Master of Science Thesis

The effects of inbreeding, crossbreeding and stress on metabolic rate in *Drosophila littoralis*

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TIIVISTELMÄ

Yleisesti yksilön kelpoisuuden mittarina käytetään tuotettujen jälkeläisten määrää ja laatua. Koska kelpoisuus on kuitenkin monesta eri tekijästä muodostuva kokonaisuus, on sen havaittu olevan yhteydessä lisääntymisen lisäksi myös muihin ominaisuuksiin kuten esimerkiksi metabolian tasoon. Metaboliatason ja yksilön laadun välisestä yhteydestä on olemassa kaksi päinvastaista näkemystä: resurssiallokaatioteorian mukaan hyvälaatuisilla yksilöillä on alhainen perusmetabolia, kun taas vastakkaisen näkemyksen mukaan alhainen metaboliataso on yhteydessä huonoon resurssinhankintakykyyn ja sitä kautta huonoon laatuun ja kelpoisuuteen. Yksilöt poikkeavat geneettisesti toisistaan ja tästä johtuvat erot myös kelpoisuudessa. Sisäsiitos on merkittävä geneettisten erojen aiheuttaja lisäten homotsygotian määrää. Tällöin resessiivisten alleelien mahdolliset haitalliset vaikutukset tulevat homotsygoottisina näkyviin kelpoisuuteen yhteydessä olevissa ominaisuuksissa aiheuttaen yksilön kelpoisuuden alenemisen. Tästä johtuu sisäsiittoisten yksilöiden huonompi kelpoisuus ei-sisäsiittoisiin verrattuna. Ristisiitoksen vaikutus kelpoisuuteen on päinvastainen. Sen seurauksena heterotsygotian määrää lisääntyy, jolloin jälkeläisten kelpoisuuden oletetaan olevan korkeampi kuin vanhempiensa. Sisäsiitoksesta johtuvan sisäisen stressin lisäksi myös ulkoiset tekijät kuten kuivuus ja monet kemikaalit voivat aiheuttaa eliöille stressiä. Sisäisillä ja ulkoisilla stressitekijöillä saattaa olla myös yhdysvaikutuksia, minkä seurauksena sisäsiittoiset yksilöt kärsivät ulkoisesta stressistä enemmän kuin ei-sisäsiittoiset. Pyrin tutkimuksessani määrittämään kelpoisuuden ja metaboliatason välistä yhteyttä muuntelemalla *Drosophila littoralis* kärpästen laatua kahden eri sisäsiitoskäsittelyn, ristisiitoksen ja suolastressin avulla. Sisäsiitetyissä perheissä oli vähemmän jälkeläisiä ja mitatun naaraan paino oli alhaisempi kuin ristisiitetyillä tai peruspopulaation kärpäsillä. Sisäsiitos siis alensi kärpästen kelpoisuutta tunnetuilla kelpoisuusmittareilla mitattuna. Sisäsiitettyjen naaraiden metabolian taso oli alhaisempi kuin mitä ristisiitetyillä. Sisäsiitettyjen kärpästen alhainen metaboliataso siis tukee teoriaa, jonka mukaan alhainen perusmetabolia on yhteydessä alhaiseen kelpoisuuteen. Ristisiitos palautti oletetusti sisäsiitettyjen kärpästen alentuneen kelpoisuuden takaisin peruspopulaation tasolle jokaisella kelpoisuusmittarilla mitattuna. Suolastressi ei vaikuttanut metaboliatasoon yksin, eikä yhdysvaikutuksia sisäsiitoksen ja suolastressin välillä ei havaittu yhdenkään mitatun ominaisuuden suhteen.

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ABSTRACT

Fitness of an individual is commonly measured through the number and quality of offspring. Fitness is, however, the final outcome of all physiological and developmental processes and thus connected also to other features than reproduction, for example to metabolic rate. There are two opposite views about the connection between metabolic rate and quality: according to resource allocation theory low metabolic rate is a sign of high individual quality when the opposite view states low metabolic rate is connected to low ability to obtain resources which further leads to poor quality and fitness. Genetic differences between individuals lead also to differences in fitness. Inbreeding increases these genetic differences by increasing the amount of homozygosity. This leads to decreased fitness when possible deleterious effects of recessive alleles become visible in a homozygous state in fitness-related traits. Because of this effect, commonly known as inbreeding depression, inbred individuals have lower fitness compared to that of outbred individuals. Crossbreeding may cancel out the effects of inbreeding as it results to increased heterozygosity and thus possibly to higher fitness compared to the inbred parents. Added to the internal stress caused by inbreeding, also external factors such as drought and various chemicals can cause stress to the organism. Internal and external stressors may also have interactions, so that inbred individuals suffer more from external stress than outbred individuals. My goal in this study was to specify the connection between fitness and metabolic rate by manipulating the quality of the *Drosophila littoralis* flies through inbreeding, crossbreeding and salt stress. There were less offspring in inbred families and the body mass of the measured females was lower than in crossbred or base population flies. Thus inbreeding had lowered the fitness of the flies when measured with commonly known fitness traits. Inbred females had lower metabolic rate than that of crossbred females. Based on this, the low metabolic rate of inbred flies observed supports the theory of low total amount of resources indicating poor fitness. Like expected, crossbreeding returned the lowered fitness back to the level of the base population with all fitness measures. Salt stress did not have an effect on metabolic rate alone nor were there interactions between breeding treatment and salt stress in any measured character.

