

Master of Science Thesis

**The effects of inbreeding on mating success and offspring  
production of males and females in a *Drosophila littoralis*  
population**

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## TIIVISTELMÄ

Sisäsiitoksella, eli pariutumisella lähisukulaisten kesken, on monia negatiivisia vaikutuksia sekä populaation että yksilön kelpoisuudelle. Sisäsiitos lisää homotsygotiaa ja mahdollistaa siten väistyvien haitallisten mutaatioiden ilmentymisen fenotyypissä sekä estää heterotsygotiasta johtuvaa paremmuutta lokuksissa, joissa on ylidominanssia. On osoitettu, että sisäsiittoisuus voi vaikuttaa koiraisiin ja naaraisiin eri tavalla siten, että toinen sukupuolista kärsii sisäsiitoksesta toista enemmän. Jotkut tutkimukset ovat näyttäneet, että sisäsiittoisuudella on voimakkaampi negatiivinen vaikutus koiraiden kuin naaraiden lisääntymiskykyyn, kun taas toiset osoittavat että naaraat kärsivät enemmän sisäsiitoksen seurauksena. Tutkimukseni tarkoituksena on selvittää löytyykö sukupuolten väliltä eroja sisäsiitoksen vaikutuksessa lisääntymismenestykseen. Tutkin sisäsiitoksen vaikutuksia pariutumismenestykseen ja jälkeläistuottoon *Drosophila littoralis* – mahlakärpäs populaatiossa kahdessa erillisessä kokeessa. Sisäsiitos alensi merkittävästi jälkeläistuottoa naarailta, kun taas sisäsiitoksen vaikutukset koiraiden jälkeläistuottoon olivat lievempiä, joskin negatiivisia. Tämä viittaa siihen, että naaraiden lisääntymismenestys kärsii enemmän sisäsiitoksen vaikutuksesta kuin koiraiden. Nämä tulokset ovat yhteneväisiä sen kanssa, että naaraat panostavat enemmän lisääntymiseen ja sisäsiitoksella voi siten olla enemmän negatiivisia vaikutuksia naaraiden kuin koiraiden lisääntymiseen. Myös sisäsiittoisten naaraiden pariutumismenestys oli heikompi kuin ei-sisäsiittoisten naaraiden, mikä vahvistaa päätelmää että naaraiden lisääntymiskyky vaarantuu sisäsiitoksen negatiivisten vaikutusten takia. Sisäsiittoisten naaraiden alhaisempi lisääntymismenestys tuo esille mielenkiintoisen näkökulman sukupuolten rooleista pariutumisessa. Vaikka naaraita pidetään usein koiraita valikoivampana sukupuolena pariutumistilanteessa, myös koiraat voivat valita parinsa naaraiden ominaisuuksien mukaan. Koiraat voivat yrittää maksimoida oman lisääntymismenestyksensä olemalla valikoivampia parinvalinnan suhteen kuin mitä aiemmin on ajateltu. Koiraat voivat myös hyötyä valikoivasta käyttäytymisestä oletettua enemmän. Sisäsiittoisuus ei heikentänyt merkittävästi koiraiden pariutumismenestystä. Nämä tulokset herättävät monia mielenkiintoisia kysymyksiä sukupuolten rooleista lisääntymisessä ja sisäsiitoksen sukupuoleen perustuvista eroista.

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## ABSTRACT

Inbreeding, mating between close relatives, has various negative effects on both population and individual fitness. Inbreeding increases homozygosity, allowing recessive deleterious mutations to be expressed and preventing superiority arising from heterozygous overdominant loci. Lower fitness of inbred individuals can lead to decreased mating success and offspring production. There is some evidence that inbreeding can affect sexes differently, so that one of the sexes suffers more from inbreeding than the other one. Some studies suggest that inbreeding has more severe negative effects on male reproduction, while others have indicated that females suffer more as a consequence of inbreeding. The aim of my study was to find out if there are any differences between sexes in the effects of inbreeding on reproduction success. I studied the effects of inbreeding on mating success and offspring production in a *Drosophila littoralis* population in two separate experiments. Inbreeding significantly decreased the offspring production of females, while the effects of inbreeding on male offspring production were milder, yet also negative. This indicates that the reproduction success of females suffers more from inbreeding than that of males. These results are consistent with the fact that females invest more in reproduction and inbreeding reduces the limited resources of the females available for reproduction. Inbred females had also lower mating success than non-inbred females, showing further evidence that the reproductive ability of females is compromised by negative effects of inbreeding. The fact that inbred females had lower mating success raises also an interesting point of view about the roles of sexes in sexual selection. Even though females are often considered to be the choosier sex in mating and males are considered to be less choosy, also males are choosy and can base their choice on the fitness of the females. Thereby males might try to maximize their reproduction by being choosier about their mates than previously has been thought. Also, males might benefit more from choosing than has been assumed before. Inbreeding did not significantly lower the mating success of the males. These results raise many interesting questions about the sex roles in reproduction and the sex-based differences of inbreeding.

