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Measuring the effect of inbreeding on
reproductive success in a population of fruit
flies (*Raleigh*)

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Introduction

Reproductive success or fitness is equal to the ability of a population, species or individual organisms to survive in the environment and complete their life cycle to reproduce. Mainly two forms of fitness are being considered in genetic studies: absolute fitness which is a statistic that addresses a genotype's expected total fitness and includes viability of individuals from egg to adult and their reproductive success, the second form is relative fitness which is mostly being used in evolutionary studies and equals with absolute fitness that is normalized in an analytical way. In evolutionary genetics, usually it is the relative fitness of a genotype that mostly being considered. Hence in the concept of evolution successful and unsuccessful individuals always matter, the difference in fitness is typically important (Orr 2009).

Among different individuals of a specific population, fitness can be varied as a consequence of genetic drift or different function of genetic components among genders. These differences in fitness create adaptation and cause natural selection (Chippindale et al. 2001), (Futuyma 2009). Additively, differences in fitness within populations can happen based on different preferences in males and females during mating (Orr 2009). However in terms of sexual antagonism, we don't have any biological reason to assume the same dominance of male or female-benefit alleles for both genders. Although the expression levels of alleles can differ from one individual to another, those which are highly expressed might have the dominant effects on fitness. This allows sexual antagonism in a wider region for even autosomal genes rather than x-linked genes (Jordan & Charlesworth 2011).

Since males and females have different characteristics in reproduction, their optimal phenotypes for being selected by the opposite sex or choosing mate are different. Under some conditions females and males show dissimilar levels of preferences toward the same alleles and this phenomenon causes intralocus sexual conflicts. The sexual conflicts; in the fitness aspect, can be used to assess the level of sexual ontogenetic conflicts. These conflicts mostly appear when a homologous trait is preferred by only one gender (Chippindale *et al.* 2001). When a shared gene pool is more preferred by one sex,

the other sex can be prevented to reach out to its sex-specific optima and leads to sexual conflicts (Van Doorn 2009).

Sexual conflict is one of the major causes for speciation. Even if natural selection guides some penetrant alleles to become highly preferred, sexual conflicts can cause the reduction of the fitness in the same alleles in another sex (Mayr *et al.* 2005). This antagonistic interaction is detectable not only in the regions of sex chromosomes, but also on autosomal loci with the possibility of different dominance expression among genders. Although it is the sex chromosome which evolves an excess of genes and influences the fitness of both genders, some other alleles shows linkage disequilibrium with a sex-specific impacts on fitness, or even they are shown to be lethal for the other gender. Still there are some controversial subjects needed to be explored: 1) is genetic variation necessarily correlated with the fitness, 2) How important it is the size of population to reproductive success, and 3) what kind of genetic scales and markers are the most foretelling for the fitness (Jordan & Charlesworth 2011). Some studies are indicating that the X chromosome has many enriched regions of genes which are responsible for reproduction, as well as thousands of genetic particles that create separate sex-specific phenotypes (Mank 2009), (Ritchie 2000). Although the exact linkage between X chromosome and fitness variation is still unknown, it is highly expected that this chromosome is polymorphic for fitness and its sexually antagonistic variation (Rice & Chippendale 2001). Because of X-linked sexually antagonistic variation, sexual selection is influenced by the structure of genome and will result the negative sexual heritability for fitness. Therefore, high fitted females/ males will produce low fitness sons/ daughters (Gibson *et al.* 2002). In natural populations sexually antagonistic variation for quantitative traits is ubiquitous, although at the same time in evolutionary quantitative genetics the chemical mechanisms and genetic interactions which maintain this variation are still unknown (Mackay *et al.* 1996). But still it is believed that sex chromosome facilitate the evolution of sexual variation because it is the main part of genome that differ between genders (Mank 2009).

Sex differences in the fitness aspect have several evolutionary outcomes. The evolution of depressed or suppressed recombination between sex chromosomes is one of the consequences which causes polymorphism. In natural selection, polymorphism appears to be maintained selectively in generations (Galkin *et al.* 1994). Under this condition, the benefits of one functional allele are accompanied by pleiotropic disadvantages in another organism function. Therefore such that alleles which improve functions of one gender can costs in the other gender without the same advantages (Jordan & Charlesworth 2011). Indeed, intralocus sexual conflict is a highly dynamic genetic event, which has been

described as a potential adaptor for speciation and evolution (Christiansen & Feldman 1975).

Selection itself can be sexually antagonistic when a specific trait and its relative fitness covariantly are different in sexes (Bonduriansky & Chenoweth 2009) and as the result, different allele frequencies in both genders of each generation are produced by this type of selection (Li & Nei 1974). There are several implications for sexual preferences and mate choice behavior. The genetic effect of a male on the female's offspring is correlated to the female's genotype. Several females in a population have different optimal males for mating with, suggesting that differences in preferred alleles by the other sex along with the compatibilities in sexual selection are the matter of fitness variation (Merila & Sheldon 1999).

Sexual dimorphism is the result of sexual antagonism (Bedhomme & Chippindale 2007). However it has been believed that during the evolutionary sexual dimorphism, intralocus sexual conflicts would be resolved because males and females have some divergence requirements and mating strategies (Cox & Calsbeek 2009), recently it is known that only some loci experience the fully resolved sexual conflicts to affect sex traits and unlikely resolute throughout the whole genome (Bedhomme & Chippindale 2007).