Contents

1. INTRODUCTION

Fitness is commonly measured through its two main components: the total number of offspring produced and the quality of these offspring (Falconer & Mackay 1996). However, as fitness is the final outcome of organisms all developmental and physiological processes, it is influenced also by other characters than the ones directly related to reproduction. These are for example somatic maintenance and basal metabolism (Hayes & O´Connor 1999, Jackson et al. 2001). In addition, some characters, like body size, have less direct influence on fitness but may nevertheless be correlated to some more direct fitness components (Falconer & Mackay 1996).

Metabolism is the sum of all chemical transformations taking place in an organism. In these processes energy is released or absorbed depending on the reaction (Campbell & Reece 2002, Nelson & Cox 2005). Aerobic organisms use oxygen and produce CO_2 in their metabolic processes and by measuring the amount of oxygen used or carbon dioxide produced, the amount of energy used in metabolic processes can be obtained. The connection of metabolism to fitness has been detected at molecular level: inbreeding has an effect on fitness related traits (see below) and inbred individuals have been noted to have different expression of metabolism genes than those from outbred individuals (Kristensen et al. 2005, 2006, Pedersen et al. 2008, Ayroles et al. 2009). However, how resting metabolic rate relates to individual fitness is an unresolved question. According to resource-allocation theory an organism has only a limited amount of resources to allocate to all activities related to survival and reproduction (Rowe & Houle 1996). According to this idea, low resting metabolic rate might be positively associated with fitness, as more resources are then left for allocation to other fitness-related traits (Hawkins & Day 1999, Ketola & Kotiaho 2009). However, another theory states that low resting metabolic rate is a sign of poor individual quality, as low resting metabolic rate is expected to be associated with low total amount of resources (Konarzewski & Diamond 1995, Reinhold 1999). Selection experiments that have tried to solve this matter have yielded contradictory results (Hayes & O´Connor 1999, Jackson et al. 2001, Artacho & Nespolo 2009).

External conditions such as temperature, humidity and various chemicals constitute the environment in which an organism needs to survive and reproduce. What constitutes a favourable environment varies from organism to organism. When a change in the environment reduces the fitness of the organism, the new environment can be considered to be stressful (Armbruster & Reed 2005). Environment may be considered stressful due to various factors, for example noxious or toxic chemicals, nutrient deprivation, temperature and desiccation stress, and the effects of competition and parasitism (Armbruster & Reed 2005). Common chemicals that are essential nutrients in small amounts can also create a harmful environment in large amounts (Sang 1956). One such chemical is salt (NaCl). When salt is ingested, extracellular solute concentration becomes higher than inside the cells and this makes water to flow osmotically out from the cells (Hill & Wyse 1989, Campbell & Reece 2002). This loss of water disturbs the cellular balance and can be considered as stress to the organism because it forces the organism to use its resources to regulate the osmotic balance.

In addition to the quality of the external environment, the “internal” quality of the individual affects its reproduction and survival. Mating between close relatives is generally known to result in inbreeding depression, i.e., reduction in offspring fitness (Keller & Waller 2002, Fox et al. 2007). Inbreeding increases the frequency of homozygous loci in offspring and reduces heterozygosity (Falconer & Mackay 1996). Two competing hypotheses about the

genetic mechanism of inbreeding depression are dominance hypothesis and overdominance hypothesis (Charlesworth & Charlesworth 1987, Lynch & Walsh 1998). According to the dominance hypothesis inbreeding depression is caused by the expression of deleterious recessive genes in homozygous individuals (Lynch & Walsh 1998). The idea in overdominance hypotesis is that something special in the heterozygous state causes increased fitness relative to both homozygotes (Lynch & Walsh 1998). Regardless of the genetic mechanism involved, according to these theories the mean phenotypic values of the traits closely connected to fitness tend to move away from the optimum with inbreeding (Charlesworth & Willis 2009).

