JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE
237

Nina Pekkala

Fitness and Viability of Small Populations

The Effects of Genetic Drift, Inbreeding, and Interpopulation Hybridization

JYVÄSKYLÄN YLIOPISTO

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Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston vanhassa juhlasalissa S212
maaliskuun 23. päivänä 2012 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Mathematics and Science of the University of Jyväskylä,
in auditorium S212, on March 23, 2012 at 12 o'clock noon.

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2012

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Publishing Unit, University Library of Jyväskylä

Jyväskylä Studies in Biological and Environmental Science

URN:ISBN:978-951-39-4684-5
ISBN 978-951-39-4684-5 (PDF)

ISBN 978-951-39-4683-8 (nid.)
ISSN 1456-9701

Jyväskylä University Printing House, Jyväskylä 2012

ABSTRACT

Pekkala, Nina
Fitness and viability of small populations: the effects of genetic drift, inbreeding, and interpopulation hybridization
Jyväskylä: University of Jyväskylä, 2012, 46 p.
(Jyväskylä Studies in Biological and Environmental Science
ISSN 1456-9701; 237)
ISBN 978-951-39-4683-8 (nid.)
ISBN 978-951-39-4684-5 (PDF)
Yhteenveto: Geneettisen satunnaisajautumisen, sisäsiitoksen ja populaatioiden välisen risteytymisen vaikutukset pienten populaatioiden kelpoisuuteen ja elinkykyyn
Diss.

Reduced population size and isolation from other populations of the same species are a threat for population persistence, for both demographic and genetic reasons. In my thesis, I have studied how the genetic processes in small populations influence the fitness of individuals and viability of populations. In addition, I have studied how a potential conservation tool, hybridization between isolated populations, affects individual fitness and population viability. To study these questions, I used an experimental approach and a model species, *Drosophila littoralis*. The results show that although natural selection can to some extent counteract the deleterious effects of inbreeding and genetic drift in small populations, in the long term, selection is inefficient against the continuous accumulation and fixation of deleterious alleles. The results further show that the effects of inbreeding and genetic drift are less harmful when the rate of inbreeding is slower, i.e., when the effective size of a population is larger. This is likely due to more efficient selection in larger populations. However, also the larger populations showed a decrease in fitness because of the increased magnitudes of inbreeding and genetic drift, suggesting that slower rate of inbreeding does not protect the populations against the deleterious effects of inbreeding and drift. I also found that interpopulation hybridization can increase the long-term viability of small populations, but the benefits are reduced when the genetic divergence between populations is high. Further, at the same levels of inbreeding, populations with smaller historical size (i.e. with faster inbreeding) are likely to benefit more from interpopulation hybridization. The results have implications for the conservation of natural populations.

Keywords: *Drosophila littoralis*, drift load, fitness surrogates, heterosis, inbreeding depression, outbreeding depression, purging.

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I-IV.

I Pekkala, N., Kotiaho, J.S. & Puurtinen, M. 2011. Laboratory relationships between adult lifetime reproductive success and fitness surrogates in a *Drosophila littoralis* population. *PLoS ONE* 6(9): e24560.

II Pekkala, N., Knott, K.E., Kotiaho, J.S. & Puurtinen, M. 2012. Inbreeding rate modifies the dynamics of genetic load in small populations. Submitted manuscript.

III Pekkala, N., Kotiaho, J.S., Nissinen, K. & Puurtinen, M. 2012. The benefits of interpopulation hybridization diminish with increasing divergence of small populations. Submitted manuscript.

IV Pekkala, N., Knott, K.E., Kotiaho, J.S., Nissinen, K. & Puurtinen, M. 2012. Effect of inbreeding rate on the magnitudes of drift load, inbreeding depression, and heterosis. Manuscript.

The table shows the contributions to the original papers. Smaller contributions are stated in the acknowledgements of the original papers. NP = Nina Pekkala, MP = Mikael Puurtinen, JSK = Janne S. Kotiaho, KEK = K. Emily Knott, KN = Kari Nissinen.

	I	II	III	IV
Original idea	NP, MP, JSK	NP, MP	NP, MP	NP, MP
Data	NP, MP	NP, MP, KEK	NP, MP	NP, MP, KEK
Statistics	NP, MP, JSK	NP, MP, JSK	NP, MP, KN	NP, MP, KN
Writing	NP, MP, JSK	NP, MP, JSK, KEK	NP, MP, JSK, KN	NP, MP, JSK, KEK, KN

1 INTRODUCTION

A growing number of animal and plant populations are decreasing in size and becoming isolated from each other. The main reasons for this phenomenon are anthropogenic, such as destruction and fragmentation of habitats, and overexploitation of populations (Ewers & Didham 2006, Avise et al. 2008). Reduced population size and isolation from other populations of the same species are a threat for population persistence, for both demographic and genetic reasons (Lande 1988, Saccheri et al. 1998, Amos & Balmford 2001, Spielman et al. 2004, Frankham 2005, O'Grady et al. 2006). Genetic threats, which are the subject of my thesis, are caused by inbreeding and genetic drift. There has been a debate among scientists about the relative importance of the demographic and genetic reasons in driving populations to extinction (see e.g. Lande 1988, Spielman et al. 2004, Frankham 2005). Today, there is a plenty of evidence for the importance of genetics for the survival of populations. It has been shown that inbreeding and genetic drift can depress the fitness and adaptive potential of populations in a way that will increase the risk of extinction in the wild (e.g. Saccheri et al. 1998, Keller & Waller 2002, O'Grady et al. 2006). Further, genetic factors are an important part of management and conservation of threatened species, for example in captive breeding, reintroduction, and translocation programs (Frankham 2010). One possible management action is to artificially increase gene flow between populations, i.e., to induce hybridization between isolated populations (Hedrick et al. 2011). However, in addition to the potential benefits, the method includes risks.

Even though the importance of genetics for the evolution and conservation of small populations has been acknowledged and, consequently, the subject has become an important area of biological research, many uncertainties and open questions remain (Pertoldi et al. 2007, Frankham 2010, Ouborg 2010). In my thesis, the main questions are: 1) How does the rate of inbreeding affect the fitness and viability of small populations at different levels of inbreeding?, and 2) How do the rate of inbreeding, the level of population divergence, and the amount of introduced genetic variation affect the consequences of interpopulation hybridization?

1.1 Small population size and drift load

In small populations, two important genetic factors affecting the fitness of individuals are inbreeding (mating between close relatives) and genetic drift (random fluctuation in allele frequencies due to finite population size). When compared to a large population, matings between close relatives in a small population are inevitably more common even when mating is random, because of the limited number of individuals contributing to each generation. Inbreeding and genetic drift lead to an increase in the level of homozygosity. Homozygosity can depress the fitness of individuals because recessive deleterious alleles are expressed in a homozygous state (*the dominance hypothesis*) and because heterozygosity is lost in loci with overdominant effects on fitness (*the overdominance hypothesis*) (Charlesworth & Willis 2009). The dominance hypothesis has received support as the main cause of reduced fitness due to an increased level of homozygosity, but overdominance can be important in some circumstances (Kärkkäinen et al. 1999, Charlesworth & Willis 2009). Also, the exposure of harmful epistatic interactions between homozygous loci may act to reduce the fitness of individuals (Lynch 1991).

The smaller the population, the more important genetic drift is in determining the allele frequencies and, correspondingly, the less efficient is natural selection (Falconer & Mackay 1996, Allendorf & Luikart 2007). The relaxation of natural selection will lead to accumulation and fixation of harmful alleles in small populations (Whitlock 2000, Whitlock et al. 2000). As a consequence of increased homozygosity and relaxation of natural selection, the average fitness in small populations is expected to decrease, i.e., the populations are expected to suffer from drift load. The reduction in fitness and the loss of genetic variation through genetic drift can also compromise the potential of the populations to adapt to changes in the environment (Bijlsma & Loeschcke 2005, Willi et al. 2006). Thus, increased levels of inbreeding and genetic drift will threaten the current and future persistence of small populations.

Predicting the effects of reduced population size on fitness and viability of populations is not simple, however. As homozygosity and, therefore, the expression of recessive (or partially recessive) deleterious alleles increases, selection can more efficiently act against them. Removal of deleterious alleles from a population through selection, generally called *purging*, can thus counteract the harmful effects of inbreeding and genetic drift (Hedrick 1994, Wang et al. 1999, Kirkpatrick & Jarne 2000, Hedrick 2002, Glemin 2003). Two important factors predicted to determine the effectiveness of purging are the harmfulness of mutations and the rate of inbreeding. The most harmful mutations are most easily purged, whereas selection against mildly harmful mutations is less efficient (Hedrick 1994, Wang et al. 1999, Glemin 2003). Therefore, highly deleterious alleles are not predicted to pose a serious threat to small populations, if the populations manage to survive the early stages of

reduced population size (Wang et al. 1999, Theodorou & Couvet 2006). In contrast, the accumulation and fixation of mildly deleterious alleles may cause a serious threat to the long-term persistence of small populations (Kimura et al. 1963, Lande 1994, 1998, Wang et al. 1999).

The effectiveness of purging can further depend on the rate of inbreeding. The term *rate of inbreeding* refers to the rate of increase in the level of homozygosity in a population due to finite population size: inbreeding rate is faster in smaller compared to larger populations. In very small populations, selection is predicted to be efficient only against highly deleterious mutations, whereas in larger populations, selection may be efficient also against less harmful alleles (Wang et al. 1999, Glemin 2003, Theodorou & Couvet 2006). Further, in larger populations there is more time for selection to act before a certain level of inbreeding (i.e., a certain level of homozygosity due to finite population size) is reached (Wang et al. 1999, Theodorou & Couvet 2006). Therefore, the frequency of deleterious alleles is expected to be lower in populations with slower inbreeding rate, when the populations are compared at the same levels of inbreeding. As a consequence, the mean fitness should be higher, i.e. drift load should be lower, in populations with slower inbreeding rate when comparing populations at the same levels of inbreeding (Wang et al. 1999, Theodorou & Couvet 2006).

Removal of deleterious alleles through selection can, however, lead to recovery in fitness only if the fitness reduction is due to increased expression of deleterious recessive alleles. If the fitness reduction is caused by loss of heterozygosity in overdominant loci it cannot be counteracted by purging, because the continuous increase in homozygosity in overdominant loci will always lead to reduction in fitness. It is, however, possible that balancing selection to maintain heterozygosity reduces the effects of drift in overdominant loci (Kristensen et al. 2005, Demontis et al. 2009). Like purging of recessive deleterious alleles, balancing selection is expected to be more efficient with a slower rate of inbreeding.

The various factors that interact to determine the harmfulness of inbreeding and genetic drift make it difficult to predict the long-term effects of reduced population size. The theoretical predictions about long-term viable population sizes are highly sensitive to the assumptions of the models, such as the mutation parameters assumed (e.g. Lande 1994, Lynch et al. 1995b, a, Lande 1998, Wang et al. 1999, Whitlock 2000, Glemin 2003, Theodorou & Couvet 2006). These assumptions may be violated in real populations (Eyre-Walker & Keightley 2007). Thus, we cannot rely on predictions of theoretical models in determining the long-term viability of populations with reduced population size, and long-term experiments over different population sizes are needed (Lynch et al. 1995b).

Current empirical evidence for the effectiveness of purging in small populations has been inconsistent (reviewed in Ballou 1997, Byers & Waller 1999, Crnokrak & Barrett 2002, Leberg & Firmin 2008). Experimental studies on the effects of inbreeding rate on fitness and population viability also have shown inconsistent results (Ehiobu et al. 1989, Bijlsma et al. 2000, Day et al.

2003, Pedersen et al. 2005, Swindell & Bouzat 2006, Mikkelsen et al. 2010, Kristensen et al. 2011). Further, although several studies have examined the relationship between the level of inbreeding and population mean fitness (e.g. Saccheri et al. 1998, Rowe et al. 1999, Bijlsma et al. 2000, Reed & Frankham 2003, Puurtinen et al. 2004, Spielman et al. 2004), and fitness has been compared between populations bred to a certain level of inbreeding with different inbreeding rates (e.g. Ehiobu et al. 1989, Day et al. 2003, Swindell & Bouzat 2006, Mikkelsen et al. 2010, Kristensen et al. 2011), very few studies have followed the fitness of different sized populations over a range of inbreeding levels (but see Reed et al. 2003). Following the changes in population mean fitness from low to high levels of inbreeding in different sized populations can increase our understanding of the temporal dynamics of drift load in small populations. In addition, we have little knowledge of the effects of very low levels of inbreeding, as most studies have focused on relatively high inbreeding levels (most often $f \geq 0.25$) (but see e.g. Bijlsma et al. 2000).

1.2 Inbreeding by non-random mating

The level of homozygosity in a population can increase also as a consequence of non-random mating, if the breeding individuals are more closely related to each other on average than they are to other individuals in the population. Increased homozygosity through non-random mating will often lead to reduced fitness of individuals, for the same reasons as increased homozygosity caused by small population size (Charlesworth & Willis 2009). The reduced fitness of offspring produced by mating between close relatives, in relation to the fitness of offspring produced by random mating within a population, is generally termed *inbreeding depression* (Templeton & Read 1994, Kirkpatrick & Jarne 2000, Charlesworth & Willis 2009). In conservation biology, however, the term inbreeding depression is often used in another context: to describe the reduction in the average performance of a small population in relation to a large or infinite population of the same species (Templeton & Read 1994, Kirkpatrick & Jarne 2000). As explained earlier, when compared to a large population, individuals in a population with small historical size are indeed more related to each other, even when mating within the population is random. Thus, the only difference between the two ways of using the term inbreeding depression is whether individuals are compared within or between populations (Templeton & Read 1994, Kirkpatrick & Jarne 2000). In the original papers of this thesis, the term inbreeding depression has been used in both contexts (in papers II and III the term is used in the context of the mean population fitness, whereas in paper IV the term is used to describe the reduction in fitness of inbred individuals within a population). In this summary of the thesis, I will use the term inbreeding depression only to refer to the reduced fitness of inbred individuals relative to the fitness of individuals produced by random mating within a

population. When referring to the reduced fitness of a small population relative to a large population I will use the term drift load (see the previous chapter).

In contrast to drift load, inbreeding depression is expected to diminish with proceeding generations in small populations (Wang et al. 1999, Bataillon & Kirkpatrick 2000, Kirkpatrick & Jarne 2000). In small populations the level of homozygosity of all individuals increases over time and, consequently, the difference in homozygosity between offspring from inbred and random mating decreases. As a result, the magnitude of inbreeding depression decreases over generations.

The effect of inbreeding rate on inbreeding depression is predicted to change with increasing levels of inbreeding. At low inbreeding levels, populations with slower inbreeding can be expected to show less inbreeding depression, due to more efficient purging of recessive deleterious alleles at the early stages of reduced population size. At higher levels of inbreeding, however, the pattern may be reversed, so that populations with fast inbreeding show less inbreeding depression than populations with slow inbreeding (Wang et al. 1999, Theodorou & Couvet 2006). This effect may follow from larger populations with slow inbreeding having more loci heterozygous for newly arising mutations. In small populations with fast inbreeding, mutations are less frequent and new mutations are quickly fixed or lost, resulting in low inbreeding depression (Wang et al. 1999).

Another reason why slow inbreeding might result in more inbreeding depression than fast inbreeding is more efficient selection with slow inbreeding to maintain heterozygosity in overdominant loci (Kristensen et al. 2005, Demontis et al. 2009). Selection for heterozygosity in overdominant loci would mainly act on the pre-existing genetic variation. Thus, this form of selection should act to increase the magnitude of inbreeding depression with slow compared to fast rate of inbreeding at all levels of inbreeding. By studying inbreeding depression in different sized populations at several levels of inbreeding, information can be gained on the nature and amount of genetic variability affecting fitness, and on the genetic processes underlying fitness in small populations.

1.3 Interpopulation hybridization

1.3.1 Heterosis

Small populations are expected to accumulate genetic differences when in isolation from each other: due to the effects of genetic drift and natural selection, different alleles and allele combinations become common or fixed in different populations (Falconer & Mackay 1996, Allendorf & Luikart 2007). Hybridization between genetically differentiated populations can alleviate the genetic problems of small populations (reviewed in Tallmon et al. 2004, Hedrick 2005, Edmands 2007, Frankham et al. 2011). The increased fitness of hybrid

offspring produced by matings between individuals from different populations, as compared to the fitness of offspring produced by random matings within the parental populations, is termed heterosis (or sometimes, hybrid vigour). Heterosis is believed to result mainly from the increased heterozygosity in the hybrid offspring; in heterozygous genotypes, recessive deleterious alleles are masked and heterozygosity in overdominant loci is restored (Lynch 1991, Whitlock et al. 2000, Charlesworth & Willis 2009). Further, hybridization can bring together new beneficial combinations of alleles, or disrupt deleterious allele combinations that may have become fixed in the small populations through genetic drift (Lynch 1991, Erickson & Fenster 2006, Edmands et al. 2009).

1.3.2 Outbreeding depression

Interpopulation hybridization can, however, have also detrimental effects on individual fitness. Reduced fitness of hybrid offspring, compared to the fitness of offspring from random matings within the parental populations, is termed outbreeding depression (Edmands 2007). Outbreeding depression can follow from disruption of local adaptations, if the parental populations have adapted to different environmental conditions (Templeton 1986). However, divergent selection pressures are not necessary for the evolution of outbreeding depression. In isolated populations, the combined actions of genetic drift and selection can lead to fixation of different alleles that cause little or no harm individually, but cause a reduction in fitness when brought together by hybridization between the diverged populations (e.g. Phillips & Johnson 1998, Orr & Turelli 2001). Further, natural selection can favour the evolution of beneficial multilocus combinations of alleles, so called co-adapted gene complexes (Templeton 1986, Lynch 1991, Fenster et al. 1997). If different co-adapted gene complexes have evolved in different populations because of the combined actions of drift and selection, interbreeding between the populations can cause outbreeding depression by disrupting these complexes (Templeton 1986, Lynch 1991).

