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How does gene flow limit local adaptation at a species range-edge? An artificial selection Drosophila model

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A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

HOW DOES GENE FLOW LIMIT LOCAL ADAPTATION AT A SPECIES RANGE-EDGE? AN ARTIFICIAL SELECTION *DROSOPHILA* MODEL

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Abstract

Gene flow has the potential to create species range limits by impeding adaptation to selective pressures at the range-edge, but it is unclear whether there is a threshold level of gene flow that causes this effect. This gene swamping hypothesis was tested using laboratory populations of *Drosophila melanogaster* under selection for desiccation resistance, and subject to a gradient of migration from unselected populations. Desiccation tolerance was impeded across the entire migration gradient, and populations receiving intermediate levels of migration exhibited no tolerance for desiccation stress, following twelve selection events. Female, but not male, flies increased desiccation tolerance following selection by reducing water loss rates, but not by carrying more water or becoming more tolerant of dehydration. This pattern is likely due to selection for increased female body size. Thus, intermediate levels of gene flow, in particular, have the potential to establish a species range-limit by confounding the response to selection.

Key words:

Drosophila melanogaster, gene flow, migration, range-edge, range limit, artificial selection, desiccation resistance, local adaptation, experimental evolution.

Dedications

This thesis is dedicated, in part, to the memory of my grandfather, Kenneth Orwald, who whole-heartedly believed in me every step of the way. This one is for you Grandpa.

I would also like to dedicate this thesis to my parents, Paul and Gayle Saindon, my stunning fiancée, Michele Brown, as well as my exceptionally supportive grandmother, Delores Saindon. Each of you consistently encouraged me throughout my studies and I cannot thank you enough for instilling motivation and simply having faith in me when I did not.

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List of Abbreviations

WC: water content

WLR: water loss rate

N_e: effective population size

m: migration rate, the probability that each individual is an immigrant (%)

$N_e m$: number of migrant individuals that move from range-core to range-edge each generation

CHAPTER 1: Introduction

Every species occupies an ecological niche or range whose bounds reflect the breadth of environmental conditions that each species can tolerate. While individuals can be highly mobile within their geographic range, they are mostly restricted to that distribution because they are unable to physiologically tolerate the extreme environmental conditions (e.g. drought) beyond the range-edge boundary (Spicer and Gaston, 1999). Aside from the obvious physical barriers or sharp environmental transitions that impede dispersal (e.g. large bodies of water, land masses, or mountain ranges), range-limits can be imposed along an ecological gradient where habitats become increasingly less suitable towards the periphery of the species range (Holt and Keitt, 2005; Kawecki, 2008). It is widely accepted that species' poleward range limits are set primarily by abiotic factors like water availability and temperature, and equator-ward limits are set by biotic interactions such as migration (gene flow), interspecific competition and parasitism (Slatkin, 1973; Slatkin, 1987; Garcia-Ramos and Kirkpatrick, 1997; Kirkpatrick and Barton, 1997; Case and Taper, 2000; Gaston, 2003; Bridle and Vines, 2007; Thomas, 2010). It is also accepted that population densities, reproduction, and survival typically decline from the core to the periphery of a range due to reductions in environmental stability, stochastic forces (e.g. genetic drift, bottlenecks), and habitat favourability (Safriel *et al*., 1994; Vucetich and Waite, 2003; Sagarin *et al*., 2006, Kawecki, 2008).

The study of local adaptation (the compatibility between the phenotypes and the local environment) at a species' range-edge has been a main focus in the field of evolutionary ecology for several decades, and the overarching goal for range-limit

research has been to understand why natural selection is unable to act on individuals at range-edges to allow further local adaptation and expansion into new regions beyond their current range-edge boundary (Mayr, 1954; Haldane, 1956; Gaston, 2003; Holt and Keitt, 2005; Bridle and Vines, 2007; Gaston, 2009; Thomas, 2010). Haldane (1956) reasoned that conditions towards the edge of a species' range become successively less optimal; hence, one of the main assumptions employed when studying the dynamics of core-peripheral populations is that peripheral (range-edge) populations experience less optimal conditions, and therefore different selection pressures to those experienced by populations in the core of the range (Sagarin *et al*., 2006; Hardie and Hutchings, 2010). Range-edge dynamics and the study of trait variability across an environmental cline can provide an ideal system for deeper investigation of many evolutionary questions pertaining to selection processes, speciation, as well as adaptation and its limitations to sustainable evolution (Holt and Gomulkiewicz, 1997; Gaston, 2003; Angert, 2009).

1.1 Gene flow drives limits to local adaptation

Range-edge populations naturally possess low genetic variation and are often genetically divergent from range core populations because of strong selection pressures at the range margin or because of genetic drift, bottleneck effects, and low mutation rates in small populations (Kirkpatrick and Barton, 1997; Kawecki and Ebert, 2004; Morjan and Rieseberg, 2004; Geber, 2008; Hardie and Hutchings, 2010). A population that has low levels of genetic variation for ecologically-relevant traits would have a reduced ability to adapt to adverse environmental conditions because genetic variation is a prerequisite for adaptive evolution by natural selection (e.g. Slatkin, 1987; Hoffmann and Blows, 1994; Gomulkiewicz *et al*., 1999; Barton, 2001; Lenormand, 2002; Blows and Hoffmann, 2005;

Kellermann *et al*., 2009). Because immigration also can increase standing genetic variation within a population, these migrants can enhance the selection response in peripheral populations thereby creating a situation where resident species are under pressure to adapt to the changing environment (e.g. Colautti *et al*., 2010). In the case where gene flow can have a facilitating, rescue effect on adaptation, it is possible that the negative effects of gene flow (accumulation of deleterious mutations under stressful conditions) are masked by the genetic variation and beneficial mutations provided by the same dispersers, thus helping to maintain adaptive potential (Lande, 1995; Holt and Gomulkiewicz, 1997; Gomulkiewicz *et al*., 1999; Holt, 2003; Garant *et al*., 2006; Holt *et al*., 2011).

Gene flow, however, can be the principal factor constraining adaptive divergence in heterogeneous range-edge environments by preventing a response to selective pressures (Slatkin, 1987; Lenormand, 2002; Hartl and Clark, 2007; Räsänen and Hendry, 2008; Thomas, 2010). Continued adaptation to unfavourable peripheral conditions can be countered by incoming gene flow from the range core (where individuals are not subject to strong selection pressures), which is made up of primarily non-selected alleles that are likely deleterious in the range-edge environment (Bridle and Vines, 2007). This influx of deleterious alleles at the range-edge can create a disparity in fitness between the migrants (genotypes from other habitats) and the residents (local genotypes) living in their local environment (Lind *et al*., 2011). This asymmetrical pattern of gene flow can offset natural selection by altering the normal migration-selection equilibrium, which in turn can create a 'migration load' or an accumulation of potentially harmful alleles in the range-edge population(s). This scenario is also known as gene swamping, which can

cause maladaptation at the periphery (Kirkpatrick and Barton, 1997; Case and Taper, 2000; Lenormand, 2002; Alleaume-Benharira *et al*., 2006; Bolnick and Nosil, 2007; Bridle and Vines, 2007). Local selective pressures at the periphery will proceed to remove maladaptive alleles, which not only reduce overall population density and suppress population growth, but also intensify the asymmetrical swamping effect of gene flow in these range-edge populations (Case and Taper, 2000, Bridle and Vines, 2007).

Gene swamping is more likely if range-edge populations are in close proximity to core populations or if there is a large amount of dispersal from the core (Bridle and Vines, 2007; Angert, 2009). In most cases, it is thought that these low densities retard local adaptation primarily because locally fit alleles are less likely to become fixed in the population due to depleted genetic variation (Bridle and Vines, 2007). Range-edge populations that are prevented from reaching their ecological fitness optimum due to gene flow from the core of a species' range may experience persistent directional selection to which they cannot adapt, resulting in reduced fitness and in some instances, a population crash (Garcia-Ramos and Kirkpatrick, 1997; Bridle *et al*., 2009). The interaction between the homogenizing effect of gene flow on neutral alleles and diversifying selection in range-edge populations ultimately leads to a reduction in the independence of their gene pools (Räsänen and Hendry, 2008) and an increase in fitness variance (Bolnick and Nosil, 2007). Kirkpatrick and Barton (1997) demonstrated that the genetic diluting effects of gene flow must be offset by local selective forces and this is most likely at borders of a species range where gene flow is typically unidirectional – that is, from the core to the periphery, primarily due to uneven population densities across space (Lenormand, 2002).

1.2 Theoretical models for the effect of gene flow on adaptation

Since Haldane (1948) and Mayr (1963), several theoretical models have explored the effects and consequences of gene flow on adaptive evolution (e.g. Hoffmann and Blows, 1994; Garcia-Ramos and Kirkpatrick, 1997; Gomulkiewicz *et al*., 1999; Case *et al*., 2005; Alleume-Benharira *et al.*, 2006). While a great deal of this theoretical work was driven by differing range-limit research interests (e.g. demographic processes, adaptive differentiation, phenotypic plasticity, dispersal effects, genetic polymorphism), several predictions concerning local adaptation have arisen from these models (Kawecki and Ebert, 2004). Such predictions include the ecological factors that are expected to encourage local adaptation in range-edge populations, for example low gene flow in combination with strong selection against migrant genotypes, minimal differences between habitats (size and quality), and relatively no variation in selection type and intensity (Kawecki and Ebert, 2004). The theoretical frameworks of these predictions as well as others have become the foundation for empirical studies interested in determining how range limits arise and how gene flow could affect the formation and persistence of a range limit.

There are several interacting factors that can either retard or accelerate local adaptation to novel selection pressures in range-edge populations, such as dispersal rate, genetic correlations, and demographic constraints (Wade and McCauley, 1988; Räsänen and Hendry, 2008; reviewed in Kawecki, 2008). In order to tease apart these factors to understand which are responsible for causing changes in the pattern and amount of genetic variation and differentiation among a subset of populations, an appropriate model for estimating gene flow should first be determined. The continent-island model is one

model that is most applicable to controlled, laboratory-based studies making it a reasonable approximation method for investigating the effect of gene flow on adaptation by artificial selection in a simple population structure (Hedrick, 2005; Hartl and Clark, 2007; Fig. 1); however, the properties can be applied to theoretical models as well. The continent-island model describes a pattern of gene flow that is unidirectional – typically from a continent (core) population to an outward, island (peripheral) population (Fig. 1). While the pattern of gene flow is typically stochastic in nature (Slatkin, 1985), a unidirectional pattern of gene flow is necessary to dissect the underlying interactions in a controlled laboratory setting.

The amount of genetic variation maintained by the balance between the level of migration (gene flow) and the amount of directional selection will have a direct bearing on the ability for a trait to become locally adapted (Garant *et al.*, 2006). For example, more genetic variance is generated under theoretical conditions involving weak selection and low migration than with strong selection (i.e. only individuals with the optimal phenotype will survive) and high migration (Phillips, 1996). Consequently, if the strength of migration is too high, then most of the associated variance would be eliminated by selection (Phillips, 1996), leading to reduced fitness in peripheral populations as they are unable to reach their ecological optimum (Garcia-Ramos and Kirkpatrick, 1997). Sufficiently strong gene flow from large, well-adapted core populations can lead to maladaptation in peripheral populations driving them to become demographic sinks, indicating a negative local growth rate and ultimately, a constraint to adaptive evolution (Kirkpatrick and Barton, 1997). Given that sink-like environments tend to have relatively lower species abundance and density, they are less likely to

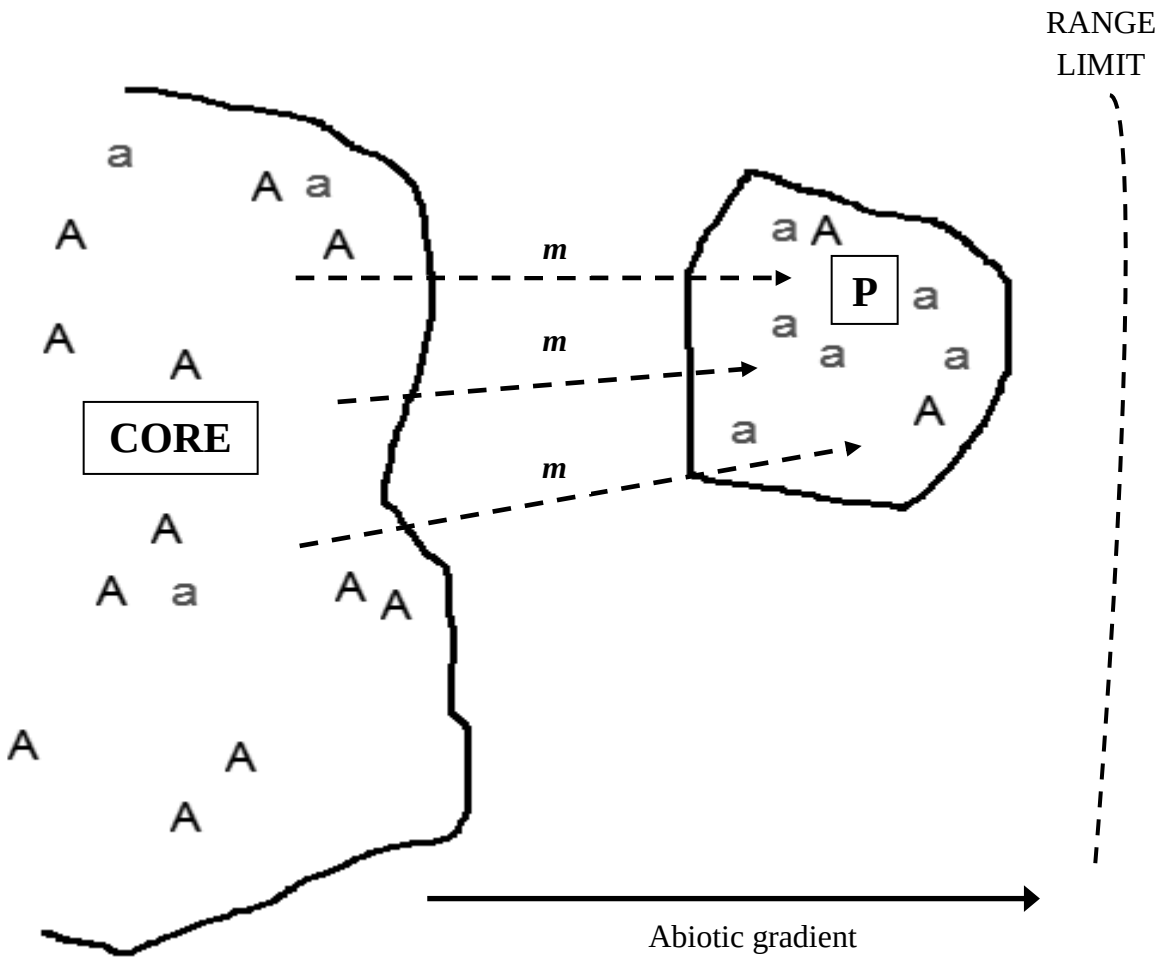

Figure 1. Hypothetical continent-island model depicting unidirectional dispersal (gene flow, *m*) composed primarily of unselected alleles (favoured 'A' allele) from the larger, continent (core) population typically at the center of a species range to an island population at the periphery (P) along an ecologically important gradient (e.g. temperature, water availability). In this schematic, the "A" alleles swamp selection for the "a" alleles at the periphery (although not depicted, over time, P would be composed mainly of 'A' alleles as 'a' alleles are removed), thereby impeding local adaptation and establishing a range limit. Adapted from Sexton *et al*. (2009).

maintain genetic variation, and are vulnerable to the swamping effects of gene flow (Kirkpatrick and Barton, 1997; Ronce and Kirkpatrick, 2001). Therefore, in order for a trait to evolve to its local optimum and therefore for local adaptation to occur, there has to be a balance between the strength of the selection pressure and the amount of genetic variance contributed by gene flow (Garcia-Ramos and Kirkpatrick, 1997; Kirkpatrick and Barton, 1997).

Thus, the current consensus model of gene flow at the species range-edge states that high levels of gene flow can cause homogenization (gene swamping) of phenotypes in small range-edge populations, potentially leading to an increase in stochastic events such as genetic drift (e.g. Alleume-Benharira *et al*., 2006). Conversely, since density typically decreases towards the range-edge, low levels of gene flow from the range core have been found to be enough to equalize the effect of drift in range-edge populations because genetic variance is replenished and maladapted alleles are removed (Alleume-Benharira *et al*., 2006). While genetic variance declines towards the range periphery, and increased gene flow into these populations can offset this deficit, genetic drift can cause stochastic variation in the mean phenotype, which may explain the lower fitness in such populations (Butlin *et al*., 2003; Alleume-Benharira *et al*., 2006; Bridle and Vines, 2007). However, stochastic variation in the mean phenotype is more likely to occur in relatively smaller (range-edge) populations and may be compensated for by mutation, thus restoring the necessary genetic variation required for adaptation (Alleume-Benharira *et al*., 2006).

1.3 Empirical evidence for the effect of gene flow on adaptation

Theoretical models have informed empirical studies, which have tried to understand the factors that create and maintain range limits by exploring the relationship

between gene flow, selection, and adaptation over a broad range of taxa. Most of the empirical research conducted on this topic has been field-based, often encompassing large geographic areas over which different traits are measured and applied to evolutionary questions initially forecasted by theoretical models (e.g. Singh and Rhomberg, 1987; Bossart and Scriber, 1995; Michalak *et al*., 2001; Paul *et al*., 2011). Furthermore, field studies often work with putative environmental gradients, and the traits measured are simply assumed to be under selection (Bridle *et al*., 2009). As a result, there are gaps of knowledge for empirical studies regarding how gene flow affects local adaptation in range-edge populations (Kawecki and Ebert, 2004; van Heerwaarden *et al*., 2009).