As the problems that arise from inbreeding are caused by the homozygous genotypes, the problems are relieved by crossing inbred lines. Crossing inbred lines that have different alleles in the same gene loci increases heterozygosity. First generation offspring performance that exceeds the average parental performance is generally referred to as heterosis or hybrid vigor (Lynch & Walsh 1998). Conflicting theories about the reversive impact of heterosis to inbreeding depression exist (Crow 1948, Falconer & Mackay 1996, Lynch & Walsh 1998, Whitlock et al. 2000). Older theory states that crossing inbred lines cancels out the effects of inbreeding depression because same alleles are involved in both (Crow 1948). However, according to the newer theory inbreeding depression is considered to be influenced mainly by alleles of large effect while heterosis is thought to results from the accumulation of different alleles of small effect (Lynch & Walsh 1998, Whitlock et al. 2000). In both of these theories, however, the superiority of crosses is expected when detrimental effects of homozygotes are broken down by heterozygosity.

External stressors and internal quality of the individual may have an interaction effect on fitness and indeed, inbreeding depression has often been found to be greater in stressful environments (Armbruster & Reed 2005, Kristensen et al. 2002, 2006, Nowak et al. 2007, Pedersen et al. 2008). However, in some studies no connection between inbreeding depression and environmental stress has been detected and there are also studies in which inbreeding depression has decreased in stressful environment (Armbruster & Reed 2005).

I tested the effects of inbreeding, crossbreeding, stress and their interactions on resting metabolic rate in *Drosophila littoralis* flies. I compared the effects of four different breeding treatments from severe inbreeding to crossbreeding reared either in normal conditions or in salt stress. To examine the effect of breeding treatment on other direct and indirect fitness characters I also measured the number of offspring and body mass of the flies.

2. MATERIALS AND METHODS

2.1. Breeding treatments

A large and genetically diverse (11 out of 14 microsatellite loci tested were polymorphic) population of *Drosophila littoralis* flies was founded in year 2006 from 147 males and 99 females collected from River Tourujoki in Jyväskylä, Finland. In the 7th laboratory generation a base population of 500 individuals (sex ratio 1:1) and several inbred populations of 10 individuals (sex ratio 1:1) were founded from this large population. Eleven generations after this (referred to as F0 in Figure 1), four different breeding treatments were created for this study. First treatment was the **base population** and the second treatment was **inbred N10**. The third treatment, **inbred full-sib**, was created by brother-sister matings from the base population flies. For the fourth breeding treatment flies from ten inbred N10 populations

(labelled A-J in Figure 2) were crossed with each other systematically so that both sexes from each population were crossed with opposite sex of one other population (Figure 2). This breeding treatment I will call the **cross N10** populations. The breeding treatments were created so that the measurements for all breeding treatments could be conducted at the same time (Figure 1), thus reducing the possible effects of temporal fluctuations in the laboratory conditions.

The breeding treatments can be described by inbreeding coefficient (f), which describes the probability that two genes of an individual at a locus are identical by descent (Wright 1922). Inbreeding coefficient compares the population in question to some specified base population which has inbreeding coefficient of zero (Falconer & Mackay 1996). In this study inbred N10 treatment (f=0.47) and inbred full-sib treatment (f=0.25) were compared to the base population (f=0) which was used as a control. The inbreeding coefficient of the inbred N10 treatment was calculated by equation $f_t = f_{t-1}+(1-2f_{t-1}+f_{t-2})/2N$ (Crow & Kimura 1970). Comparisons between inbred N10 and cross N10 treatments, and cross N10 and base population were also made as crossing was expected to reset the effects of inbreeding back to the level of base population.

Earlier generations of the base population and the inbred N10 treatment were raised in Erlenmayer flask shaped plastic bottles, but to make the living conditions of different breeding treatments comparable, all the flies used in the experiments and their parents were reared in smaller cylinder shaped plastic vials (diameter 23.5 mm, height 75.0 mm). In the case of inbred full-sib treatment also the grandparents of the experimental flies were reared in vials. Flies were reared at the facilities of University of Jyväskylä in conditions of 19˚C with relative humidity of 60 %, constant light, and malt medium available continuously. Due to practical constraints (measuring capacity and time constraints) both sexes could not be measured in the experiment. I decided to analyze only females because their fitness is more important to the viability of the population than the fitness of males (Pekkala unpublished data).