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## 1. INTRODUCTION

From the evolutionary perspective reproduction is the most important issue in an individual's life, as it determines the number of genes an individual can transfer to next generations via offspring. Different reproductive strategies have evolved for different organisms, such as asexual reproduction which is most typical for many plants, and sexual reproduction with several types of variations (Emlen & Oring 1977). Regardless of the type of reproductive strategy, reproduction comes with variable risks, for example injuries from competing over mates or from mating itself (Bean & Cook 2001, Blanckenhorn et al. 2002). As reproduction is crucial for individual fitness, selection should have favored reproductive strategies that maximize reproduction. This is why in many species the sex that makes larger investments to reproduction is also choosier about whom they mate with (Reynolds 1996, Kokko et al. 2003). Commonly the major investor is female. She usually produces the larger gametes, in mammals carries the embryo in her womb and lactates the young, and in many species takes care of the offspring. As a result of these large investments, in many species females tend to seek the most qualified mate for themselves.

The costs of mating are distributed unevenly between the sexes. In many cases mating costs are more severe to males than to females. This is mainly because females are generally the limiting sex for matings. Males often have to compete to maintain good territories and to gain access to females (Reynolds 1996, Bean & Cook 2001). Once they manage to do that, there are still additional obstacles to cross. Females can be choosy and reject the males attempting to mate with them (Spieth 1974, Kokko et al. 2003, Mackay et al. 2005). In most severe cases males get killed by the females that feed on them. This sexual cannibalism has been detected on some insects such as the praying mantis and various spider species (Bushkirk et al. 1984). In less deadly mating attempts the males have nonetheless spent their resources in vain if the mating attempt is unsuccessful.

The concept of sexual selection is commonly considered to consist of two main points: the male competition and the female choice. Females look for quality in their mates and the role of males has been understood to be more about seeking quantity in matings and competing with each others for mates while females have been regarded as the selective sex (Reynolds 1996, Kokko et al. 2003). However, choosiness might not just be a privilege reserved for females. Male choosiness can also be expected in certain situations when potential female mates are abundant, when the male mating investments are notable, and in species with reversed sex roles (Bonduriansky 2001). Evidence for male choosiness has been found in several species, such as house finches (*Carpodacus mexicanus*), sticklebacks (*Gasterosteus aculeatus*), two-spotted gobies (*Gobiusculus flavescens*), and orb-weaving spiders (*Zygiella x-notata*) (Hill 1993, Kraak & Bakker 1998, Amundsen & Forsgren 2001 and Bel-Venner et al. 2008, respectively).

Inbreeding has various known negative effects to the fitness of individuals and also to the fitness of populations. Inbreeding increases the proportion of homozygous genotypes in a population, allowing deleterious recessive mutations to be expressed, and reducing heterozygosity in overdominant loci (Keller & Waller 2002). According to reviews by Keller & Waller (2002) and Charlesworth & Willis (2009), inbreeding depression (i.e. reduced fitness due to inbreeding) occurs commonly in nature and can seriously affect the viability of populations. Effects of inbreeding are especially severe if populations are small and isolated. Stressful and unsteady conditions often tend to increase the negative effects of inbreeding (Bijlsma et al. 1999, Meagher et al. 2000, Joron & Brakefield 2003, Enders & Nunney 2010).

The effects can be also long lasting; it can take at least 50 generations for a population to recover from inbreeding depression (Bijlsma et al. 2000). Nevertheless the effects of inbreeding might in some cases be surprisingly positive, such as in the case of *Drosophila melanogaster*, where females that did better in a resource location test at cold temperatures (Kristensen et al. 2008). So in some cases inbreeding might actually give individuals some advantage; although the advantage is usually environmental dependent (Hedrick 1994, Bijlsma et al. 1999, Dahlgaard & Hoffmann 2000).

Inbreeding has been shown to result in lowered offspring production in both sexes (Meagher et al. 2000, Tregenza & Wedell 2002, Kristensen et al. 2008) suggesting that decreased heterozygosity has negative effects on reproduction. This was shown in the study by Saccheri et al. (1998) in a Glanville fritillary butterfly (*Melitaea cinxia*) metapopulation. Recently it has been found that inbreeding also affects the immune response, leaving the inbred individuals particularly exposed to parasites (Reid et al. 2007). Since inbreeding has such negative effects for individuals, they have several mechanisms to avoid it, such as dispersal, extra-pair copulations, recognition and avoidance of kin as mates as well as delayed maturation (Pusey & Wolf 1996, Brown 1997, Tregenza & Wedell 2000, Foerster et al. 2003, Kempenaers 2007).

There might be some differences in the effects of inbreeding on mating and reproduction between males and females. This may be because males and females have different roles in sexual reproduction and hence uneven costs of intersexual competition, reproduction and providing for offspring. Thus males and females might have unequal amounts of benefits to gain from the choice of a mating partner. The ability to produce viable offspring may be compromised more in females, since they invest more on reproduction while male mating success could be poorer because of male competition and female choosiness. Several studies have indicated that inbreeding has more severe consequences on male than female fertility, offspring production and survival (Jiménez et al. 1994, Meagher et al. 2000, Okada et al. 2011, Simmons 2011). Then again Keller (1998) and Charpentier et al. (2006) show that females suffer more from inbreeding than males. Sex-dependent differences have also been found in immune response (Reid et al. 2007) and survival rates (Coulson et al. 1999, Rossiter et al. 2001 and Rioux-Paquette 2011).