For an adaptation to evolve there must be selection acting on the particular trait(s). While there is considerable variation in the type of selective pressure employed in the lab or observed empirically in the field with temperature being the most common, the target and intensity of selection is unclear for a large portion of this work. This lack of awareness and control over the selective pressure is particularly concerning for those studying range limits and the heritability of traits because selection (e.g. on morphological traits) is typically differential across a species' range (e.g. Paul *et al*., 2011), and therefore the rate of adaptive evolution can also vary across a range. For example, Hendry and Taylor (2004) investigated the amount of variation in adaptive divergence that could be attributed to gene flow by using multiple natural populations of the three-spine stickleback, *Gasterosteus aculeatus*. While habitat features of the ecosystem differed between the populations sampled, the selective pressures acting in these populations were not measured. The authors were therefore unable to infer whether

the correlation between gene flow and adaptive divergence was strictly due to the strength of divergent selection or if there were other factors contributing to the response, which could explain the observed large amount of variation in adaptive divergence that can be explained by gene flow (Hendry and Taylor, 2004).

The most common environmental gradients over which selective traits are measured in the field (often those that were specific to the organism studied) are based on either latitude or temperature, likely because they can be relatively simple to measure and they have a substantial impact on biological systems (Hochachka and Somero, 2002). For example, Colautti *et al*. (2010) explored the genetic constraints that set geographical range limits of the invasive plant, purple loosestrife (*Lythrum salicaria*), sampled along a latitudinal gradient. They found that life-history traits (e.g. reduced genetic variance) and increased temperature selection compared to range-central populations, generated fitness trade-offs, which compromised local adaptation at the range-edge of purple loosestrife (Colautti *et al*., 2010).

Movement of individuals among populations affects the potential for population persistence and adaptive evolution in complex landscapes; hence, it is essential that a precise method is used to estimate gene flow and the amount of genetic variation among populations (Whitlock and McCauley, 1999; Balloux and Lugon-Moulin, 2002). Gene flow in natural populations can be stochastic, and the reliability of methods for estimating gene flow varies depending on the complexity of the population dynamics of the system under study (Slatkin, 1985). Indirect estimates of gene flow include Wright's (1931) *F*-statistic,

$$F_{ST} \approx 1/(1+4N_e m) \quad [1]$$

where F_{ST} is an approximation of the amount of genetic differentiation in a population (0 = no differentiation, 1 = complete differentiation), N_e = effective population size, m = migration rate, and $N_e m$ = the number of migrants moving into a population each generation (Hartl and Clark, 2007). F_{ST} has been used most commonly to compare levels of genetic differentiation and strengths of gene flow among populations and it has proven to be a robust method for providing a holistic picture of the cumulative effects of gene flow (Slatkin, 1985; Neigel, 2002, Beaumont, 2005). However, there are also limitations to the use of F_{ST} (Bossart and Prowell, 1998; Whitlock and McCauley, 1999). For example, Slatkin (1985) found that estimates of F_{ST} are sensitive to weak selection when there is no gene flow, and that it is most affected by common, rather than rare alleles. While there are apparent limitations such as when gene flow is high, F_{ST} overestimates it, the values of F_{ST} are aligned with biologically-informed expectations as a robust comparative measure of the average effects of gene flow in populations at equilibrium (Balloux and Lugon-Moulin, 2002; Neigel, 2002; Magiafoglou, 2002; Beaumont, 2005; Kisel and Barraclough, 2010).

Field studies often use microsatellite markers to determine the role of gene flow on genetic variation of trait means tracked over time in natural populations, and use these data to calculate an estimate of gene flow, such as an indirect measure of genetic differentiation (e.g. F_{ST}), in order to compare among populations. Singh and Rhomberg (1987) studied over one hundred gene loci in several geographically distant populations of *Drosophila melanogaster* in the wild and found that approximately two thirds of the observed frequency of polymorphic loci are concentrated at low F_{ST} values (mode = 0.1).

These results indicate that those loci are, at best, minimally differentiated and experience high rates of gene flow (Singh and Rhomberg, 1987). While Singh and Rhomberg (1987) failed to detect any genetic clines among the 15 populations tested, they were able to conclude that these natural populations experienced high amounts of gene flow, so minimal within-locus geographic differentiation among populations should be expected.

Most empirical studies generally describe a correlative effect of gene flow on adaptation. Surprisingly, there is relatively equal evidence for both a facilitating and a limiting effect of gene flow on local adaptation; however the relationship of this effect is semi-dependent on the type of study. As a result, there is currently not enough empirical evidence of gene flow impeding local adaptation at the range-edge to conclude a dominant swamping or assisting effect of gene flow (Moore and Hendry, 2009). In general, field studies that measure dispersal commonly demonstrated a facilitating effect of gene flow (e.g. Saint-Laurent *et al*., 2003; Budd and Pandolfi, 2010), whereas gene flow in lab-based studies typically impedes local adaptation (e.g. Dey and Joshi, 2006; Forde *et al*., 2007). This mixed effect of gene flow observed empirically is therefore, half supported by the current consensus model for when gene flow impedes local adaptation.

1.4 Artificial selection in Drosophila

Studies of experimental evolution and artificial selection on model organisms, such as *Drosophila*, have proven to be an effective means of establishing causal links between controlled selective pressures and evolutionary responses, thereby bolstering our understanding of such processes (e.g. Gibson *et al*., 1979; Rose and Charlesworth, 1981; Chippindale *et al*., 1998; Gibbs, 1999; Hercus and Hoffmann, 1999; Hoffmann and Harshman, 1999; Archer *et al*., 2003). For example, Djawdan *et al*. (1997) explored

whether *D. melanogaster* artificially selected for desiccation resistance exhibit lower metabolic rate under stressful (desiccation, starvation) conditions relative to non-stressful conditions. The metabolic rate of *D. melanogaster* from selected populations did not significantly differ from flies from control populations, suggesting a lower metabolic rate is not required for increased tolerance to desiccation (Djawdan *et al*., 1997). Swindell and Bouzat (2006) explored the changes in adaptive potential (the selection response to sternopleural bristle number) that occur as a result of gene flow in laboratory-reared populations of *D. melanogaster*. The authors found low levels of gene flow ($m = 0.05$; $N_e m = 1$) to increase adaptive potential by increasing bristle number following only three generations of artificial selection.

Drosophila spp. have been used as a model organism in several experimental evolution studies examining adaptive responses to desiccation selection (e.g. increased desiccation resistance) primarily because it can be executed effectively and efficiently since *Drosophila* have short generation times, high breeding success, can be easily reared and manipulated in the laboratory, and have sufficient genetic markers to measure variance in fitness-related traits (Hoffmann and Parsons, 1993; Gibbs *et al.*, 1997; Chippindale *et al*., 1998; Hercus and Hoffmann, 1999; Hoffmann *et al*., 2003; Gefen *et al*., 2006). In addition, due to the small size of most terrestrial insects including *Drosophila*, one of the biggest challenges for them in the wild is to resist desiccation stress owing to their large surface area to volume ratio (Gibbs, 2002b). Therefore, desiccation risk is of significant biological importance as it is a key factor for predicting the abundance and distribution of *Drosophila* species in the wild – species that are

restricted to the tropics show low levels of desiccation resistance relative to their temperate counterparts (Kellermann *et al*., 2009; Van Heerwaarden *et al*., 2009).

1.5 Physiological strategies to increase desiccation tolerance in Drosophila

Physiologists have long been interested in how organisms maintain water balance in order to thrive in extreme desert-like environments (Hadley, 1994; Gibbs, 2002b). The physiological means of surviving water loss under desiccating conditions, and therefore the strategies to increase desiccation tolerance in insects are relatively well-understood (Chown and Nicholson, 2004). In the wild, insects from warmer, drier environments are known to exhibit adaptive differences in water balance compared to their mesic counterparts, such as reduced cuticular permeabilities and reduced excretory water loss (reviewed by Hadley, 1994). In *Drosophila melanogaster*, these mechanisms of surviving water loss are not mutually exclusive and may consist of 1) carrying more water (as bulk or metabolic water), 2) tolerating losing more water, or 3) reducing the rate at which water is lost (Gibbs *et al*., 1997; reviewed by Archer *et al*., 2007). Variation in desiccation resistance among *Drosophila* species has been attributed to differences in body size, rates of water loss, as well as glycogen reserves (Hoffmann and Parsons, 1989a; Hercus and Hoffmann, 1999). *Drosophila melanogaster* in the wild show a substantial amount of variation in desiccation resistance as well as in the strategies of water balance (e.g. Kellermann *et al*., 2009), and *D. melanogaster* that have evolved resistance to desiccation stress as a result of intense artificial selection increase bulk water content before and reduce water loss rates during exposure to desiccation stress to evade impending water loss (e.g. Gibbs *et al*., 1997; Chippindale *et al*., 1998; Bazinet *et al*., 2010).

Increasing initial water content (carrying more water) is achieved in part by increasing hemolymph volume (Hadley, 1994) and by accumulating glycogen stores as one molecule of glycogen can bind 3-5 times its mass in water, and therefore by storing more glycogen, a fly would be able to store more water (Gibbs *et al*., 1997; Folk *et al.*, 2001; Gibbs, 2002b). While water that is bound to glycogen is expected to be a more important water resource than water found in lipids and proteins (Gibbs *et al*., 1997), bound water can only be used by the fly to extend survival under desiccation stress if glycogen is metabolized (Gibbs, 2002b). Glycogen catabolism generates metabolic water under desiccating conditions, which can also be used by the fly to extend survival, and thus the preferential metabolism of glycogen is considered an indirect mechanism of coping with dehydration stress (Gibbs, 2002b).

Increased tolerance for water loss is achieved by having less water content at death due to desiccation stress. *Drosophila* from mesic environments are expected to be less tolerant of dehydration compared to *Drosophila* from xeric environments, but dehydration tolerance in general, has received little attention (Gibbs and Matzkin, 2001). However, studies that have measured water content at death in the laboratory between flies selected for desiccation and flies that were not selected commonly did not find a significant difference in the ability to tolerate more water loss, suggesting dehydration tolerance is not plastic in *D. melanogaster* (Hoffmann and Parsons, 1993; Gibbs *et al*., 1997; Gibbs and Matzkin, 2001; Bazinet *et al*., 2010). Nevertheless, flies that show increased tolerance for water loss as a strategy for increasing desiccation resistance should have increased survival under desiccating conditions (Gibbs and Matzkin, 2001).

To lose water at a slower rate, a fly could reduce excretory water loss, moderate their cuticular permeability, or lower their respiratory water loss by reducing the metabolic rate or modifying their spiracle opening patterns (Gibbs and Matzkin, 2001; Chown, 2002; Chown and Nicholson, 2004; Bazinet *et al*., 2010; Williams *et al*., 2010). The regulation of cuticular permeability is driven by the waterproofing capabilities of the epicuticular hydrocarbons, such that longer chain hydrocarbons lead to higher melting temperatures, and thus decreased permeability and evaporative water loss (Gibbs, 2002a; Chown and Nicholson, 2004; Bazinet *et al*., 2010). For example, Gefen and Gibbs (2009) demonstrated a reduction in metabolic rate (as measured by CO_2 production) in flies exposed to acute desiccation stress, which prolonged survival under xeric conditions.

1.6 Study design and objectives

While theoretical models have generally suggested that gene flow can limit local adaptation through gene swamping (e.g. Kirkpatrick and Barton, 1997) and field studies have demonstrated that high gene flow is correlated with a lack of differentiation in range-edge populations (e.g. Bossart and Scriber, 1995; Magiafoglou *et al*., 2002), the causal links between gene flow and local adaptation have not been identified in biological systems (e.g. Slatkin, 1973). I am not aware of any study that has shown empirical evidence of how much gene flow from the range core is required to impede local adaptation to a strong abiotic stressor at the range-edge. Through experimental evolution, I addressed this question using large desiccation-selected, laboratory-reared populations of the common fruit fly, *Drosophila melanogaster* as a model system.

The primary objective of this thesis is to identify causal links between gene flow and local adaptation by selection in range-edge populations. I explored the effect that

varying levels of gene flow have on a response to desiccation stress in *D. melanogaster* using the continent-island model of gene flow where non-selected and selected populations are analogues of core and peripheral populations respectively. I assayed survival under desiccating conditions in each of the selected and unselected populations experiencing a gradient of gene flow to compare and track changes in desiccation tolerance over time. I concurrently measured the physiological strategies to increase desiccation tolerance, and therefore reduce water loss (initial water content, water content at death, and water loss rates), in response to selection. I used estimates of F_{ST} and gene flow from published field studies of wild *D. melanogaster* populations as well as modelling studies (e.g. Singh and Rhomberg, 1987; Michalak *et al*., 2001; Magiafoglou *et al*., 2002; Alleaume-Benharira *et al*., 2006) to inform the migration rates, and to test five levels (0% to 13% of the total effective population size) of migration between non-selected and selected populations under a constant strong selection pressure.

I tested the hypothesis that gene flow impedes local adaptation in selected, range-edge populations. I predicted that high levels of gene flow (e.g. m = 13 %) from core (non-selected) populations will prevent a response to selection in peripheral populations, and low levels of gene flow (e.g. m = 0.7 %) will fuel a response to selection. Between m = 6.7 - 13 %, I predicted that a threshold level of gene flow, above which no adaptation to an environmental selection pressure will occur and when the level is exceeded, capacity for adaptation will likely remain constant (Hartl and Clark, 2007; Gomulkiewicz *et al*., 1999). Therefore, above a certain level of gene flow, the beneficial effects (e.g. through increased genetic variation) in response to selection will likely be overcome by the

negative effects (e.g. through gene swamping) thereby inhibiting local adaptation (Lenormand, 2002).

CHAPTER 2: Materials & Methods

2.1 Fly rearing

Thirty-five isofemale lines of *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) were collected from London, Ontario, Canada in summer 2007 (Marshall and Sinclair 2010). These lines were combined into a large panmictic population (N ≈ 5,000) to maximize standing genetic variation, and to minimize the occurrence of inbreeding and genetic drift. *Drosophila melanogaster* were reared in a Percival I36VL incubator (Percival Scientific Inc., Perry, IA) at the University of Western Ontario on a three-week schedule for 17 non-overlapping generations under summer conditions (22 °C, 50 ± 5 % RH, 14L:10D light cycle) until January 2010. The population was then transferred to Sanyo MR-153 incubators (Sanyo Scientific, Bensenville, IL) at 27 °C (60 ± 5 % RH, 14L:10D), reducing generation time to eight days for the remainder of the experiments.

Flies were mass-reared following methods described by Gefen *et al*. (2006). Pre-adult stages were reared at densities of 70-90 larvae per 35 ml vial on ~10 ml of a cornmeal-sucrose-yeast medium (see Appendix 1 for composition). On the eighth day following egg collection, adult flies (approximately 1-2 days post-eclosion) were transferred to a 3.8 L clear plastic population cage (23 cm × 15 cm × 13 cm) with a medical stockinette closure to allow access to the cage. The population cage was supplied with daily changes of Petri dishes containing approximately 35 ml food medium supplemented with a small amount (~ 7 ml) of a paste of active yeast mixed with distilled water to encourage oviposition. On the third day after transferring flies to the cage, the cage was prepared for egg collection by cutting the food in the Petri dish into six equal

pieces, putting half of the pieces onto the lid of the Petri dish, to increase surface area for egg laying and because flies preferentially lay eggs on the vertical surfaces of the food. After approximately 16 hours, eggs were collected. The food was sliced into cubes each with 70-90 eggs and placed into fresh media vials (1 cube per vial and 70-100 vials per population) to found the next generation. Initially, eggs were counted under a dissecting microscope, but afterwards numbers were checked regularly by counting pupal cases from 5-10 vials/population/generation.

2.2 Experimental design overview

The experiments and the study design were intended to determine the extent to which gene flow can limit local adaptation in model range-edge populations, while controlling for the selection intensity, the level of gene flow, and the environmental conditions under which *D. melanogaster* was reared. Populations selected for desiccation resistance received varying levels of gene flow from an unselected (core) population, which permitted for direct comparisons of the effect of gene flow on the response to selection among all populations (selected and unselected). To measure desiccation tolerance and compare the responses of gene flow and selection treatments among the tested populations, survival under desiccating conditions was tracked over the course of the experiment in range-edge populations receiving a gradient of migration from the unselected core population. The physiological strategies of surviving water loss and therefore the strategies of increasing desiccation tolerance were concurrently assessed via measures of initial water content, water content at death, and water loss rates. In addition, the potential for females exhibiting a mate-choice preference among migrant and resident flies was also assessed for each selected population.

The initial large outbred population was used to found seven new populations. Twenty vials (70-90 eggs per vial) were used to start each new population. Each population was then expanded over 2-3 generations to a population size of approximately 5000 flies before initiation of experiments and sampling. These seven separate, isolated populations were allocated to one of five treatment (T) populations and two control populations (C; see Table 1). One of the control populations (0C) experienced no migration or selection, and acted as the source ('core') population for migrants. The second control population (13C) experienced high (13 %) migration and no selection. The five treatment populations were selected for desiccation resistance in alternate generations, coupled with migration from the core population. The males and females from each population experiencing migration were representative of those in a peripheral population that is subject to elevated selection pressures. Throughout the experiment, populations were maintained at 4500 ± 500 flies per population by adding the same number of vials containing approximately equal number of flies to each population cage. Since it takes approximately eight laboratory generations or selection events for desiccation resistance to be detected with 85 % intensity (Hoffmann and Parsons, 1989b), populations were followed over 12 desiccation selection events (over 24 generations; Fig. 2). Afterwards, populations were maintained without selection for three additional generations and then measures quantifying desiccation tolerance and the accompanying strategies of reducing water loss were performed again to control for any maternal effects (Hoffmann and Parsons, 1989b; Gibbs, 1999; Fig. 2). Populations were then maintained for an additional five generations without selection (32 generations total) and desiccation survival as well as the responses to selection were measured again for a

Table 1. Experimental design of control and treatment *D. melanogaster* populations as outlined for the migration gradient following an initial selection event causing mortality to 85 % of the population (N ≈ 5000). Rates of migration are fixed, but number of migrants moving into a given population per generation (N_em), and F_{ST} , the measure of genetic differentiation in populations, vary depending on the effective population size from the previous generation. Values of N_e, N_em, and F_{ST} are hypothetical estimates (grey) based on precise, 85 % selection and a population size of exactly 5000 individuals. Values of N_e, N_em, and F_{ST} from selected populations are actual estimates (black, last three columns) based on generation one mass selection data (varying N).

