring which were grown up in communally reared litters: female offspring showed higher levels of maternal care and reduced anxiety-like behavior. Another study with laboratory mice (CD1 mice) showed that offspring from communal nests displayed relevant changes in brain function and behavior (Branchi 2009).

Contrary to the above mentioned higher reproductive success for females grown up in communal litters, I observed a significantly higher proportion of communally reared individuals with complete breeding failure. Due to the fact that communal litters were increasing in frequency towards the end of the experiment, I consider this result might be a bias, since animals born towards the end of the experiment had only a small possibility to raise offspring.

Out of 10 sister – sister breeding pairs, only one was of half sibs, the others were full sibs. Something similar was reported by Evans & Kelley (2008), who found in guppies that pairs of full siblings spent significantly more time shoaling than pairs of half siblings. The authors suggested that this finding presents a potential cost of polyandry: a reduction in within-brood relatedness with potentially important

implications for offspring social behavior. The preference to relatives as partners was already reported in other studies: König (1994) showed in a laboratory setting that females nursing communally with sisters had a higher reproductive success than females nursing with unrelated but familiar females, and Manning et al. (1992) showed that females preferred communal nursing partners with a similar MHC. Weidt et al. (2008) reported that females in pairs with a preferred social partner had a higher reproductive success than females in non-preferred pairs.

3.4.2 Multiple paternity

The here reported frequencies of multiple paternity support the assumption of multiple mating as a common strategy. The frequencies did not differ between different population backgrounds or experiments (see also chapter 2) and showed for all cases higher values correlating with higher population densities. Litters in which mating events between relatives were involved showed a significantly higher frequency of multiple paternity. Reproductive success was slightly elevated for females which had at least once a multiple paternity litter. Enhanced reproductive success for sons, supporting the sexy sperm hypothesis and heritability testing of multiple mating were not statistically significant. All results were consistent for the different population backgrounds. Costs for females in form of a reduced survivorship or inferior individual condition were not detected.

It has to be mentioned that in all the above documented results, the analysis of multiple paternities underestimated frequencies of multiple mating. Effects of sperm competition and cryptic female choice can bias the results.

Frequency of multiple paternity

Analyzing all experiments together, 29% of litters were sired by multiple males. No statistically significant differences in multiple paternity frequencies were detected between the different populations (Table 3.1). By this, a similar multiple mating frequency for both populations can be assumed, and also postcopulatory mechanisms like sperm competition and cryptic female choice might be similar. Considering the strongly varying frequencies of multiple paternity found in other enclosure studies (e.g., 19.4% in Carroll et al. (2004) and 64% in Ehman & Scott (2004)) and wild populations (e.g., 6-43% found for 7 island populations by Firman & Simmons (2008)), the results found here indicate a similar multiple mating frequency for females depending on current environmental conditions. This assumption is

supported by the elevation of multiple paternity frequencies towards the end of the experiment, when population densities increased considerably in all replicates and population backgrounds (Table 3.4 and Table 3.5). The increase of multiple paternity in high density areas was also reported by Dean et al. (2006). These authors explained this finding with the higher probability of a female to encounter more than one male in higher density populations. In the enclosure setting, however, this explanation is not very likely, since densities were high enough from the beginning of the experiment that individuals could encounter each other. Another explanation would be a targeted strategy of females depending on population density (e.g. to reduce male harassment).

Benefits of multiple mating

Analyzing the reproductive success showed that females which had at least one multiple paternity litter had a higher overall reproductive success than females which only had litters sired by a single male (Figure 3.8) and litters were bigger when sired by multiple males (Figure 3.7). This result was observed for all experiments, as well as for females of different population background (Table 3.3), although differences were often not statistically significant. This is in line with results of Firman & Simmons (2008a) who showed that females mated to 3 different males during one reproductive cycle had greater post birth pup survival than females mated 3 times to the same male.