1.3.3 The net outcome of interpopulation hybridization

Several factors can influence the magnitudes of the positive and negative effects, and thus the net outcome, of interpopulation hybridization. One of the key factors is the level of genetic divergence between the populations (Lynch 1991, Falconer & Mackay 1996, Lynch & Walsh 1998, Whitlock et al. 2000). In the absence of selection, heterosis from masking of recessive deleterious alleles should increase linearly with the divergence of the populations. In contrast, outbreeding depression due to interactions between different genetic loci is expected to evolve slowly in the first stages of population divergence, but then develop at an accelerating rate as populations become increasingly differentiated (Orr 1995, Orr & Turelli 2001). Thus, assuming that heterosis results mainly from divergence in single-locus genotypes whereas outbreeding

depression involves divergence at two or more interacting loci, the positive effects of hybridization should predominate at low to intermediate population divergence, whereas at higher divergence there is an increasing risk of outbreeding depression.

The outcome of interpopulation hybridization can further depend on the rate of inbreeding in the parental populations. When compared at the same levels of inbreeding, there should be less potential for heterosis between populations with slow inbreeding rate than between populations with fast inbreeding rate. This expectation follows from the prediction of more effective selection and, consequently, lower drift load when the rate of inbreeding is slower (Wang et al. 1999, Whitlock et al. 2000, Theodorou & Couvet 2006). Further, it can be expected that more effective selection with slower inbreeding will enhance the development of co-adapted gene complexes, and thus lead to outbreeding depression in interpopulation crosses. It follows that, when comparing populations at the same level of inbreeding, hybridization is expected to be more beneficial between populations with fast inbreeding than between populations with slow inbreeding.

Another factor that can have an influence on the outcome of interpopulation hybridization is the amount of genetic variation introduced with hybridization, which depends on the number and genetic variability of the introduced individuals. As hybridization can have both positive and negative effects on population viability, determining the optimal amount of introduced genetic variation is difficult. In general, it appears that rather low levels of immigration are enough to cause an increase in fitness of a small population (reviewed in Mills & Allendorf 1996, Tallmon et al. 2004). However, even low levels of immigration from a genetically incompatible population can potentially cause considerable damage (Mills & Allendorf 1996, Edmands & Timmerman 2003).

The effects of interpopulation hybridization can also vary between generations following the hybridization. Heterozygosity peaks in the first hybrid generation and is diluted thereafter, suggesting that heterosis should also peak in the first generation and decrease in subsequent generations following hybridization (Tallmon et al. 2004). Fitness may be reduced in generations following hybridization also because of increased potential for outbreeding depression, as recombination continues to break up the parental gene combinations, and harmful epistatic interactions involving recessive alleles are exposed (Lynch 1991, Tallmon et al. 2004). On the other hand, outbreeding depression may also be a temporary phenomenon, as it is possible that natural selection favours beneficial alleles and allele combinations and removes the unfit genotypes from a population (Templeton 1986). However, overcoming outbreeding depression by natural selection requires that selection coefficients are high enough and population size large enough so that the population is not faced with extinction before fitness is recovered (Templeton 1986). Further, selection might not be very efficient in removing outbreeding depression if the fitness reduction is caused by disruption of genetic complexes that involve many loosely linked loci (Edmands 1999).

In summary, the net outcome of interpopulation hybridization can be affected by several factors, such as the level of divergence between the populations, the rate of inbreeding in the populations, and the amount of genetic variation introduced. Further, due to various reasons, the effects can change between generations. Consistent with the expectations, some previous studies on the effects of population divergence on the outcome of interpopulation hybridization have found either an intermediate optimum or a negative relationship between the level of parental divergence and fitness of the hybrid offspring (reviewed in Edmands 2002, 2007). However, positive relationships are also known, and predicting the optimum level of divergence has proven difficult (Edmands 2002, 2007, Willi et al. 2007). Studies on the effect of population divergence have focused on geographically separated natural populations (e.g. Edmands 1999, Fenster & Galloway 2000, Galloway & Etterson 2005, Schiffer et al. 2006, Demuth & Wade 2007, Willi et al. 2007), making it difficult to disentangle the effects of local adaptation and processes independent of the environment in the evolution of genetic divergence between the populations. The few studies on the effect of population size (i.e. inbreeding rate) on the outcome of interpopulation hybridization have not separated the effects of population size and the level of inbreeding in the populations (Paland & Schmid 2003, Busch 2006, Willi et al. 2007, Escobar et al. 2008, Coutellec & Caquet 2011). Studies on the long-term effects of interpopulation hybridization are also scarce; very few previous studies have been continued beyond the second or third generation after hybridization (but see Edmands et al. 2005, Erickson & Fenster 2006, Bijlsma et al. 2010, Hwang et al. 2011).

1.4 Measuring fitness in empirical studies

Measuring the fitness of particular phenotypes (or genotypes) is an essential part of evolutionary biology research. Measuring fitness is, however, not a simple task, and the best measure of fitness can differ depending on the biology of the study system. For species with non-overlapping generations and for populations at constant population size, the best measure of individual fitness is lifetime reproductive success: the number of viable zygotes produced over the whole lifetime of an individual (Stearns 1992, Hunt & Hodgson 2010). Even when model species with a relatively short life span are used, measuring the total fitness of individuals is often not practical because of constraints in time and other resources. Instead, researchers use various surrogates of fitness; traits that are thought to reflect the true fitness of individuals and are relatively easy to measure. These surrogates can be different components of fitness, such as fecundity or offspring survival, or proxies more uncertainly related to fitness, such as body size (Hunt & Hodgson 2010). Ideally, fitness would be measured over the lifetime of individuals, but more convenient short-term measures are often used. The choice of the fitness measure to be used is a significant part of

empirical research, since using fitness surrogates that are poor indicators of true fitness may lead to erroneous conclusions about the phenomena under study.

1.5 Aims of the thesis

In my thesis, my goal was to study how reductions in population size and isolation from other populations of the same species affect the fitness and survival of populations. In addition, I aimed to study how a potential conservation tool, artificially increased hybridization between isolated populations, affects the fitness of individuals and the long-term viability of populations. More specifically, my aims were to study the effect of inbreeding rate on the magnitudes of drift load and inbreeding depression over a range of inbreeding levels, and the effects of inbreeding rate, population divergence, and the amount of genetic variation introduced on the outcome of interpopulation hybridization.

To study these questions, I used an experimental approach and a model species, *Drosophila littoralis*. Although examination of real conservation applications in natural populations of threatened species can provide important information about natural situations, the lack of replication and control makes it difficult to disentangle the possible factors influencing the results (Tallmon et al. 2004). Adequately replicated experimental studies are an invaluable means for studying the role of specific factors, such as inbreeding rate, on the fitness of individuals and populations. By using a model species that is easy to rear under laboratory conditions, I was able to manipulate the factors that I wanted to study, and to control for other factors, such as changes in the environment, that otherwise could have confounded the results. *D. littoralis* is a small fly that is easy to rear in large numbers and has a relatively short life span, allowing me to conduct studies over several generations and to have sufficient numbers of replicates for drawing conclusions about the importance of the studied factors. In addition, with the genetic markers available for *D. littoralis*, I was able to estimate the realized inbreeding rates in the study populations.

The effect of inbreeding rate on the magnitude of drift load was studied in two of the original papers (II, IV). In the second original paper (II), the relationship between population viability and the level of inbreeding was compared between isolated *D. littoralis* populations replicated in two sizes (N = 10 and N = 40). Viability (offspring production and extinction) of the populations was followed over 25 generations in relation to a contemporary large control population (N = 500). In the fourth original paper (IV), individual fitness (egg-to-adult survival and fecundity) was measured in populations replicated in three different sizes (N = 2, N = 10, and N = 40). Again, the fitness of individuals in these populations was measured in relation to the large control population (N = 500), and was compared between the different population sizes over a range of inbreeding levels.

The effect of inbreeding rate on the magnitude of inbreeding depression was studied in the fourth original paper (IV). In populations replicated in two sizes (N = 10 and N = 40), inbreeding depression was measured as the fitness of offspring from full-sib crosses in relation to the fitness of offspring from random crosses within the same populations. Inbreeding depression was measured at several levels of inbreeding.

The effects of hybridization between isolated populations were studied in the original papers III and IV. In the third paper (III), the dependence of the long-term effects of interpopulation hybridization on the level of population divergence and the amount of genetic variation introduced was studied by comparing viability between isolated populations (N = 10) and hybrid populations established from the isolated populations. In the fourth paper (IV), the effects of inbreeding rate and the level of population divergence on the outcome of interpopulation hybridization were studied by measuring fitness of the first generation offspring from interpopulation crosses in relation to the mean fitness of offspring from crosses within the parental source populations. The effects of hybridization were compared between three population sizes (N = 2, N = 10, and N = 40) over a range of inbreeding levels (which equal the level of genetic divergence between populations of the same size).

In the first original paper (I), phenotypic correlations between adult lifetime reproductive success (adult LRS) and various morphological and life history traits were explored in order to evaluate the reliability of various commonly used fitness surrogates, and to explore the potential fitness surrogates to be used in the measurements of individual fitness in the experiments of this thesis (IV). Adult LRS was measured as the total number of offspring produced over the lifetime of individual females. Adult LRS is very likely to be closely related to total fitness of individuals, as it combines several components of fitness, such as longevity, fecundity, and offspring viability. It is, however, difficult to measure and therefore rarely included in fitness estimation in experimental studies.

2 MATERIAL AND METHODS

2.1 Study species and the origin of the study population

Drosophila littoralis is a boreal drosophilid belonging to the *D. virilis* species group (Morales-Hojas et al. 2011). It is found in humid habitats such as lake- and riversides (Aspi et al. 1993). In northern Fennoscandia, the species overwinters as adults and is practically univoltine with only slightly overlapping generations (Aspi et al. 1993).

A laboratory population of *D. littoralis* was founded with 157 males and 99 females collected from a natural population at the Tourujoki River in Jyväskylä, Central Finland, in May 2006. Thirty-four of the 99 females had been inseminated in the wild and produced fertile eggs after transfer to the lab. The rest of the females were mated randomly with the wild-caught males. The flies were maintained in the laboratory at 19 ºC and relative humidity of 60 % with constant light and malt medium (Lakovaara 1969) available *ad libitum*. For the first five generations (P-F4; P refers to the wild-caught flies) the population was maintained in a pedigree. Inbreeding was reduced by excluding matings between full siblings. Population size was increased to 419 breeding pairs in the second laboratory generation, and maintained as 396 pairs in the third and 368 pairs in the fourth laboratory generation. In a sample of 20 individuals from the fourth laboratory generation, 11 out of 14 nuclear microsatellite loci were polymorphic, and the mean number of alleles in the polymorphic loci was 6.8 with mean observed heterozygosity of 0.55 (Routtu et al. 2007). For the next two generations (F5-F6) the flies were allowed to mate randomly (with separate generations) as a population of approximately 500 breeding pairs.

2.2 Establishment and maintenance of the experimental populations

To manipulate the rate of inbreeding, experimental populations were established in three different sizes: $N = 2$, $N = 10$, and $N = 40$ (see Fig. 1). At the seventh laboratory generation, 16 replicate populations of 10 individuals (N10; sex ratio 1:1), and 12 replicate populations of 40 individuals (N40; sex ratio 1:1) were established. Also, a large population consisting of 500 individuals (N500, sex ratio 1:1) was established to serve as a control against possible environmental variation in time. The populations were maintained in plastic bottles containing 50 ml of malt medium at density of five pairs per bottle. Thus, the N10 populations consisted of one bottle per replicate, the N40 populations consisted of four bottles per replicate, and the control population consisted of 50 bottles. Each generation, the parental flies were allowed to mate and lay eggs in the bottles for 5 days, after which they were removed and stored in ethanol for genetic analysis. The first eclosing offspring from all populations were discarded. Seven days later, the newly eclosed offspring were collected and separated according to sex under CO_2 anaesthesia. For the N40 populations, offspring from all bottles in the same replicate were mixed each generation prior to collecting the flies. Likewise, offspring from all bottles in the control population were mixed each generation. When mature, the parental flies for the next generation were randomly picked among the offspring produced by each replicate population.

At the 12th laboratory generation, the smallest populations of one male-female pair (N2) were established with 96 randomly chosen pairs from the control population (Fig. 1). The N2 populations were maintained in plastic vials with 8 ml of malt medium. Each generation, the parental pair was allowed to mate and lay eggs for 10 days. The pair was changed into a new vial first after 4 days and then every second day to prevent crowding of the larvae. After the 10 days, the parental flies were removed and stored in ethanol for genetic analyses. The first eclosing offspring from all populations were discarded. Seven days later, the newly eclosed offspring were collected and separated according to sex under CO_2 anaesthesia. When mature, the parental flies for the next generation were randomly picked among the offspring produced by each replicate population.

To study the effects of interpopulation hybridization on population viability (see 2.6 Long-term effects of interpopulation hybridization), hybrid populations were established with offspring from the isolated N10 populations (Fig. 1). All hybrid populations were founded with five males and five females. The populations were maintained for 7 generations keeping population size constant with a procedure analogous to the isolated N10 populations (see above).

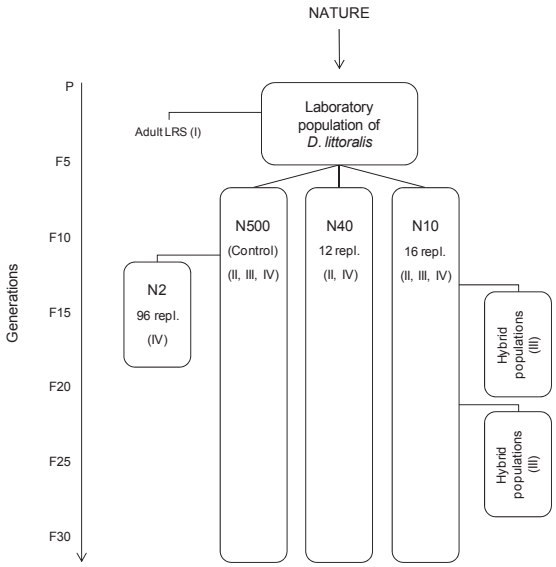

FIGURE 1 Establishment of the populations for the different experiments of the thesis (the Roman numerals I-IV refer to the original papers of the thesis). The N500 (control), N40, and N10 populations were maintained for a total of 26 generations. The N2 and the hybrid populations were maintained for 7 generations.

2.3 Genetic analyses

The inbreeding levels at different generations in the isolated experimental populations (N2, N10, and N40) were measured as the inbreeding coefficient (f), which describes the increase in homozygosity due to finite population size. To estimate the inbreeding coefficient, information of the effective population size (N_e), was needed. N_e describes the size of an ideal population that would result in the same magnitude of increase in homozygosity as in the observed population (Crow & Kimura 1970). Since the effective population size may deviate from the census size (N) due to non-random contribution of the parents to the next generation, effective population sizes of the populations were estimated based on genetic variation at eight nuclear microsatellite loci. The eight loci chosen for the study (Vir4, Vir11, Vir32, Vir38, Vir99, Mon6, Mon17, Mon26) were polymorphic in the original large population (Routtu et al. 2007).

Samples of individuals from all population sizes, including the large control population (N500), were genotyped at multiple generations (for further details, see original paper II). The inbreeding coefficients for each population size at the genotyped generations were determined pooling the data from the replicate populations, as

$$f_{observed} = 1 - H_o \,/\, H_{e(N500,1)}$$

where H_o is the observed heterozygosity in the pooled samples, and $H_{e(N500,1)}$ is the expected heterozygosity in the control population after one generation from the establishment of the population. The effective population sizes (N_e) that would produce the observed inbreeding coefficients were calculated using the equation

$$f_t = f_{t-1} + (1 - 2 f_{t-1} + f_{t-2}) \,/\, 2N \text{ (Crow \& Kimura 1970 p. 102)}$$

replacing N with different values of N_e and assuming that the parental flies at the generation of the establishment of the populations were not related. The estimated N_e was 23.2 for the N40 populations, 8.1 for the N10 populations, and 1.9 for the N2 populations. The control population (N500) sustained a high level of heterozygosity throughout the experiment. The estimated N_e for the control population was 342 individuals.

The inbreeding coefficients in the populations for all experimental generations were then calculated using the same equation as above (Crow & Kimura 1970 p. 102), replacing N with the estimated N_e and assuming that the parental flies at the generation of the establishment of the populations were not related (see Fig. 2 for the estimated inbreeding coefficients). The inbreeding coefficients were used also as estimates of population divergence (III, IV): assuming random mating within populations, the inbreeding coefficient in the isolated populations is equal to the level of differentiation in allele frequencies between populations of the same size (in other words, the inbreeding coefficient relative to the starting population equals F_{ST} between the subpopulations).

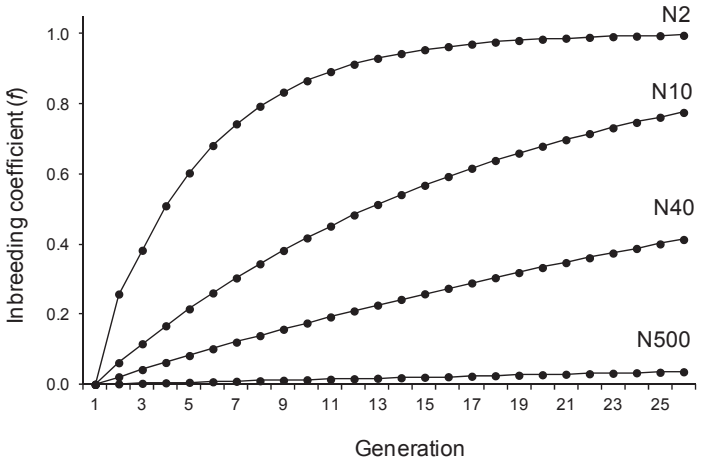

FIGURE 2 The estimated inbreeding coefficients in the isolated experimental populations (N2, N10, and N40) and in the control population (N500) over generations following the establishment of the populations.

2.4 Relationship between adult LRS and fitness surrogates (I)

To explore the phenotypic correlations between female lifetime reproductive success (adult LRS) and different surrogates of fitness, the reproductive output of *D. littoralis* females was followed from 5 days after eclosion until death. At the third laboratory generation (Fig. 1), 84 females at age of 5 days after eclosion were introduced to mature males. One female and one non-sib male were placed into a plastic vial with 8 ml of malt-yeast medium to mate and lay eggs. The pair was placed into a new vial every second day until the death of the female. The male was replaced with a new one every second week, or immediately if the male was found dead or if it escaped during handling.

Adult LRS was measured as the number of eclosing offspring produced over the lifetime of the females (from the age of 5 days after eclosion until death). Noting that survival is usually higher in laboratory conditions than in nature, where individuals are subject to predation and other hazards (Rosewell & Shorrocks 1987), adjusted adult LRS -measures that give more weight to early reproduction were also calculated, by assuming additional values of daily mortality risk of 2, 4, 6, 8, 10, and 12 % for the females.