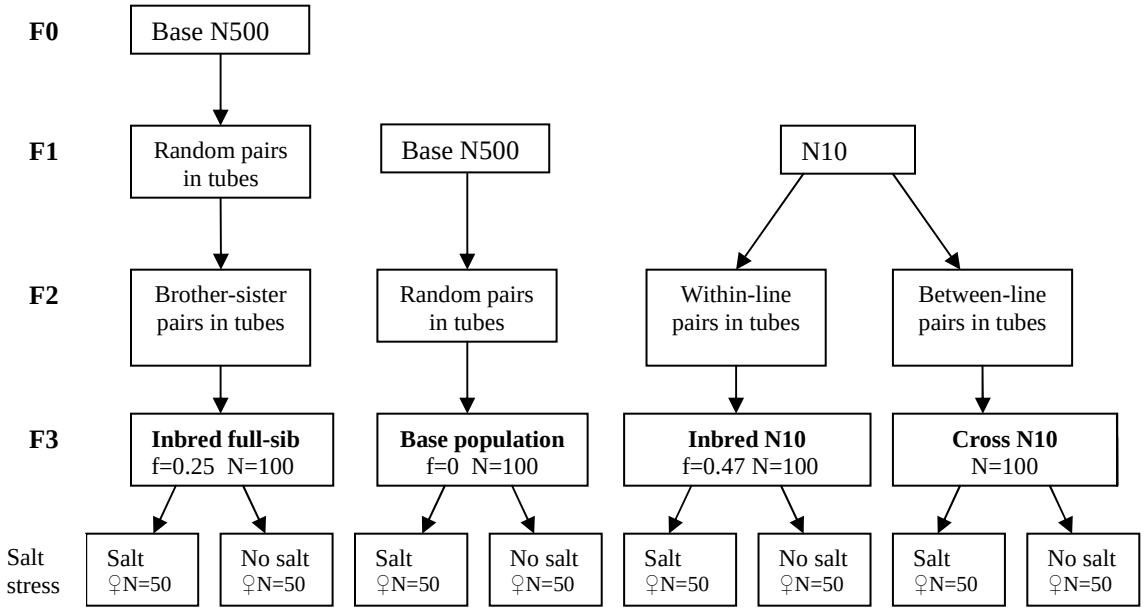

Figure 1. Experimental design from generation to generation in different breeding treatments. On the bottom row is the division of individuals to salt stress. f represents the inbreeding coefficient (see text) and N is the number of individuals.

♀ x ♂
A x B
B x C
C x D
D x E
E x F
F x G
G x H
H x I
I x J
J x A

Figure 2. Crossed pairs of inbred N10 populations resulting to flies of cross N10 treatment. Letters A-J indicate different inbred N10 populations, each cross is in its own row.

2.2. Salt stress

Half of the experimental flies from all four breeding treatments were exposed to normal conditions and the other half to salt stress for one week before the measurement of the metabolic rate. Salt stress environment was created by adding salt to the malt medium, so that the salt concentration of the medium was 1 %. This salt concentration has been found to affect the reproduction of flies, but not to be lethal (Pekkala, Puurtinen and Kotiaho unpublished data).

2.3. Offspring production and body mass

Parent flies were allowed to copulate and lay eggs for 3 days. The number of hatched flies 21-24 days after mating was counted. It has been noted that base population females produce the same number of eggs when mated with a random male picked from the base population and when mated with its brother (i.e. whether outbred or inbred) (Pekkala, Puurtinen and Kotiaho, unpublished data). However, the number of hatched flies is lower when inbred than when outbred. Therefore as inbreeding does not have an effect on the number of eggs, but to whether eggs hatch, the number of offspring in a family can be considered as a measure of survival in that family.

From each of these families, one fly was taken further to the measurement of the metabolic rate. Eclosed offspring were transferred singly to plastic vials with regular malt medium (with no added salt). Seven days after isolation the body mass of the flies was measured to see the effect of breeding treatment on body mass (called body mass at 7 days from here on). After this, half of the experimental flies in each treatment were exposed to salt for one week. After that the body mass of the flies was measured again just before measurement of metabolic rate (called body mass at 14 days from here on). The change in body mass was calculated for each fly as body mass at 14 days minus body mass at 7 days. Body mass change was calculated to see whether salt stress and breeding treatment had an effect on the body mass change between days 7 and 14. Effect of salt stress to a change in body mass can indicate stressfulness of the environment.