An indirect possible benefit of multiple mating for females is the reduction of inbreeding risk. Whether mating with relatives favors multiple mating can be tested indirectly via comparing multiple paternity frequencies in litters of unrelated couples with litters where mating events between relatives were involved. Analyzing all mating events together showed that the frequency of multiple paternity was significantly higher for litters in which mating events between relatives were involved. When testing pairs of different relatives (mother-son, daughter-father, half-sibs and full-sibs), it was shown that the litters where mother – son matings were involved showed the highest frequency of multiple paternity (Figure 3.6) compared to litters from non related couples, and in this cases significantly more litters are sired by several males than by one male. The results were consistent when analyzing all litters together as well as for the separate analysis of litters with different population background (Table 3.2). This finding is an additional hint for a general mating strategy of house mouse females to reduce the risk of inbreeding. It supports the result of Firman & Simmons (2008) who showed that polyandry may provide an

opportunity for females to avoid the cost of inbreeding by exploiting postcopulatory mechanisms that bias paternity towards unrelated male gametes.

Another assumed benefit of multiple mating is related to the “sexy sperm” hypothesis, where females give birth to highly competitive sons through enabling sperm competition (Harvey & May 1989; Keller & Reeve 1995). Following this theory, females have an indirect benefit for their offspring if the most competitive sperm gain fertilization of their ova. Related to this hypothesis is the assumption that female benefits result from “good genes” which enhance fitness of offspring. Evidence for female benefits of multiple mating through a higher reproductive success of sons had been demonstrated by Klemme et al. (2008) who showed a higher reproductive success for bank vole males born in multiple paternity litters. Unfortunately, in my experiment only few males (N=5) from multiple paternity litters achieved offspring. This is due to the fact that most multiple paternity litters were born towards the end of the experiment, and offspring from these litters were too young to fertilize a considerable number of females. However, the reproductive success of males from mothers which had at least one multiple paternity litter was slightly higher than for sons of females which only had litters sired by one male (Figure 3.9 and Figure 3.10). Considering the elevated frequency of multiple paternity towards the end of the experiment, it is more likely that the sexy sperm hypothesis does not play a major role to counterbalance costs through multiple mating, as it would be expected that benefits through this mechanism would be effective also in low density populations.

Support for heritability of multiple mating was tested. Indeed, daughters of females which had multiple paternity litters showed a higher probability to have multiple paternity litters than females whose mothers did not have multiple paternity litters (Table 3.6), but this result was not statistically significant.

Costs for females in form of a reduced survivorship or inferior individual condition could not be detected comparing females which had multiple paternity litters versus females which had only litters sired by a single male.

3.4.3 Effects of the t Haplotype

t haplotype frequencies in the enclosures were around 20-30% and mostly decreased towards the end of the experiment. Male transmission ratio distortion was, compared to other studies, rather low.

The effect of the t haplotype on reproductive success was restricted to the number of offspring in mating events between two individuals heterozygous for the t haplotype. Although this finding should present a selection pressure towards a strategy for t/wt females to avoid mating with t/wt males or an increased frequency of multiple mating, no significant evidence was found for this. The consequences for t/wt females to mate with t/wt males seemed to be low, as no other effects on reproductive success or individual fitness were found. These findings were the same for the different population backgrounds (Table 3.7 and Table 3.8).

t haplotype frequencies and TRD

21.4 % of animals from the long-term experiment were heterozygous for the t haplotype. The frequencies differed considerably in the four experimental replicates, which is partially explained by different initial frequencies. In three experiments frequencies declined towards the end of the experiment, while experiment 4 showed a relative increase in t frequency (Figure 3.11 and Figure 3.12) and is outstanding also in other aspects related to the t haplotype.

The observed male transmission ratio distortion (TRD) was 69.3 %, which is low, compared to other studies (Carroll et al. 2004). This low value is possibly biased, as in many litters, especially towards the end of the experiment, only few offspring were found, possibly due to infanticide. However, female transmission distortion follows with 45.3% the expectations (43% reported by Carroll et al. 2004).

Mate choice

The predicted preference of females heterozygous for the t haplotype for mate partners without a t haplotype to encounter homozygous lethal or sterile effects in the offspring was not supported by the results of the experiment. Through analysis of 252 pairs no significant preferences for or against the t haplotype were found (Figure 3.13 and Figure 3.14).

The frequency of multiple paternity was lower in t/wt females (39.3%) than in wt/wt females (48.4%). This is not expected as it is assumed that multiple mating could be a female strategy to encounter deleterious effects of t haplotype (Haig & Bergstrom 1995). However, it has to be recalled that frequencies of multiple paternity are only an indicator for multiple mating and underestimate the real degree of polyandry, i.e., a female which biases fertilization 100% towards wt/wt male sperm, would not be

detected as a multiple mating female. The effect of cryptic choice could present a potent force.