Population	**Population Label**	**Selection (Y/N)**	**Migration rate (*m*)**[a]	N_e	N_em[b]	F_{ST}	N_e	N_em[b]	F_{ST}
1	0C	N	0	5000	0	1	5000	0	1
2	13C	N	0.13	5000	650	0.000384	5000	650	0.000384
3	0TS	Y	0	750	0	1	645	0	1
4	0.7TS	Y	0.007	750	5	0.0476	435	3	0.0769
5	3.3TS	Y	0.033	750	25	0.00991	471	16	0.0154
6	6.7TS	Y	0.067	750	50	0.00498	1239	83	0.00310
7	13TS	Y	0.13	750	98	0.00254	1349	175	0.00143

[a] – Based on initial effective population size, N_e = 750, for selected populations.

[b] – Values were rounded to the nearest whole number.

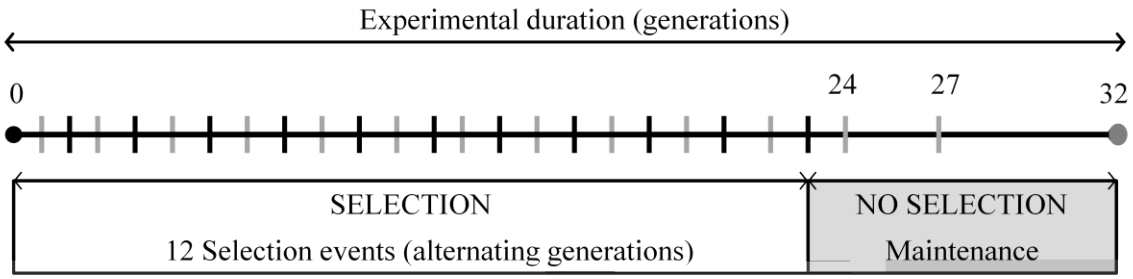

Figure 2. Timeline of study design highlighting when the selection events (black tick marks) took place beginning with generation zero, as well as when sampling for desiccation tolerance and migrant introductions happened (grey tick marks) culminating with generation 32. Numbers on top of timeline represent generations. Grey line at generation 27 represents sampling for maternal effects.

final time using a larger sample size (n = 50 flies/sex; Fig. 2). Thus, the final measurements reflected genetic local adaptation rather than phenotypic plasticity.

Desiccation tolerance was assayed using the descendants of the survivors from a previous desiccation selection event for each selected population, where 4-5 randomly chosen vials containing adult flies were set aside. Desiccation tolerance was quantified via individual (20-22 flies/sex/generation/population) measures of survival under desiccating conditions, in alternate generations to the selection events, but before the introduction of migrants. To determine what the physiological response to selection was, initial water content, water content at death, and rates of water loss were measured gravimetrically.

Mate choice assays were conducted to ensure that there was no component of sexual selection acting on the mate-preference of migrants, which could confound the effect of selection for desiccation resistance. Briefly, this procedure determined if the migrant (an unselected female) shows mate preference for selected or unselected male flies based on whether or not selected flies were no more or less attractive than unselected flies. An assay using selected females instead of unselected females was also performed to determine if selected females exhibit male mate preference.

2.3 Desiccation selection

Before initial fly populations were divided for experimental use, a brief experiment was performed to ensure that populations of flies used in the desiccation selection methods described below were dying as a result of dehydration and not due to starvation. Two separate populations were created from two subsets of 2-3 day old adult flies and expanded (N = 4500 ± 500 per population) to assess survival of a population that

is starved compared to one that is dehydrated. The starved population was given non-nutritive agar, while the dehydrated population was exposed to silica gel desiccant as per the desiccation selection protocol, and mortality was assessed hourly until approximately 85 % of each population was dead.

Populations of 3-4 day old *D. melanogaster* were subjected to desiccation selection two days after flies were transferred to population cages as performed after Gefen *et al*. (2006). Food plates were removed, and a stockinette-covered dish with ~200 g of silica gel (4-10 mesh; J.T. Baker, Phillipsburg, NJ, USA) desiccant was added. The open end of the cage was loosely covered with Parafilm (Pechiney Plastic Packaging, Menasha, WI, USA) to allow some gas exchange, but preserve low humidity. In the first selection event for each treatment population, approximately 85 % of the population (initially $N \approx 5000$ flies) was killed (presumably by desiccation), at which point the desiccant was immediately removed and replaced with a Petri dish of food and yeast paste. The time taken for 85 % mortality to occur was recorded for each population and was used for subsequent selection events for the remainder of the test generations. Thus, the ability to survive desiccation stress was not becoming proportionately more difficult for migrants introduced into an already-adapted population, over time.

At the conclusion of each selection event, dead flies were extracted from the population cage using an aspirator, transferred into pre-weighed micro-centrifuge tubes and weighed (± 0.5 μg; MX5 microbalance, Mettler Toledo, Columbus, OH, USA). The total mass of the dead flies ('non-survivors') divided by the mean mass of a fly that was killed by desiccation (0.570 mg, determined from preliminary experiments), provided the approximate total number of flies killed by the imposed selection pressure assuming a 1:1

sex ratio. A random sample of the extracted, dead flies (n = 100) were then sexed to determine the actual sex ratio, which in addition to the estimated total number of flies killed by selection, was used to estimate the proportion of each sex killed following each time-constant selection event.

Following selection, flies were given one day to recover with access to food before eggs were collected to found the next generation. Once eggs were collected, population cages were placed in a freezer to kill the surviving flies. The approximate number of flies that survived the selection process ('survivors') as well as the sex ratio was determined (as per the methods used with the non-survivors above), and used to determine N_e, and later, N_em, in subsequent generations. Prior to selection, all populations were assumed to have an equal sex ratio (Bodmer and Edwards, 1960). However, after selection, a non-Fisherian model for unequal sex ratios was used to calculate N_e, the effective population size, in each generation for each population,

$$N_e = 4N_mN_f/(N_m+N_f) \quad [2]$$

where N_m and N_f are the number of males and females respectively (Hartl and Clark, 2007). The mass of a random sample (n = 100) of male and female flies from the surviving population were again calculated providing an estimate of mass as well as the sex ratio.

2.4 Migrant introduction

Gene flow, as a result of dispersal, was calculated as the absolute number of migrant individuals (N_em) that have moved from range core to range-edge populations, where the migration rate (m) is the probability that an individual is an immigrant and N_e is

dependent on the sex ratio of the flies that survived each selection event (Hartl and Clark, 2007). To establish the migration gradient, the fixation index (Wright, 1931), F_{ST} was used to ensure that the range of migration rates spanned the range of known F_{ST} values for *Drosophila* (Singh and Rhomberg, 1987; Hartl and Clark, 2007). Estimates of F_{ST} values for natural populations of *Drosophila* species, such as *D. melanogaster* and *D. pseudoobscura,* are low (between 0.04 and 0.2; Singh and Rhomberg, 1987). Under the island model of migration where a large population splits into several subpopulations and there is random migration between the separate populations, this observed pattern of low F_{ST} values is, in part, explained by the strong exponential decay relationship between F_{ST} and the number of migrant organisms per generation, N_em (Hartl and Clark, 2007). This relationship between gene flow and F_{ST} was taken into account when designing the migration gradient in this study by having smaller increments between the low levels of gene flow compared to high levels to account for large variation in F_{ST} with small fluctuations in N_em (Table 1; Morjan and Rieseberg, 2004; Hartl and Clark, 2007). Under ideal conditions the upper limit of gene flow (m = 0.13) should approximate an F_{ST} of zero and there should be genetic homogeneity between core and peripheral populations (Alleaume-Benharira *et al*., 2006). The island model does not incorporate selection (Hedrick, 2005), and therefore was only used to initially inform the migration gradient and was not used to compare values of F_{ST} among selected lines.

Migrants were the virgin females from the unselected, core (0C) population. For each migrant, three virgin males were randomly extracted from a subset of vials from each population experiencing migration, before the remaining vials were transferred to their respective cage, to introduce to a fresh food vial each containing a single unselected

virgin female (the migrant) from the core population and allowed for mating to occur over two days. A mating ratio of 3:1 (M:F) was used to allow and satisfy female choice. For higher levels of gene flow (> 3.3 %), the same mating ratios (3:1 M:F) were set up in population cages instead of vials primarily because cage rearing is more manageable with a large number of flies (> ~200 flies). After two days, parents (the migrants) were discarded and eggs are collected from the population. When cages were used, the full reproductive effort in eggs produced was collected and combined with a small proportion of eggs (10-15) apportioned from the respective selected population to ensure consistent egg densities in every food vial (see Fig. 3), but still guaranteeing that the bulk of the offspring were offspring of the migrants. Depending on the number of migrants, 15 - 35 vials worth of eggs were collected, such that populations with a relatively large number migrants in a given generation would require more vials (e.g. 35) for migrant egg collection than a population with a lower respective number of migrants (e.g. 15 vials). After eight days of incubation, the newly-eclosed adult flies along with the newly-eclosed offspring of the migrant matings were transferred to a population cage for the second selection event thus ensuring the introduction of migrant genes. After two days of allowing the migrant flies to mate with the resident population flies, mass desiccation selection was performed as above (Fig. 3).

2.5 Response to desiccation selection and analysis of resistance

The change in desiccation tolerance and the physiological strategies responsible for increasing desiccation tolerance by reducing water loss (initial water content, water content at death, and water loss rates) were measured for each population over time. Initial water content was assayed gravimetrically before each desiccation selection event

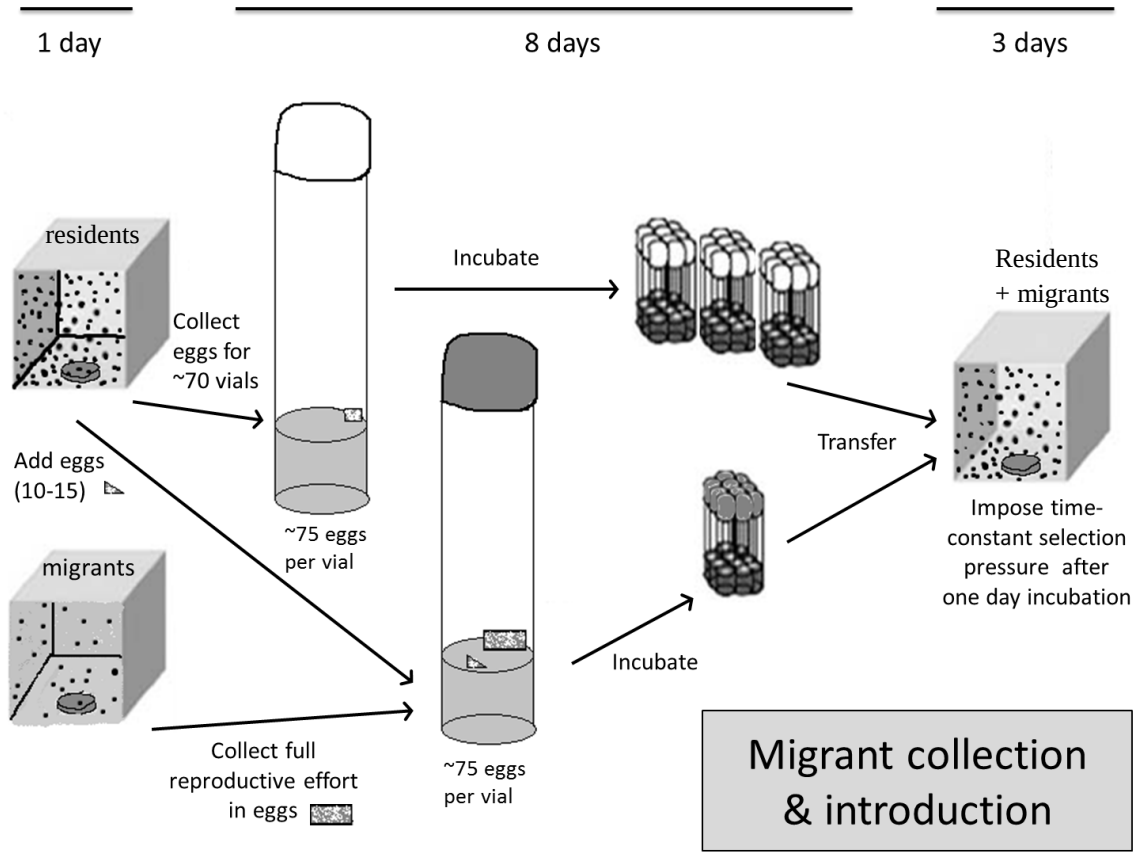

Figure 3. Experimental protocol and timeframe for migrant collection and introduction of *D. melanogaster* into populations (light grey boxes) receiving migrants. Flies always remained in cages for three days with selection performed every other generation on day two (see text for full description). One hundred vials of eggs were collected for each population regardless of the amount of migrant eggs produced. Dark grey tops indicate migrant vials, while white tops indicate resident population vials. Flies were incubated at 27 ± 0.5 °C.

using different flies than those used in the desiccation assay to determine final water content. Water content at death was measured using the same flies subjected to the desiccation assay where survival time was measured. Predicted water loss rates were calculated for each fly by first performing a linear regression between initial water content and dry mass for each population and sex in order to predict initial water content for every fly used in the desiccation assay. Predicted initial water content (y) was calculated for each fly using a standard linear equation ($y = mx+b$), where m is the slope from the regression between initial water content and dry mass for each population, x is the dry mass from each fly used to determine water content at death, and b is the intercept from the initial water content – dry mass regression. An estimated amount of water lost (predicted initial water content – water content at death) as well as an estimate of the water loss rate (amount of water lost/survival time) was then calculated.

To determine initial water content, a few randomly chosen vials of 1-2 day old adult flies from each population and for each generation were combined in food vials and snap frozen in liquid nitrogen vapour thereby killing the flies and preventing freezer burn. Flies were then thawed to room temperature, sexed (20 flies/sex), and immediately weighed (wet mass) for measurements of initial water content as described by Gibbs *et al*. (1997). The flies were then dried overnight at 60 °C in an oven (Thelco Model 15, Chicago, IL, USA) and weighed again (dry mass). Initial water content was calculated as the difference between wet mass and dry mass (Gibbs *et al*., 1997).

Desiccation resistance was measured after the method of Gibbs *et al*. (1997). A subset of 4-5 vials containing adult flies were individually transferred and separated by sex under light CO_2 to food vials (1 fly/vial × 20 replicates) between 12-20 hours

following eclosion. Flies were allowed 48 hours to recover from CO_2 anaesthesia (Nilson *et al*., 2006) and then were transferred to empty 35 ml plastic fly vials where they were restricted to the bottom half of the vial with foam stoppers. Approximately three grams of fresh silica gel was added above the stoppers, and the vials were then sealed with Parafilm to establish and maintain low humidity (Gibbs *et al*., 1997; Gefen *et al*., 2006). An iButton hygrochron (Maxim Integrated Products, Sunnyvale, CA, USA), revealed that, after being sealed, relative humidity of a vial steadily dropped to 30 % within the first 30 minutes, and 5 % within 90 minutes. Vials were placed in an incubator set at 29 °C and mortality was assessed every hour for the first four hours and then every 30 min until all flies were dead. Survival assays were consistently performed at 29 °C instead of 27 °C for feasibility purposes. The first time to death for each fly was also recorded. Flies that could not stand or right themselves when the vial was shaken were scored as dead (Gibbs *et al*., 1997; Gefen *et al*., 2006). Dead flies were immediately transferred to individual 1.7 ml micro-centrifuge tubes and snap frozen in liquid nitrogen vapour. Approximately three minutes later they were thawed to room temperature, and gravimetric water content was determined as described above providing water content at death (Gibbs *et al*., 1997).

2.6 Mate-choice assays

Female mate-choice assays were designed to mimic the conditions experienced in a population cage prior to and after desiccation since mating rarely occurs during desiccation stress (Chippindale *et al*., 1998; Kwan *et al*., 2008). Subsets of flies from 5-6 vials were set aside following the tenth selection event of each population. Flies were sexed as virgins under light CO_2 anaesthesia and during this time, the distal part of the

male wing was clipped using a scalpel in a cross-pattern in order to differentiate between the two males in the vial (Averhoff and Richardson, 1974). To control for potential bias in female preference for wing clipping, both males (selected and unselected) were used such that half of each group had clipped wings (Skroblin and Blows, 2006). For each population, 20 replicate vials were used, where each replicate consisted of one unselected virgin female (the migrant), one selected virgin male, and one unselected virgin male. To ensure flies had not mated prior to the experiments, all flies used in the mate-choice assays were isolated by sex <12 h post-eclosion (i.e. before they were reproductively viable), and were maintained in food vials in an incubator at 27 °C until flies were 5-6 days old. The twenty 35 ml glass vials for each treatment population were prepared by heating them at 80 °C for 3 h to ensure they were hydrocarbon-free and sterile (Chenoweth and Blows, 2003). Flies (1 unselected female, 1 selected male, 1 unselected male) were transferred into each vial, plugged with a cotton ball, and the start time recorded. The proportion of selected vs. unselected flies chosen as a mate by the unselected female was measured. Females were observed for 45 minutes or until a male successfully mated. When copulation commenced, the copulating pair (or at least, the copulating male) was aspirated out of the vial to determine which male was chosen and which male was rejected (by the presence or absence of a wing clip). If mating did not occur within 45 min, the replicate was discarded. Mate-choice preference was also examined in selected females under the same experimental protocol as described above.

2.7 Statistical analysis

All statistical analyses were conducted in R (v. 2.13.2, R Development Core Team 2009). All analyses were performed separately on each sex to simplify interaction terms

in the models since female flies have much longer survival time and greater water content compared to males (e.g. Gibbs *et al*., 1997). A minimally adequate model (Crawley, 2005) was produced for each analysis by dropping terms when $P > 0.05$, except when comparing models with the same terms, but different distributions, as with the models of survival time. Data from a final desiccation assay (50 flies/sex instead of 20 flies/sex) performed in generation 32 (after 12 selection events performed every second generation and 8 generations of maintenance; Fig. 2) was used to determine the effect of gene flow and selection on desiccation tolerance and the strategies for reducing water loss.