In this study I concentrated on the effects of inbreeding on mating success and offspring production in *Drosophila littoralis* flies. Inbreeding has various known effects on *Drosophila* flies, such as decreased male-male competitive ability (Sharp 1984), decreased stress resistance (Bijlsma et al. 2000, Dahlgaard & Hoffmann 2000) and decreased productivity (Okada et al. 2011). Aspi (2000) showed inbreeding depression in *Drosophila montana* male courtship song characters. There has also been reports on some sex-dependent effects of inbreeding in *Drosophila melanogaster* flies, related to heat and cold tolerance (Kristensen et al. 2008 and Mikkelsen et al. 2010) and resource location (Kristensen et al. 2008). I was interested about the possible differences in the effects of inbreeding between the sexes. There were two main questions: 1) Does inbreeding of males and females have different effects on offspring production of the two sexes (measured as number of eggs and adult offspring)? 2) Does inbreeding of males and females have different effects on mating success of the two sexes? These questions were studied with two separate experiments. My hypothesis was that because of the larger maternal investments the effects of inbreeding on offspring production would be stronger in females than males. Male mating success would in turn suffer more from the inbreeding because of male competition and female choice.

## 2. MATERIALS AND METHODS

### 2.1 Study species

The study species is *Drosophila littoralis*. Courtship behavior of *Drosophila* fruit flies has been studied for a long time and is well known (Spieth 1974, Liimatainen et al. 1992, Mackay 2005). First the male finds the female by using visual and olfactory signals and then aligns himself with the female. This is followed by physical contact where the male will recognize the species of the female by using pheromonal cues. This contact is usually performed as tapping the female's abdomen with the foreleg. Then the male starts to vibrate his wing or wings to create a species-specific courtship song. After this the male licks the female's genitalia for further pheromonal cues and then tries to copulate. The female can accept the male and raise her wings to allow him to copulate or reject him by moving away (Geer & Green 1962, Spieth 1974, Mackay et al. 2005). The courtships song is a particularly important and well studied part of *Drosophila* mating and has been identified and recorded for a number of species, including *Drosophila littoralis* (Aspi & Hoikkala 1995, Hoikkala et al. 1998).

### 2.2 Inbreeding treatments

A laboratory population of *Drosophila littoralis* was founded from 157 males and 99 females that had been collected by the Tourujoki River in Jyväskylä, Finland. During the first six generations the population was kept at a size of approximately 360-500 breeding pairs. The base population consisting of 500 individuals (250 pairs) was established from the 7<sup>th</sup> laboratory generation. The flies were kept in plastic bottles in constant conditions of 19°C, humidity of 60%, permanent light and continuously available malt medium (Lakovaara 1969) for 24 generations before the experiments.

There were three different inbreeding treatments for the experiments resulting in three different inbreeding coefficients:  $f = 0.00$ ,  $f = 0.25$  and  $f = 0.38$  (see Figure 1.). The inbreeding coefficient was calculated as  $f_i = \frac{1}{4} (1 + 2 f_{i-1} + f_{i-2})$  (Falconer & Mackay 1996). The inbreeding coefficient denotes the expected proportion of loci that have alleles identical by descent in an individual. The control flies were taken from the base population and randomly mated in vials for one generation: individuals in this control group had  $f = 0.00$ . The two groups of inbred flies, having  $f = 0.25$  and  $f = 0.38$ , were created from the base population flies by one generation (for the  $f = 0.25$ ) or two generations (for the  $f = 0.38$ ) of full-sib matings. Inbreeding treatments were made in such a way that all the groups reached the desired level of inbreeding at the same generation. Thus the matings for the group  $f = 0.38$  started two generations before the matings for the group  $f = 0.00$  flies (the control group). All the flies were collected before they reached maturity, at the age of 0-5 days after eclosion, and males and females were separated under CO<sub>2</sub> anesthesia and kept in different vials to ensure that the flies used in the experiments were virgins. The matings took place in small plastic vials (diameter 23.5mm, height 75.0mm) with 8ml of malt medium, which the flies used as nourishment and also laid eggs in it. Later, the hatching larvae consumed the medium.