Lifetime fecundity of the females was measured as the number of eggs produced from the age of 5 days after eclosion until death. Offspring viability was measured for each female by dividing the total number of offspring produced (i.e. adult LRS) by the total number of eggs produced (i.e. lifetime fecundity). Short-term estimates of offspring production and fecundity were calculated as sliding windows throughout female life in three different time frames (2, 4 and 10 days).

The females were weighed before introduction to the male. After death, females were preserved in 70 % ethanol. Several morphological measurements were taken from the preserved samples. Distance between nine cross points of the wing veins and the length of femur, tibia, and the five segments of the tarsus of the hind legs were measured from digital images (the average of the left and right measurements were used when possible). To obtain a single size component for wings and legs, the first principal component was extracted from the correlation matrix of the measurements. Length of thorax (longest distance between neck and tip of scutellum measured from the side of the fly), length of scutellum (longest dorsoventral distance), and width of head (distance between eyes through ocelli) were measured using a light microscope.

2.5 Effects of inbreeding and genetic drift on population viability (II)

To study the effects of the level and the rate of inbreeding on population viability, viability of the isolated N10 and N40 populations was measured for 25

generations following the establishment of the populations. During that time, the N40 populations reached an inbreeding coefficient (f) = 0.41 and the N10 populations reached f = 0.78. Viability was compared between the two population sizes at inbreeding coefficients ranging from f = 0.06 until f = 0.41 (in the N40 populations) or f = 0.42 (in the N10 populations).

Population viability was measured as extinction and *per capita* offspring production. A population was considered extinct if fewer female and/or male offspring eclosed during the seven-day collection period than required for founding the subsequent generation at the defined population size (see 2.2 Establishment and maintenance of the experimental populations). Offspring production was counted for 28 days after removing the parental flies from the bottles. The *per capita* offspring production was obtained by dividing the total number of offspring with the number of bottles in the replicate. To control for possible environmental variation over time, and to facilitate comparisons between the two population sizes at different points in time, offspring production was measured in relation to the large control population (N500), measured at the same generation. When a population was considered extinct, offspring production was recorded as zero from the extinction onwards.

2.6 Long-term effects of interpopulation hybridization (III)

To study the effects of population divergence and the amount of genetic variation introduced on the long-term effects of interpopulation hybridization, hybrid populations were established with offspring from the isolated N10 populations (Fig. 1). To manipulate the level of population divergence, the hybrid populations were founded at two points in time: after 7 generations and after 15 generations following establishment of the isolated populations. The inbreeding coefficient in the isolated N10 populations, based on the estimated N_e, was 0.30 after 7 generations and 0.57 after 15 generations of isolation. To manipulate the amount of genetic variation introduced, the hybrid populations were founded with individuals from either two or four different isolated N10 populations. The combinations of isolated populations were assigned randomly, while assuring that each combination was different from the others. Only males or females were taken from any isolated population when constructing a hybrid population, so that all first generation offspring produced in the hybrid population were hybrids. The viability (offspring production and extinction) of the hybrid populations was measured for 7 generations after their establishment as was done for the isolated N10 populations (see the previous chapter). Viability of the hybrid populations was compared to the viability of the contemporaneous isolated N10 populations, and the large control population (N500).

2.7 Effects of genetic drift, inbreeding, and interpopulation hybridization on fitness of individuals (IV)

In the fourth original paper (IV), the effects of reduced population size and interpopulation hybridization were studied at the level of individual fitness. To study the effects of the level and the rate of inbreeding on drift load and inbreeding depression, and the effects of inbreeding rate and population divergence on the outcome of interpopulation hybridization, controlled crosses within and between the isolated populations were made. Within the experimental populations (N2, N10, and N40), crosses were made using randomly chosen and full-sib pairs (note that for the N2 populations these are equivalent). Crosses between populations of the same size were made using reciprocal randomly chosen pairs. In addition, crosses were made with randomly chosen pairs within the large control population (N500). All cross types were done at several generations following the establishment of the populations, i.e. at several levels of inbreeding.

From each cross, fitness of the first generation offspring was measured as egg-to-adult survival, fecundity, and total fitness. The male and female of the experimental cross were placed in a plastic vial with 8 ml of malt medium when mature (age 13 to 26 days from eclosion). The pair was maintained together for 10 days and changed into a new vial first after 4 days and then every second day to prevent crowding of the larvae. The first 4 days were considered as a familiarization period and were not used for the fitness measurements. From the following six-day period, egg-to-adult survival of the offspring was calculated as the ratio of the number of eclosed adult offspring to the number of eggs laid by the female.

When mature (age 13 to 24 days from eclosion), one female offspring from each experimental cross (if possible) was introduced to a male collected from the control population. Again, the pair was maintained together for 10 days, and was changed into new vials first after 4 days and then every second day. Offspring fecundity was measured as the average number of eggs laid in a vial during the last six-day period. Total fitness of the offspring was calculated by multiplying offspring fecundity with egg-to-adult survival.

Drift load was estimated as the fitness of offspring from random crosses within the experimental populations (N2, N10, and N40) relative to the fitness of offspring from random crosses within the large control population (N500), measured at the same generation. Drift load was estimated at several levels of inbreeding in each population size (f = 0.26 – 0.74 in the N2, f = 0.17 – 0.70 in the N10, and f = 0.21 – 0.39 in the N40 populations), and the magnitude of drift load was compared between the different sized populations over similar levels of inbreeding.

Inbreeding depression was estimated for the N10 and N40 populations as the fitness of offspring from full-sib crosses within a population relative to the fitness of offspring from random crosses within the same population, measured

at the same generation. Like drift load, inbreeding depression was measured at several inbreeding levels (f = 0.30 – 0.70 in the N10, and f = 0.23 – 0.39 in the N40 populations), and the magnitude of inbreeding depression was compared between the different sized populations over similar levels of inbreeding.

Effects of interpopulation hybridization were estimated as the performance of interpopulation crosses relative to the mean of within-population random crosses in the parental source populations, measured at the same generation. The effects of hybridization were studied in the three population sizes (N2, N10, and N40) at several levels of inbreeding (f = 0.38 – 0.68 for the N2, f = 0.17 – 0.70 for the N10, and f = 0.23 – 0.39 for the N40 populations), and the effects were compared between the different sized populations over similar inbreeding levels (which equal the level of genetic divergence between populations of the same size).

3 RESULTS AND DISCUSSION

3.1 Relationship between adult LRS and fitness surrogates (I)

The lifetime measures of fecundity, longevity, and offspring viability were all relatively highly correlated with adult LRS ($r = 0.81$, $r = 0.63$, and $r = 0.51$, respectively). In contrast, the correlations of the short-term measures of fecundity and offspring production with adult LRS were highly dependent on the time of measurement: for young females the correlations were low, but when these were measured from older females, the correlations were much higher (up to 0.67 for short-term fecundity and 0.83 for short-term offspring production). For both short-term fecundity and short-term offspring production the highest correlations with adult LRS were reached when the female age was approximately 50 to 80 days from eclosion. The highest correlations between adult LRS and the size measures were found for leg size ($r = 0.38$) and for female weight ($r = 0.32$). Similar observations of strong correlation between adult LRS and other life history traits measured over the whole lifetime of individuals have been made in other species as well, both in laboratory (Partridge 1988, Reed & Bryant 2004) and in field studies (Bryant 1988, Newton 1988, Smith 1988, Bercovitch & Berard 1993, Kruuk et al. 1999). In contrast, previous results on the relationship between adult LRS and short-term measures of life history traits (Smith 1988, Reed & Bryant 2004), and between adult LRS and size measures (Partridge et al. 1986, Bryant 1988, Scott 1988, Wauters & Dhondt 1989, Ribble 1992), are more variable.

To explore the effects of higher mortality rates likely to exist under natural conditions, adjusted adult LRS -measures with additional daily mortality risk of the females were calculated. In contrast to what was found for the unadjusted adult LRS -measure, the short-term measures of fecundity and offspring production correlated better with mortality-adjusted adult LRS when measured from younger rather than older flies. In general, the highest correlations with the adjusted adult LRS -measures, for both short-term fecundity and short-term offspring production, were reached when the female age was approximately 5 to 30 days from eclosion. Mortality-adjustment to adult LRS did not have a

strong effect on the correlations between lifetime offspring production and the size measures.

To estimate the fitness of offspring from the experimental crosses (IV), reproductive output of the female offspring was measured as fecundity over a period of 6 days. During the six-day period, female age ranged from 17 to 34 days after eclosion. This measure was chosen on the basis of the results from the adult LRS -experiment, but also because of practical issues. In the adult LRS -experiment, correlation between the unadjusted adult LRS -measure and short-term fecundity over this age period, calculated as mean of the 10-day windows (four windows; first window at 17-27 days age, second window at 19-29 days age, third window at 21-31 days age, and the last window at 23-33 days age from eclosion), was rather modest; r = 0.20. Correlation between the mortality-adjusted adult LRS -measures and short-term fecundity over this age period (calculated as mean of the 10-day windows; same windows as above) was higher, ranging from r = 0.40 with 2 % and 12 % additional mortality risks to r = 0.53 with 6 % additional daily mortality risk.

The adult LRS -measures adjusted for mortality give more weight to early reproduction than to later reproduction and are therefore more likely to reflect fitness in natural conditions where the flies have evolved (Rosewell & Shorrocks 1987). Indeed, the peak in egg and offspring production of the females in the adult LRS -experiment was reached before 45 days age (at 20-45 days age for egg production, and at 20-25 days age for offspring production). It can be expected that the effects of inbreeding and drift are more pronounced in those traits that have been the targets of natural selection in the history of the population. Thus, measuring the fecundity of the offspring from the experimental crosses (IV) at a rather young age was considered reasonable.

In the adult LRS -experiment, correlates of adult lifetime reproductive success were studied without considering possible differential mortality before the females reached adulthood. Early mortality can, however, be a significant source of variation in fitness. Therefore, in estimation of offspring fitness from the experimental crosses (IV), the contribution of early mortality (egg-to-adult survival) was included in the fitness estimation, together with estimation of the reproductive output (fecundity of the female offspring).

3.2 Small population size and drift load

3.2.1 Drift load in population viability (II)

The increasing level of inbreeding affected offspring production of the populations differently depending on population size. In the smaller populations (N10), i.e. with a faster inbreeding rate, there was a steep decline in offspring production already at low levels of inbreeding, followed by a rebound to the level of the control population. The recovery of offspring production was, however, only temporary, as offspring production decreased again in later

generations. In the larger populations (N40), i.e. with a slower rate of inbreeding, offspring production decreased only after the populations reached higher levels of inbreeding.

The higher offspring production in the larger populations at low levels of inbreeding was likely due to more effective selection with slower inbreeding against deleterious recessive alleles (Wang et al. 1999, Theodorou & Couvet 2006), and possibly also for maintaining heterozygosity in overdominant loci (Kristensen et al. 2005, Demontis et al. 2009). Slower inbreeding has been shown to be less harmful to the fitness of individuals or viability of populations in other studies as well (Ehiobu et al. 1989, Day et al. 2003, Reed et al. 2003, Pedersen et al. 2005). However, in some studies no effects of inbreeding rate on fitness has been found (Kristensen et al. 2011), and in others, the effect of inbreeding rate has varied from negative to positive depending on the trait and the environment (Bijlsma et al. 2000, Swindell & Bouzat 2006, Mikkelsen et al. 2010). Despite high fitness at low to intermediate levels of inbreeding, offspring production of the larger populations did decrease at higher inbreeding levels, suggesting that the slower rate of inbreeding did not protect the populations from the harmful effects of inbreeding and genetic drift in the long term.

The initial decrease in offspring production of the smaller populations, and the rebound to the level of the control population, can be explained by increased expression and subsequent purging of recessive deleterious alleles (Lynch et al. 1995b, Wang et al. 1999, Theodorou & Couvet 2006). In later generations, the continuous decrease in offspring production was likely due to accumulation and fixation of mildly deleterious alleles that cannot be efficiently purged (Lande 1994, Lynch et al. 1995b, Wang et al. 1999, Whitlock 2000, Glemin 2003). Such fluctuations of fitness in populations of limited size have not been reported often, but this may be due to the fact that only a few studies have followed the fitness of small populations over a range of inbreeding coefficients. However, a similar observation of an initial decrease in fitness, followed by a recovery, was recently made by Larsen et al. (2011) with guppy (*Poecilia reticulata*) populations consisting of five pairs of fish.

Only a few extinctions happened before f = 0.42 was reached, and the difference in the proportion of extinct replicate populations between the two population sizes at f = 0.06 – 0.42 was not significant. However, by the end of the experiment, 67 % of the N10 replicate populations faced extinction. A similar threshold relationship between extinction and the level of inbreeding has been observed in other experimental studies (see Frankham 1995), and is also expected on theoretical grounds (Lynch et al. 1995b, a, Theodorou & Couvet 2006). It was also found that the time to extinction of the smaller populations could be predicted by offspring production at the generation at which the offspring production recovered after the initial decrease. This suggests that the magnitude of the fitness rebound, i.e. the degree to which the population was able to purge deleterious alleles, affected the survival of the populations in later generations.

3.2.2 Drift load in individual fitness (IV)

In the fitness measurements of the first generation offspring from the experimental crosses, smaller populations showed a higher magnitude of drift load (lower mean fitness) compared to larger populations over similar levels of inbreeding (compared between the three population sizes: N2, N10, and N40). Further, the increase in the magnitude of drift load with increasing level of inbreeding was stronger in smaller populations. These findings are consistent with the study on the effects of genetic drift on population viability (II), and further support the inference of more effective selection against the deleterious effects of drift with slower rate of inbreeding. In the two smallest population sizes (N2 and N10), the frequency of deleterious alleles and allele combinations may have been reduced also through selective elimination of the populations. On the other hand, the extinction rate was highest in the smallest (N2) populations, and still a significant decreasing trend in fitness was observed in these populations. This shows the inefficiency of selection against the harmful effects of inbreeding and genetic drift when the rate of inbreeding is very fast. A negative relationship between the level of homozygosity and population fitness is a common observation both in experimental studies and in the wild (e.g. Saccheri et al. 1998, Rowe et al. 1999, Bijlsma et al. 2000, Reed & Frankham 2003, Puurtinen et al. 2004, Spielman et al. 2004), but very few studies have followed the fitness of different sized populations over a range of inbreeding coefficients. However, my findings are consistent with those of Reed et al. (2003), who used different sized *D. melanogaster* populations and showed that the decrease in population survival with increasing level of inbreeding was faster in smaller populations, i.e. with a faster rate of inbreeding.

3.3 Inbreeding by non-random mating (IV)

In the experimental crosses, there was significant inbreeding depression (reduced fitness of offspring from full-sib matings relative to random matings within a population) in the larger (N40), but not in the smaller (N10) populations. However, the mean fitness of offspring from the full-sib crosses compared to random crosses within the populations was often less than one also in the smaller populations, indicating harmful effects of inbreeding. Reduced fitness following mating between close relatives has been observed practically in all examined taxa both in captivity and in natural populations (see e.g. Charlesworth & Charlesworth 1987, Crnokrak & Roff 1999, Keller & Waller 2002). A possible explanation for finding significant inbreeding depression only in the larger and not in the smaller populations is that at similar estimated levels of inbreeding, the larger populations were in fact more heterozygous at loci under selection. This difference could be caused by larger populations having more loci heterozygous for newly arising mutations (Wang et al. 1999). It is also possible that selection to maintain the pre-existing heterozygosity in

overdominant loci was more effective in the larger populations (Kristensen et al. 2005, Demontis et al. 2009). However, the overall difference between the two population sizes was not significant, and the data does not allow firm conclusions about the effects of inbreeding rate on the magnitude of inbreeding depression.

3.4 Interpopulation hybridization

3.4.1 Long-term effects of hybridization on population viability (III)

Overall, hybridization between isolated populations increased population viability, and the positive effects of hybridization lasted for the duration of the experiment (for at least 7 generations). However, the effects of hybridization differed between the two levels of population divergence. At the lower level of divergence (f = 0.30), hybrid populations were significantly more viable than the isolated source populations: offspring production was higher and extinction probability was lower in the hybrid populations than in the isolated populations. Further, offspring production in the hybrid populations was higher than in the large control population. At the higher level of divergence (f = 0.57), the hybrid populations were not significantly more viable than the isolated source populations, and offspring production was lower than in the control population. The number of source populations had no significant effect on population viability, although there was a tendency for higher offspring production in hybrid populations established from two source populations as compared to hybrid populations established from four source populations.

The significantly improved viability of the hybrid populations compared to the isolated source populations at the lower level of population divergence was likely caused by masking of recessive deleterious alleles in heterozygous genotypes, but increased heterozygosity in overdominant loci and interactions between loci may also have had an effect (Lynch 1991, Lynch & Walsh 1998, Whitlock et al. 2000, Erickson & Fenster 2006, Edmands et al. 2009). Fitness increase following hybridization between populations suffering from inbreeding and drift has been observed before in several taxa (see e.g. Edmands 1999, Fenster & Galloway 2000, Richards 2000, Marr et al. 2002, Rhode & Cruzan 2005, Hogg et al. 2006, Coutellec & Caquet 2011). In many of the studies that have followed performance beyond the first generation after hybridization, heterosis observed in the first hybrid generation has turned into outbreeding depression in the following generations (see Edmands 2007). My findings are consistent with those of some previous studies that have found the positive effects of hybridization to persist longer (e.g. Moll et al. 1965, Spielman & Frankham 1992, Willi et al. 2007). Very few studies have been continued beyond the second or third generation after hybridization. However, in a recent study using experimental *D. melanogaster* populations, Bijlsma et al. (2010) found that introducing 10 % immigration into small populations increased fitness of the

populations for at least ten generations. Further, some long-term studies have found outbreeding depression in early hybrid generations, but fitness recovery in later generations (Erickson & Fenster 2006, Hwang et al. 2011). It is possible that selection against deleterious alleles and allele combinations, and positive selection favouring beneficial allele combinations, to some extent accounts for the long-lasting positive effects of hybridization (Templeton 1986).