Sexual antagonism stabilized the association between typically transient loci which is called linkage disequilibrium. Although the majority of genes are not influenced by differences of sexes, still under some circumstance antagonistic selection have different effects on each sex loci and consequently the selected alleles in males create good male phenotype and bad female phenotype. Oppositely, when a set of alleles is preferred by females, it causes the good female phenotype and bad male phenotype to create. Therefore, an optimal genotype for both genders can't be reached out and mostly this effect causes the genetic diversity (Innocenti & Morrow 2010).

Due to the effects of sexual antagonism, the turnover of sex chromosomes can be promoted and as a consequence, some existing or ancestral loci might be replaced by new sex-determining loci. This can even happen to a locus that is located far from the original sex-determining regions. Moreover, the loss of recombination between sex chromosomes can be favored due to the effects of polymorphism (Jordan & Charlesworth 2011).

Genetic variation is necessary for evolution to occur (Vrijenhoek et al. 1977). Genetic variation along with ecological diversity and speciation can form the biodiversity. Within a population, genetic variation needs to be well-maintained since it controls the correlation between reproduction success and heterozygosity in that population. In the

concepts related to the theory of breeding, the evolution of selection and its genetic consequences have a significant role (Hill & Caballero 1992). Inbreeding influences the genetic variation. On the other hand it reduces reproductive success. Therefore, a rational link between heterozygosity and the ability of producing offspring can be expected which demonstrates a new different function of some alleles (Chakraborty 1981). Interestingly, the correlation between genetic variation and fitness can be vanished due to the removal of deleterious alleles as the consequence of amplified selection against homozygotes (Reed & Frankham 2003). For instance in small inbred populations, most of the alleles likely to be deactivated and the deleterious alleles seem to be responsible for most of the fitness variation (Singh & Krimbas 2000).

Apparently, selection tends to eliminate the population of recessive deleterious alleles (Charlesworth 1987). Based on this theory, inbred populations can be derived from outbred progenitors and can carry higher fitness values. As the result, highly fitted inbred populations would have less genetic variation and vice versa till its size stays large enough to prevent the fixation of deleterious alleles (Reed & Bryant 2000). Genetic variation needs to be conserved with a population. It is because of its necessity for evolutionary genetic changes. Also with genetic diversity the relationship between heterozygosity and population fitness can be estimated (Reed & Frankham 2003). In the populations that have similarity in their genomic data, the amount of genetic variation correlates with evolutionary potential of heritabilities and their current fitness (Franklin 1980).

In spite of the classical population genetics theory, which argues that heritable fitness variation would be depleted via natural selection, it has been shown that fitness and the heritability of traits can be negatively correlated (Kruuk *et al.* 2000). Heritability of a trait is defined as the ratio between the additive genetic variation of a trait and its total phenotypic variance and it shows the response of a trait to selection (Suvanto 1999). When comparing fitness related and non-fitness traits, the first group appears to have higher levels of additive genetic variance. This difference basically occurs due to the higher residual variance among lower heritable traits (Merila & Sheldon 1999). The size of population will be reduced if there is a conversion of non-additive to additive genetic variance or during the efforts for getting used to the new environment. These together can influence on the fitness and obscure its linkage with heritabilities (Reed & Frankham 2003).

Based on the proved results from several earlier experiments, it can be predicted those traits which are the subject of strong directional selection during mating might have lower degree of additive genetic variance in compare to the traits that are inherited during

a weak stabilizing selection. Although the distribution of mutations, the number and the location of loci where the traits are located on need to be considered as well (Merila & Sheldon 1999). Still yet, the theory that different type of traits might display different levels of heritable variation (Falconer 1989) is under estimation. But it has been accepted that in wild population mostly there is a negative relationship between the proportion of lifetime reproductive success and a trait's heritability (Merila & Sheldon 1999). This idea has been confirmed also from an outbred population of *Drosophila* (Roff & Mousseau 1987). If in a large outbred population, a random mating occurs among the most fitted adults, loss of recessive deleterious alleles will occurs exclusively in males and mostly it happens in the haploid state (Thornhill 1993).

The fitness variation and its different ranks, is associated with behavioral and physiological differences. Although sexual antagonism and fitness variation have been explored in recent years, not many experiments have been conducted to study the genetic correlation among them. Most of studies focus on either through male-male competition or female choice of mating, and generally don't consider the possibilities of sexual antagonistic effects on fitness variation or the genomic components which are responsible for sexual antagonism (Promislow *et al.* 1998). Males and females characterize different mating situations. Therefore based on their mating preferences, homologous fitness-related traits or heterologous fitness-related traits can be more preferred due to the functions of antagonistic alleles (Chippindale *et al.* 2001).

The aim of this project was to compare the result of fitness data analysis from outbred populations derived from inbred lines which have been assayed earlier in a separated project. The effect of inbreeding on relative fitness values for outbred males and the possible reasons for any observed changes in values have been studied. These data tell us whether inbreeding has an effect on male fitness and also whether it is likely to change the relationship between male and female fitness seen previously in the inbred lines.