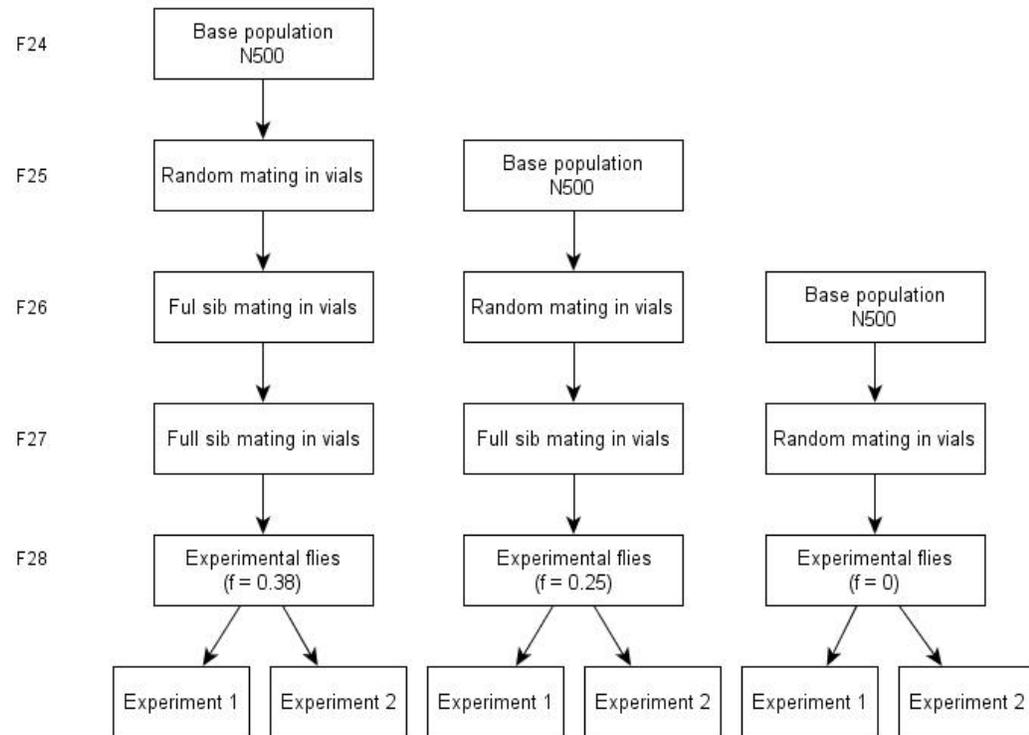


Figure 1. Inbreeding treatments. Matings for the experimental groups started from the generation 24 of the laboratory population and the experiments were conducted at F28.

### 2.3 Experiment 1: Offspring production

In Experiment 1 the aim was to study whether inbreeding of males and females has different effects on offspring production of the two sexes. The effects of inbreeding on the number of eggs and offspring sired by males was studied by mating  $f = 0.00$ ,  $f = 0.25$  and  $f = 0.38$  males to  $f = 0$  females. Likewise, the effects of inbreeding on female egg and offspring production was studied by mating  $f = 0.00$ ,  $f = 0.25$  and  $f = 0.38$  females to  $f = 0.00$  males (Figure 2). Each type of mating was done with 30 pairs of flies.

The flies were kept in vials containing 8ml of malt medium. Individual pairs were first kept in plastic vials for four days. After this period, the pairs were changed to new vials every two days for three times (i.e. for six days). Thus there were a total of four different vials used for each pair. After this the male and female were removed. The number of eggs and eclosing adult offspring from the vials were counted (eggs were counted from the last three vials only). All the flies were virgins and 16-21 days old (age from eclosion) at the time of introduction to the first vial. Individuals of both sexes were weighed just before introduction to the first vial to take the size of the flies into consideration in the statistical analyzes.

Inbreeding coefficient ( $f$ )		♂		
		0	0.25	0.38
♀	0	30 pairs	30 pairs	30 pairs
	0.25	30 pairs	-	-
	0.38	30 pairs	-	-

Figure 2. Design of the experiment 1. Females from the inbreeding level  $f = 0.00$  were mated with males from groups  $f = 0.00$ ,  $f = 0.25$  and  $f = 0.38$ . 30 pairs were mated for each match. Similarly, males from the inbreeding level  $f = 0.00$  were mated with females from groups  $f = 0.00$ ,  $f = 0.25$  and  $f = 0.38$ ; 30 pairs for each match.

## 2.4 Experiment 2: Mating success

In Experiment 2 I studied the effects of inbreeding on the mating success of the flies. The experiment was designed to test the effects separately on females and males (see Figure 3). The effects of inbreeding on mating success on males was studied by placing 30 individuals of  $f = 0.00$  females with thirty individuals of males from each inbreeding level  $f = 0.00$ ,  $f = 0.25$  and  $f = 0.38$  into a mating cube. Thus, the total amount of males in the cube was 90 individuals and a total of 120 flies (including males and females) were placed inside the cube at each replicate. The effects of inbreeding on mating success appeared as different mating probabilities for males from different inbreeding levels. Because the number of males exceeds the number of females, the males had to compete for mating opportunities. The effects of inbreeding on mating success of the females was studied with the same method, placing 30 individuals of  $f=0.00$  males with thirty individuals of each inbreeding level  $f = 0.00$ ,  $f = 0.25$  and  $f = 0.38$  into a mating cube.

To identify the flies from each test group Dr. Oetker edible food color was used. The color was given to the three groups of focal females and the three groups of focal males. One group of the focal individuals was given blue color, one group was given red color, and one group was left undyed. The edible food color was applied on the top of the malt medium in the vials and the flies were placed in the colored vials the day before the experiment. The food color showed in the dissection of the flies as their intestines were full of colored malt medium. To exclude the possible effects of different food color (or no color) to the matings the blue and red colors were circled between different groups for every replicate.

In each of the six replicates the flies were placed in a pairing cage, which is a 6cm x 6cm x 6cm cube made of clear plastic with a round hole at the top of it covered with a flap made of sticky-backed plastic for inserting and removing the flies. All the 120 flies in the experiment were first put in the same plastic vial and then simultaneously released inside the mating cube. Mating pairs were collected from the cage with a glass pipette. The flies were observed for a maximum of two hours, or until all individuals of the minority sex had paired. The collected pairs were placed in empty vials numbered from one to thirty in the order of capture and then taken into a freezer until the next day so that the flies would die and the dissection would be easier and more humane. After this the flies were dissected to find out if there were

differences in the number of matings between the three groups of flies (the control and the two inbred groups). All mating success trials were done between 9 am and 11 am when the flies were most active. One replicate for each of the experiment parts A and B was made in one day, and the order of the parts A and B was rotated each day. The mating cage was cleansed with water and ethanol between all replicates.