2.4. Measurement of the metabolic rate

When the measured individual was at resting state in optimal environmental conditions (19˚C) in respirometry, the resting metabolic rate (RMR) was measured. Respirometry functions in the following way: Incoming air is pushed by a pump (SS-3, Sable Systems) through the respirometric system. First air passes through moisture absorbent (Drierite, Hammond Drierite, Xenia, Ohio, USA) and after that through Ascarite II (J.T. Baker, Deventer, Holland) to remove CO_2 from the air. Next the dry, CO_2-free air is led to the measurement chamber (~ 0.45 cm^2). Steady 70 ml/min air flow through respirometric system was controlled by mass flow controller (Sierra Instruments, Monterrey, California, USA). The CO_2-analyzer was calibrated weekly with CO_2-free air and span gas. The span gas was industrial air (AGA, Finland) with a calibration gas (450 ppm CO_2 in nitrogen). The respirometric system was connected to PC and data from the measurements was acquired and further analysed by ExpeData software (Sable Systems, Henderson, Nevada, USA). Movements of the fly in the chamber (i.e. activity) were recorded by activity detectors (AD-1, Sable Systems) the function of which is based on infrared light and its reflections on the silvery cover of the chamber. Activity detectors were standardized to be equally sensitive before measurements. Another pump (SS-2, Sable Systems) system with same kind of moisture absorber but soda lime as CO_2 absorber pumped air to chambers which were not measured at that moment to prevent the accumulation of CO_2 in the measurement chambers and the suffocation of the flies.

Resting metabolic rate was calculated as the mean CO_2 production per unit time (ml/h) during a 5 minute measuring period. The experimental set was systematically randomized across treatments to equalize the possible chamber effect and the possible effect of daytime on the amount of CO_2 produced. To measure resting metabolic rate of the flies, the amount of stress caused to flies during the measurement was kept as low as possible by carrying out the measurements in same temperature in which flies were reared (19˚C). The temperature outside the chambers was controlled with Peltier effect constant temperature cabinet (PTC-1, Sable Systems) and the air inside the chambers was also 19˚C as it was taken from the room which had the same temperature. The possible activity of the fly which was recorded was taken into account in analyses. Six flies could be put to separate measurement chambers of the respirometry system simultaneously by using a 8-channel multiplexer (Sable Systems). One empty chamber was used as a baseline level and measured before and after every chamber. Chambers were measured one after another and measuring one set of 6 flies took about 60 minutes.

2.5. Statistical analyses

Statistical analyses were performed with SPSS 15.0. Analysis of variance (ANOVA) and multiple comparisons with least significant difference (LSD) -test were used in the analyses. The effect of breeding treatment on the number of offspring and body mass at 7 days was calculated with 1-way ANOVA. 2-way ANOVA was used to test the effect of salt stress and breeding treatment on body mass change and on metabolic rate. Body mass was ln-transformed before calculating the change in body mass because by using this transformation the body mass change became relative instead of being absolute (Kotiaho 1999). This is of importance when the body mass of the flies from different breeding treatments may differ.

As a dependent variable I used a residual CO_2 production that was obtained by removing variance due to body mass, chamber effect and activity of the flies. First I calculated unstandardized residuals from a regression between CO_2 production and the body mass at 7

days. The body mass at 7 days was used in the calculations instead of body mass at 14 days because the body mass at 7 days was not effected by salt stress, like the body mass at 14 days was. Next, a standardized residual between the residual CO_2 production and the activity of each fly was calculated. For this analysis, the data was split by chamber to simultaneously remove the mean differences in CO_2 production between chambers. Finally, the obtained residuals were analyzed in 2-way ANOVA to determine the effects of salt stress and breeding treatments on metabolic rate.

Equalities of variances were tested with Levene´s test and no significant heterogeneity was detected. Analyses were made even though all samples were not normally distributed as ANOVAs are quite robust to deviations from normality as long as sample sizes are large and equal (Zar 1999) as they were in this case.

3. RESULTS

Breeding treatment had an effect on the number of offspring (ANOVA $F_{3, 407}$=9.64, P <0.001) such that the number of offspring was lower in the inbred full-sib treatment compared to the other treatments (Table 1, Figure 3a). However, the number of offspring in the inbred N10 treatment did not differ from that of the base population or that of the cross N10 treatment. There was also no difference between the cross N10 treatement and the base population (Table 1, Figure 3a).

Breeding treatment had also an effect on the body mass at 7 days (ANOVA $F_{3, 411}$=4.37, P=0.005). Flies from the inbred N10 treatment were lighter than the flies in other treatments (Table 1, Figure 3b). Body mass of flies from the inbred full-sib treatment did not differ from that of the cross N10 treatment or from that of the base population. What is of interest, however, is that the body mass of flies from the cross N10 treatment was higher than the inbred N10 treatment but was not different from the base population (Table 1, Figure 3b).