The higher offspring production of the hybrid populations established at the lower level of population divergence compared to the large control population suggests that selection had removed (purged) at least some deleterious alleles from the isolated populations. Had there been no selection, average genotypes in the hybrid populations should be similar to genotypes in the control population, and the mean fitness of the hybrid populations should equal the fitness in the control population (Falconer & Mackay 1996, Crnokrak & Barrett 2002). The occurrence of purging in the isolated populations is supported also by the study on the magnitude of drift load in the isolated populations (II). Previous empirical evidence for purging has been equivocal (Ballou 1997, Byers & Waller 1999, Crnokrak & Barrett 2002, Leberg & Firmin 2008). In particular, purging by small population size, as opposed to purging by non-random mating, has been considered relatively inefficient (Glemin 2003). The results of my thesis show that some purging of genetic load can occur also in populations of very small size.

The reduced improvement in viability of the hybrid populations at the higher level of population divergence can be due to several non-exclusive genetic mechanisms. First, it is possible that recessive deleterious alleles have been purged from the isolated populations to a large extent before the hybrid populations were established. Naturally, hybridization cannot mask the effects of recessive deleterious alleles if these alleles do not exist. A second possible mechanism is accumulation of mildly deleterious alleles in the isolated populations (Lande 1994, Lynch et al. 1995b, Wang et al. 1999, Whitlock 2000, Glemin 2003). As mildly deleterious alleles typically are only weakly recessive, their effects are expected to be masked only to a slight degree in the heterozygous genotypes (Whitlock et al. 2000). A third possible explanation is an increased opportunity for negative effects of hybridization with a higher level of population divergence, through breakage of coadapted gene complexes that may have developed in the isolated populations (Templeton 1986, Lynch 1991), or through formation of deleterious allele combinations in the hybrid populations (Phillips & Johnson 1998, Orr & Turelli 2001). The higher offspring production of the hybrid populations at the lower level of population divergence, as compared to the control population, suggests that highly deleterious recessive alleles were to some degree purged from the isolated populations. The results from the experiment on the magnitude of drift load in the isolated populations (II) also support this conclusion, and further, suggest that the isolated populations had accumulated mildly deleterious alleles by the time the later hybrid populations were established. Both of these factors probably contribute to the reduced improvement in viability of the hybrid

populations at the higher level of population divergence. However, increased expression of negative effects of hybridization is also possible.

3.4.2 Short-term effects of hybridization on individual fitness (IV)

In the experimental crosses, interpopulation hybridization increased offspring fitness (i.e. induced heterosis) in the two smallest population sizes (N2 and N10), but not in the largest populations (N40). When compared over similar levels of inbreeding, heterosis was higher in smaller populations, i.e. with a faster rate of inbreeding. This is not surprising, taking into account that the detrimental effects of genetic drift were also stronger with faster inbreeding rate. The observation gives further evidence for more effective selection with slower inbreeding: if the larger populations have more efficiently purged recessive deleterious alleles and harmful allele combinations, or more efficiently maintained heterozygosity in overdominant loci, there simply is not that much to gain from hybridization. Higher heterosis in smaller compared to larger populations has been reported before (Paland & Schmid 2003, Busch 2006, Willi et al. 2007, Escobar et al. 2008, Coutellec & Caquet 2011), but these studies have not separated the effects of population size and the level of inbreeding: the observations of more heterosis in smaller populations in these studies are likely caused by smaller populations being more inbred than the larger populations. To my knowledge, no previous studies exist that would have studied the effect of inbreeding rate on the outcome of interpopulation hybridization while controlling for the effect of the level of inbreeding in the populations.

Population divergence had no significant effect on the outcome of interpopulation hybridization. However, judged from the confidence intervals, the N10 populations expressed significant heterosis in total fitness at intermediate (f = 0.30 and f = 0.34), but not at low (f = 0.17), or at high levels of population divergence (f = 0.45, f = 0.57, and f = 0.70). Not finding heterosis at a low level of divergence can be explained by the fact that the potential for heterosis through increased heterozygosity is low before some level of divergence between populations is reached. Not finding heterosis at higher levels of population divergence could be due to purging of highly deleterious recessive alleles in the isolated populations (Lande 1994, Lynch et al. 1995b, Wang et al. 1999, Whitlock 2000, Glemin 2003), or due to an increased risk for the negative effects of hybridization (Templeton 1986, Lynch 1991, Orr 1995, Phillips & Johnson 1998, Orr & Turelli 2001). Based on the other results from the thesis (II-III; see above), purging of deleterious recessive alleles from the isolated populations seems a plausible explanation, although the expression of negative effects of hybridization is also possible. An intermediate optimum between the level of parental divergence and fitness of the hybrid offspring has been observed in other experimental studies of hybridization (reviewed in Edmands 2002, 2007). However, the mechanism of population differentiation may be different in the previous studies compared to this study, because the previous studies have focused on geographically isolated natural populations that may harbour adaptations to local environmental conditions.

4 CONCLUSIONS AND IMPLICATIONS

The results from my thesis show that selection can, to some extent, counteract the deleterious effects of inbreeding and genetic drift even in populations with an effective population size as small as eight individuals (II, III). However, selection is inefficient against the continuous accumulation and fixation of deleterious alleles if population size remains small. Even if small populations are able to survive past the initial reductions in fitness at the early stages of reduced population size, it is likely that they will suffer from decreased offspring production and increased risk of extinction in future generations.

Furthermore, the results show that the effects of inbreeding and genetic drift are less harmful when the rate of inbreeding is slower (II, IV). This is likely due to more efficient selection with slower inbreeding against deleterious recessive alleles and for maintenance of variation in loci with overdominant effects on fitness. However, also the largest populations used in the experiments (effective population size 23 individuals) did show a decrease in fitness due to increased magnitudes of inbreeding and genetic drift (IV), and offspring production of the populations was reduced at higher levels of inbreeding (II). This suggests that a slower rate of inbreeding might not protect larger populations against the deleterious consequences of inbreeding and genetic drift, although it has to be noted that the effective population size was rather small also in the largest populations used in the experiments.

The results on the effects of hybridization between isolated populations show that interpopulation hybridization can increase the long-term viability of small populations, but the benefits of hybridization are reduced when the genetic divergence between populations is high (III). Furthermore, at the same levels of inbreeding, smaller populations with faster rate of inbreeding are likely to benefit more from interpopulation hybridization (IV).

Implications for conservation

The existence of natural populations that thrive in spite of severe reductions in population size in their history has been suggested as evidence that small populations can overcome problems caused by inbreeding and genetic drift

through selective elimination of deleterious alleles (Ellegren et al. 1993, Hoelzel et al. 1993, Visscher 2001, Windig et al. 2004, Facon et al. 2011). However, these populations might represent only a small fraction of populations with similar histories, with the majority of them being extinct today. Additionally, the success of these populations may rely heavily on a substantial increase in population size after the temporary reductions (Bryant et al. 1990, Saccheri et al. 1996, Miller & Hedrick 2001, Reed & Bryant 2001, but see Leberg & Firmin 2008). The results from my thesis show that small population size and isolation from other populations of the same species is a threat to population persistence. Although the harmful effects of reduced population size can be to some extent counteracted by natural selection, permanent recovery of fitness is not likely to happen in populations of consistently small size. The results also show that although populations experiencing slow inbreeding are likely to be more fit than populations experiencing fast inbreeding, when compared at the same level of inbreeding, slow inbreeding rate cannot be relied on to protect the populations against the deleterious consequences of inbreeding and genetic drift. Furthermore, although the effects of genetic drift on the current fitness of a population can be milder when the rate of inbreeding is slower, it has to be recognized that the loss of genetic variation through drift may still compromise the ability of the population to adapt to future changes in the environment (Bijlsma & Loeschcke 2005, Willi et al. 2006).

Introduction of genetic material from other populations has been suggested as a strategy to improve the viability of inbred populations (reviewed in Tallmon et al. 2004, Hedrick 2005, Edmands 2007, Frankham et al. 2011). On the other hand, scientists have been inconsistent in their recommendations for using interpopulation hybridization as a management tool. Some recommend a more cautious approach because of the risks of outbreeding depression (Edmands 2007) while others call for a more active approach (Frankham et al. 2011). The results from my thesis indicate that hybridization between isolated populations living in the same environment can yield long-lasting fitness benefits for populations suffering from inbreeding and drift. However, the results also show that the benefits of hybridization may diminish with greater genetic divergence of the populations. Thus, even though the results indicate the benefits of hybridization at low to moderate genetic divergence, they call for caution when populations are more diverged, even with populations living in similar environments. It is possible that hybridization between such populations causes negative epistatic effects that reduce the fitness of the hybrid individuals. On the other hand, if highly inbred small populations have purged recessive detrimental alleles and accumulated only partially recessive mildly detrimental alleles, as the results suggest, the populations may benefit from introduced gene flow only if immigrants are available that do not suffer from accumulation of mildly deleterious alleles, i.e., that come from a large population. If augmentation of gene flow is considered necessary, it is better to act sooner rather than later, especially if the existing populations of the species are small in size.

Captive breeding and conservation programs can benefit further from the knowledge that at the same levels of inbreeding, populations with smaller historical size (faster rate of inbreeding) are likely to gain more from artificially increased gene flow between populations, than are populations with larger historical size (slower rate of inbreeding). To my knowledge, the results from my thesis are among the first to show the effect of inbreeding rate on the magnitude of benefits gained from interpopulation hybridization, while controlling for the level of inbreeding in the populations.

Managers are also faced with a dilemma of how many individuals and how many populations to use in translocation and re-introduction programs. When using too little, there is a risk that the desired benefits are not achieved, whereas using too many might be risky because of the higher potential for outbreeding depression. In my thesis, the number of source populations did not have a significant effect on the viability of the hybrid populations. However, there was a general tendency for higher offspring production in hybrid populations established from two source populations, as compared to hybrid populations established from four source populations. This might imply that using more source populations will increase the risk of negative effects of hybridization. However, since the effect of the number of source populations was not statistically significant, the results must be interpreted cautiously.

When considering the implications of the results from my thesis, it must be taken into account that the populations under study originated from a single source population quite recently, and were reared in the same, benign environment. In the conservation of natural populations and species, it is important to consider the possibility of divergent local adaptations (Templeton 1986), the influence of stressful or fluctuating environments (Swindell & Bouzat 2006, Edmands 2007, Mikkelsen et al. 2010, Kristensen et al. 2011), and the potential of the populations to adapt to future environmental challenges (Bijlsma & Loeschcke 2005, Hedrick 2005, Willi et al. 2006). Furthermore, when considering the potential benefits gained from interpopulation hybridization, if time frames of several tens or hundreds of generations are considered, it is likely that hybridization can benefit a population only if population size is allowed to increase (Liberg et al. 2005, Bijlsma et al. 2010). If the population remains small and without constant gene flow, for example due to restricted availability of suitable habitat, it can be expected that inbreeding and drift will continue to decrease fitness of the population after the hybridization event.

Acknowledgements

First of all, I want to thank my supervisor, Mikael. Our collaboration begun when I did my master's thesis under your supervision. I think it was already then when you said to me, that you like to think of us as colleagues, not as a student and a supervisor. I think that says a lot about you. You have had a great influence on this project, and you have always, always been there for me, giving your advice whenever needed. Besides your intelligence and talent, you are most definitely one of the kindest people I know. I'm not sure if any other supervisor would offer to help in counting *Drosophila* eggs outside the office hours, and cheer up the tired workers with a bag of candy! Thank you Mikael, for being such a great colleague.

Janne "The First", you have also been my supervisor since my master's thesis. I value the way you have so intensively put your mind into the discussions we've had, no matter how many threads you have been holding in your hands. As so many other people, I admire your great energy and enthusiasm, as well as your ability to believe that anything is possible. I want to thank you for your guidance and for your belief in me during these years. I appreciate that a lot.

Emily, although you weren't involved in the project from its beginning, your contribution has been prominent. You have given your time and effort to make the DNA analyses happen, but you have also helped me in many other ways, with the thesis and outside it. Thank you for your perceptive comments, and for correcting my English. I especially want to thank you for being the person you are. Your warmth and kindness, and your positive way of encountering people (including me) is special.

I also want to thank Anneli Hoikkala, for building up the evolutionary genetics studies in the University of Jyväskylä. Without the new major, this thesis would probably not have happened (I almost became a molecular biologist instead!). Moreover, Anneli, I want to thank you for all the support you have given me throughout these years.

Kari, thank you for your priceless (and amazingly patient!) contribution to the statistical analyses of the data. Jari, thank you for the editorial work, and for the supportive comments you never spare. Atte, thanks for being the "Finnish language -police"! Anna-Liisa Laine and Jacob Höglund, many thanks for your work as the pre-examiners of the thesis, and for the suggestions to improve the work.

Sari Mikkelsson, your work in the maintenance of the flies and in acquiring the data has been indispensable. You have been the person I have been able to count on, to always do the job with precision and thought. Thank you for the years you spent with the flies. I hope you don't miss them too much!

Eliisa and Laura, thank you for all your help in the maintenance of the flies. Your work is essential; the animal center would not run without you! Elina and Sari, thank you for the advice and assistance in the DNA-lab. Juha, Tarmo, and Ahti, warm thanks for the help you have provided in various

practical matters. Warm thanks also to the master's students, Marja, Lily, and Terhi, who have been working with the flies on diverse questions, and also helped in acquiring data for my thesis. Jouni, thank you for teaching me how to fix the wings and legs of the flies, and Lauri, thank you for your help in editing the pictures. Many thanks also to Anu, Ilari, Susan, Antti, Tony, and Hannah; your involvement in the project has been important and much appreciated.

To all the people in the MCC, I truly appreciate the time we have spent together, and the lively conversations we've had; thank you all for that. I think you are all amazing personalities, and I will always remember you with warmth. I especially want to thank Anne, for your friendship and for the APO-year we sheared. Warm thanks also to Satu, Katja, Elisa, and Merja for taking part in my APO-project. Carita, it was really nice to work with you on the ecological genetics workshop! All Sauna & Support actives, especially Jonna, Aapo, and Mika, thank you for the invaluable support and for your friendship during these years. Saija and Rocío, and all the other people I have shared the office with during these years, thank you for the nice company.

My dear "fly girls": Hannele, Laura, Jenni, Tiina, and Venera, thank you for all the funny moments we have shared. I will always remember our chats during the coffee breaks, the (sometimes too) warm get-togethers in palju (thank you Hannele for being such a great host!), and the delicious chocolate fondue (well, especially the not-so-successful cheese fondue trial; thank you Venera!). I especially want to thank Hannele, for the time we spent in the same office, and for our shared journey to the life as a lecturer.

Many more people have affected my work in one way or another. I want to thank all the people in the department, especially in the Ecology and Evolutionary Biology division, for creating such a great atmosphere. It has been a pleasure to work with you all. For funding the project and for the great opportunities to meet students and scientists outside Jyväskylä, I want to thank the BIOINT graduate school and all the people involved. The project was funded also by the Academy of Finland, the CoE in Evolutionary Research, the Emil Aaltonen Foundation, and the CoE in Biological Interactions.

I have been lucky to have many great friends also outside work. I'm not going to name you, because I'm afraid I would forget somebody, but I'm sure you know who you are. You are the reason I have had life also outside the office. Your friendship means the world to me. I love you all.

I also want to thank my family: Sirpa, Kari, Reino and Sirpa, Seija and Risto, as well as all my cousins. Ritva, Mira, Sami, and Janna, you have been my family in Jyväskylä. You have been an important part of my life, a safety-net I could always count on. Thank you for all the nice moments we have had.

Arto, we have shared the last seven years of our lives. You have been there for me when I have felt down. With you, I also have shared some of the happiest moments of my life. Thank you for your presence, and for your love.

Finally, and most of all, I want to thank my mom, Merja. Without your love and support, from the very beginning of my life until this day, I would not be where I am today. Kiitos, äiti.

YHTEENVETO (RÉSUMÉ IN FINNISH)

Geneettisen satunnaisajautumisen, sisäsiitoksen ja populaatioiden välisen risteytymisen vaikutukset pienten populaatioiden kelpoisuuteen ja elinkykyyn

Yhä useammat eläin- ja kasvipopulaatiot ovat pienikokoisia ja eristyneitä toisista saman lajin populaatioista, erityisesti ihmisen toiminnasta johtuvan elinympäristöjen tuhoutumisen ja pirstoutumisen seurauksena. Tämä on ongelma populaatioiden ja lajien suojelulle, koska pienet populaatiot ovat vaarassa hävitä sekä demografisista että geneettisistä syistä. Pienten populaatioiden selviytymistä uhkaavat geneettiset tekijät aiheutuvat lisääntyneestä sisäsiitoksesta ja geneettisestä satunnaisajautumisesta, jotka johtavat kelpoisuutta heikentävän homotsygotian lisääntymiseen sekä haitallisten alleelien yleistymiseen ja geneettisen muuntelun häviämiseen populaatiosta. Vaikka voimakkaasta sisäsiitoksesta aiheutuvat välittömät haitat yksilöiden ja populaatioiden kelpoisuudelle ovat hyvin tunnettuja, kelpoisuuden laskun ennustaminen pitkälle tulevaisuuteen sisäsiitosasteen vähittäisen kasvun myötä ei ole yksinkertaista. Homotsygotian lisääntyessä on mahdollista, että luonnonvalinta poistaa haitallisia resessiivisiä alleeleja populaatiosta. Valinnan tehokkuus voi kuitenkin riippua monista eri tekijöistä, kuten haitallisten mutaatioiden luonteesta ja sisäsiitoksen nopeudesta. On ennustettu, että valinta toimii tehokkaammin suuremmissa populaatioissa, joissa sisäsiitosnopeus on pienempi. Kokeelliset tulokset valinnan tehokkuudesta sisäsiitoksen ja geneettisen satunnaisajautumisen haitallisia vaikutuksia vastaan ovat kuitenkin ristiriitaisia.

Sisäsiitoksesta ja geneettisestä satunnaisajautumisesta kärsivien populaatioiden suojelemiseksi on esitetty, että geenivirtaa eristyneiden populaatioiden välillä lisättäisiin keinotekoisesti, esimerkiksi siirtämällä yksilöitä populaatiosta toiseen. Eri populaatioista tulevien yksilöiden risteytyminen johtaa usein jälkeläisten kelpoisuuden kasvuun verrattuna populaatioiden sisällä tapahtuvaan pariutumiseen, pääasiassa jälkeläisten lisääntyneen heterotsygotian takia. Populaatioiden välinen risteytyminen voi kuitenkin olla myös haitallista. Risteytymisen hyödyllisyys tai haitallisuus voi riippua monista eri tekijöistä, kuten geneettisen erilaistumisen tasosta ja sisäsiitosnopeudesta eristyneissä populaatioissa. Erityisesti pitkäaikaisten vaikutusten arviointi on vaikeaa.