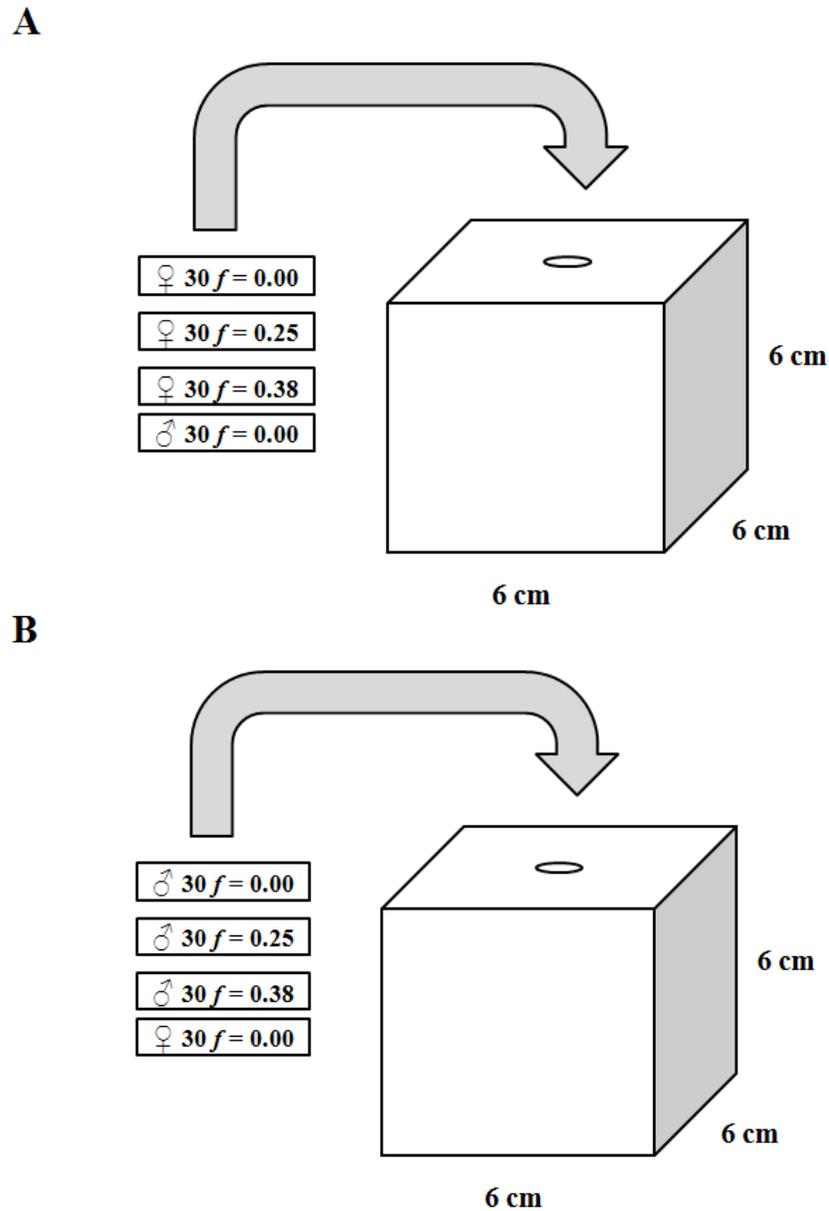


Figure 3. Design of the experiment 2. In part **A** the experiment contained 30 individuals of  $f = 0.00$  males and 30 individuals of females from each of the inbreeding levels  $f = 0.00$ ,  $f = 0.25$ , and  $f = 0.38$ . In part **B** the experimental flies consisted of 30 females from  $f = 0.00$  inbreeding level and 30 males from each of the inbreeding levels  $f = 0.00$ ,  $f = 0.25$ , and  $f = 0.38$ .

## 2.5 Statistical analyses

Statistical analyses were performed with PASW Statistics 18. The effect of the inbreeding coefficient of the sire or dam on offspring production (Experiment 1) was analyzed using ANCOVA with female mass as a covariate for both female and male data to exclude possible female mass-dependent effects. Post-hoc Tukey test was applied to analyze the differences between inbreeding levels. Effect of inbreeding coefficient on mating success (Experiment 2) was analyzed using the Pearson chi-square test for females and males separately, pooling data over replicates. This was done because independent Pearson chi-square tests did not show differences between test groups, most probably because the relatively low number of observations in each individual replicate.

## 3. RESULTS

### 3.1 Experiment 1: Offspring production

For the females, there was a slight decreasing trend on the egg production with increasing inbreeding coefficient, but there was not a significant difference between the inbreeding levels (Figure A; Table 1). Inbreeding coefficient affected female offspring production negatively so that inbred females had fewer offspring than non-inbred females (Figure 4 B; Table 1). In both inbred groups offspring production was significantly lower than in the control group (Tukey test,  $P=0.004$  in  $f=0.25$  and Tukey test,  $P=0.001$  in  $f=0.38$ ), but the inbred groups did not differ significantly from each other (Tukey test,  $P=0.641$ ).

The inbreeding level of males did not have a significant effect on the number of eggs sired (Figure 5 A; Table 2), or on the number of offspring sired (Table 2), although there was a slight decreasing trend in the number of offspring with increasing inbreeding coefficient (Figure 5 B). Female mass was used as a covariate in all the analyses and it did not have statistically significant effect in any case.