Material and Methods

Experimental method

Before conducting the current assay, in an earlier project on measuring sex-specific fitness over a set of inbred lines of *drosophila*, a non-negative correlation between sexes was observed (Mohebbi 2011). To address this problem, for current study a new set of *Drosophila melanogaster* strains has been derived from the first assayed population and was established after inbreeding with the aim of exploring which lines of the population

can be used to investigate genetic basis of higher fitness. Also in this study, it is mainly focused on the consequences of inbreeding on sexual antagonistic alleles and detecting the fitness values changes when there is a shift from inbred to outbred population.

To design the current assay, results from two previous studies were used as the background data. In the first assay (Innocenti & Morrow 2010) which under competitive conditions, adult fitness was measured in regards of females fecundity and males fertilization power, the base population was sampled from a hundred hemiclinal haplotypes of fruit flies. Fitness data analysis showed a definite effect of sexually antagonistic selection on fitness-related genes. The provided data from this experiment, lead to a better understanding of sexual conflicts function. Hence, the second assay was designed with the focus on measuring male fitness which were sampled from a set of 37 inbred lines (Raleigh population) under competitive condition with *LHm* male flies (supplementary material). The comparison of results from this study to the female fitness values from the earlier experiment (Innocenti & Morrow 2010); strangely showed a non-negative correlation between sexes. These results together, formed the changes on the last protocol and formed the current assay.

For male assay, the base population used in this study was over 32 outbred lines that had been created via crossing *LHm* males with virgin females sampled from 32 different species of Raleigh population. Females were allowed to oviposit in vials for 18h. After trimming and reducing the number of laid eggs to 150 viable zygotes, the incubation time for 14 days was kept and flies emerged. From each lines 5 males were selected as heterozygote targets and located with 10 males of *LHm-bw⁻* replica which were marked with *bw⁻* eye-color (recessive for *bw⁻* allele). The recessive population was considered as homozygous competitors. The total 15 males of each vial were located with 15 virgin females from *bw⁻* population. Flies were reared in 25 mm yeasted vials containing cornmeal-molasses-agar media for 2 days. Females were collected and isolated in single test tubes, being allowed to oviposit for 18 hours. Progeny produced by each female was observed after 12 days and scored for the eye colors. This assay was repeated four times to reach out to 4 replications.

For female assay, the same protocol was followed but after crossing the Raleigh lines with *LHm* flies and creating two heterozygous replica of each line. The target population consisted of five heterozygous virgin females which were housed with 10 *LHm-bw⁻* virgin females as competitors and 15 *LHm-bw⁻* males in yeasted vials kept for two days. Target females were then placed in single test tubes for 18h to lay eggs; the relative adult fitness for each line was measured by averaging the mean number of eggs from each test tube divided by the maximum fecundity across the 32 lines.

Fitness Data Analysis

All data were exported to *R* software ([http:// www.R-project.org](http://www.R-project.org) 2009) and statistical analyzes performed by fitting data into a linear mixed model.

Results

Adult fitness was measured in terms of females' fecundity and males' fertilization under competitive conditions. Relative fitness for males was measured among 35 different species of *Raleigh* lines. Line number 360, showed the highest fitness value (Figure 1, 2).

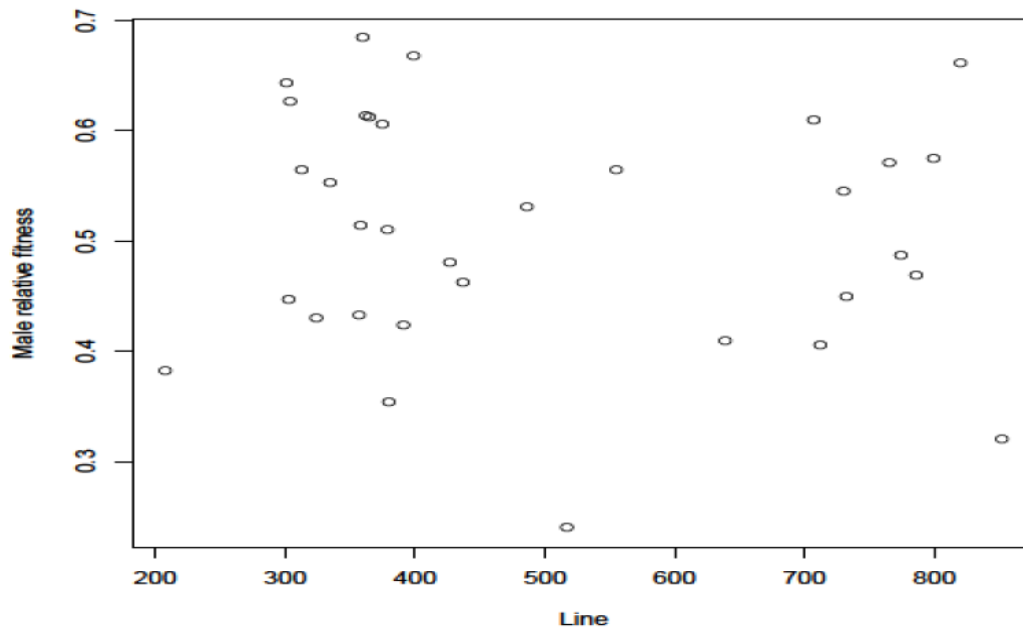


Figure1. Result for male relative fitness. The line number 360 has the highest value = 0.68