Väitöskirjatyössäni tutkin pienen populaatiokoon ja eristyneiden populaatioiden välisen risteytymisen vaikutuksia yksilöiden kelpoisuuteen ja populaation elinkykyyn. Tavoitteeni oli selvittää, miten sisäsiitosnopeus (populaation efektiivinen koko) vaikuttaa geneettisen satunnaisajautumisen ja sisäsiitoksen seurauksiin eri sisäsiitosasteissa. Lisäksi tutkin, miten geneettisen erilaistumisen taso, sisäsiitosnopeus eristyneissä populaatioissa ja risteytettävien populaatioiden määrä vaikuttavat populaatioiden välisen risteytymisen seurauksiin. Käytin työssäni kokeellista lähestymistapaa mallilajina mahlakärpänen *Drosophila littoralis*. Kokeellinen lähestymistapa mahdollistaa erilaisten vaikuttavien tekijöiden kontrolloinnin sekä riittävän toistojen määrän, mikä on usein mahdo-

tonta uhanalaisilla lajeilla luonnossa tehtävissä tutkimuksissa. Mallilajin avulla olen toteuttanut laajamittaisia ja kontrolloituja kokeita, joiden avulla olen voinut selvittää tiettyjen yksittäisten tekijöiden vaikutuksia yksilöiden ja populaatioiden kelpoisuuteen. Tutkimuspopulaatioiden sisäsiitosnopeuden arvioin tutkimuslajille soveltuvien merkkigeenien avulla.

Väitöskirjatyöni tulokset osoittivat, että luonnonvalinta voi jossain määrin vähentää geneettisistä tekijöistä aiheutuvia haittoja pienissä populaatioissa, jopa silloin kun populaation efektiivinen koko on vain noin 8 yksilöä. Luonnonvalinta ei kuitenkaan ole riittävän tehokasta estämään vähemmän haitallisten alleelien yleistymistä sisäsiitosasteen kasvaessa, jos populaatiokoko säilyy pienenä. Vaikka populaatiot kykenisivät selviämään pienentyneen populaatiokoon aiheuttamasta välittömästä kelpoisuuden laskusta, on todennäköistä, että ne tulevat kärsimään vähentyneestä jälkeläistuotosta ja lisääntyneestä sukupuuttoriskistä myöhemmissä sukupolvissa.

Tutkimuksissani havaitsin myös, että sisäsiitoksen ja geneettisen satunnaisajautumisen vaikutukset ovat vähemmän haitallisia jos sisäsiitosnopeus on pienempi: kun erikokoisia populaatioita (N_e = 1.9, N_e = 8.1 ja N_e = 23.2) verrattiin samoissa sisäsiitosasteissa, kelpoisuus oli parempi suuremmissa kuin pienemmissä populaatioissa. Tulos johtuu todennäköisesti eroista luonnonvalinnan tehokkuudessa: suuremmissa populaatioissa valinta toimii tehokkaammin haitallisten alleelien yleistymistä vastaan. Lisääntynyt sisäsiittoisuus ja geneettinen satunnaisajautuminen johtivat kuitenkin kelpoisuuden ja jälkeläistuoton laskuun myös suuremmissa populaatioissa. Näyttäisikin siltä, että pienempi sisäsiitosnopeus ei suojaa populaatioita sisäsiitoksen ja geneettisen satunnaisajautumisen haitallisilta vaikutuksilta. On kuitenkin otettava huomioon, että suurimmatkin tutkimuksissani käyttämät populaatiot olivat efektiiviseltä kooltaan hyvin pieniä, vain noin 23 yksilöä.

Tulokset eristyneiden populaatioiden välisen risteytymisen seurauksista osoittivat, että populaatioiden välinen risteytyminen voi parantaa pienten populaatioiden pitkäaikaista kelpoisuutta ja elinkykyä. Risteytymisen hyödyt kuitenkin vähenivät huomattavasti, kun populaatioiden geneettinen erilaistuminen lisääntyi. Tämä johtui todennäköisesti siitä, että kun sisäsiitosaste eristyneissä populaatioissa kasvoi, erittäin haitallisten resessiivisten alleelien määrä väheni luonnonvalinnan seurauksena. Toisaalta vähemmän haitallisten alleelien määrä populaatioissa todennäköisesti lisääntyi, koska valinta ei pienissä populaatioissa toimi tehokkaasti niitä vastaan. Vähemmän haitalliset alleelit ovat vaikutuksiltaan vain osittain resessiivisiä, ja siksi risteytymisen seurauksena lisääntynyt heterotsygotia ei peitä niiden haitallisia vaikutuksia. Onkin mahdollista, että erittäin sisäsiittoisille populaatioille lisääntyneestä geenivirrasta on merkittävää hyötyä vain, jos geenivirta on suuresta populaatiosta, joka ei kärsi geneettisen satunnaisajautumisen seurauksista. Lisäksi havaitsin, että verrattaessa risteytymisen hyötyjä erikokoisten populaatioiden välillä samoissa sisäsiitosasteissa, pienemmät populaatiot hyötyivät risteytymisestä enemmän kuin suuremmat populaatiot. Pienempi sisäsiitosnopeus vähensi risteytymisen hyötyjä todennäköisesti siksi, että tehokkaampi valinta vähensi haitallisten alleelien

fiksoitumista populaatioissa. Populaatioiden määrällä ei ollut merkitsevää vaikutusta risteytymisen seurauksiin.

Väitöskirjani tulokset voivat vaikuttaa käytännön luonnonsuojelutyöhön monella tavalla. Arvioitaessa pienen populaatiokoon ja populaatioiden välisen risteytymisen vaikutuksia luonnonpopulaatioissa on kuitenkin otettava huomioon myös mahdolliset ympäristötekijät, joiden vaikutuksia en ole väitöskirjatyössäni käsitellyt. Vaativat tai vaihtelevat ympäristöolot sekä populaatioiden sopeutuminen erilaisiin ympäristöihin voivat vaikuttaa niin sisäsiitoksen ja geneettisen satunnaisajautumisen kuin populaatioiden välisen risteytymisen seurauksiin. On myös pyrittävä mahdollistamaan populaatioiden sopeutuminen tuleviin ympäristön muutoksiin. Lisäksi on huomioitava, että populaatioiden välinen risteytyminen voi todella hyödyttää populaatioita vain, jos populaatiokoon kasvu turvataan. Jos populaatiokoko säilyy pienenä, esimerkiksi sopivan elinympäristön vähäisyyden vuoksi, on todennäköistä, että ilman jatkuvaa geenivirtaa populaatio kärsii sisäsiitoksen ja geneettisen satunnaisajautumisen aiheuttamista ongelmista tulevissa sukupolvissa.

REFERENCES

Allendorf F.W. & Luikart G. 2007. *Conservation and the genetics of populations*. Blackwell Publishing, Malden.

Amos W. & Balmford A. 2001. When does conservation genetics matter? *Heredity* 87: 257-265.

Aspi J., Lumme J., Hoikkala A. & Heikkinen E. 1993. Reproductive ecology of the boreal riparian guild of *Drosophila*. *Ecography* 16: 65-72.

Avise J.C., Hubbell S.P. & Ayala F.J. 2008. In the light of evolution II: Biodiversity and extinction. *PNAS* 105: 11453-11457.

Ballou J.D. 1997. Ancestral inbreeding only minimally affects inbreeding depression in mammalian populations. *J. Hered.* 88: 169-178.

Bataillon T. & Kirkpatrick M. 2000. Inbreeding depression due to mildly deleterious mutations in finite populations: size does matter. *Genet. Res.* 75: 75-81.

Bercovitch F.B. & Berard J.D. 1993. Life history costs and consequences of rapid reproductive maturation in female rhesus macaques. *Behav. Ecol. Sociobiol.* 32: 103-109.

Bijlsma R. & Loeschcke V. 2005. Environmental stress, adaptation and evolution: an overview. *J. Evol. Biol.* 18: 744-749.

Bijlsma R., Bundgaard J. & Boerema A.C. 2000. Does inbreeding affect the extinction risk of small populations?: predictions from *Drosophila*. *J. Evol. Biol.* 13: 502-514.

Bijlsma R., Westerhof M.D.D., Roekx L.P. & Pen I. 2010. Dynamics of genetic rescue in inbred *Drosophila melanogaster* populations. *Conserv. Genet.* 11: 449-462.

Bryant D.M. 1988. Lifetime reproductive success of house martins. In: Clutton-Brock T.H. (ed.), *Reproductive success - studies of individual variation in contrasting breeding systems,* The University of Chicago Press, Chicago, pp. 173-188.

Bryant E.H., Meffert L.M. & McCommas S.A. 1990. Fitness rebound in serially bottlenecked populations of the house fly. *Am. Nat.* 136: 542-549.

Busch J.W. 2006. Heterosis in an isolated, effectively small, and self-fertilizing population of the flowering plant *Leavenworthia alabamica*. *Evolution* 60: 184-191.

Byers D.L. & Waller D.M. 1999. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annu. Rev. Ecol. Syst.* 30: 479-513.

Charlesworth D. & Charlesworth B. 1987. Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.* 18: 237-268.

Charlesworth D. & Willis J.H. 2009. The genetics of inbreeding depression. *Nat. Rev. Genet.* 10: 783-796.

Coutellec M.-A. & Caquet T. 2011. Heterosis and inbreeding depression in bottlenecked populations: a test in the hermaphroditic freshwater snail *Lymnaea stagnalis*. *J. Evol. Biol.* 24: 2248-2257.

Crnokrak P. & Roff D.A. 1999. Inbreeding depression in the wild. *Heredity* 83: 260-270.

Crnokrak P. & Barrett S.C.H. 2002. Perspective: Purging the genetic load: A review of the experimental evidence. *Evolution* 56: 2347-2358.

Crow J. & Kimura M. 1970. *An introduction to population genetics theory*. Harper & Row, New York.

Day S.B., Bryant E.H. & Meffert L.M. 2003. The influence of variable rates of inbreeding on fitness, environmental responsiveness, and evolutionary potential. *Evolution* 57: 1314-1324.

Demontis D., Pertoldi C., Loeschcke V., Mikkelsen K., Axelsson T. & Kristensen T.N. 2009. Efficiency of selection, as measured by single nucleotide polymorphism variation, is dependent on inbreeding rate in *Drosophila melanogaster*. *Mol. Ecol.* 18: 4551-4563.

Demuth J.P. & Wade M.J. 2007. Population differentiation in the beetle *Tribolium castaneum*. I. Genetic architecture. *Evolution* 61: 494-509.

Edmands S. 1999. Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. *Evolution* 53: 1757-1768.

Edmands S. 2002. Does parental divergence predict reproductive compatibility? *TREE* 17: 520-527.

Edmands S. 2007. Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Mol. Ecol.* 16: 463-475.

Edmands S. & Timmerman C.C. 2003. Modeling factors affecting the severity of outbreeding depression. *Conserv. Biol.* 17: 883-892.

Edmands S., Northrup S.L. & Hwang A.S. 2009. Maladapted gene complexes within populations of the intertidal copepod *Tigriopus californicus*? *Evolution* 63: 2184-2192.

Edmands S., Feaman H.V., Harrison J.S. & Timmerman C.C. 2005. Genetic consequences of many generations of hybridization between divergent copepod populations. *J. Hered.* 96: 114-123.

Ehiobu N.G., Goddard M.E. & Taylor J.F. 1989. Effect of rate of inbreeding on inbreeding depression in *Drosophila melanogaster*. *Theor. Appl. Genet.* 77: 123-127.

Ellegren H., Hartman G., Johansson M. & Andersson L. 1993. Major histocompatibility complex monomorphism and low levels of DNA fingerprinting variability in a reintroduced and rapidly expanding population of beavers. *PNAS* 90: 8150-8153.

Erickson D.L. & Fenster C.B. 2006. Intraspecific hybridization and the recovery of fitness in the native legume *Chamaecrista fasciculata*. *Evolution* 60: 225-233.

Escobar J.S., Nicot A. & David P. 2008. The different sources of variation in inbreeding depression, heterosis and outbreeding depression in a metapopulation of *Physa acuta*. *Genetics* 180: 1593-1608.

Ewers R.M. & Didham R.K. 2006. Confounding factors in the detection of species responses to habitat fragmentation. *Biol. Rev.* 81: 117-142.

Eyre-Walker A. & Keightley P.D. 2007. The distribution of fitness effects of new mutations. *Nat. Rev. Genet.* 8: 610-618.

Facon B., Hufbauer R.A., Tayeh A., Loiseau A., Lombaert E., Vitalis R., Guillemaud T., Lundgren J.G. & Estoup A. 2011. Inbreeding depression is purged in the invasive insect *Harmonia axyridis*. *Curr. Biol.* 21: 424-427.

Falconer D.S. & Mackay T.F.C. 1996. *Introduction to quantitative genetics*. Prentice Hall, Harlow.

Fenster C.B. & Galloway L.F. 2000. Population differentiation in an annual legume: Genetic architecture. *Evolution* 54: 1157-1172.

Fenster C.B., Galloway L.F. & Chao L. 1997. Epistasis and its consequences for the evolution of natural populations. *TREE* 12: 282-286.

Frankham R. 1995. Inbreeding and extinction - A threshold effect. *Conserv. Biol.* 9: 792-799.

Frankham R. 2005. Genetics and extinction. *Biol. Conserv.* 126: 131-140.

Frankham R. 2010. Challenges and opportunities of genetic approaches to biological conservation. *Biol. Conserv.* 143: 1919-1927.

Frankham R., Ballou J.D., Eldridge M.D.B., Lacy R.C., Ralls K., Dudash M.R. & Fenster C.B. 2011. Predicting the Probability of Outbreeding Depression. *Conserv. Biol.* 25: 465-475.

Galloway L.F. & Etterson J.R. 2005. Population differentiation and hybrid success in *Campanula americana*: geography and genome size. *J. Evol. Biol.* 18: 81-89.

Glemin S. 2003. How are deleterious mutations purged? Drift versus nonrandom mating. *Evolution* 57: 2678-2687.

Hedrick P.W. 1994. Purging inbreeding depression and the probability of extinction: full-sib mating. *Heredity* 73: 363-372.

Hedrick P.W. 2002. Lethals in finite populations. *Evolution* 56: 654-657.

Hedrick P. 2005. 'Genetic restoration': a more comprehensive perspective than 'genetic rescue'. *TREE* 20: 109-109.

Hedrick P.W., Adams J.R. & Vucetich J.A. 2011. Reevaluating and Broadening the Definition of Genetic Rescue. *Conserv. Biol.* 25: 1069-1070.

Hoelzel A.R., Halley J., Obrien S.J., Campagna C., Arnbom T., Leboeuf B., Ralls K. & Dover G.A. 1993. Elephant seal genetic variation and the use of simulation models to investigate historical population bottlenecks. *J. Hered.* 84: 443-449.

Hogg J.T., Forbes S.H., Steele B.M. & Luikart G. 2006. Genetic rescue of an insular population of large mammals. *Proc. R. Soc. B-Biol. Sci.* 273: 1491-1499.

Hunt J. & Hodgson D. 2010. What is fitness, and how do we measure it? In: Westneat D.F. & Fox C.W. (eds.), *Evolutionary behavioral ecology,* Oxford University Press, New York.

Hwang A.S., Northrup S.L., Alexander J.K., Vo K.T. & Edmands S. 2011. Long-term experimental hybrid swarms between moderately incompatible *Tigriopus californicus* populations: hybrid inferiority in early generations

yields to hybrid superiority in later generations. *Conserv. Genet.* 12: 895-909.

Keller L.F. & Waller D.M. 2002. Inbreeding effects in wild populations. *TREE* 17: 230-241.

Kimura M., Maruyama T. & Crow J.F. 1963. The mutation load in small populations. *Genetics* 48: 1303-1312.

Kirkpatrick M. & Jarne P. 2000. The effects of a bottleneck on inbreeding depression and the genetic load. *Am. Nat.* 155: 154-167.

Kristensen T.N., Knudsen M.R. & Loeschcke V. 2011. Slow inbred lines of *Drosophila melanogaster* express as much inbreeding depression as fast inbred lines under semi-natural conditions. *Genetica* 139: 441-451.

Kristensen T.N., Sørensen A.C., Sorensen D., Pedersen K.S., Sørensen J.G. & Loeschcke V. 2005. A test of quantitative genetic theory using *Drosophila* – effects of inbreeding and rate of inbreeding on heritabilities and variance components. *J. Evol. Biol.* 18: 763-770.

Kruuk L.E.B., Clutton-Brock T.H., Rose K.E. & Guinness F.E. 1999. Early determinants of lifetime reproductive success differ between the sexes in red deer. *Proc. R. Soc. Lond. Ser. B-Biol. Sci.* 266: 1655-1661.

Kärkkäinen K., Kuittinen H., van Treuren R., Vogl C., Oikarinen S. & Savolainen O. 1999. Genetic basis of inbreeding depression in *Arabis petraea*. *Evolution* 53: 1354-1365.

Lakovaara S. 1969. Malt as a culture medium for *Drosophila* species. *Drosophila Inf. Serv.* 44: 128.

Lande R. 1988. Genetics and demography in biological conservation. *Science* 241: 1455-1460.

Lande R. 1994. Risk of population extinction from fixation of new deleterious mutations. *Evolution* 48: 1460-1469.

Lande R. 1998. Risk of population extinction from fixation of deleterious and reverse mutations. *Genetica* 102-3: 21-27.

Larsen L.K., Pelabon C., Bolstad G.H., Viken A., Fleming I.A. & Rosenqvist G. 2011. Temporal change in inbreeding depression in life-history traits in captive populations of guppy (*Poecilia reticulata*): evidence for purging? *J. Evol. Biol.* 24: 823-834.

Leberg P.L. & Firmin B.D. 2008. Role of inbreeding depression and purging in captive breeding and restoration programmes. *Mol. Ecol.* 17: 334-343.

Liberg O., Andren H., Pedersen H.C., Sand H., Sejberg D., Wabakken P., Akesson M. & Bensch S. 2005. Severe inbreeding depression in a wild wolf (*Canis lupus*) population. *Biol. Lett.* 1: 17-20.

Lynch M. 1991. The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution* 45: 622-629.