Table 1. Analysis of the number of eggs (vials 2-4) and offspring (vials 1-4) produced by females in each inbreeding level ( $f=0.00$ ,  $f=0.25$  and  $f=0.38$ ), using female mass as a covariate.

Source	Egg production				Offspring production			
	df	Mean Square	F	Sig.	df	Mean Square	F	Sig.
Corrected Model	3	10059	2.552	.062	3	49511	6.124	.001
Intercept	1	1019	.259	.612	1	3117	.385	.536
Female Mass	1	12649	3.209	.077	1	5413	.669	.416
Female $f$	2	7249	1.839	.166	2	66832	8.266	.001
Error	78	3941			79	8085		
Total	82				83			
Corrected Total	81				82			

Table 2. Analysis of the number of eggs (vials 2-4) and offspring (vials 1-4) sired by males in each inbreeding level ( $f=0.00$ ,  $f=0.25$  and  $f=0.38$ ), using female mass as a covariate.

Source	Egg production				Offspring production			
	df	Mean Square	F	Sig.	df	Mean Square	F	Sig.
Corrected Model	3	5556	2.012	.119	3	5498	.856	.468
Intercept	1	2530	.916	.341	1	8332	1.297	.258
Female Mass	1	10842	3.927	.051	1	3996	.622	.433
Female $f$	2	4638	1.680	.193	2	5728	.892	.414
Error	81	2761			81	6425		
Total	85				85			
Corrected Total	84				84			

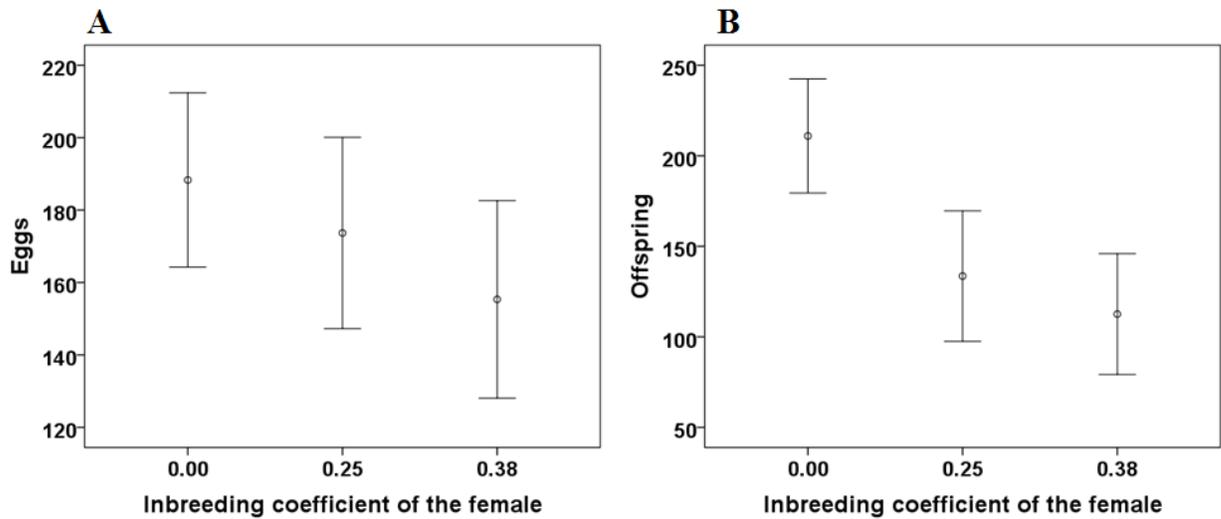


Figure 4. Number of eggs and offspring produced in relation to the inbreeding level of the female. **A** mean egg production (vials 2-4) with 95% confidence interval in relation to the female inbreeding level. **B** mean offspring production (vials 1-4) with 95% confidence interval in relation to the female inbreeding level.