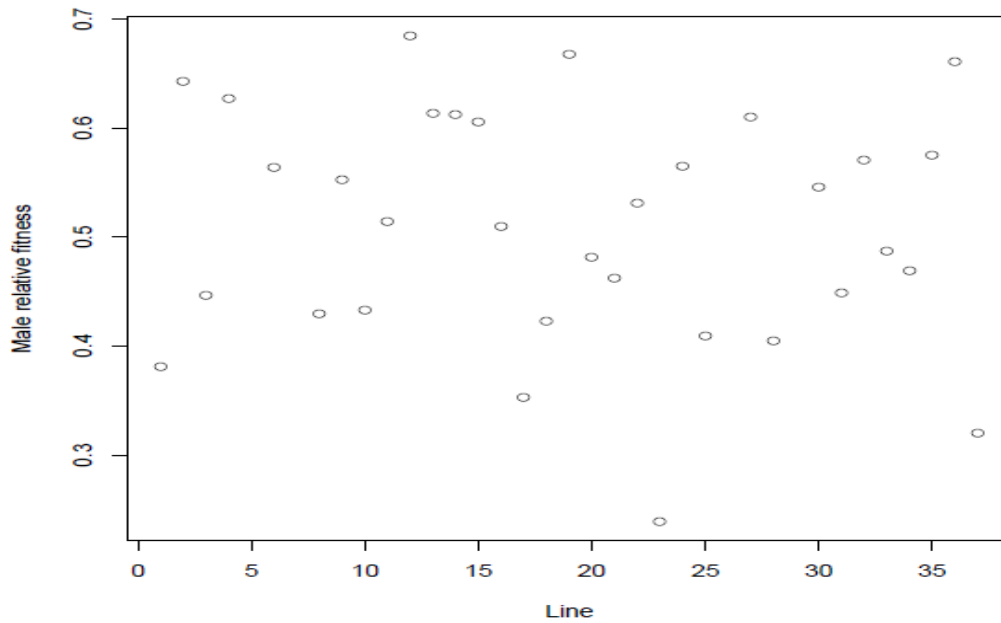


Figure2. Result for male relative fitness among 35 different Genotypes.

In the next step, relative fitness for females among 35 different species of *Raleigh* lines was assayed. Line number 555, showed the highest fitness value (Figure 3).

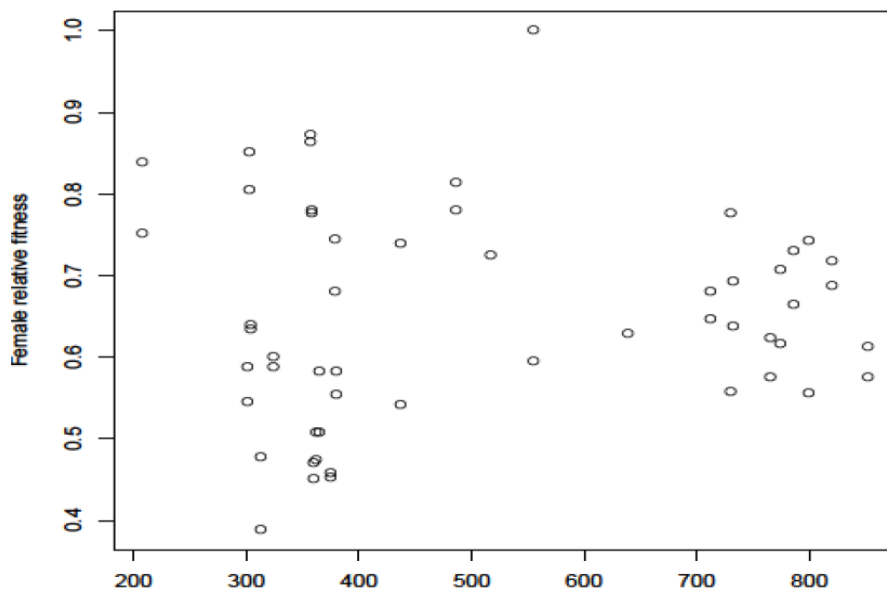


Figure3. Result for female relative fitness, the line number 555 has the highest value = 0.87.

Then, male's fertilization (as the measure for adult fitness) was analyzed and compared to female's fecundity. Result demonstrated a negative correlation between males and females (Figure 4). Statistic values were analyzed and the measured correlation coefficient for the negative sexual relationship was -0.3728465 . The P value was 0.05069 and nearly significant.