Lynch M. & Walsh B. 1998. *Genetics and analysis of quantitative traits*. Sinauer Associates Sunderland, Massachusetts.

Lynch M., Conery J. & Burger R. 1995a. Mutational meltdowns in sexual populations. *Evolution* 49: 1067-1080.

Lynch M., Conery J. & Burger R. 1995b. Mutation accumulation and the extinction of small population. *Am. Nat.* 146: 489-518.

Marr A.B., Keller L.F. & Arcese P. 2002. Heterosis and outbreeding depression in descendants of natural immigrants to an inbred population of song sparrows (*Melospiza melodia*). *Evolution* 56: 131-142.

Mikkelsen K., Loeschcke V. & Kristensen T.N. 2010. Trait specific consequences of fast and slow inbreeding: lessons from captive populations of *Drosophila melanogaster*. *Conserv. Genet.* 11: 479-488.

Miller P.S. & Hedrick P.W. 2001. Purging of inbreeding depression and fitness decline in bottlenecked populations of *Drosophila melanogaster*. *J. Evol. Biol.* 14: 595-601.

Mills L.S. & Allendorf F.W. 1996. The one-migrant-per-generation rule in conservation and management. *Conserv. Biol.* 10: 1509-1518.

Moll R.H., Lonnquist J.H., Fortuno J.V. & Johnson E.C. 1965. Relationship of heterosis and genetic divergence in maize. *Genetics* 52: 139-144.

Morales-Hojas R., Reis M., Vieira C.P. & Vieira J. 2011. Resolving the phylogenetic relationships and evolutionary history of the *Drosophila virilis* group using multilocus data. *Mol. Phylog. Evol.* 60: 249-258.

Newton I. 1988. Age and reproduction in the sparrowhawk. In: Clutton-Brock T.H. (ed.), *Reproductive success - studies of individual variation in contrasting breeding systems,* The University of Chicago Press, Chicago, pp. 201-219.

O'Grady J.J., Brook B.W., Reed D.H., Ballou J.D., Tonkyn D.W. & Frankham R. 2006. Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. *Biol. Conserv.* 133: 42-51.

Orr H.A. 1995. The population genetics of speciation: the evolution of hybrid incompatibilities. *Genetics* 139: 1805-1813.

Orr H.A. & Turelli M. 2001. The evolution of postzygotic isolation: Accumulating Dobzhansky-Muller incompatibilities. *Evolution* 55: 1085-1094.

Ouborg N.J. 2010. Integrating population genetics and conservation biology in the era of genomics. *Biol. Lett.* 6: 3-6.

Paland S. & Schmid B. 2003. Population size and the nature of genetic load in *Gentianella germanica*. *Evolution* 57: 2242-2251.

Partridge L. 1988. Lifetime reproductive success in *Drosophila*. In: Clutton-Brock T.H. (ed.), *Reproductive success - studies of individual variation in contrasting breeding systems,* The University of Chicago Press, Chicago, pp. 11-23.

Partridge L., Fowler K., Trevitt S. & Sharp W. 1986. An examination of the effects of males on the survival and egg-production rates of female *Drosophila melanogaster J. Insect Physiol.* 32: 925-929.

Pedersen K.S., Kristensen T.N. & Loeschcke V. 2005. Effects of inbreeding and rate of inbreeding in *Drosophila melanogaster* - Hsp70 expression and fitness. *J. Evol. Biol.* 18: 756-762.

Pertoldi C., Bijlsma R. & Loeschcke V. 2007. Conservation genetics in a globally changing environment: present problems, paradoxes and future challenges. *Biodiversity Conserv.* 16: 4147-4163.

Phillips P.C. & Johnson N.A. 1998. The population genetics of synthetic lethals. *Genetics* 150: 449-458.

Puurtinen M., Knott K.E., Suonpaa S., van Ooik T. & Kaitala V. 2004. Genetic variability and drift load in populations of an aquatic snail. *Evolution* 58: 749-756.

Reed D. & Bryant E. 2001. Fitness, genetic load and purging in experimental populations of the housefly. *Conserv. Genet.* 2: 57-61.

Reed D.H. & Frankham R. 2003. Correlation between fitness and genetic diversity. *Conserv. Biol.* 17: 230-237.

Reed D.H. & Bryant E.H. 2004. Phenotypic correlations among fitness and its components in a population of the housefly. *J. Evol. Biol.* 17: 919-923.

Reed D.H., Lowe E.H., Briscoe D.A. & Frankham R. 2003. Inbreeding and extinction: Effects of rate of inbreeding. *Conserv. Genet.* 4: 405-410.

Rhode J.M. & Cruzan M.B. 2005. Contributions of heterosis and epistasis to hybrid fitness. *Am. Nat.* 166: 124-139.

Ribble D.O. 1992. Lifetime reproductive success and it's correlates in the monogamous rodent, *Peromyscus californicus*. *J. Anim. Ecol.* 61: 457-468.

Richards C.M. 2000. Inbreeding depression and genetic rescue in a plant metapopulation. *Am. Nat.* 155: 383-394.

Rosewell J. & Shorrocks B. 1987. The implication of survival rates in natural populations of *Drosophila*: capture-recapture experiments on domestic species. *Biol. J. Linn. Soc.* 32: 373-384.

Routtu J., Hoikkala A. & Kankare M. 2007. Microsatellite-based species identification method for *Drosophila virilis* group species. *Hereditas* 144: 213-221.

Rowe G., Beebee T.J.C. & Burke T. 1999. Microsatellite heterozygosity, fitness and demography in natterjack toads *Bufo calamita*. *Anim. Conserv.* 2: 85-92.

Saccheri I.J., Brakefield P.M. & Nichols R.A. 1996. Severe inbreeding depression and rapid fitness rebound in the butterfly *Bicyclus anynana* (Satyridae). *Evolution* 50: 2000-2013.

Saccheri I., Kuussaari M., Kankare M., Vikman P., Fortelius W. & Hanski I. 1998. Inbreeding and extinction in a butterfly metapopulation. *Nature* 392: 491-494.

Schiffer M., Gilchrist A.S. & Hoffmann A.A. 2006. The contrasting genetic architecture of wing size, viability, and development time in a rainforest species and its more widely distributed relative. *Evolution* 60: 106-114.

Scott D.K. 1988. Breeding success in Bewick's swans. In: Clutton-Brock T.H. (ed.), *Reproductive success - studies of individual variation in contrasting breeding systems,* The University of Chicago Press, Chicago, pp. 221-236.

Smith J.N.M. 1988. Determinants of lifetime reproductive success in the song sparrow. In: Clutton-Brock T.H. (ed.), *Reproductive success - studies of individual variation in contrasting breeding systems,* The University of Chicago Press, Chicago, pp. 154-172.

Spielman D. & Frankham R. 1992. Modeling problems in conservation genetics using captive *Drosophila* populations - improvement of reproductive fitness due to immigration of one individual into small partially inbred populations. *Zoo Biol.* 11: 343-351.

Spielman D., Brook B.W. & Frankham R. 2004. Most species are not driven to extinction before genetic factors impact them. *PNAS* 101: 15261-15264.

Stearns S.C. 1992. *The evolution of life histories*. Oxford University Press, New York.

Swindell W.R. & Bouzat J.L. 2006. Selection and inbreeding depression: Effects of inbreeding rate and inbreeding environment. *Evolution* 60: 1014-1022.

Tallmon D.A., Luikart G. & Waples R.S. 2004. The alluring simplicity and complex reality of genetic rescue. *TREE* 19: 489-496.

Templeton A.R. 1986. Coadaptation and outbreeding depression. In: Soulé M.E. (ed.), *Conservation biology: the science of scarcity and diversity,* Sinauer Assoc., Sunderland, MA, pp. 105-116.

Templeton A.R. & Read B. 1994. Inbreeding: one word, several meanings, much confusion. In: Loeschcke V., Tomiuk J. & Jain S.K. (eds.), *Conservation Genetics,* Birkhäuser, Verlag, Basel, pp. 91-105.

Theodorou K. & Couvet D. 2006. On the expected relationship between inbreeding, fitness, and extinction. *Genet. Sel. Evol.* 38: 371-387.

Wang J.L., Hill W.G., Charlesworth D. & Charlesworth B. 1999. Dynamics of inbreeding depression due to deleterious mutations in small populations: mutation parameters and inbreeding rate. *Genet. Res.* 74: 165-178.

Wauters L. & Dhondt A.A. 1989. Body weight, longevity and reproductive success in red squirrels (*Sciurus vulgaris*). *J. Anim. Ecol.* 58: 637-651.

Whitlock M.C. 2000. Fixation of new alleles and the extinction of small populations: Drift load, beneficial alleles, and sexual selection. *Evolution* 54: 1855-1861.

Whitlock M.C., Ingvarsson P.K. & Hatfield T. 2000. Local drift load and the heterosis of interconnected populations. *Heredity* 84: 452-457.

Willi Y., Van Buskirk J. & Hoffmann A.A. 2006. Limits to the adaptive potential of small populations. *Annu. Rev. Ecol. Evol. Syst.* 37: 433-458.

Willi Y., Van Kleunen M., Dietrich S. & Fischer M. 2007. Genetic rescue persists beyond first-generation outbreeding in small populations of a rare plant. *Proc. R. Soc. B-Biol. Sci.* 274: 2357-2364.

Windig J.J., Veerkamp R.F. & Nylin S. 2004. Quantitative genetic variation in an island population of the speckled wood butterfly (*Pararge aegeria*). *Heredity* 93: 450-454.

Visscher P.M. 2001. A viable herd of genetically uniform cattle. *Nature* 409: 303.

ORIGINAL PAPERS

I

LABORATORY RELATIONSHIPS BETWEEN ADULT LIFETIME REPRODUCTIVE SUCCESS AND FITNESS SURROGATES IN A *DROSOPHILA LITTORALIS* POPULATION

by

Nina Pekkala, Janne S. Kotiaho & Mikael Puurtinen 2011

PLoS ONE 6(9): e24560.

OPEN ACCESS Freely available online

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Laboratory Relationships between Adult Lifetime Reproductive Success and Fitness Surrogates in a *Drosophila littoralis* Population

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Abstract

The difficulties in measuring total fitness of individuals necessitate the use of fitness surrogates in ecological and evolutionary studies. These surrogates can be different components of fitness (e.g. survival or fecundity), or proxies more uncertainly related to fitness (e.g. body size or growth rate). Ideally, fitness would be measured over the lifetime of individuals; however, more convenient short-time measures are often used. Adult lifetime reproductive success (adult LRS) is closely related to the total fitness of individuals, but it is difficult to measure and rarely included in fitness estimation in experimental studies. We explored phenotypic correlations between female adult LRS and various commonly used fitness components and proxies in a recently founded laboratory population of *Drosophila littoralis*. Noting that survival is usually higher in laboratory conditions than in nature, we also calculated adjusted adult LRS measures that give more weight to early reproduction. The lifetime measures of fecundity, longevity, and offspring viability were all relatively highly correlated with adult LRS. However, correlations with short-time measures of fecundity and offspring production varied greatly depending on the time of measurement, and the optimal time for measurement was different for unadjusted compared to adjusted adult LRS measures. Correlations between size measures and adult LRS varied from weak to modest, leg size and female weight having the highest correlations. Our results stress the importance of well-founded choice of fitness surrogates in empirical research.

Citation: Pekkala N, Kotiaho JS, Puurtinen M (2011) Laboratory Relationships between Adult Lifetime Reproductive Success and Fitness Surrogates in a *Drosophila littoralis* Population. PLoS ONE 6(9): e24560. doi:10.1371/journal.pone.0024560

Editor: Daniel Ortiz-Barrientos, The University of Queensland, Australia

Received December 17, 2010; **Accepted** August 14, 2011; **Published** September 9, 2011

Funding: The study was financed by Academy of Finland's Centre of Excellence in Evolutionary Research, and Academy of Finland's grant no. 7121616 to MP. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Fitness can be defined as a property of a phenotype (or genotype) that predicts its representation in future generations [1–6]. Evolutionary biologists often seek to measure the fitness of particular phenotypes (or genotypes) in order to understand and predict changes in the constitution of populations. Measuring fitness is not a simple task, and the best measure of fitness can differ depending on the biology of the study system. Particularly, the strength of genotype-by-environment interactions on fitness [3,4] and, in species with overlapping generations, the rate of reproduction [3,7–9], need to be considered when measuring fitness. For species with non-overlapping generations, and for populations at constant population size, the best measure of individual fitness is the lifetime reproductive success, i.e. the number of viable zygotes produced over the whole life-cycle of the individual [3,6].

Measuring the total fitness of individuals is often unfeasibly demanding. Instead, researchers use various fitness surrogates, traits that are thought to reflect fitness and are relatively easy to measure. Fitness components, such as fecundity and survival, are by necessity related to fitness [6], and are thus often preferred as fitness surrogates in empirical studies [10–13]. However, these traits are seldom measured over the whole lifetime of individuals, but only over a restricted time frame that is most feasible for the study system. Besides different components of fitness, morphological and behavioral traits such as body size, growth rate, dominance, and mating success, are often used as surrogates of fitness [12,14,15]. The association between these so called fitness proxies and total fitness of individuals is more uncertain than that between fitness components and total fitness, but they are often measured due to their convenience [6]. Using any fitness surrogate without empirical knowledge about the true relationship of the surrogate and total fitness may lead to erroneous conclusions.

Adult lifetime reproductive success (adult LRS) is likely to be closely related to total fitness of individuals, as it combines several components and proxies of fitness (longevity, fecundity, offspring viability, mating success, etc.). Brommer et al. [16] have shown adult LRS to be a good predictor of long-term genetic contribution to the population in natural populations of two bird species. Adult LRS is, however, difficult to measure and therefore rarely included in fitness estimation in experimental studies.

To evaluate the reliability of various commonly used fitness surrogates, we explored phenotypic correlations between adult LRS, measured as the total number of offspring produced over the adult lifetime of individual females, and various morphological and life history traits, in a recently founded *Drosophila littoralis* laboratory population. *D. littoralis* is a boreal drosophilid belonging

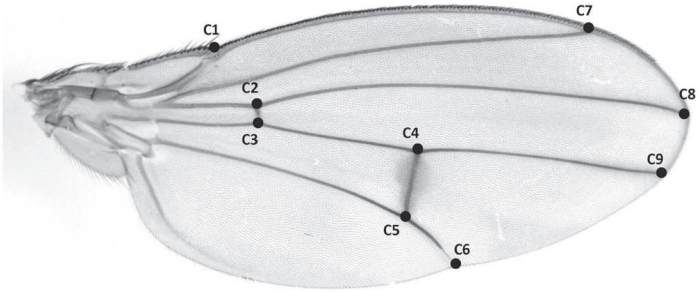

Figure 1. Landmarks for measurement of wing size (C1–C9).
doi:10.1371/journal.pone.0024560.g001

to the *D. virilis* species group. In northern Fennoscandia *D. littoralis* overwinters as adult, reproduces in the spring, and the next generation (summer generation) emerges before autumn [17]. The overwintered and summer generations overlap only slightly and only a small proportion of the summer generation reproduces during the ongoing summer [17]. The species is thus practically univoltine, with only slightly overlapping generations. However, noting that survival is usually higher in laboratory conditions than in nature where individuals are subject to predation and other hazards, we also calculated adjusted adult LRS measures that give more weight to early reproduction. Comparing the correlations of other fitness surrogates to adjusted and unadjusted adult LRS measures provides insight about the sensitivity of laboratory-derived fitness correlations to the higher mortality rates likely to exist in natural conditions. We explored phenotypic correlations between the adult LRS measures and fitness components measured over the lifetime of the females (longevity, lifetime fecundity, and lifetime egg-to-adult viability of offspring), fitness components measured over shorter periods throughout female life (short-time fecundity and short-time offspring production), and size measures often used as proxies of individual fitness (weight and several morphological measures of the females).

Methods

Ethics Statement

No permits are required for collecting flies by the Tourujoki River in Jyväskylä, Finland.

A laboratory population of *D. littoralis* was founded in spring 2006 from 157 males and 99 females collected from a natural population by the Tourujoki River in Jyväskylä, Finland. Thirty-four of the 99 females had been inseminated in the wild and produced fertile eggs after transfer to the lab. The rest of the females were mated randomly in the lab with the wild-caught males. Population size was increased to 419 breeding couples in F2. The parental flies were assigned randomly each generation, but inbreeding was reduced by preventing full-sib matings. In a sample of 20 individuals from F4, 11 out of 14 nuclear microsatellite loci were polymorphic [18]. In the polymorphic loci, the mean number of alleles was 6.8 and the mean observed heterozygosity was 0.55. The flies were kept in plastic vials (diameter 23.5 mm, height 75.0 mm) with malt-yeast medium [19], at 19°C and relative humidity of 60% with constant light. Generation length of the flies under these conditions is approximately 35 days.

In F3, we measured egg and offspring production for 84 females from 5 days after eclosion until death. Based on a pilot experiment, females don't produce eggs before this age (data not shown). All females were from different families. One female and one non-sib male (age 13–22 days from eclosion) were placed into a plastic vial with 8 ml of malt-yeast medium to mate and lay eggs. The couples were placed into a new vial every second day, which is sufficient to prevent crowding of the larvae (see Results). To make sure that female reproduction was not limited by male quality, the male was replaced with a new one (age 13–22 days) every second week, or immediately if it was found dead or if it escaped during handling. The number of eggs laid and the number of eclosing flies were counted from each vial. Mould or bacterial growth in vials was rare, and was not observed more often in vials with small number of eggs compared to vials with more eggs (personal observation).