The effect of selection for desiccation resistance (mean time to death under desiccating conditions) was compared among gene flow treatments using accelerated failure time (AFT) models built in R using the survreg() function in the Survival package. Mean survival time predicted from AFT models take into account non-normality of distributions. Models using exponential, extreme, Gaussian, logistic, and Weibull distributions were compared and the best-fitting model for the survival distribution was chosen using Akaike's Information Criteria (AIC). For every survival comparison the Gaussian distribution always had the lowest AIC. Survival time for each of the seven populations was directly compared to each other as a single level predictor by grouping the model factors gene flow and selection. Once the model distribution with the best fit to the data was determined, population effects on survival time were compared for each sex. All *post-hoc* comparisons of mean survival time were performed using Tukey's HSD with the glht() function in the Multcomp package (Hothorn *et al*., 2008).

Initial water content, water content at death, and rates of water loss were separately compared among populations and between the sexes with general linear

models. Since dry mass (body size) was strongly correlated to all measures of water content (pre- and post-desiccation) and because dry mass differed significantly among populations leading to significant, non-interpretable, higher order interactions with population, all analyses of water content (including predicted water loss rates) were performed using the residuals of a regression between water content and dry mass. As above, measures of water content for each of the seven populations was directly compared to each other as a single level predictor by grouping the model factors gene flow and selection. Tukey's HSD was used to make all *post-hoc* comparisons of initial water content, water content at death, and water loss rates with the glht() function in the Multcomp package (Hothorn *et al*., 2008).

The mean survival time under desiccating conditions, mean initial water content, and mean water content at death were compared between the sexes by a two-sample unpaired Students *t*-test using data from the final generation of sampling for desiccation resistance to show how much more tolerant of desiccation female flies are relative to males.

Female (unselected and selected) mate-choice was compared separately among the categorical variables gene flow and selection, as well as gene flow and wing-clipped, using binomial regressions. There was no *a priori* reason to suspect gene flow would have an effect on migrant mate-choice preference in this experimental design so gene flow served a replication role in this analysis.

Maternal effects were examined by comparing survival time between generation 24 and 27 to see if the phenotype expressed in generation 27 reflected that expressed in

generation 24 following three consecutive unselected generations (Fig. 2). Survival time was compared between the two generations for each population and sex using the same AFT model and procedure described above.

CHAPTER 3: Results

When exposed to starving conditions, it took a single population (N = 4500 ± 500) of flies 450 min longer to reach 85 % mortality (840 min) than flies from another population subjected to desiccating conditions (390 min) as per the methods of selection.

For each of the five selected populations, the time taken for approximately 85 % of the flies to die in the initial generation (Table 2) ranged from 385 min to 405 min (mean = 390 ± 5 min). The proportion of females and males that survived desiccation did not significantly differ over 24 generations for the population experiencing gene flow of 3.3% ($F_{1,10}$ = 3.43, P = 0.094), 6.7% ($F_{1,11}$ = 4.73, P = 0.053), or 13% ($F_{1,9}$ = 0.106, P = 0.752); however, there was a significant increase in sex ratio for the population experiencing 0% gene flow ($F_{1,10}$ = 6.70, $P < 0.05$) and 0.7% ($F_{1,10}$ = 8.13, $P < 0.05$).

In all cases, female flies survived desiccation significantly longer than male flies (Fig. 4). For example, in the final generation (generation 32) of sampling for desiccation resistance, the mean survival time under desiccating conditions of females (505 ± 5 min) from the core (no selection, no gene flow) population was significantly greater than the mean survival time of males (346 ± 5 min) from the same population (t_{96} = 10.9, $P < 0.001$). Similarly, the mean survival time for a selected male (365 ± 2 min) from 0TS was significantly less than the mean survival time for a selected female (543 ± 2 min) from the same population after 12 selection events (t_{94} = 11.3, $P < 0.001$). In a desiccation survival assay performed in the final generation of sampling for desiccation resistance, 25-35 % of females from each population remained alive when all the males had died after 7.5 hours of exposure to desiccation stress (Fig. 4). Prior to the desiccation

Table 2. The time taken to reach approximately 85 % selection (death by desiccation) for each *D. melanogaster* population (n=5) experiencing a gradient of gene flow (% of the effective population size) in generation zero. The estimated absolute population size is also presented for each population.

Gene flow (%)	**0**	**0.7**	**3.3**	**6.7**	**13**
Population Size (N)	4500	3500	3195	3566	4000
Time to 85 % selection (min)	405	375	390	390	390

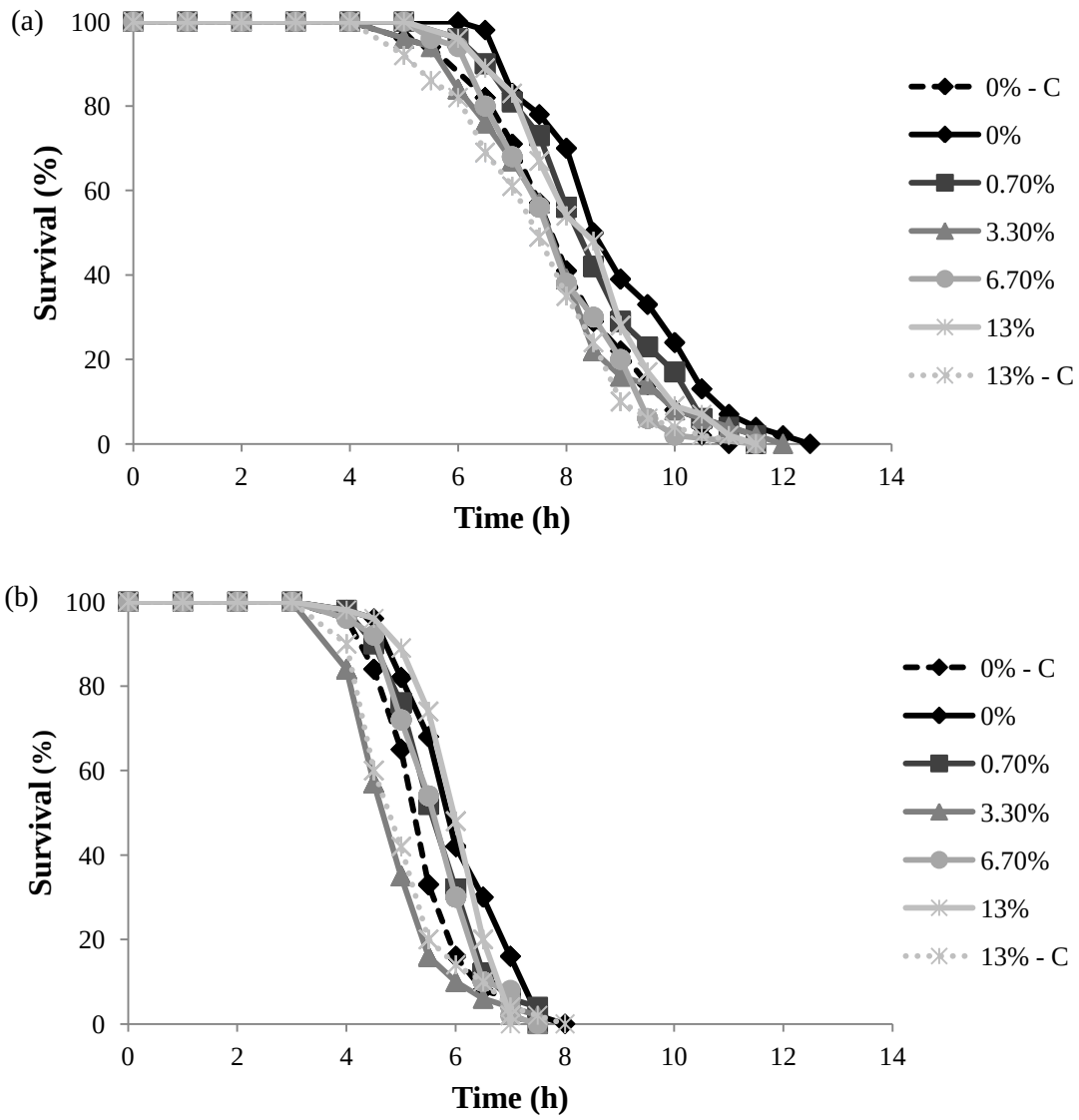

Figure 4. Survival for female (a) and male (b) *D. melanogaster* while under desiccating conditions for each selected and unselected, control (C) population experiencing a gradient of gene flow (% N_e). Data (n = 48-50 flies/sex/population) shown here were collected in the final generation of sampling for desiccation resistance (generation 32).

assay in the final generation of sampling for desiccation resistance, the mean initial water content of a female fly from the population experiencing 0 % gene flow and selection (0TS; 0.710 ± 0.014 mg) was significantly greater than the mean initial water content for a selected male fly from the same population (0.523 ± 0.008 mg; $t_{74} = 11.47$, $P < 0.001$). Following the desiccation assay in the final generation of sampling for desiccation resistance, the mean water content at death of a female fly from 0TS (0.373 ± 0.0075 mg) also had significantly greater than the mean water content at death for a selected male fly from the same population (0.230 ± 0.005 mg; $t_{107} = 16.14$, $P < 0.001$).

3.1 Desiccation survival

There was no significant effect of post-desiccation dry mass (body size) on survival time for females ($Z_{7,309} = 0.771$, $P = 0.441$) or males ($Z_{13,309} = 1.52$, $P = 0.128$) in the final of sampling. As a result, it was not necessary to control for body size in subsequent analyses of survival time and for that reason dry mass was not included in later models.

Desiccation tolerance as measured by mean survival time was significantly greater for female (by 57 ± 17 min) and male (by 39 ± 10 min) flies from 0TS relative to those from the core population (Table 3; Fig. 5). By comparison, the mean survival time of females from 0TS after only one generation of selection was 15 ± 6 min and for males was 6 ± 8 min greater than the core population. The mean survival time under desiccating conditions was not significantly different from that of the core population for populations experiencing 0.7, 3.3, and 6.7 % gene flow (Table 3; Fig. 5). However, the mean survival time under desiccating conditions for 13TS was significantly higher than the core population in males, but not in females (Table 3; Fig. 5). The population

Table 3. Results from accelerated failure time (AFT) models of survival analysis under desiccating conditions for male and female *D. melanogaster* selected for desiccation resistance from populations experiencing varying levels of gene flow. AFT models compared mean survival time for each population and sex relative to the core (0C) population. Data (n = 50 flies/sex/population) were collected in the final generation of sampling. Significant differences are indicated in bold typeface.

	FEMALES			**MALES**		
Population (% gene flow)	**Z-value**	***df***	***P***	**Z-value**	***df***	***P***
0	3.24	6,336	**<0.01**	3.87	6,336	**<0.005**
0.7	1.84	6,336	0.523	1.84	6,336	0.519
3.3	-0.425	6,336	0.999	-2.72	6,336	0.093
6.7	-0.282	6,336	0.999	1.55	6,336	0.717
13	1.53	6,336	0.725	3.49	6,336	**<0.01**
13C	-1.52	6,336	0.732	-1.85	6,336	0.515

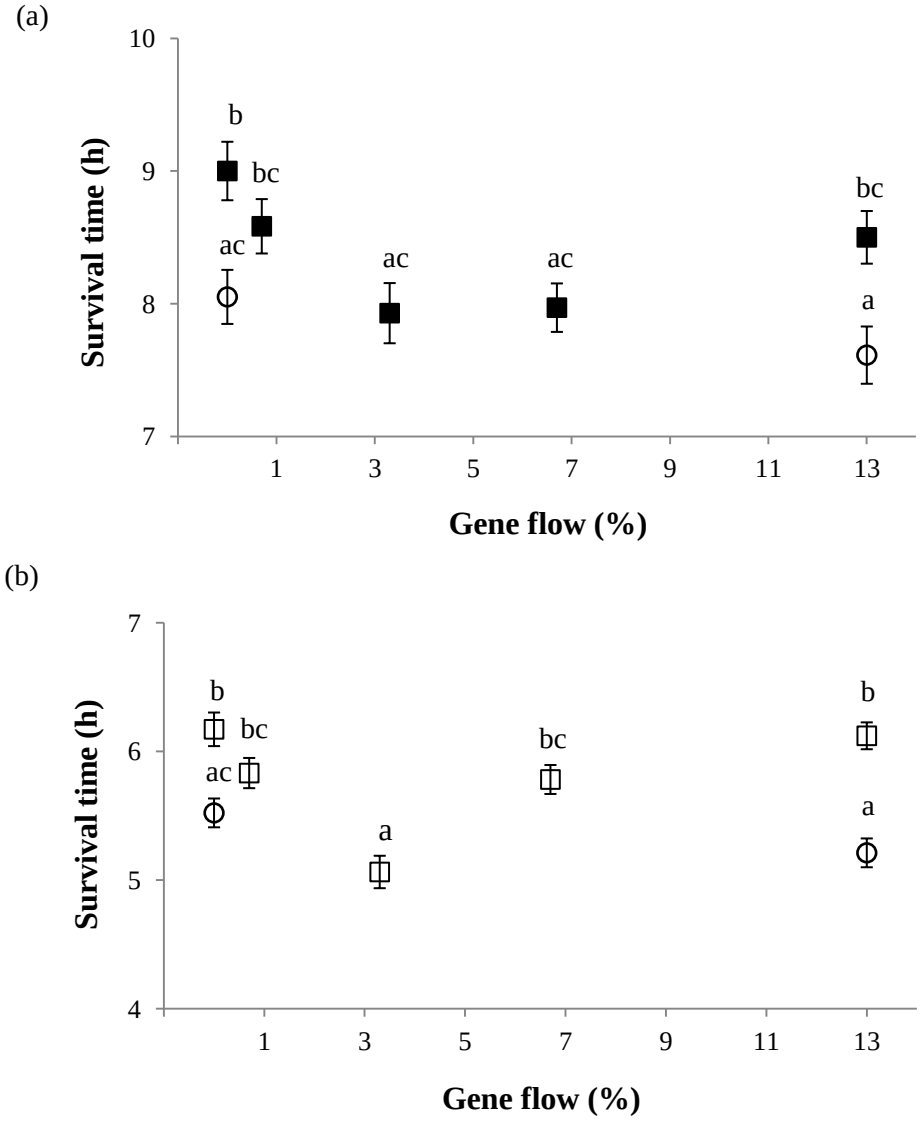

Figure 5. Survival time (mean ± SE) under desiccating conditions as a function of gene flow (% N_e) for female (a) and male (b) *D. melanogaster*. Samples (n = 50 flies/population) were collected for this analysis in the final generation of sampling for desiccation resistance. Selected populations (squares) were selected for desiccation resistance for 12 generations. Control populations (open circles) experienced no selection. Populations with the same letter are not significantly different after accelerated failure time model analysis with Tukey's HSD *post-hoc* test; $P<0.05$.

experiencing 3.3 % gene flow exhibited the lowest mean survival time for a selected population for both sexes, but its survival time was not significantly lower than either control population (Fig. 5). Lastly, there were no significant differences in survival time between the two unselected populations for males or females (Table 3; Fig. 5).

Females from populations for which mean survival time increased (particularly those receiving 0 and 0.7 % gene flow) had a distribution of survival time that was more normally distributed, while populations where survival time did not increase largely had a right-skewed distribution of survival time (Fig. 6a). This pattern held for males, although populations experiencing high gene flow (6.7 and 13 %) had an increasingly left-skewed distribution of survival time (Fig. 6b). However, despite this difference, these two populations did not survive desiccation for as long as populations experiencing low gene flow (Fig. 6b). This pattern indicates that the start and end points of death are shifting with the mean values (Fig. 4, 6). To that end, the shape of the survival curves did not differ among populations, which was a consistent pattern for both sexes; however, the time at which the first fly and the last fly died were different among the populations (Fig. 4). For example, female flies from 0TS, 0.7TS, and 13TS (three populations that exhibited high desiccation tolerance), started dying later, and survived longer than other populations (Fig. 4a).

3.2 Initial water content

There was significant variation in initial (pre-desiccation) dry mass among populations as shown by the original significantly positive relationship between initial water content and dry mass for males ($F_{6,329} = 31.4$, $P < 0.001$; Fig. 7a) and females ($F_{6,331} = 12.3$, $P < 0.001$; Fig. 7b). There was a significant effect of selection on initial

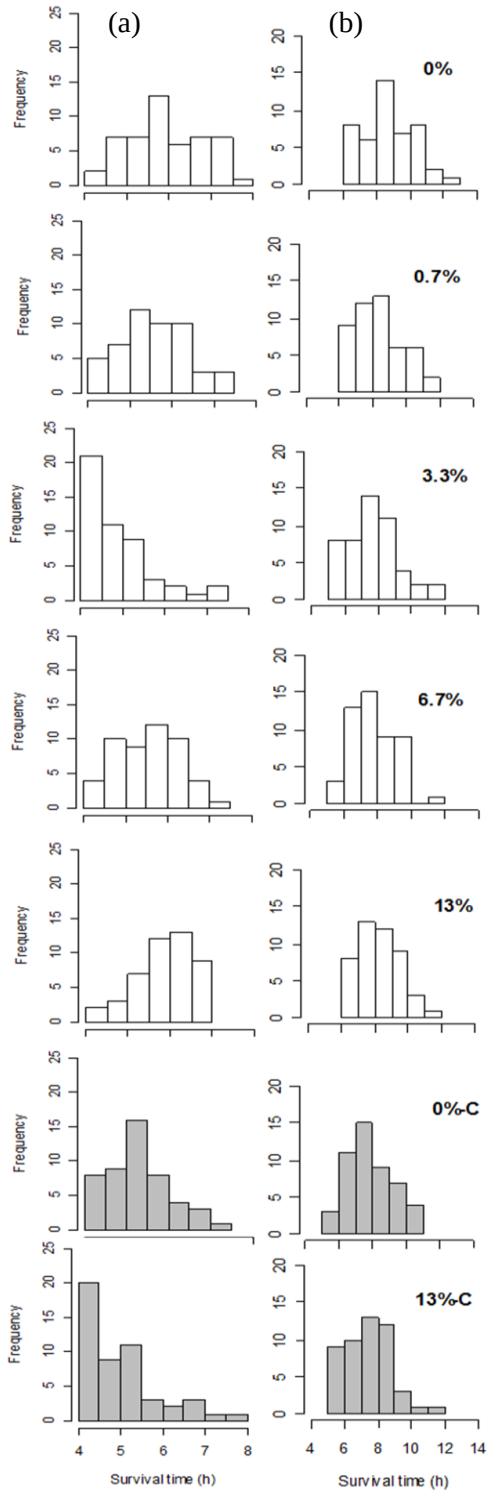

Figure 6. Distribution of mean survival time for male (a), and female (b) *D. melanogaster* under desiccating conditions for each population experiencing varying levels of gene flow (% N_e) tested in generation 32. Grey bars are unselected control (C) populations. N = 48-50 flies/sex/population.