Individual reproductive success

In an extensive enclosure study on the ecological effects of the t haplotype, Carroll et al. (2004) found a decrease in reproductive success for heterozygous individuals. My experiment did not support their findings: While the aforementioned authors reported a higher breeding failure for t heterozygous females, in three of the four experiments no significant differences in the proportion of successful animals were found, and in experiment 4, significantly more males and females heterozygous for t were reproductively successful than wt/wt animals (Figure 3.16).

The examination of consequences for t/wt females which mated with t/wt males is confounding: although offspring number was reduced in mating events where both partners are heterozygous for the t haplotype, t/wt females did not show a lower overall offspring number. This result suggests the presence of a mating strategy of heterozygous females biasing their mate choice towards males without the t haplotype or to increase multiple mating frequencies; nevertheless, analysis of the mentioned parameter did not reflect this.

Since litter size in t/wt females is not reduced significantly, the possibility of a fertilization bias of t/wt females in favor of wt/wt males is assumed. Nevertheless, the analysis of the relative fertilization success for t/wt vs. wt/wt males in multiple paternity litters showed no fertilization bias dependent on the t haplotype.

Individual fitness

No difference of individual fitness was found between t/wt and wt/wt animals. This is contrary to the observation reported by Carroll et al. (2004), who showed that t/wt males have significantly higher difficulties to maintain territories.

3.4.4 Parameters correlating with reproductive success

There is some correlation of RTW with reproductive success: males which had no offspring had significantly lower testis size. However, relative and absolute numbers of offspring do not correlate with RTW (Figure 3.20). It is important to consider that the RTW value for each individual male is only a snap-shot from the day the males were dissected, which could change with the condition of the male: Schulte-Hostedde et al. (2005) showed in a study with three small mammal species that testis size was

positively related to body condition which suggests that males in good condition are capable of investing more in ejaculates than males in poor condition.

3.5 Conclusion

The above discussed results indicate that the French and German populations do not differ in effects of the t haplotype on mate choice. In addition, frequencies of communal breeding and multiple mating in females are similar in both populations and respond equally to environmental changes (in terms of population density) and inbreeding risk.

Considering these findings, there is no hint towards a different population history which would shape mate choice and reproductive behavior differently in the German and French population. However, the highly significant *father related assortative mating pattern* indicates that some traits are recognized by the individuals as “own” and “foreign”. Genomic imprinting is only one possible explanation, and the screening of the influence of other parameters, e.g. major urinary proteins or MHC alleles may identify the divergence between the populations.

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Supplement

Supplement 1: Description Access Database

Overview tables

Name	Description
tblCheckdate	Information on which days the spatial data were taken.
tblChecklist	Spatial data taken on different “checkdates”
tblLocality	Information about different localities and primary key for locality (psLoc). Localities with “I” or “II” in the name refer to Room 1 or 2 respectively
tblMice	Individual mice with constant characters (e.g. sex, birth, death). Includes also results of paternity testing and t haplotype determination (Items are also explained in the design view in Access).
tblMonitoring	Information gathered during monitoring events (each 3 – 4 weeks): weight, pregnancy, lactation, general condition, mites, ..)
tblPups	Information about samples taken from newborn mice (still in litters). These help to find birth dates of mice by identity-matching between “mice” and “pups” - genotypes

Detailed description tblMice

Items	**Information**
psMouse	Primary key: unique number for all mice, serves to connect table with other data (e.g. data from localization check, offspring, partner, monitoring etc.)
Experiment	Number of experiment where mouse was involved
TubeNo	Sample number / number of tube where mouse is stored after death (or end of the experiment): all samples (extracted DNA, backup DNA, stored carcass, shock frozen tissue, etc…) are labeled with this number.
RunningID	Name of mouse during the experiment, useful especially at the beginning of the experiments to distinguish between mice of the populations, no need for consequent use
Animal	General information about the mouse, e.g.: - Parent (F0 generation) - Offspring (later generations) - Dead Pup (animal which was sampled dead during the experiment) - “E” with number (Embryo of certain female with embryo number, e.g. E_IV_141-2: Second Embryo from mother with TubeNo IV_141) - Newborn (mice born few days before end of experiment) - Temporary mice (mainly from F0 who lost their transponders) - pups from tblpups: without identity match in tblmice (were sampled but not found dead or at the end of the experiment)
Transponder	Number of the transponder, is not unique, as in some cases mice lost their transponders and had to be re-transpondered during the experiment. Some transponder numbers were used more than once (in different experiments)
TPDate	Day when mouse was transpondered
MouseHouse ID	ID from the stock collection (only existent for parent generation)
fsMother	psMouse of Mother