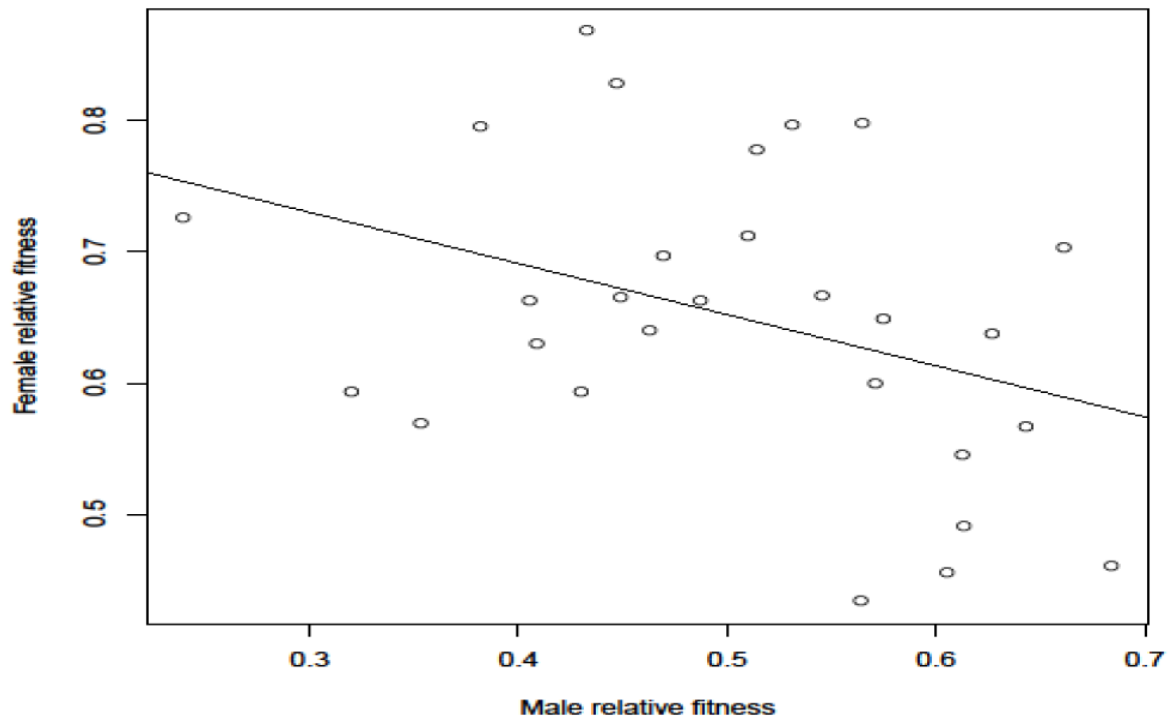


Figure 4. The correlation of relative fitness values for outbred males and outbred females. Result analyzing showed significant negative correlation in relative fitness values between sexes. Correlation coefficient = -0.3728465 . P-value = 0.05069 .

Finally, results were compared to the result of the previous project, which relative fitness values for inbred population were analyzed (Figure 5). These new strains appeared to possess a significant genetic variation and a high rate of changes in their reproductive success. The non-negative relationship between inbred males of *Raleigh* lines versus

hemiclone females in comparison to the negative correlation in fitness of outbred lines indicated the total change of sexual conflicts pattern among these different populations.

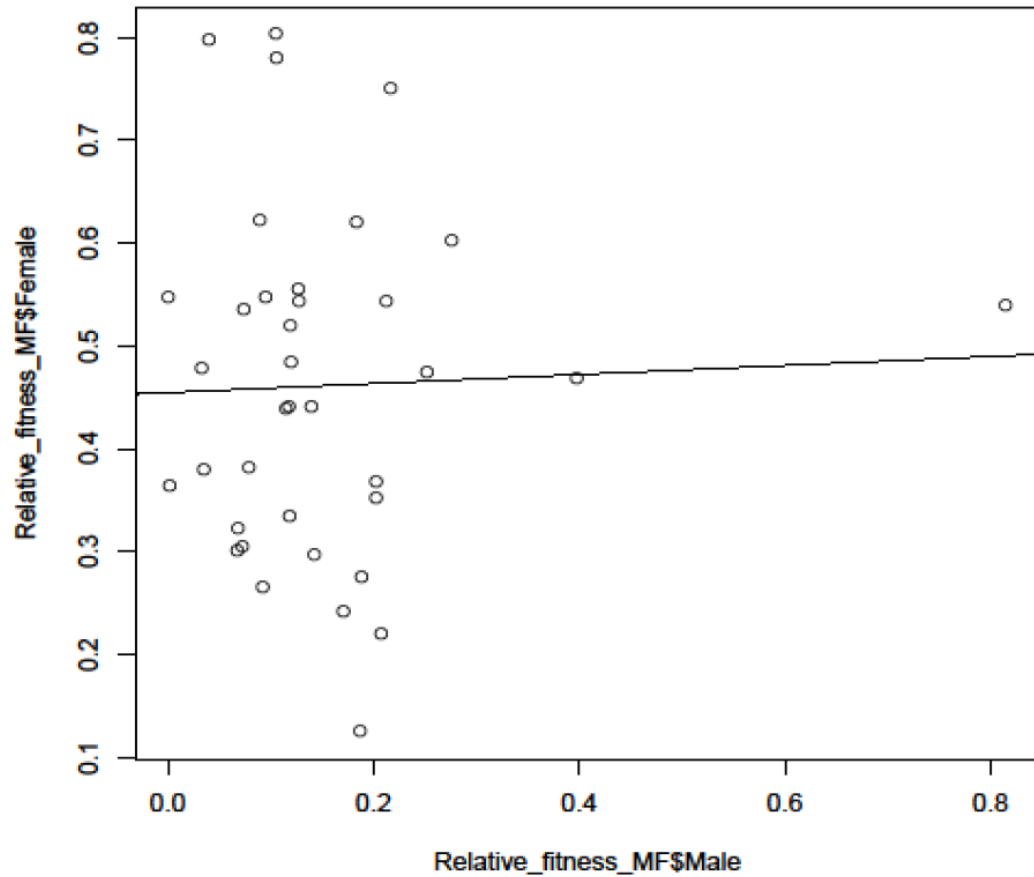


Figure 5. The correlation of relative fitness values for inbred males assayed in previous project with relative fitness values for hemiclone females assayed by Innoceni and Morrow (2010). Result analyzing showed no negative relationship between sexes. Correlation coefficient = 0.03651192. P-value = 0.8301.