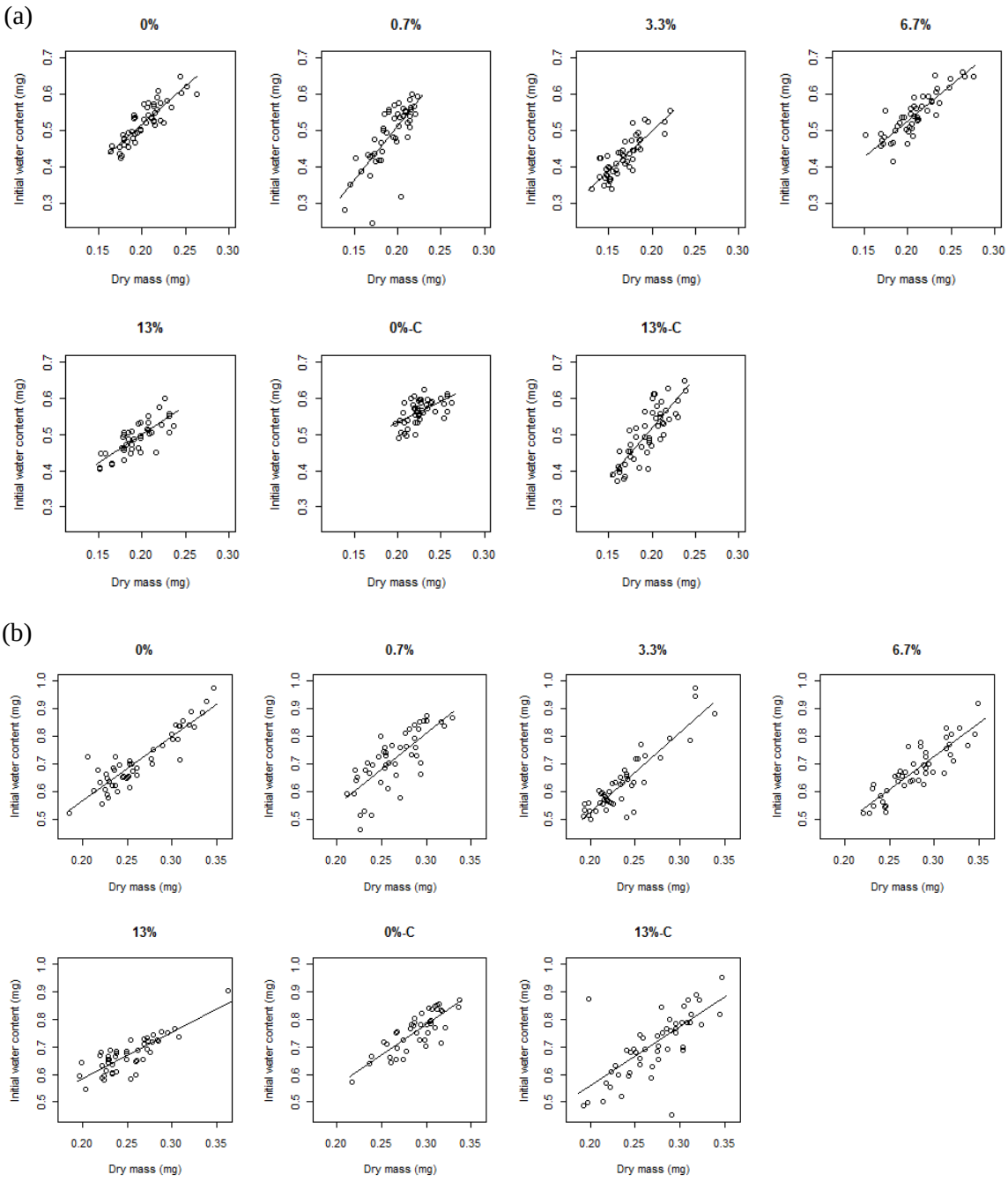

Figure 7. Linear regressions between male initial water content (a), and female initial water content (b) and dry mass. Data (n = 48-50 flies per population) displayed were collected in the final generation of assaying for desiccation tolerance (generation 32). Control populations (C) received no selection. Initial water content significantly increased with increasing dry mass for each population and sex ($P << 0.001$).

dry mass (body size) in the final generation of sampling for desiccation tolerance, such that the mean body size of females from 0TS was significantly less than the mean body size of females from the core population prior to a desiccation assay ($P < 0.001$; Fig. 8a). The body size of males from 0TS was also significantly less than the body size of males from the core ($P < 0.001$; Fig. 8b).

Initial water content did not significantly differ with selection treatment for females ($F_{1,316} = 0.482$, $P = 0.488$; Fig. 9a) or males ($F_{1,333} = 0.140$, $P = 0.708$; Fig. 9b). There was a significant decrease in dry mass-specific initial water content as the rate of gene flow increased for female flies ($F_{1,316} = 11.48$, $P < 0.001$; Fig. 9a), which was due to 6.7TS having significantly reduced initial water content relative to all other populations ($P < 0.001$). Gene flow treatments did not significantly affect dry mass-specific initial water content in male flies ($F_{1,333} = 0.470$; $P = 0.274$; Fig. 9b). With the exception of the decrease in initial water content with 6.7TS, there was no significant effect of gene flow on initial water content for females (Fig. 9a). Likewise, there was no significant effect of gene flow on initial water content for males, although 6.7TS also had increased initial water content relative to 3.3TS ($P < 0.05$; Fig. 9b). Values for mean dry mass-specific initial water content were generally unimodal and normally distributed for each sex, reflecting the lack of response of selection on initial water content (Fig. 10a, b).

3.3 Water content at death

Post-desiccation dry mass significantly varied among populations as illustrated by the significant positive relationships between water content at death and dry mass for

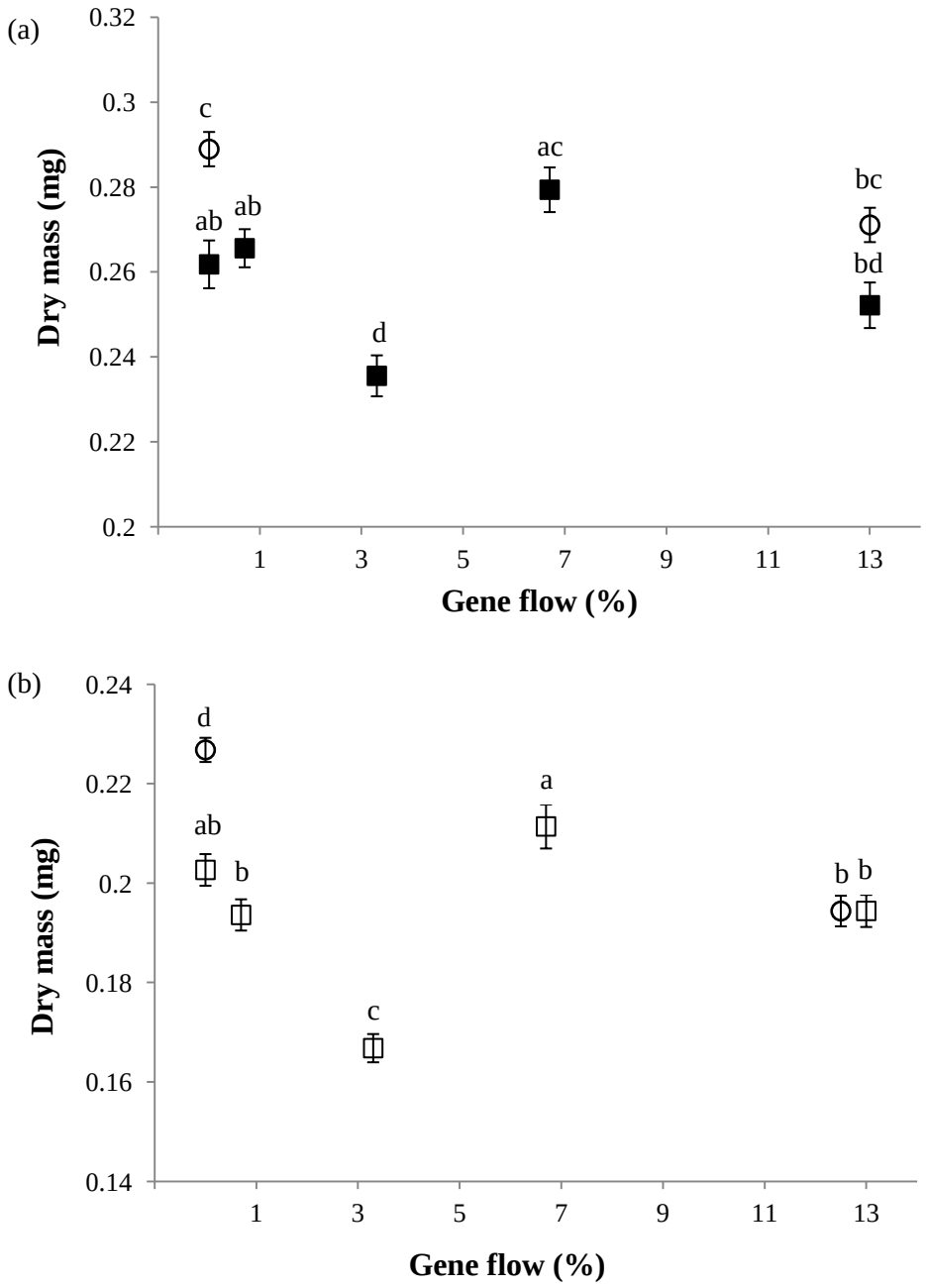

Figure 8. Female (a) and male (b) *D. melanogaster* initial dry mass (body size) prior to a desiccation assay in the final generation of assaying for desiccation tolerance for each population experiencing a gradient of gene flow (% N_e). Selected populations (squares) were selected for desiccation resistance for 12 generations. Control populations (open circles) experienced no selection and 13C (males) is offset here by -0.5 % for illustrative purposes. Populations with the same letter are not significantly different.

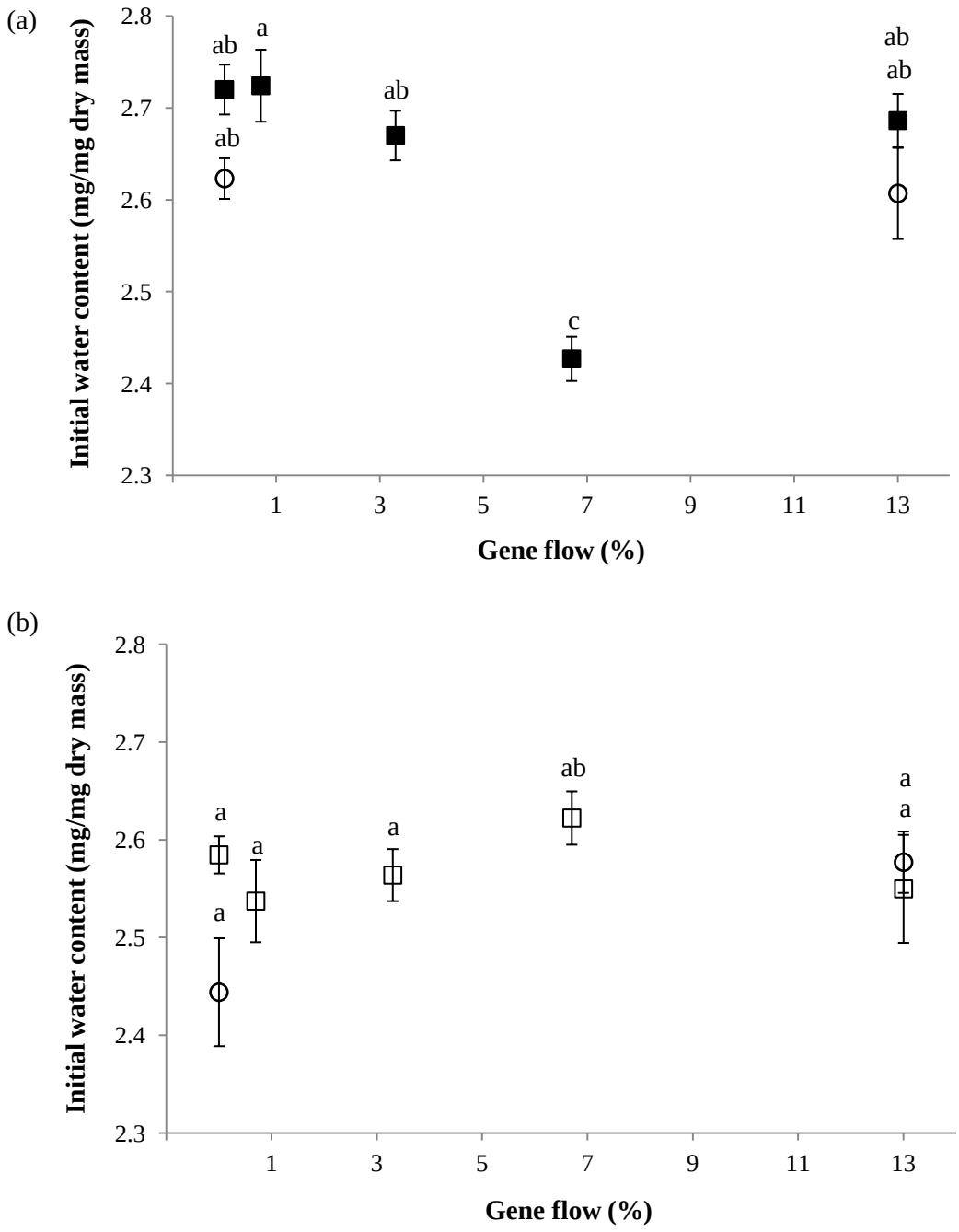

Figure 9. Mean (± SE) initial water content (mg/mg dry mass) for female (a) and male (b) *D. melanogaster* as a function of the level of gene flow (% N_e). Samples (n = 50 flies/population) were collected for this analysis in the final generation of sampling for desiccation tolerance. Selected populations (squares) were selected for desiccation resistance for 12 generations. Control populations (open circles) experienced no selection. Populations with the same letter are not significantly different.

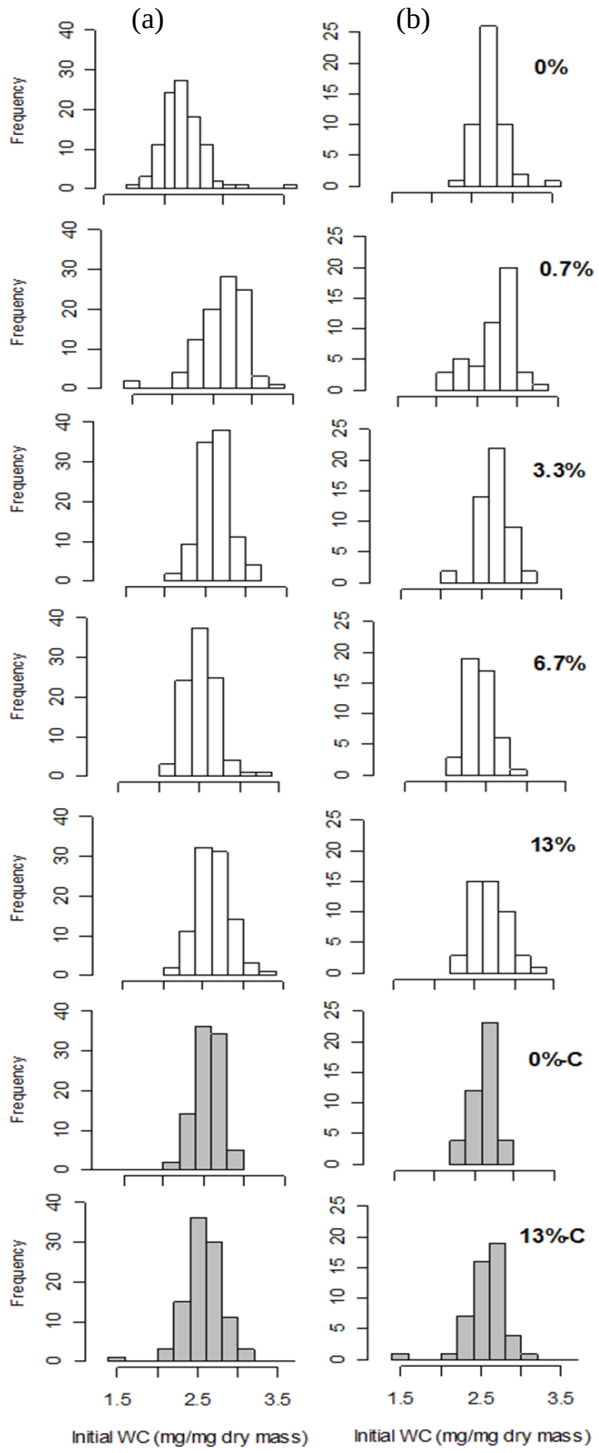

Figure 10. Distribution of mean initial water content for male (a), and female (b) *D. melanogaster* prior to a desiccation assay for each population experiencing varying levels of gene flow (% N_e) tested in generation 32. Grey bars are unselected control (C) populations. N = 48-50 flies/sex/population.

males ($F_{6,333}$ = 25.3, P < 0.001; Fig. 11a) and females ($F_{6,302}$ = 18.3, P < 0.001; Fig. 11b). Female flies from 0TS exhibited significantly greater dry mass at death compared to unselected populations following a desiccation assay (P < 0.001; Fig. 11a). There was no significant effect of selection on body size for males (P = 0.078; Fig. 12b). Further, as the rate of gene flow increased, there was a trend for decreased female body size, such that populations experiencing low (0, 0.7, 3.3 %) levels of gene flow had more dry mass following desiccation relative to populations experiencing high (6.7, 13 %) levels of gene flow (Fig. 12a).