We measured adult LRS as the number of eclosing offspring produced by an adult female over its lifetime. In optimal laboratory conditions with continuous availability of food and no predators the lifetime of *Drosophila* is much longer than in natural populations [20]. Thus, the lifetime reproductive success reached in laboratory conditions is rarely realized in nature. To further explore the possible consequences of higher mortality on the

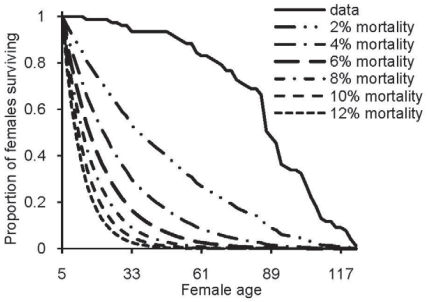

Figure 2. Female survival. Proportion of females surviving in the experiment (solid line), and expected survival probability with additional daily mortality risk of 2, 4, 6, 8, 10, and 12% (dashed lines) for different female ages (the dashed lines combine natural deaths with the additional mortality risk). Female age (in days) is scored according to the last day in a vial.
doi:10.1371/journal.pone.0024560.g002

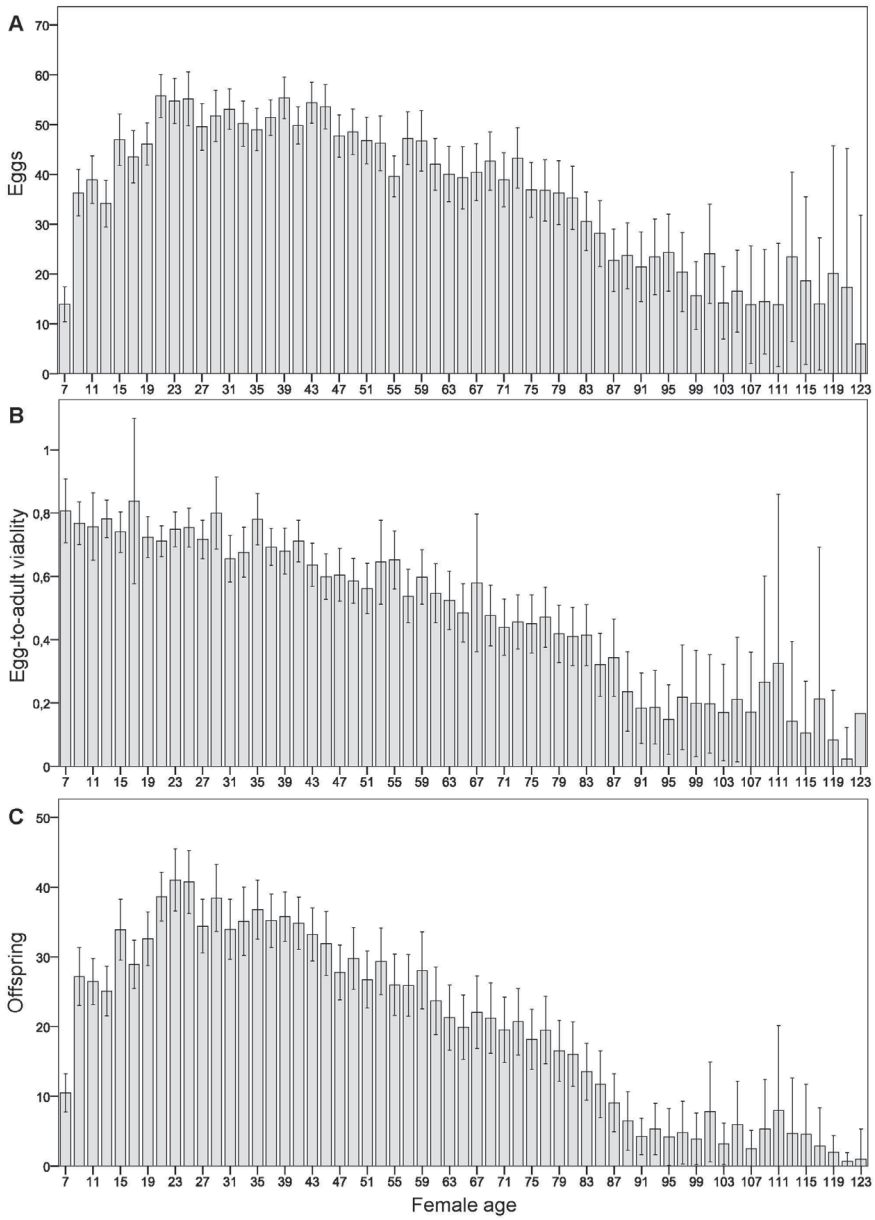

Figure 3. Effect of age on female reproduction. A) mean egg production, B) mean egg-to-adult viability of offspring, and C) mean offspring production, in relation to female age. Error bars indicate 95% confidence interval. Female age (in days) is scored according to the last day in a vial.
doi:10.1371/journal.pone.0024560.g003

fitness surrogates, we calculated adjusted adult LRS measures by assuming additional values of daily mortality risk of 2, 4, 6, 8, 10, and 12% for the females. The offspring number in each vial was multiplied by the calculated survival probability to the specified age, and the adjusted offspring numbers of all the vials for each female were then summed together. The adjusted adult LRS thus equals the expected number of offspring a female with a certain reproductive history in laboratory would produce if there was some external factor, e.g. predation, inflicting a constant daily risk of mortality. Lifetime fecundity was measured as the number of eggs produced by an adult female over its lifetime. Offspring viability was measured for each female by dividing the total number of offspring produced (i.e. adult LRS) by the total number of eggs produced (i.e. lifetime fecundity; note that the fertilization rate of the eggs is not known).

The short-time estimates of offspring production and fecundity were calculated as sliding windows throughout female life. To be able to compare estimates based on time frames of different length, we used three different time frames: 2, 4 and 10 days. We also present the correlations of cumulative offspring production and cumulative fecundity with adult LRS. Comparing the correlations of the cumulative measures and the short-time measures may reveal the possible benefit of measuring offspring production or fecundity of individuals from sexual maturity to some specific age (i.e. cumulative measurement), versus measuring these traits only for a short period at a specific age.

The females were weighed in the beginning of the experiment (5 days after eclosion). After death, females were preserved in 70% ethanol. Several morphological measurements were taken from the preserved samples. The wings and hind legs of the flies were fixed on microscope slides and digitally photographed. Distance between nine cross points of the wing veins (Fig. 1) and length of femur, tibia, and the five segments of tarsus of hind legs were measured from the images. When measurements could be taken from both left and right wings or legs, we averaged the left and right measurements to get one estimate for each measurement for each fly. When only one measurement was possible due to damaged wings or legs (note that the flies had died of old age and were thus rather worn), the single available measurement was used. To obtain a single size component for wings and legs, we extracted the first principal component from the correlation matrix of the measurements. The size component for wing explained 78.5% of total variance with initial eigenvalue of 28.3. The size component for leg explained 50.3% of total variance with initial eigenvalue of 3.5. Length of thorax (longest distance between neck and the tip of scutellum measured from the side of the fly), length of scutellum (longest dorsoventral distance), and width of head (distance between eyes through ocelli) were measured using light microscope. Each fly was measured twice, and the mean of the two measurements was used in the analyses to reduce the measurement error.

Measurements done with light microscope had fairly low repeatabilities (thorax 0.85, scutellum 0.58 and head width 0.54). Using the average of two measurements of the same trait however reduces the measurement error. Measurements from wings and legs were taken from digital photographs and are less affected by measurement error. Calculating the repeatability from left and right measurements includes variance due to asymmetry, in addition to variance due to measurement error. Excluding the two most asymmetric individuals from analysis, distance measurements from left and right wings had average repeatability of 0.93, distance measurements from left and right legs had average repeatability of 0.60, and left and right measurements of tibia had repeatability 0.86. As pointed out above, these repeatabilities are affected by real within-individual asymmetry. As we used the average of the left and right-side measurements in all analysis, we were able to obtain individual estimates that were less affected by both asymmetry and measurement error.

Except for female longevity, all the variables were normally distributed (one-sample Kolmogorov-Smirnov test). Thus, we analyzed the parametric correlation coefficients between variables other than longevity, and both parametric and non-parametric correlation coefficients between longevity and the other variables. We corrected for multiple testing using the Benjamini & Hochberg correction for false discovery rate at 0.01 and 0.05 significance levels [21]. To examine the possible effect of crowding on offspring emergence, we tested the effect of number of eggs in a vial on egg-to-adult offspring viability with linear regression. All the analyses were performed with PASW Statistics 18.

Results

After removing females that accidentally escaped or died during handling, a total of 77 females remained in the analyses. The last female in the experiment was found dead at the age of 125 days (Fig. 2). Offspring production of the females decreased with aging, and this was due to combined effects of senescence on both female fecundity and on egg-to-adult viability of offspring (Fig. 3). Mean number of eggs laid by the females began to decrease approximately from the age of 45 days onwards. Mean egg-to-adult offspring viability showed a continuous decrease as the females aged. The peak in mean number of offspring produced was at the age of 21 to 25 days. Negative effect of senescence on female fecundity and offspring viability have been reported before e.g. in *D. melanogaster* [22,23].

The possible effect of crowding on egg-to-adult viability of the offspring was tested for vials collected from the beginning of the experiment until the females were 35 days old, so that the effect of female aging on offspring viability could be minimized. Number of eggs in a vial did not affect egg-to-adult viability of the offspring (Fig. 4).

Phenotypic correlations between the adult LRS measures, fitness components measured over the lifetime of the females, and size measures, together with means and standard deviations of the variables, are shown in Table 1. Figure 5 displays correlations of the variables graphically (not shown for the adjusted LRS measures). From the fitness components measured over the

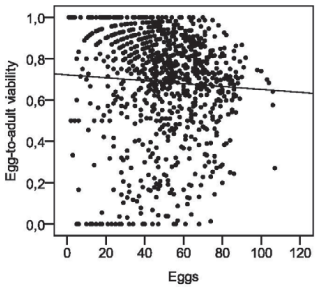

Figure 4. Egg-to-adult viability of offspring plotted against number of eggs in a vial. Number of eggs in a vial did not affect egg-to-adult viability of the offspring (linear regression of egg-to-adult viability on egg number: $F_{1,966} = 2.997$, $R^2 = 0.003$, $p = 0.084$).
doi:10.1371/journal.pone.0024560.g004

Table 1. Correlations between adult LRS measures, lifetime fitness components, and size measures, with means and standard deviations of the variables.

	Adult LRS	Adult LRS with 2% mortality	Adult LRS with 4% mortality	Adult LRS with 6% mortality	Adult LRS with 8% mortality	Adult LRS with 10% mortality	Adult LRS with 12% mortality	Lifetime fecundity	Lifetime offspring viability	Longevity (parametric)	Longevity (non-parametric)	Female weight (mg)	Wing size	Leg size (mm)	Tibia (mm)	Thorax (mm)	Scutellum (mm)	Head (mm)
Mean; SD	1027; 48	538; 193	323; 110	214; 75	152; 56	113; 45	88; 37	1689; 654	.61; .16	86; 25	86; 25	3.33; .40	--	--	.97; .04	1.54; .08	.45; .03	.53; .03
Adult LRS		.94** (77)	.80** (77)	.66** (77)	.55** (77)	.48** (77)	.42** (77)	.81** (77)	.51** (73)	.63** (77)	.55** (77)	.32** (77)	.24 (47)	.38* (48)	.24 (70)	.22 (74)	.24 (74)	.12 (75)
Adult LRS with 2% mortality			.95** (77)	.86** (77)	.78** (77)	.71** (77)	.65** (77)	.78** (77)	.51** (73)	.55** (77)	.43** (77)	.36** (77)	.32* (47)	.42** (48)	.28* (70)	.26* (74)	.25* (74)	.17 (75)
Adult LRS with 4% mortality				.97** (77)	.92** (77)	.87** (77)	.83** (77)	.67** (77)	.44** (73)	.44** (77)	.31** (77)	.36** (77)	.38* (47)	.44** (48)	.29* (70)	.28* (74)	.24 (74)	.20 (75)
Adult LRS with 6% mortality					.99** (77)	.96** (77)	.93** (77)	.56** (77)	.37** (73)	.33** (77)	.23* (77)	.36** (77)	.41** (47)	.44** (48)	.28* (70)	.28* (74)	.22 (74)	.22 (75)
Adult LRS with 8% mortality						.99** (77)	.98** (77)	.47** (77)	.30* (73)	.25* (77)	.16 (77)	.31* (77)	.42** (47)	.44** (48)	.25 (70)	.27* (74)	.21 (74)	.23 (75)
Adult LRS with 10% mortality							.99** (77)	.41** (77)	.25* (73)	.20 (77)	.18 (77)	.28* (77)	.43** (47)	.42** (48)	.23 (70)	.26* (74)	.20 (74)	.23 (75)
Adult LRS with 12% mortality								.36** (77)	.22 (73)	.16 (77)	.07 (77)	.26* (77)	.43** (47)	.41** (48)	.20 (70)	.26* (74)	.21 (74)	.23 (75)
Lifetime fecundity									−.05 (73)	.78** (77)	.71** (77)	.30* (77)	.26 (47)	.30 (48)	.17 (70)	.12 (74)	.17 (74)	.10 (75)
Lifetime offspring viability										−.02 (73)	−.06 (73)	.11 (73)	−.05 (45)	.20 (44)	.12 (66)	.18 (70)	.08 (70)	.12 (71)
Longevity (days; parametric)											--	.20 (77)	.00 (47)	.11 (48)	.06 (70)	.03 (74)	.07 (74)	.08 (75)
Longevity (days; non-parametric)												.16 (77)	−.03 (47)	.13 (48)	.01 (70)	.01 (74)	.02 (74)	.16 (75)
Female weight (mg)													.67** (47)	.73** (48)	.64** (70)	.71** (74)	.57** (74)	.38** (75)
Size component for wing														.88** (34)	.86** (46)	.78** (46)	.73** (46)	.47** (47)
Size component for leg															.92** (48)	.80** (47)	.68** (47)	.58** (48)
Tibia																.77** (69)	.61** (69)	.54** (70)
Thorax																	.71** (74)	.48** (74)
Scutellum																		.45** (74)

**significant at the 0.01 level; * significant at the 0.05 level; significance levels are adjusted by Benjamini & Hochberg correction for false discovery rate.

Pearson's correlation coefficients (sample size in parentheses) between the adult LRS measures, lifetime fecundity, lifetime offspring viability, longevity (also non-parametric results shown), and the size measures of the females. On the uppermost row mean and standard deviation of the variables.

doi:10.1371/journal.pone.0024560.t001

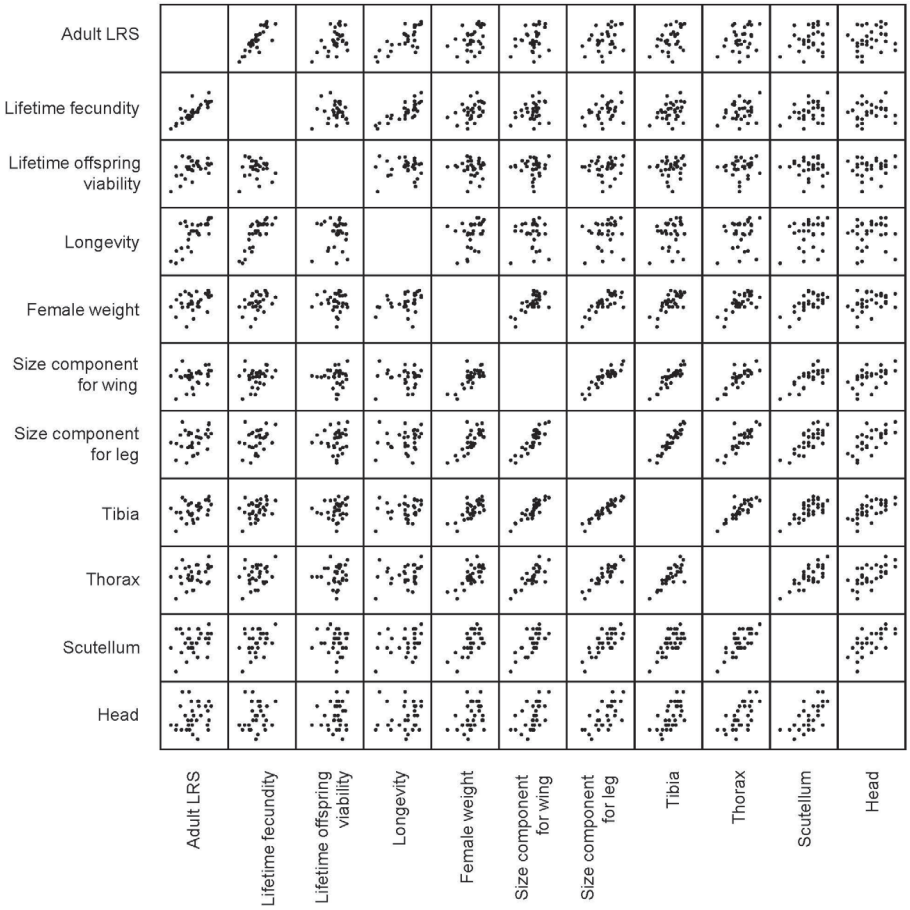

Figure 5. Scatterplots of adult LRS, lifetime fitness components, and size measures. Data is shown only for individuals to whom measurements for all the variables were available.
doi:10.1371/journal.pone.0024560.g005

lifetime of the females, fecundity had the highest correlation with adult LRS ($r = 0.81$). Female longevity and offspring viability were also relatively highly correlated with adult LRS ($r = 0.63$, and $r = 0.51$, respectively). Longevity and fecundity correlated positively with each other, but offspring viability correlated with neither longevity nor fecundity. Size measures had modest to weak correlations with adult LRS. Leg size, based on measurements of all segments of the hind legs, had the highest correlation ($r = 0.38$), followed by female weight ($r = 0.32$).

Correlations between adult LRS and cumulative and short-time measures of fecundity and offspring production are shown in Figure 6. Correlations of the short-time measures of fecundity and offspring production with adult LRS were highly dependent on the time of measurement: for young females the correlations were low, but when measured from older females, the correlations were much higher (up to 0.67 for short-time fecundity and 0.83 for short-time offspring production). For both short-time fecundity and short-time offspring production the highest correlations with adult LRS were reached when the female age was about 50 to 80 days. The length of the time frame had only a minor effect: the correlation of the 10-day measure with adult LRS was generally only slightly higher than that of the 2-day measure. The short-time measures performed well in comparison to the cumulative measures of fecundity and offspring production.