Discussion

This project has provided the data for relative fitness of an outbred population. The assayed population was derived from the basic population of *Raleigh* lines. Results emphasize that females and males show different fitness values even if they are sampled from the same population. Sexual conflicts can run the rapid evolution of genes and at the same time can create reproductive barriers in both genders. Under environmental changes or by random genomic interactions some new contingent traits may appear in one sex and effect on fitness. However it can be assumed that fitness variation is not necessarily caused by genetic changes in a large scale. Even specific function of an individual allele or elimination of a few deleterious homozygous alleles can trigger significant changes in heritable traits (Wright 1940). This definition explains why different fitness values were observed in males and females from each line of the same population.

Sexual antagonism is usually used to mean alleles with the opposite effects of reproductive success in two genders (Hansson & Westerberg 2002). One sex has advantages of some alleles that their effects couldn't be expressed in the other sex (Jordan & Charlesworth 2011). In the previous project (Mohebbi 2011), a set of inbred lines from *Raleigh* population was assayed and then the results for fitness of inbred males were compared to the results for hemiclone females which previously had been assayed (Innocenti & Morrow 2010). Then, the results of those two studies were analyzed and compared to one another (supplementary material). By considering the different genetic characters of each assayed population, it could be concluded the non-negative sexual relationship among them was due to new genetic structure which had been formed after the hybridizations. The rate of inbreeding is determined by long-term effective population size and the constancy of the environment which itself is correlated with fitness (Reed & Frankham 2003). In the first assay *Raleigh* population had been inbreeding constantly under laboratory condition for a long term. Therefore the effect of inbreeding depression on reproductive success of such population in compare to a new outbred population can't be deniable.

The pattern of the inheritance of sexual traits to the offspring is instable in the level of genome-wide basis (Hansson & Westerberg 2002). This instability can be also another reason for the non-negative correlation among genders in the previous studies. Generally, a hemiclone is the offspring that has been created by a random selection of a group of eggs from basic populations. These eggs are meant to be fertilized with a cloned copy of the same sperm. It means that hemiclones share half of their genetic variation. Therefore, in the base population the heritability of hemiclone traits is two times the additive genetic variation divided by total phenotypic deviation (Mayr *et al.* 2005). Despite of any

reduction in population size, additive genetic variation and also heritability can constantly increase or at least stay high (Reed & Frankham 2003).

Due to the heterozygosity of hemiclones, the negative correlation of genders is more significant among them. In the first assay, hemiclone females carried large number of genes responsible for a successful reproduction whereas inbred males had a total different pattern of fitness due to their homozygosity. Inbreeding leads to the decrease of heterozygosity. Mating individuals that are related mutually by descent display a total reduction in their fitness and this phenomenon is the major concern of experimental inbreeding (Frankham *et al.* 1993). It has been shown that in comparison to an outbred population of *Drosophila*, inbred individuals which were subject to one generation of full sibling mating demonstrated serious deleterious recessives and the decline in allozyme heterozygosity with 4 times greater heritability (Thelen & Allendorf 2001). Besides, regarding the chromosomal interactions, inbreeding causes a severe decrease in egg-to-adult viability (Lopez-Fanjul & Villaverde 1989).

When alleles are made homozygous, most of deleterious mutations will confer reduction in fitness (around 2%). Then if through mating between relatives and ancestors most of the loci are made homozygous, the reduction of fitness would reach out 200% (Lynch 1991). *Raleigh* lines are the non-random species of *Raleigh* source population which initially was over 1500 lines and by inbreeding process gradually were reduced up to 300 survival lines. Therefore, in a homozygous population that experiences the loss of heterozygosity benefits (Hansson & Westerberg 2002), lower fitness and less genetic variations cause the decline in values of sexual conflicts when compared to hemiclones. The phenomenon of heterosis is more observed in fitter populations. Organisms that reproduce sexually carry two copies of genes at each chromosomal locus. Homozygote individuals have the same gene occur twice whereas heterozygous individuals that have different genes. Then when two heterozygotes mate, some stronger genes can contribute matching in a better proportion to a fitter offspring. As the result, the viable traits would be fixed and the other traits would be vanished. Oppositely, homozygote populations are more exposed to be aborted because they can't have the benefits of blending inheritance and lethal types intermediate between two mating homozygotes. Although it is interesting in spite of the fact that negative selection is against homozygous population and their fitness is low, still they reproduce continuously (Matthen 2002). In the genomic level, it has been shown that multilocus heterozygosity- fitness at a specific loci in heterozygotes are correlated with the functions of some enzyme with different expression or catalytic properties in compare homozygotes. Indeed the biochemical system of heterozygotes and their allozymes

display many dissimilarities than homozygotes in respect of fitness, appear more efficient (Kruuk & Coltman 1998).

All these information together, lead us to create a new population which has some basic similarity with the basic *Raleigh* population and also has the heterozygosity benefits and consequently higher fitness. Since by molecular markers it has been shown that the level of heterozygosity is firmly related to the size of population and fitness (Frankham et al. 1993), heterozygosity can indicate the level of fitness in a certain sized population.