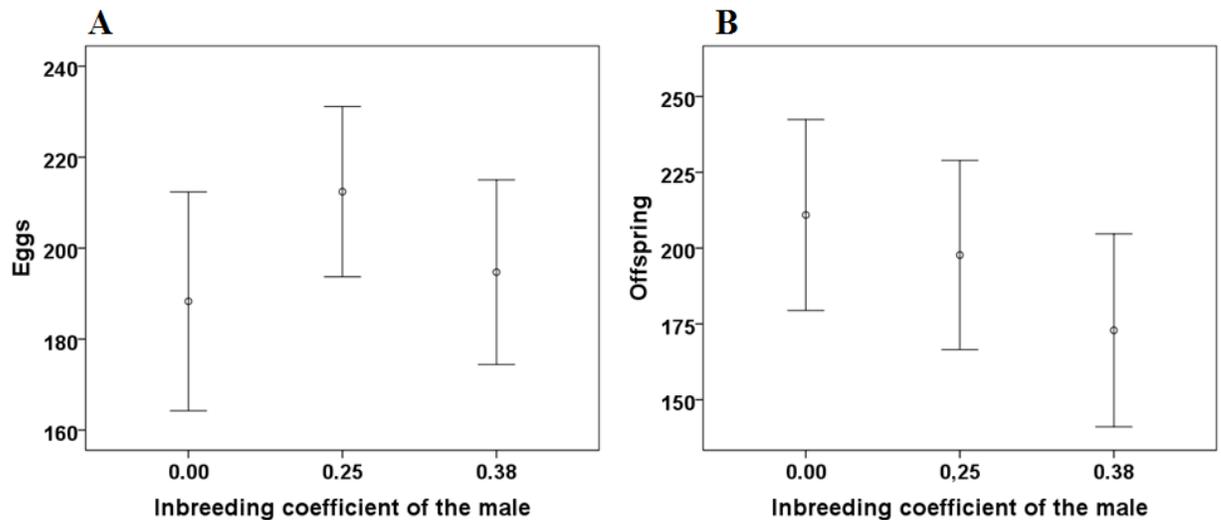


Figure 5. Number of eggs and offspring sired in relation to the inbreeding level of the male. **A** mean egg production (vials 2-4) with 95% confidence interval in relation to the male inbreeding level. **B** mean offspring production (vials 1-4) with 95% confidence interval in relation to the male inbreeding level.

### 3.2 Experiment 2: Mating Success

Inbreeding had a significant decreasing effect on the mating success of the females (Pearson  $\chi^2_2 = 6.086$ ,  $p = 0.048$ ), but did not significantly affect the mating success of the males (Pearson  $\chi^2_2 = 2.855$ ,  $p = 0.240$ ) (Figure 6). However, the trend in the male mating success was slightly decreasing with increasing inbreeding coefficient.

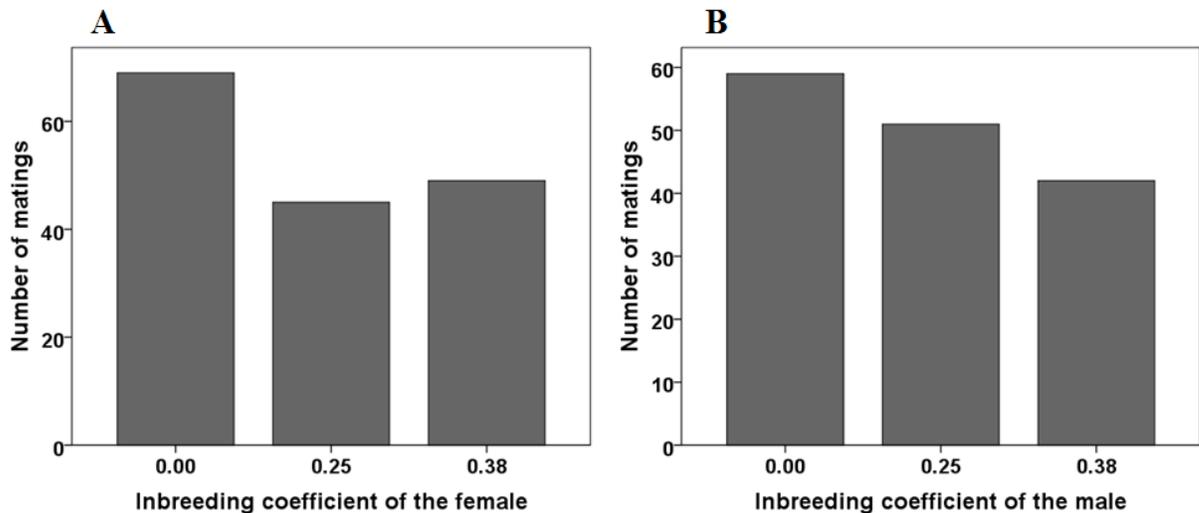


Figure 6. Number of matings in relation to the inbreeding level of **A** the male, and **B** the female.

## 4. DISCUSSION

Inbred females produced significantly fewer offspring than the non-inbred females, but inbreeding had no significant effect on offspring production of the males. This suggests that inbreeding has more severe consequences on female reproduction than on male reproduction. Perhaps inbred females must allocate their limited resources to basic survival and the amount that is left to invest to offspring production is scarce. Since the number of eggs produced by inbred females did not show equal decrease compared to number of offspring, it is likely that the inbred females produced higher proportions of unfertilized or inviable eggs. It can also be that the mortality of larvae was higher among the offspring of inbred females, or both of these reasons together may have caused the decreased offspring production of inbred females. The results from a study by Enders & Nunney (2010) suggest that the larval mortality has a major part in the poorer offspring production in *Drosophila melanogaster*. However, in their study the offspring in question were inbred contrary to my study where the offspring from the experiment were not inbred. Inbreeding did not have so severe effects on the amount of eggs or offspring sired by males, but there was a slightly decreasing trend detected.

Earlier studies are contradictory about whether the effects of inbreeding are more severe on males or females. Okada et al. (2011) found out that inbreeding decreases the amount of viable offspring produced by males in *Drosophila simulans*. Other studies have found that inbreeding has more severe consequences on male than female fitness in white-footed mice

*Peromyscus leucopus noveboracensis*, (Jiménez et al. 1994) wild house mice *Mus domesticus* (Meagher et al. 2000), greater horseshoe bats *Rhinolophus ferrumequinum* (Rossiter et al. 2001) and cricket *Teleogryllus oceanicus* (Simmons 2011). On the other hand Keller's study (1998) gave opposite results from the effects of inbreeding among song sparrows; eggs produced by inbred females had lower hatching rates than those that were produced by non-inbred females. and Charpentier et al. (2006) found out that inbred females were smaller and started reproducing earlier than non-inbred females, the latter not necessarily being a fitness advantage. Also, a recent study by Rioux-Paquette et al. (2011) show that inbreeding reduces the overwinter survival of female bighorn sheep *Ovis canadensis*, but does not impact the survival of males. Coulson et al. (1999) had similar results studying red deer (*Cervus elaphus*).