Correlations between the adjusted adult LRS measures and 10-day measures of fecundity and offspring production are shown in Figure 7. As expected, correlations between the short-time measures of fecundity and offspring production with adjusted

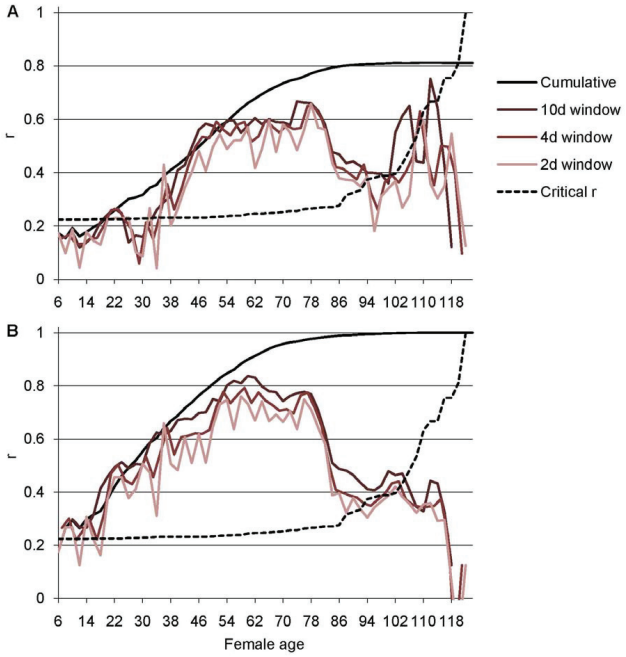

Figure 6. Correlations between adult LRS and cumulative and short-time fecundity and offspring production. Pearson's correlation coefficients (r) between adult LRS and A) cumulative fecundity, and fecundity in sliding windows of 2 days, 4 days, and 10 days, and B) cumulative offspring production, and offspring production in sliding windows of 2 days, 4 days, and 10 days. Above critical r (dashed line) correlations are significant at $\alpha = 0.05$ level (two-tailed; note that the critical effect size for significance increases with increasing female age because of decreasing sample size). Female age is scored according to the midpoint of the time frame in question.
doi:10.1371/journal.pone.0024560.g006

adult LRS were generally higher when measured from younger than when measured from older flies; the effect was more pronounced with higher levels of additional mortality risk. Adjusting adult LRS with additional mortality risk also increased variation between the different lengths of time frames: the 10-day measure outperformed the shorter time frames by giving more consistent correlations (data from shorter windows is not shown).

Mortality-adjustment to adult LRS did not have a strong effect on the correlations with size measures (Table 1). If anything, the correlations of size measures were stronger with the adjusted adult LRS measures than with unadjusted adult LRS. This effect was due to generally higher correlation of size measures with early fecundity and offspring production than with late fecundity and offspring production (analysis not shown).

Discussion

We explored phenotypic correlations between adult LRS, measured as the total number of offspring produced over the adult lifetime of individual *D. littoralis* females in laboratory, and various morphological and life history traits commonly used as fitness surrogates. As could be expected, the lifetime measures of fecundity, longevity, and offspring viability were all relatively highly correlated with adult LRS. Previous research on correlations between adult LRS and other fitness surrogates is rather scarce. However, strong positive correlation between longevity and adult LRS has been documented also in *D. melanogaster* [24] and in the house fly (*Musca domestica*) [25] in laboratory and in some bird [26–28] and mammal species [29,30] in the field. In the housefly [25], the song sparrow (*Melospiza melodia*) [26], and the house martin (*Delichon urbica*) [27], strong correlation was also found between lifetime fecundity and total number of offspring produced.

Correlation of the short-time measures of fecundity and offspring production with adult LRS depended greatly on the time of measurement: when measurements were from older rather than from younger females correlations were surprisingly high. The short-time measures performed well also in comparison to the cumulative measures of fecundity and offspring production. It seems that, if timed correctly, the more practical short-time measures could give as good estimates of adult LRS as can more laborious and time-consuming cumulative measurements. In contrast to our findings, Reed and Bryant [25], exploring the relationship between adult LRS and seven other fitness surrogates in pairs of the housefly, ended up recommending only fitness surrogates covering the entire lifetime of the organism. However, the argument of Reed and Bryant [25] is based on the weak performance of three fitness surrogates measured at the very beginning of the reproductive lifetime of the housefly pairs (age at

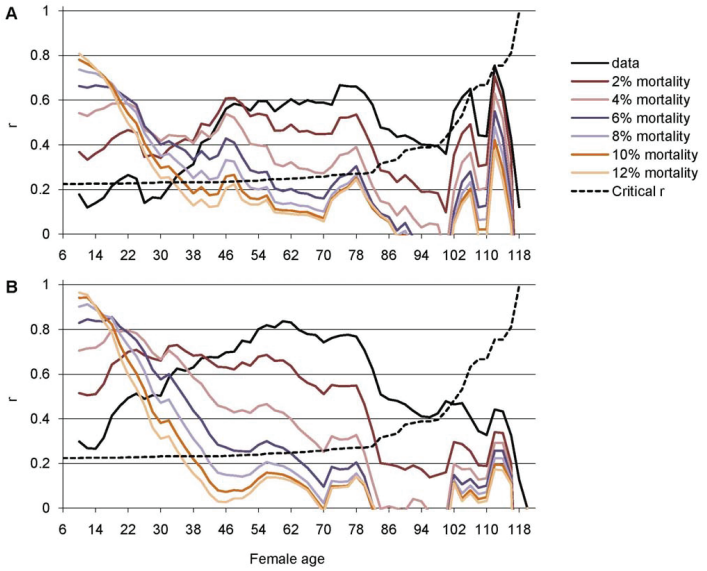

Figure 7. Effect of additional mortality on correlations between adult LRS and 10-day measures of fecundity and offspring production. Pearson's correlation coefficients (r) between adult LRS measures (unadjusted adult LRS, and adult LRS adjusted for additional daily mortality risk of 2, 4, 6, 8, 10, and 12%) and A) fecundity in sliding windows of 10 days, and B) offspring production in sliding windows of 10 days. Above critical r (dashed line) correlations are significant at $\alpha = 0.05$ level (two-tailed; note that the critical effect size for significance increases with increasing female age because of decreasing sample size). Female age is scored according to the midpoint of the time frame in question.
doi:10.1371/journal.pone.0024560.g007

first reproduction, the size of the first egg clutch, and egg-to-adult viability of the first clutch). We measured short-time fecundity and offspring production of individual females throughout the female lifetime and, as said, discovered that when measured from individuals well into their reproductive life, short-time measures predicted adult LRS surprisingly well. Measuring fitness surrogates from older individuals is of course justifiable only when mortality is negligible; if mortality is high, the older age-classes comprise only a selected subset of the population.

Correlation between adult LRS and short-time components of fitness may depend greatly on the short-time measure used. In the song sparrow, a strong correlation was found between the number of young raised in the first breeding year and total number of young reared by females in their lifetime ($r = 0.82$) [26]. However, correlation between the number of eggs laid in the first breeding year and the total number of young reared was relatively poor ($r = 0.32$) [26].

Correlations between adult LRS and size measures were generally weaker than those between adult LRS and measures of life history traits (longevity, lifetime or short-time fecundity, short-time offspring production, and lifetime egg-to-adult viability of offspring). However, two of the size measures correlated reasonably well with adult LRS: leg size and female weight. In fact, by simply weighing the female one can get a better estimate for adult LRS than with an unfavorably timed measurement of fecundity. Tibia length, a commonly used size measure [14,31,32], had a lower correlation with adult LRS than the size measure combining all leg segments.

There seems to be a lot of variation in how size measures relate to offspring production between different species studied. Partridge *et al.* [23] documented a strong positive correlation between thorax length and adult LRS in *D. melanogaster* ($r = 0.67$). This correlation is much stronger than what was found in our study ($r = 0.22$), in spite of the similar study systems. Strong correlations between offspring production and weight have been documented e.g. in red squirrels (*Sciurus vulgaris*) [33] and in a monogamous rodent (*Peromyscus californicus*) [34]. Scott [35] studied these relationships in Bewick's swans (*Cygnus columbianus bewickii*), and found only moderate to weak correlations between total number of young and female weight and morphological measures. In the house martin, body mass, keel length, and wing length were all very poor indicators of total young reared [27].

In addition to the adult LRS realized in laboratory conditions, we used adjusted measures with additional daily mortality risk of the females. Thus, the adult LRS measures adjusted for mortality weight early reproduction more than later reproduction, and therefore more closely reflect fitness in natural conditions where the flies have evolved. It is well known that predation and other hazards in nature result in shorter lifespan in nature than in laboratory [20], and that mortality caused by predation affects the evolution of life-histories [36,37]. Estimates of daily mortality risk in natural populations of various *Drosophila* species range from 15% to 55% [20]. Thus, although a lifetime in *D. littoralis* is somewhat longer than in the species used in these studies, the daily mortality estimates used here (2, 4, 6, 8, 10, and 12%, in addition to natural death of the females in the experiment) can be considered

conservative. Predictably, and in contrast to what was found for unadjusted adult LRS, the short-time measures of fecundity and offspring production correlated better with mortality-adjusted adult LRS if measured from younger flies than if measured from older flies. Thus, assuming additional mortality risk in nature changes the optimal time frame for short-time measurements of fecundity and offspring production. Interestingly, size measures tended to correlate more strongly with adjusted adult LRS than with unadjusted adult LRS, suggesting that size might predict fitness better in environments where mortality rates are higher.

Because adult LRS combines several fitness components, it is likely to be closely related to the total fitness of individuals. Using adult LRS as a surrogate for total fitness is not, however, totally unambiguous. The number of adult offspring eclosing from the eggs laid by a female is not solely the property of the female, but also that of the offspring themselves, as the offspring have unique genotypes different from their mother. Assigning offspring fitness to the mother may thus lead to erroneous conclusions, especially if the impact of offspring genotype on offspring viability is large in comparison to maternal effects [38]. In this light, lifetime fecundity might be considered a better estimate of female fitness than lifetime offspring production, as fecundity can more clearly be considered a property of the female itself. While achieving consensus on the best fitness measure (total number of eggs vs. total number of adult offspring) is beyond the scope of the current paper, we argue that researchers should always carefully consider how they define individual fitness.

The possible effects of competition are excluded in our study, as the availability of food was not a limiting factor, and only one male and one female fly were introduced to each other. Competition over resources such as food and shelter may not be strong in the natural habitat of the flies, as the population density seemed low at the Tourujoki River area (personal observation). However, other evolutionary processes such as sexual selection might potentially contribute to the reproductive success of the flies [39]. A recent study showed that increased exposure to males changes rate-sensitive fitness estimates of females in *D. melanogaster*, and the direction of the change depends on whether the population is expanding or declining [7]. The effects of competition can thus be complex and dependent on population dynamics.

In summary, the best surrogates for adult LRS of *D. littoralis* females in this study were lifetime fecundity and well-timed short-time measures of fecundity and offspring production. The great variation found in the strength of the relationship between adult LRS and the other surrogates of fitness shows the importance of careful choice of fitness surrogates in empirical research. With short-time measures, it is crucial to pay attention to the timing of the measurements.

Acknowledgments

We thank the editor and three anonymous referees for their constructive comments. We also thank Sari Mikkelsson for her contribution on the maintenance of the flies and measurements of egg and offspring production.

Author Contributions

Conceived and designed the experiments: NP JSK MP. Performed the experiments: NP. Analyzed the data: NP JSK MP. Wrote the paper: NP JSK MP.

References

1. Endler JA (1986) Natural selection in the wild. Princeton, N.J. : Princeton University Press.
2. Tantawy AO, Rakha FA (1964) Studies on natural populations of *Drosophila*. IV. Genetic variances of and correlations between four characters in *D. melanogaster* and *D. simulans*. Genetics 50: 1349–1355.
3. Stearns SC (1992) The evolution of life histories. New York: Oxford University Press.
4. Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics. Harlow, UK: Prentice Hall.
5. Maynard Smith J (1998) Evolutionary genetics. New York: Oxford University Press.
6. Hunt J, Hodgson D (2010) What is fitness, and how do we measure it? In: Westneat DF, Fox CW, eds. Evolutionary behavioral ecology. New York: Oxford University Press.
7. Edward DA, Fricke C, Gerrard DT, Chapman T (2011) Quantifying the life-history response to increased male exposure in female *Drosophila melanogaster*. Evolution 65: 564–573.
8. McGraw JB, Caswell H (1996) Estimation of individual fitness from life-history data. Am Nat 147: 47–64.
9. Brommer JE, Merila J, Kokko H (2002) Reproductive timing and individual fitness. Ecol Lett 5: 802–810.
10. Fincke OM, Hadrys H (2001) Unpredictable offspring survivorship in the damselfly, *Megaloprepus coerulatus*, shapes parental behavior, constrains sexual selection, and challenges traditional fitness estimates. Evolution 55: 762–772.
11. Simmons LW, Emlen DJ (2008) No fecundity cost of female secondary sexual trait expression in the horned beetle *Onthophagus sagittarius*. J Evol Biol 21: 1227–1235.
12. Kolss M, Vijendravarma RK, Schwaller G, Kawecki TJ (2009) Life-history consequences of adaptation to larval nutritional stress in *Drosophila*. Evolution 63: 2389–2401.
13. Cotton S, Small J, Hashim R, Pomiankowski A (2010) Eyespan reflects reproductive quality in wild stalk-eyed flies. Evol Ecol 24: 83–95.
14. Zhou Y, Gu H, Dorn S (2007) Effects of inbreeding on fitness components of *Cotesia glomerata*, a parasitoid wasp with single-locus complementary sex determination (sl-CSD). Biol Control 40: 273–279.
15. Janicke T, Scharer L (2009) Determinants of mating and sperm-transfer success in a simultaneous hermaphrodite. J Evol Biol 22: 405–415.
16. Brommer J, Gustafsson L, Pietiäinen H, Merilä, J (2004) Single-generation estimates of individual fitness as proxies for long-term genetic contribution. The American Naturalist 163: 505–517.
17. Aspi J, Lumme J, Hoikkala A, Heikkinen E (1993) Reproductive ecology of the boreal riparian guild of Drosophila. Ecography 16: 65–72.
18. Routtu J, Hoikkala A, Kankare M (2007) Microsatellite-based species identification method for *Drosophila virilis* group species. Hereditas 144: 213–221.
19. Lakovaara S (1969) Malt as a culture medium for *Drosophila* species. Drosophila Inf Serv 44: 128.
20. Rosewell J, Shorrocks B (1987) The implication of survival rates in natural populations of *Drosophila*: capture-recapture experiments on domestic species. Biol J Linn Soc 32: 373–384.
21. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B-Methodol 57: 289–300.
22. Kern S, Ackermann M, Stearns SC, Kawecki TJ (2001) Decline in offspring viability as a manifestation of aging in *Drosophila melanogaster*. Evolution 55: 1822–1831.
23. Partridge L, Fowler K, Trevitt S, Sharp W (1986) An examination of the effects of males on the survival and egg-production rates of female *Drosophila melanogaster*. J Insect Physiol 32: 925–929.
24. Partridge L (1988) Lifetime reproductive success in *Drosophila*. In: Clutton-Brock TH, ed. Reproductive success - studies of individual variation in contrasting breeding systems. Chicago: The University of Chicago Press. pp 11–23.
25. Reed DH, Bryant EH (2004) Phenotypic correlations among fitness and its components in a population of the housefly. J Evol Biol 17: 919–923.
26. Smith JNM (1988) Determinants of lifetime reproductive success in the song sparrow. In: Clutton-Brock TH, ed. Reproductive success - studies of individual variation in contrasting breeding systems. Chicago: The University of Chicago Press. pp 154–172.
27. Bryant DM (1988) Lifetime reproductive success of house martins. In: Clutton-Brock TH, ed. Reproductive success - studies of individual variation in contrasting breeding systems. Chicago: The University of Chicago Press. pp 173–188.
28. Newton I (1988) Age and reproduction in the sparrowhawk. In: Clutton-Brock TH, ed. Reproductive success - studies of individual variation in contrasting breeding systems. Chicago: The University of Chicago Press. pp 201–219.
29. Kruuk LEB, Clutton-Brock TH, Rose KE, Guinness FE (1999) Early determinants of lifetime reproductive success differ between the sexes in red deer. Proc R Soc Lond Ser B-Biol Sci 266: 1655–1661.
30. Bercovitch FB, Berard JD (1993) Life history costs and consequences of rapid reproductive maturation in female rhesus macaques. Behav Ecol Sociobiol 32: 103–109.

31. LeBas NR, Hockham LR, Ritchie MG (2004) Sexual selection in the gift-giving dance fly, *Rhamphomyia sulcata*, favors small males carrying small gifts. Evolution 58: 1763–1772.
32. Urrutia MA, Wade MR, Phillips CB, Wratten SD (2007) Influence of host diet on parasitoid fitness: unravelling the complexity of a temperate pastoral agroecosystem. Entomol Exp Appl 123: 63–71.
33. Wauters L, Dhondt AA (1989) Body weight, longevity and reproductive success in red squirrels (*Sciurus vulgaris*). J Anim Ecol 58: 637–651.
34. Ribble DO (1992) Lifetime reproductive success and it's correlates in the monogamous rodent, *Peromyscus californicus*. J Anim Ecol 61: 457–468.
35. Scott DK (1988) Breeding success in Bewick's swans. In: Clutton-Brock TH, ed. Reproductive success - studies of individual variation in contrasting breeding systems. Chicago: The University of Chicago Press. pp 221–236.
36. Reznick DA, Bryga H, Endler JA (1990) Experimentally induced life-history evolution in a natural population. Nature 346: 357–359.
37. Reznick DN, Butler MJ, Rodd FH, Ross P (1996) Life-history evolution in guppies (*Poecilia reticulata*).6. Differential mortality as a mechanism for natural selection. Evolution 50: 1651–1660.
38. Wolf JB, Wade MJ (2001) On the assignment of fitness to parents and offspring: whose fitness is it and when does it matter? J Evol Biol 14: 347–356.
39. Aspi J, Hoikkala A (1995) Male mating success and survival in the field with respect to size and courtship song characters in *Drosophila littoralis* and *D. montana* (Diptera: Drosophilidae). J Insect Behav 8: 67–87.

II

INBREEDING RATE MODIFIES THE DYNAMICS OF GENETIC LOAD IN SMALL POPULATIONS

by

Nina Pekkala, K. Emily Knott, Janne S. Kotiaho & Mikael Puurtinen

Submitted manuscript

III

THE BENEFITS OF INTERPOPULATION HYBRIDIZATION DIMINISH WITH INCREASING DIVERGENCE OF SMALL POPULATIONS

by

Nina Pekkala, Janne S. Kotiaho, Kari Nissinen & Mikael Puurtinen

Submitted manuscript

IV

EFFECT OF INBREEDING RATE ON THE MAGNITUDES OF DRIFT LOAD, INBREEDING DEPRESSION, AND HETEROSIS

by

Nina Pekkala, K. Emily Knott, Janne S. Kotiaho, Kari Nissinen & Mikael Puurtinen

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