As deleterious alleles appear to be expressed in recessive status, by crossing individuals from different populations, the recessive alleles are masked and a fitter offspring can be created. Interestingly if in a population many recessive alleles be segregated, even after a sibling-cross and creation of a homozygous offspring their relative fitness values remain higher in compare to their parents. It is because of the total reduction in the number of deleterious alleles which are expressed in recessive status (Lynch 1991).

To validate this interpretation, in current experiment we created two replicates of new outbred populations for both sexes which were derived from inbred lines. Data also included the results from our previous studies in which the reproductive success from the original populations was measured. Interestingly, all new replicates showed a total shift from a non-negative sexual relation for fitness to a completely negative correlation and results were nearly significant for both replicas. Apparently, in order to obtain the best estimation of intersexual genetic correlation for relative fitness we should have examined the same genotype for each gender which is demonstrating similar juvenile viability and cost of mating.

By combining all results together, it can be interpreted that the severity of recessive status of traits and also the number of deleterious alleles can decline the relative fitness values. Deleterious alleles are much more expressed among homozygous populations and therefor inbreeding can reduce the number of homozygous individuals. Sometimes this reduction is more observable among only one gender and it causes the pattern of sexual conflicts to be changed. Then the pattern of intersexual conflicts totally depends on the level of heterozygosity.

Conclusion

In his study, the average fitness values for an outbred population were measured and compared to the fitness values for the inbred lines from the same population. This population was derived from the cross of *Raleigh* population with *LHm* flies. Results confirm that reproductive success is formed by opposing selection on males and females. On the other hand besides the selection, it is confirmed that the intralocus sexual conflict is significantly effective on the fitness-related heritable traits. However it had been shown that nonadditive genetic variance and also environmental effects are the most common elements that influence fitness in nature (Merila & Sheldon 1999). Based on the experiment, the genetic construction governs the sexual preferences as well as attractiveness and mating behavior. Also, results indicate that in the case of shifting from homozygosity to heterozygosity the pattern of sexual conflicts entirely changes among genders and influences their reproductive success. Finally, it is highly probable that fitness traits harbor high absolute degrees of additive genetic variation due to the accumulation of mutant alleles which are determinant for the fitness.

Plans for future studies

This experiment is the start point for future investigations on evolutionary genetic studies with the main focus on fitness variation and the effect of inbreeding depression on reproductive success. The knowledge gained would be the start point to evaluate heterozygosity-fitness in the correlation with sexual conflicts. There are several experimental approaches that can be taken to a better understanding for this subject. For instance, the protocol of our experiment can be more completed by studying the responsible genes for the observed variations in the highest and the lowest fitted assayed individual. Any genetic linkages which cause fitness variation in our population and any possibility of existence the grope genes that are expressed or depressed together to create such different fitness patterns can be explored. Also studying the exact locations and numbers of antagonistic genes and their products, and also existence of specific markers on the genome reveals many key concepts of fitness variation among genders in the molecular genetic level. Conduction of some more direct assays to study the life history and morphological traits and detection of any evidence for different functions and genomic architectures of such traits also seem required.

References

- Bedhomme, S. & Chippindale, A.K., 2007. Irreconcilable differences: when sexual dimorphism fails to resolve sexual conflict. *Sex, size and gender roles: evolutionary studies of sexual size dimorphism*. Oxford University Press, Oxford, pp.185–194.
- Bonduriansky, R. & Chenoweth, S.F., 2009. Intralocus sexual conflict. *Trends Ecol Evol*, 24(5), pp.280-288.
- Chakraborty R., 1981, The distribution of the number of heterozygous loci in an individual in natural populations. *Genetics*, 98.pp 461-466.
- Charlesworth, D. & Charlesworth, B., 1987. Inbreeding Depression and its Evolutionary Consequences. *Annual Review of Ecology and Systematics*, 18, pp.237-268.
- Chippindale, A.K., Gibson, J.R. & Rice, W.R., 2001. Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*. *PNAS*, 98(4), pp.1671-1675.
- Christiansen F. & Feldman W.M, 1974, Subdivided populations: A review of the one- and two-locus deterministic theory. *Elsveir, Department of biological science, Stanford University*. 7(1),pp.13-38.
- Cox, R.M. & Calsbeek, R., 2009. Sexually antagonistic selection, sexual dimorphism, and the resolution of intralocus sexual conflict. *The American Naturalist*, 173(2), pp.176-187.
- Falconer, D. S., 1989, Introduction to quantitative genetics. *Longman Scientific Techn.*, New York. ISBN 0 470 21162 8.
- Frankham, R., Smith, G. & Briscoe, D., 1993. Effects on heterozygosity and reproductive fitness of inbreeding with and without selection on fitness in *Drosophila melanogaster*. *School of Biological Sciences, Macquarie University, Sydney*, 86(8), pp.1023-1027.
- Franklin, I., 1980. Evolutionary change in small populations. *Conservation biology: an evolutionary-ecological perspective*. Sinauer Associates, Sunderland, Massachusetts, pp. 135–150.
- Futuyma D. 2009. Natural selection and adaptation . *Evolution, Sinauer Associates press. Chapter 11*.
- Galkin A., Boshakov V. & Kaidanov Z.,1994. Closely related *Drosophila melanogaster* strains with altered fitness also show changes in their hobo element properties. *Elsevier/INRA*. 26. pp.205-216.
- Gibson JR, Chippindale AK, Rice WR. 2002. The X chromosome is a hot spot for sexually antagonistic fitness variation. *Proc R Soc Lond*. 269,pp.499–505.
- Hansson, B. & Westerberg, L., 2002. On the correlation between heterozygosity and fitness in natural populations. *Molecular Ecology*, 11(12), pp.2467-2474.
- Hill WG. & Caballero A., 1992. Artificial selection experiments. *Annual Review on Ecological Systems*. 23, pp.287-310.