Salt stress had an effect on the change of the body mass (Table 2). Flies exposed to salt stress lost less mass than the flies which were not exposed to salt (Figure 4). However, breeding treatment had no effect on the change of the body mass, nor were there interactions between breeding treatment and salt stress (Table 2, Figure 4).

Breeding treatment had an effect on the resting metabolic rate (Table 3) such that flies from both the inbred full-sib and the inbred N10 treatments had lower metabolic rate than the flies from the cross N10 treatment (Table 1, Figure 5). However, there was no difference between the two inbreeding treatments or between the cross N10 treatment and the base population (Table 1). Salt stress had no effect on metabolic rate and there was no interaction between salt stress and breeding treatment on metabolic rate (Table 3).

Table 1. LSD-comparisons between breeding treatments on measured characters. Mean difference (MD), probablity (P)

	Number of offspring		Body mass at 7 days (mg)		Metabolic rate, standardized residuals (see text)	
	MD	P	MD	P	MD	P
Base – Inbred full-sib	7.26	<0.001	0.05	0.213	0.20	0.141
Base – Inbred N10	1.82	0.193	0.13	0.001	0.15	0.254
Base – Cross N10	2.54	0.068	0.01	0.792	-0.16	0.230
Inbred full-sib – Inbred N10	-5.44	<0.001	0.08	0.042	-0.04	0.765
Inbred full-sib – Cross N10	-4.71	0.001	-0.04	0.352	-0.36	0.011
Inbred N10 – Cross N10	0.72	0.622	-1.12	0.004	-0.31	0.026

Table 2. Analysis of variance on relative body mass change between days 7 and 14. Degrees of freedom (df), mean square (MS), test statistic (F), probability (P).

	df	MS	F	P
Breeding	3	0.010	1.06	0.364
Stress	1	0.077	8.21	0.004
Breeding*stress	3	0.004	0.39	0.761
Error	407	0.009		

Table 3. Analysis of variance on resting metabolic rate (standardized residuals; see methods).

	df	MS	F	P
Breeding	3	2.645	2.75	0.042
Stress	1	2.105	2.19	0.140
Breeding*stress	3	0.789	0.82	0.483
Error	407	0.961		

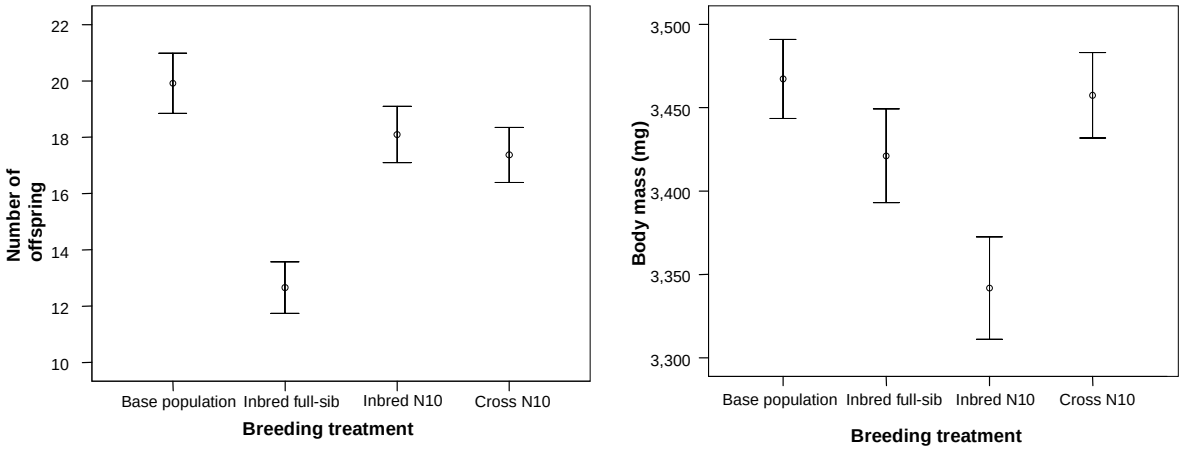

Figure 3. a) Number of offspring (+- 1 SE) and b) body mass at 7 days in different breeding treatments.