*Drosophila melanogaster* has also shown sex-dependent inbreeding effects: Mikkelsen et al. (2010) showed that inbred females had lower heat resistance, but better cold tolerance than inbred males. Females also tolerated desiccation stress better than males. Kristensen et al. (2008) found out that inbred *Drosophila melanogaster* males had decreased heat resistance and increased cold resistance compared to non-inbred males, whereas inbred females did not suffer significantly from heat stress. The likelihood of locating a resource in nature was also tested in a release experiment in wild. At cold temperatures inbred females did better than non-inbred females. At warm temperatures, however, both sexes suffered from inbreeding depression and did not perform nearly as well as the non-inbred individuals. The researchers suggest that these results might be a consequence of environment-dependent inbreeding depression, the warmer temperatures favoring non-inbred individuals with reduced energy expenditures for maintenance metabolism, and that this advantage would disappear in low temperatures where metabolism is slower. So there are clearly many sex-dependent effects of inbreeding affecting individual fitness at different life stages and in different environmental conditions. Therefore there is no conclusive explanation for how inbreeding impacts different sexes.

My results show that inbreeding reduces the amount of offspring produced by females more than the amount of offspring sired by males. The reason for a statistically insignificant result of males might be that the effects of choice and competition were excluded from this part of the experiment. Males and females were assigned for each other as mates and no opportunity for mate choice was given. Also competition was entirely excluded from the mating situation. Consequently, individuals that would not have an opportunity to mate and produce offspring if mate choice and competition would have been allowed could now do so. This might have contributed to the non-significant effect of inbreeding on male reproductive success in this part of the experiment. In some of the previous studies this phenomenon could have been partly hidden underneath the effects of mate choice and competition, allowing only the fittest males to mate in the first place and thus giving results that consider also the influence of choice and competition. The results from my study thereby show that, giving equal mating chances, the negative effects of inbreeding are more severe for female than male reproduction. This result implies that there is a greater importance for males to have a non-inbred mating partner than there is for females.

Inbreeding decreased significantly the mating success of the females, but only slightly the mating success of the males. This is an interesting result, because it is opposite to the common belief that females are the choosier sex in mating events (Reynolds 1996, Kokko et al. 2003, Mackay et al. 2005). The result seems, however, reasonable in the light of the results from the offspring production experiment, showing stronger effect of inbreeding on female than on male reproduction. It seems that either the males prefer non-inbred females over

inbred females to mate with, or the inbred females are not so active in accepting male mating attempts and thus gain fewer matings. When compared to the results from the offspring production experiment it is obvious that the males would benefit from choosing a non-inbred female to mate with, because mating with these females has a significant advantage in form of greater number of offspring for males.

Male mating success showed a decreasing trend with increasing level of inbreeding, but less severe than the steep decrease in the mating success of the inbred females. Even though this decrease was not statistically significant, females might still be choosing their mates, but not be as selective as might be expected based on studies with other *Drosophila* species (Geer & Green 1962, Cobb & Ferveur 1996). Since the base population of the laboratory flies had been kept under optimal conditions for already 24 generations before the first experimental matings began, and the experiment took place at the 28<sup>th</sup> laboratory generation, they might have adapted to those stable conditions. Another possible explanation for the results of the mating success experiment may also be found. The steady laboratory conditions might have masked the effects of inbreeding depression, compared to the more stressful and unpredictable conditions often common in nature. Stressful conditions have been shown to have more negative effects on inbred males than females (Jiménez et al. 1994, Meagher et al. 2000), supporting this possibility. The effect of benign laboratory conditions diluting the effects of inbreeding has been shown to happen in butterflies by Joron & Brakefield (2003) as well as in *Drosophila melanogaster* by Bjlsma et al. (1999). And of course one can never totally rule out the possibility of chance either.

Darwin (1871) stated: “The exertion of some choice on the part of the female seems almost as general a law as the eagerness of the male”, basing his conclusions on the appearance and evolution of the sexual ornaments and dimorphism. Since then, much of the research considering sexual selection has focused mainly on two main mechanisms: male competition and female choice. After Darwin's first proposal scientists have found out numerous examples from both mechanisms. Male competition is fierce among many species (Emlen & Oring 1977, Reynolds 1996, Meagher et al. 2000, Bean & Cook 2001, Blanckenhorn et al. 2002,) and female choice is generally considered the dominant mechanism affecting male mating success (Emlen & Oring 1977, Bischoff et al. 1985, Brown 1998, Reynolds 1996, Watson 1998, Kokko et al. 2003, Kempenaers 2007). In the light of this study it seems that the choosiness for mate is a more complex mechanism than previously thought to be. In future studies more interest should be paid on male choosiness and the underlying mate-choice mechanisms as well as investigating sex-based differences in the mating success of inbred individuals. Research under natural or semi-natural conditions could bring forth even more radical outcomes than found in this study and thus show the realistic effects of inbreeding on mating success and offspring production as well as possible differences on these among males and females.

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