Water content at death did not significantly differ with selection treatment for females ($F_{1,306}$ = 0.597, P = 0.44; Fig. 13a); however, water content at death was significantly higher for males from populations experiencing selection ($F_{1,337}$ = 8.06, P < 0.01; Fig. 13b), which was due to 6.7TS having significantly more water content at death relative to all other populations (P < 0.01; Fig. 13b). Dry mass-specific water content at death significantly decreased with increasing gene flow treatments for female flies ($F_{1,306}$ = 4.49, P < 0.05; Fig. 13a) and significantly increased with increasing gene flow for male flies ($F_{1,337}$ = 9.47, P < 0.005; Fig. 13b). These effects, however, were driven primarily by females from 6.7TS, which had significantly reduced water content at death and males from 6.7TS, which had significantly more water content at death relative to all other populations (Fig. 13a, b). The distribution of mean dry mass-specific water content at death was unimodal for males and females; however, there was a trend for populations that had increased survival (in particular, 0, 0.7, and 13TS) to shift from a left-skewed distribution to a normal distribution (Fig. 14a, b).

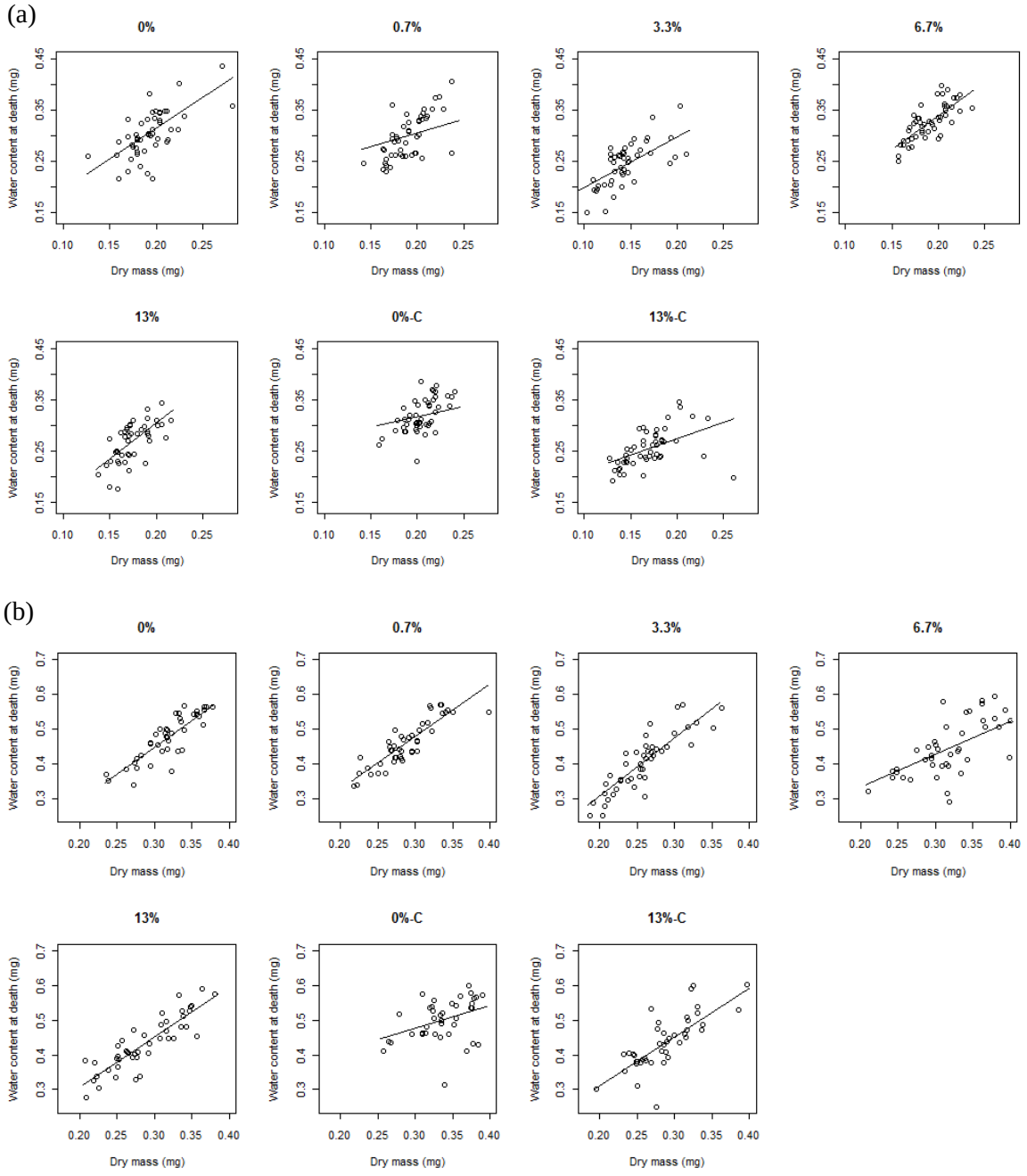

Figure 11. Linear regressions between male water content at death (a), and female water content at death (b) and dry mass. Data (n = 48-50 flies/population) displayed are from the final generation (generation 32) of assaying for desiccation tolerance. Control populations (C) received no selection for the duration of the experiments. Water content at death significantly increased with increasing dry mass for each population and sex ($P < 0.001$), with the exception of males from 0.7TS ($P < 0.05$), and females from 0C ($P < 0.05$), where there was less of an effect of dry mass on water content at death.

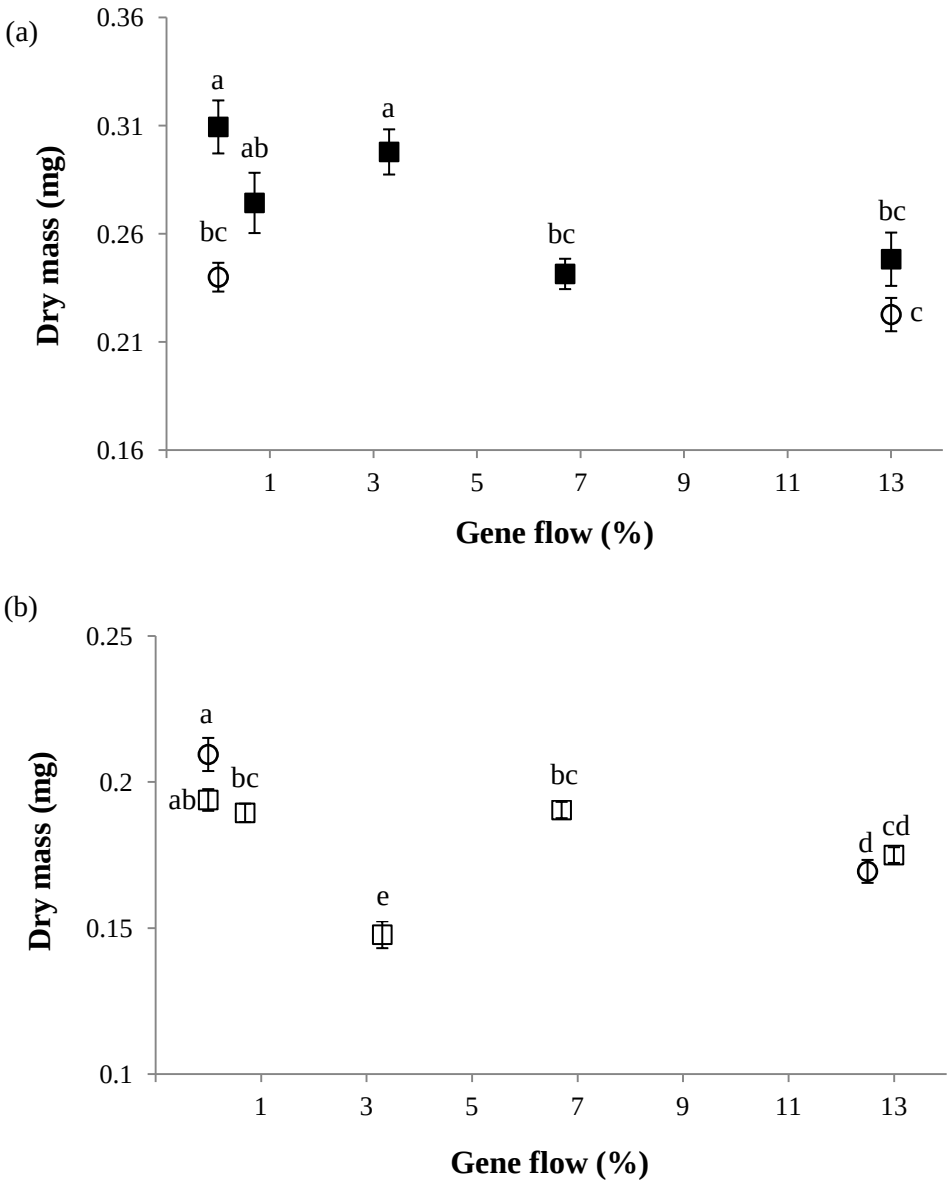

Figure 12. Female (a) and male (b) *D. melanogaster* final dry mass (body size) following a desiccation assay for each population experiencing a gradient of gene flow (% N_e). Flies were sampled (n = 50 flies/population) in the final generation of assaying for desiccation tolerance. Selected populations (squares) were selected for desiccation resistance for 12 generations. Control populations (open circles) experienced no selection and 13C (males) is offset here by -0.5 % for illustrative purposes. Populations with the same letter are not significantly different.

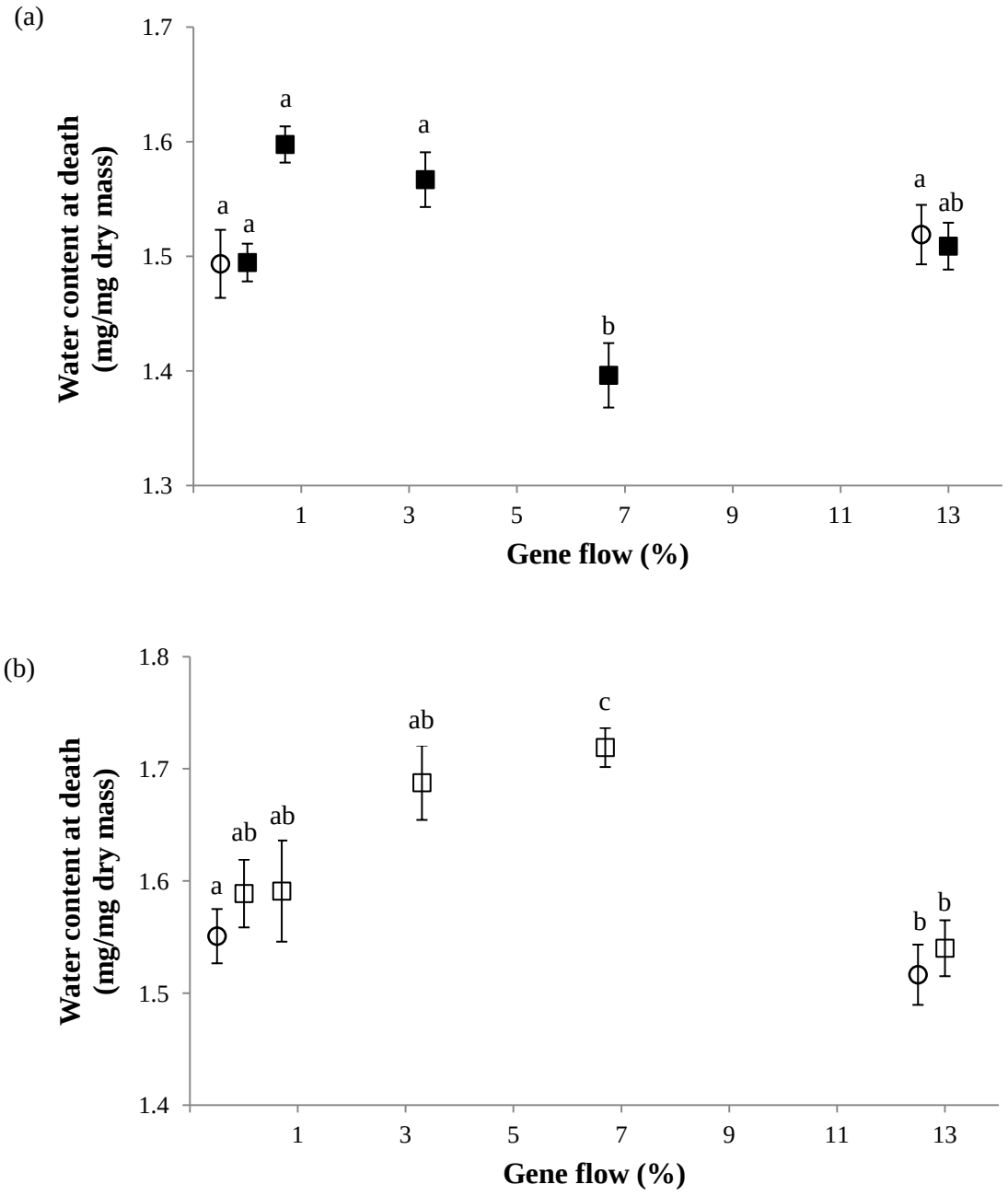

Figure 13. Mean (± SE) water content at death (mg/mg dry mass) for female (a) and male (b) *D. melanogaster* as a function of the level of gene flow (% N_e). Samples (n = 50 flies/population) were collected for this analysis in the final generation of sampling for desiccation resistance. Selected populations (squares) were selected for desiccation resistance for 12 generations. Control populations (open circles) experienced no selection and are offset here by -0.5 % for illustrative purposes. Populations with the same letter are not significantly different.

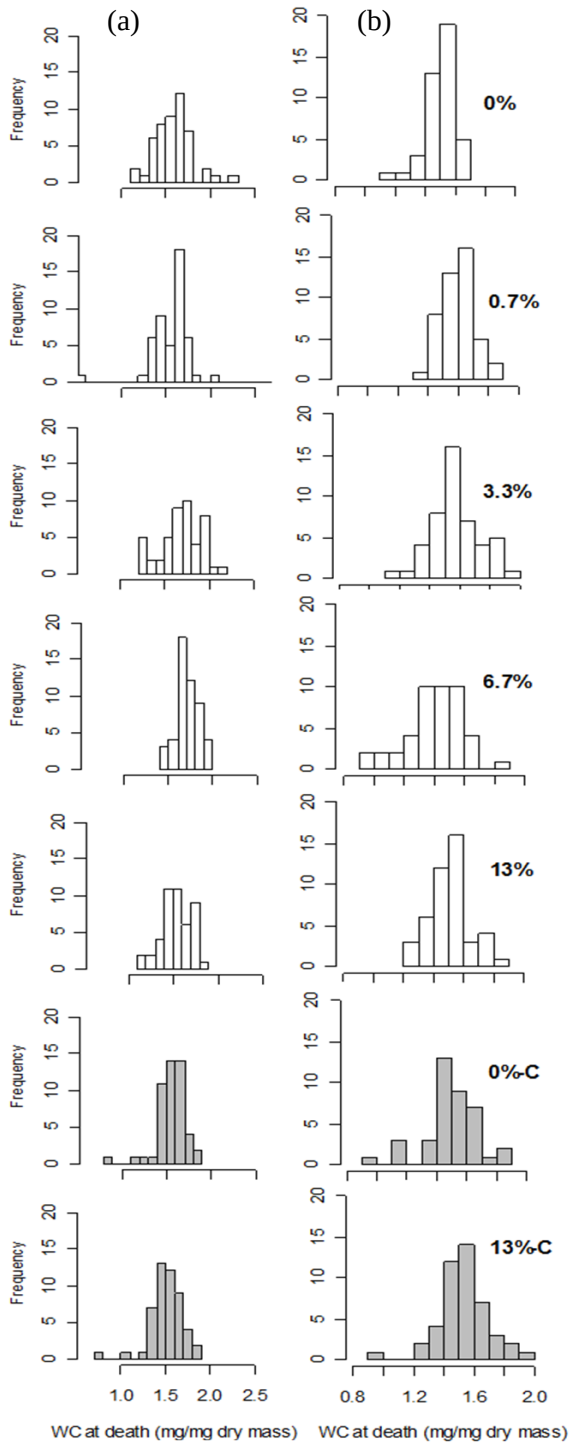

Figure 14. Distribution of mean water content at death for male (a), and female (b) *D. melanogaster* following a desiccation assay for each population experiencing varying levels of gene flow (% N_e) tested in generation 32. Grey bars are unselected control (C) populations. N = 48-50 flies/sex/population.

3.4 Rates of water loss

Predicted rates of water loss were calculated using gravimetric data collected in the final generation of assaying for desiccation tolerance. Female flies that had been selected for desiccation tolerance significantly reduced their water loss rate in all populations except 3.3TS, 6.7TS, and 13TS (Fig. 15a). This pattern was especially clear for females from populations experiencing no gene flow (0TS and 0C) – the mean predicted water loss rate for 0TS (0.0402 ± 0.018 mg/mg dry mass/h) was significantly lower than the mean predicted water loss rate for 0C (0.0459 ± 0.022 mg/mg dry mass/h; $P < 0.001$; Fig. 15a), which corresponds to the significantly lower mean time to death for females in the final generation of sampling from 0TS (540 ± 13 min) vs. 0C (486 ± 12 min). This effect of water loss rate correlating to the phenotype of desiccation survival was not apparent in the males – there was no significant difference in predicted rates of water loss between the two populations receiving no gene flow for males ($P = 0.996$), although 6.7TS displayed significantly greater rates of water loss compared to all other populations ($P < 0.05$; Fig. 15b).