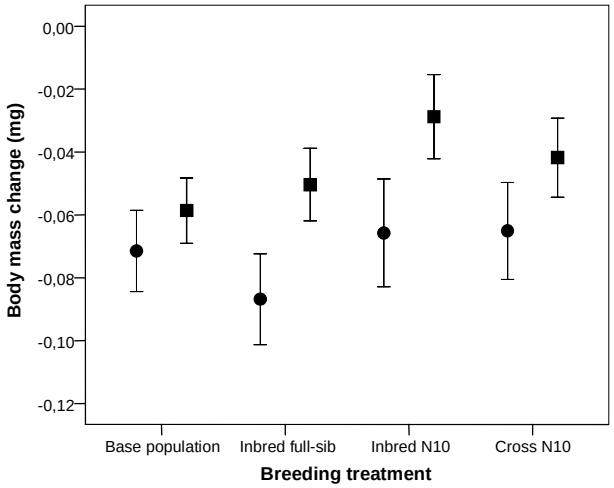

Figure 4. Body mass change (+- 1 SE) (ln-transformed mg) during salt stress in different breeding treatments. Flies exposed to salt stress (■), flies not exposed to salt stress (●)

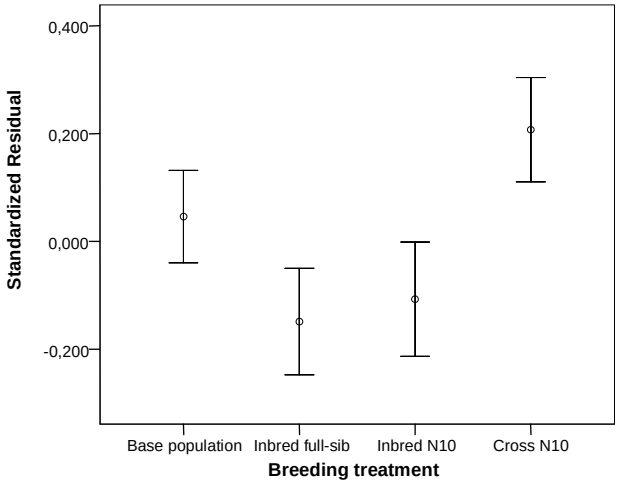

Figure 5. Standardized residuals (+- 1 SE) of resting metabolic rate (see text) in different breeding treatments. Flies exposed to salt and no salt environments are grouped together inside each treatment as stress did not have an effect on metabolic rate (Table 3).

4. DISCUSSION

Both inbreeding treatments (inbred full-sibs and inbred N10) had lower resting metabolic rate than that of the cross N10 treatment. This result contrasts with results from earlier studies, where resting metabolic rate of male crickets either did not differ between inbred individuals and crosses (Rantala & Roff 2006), or inbred individuals had greater resting metabolic rate (Ketola & Kotiaho 2009). I measured females while males were studied in the earlier studies and one explanation for contrasting results can be different selection pressures acting on different sexes. In general, large adult body size is favoured as it contributes positively to mating success, fecundity and survival (Kingsolver & Huey 2008). In females the large size is specifically favored as it allows them to lay more or larger eggs (Ricklefs & Miller 1999).

Body size has been noted to correlate with many fitness characters (Falconer & Mackay 1996), also with metabolic rate (Broggi et al. 2007, Nilsson 2009 but see also Van Voorhies 2004). This can be seen also from my results as inbred N10 flies, which had low level of resting metabolic rate, had also low body mass. It has also been discussed that it is actually metabolic rate which determines the body size and not the other way around (Yamamoto 1998). The connection between low metabolic rate and low body mass which I observed supports the theory that low metabolic rate is a sign of a poor fitness (Konarzewski & Diamond 1995, Reinhold 1999). This is because when large body mass is favored, as it was in female flies in my study, inbreeding leads to low metabolic rate and further to low body mass which is selected against. This theory applies to physically active species in optimal environment, as flies in my study. Active species have higher resting metabolic rate and thus also higher energy expenditure than less active species (Reinhold 1999). When food supply is sufficient to meet this energy demand, as it is in optimal environment, high energy expenditure can be afforded. However, if environment changes in the way that food supply declines - a situation faced often in the wild - individuals with low metabolic rate would require less food, would be less likely to starve and would in turn have the selective advantage. Because of this, different results may appear from laboratory studies when compared to studies made in the wild (Álvarez & Nicieza 2005). The environment in which a species or a population has evolved affects its metabolic strategy: low metabolic rate is favored in areas where food supply is scarce while in environment of abundant food individuals with high metabolic rate can have selective advantage (Mueller & Diamond 2001). Thus even a laboratory experiment made with individuals from high productivity area would probably give different results about the quality of low metabolic rate than an experiment made with individuals from low productivity environment (Mueller & Diamond 2001). Flies used in my experiment have been reared 21 generations in laboratory with abundant food. During these generations some adaptation to food abundancy may have happened favoring flies with high resting metabolic rate and energy usage, possibly contributing to my results of low resting metabolic rate of poor quality individuals.