<http://www.R-project.org>, R., 2009. R. *Development Core Team*. Available at: <http://freecode.com/projects/r> [Accessed December 25, 2011].

Innocenti, P. & Morrow, E.H., 2010. The sexually antagonistic genes of *Drosophila melanogaster*. *PLoS Biol*, 8(3), p.e1000335.

Jordan, C.Y. & Charlesworth, D., 2011. The Potential for Sexually Antagonistic Polymorphism in Different Genome Regions. *Evolution*. Available at: <http://onlinelibrary.wiley.com/doi/10.1111/j.1558-5646.2011.01448.x/abstract> [Accessed January 6, 2012].

Kruuk, L.E.B. 2000. Heritability of fitness in a wild mammal population. *Proceedings of the National Academy of Sciences*, 97(2), pp.698 -703.

Li, W. & Nei, M., 1974. Stable linkage disequilibrium without epistasis in subdivided populations. *Theoretical Population Biology*, 6, pp.173-183.

Lopez-Fanjul, C. & Villaverde, A., 1989. Inbreeding Increases Genetic Variance for Viability in *Drosophila melanogaster*. *Evolution*, 43(8), pp.1800-1804.

Lynch, M., 1991. The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution*, 45(3), pp.622-629.

Mackay, T. F. C., Hackett, J. B., Lyman, R. F., Wayne, M. L. & Anholt, R. R. 1996 Quantitative genetic variation of odor-guided behavior in a natural population of *Drosophila melanogaster*. *Genetics* 144, pp.727-735.

Mank, J.E. 2009. Sex chromosomes and the evolution of sexual dimorphism: lessons from the genome. *Am Nat*.173, pp.141–150.

Matthen, M., 2002. two ways of thinking about fitness and natural election. *The journal of philosophy*. 99(2), pp.55-83. Available at : <http://www.jstor.org/stable/3655552>.

Mayr, E. 2005. *Systematics and the origin of species: on Ernst Mayr's 100th anniversary*, National Academies Press.

Merila, J. & Sheldon, B.C., 1999. Genetic architecture of fitness and nonfitness traits: empirical patterns and development of ideas. *Heredity*, 83(2), pp.103-109.

Mohebbi, S., 2011. Study of intralocus sexual conflicts in a male population of *drosophila melanogaster* (RALM lines). Research training project. Dept of animal biology. Evolutionary biology center. Uppsala university

Orr, H.A., 2009. Fitness and its role in evolutionary genetics. *Nat Rev Genet*, 10(8), pp.531-539.

Promislow, D.E., Smith, E.A. & Pearse, L., 1998. Adult fitness consequences of sexual selection in *Drosophila melanogaster*. *PNAS*, 95(18), pp.10687-10692.

Reed, D.H. & Bryant, E.H., 2000. Experimental Tests of Minimum Viable Population Size. *Animal Conservation*, 3(01), pp.7-14.

- Reed, D.H. & Frankham, R., 2003. Correlation between Fitness and Genetic Diversity. *Conservation Biology*, 17(1), pp.230-237.
- Rice, W. R. & Chippindale, A. K., 2001. Intersexual ontogenetic conflict. *Evol. Biol.* 14, pp.685- 693.
- Ritchie, M. G., 2000. The inheritance of female preference functions in a mate recognition system. *Proc. R. Soc. Lond.* 267, pp.327-332.
- Roff, D. A. & Mousseau, T. A., 1987. Quantitative genetics and fitness: lessons from *Drosophila*. *Heredity*, 59, pp.103-118.
- Singh, R.S. & Krimbas, C.B., 2000. *Evolutionary genetics: from molecules to morphology*, Cambridge University Press.
- Suvanto, L., 1999. Mate choice and genetic variation in male courtship song in *Drosophila montana*. *Oulu University Library*. Available at: <http://herkules.oulu.fi/isbn9514251911/> [Accessed December 22, 2011].
- Thelen, G., Allendorf, C., 2001. Heterozygosity-fitness correlations in rainbow trout: effects of allozyme loci or associative overdominance? *Evolution*, 55(6), pp.1180-1187.
- Van Doorn, 2009. Intralocus Sexual Conflict. *Annals of the New York Academy of Sciences*, 1168(1), pp.52-71.
- Vrijenhoek, R. C., Angus R. & Schultz J., 1977. Variation and heterozygosity in sexually vs. clonally reproducing populations of *Poeciliopsis*. *Evolution*, 31(4), pp. 767-781.
- Wright, S., 1940. Breeding Structure of Populations in Relation to Speciation. *The American Naturalist*, 74(752), pp.232-248.