There was no significant difference in predicted water loss rates among any of the selected populations receiving gene flow for females or males; however, males from 6.7TS had a significantly higher rate of water loss relative to all other populations with the exception of 13C ($P = 0.285$; Fig. 15b). The rate of water loss began to decline again after 6.7 % gene flow suggesting that this amount was enough to limit the ability of female and male flies to reduce rates of water loss (Fig. 15a, b). The distribution of water loss rates in the final generation of sampling was consistent for males and females – populations that showed reduced survival under desiccating conditions (in particular,

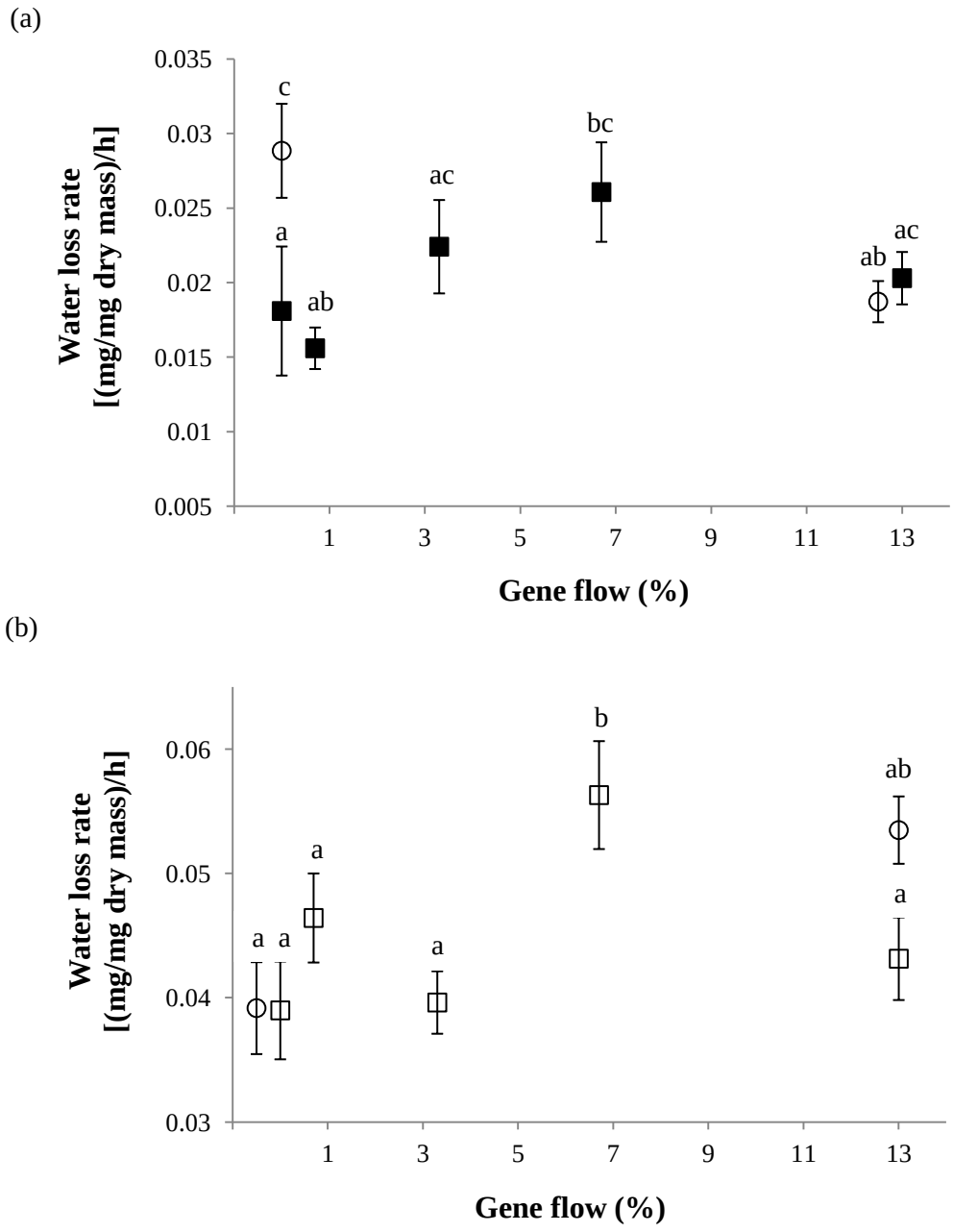

Figure 15. Estimated rate of water loss (mg/mg dry mass/h) during a desiccation assay for female (a) and male (b) *D. melanogaster* from each population experiencing a gradient of gene flow (% N_e). Data shown are from the final generation of sampling (n = 48-50 flies/ population). Selected populations (squares) were selected for desiccation resistance for 12 generations. Control populations (open circles) experienced no selection and some are offset here by -0.5 % for illustrative purposes. See text for description of how water loss rates were calculated. Populations with the same letter are not significantly different.

3.3 and 6.7TS) had a normal distribution of water loss rates, while all other populations generally had a right-skewed distribution with the exception of the two control populations (Fig. 16a, b), which together are similar to the distributions of survival time.

3.6 Maternal effects

There was no significant change in mean desiccation survival time between generation 24 and generation 27 for any of the seven populations for males or females (Table 4).

3.7 Sexual selection

Unselected female flies (migrants) did not display a significant preference when choosing a mate based on whether they were selected or not ($Z_{3,4}$ = 1.03 , P = 0.306), or whether they had clipped wings ($Z_{3,4}$ = 0.621, P = 0.534). Selected female flies also did not exhibit a significant difference in mate preference for a male fly that was selected ($Z_{3,4}$= 1.623, P = 0.105) or had its wing clipped ($Z_{3,4}$ = 0.425, P = 0.671).

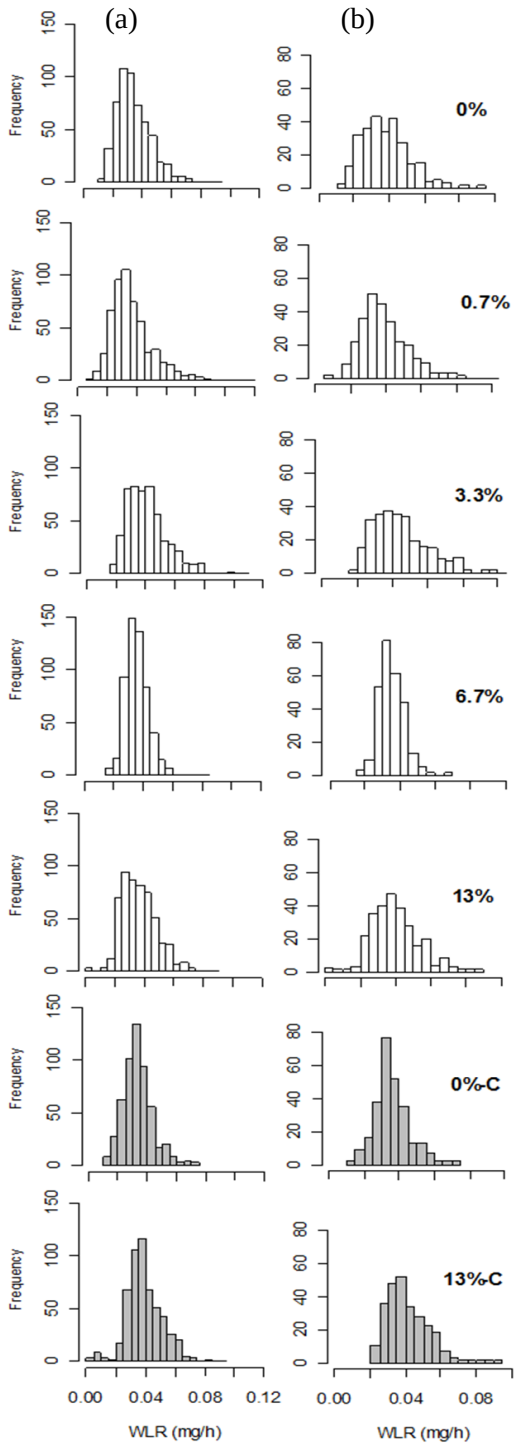

Figure 16. Distribution of predicted water loss rates (mg water/time dead) for male (a), and female (b) *D. melanogaster* following a desiccation assay for each population experiencing varying levels of gene flow (% N_e) tested in generation 32. Grey bars are unselected control (C) populations. N = 48-50 flies/sex/population.

Table 4. Results from accelerated failure time models comparing mean survival time of *D. melanogaster* under desiccating conditions for each population and sex between generation 24 and 27. C = control (unselected) population. Populations of flies were selected for desiccation resistance and experienced varying levels of gene flow (n = 20-22 flies/sex/population/generation).

	FEMALES					**MALES**				
Population (% gene flow)	**Value**	**± SE**	**Z-value**	***df***	***P***	**Value**	**± SE**	**Z-value**	***df***	***P***
0	0.0097	0.101	0.096	1,44	0.923	0.0565	0.101	0.563	1,44	0.574
0.7	0.0246	0.103	0.239	1,42	0.811	-0.0114	0.101	0.113	1,44	0.910
3.3	0.0679	0.103	0.660	1,42	0.509	0.0257	0.103	0.249	1,42	0.803
6.7	-0.0161	0.102	0.158	1,43	0.874	0.0416	0.102	0.409	1,43	0.682
13	0.0061	0.102	0.060	1,43	0.952	0.0558	0.101	0.555	1,44	0.579
13C	0.0527	0.103	0.511	1,42	0.609	0.0456	0.102	0.448	1,43	0.654
0C	-0.0677	0.102	0.578	1,44	0.563	-0.0344	0.101	0.475	1,42	0.634

CHAPTER 4: Discussion

Most of the empirical research that has explored the interaction between gene flow and local adaptation has described individual components of the bigger picture (e.g. the genetic changes associated with local adaptation; Gaston, 2003; Sexton *et al*., 2009). Previous studies that have explored this relationship are correlative and are not designed to determine causal relationships, nor the amount of gene flow required to counteract selection and limit adaptation. I explored the relationship between gene flow and local adaptation by desiccation selection in an effort to test the hypothesis that gene flow impedes local adaptation in range-edge populations and to determine the amount of gene flow required to impede a response to selection (for desiccation resistance), as well as the strategies responsible for reducing water loss during desiccation. Briefly, all levels of gene flow impaired a response to selection, but populations experiencing intermediate levels of gene flow had the strongest retarding effect on desiccation tolerance.

4.1 Desiccation survival

Male and female *D. melanogaster* from a population experiencing 0 % gene flow that had experienced 12 desiccation selection events were found to have significantly higher mean survival time while under desiccation stress relative to flies from the core, unselected population (Fig. 5). This pattern is consistent with studies that measured desiccation resistance between selected and unselected populations of *D. melanogaster* (e.g. Gibbs *et al*., 1997; Chippindale *et al*., 1998), although the magnitude of the difference in survival time between selected and unselected populations is less dramatic in this study. This difference in the magnitude of the response to selection is likely because a constant 85 % selection pressure applied consistently every generation is a

common procedure for artificial desiccation selection studies attempting to answer questions that require a rapid response to selection (e.g. Gibbs *et al*., 1997; Chippindale *et al*., 1998; Gefen and Gibbs, 2009). Typically in nature when the selection pressure is too intense, the population is at risk for being bound to a source-sink situation with minimal genetic differentiation and adaptive potential among populations (Kawecki and Ebert, 2004). For a sink population under strong selection and characteristically receiving asymmetric gene flow, alleles that enhance adaptation in the local population are unable to spread, so I used a series of time-constant selection pressures in this study to warrant an opportunity for survival for an unselected migrant coming into an increasingly selected population over time.

The effect of selection on mean survival time under desiccating conditions was significantly greater in females than males, suggesting that desiccation selection was acting differently on females than it was males. In particular, I found that the mean survival time of female flies from 0TS increased by 33 min, compared to male flies from 0TS where the mean survival time increased by 25 min following selection. Because males are inherently less tolerant of desiccation stress (Kwan *et al*., 2008), these unequal increases in mean survival time could be due to stronger selection on male than female flies. Chippindale *et al*. (1998) postulated that male flies are in fact being selected for early reproduction and not desiccation resistance since they did not observe mating to occur during desiccation exposure, which could, in part, explain why males are less responsive to and tolerant of desiccation stress. Since desiccation selection culled upwards of 90 % of the males from a given population, males likely had to channel energy into reproduction before selection was imposed because it was unlikely that they

would survive the entire duration of selection. However, male flies that did survive a selection event were afforded adequate time to allow for mating to happen before eggs were collected.

There was a non-linear relationship between desiccation tolerance and gene flow for each sex, where survival time for populations experiencing levels of gene flow intermediate on the experimental gradient were lower than those experiencing low or high levels of gene flow (Fig. 5). Only females from populations experiencing no gene flow survived desiccation stress significantly longer relative to the core population after 32 generations. Moreover, the observed shift in the distribution of survival time (Fig. 6) from right-skewed to somewhat normal in this population suggests that the greatest number of deaths occur mid-way through exposure to desiccation stress. For other less tolerant populations, the distribution of survival time was generally right-skewed indicating that the majority of deaths occur at the beginning of each desiccation survival assay.

Populations experiencing intermediate levels of gene flow (3.3 % and 6.7 %) exhibited little to no desiccation tolerance as measured by mean survival time. This implies that populations experiencing intermediate levels of gene flow were impeded from reaching their ecological optima due to persistent gene flow bringing unselected alleles into the populations. Yaemen and Guillaume (2009) demonstrated that intermediate levels of gene flow and moderate-strong selection intensity can generate high genetic skew, which is attributed to a greater response to selection than if the distribution of the genotype in a population is normally-distributed. Genetic skew arises when distributions of genetic values are skewed toward the immigrants mean trait value

(Yaemen and Guillaume, 2009). This is not supported by the low survival time in response to desiccation selection for populations experiencing 3.3 % and 6.7 % gene flow, which are intermediate in this experimental design. However, in the simulation models, intermediate levels of gene flow were set at around 10^{-3} (approximately equal to $N_e m = 1$; Yaemen and Guillaume, 2009), which is considerably lower than what was considered intermediate in this study.

Low amounts (< 1 %) have been shown to provide enough genetic variation to allow adaptation to occur (e.g. Forde *et al*., 2004; Swindell and Bouzat, 2006; Cassel-Lundhagen *et al*., 2011) and to prevent random genetic drift irrespective of population size (Slatkin, 1987; Bossart and Scriber, 1995). Low rates of gene flow (e.g. $m = 0.007$) were expected to have a favourable amount of genetic variation, and thus respond more rapidly to selection than selected populations experiencing 0 % gene flow (Guillaume and Whitlock, 2007); however, there were no significant differences in desiccation tolerance between the two populations. The apparent genetic 'rescue' effect observed for the population experiencing high (13 %) gene flow is not supported by the hypothesis that high levels of gene flow impede local adaptation since the migration rate is relatively high compared to the selection pressure, and thus gene swamping was expected to occur (Lenormand, 2002). Since rates of evolution depend on the amount of genetic variation available in a population, and because the immigrants would increase genetic diversity, this implies that the majority of the alleles from the core were advantageous in the 13TS population in order for this population to exhibit increased tolerance to desiccation stress (e.g. Garant *et al*., 2006). However, hybrids generated from the unselected female (migrant), selected male matings are expected to be less fit (less tolerant of desiccation)

than flies from the resident population, since the new combination of alleles has yet to be tested by selection (Bridle and Vines, 2007; Barrett and Schluter, 2008). Hence, there would be a reduced ability to tolerate desiccation due to the relatively large proportion of less fit hybrids generated from the 13TS population because of the high number of immigrants.

4.2 Stored water content

One physiological strategy to increase desiccation survival and reduce water loss under desiccating conditions is to store more water either as bulk water or by increasing glycogen. *Drosophila melanogaster* selected for desiccation resistance evolve a bigger body size, contain approximately 30 % more initial (bulk) water content, and have increased glycogen content compared to unselected control flies (Gibbs *et al*., 1997; Folk *et al*., 2001). A bigger body size could improve desiccation tolerance as it could allow the fly to carry more water (Folk *et al*., 2001; Kwan *et al*., 2008), and could decrease the surface area to volume ratio of the fly resulting in less water lost across its cuticle under arid conditions (Folk *et al*., 2001; Gibbs and Matzkin, 2001). Since the response to selection is greater in females than males, females would be expected to increase body size more so than males. This could lead to female body size dimorphism between unselected and selected females, but because mate-choice in *Drosophila* is dominated by females, this dimorphism would not be expected to alter mating success (e.g. Kwan *et al*., 2008).

There was a significant effect of selection on body size in both sexes, but the direction of this relationship depended on the timing of the desiccation stress. In general, body size significantly decreased as a result of selection in females and males prior to an

assay for desiccation resistance; however, body size significantly increased following a desiccation assay for females, but not males, such that flies from 0TS were significantly larger following a desiccation assay relative to the core population, which is consistent with the majority of the literature (Gibbs *et al*., 1997; Chippindale *et al*., 1998; Folk *et al*., 2001; Kwan *et al*., 2008). This three-way interaction between body size, selection, and time of exposure to desiccation stress could be because flies used to determine initial water content were not the same as those used in the desiccation survival assays or for measurements of water content at death. Flies used for the survival assay were given two days to recover from CO_2 anaesthesia used for sexing (Nilson *et al*., 2006), and therefore it is possible that the relatively small difference in age could have allowed flies to fully develop and obtain resources (food and water) from their environment. It is also possible that behavioural differences could account for the difference between selected and unselected populations (Gibbs, 1999), such that female flies from selected populations reduce locomotion while under desiccation stress resulting in reduced energy expenditure and excess glycogen to bind water.

This study demonstrated that neither sex significantly increased dry mass-specific initial water content in response to selection in the final generation of sampling, suggesting that flies were not storing more water to increase survival. This result is unlike what other studies using *Drosophila* (e.g. Gibbs *et al*., 1997) have found, but this difference may reflect the different types of selection pressures employed – constant intensity or constant duration. For example, when a constant intensity selection pressure is used instead of one that is time-constant, individuals are consistently exposed to an intense selection pressure resulting in an increase in the response to selection (e.g.

increased initial water content) relative to unselected controls. Much of the increase in initial water content that Gibbs *et al*. (1997) found was bound to glycogen, which is released when glycogen is metabolized (Chippindale *et al*., 1998), following more than 100 generations of exposure to dehydrating conditions. However, since dry mass-specific initial water content did not increase at the end of selection experiments for either sex relative to the core population, glycogen content was not measured.