Flies from the inbred full-sib treatment did not differ in their resting metabolic rate or body mass from the base population flies (these flies were compared to base population and not to flies from the cross N10 treatment, as they originated from the base population). The reason for the lowered metabolic rate and body mass in inbred N10 flies but not in inbred full-sibs is that inbred full-sibs were not as inbred (f=0.25) as N10 flies (f=0.47) were. Thus the probability that an inbred full-sib fly got two genes identical by descent at any locus (i.e. became homozygous) was lower than in inbred N10 flies (Wright 1922, Falconer & Mackay 1996). As there was less homozygosity among inbred full-sibs, also the effects of inbreeding depression on metabolic rate and body mass occurred less frequently.

Further support about the detrimental effects of inbreeding comes from lowered survival of inbred full-sib flies (measured as the number of offspring). Flies from the inbred N10 treatment with higher inbreeding coefficient did not show a reduction in survival, even though inbred full-sibs with lower inbreeding coefficient did. Possible reason for this lies in the ability of selection to eliminate recessive deleterious alleles (i.e. purging) when inbreeding is slow (Ehiobu et al. 1989, Reed et al. 2003). During the 14 generations which inbred N10 lines had been kept at adult population size of 10 individuals (5 males and 5 females), selection has had opportunities to remove recessive deleterious alleles from the population. In full-sib treatment instead, inbreeding was sudden - a result of only one generation of inbreeding. That is why selection did not have time to purge recessive deleterious alleles but the deleterious effects of

inbreeding became visible. The effectiveness of purging depends also on the magnitude of the deleterious effect of an allele on fitness (Hedrick 1994). If inbreeding depression is caused by alleles with severe detrimental effects, inbred individuals may not reproduce or they may die, and purging is efficient. However, alleles that have only a small detrimental effect reduce the fitness of an individual only slightly and thus will not be purged from the population but will persist (Hedrick 1994). As lower body mass reduces the fitness of an individual only slightly, this feature was not purged from my inbred N10 treatment contrary to deleterious alleles leading to reduced survival which were purged.

As mentioned above, inbred N10 flies had lower metabolic rate and body mass than that of the cross N10. This means that crossbreeding restored both the resting metabolic rate and body mass back to the level of base population and crossed N10 flies had better performance than the average performance of their inbred N10 parents i.e. there is evidence for heterosis (Falconer & Mackay 1996, Lynch & Walsh 1998). Similar kind of improvement in performance due to crossbreeding has been observed also in other studies concerning particularly species and characteristics of economic interest (Zhang et al. 2008).

During the week before the measurement of the metabolic rate, both control flies and flies exposed to salt stress lost some of their body mass, but salt stressed flies lost less mass. This may be explained by an accumulation of fluids to the bodies of salt exposed flies as a consequence of high salt intake. When after salt ingestion extracellular solute concentration is higher and water flows osmotically out from the cells, the organism tries to retain cellular homeostasis and accumulates water to its body making itself heavier. This shows the effectiveness of salt as a stressor. Salt stress exposure did not cause differences in body mass change between inbred and outbred flies. It also did not affect metabolic rate alone or have interactions with breeding treatment. Effects of stress on inbreeding depression are highly stress-dependent and differ with the strength of the stress (Bijlsma et al. 1999, Bijlsma et al. 2000, Dahlgaard & Hoffmann 2000, Nowak et al. 2007, Kristensen et al. 2008). Thus controversial results have been reported about interactions between internal and external stressors (Keller & Waller 2002, Armbruster & Reed 2005). The majority of cases reviewed in Armbruster and Reed (2005) showed the increase of inbreeding depression under stress. There were, however, quite a few exceptions to this trend some showing better performance in stressed conditions while to some stress did not have any effect.

In conclusion my results give further support to earlier theories about the effects of inbreeding on resting metabolic rate and its connections to body mass, and it also showed the ability of crossbreeding to relieve the negative effects of inbreeding. My results also raised an intriguing possibility that different selection pressures may act on the metabolic rate of males and females. If high metabolic rate leads to large size, it may be favored in females because their fitness is more dependent on body size than that of males. This would also explain why different results were observed in studies made with male crickets. Theory about the connection between poor fitness and low resting metabolic rate is also supported. However, it needs to be taken into account that low resting metabolic rate observed in inbred flies in this study should not be considered as an undisputed sign of low fitness because of the high dependency of the environment in which the population evolved. Instead it can be generalized as a sign of low fitness in active species which have evolved in environment of abundant resources.

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