fsFather	psMouse of Father
InfoPat	Information about paternity assignment, e.g. in case of problems
Population Background ("Herkunft")	left side: abbrev.origin mother "." , right side abbrev.origin father. In higher generations separation with "_", then "-". "unklar" in case of paternity problems
Mismatches	Information about number of mismatches (when critical)
ProbPat	"yes" in case of problem with paternity assignment (generally parents too young or assignment with more than 2 mismatches
PatSolved	Paternity assignment reliable
InfoPat	Information about paternity assignment (which phase, observation why paternity problem, etc.)
Mismatches	Number of mismatches in paternity assignment
MP/CB checked	Individual checked in litter context (same mother/how many different fathers – reliable number of fathers, reliable birth date) Only for internal use during the analysis
PatProb	"yes" when paternity could not be assigned
PatSolved	"yes" when paternity was successfully assigned. Field was useful during analysis
Nicht auswertbar	In case of missing data (generally mice who lost transponder during experiment – just "check" data, no typing)
Info Partnerchoice	In case of females: obtained from information about fathers of litter. E.g.: one (or/and) multiple father litters (number of litter: number of pups per litter, separated by comma). Was useful during analysis
OldLitterID	First assigned litter ID. Was kept to clarify eventual doubts
LitterID	ID of litter mouse is assigned to – useful for multiple paternity and communal breeding analysis. In case of communal breeding: LitterID ends with a letter (a, b, c, etc.)
Communal_breeding	animals grown up in communal breeding litters
Sex	m: male, f: female. "0": not determined
Birth	Date of Birth
Dead	Date when found dead or last day of experiment
days_in_exp	Number of days an animal had been in the experiment. Useful to calculate relative reproductive success
t Haplotype TCP1	"no" when homozygous for wildtype, "yes" when heterozygous t/wt
t Haplotype Hba4ps	"no" when homozygous for wildtype, "yes" when heterozygous t/wt
Tcp1	Allele 1 at TCP1 locus. "W" when wildtype, "T" when t haplotype
Tcp2	Allele 2 at TCP1 locus. "W" when wildtype, "T" when t haplotype
Hba-ps4_1	Allele 1 at Hba-ps4 locus. "W" when wildtype, "T" when t haplotype
Hba-ps4_2	Allele 2 at Hba-ps4 locus. "W" when wildtype, "T" when t haplotype
Hba data missing	"yes" when typing for t haplotype at locus Hba-ps4was not successful
tcp1 data missing	"yes" when typing for t haplotype at locus TCP1 was not successful
Bellycolor	Dark or bright. Information not consequently added, but data available (not systematized)
Disperser	"yes" when animal used dispersal tube to "migrate" from enclosure
DisperalDate	Date of dispersal
OfRunningID	Running Id of offspring. Not consequently used, since utility was not confirmed
nicht auswertbar	"yes" when not analyzable, e.g. due to missing microsatellite data, or no tissue samples. Generally mice which lost their transponders during the experiment. Localization data of these mice can not be

	assigned to an individual.
Alleles of microsatellite loci: per locus two columns, one for each allele. Name of loci at first positions, last position (“1” or “2”) refers to the first or second allele	Information on allele
spleen	spleen tissue available
extracted	DNA extraction done (information not constantly updated)
20ng/ul	Presence of DNA template with concentration of 20 ng/ul (information not constantly updated)
backup	presence of backup material
carcass	storage of carcass
carcass Alc	carcass in good alcohol
Info	Additional information (not systematic)

Digital Supplement

Access Database with all data gathered during the longterm-experiment (description of database see above).

Erklärung

Ich versichere, dass ich die von mir vorgelegte Dissertation selbstständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit - einschließlich Tabellen, Karten und Abbildungen -, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie noch nicht veröffentlicht worden ist, und ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde. Weiterhin versichere ich, dass die Arbeit unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft entstanden ist. Die Bestimmungen dieser Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Herrn Prof. Dr. Diethard Tautz betreut worden.

Plön, den 12. November 2010 Inka Montero

Lebenslauf

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