Gene flow did not affect the lack of response for dry mass-specific initial water content for either sex, although females from 6.7TS had significantly lower initial water content relative to all other populations (selected and unselected), which is similar to the desiccation survival phenotype for female flies. Therefore, if gene flow impeded the ability to increase desiccation tolerance at an intermediate level of gene flow (i.e. 6.7 %), then this population would likely be unable to respond to selection by storing more water. Alternatively, it is possible that 6.7TS exhibited low resistance to desiccation due to a founder effect when initially created from the core population resulting in particularly low levels of stored water content to begin with. Indeed, females from 6.7TS did start with the lowest initial water content relative to all other populations (data not shown); however, after 12 selection events, the initial water content of 6.7TS converged with the initial water content from the other populations, such that the slope of initial water content over generations was higher than other populations.

4.3 Water content at death

Another physiological strategy to resist desiccation and increase survival is tolerating losing more water; however, there is little evidence to support this strategy in response to selection in *Drosophila* (Gibbs *et al*., 1997). Previous studies that have

measured water content at death among selected and unselected populations of *Drosophila* generally did not find a significant difference in the ability to tolerate low water content (e.g. Hoffmann and Parsons, 1993; Gibbs *et al*., 1997), although this tolerance varies widely among other taxa and across species ranges in nature (Hadley, 1994; Gibbs and Matzkin, 2001). This could be because there is a lower physiological limit constraining how much water a fruit fly must retain to stay viable and resist death by dehydration (Gibbs *et al*., 1997). After several generations of selection in this study, no new mutations arose to allow the flies to tolerate losing more water, which is consistent with the majority of literature (e.g. Hoffmann and Parsons, 1989a; Hoffmann and Parsons, 1993; Gibbs *et al*., 1997; but reviewed in Archer *et al*., 2007). However, seeing as there was variation in water content at death within and among the populations and because desiccation resistance is considered highly heritable in *D. melanogaster* with a narrow-sense heritability of 0.65 (Hoffmann and Parsons, 1989a), this suggests that there was enough genetic variation for dehydration tolerance in the founding population. Moreover, there was a pattern of low dehydration tolerance for 6.7TS females and high dehydration tolerance for 6.7TS males, which is consistent with initial water contents (Fig. 9). These patterns of water content with 6.7TS cannot be explained by having a disproportionate amount of water at death at the start (i.e. generation 0) of the selection experiment relative to other selected populations (data not shown). This implies that some populations were becoming less tolerant of water loss perhaps because they are producing water by burning energy stores, such as carbohydrates, since desiccation-selected flies preferentially metabolize carbohydrates over lipids compared to their unselected counterparts (Djawdan *et al*., 1997; Chippindale *et al*., 1998).

4.4 Water loss rates

The third and final physiological strategy to increase survival under desiccating conditions is reducing the rate at which water is lost. *Drosophila* from xeric environments have been found to survive desiccation stress longer by losing water more slowly relative to other *Drosophila* from mesic environments (Gibbs and Matzkin, 2001). Gibbs *et al*. (1997) showed that *D. melanogaster* that had been intensely selected for desiccation resistance in the lab, displayed a 40 % reduction in their water loss rate relative to flies from an unselected, control population. Patterns of predicted water loss rates corresponded to patterns of female desiccation tolerance (mean survival time) in this study, where females from 0TS had a significantly lower rate of water loss relative to females from the core population, suggesting that selected female flies increased survival by reducing their water loss rate. Although not significant, there was also a trend for high rates of water loss for individuals from 6.7TS. Given that the pattern of female water loss rates among the populations generally models the pattern of female desiccation survival, it is likely that a threshold to the level of gene flow that impedes local adaptation lies at intermediate levels (6.7 %) of gene flow. In addition, patterns of female body size following desiccation among the populations strongly correlates to patterns of water loss rates and to the desiccation survival phenotype among the populations, confirming that a larger body size in response to selection is advantageous for reducing water loss rates and improving survival under desiccating conditions. Reduced rates of water loss can be largely attributed to reduced cuticular permeability in *D. melanogaster* since the majority of the waterproofing of an insects cuticle is supplied by a greater amount of longer chain cuticular hydrocarbons (Gibbs, 2002b; Chown and Nicholson, 2004; Bazinet *et al*., 2010).

4.5 Additional explanations for the observed relationships with gene flow

Non-genetic effects such as handling conditions, rearing conditions, plasticity, and maternal effects should be considered when measuring local adaptation and genetic differentiation as they can contribute to the overall adaptive potential (Kawecki and Ebert, 2004; Nosil *et al*., 2006). Maternal effects in particular can mimic local adaptation because they can create plastic responses that are adaptive in the maternal environment yet obscure the pattern of local adaptation for the offspring (Kawecki and Ebert, 2004). These non-genetic effects were minimized and the potential for confounding maternal effects was addressed in this study by comparing desiccation tolerance for each population between generation 24 and 27 (i.e. following three generations of no selection). There were no significant differences in mean survival time between the two generations of sampling, suggesting no maternal effects.

Individuals from range-edge populations can adapt to novel environments or novel selection pressures by selection on new mutations offered by migrants or selection on pre-existing (standing) genetic variation (Barrett and Schluter, 2008). Local adaptation is expected to occur more rapidly from standing genetic variation than from introduced variation offered by migrants because a beneficial allele (e.g. one that aids in desiccation resistance) or set of alleles that resides in the standing genetic variation is older and likely to have already been exposed to selection (Barrett and Schluter, 2008). In addition, alleles present in standing genetic variation are likely more abundant and exist in multiple copies compared to an allele that appears as a single new mutation in a population (Barrett and Schluter, 2008). Therefore, the probability of fixation of an allele or alleles conferring desiccation resistance is greater if it is part of standing genetic

variation, unless migration is sufficiently high to introduce equal or more novel alleles. To that end, the probability of fixation also increases with increasing effective population size and consequently, populations experiencing high (13 %) levels of gene flow should have a greater proportion of new mutations entering the environment vs. standing genetic variation. This concept is not supported by the results for desiccation survival for populations experiencing 13 % gene flow, so it is unlikely that populations experiencing high gene flow exhibited increased survival solely due to differences in fitness and preference between resident alleles present in standing genetic variation and migrant alleles generated through random mutation. Finally, because desiccation resistance is a complex adaptation (e.g. Hoffmann and Parsons, 1989a; Hoffmann and Parsons, 1989b; Djawdan *et al*., 1997; Chippindale *et al*., 1998), it is unlikely to be a single allele, but rather, multiple alleles that are responsible for increasing survival under desiccating conditions. Therefore, a greater number of hybrids (e.g. in 13TS) have the potential to introduce new or different linkage groups, which could lessen the impairing effect of gene flow on desiccation resistance.

In general, the migrant genotype is rare relative to the resident genotype, and therefore the overall effect of gene flow on a population will depend on the performance of the immigrants as well as the fitness of their offspring and descendants (Kawecki and Ebert, 2004). Since all of the offspring generated from the migrants (unselected females) were from mating with individuals from the resident population (the selected males), often these hybrids will backcross (mate with an individual similar to its parent) with the resident genotypes (Kawecki and Ebert, 2004). Given that there was a larger proportion of hybrid flies entering populations receiving high gene flow relative to those receiving

low gene flow, the fitness of these backcrossed hybrids could influence local adaptation in three ways as described by Kawecki and Ebert (2004). First, the hybrid phenotype may deviate from the resident phenotype because of epistatic interactions. Secondly, the offspring of the migrants could favour hybrid vigour (heterosis). Lastly, the hybrid genotype could suffer from outbreeding depression, which occurs if the parental genotypes reach alternative ecologically 'adaptive peaks', and therefore have lower fitness compared to resident genotypes (Kawecki and Ebert, 2004). In theory, epistatic interactions and outbreeding depression are equally likely mechanisms to have contributed to the observed trend for increased desiccation tolerance in a population experiencing high gene flow because there was a much larger proportion of migrant matings and therefore, offspring given to the next generation. Similarly, there were more migrants entering the selected population receiving 13 % gene flow every other generation, thus it is possible that a favoured hybrid vigour phenotype was created which had superior mating success compared to other migrants. As a result, the genes from this original hybrid vigour could have conferred a fitness advantage for desiccation resistance over resident and migrant phenotypes, which could explain the observed response to selection for this population (Hoffmann and Parsons, 1989a; Hoffmann *et al*., 2003; Kellermann *et al*., 2009).

A change in environmental conditions can result in differential selection pressures acting on the sexes, which can generate sexual conflict over time (Kwan *et al*., 2008). There is mixed evidence for species displaying mate-choice preference when relocated to their non-native environment, often where there is increased selection pressures (e.g. Hendry and Taylor, 2004; Plath *et al*., 2010). If a unique sexually-selected ornament or

display evolves among different populations, immigrants from that population could possibly be discriminated against by local females thereby creating sexual selection against the migrants (Kawecki and Ebert, 2004). Here, mate-choice assays were primarily conducted to determine if the migrants (unselected females) were exhibiting a preference for a mate based on whether or not they were selected for desiccation resistance. Unselected females as well as selected females did not demonstrate a preference for selected males, suggesting that females did not prefer to choose to mate with a selected vs. an unselected male and that sexual conflict was not occurring in any of the lines. Populations were therefore selected solely for desiccation resistance and maladapted flies were not discriminated against during mating.

4.6 Implications for species' range-edges

Few studies have been able to test the genetic constraints on local adaptation to explain species geographic range limits due to the inherently large spatial scales involved, as well as the intricacies of the underlying genetic architecture (e.g. Hendry and Taylor, 2004; Sagarin *et al*., 2006; Sexton *et al*., 2009; Colautti *et al*., 2010). Therefore, there is a large amount of discussion regarding why evolution fails at a species range-edge, which pivots on determining how much gene flow is required to maintain genetic variation and therefore an adaptive potential, without impeding local adaptation by introducing maladaptive alleles (Alleaume-Benharira *et al*., 2006; Bridle and Vines, 2007). Here, the response to selection was least impaired at relatively low amounts of gene flow, implying that these amounts are sufficient to maintain standing genetic variation and therefore, allow for local adaptation to occur. As anthropogenic climate change is rapidly altering abiotic conditions and imposing new suites of selection pressures, many organisms are

expected to shift their range in a poleward direction to accommodate the new conditions (Parmesan *et al*., 2005; Bridle and Vines, 2007; Budd and Pandolfi, 2010; Thomas, 2010; Hardie and Hutchings, 2010; Hoffmann and Sgrò, 2011). Given that I found intermediate levels of gene flow to impede a response to selection, these increasingly vulnerable range-edge populations are at risk for experiencing higher levels of gene flow, which could disrupt the balance between selection and gene flow thereby confounding local adaptation to the modified selection pressures and ultimately, impeding a range shift. Therefore, the results of this study have significant implications for range-edge populations that are expected to encounter adverse environmental conditions due to progressions with climate change and anthropogenic disturbances, which have the potential to impart detrimental effects on the state and viability of these already vulnerable populations (e.g. Spicer and Gaston, 1999; Alleaume-Benharira *et al*., 2006; Cassel-Lundhagen *et al*., 2011).

4.7 Limitations of this study

One limitation of this study is that water loss and metabolic rates among all populations were not directly measured (e.g. via CO_2/H_2O output with flow-through respirometry) in order to obtain a more accurate measure of how much water was lost during desiccation and to determine precisely the mechanism by which flies reduce the rate of water loss. Instead, water loss rates were estimated via gravimetric water content data. As a result, I was unable to infer the mechanism responsible for reduced water loss rates in selected populations, although it is likely due to changes in cuticular permeability since reduced water loss rate is correlated to larger body size following selection, at least for female flies.

Studies that have examined the physiological strategies to overcome desiccation stress have selected populations of *Drosophila* for upwards of 100 generations (e.g. Djawdan *et al*., 1997; Gibbs *et al*., 1997), which could explain the discrepancies observed in the response to selection between this study and those that exposed populations to far more selection events. Presumably with more selection events, there would have been a much clearer response to selection with, for example, stored water content as observed with Gibbs *et al.* (1997). However, with a time-constant selection pressure I expect it would not be necessary to perform much more than 20 selection events, since after 12, the overall desiccation survival of the population experiencing 0 % gene flow increased by approximately 25 % (data not shown).

Another limitation of this study is not having replicate experimental populations for each level of gene flow to account for biological variation among populations given that a negative linear relationship between desiccation tolerance and gene flow was expected. Replicate populations would allow for precise comparisons between populations, while ensuring experimental procedures (e.g. making sure each population consisted of 4500 ± 500 flies prior to selection, qualitatively desiccating 85 % of a population) are executed accurately. Further, replicate populations would ensure that the measured differences in desiccation tolerance and the strategies to reduce water loss were accurate, and not just an experimental artefact. Given the time-scale of this selection experiment, it is possible that environmental differences could have affected the results despite all efforts to ensure consistency and standardize conditions. However, this is difficult to conclude seeing as there is no measure of inter-population variation given that only one population per gene flow treatment was used.

The time to 85 % mortality by desiccation was separately determined for each selected population, thus some populations received longer periods of desiccation selection than others. While there is small variation in the time taken to achieve the target selection intensity in generation zero, this amount should have been generated from the core population and applied consistently to all populations. Instead, this small variation is likely due to populations with fewer flies seemingly reaching estimated 85 % mortality before larger populations, although preliminary analyses showed that the relationship between initial population size and the total proportion of a population killed by selection was not significant.

Finally, the sample size (n = 20 flies/sex) used to examine the rate of change in desiccation tolerance and the associated strategies to maintain water balance over the sampled generations was too small. This sample size was based on what others who study desiccation resistance in *Drosophila* in the laboratory have used (e.g. Gibbs *et al*., 1997; Chippindale *et al*., 1998; Gefen *et al*., 2006), but given that this study design did not include replicate populations per gene flow treatment, there was a considerable amount of variation in the measurements between generations. Fortunately, the sample size was much larger (n = 50 flies/sex) for measures quantifying desiccation tolerance and the physiological strategies to overcome water loss in the final generation of sampling, and therefore these more robust estimates were used to compare the effect of selection and gene flow among populations.

4.8 Future directions

Currently, there is conflicting evidence for the role of gene flow limiting a species range (Moore and Hendry, 2009), largely because each study that has attempted to

explore this relationship has been designed independent of the others and thus has rendered unique outcomes and limited comparability. Research on range limits should test hypotheses of range limit adaptation by first characterizing the major factors potentially influencing adaptation (e.g. gene flow, selective pressures, ecological gradients, habitat quality) as well as the population landscape over which the trait(s) is studied to cultivate a holistic picture of the interacting effects (Sexton *et al*., 2009). An experimental evolution approach is one way to tie together these suggested components to bridge the gap between theoretical population models and macro-evolutionary empirical field- and lab-based studies, as they have the potential to unravel the genetic mechanisms behind this interaction that are otherwise confounded by limitations in studies of natural metapopulations and bound by assumptions in theoretical models (Kawecki and Ebert, 2004). This research has begun to bridge this gap of knowledge in understanding species range limits using predictions generated from decades of theoretical simulation models and from empirical observations, which shed light on the role of gene flow in impeding local adaptation at the species range-edge.

Future studies could employ a similar experimental approach to the one I used, since experimental manipulations of gene flow and selection are a powerful way to infer causation (e.g. Forde *et al.,* 2004; Räsänen and Hendry, 2008), and because this approach has allowed for comparisons of adaptive responses to selection between independent populations that have evolved under different conditions (in this case, different gene flow treatments). Replicate experimental populations 'connected' by a much larger migration gradient with more levels of gene flow should also be incorporated into the experimental design for reasons discussed above. It would be interesting to see if the relationship

between the response to selection and gene flow observed here can be replicated using more levels of gene flow, which theoretically could determine more precisely where the threshold level of gene flow lies. Finally, the amount of gene flow required to impede adaptation by natural selection at a species range-edge is likely dependent on the adaptive potential of the species or populations, their sensitivity to particular conditions (e.g. intermittent dry conditions), the genetic architecture of the spatial landscape, as well as a clear understanding of the trait heritability for a complex selection pressure (Hoffmann and Sgrò, 2011). Therefore, interactions between selection, gene flow, mutation, epigenetics, and life-history factors must also be considered to be components impeding adaptive evolution at the range-edge that have not been considered in some previous work (Lenormand, 2002; Hardie and Hutchings, 2010; Holt *et al*., 2011).

4.9 Concluding remarks

All levels of gene flow impaired a response to selection to varying degrees where populations receiving no gene flow have the strongest response to selection and populations receiving intermediate (3.3 %, 6.7 %) levels of gene flow have the strongest retarding effect. This effect of gene flow on the response to selection is more pronounced in female than male flies. Of the three strategies to reduce water loss under desiccation stress, female, but not male, flies from selected populations reduce water loss rates. Lower water loss rates are attributed to bigger body size – selected flies have a smaller initial (pre-desiccation) body size, and a larger final (post-desiccation) body size relative to the unselected, core population. This difference in body size may be due to age or complex genetic correlations for traits involved with increasing desiccation tolerance and

extending longevity, which can create trade-offs confounding the response to selection. Flies are not carrying more water or tolerating losing more water following selection.

This study is the first to use experimental evolution to empirically establish a quantitative estimate of the amount of gene flow required to impede local adaptation at a model species range-edge. I was able to demonstrate the effect that varying levels of gene flow has on the response to desiccation selection for *D. melanogaster*. While I am unable to pinpoint a threshold level of gene flow that limits local adaptation, I did reveal evidence that suggests intermediate levels of gene flow, rather than high levels as predicted, can limit a response to selection in range-edge populations, thereby limiting a species range from expansion. This research can be used to help understand the impact of gene flow (e.g. of an invasive species) on local adaptation of populations at a species range-edge, as well as how this will be of increasing importance as climate change modifies selective pressures causing species to geographically shift their ranges. This research also highlights the significance and consequences of using an ecologically relevant selection pressure to infer causal relationships as well as to understand population dynamics and patterns of local adaptation in a controlled laboratory setting.

CHAPTER 5: References

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Appendices

Appendix 1. The recipe for the fly food media was obtained from the UC San Diego *Drosophila* Stock Centre.

Diet	Ingredients
Standard Cornmeal	1.5% active yeast (w/v) 4.3% sugar 2.7% cornmeal 1.0% agar 0.4% propionic acid, ACS reagent, ≥ 99.5%

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