GENETIC DIVERSITY IN EXPERIMENTAL METAPOPULATIONS

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GENETIC DIVERSITY IN EXPERIMENTAL METAPOPULATIONS

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To my parents
Voor mijn ouders

Contents

CHAPTER 1	General introduction	9
CHAPTER 2	Genetic differentiation in experimental *Drosophila melanogaster* metapopulations I. Random genetic drift	37
CHAPTER 3	Genetic differentiation in experimental *Drosophila melanogaster* metapopulations II. Stepping-stone migration	59
CHAPTER 4	Genetic differentiation in experimental *Drosophila melanogaster* metapopulations III. Local extinction and recolonization	87
CHAPTER 5	Consequences of fragmentation for the ability to adapt to novel environments in experimental *Drosophila* metapopulations	115
CHAPTER 6	Summarizing discussion	131
Bibliography		147
Samenvatting		163
Nawoord		175

CHAPTER 1

General introduction

The biological diversity of our planet is rapidly declining as a direct or indirect consequence of human activities (Lawton & May 1995, Palumbi 2001, Gaston 2005). A large number of species has gone extinct and is irreversibly lost. Because of the destruction, deterioration and fragmentation of their habitats, many other species have been reduced to small and often isolated populations that are at risk of extinction due to environmental, demographic or genetic stochasticity. In a worst-case scenario, all these factors may work negatively in concert, and further increase the extinction risk of fragmented populations (Lande 1998, Fahrig 2003, Gaggiotti 2003, Reed 2004, Ewers & Didham 2006).

The International Union for the Conservation of Nature (IUCN) recognizes the need to conserve biodiversity at three levels: genetic diversity, species diversity, and ecosystem diversity (McNeely *et al.* 1990). This thesis centres on the dynamics of genetic diversity in a metapopulation context, and the relevance of general population genetic theory for nature conservation management. I aim at integrating experimental and theoretical approaches by comparing data from experimental *Drosophila melanogaster* metapopulations and individual-based computer simulations of increasing complexity with the patterns as predicted by general population genetic theory. I focus on the implications of metapopulation structure for the proper interpretation of genetic data quantifying diversity and differentiation, and the consequences of these implications for conservation biology.

THE METAPOPULATION APPROACH

As a consequence of the increasing fragmentation of the natural habitat of many species, the "metapopulation approach" has become a mainstream approach in conservation biology over the last two decades (Hanski & Simberloff 1997, Hanski 1999). A metapopulation (Levins 1969) consists of habitat fragments harbouring small, relatively isolated local populations, the so-called subpopulations or demes. Connection of the habitat patches through migration is limited, and patches may be subject to local extinction and recolonization.

Recent research based on the metapopulation concept has provided a large body of theoretical studies contributing to the understanding of ecology at the population, landscape and community levels, of population genetics and of evolutionary biology (overview in Hanski & Gaggiotti 2004, Rousset 2004). Empirical studies providing data to test and validate the theory, however, are still limited (*e.g.*, Lambin *et al.* 2004), with a few exceptions (*e.g.*, butterflies, overview in Thomas & Hanski 2004; water fleas, Haag *et al.* 2005; plants, Antonovics 2004). The majority of empirical studies in natural systems are generally faced with the problem that the observed patterns are often unique, since they are the outcome of specific historical events, and only allow indirect comparisons when testing predictions from metapopulation theory (*e.g.*, Hanski *et al.* 2002). Moreover, even large empirical datasets are mostly descriptive and do not allow unravelling the underlying causes and mechanisms of the observed patterns. Experimental

studies aiming at validation of theoretical models commonly focus on single populations in homogeneous habitats (*e.g.*, bacteria, Dykhuizen & Dean 2004; plants, Holeski & Kelly 2006; nematodes, Cutter 2005; fruit flies, Snook *et al.* 2005), although some recent studies also include spatial structure (*e.g.*, protists, Warren 1996; nematodes, Friedenberg 2003; plants, Olson *et al.* 2005) and spatial heterogeneity (*e.g.*, fruit flies, Mery & Kawecki 2004). To disentangle the complex interaction of environmental, demographic and genetic processes, however, controlled and replicated observations and manipulations of metapopulation features such as gene flow or extinction rates are necessary. Experimental metapopulations under controlled laboratory conditions provide an obvious answer to this need, and are the focus of this thesis.

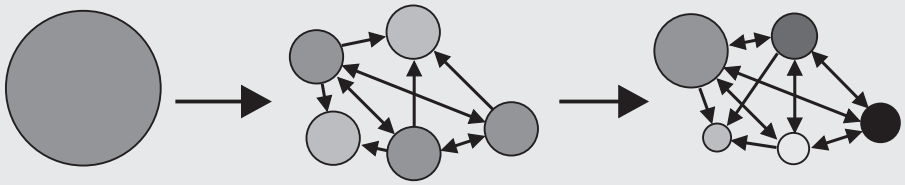
In the next section of the introduction I briefly review the theoretical results in metapopulation biology (readers familiar with metapopulation theory can skip this section). In the two subsequent sections, I introduce some population and conservation genetic concepts and parameters that are relevant for this thesis. In the final section I explain the approach taken in this study and the subsequent outline of my thesis.

A REVIEW OF METAPOPULATION THEORY

The development of basic theoretical models in both population dynamics and population genetics is often based on a hypothetical large population of equally interacting individuals that is closed to migration. Clearly, this approach is not sufficient to describe the processes occurring in most natural populations, which tend to be structured, for instance in age cohorts or by the spatial aggregation of individuals. On a larger scale, separate patches of habitat able to sustain a local breeding group during one or more generations and connected through migration, constitute a structured, subdivided population, or metapopulation. Each habitat patch sustaining a breeding group within such a metapopulation then constitutes a subpopulation, or deme. A habitat patch may be temporarily empty when a local extinction event wiped out its inhabitants, until it is recolonized by migrants from other demes within the metapopulation that succeed in founding a new deme. This succession of local extinction and recolonization events within a metapopulation is referred to as population turnover.

It is obvious that on the one hand, spatial structure and the associated demographic processes of migration between habitat patches, extinction of local demes and (re)colonization of empty patches will have profound effects on the distribution of genetic variation (*i.e.*, the variation within demes *versus* the variation among demes) in a metapopulation (box 1.1). Increasing fragmentation of the habitat will increase the genetic differentiation among demes (Hastings & Harrison 1994). Small and relatively isolated demes are subject to genetic drift leading to the loss of genetic variation and an increase of autozygosity. This may in turn result in inbreeding depression (*i.e.*, the decrease of population fitness due to a high level of expression of recessive deleterious alleles), and increase the risk of extinction (Bijlsma *et al.* 2000, Frankham 2003, Gaggiotti 2003, Reed & Frankham 2003, Spielman *et al.* 2004a). This process of deterioration due to

Box 1.1 Metapopulation development in space and time



Large, undivided population with little genetic differentiation or demographic heterogeneity

Population subdivision due to habitat fragmentation. Subpopulations (demes) are connected through migration and can be recolonized after local extinction events (light grey demes).

Over time, the demographic (size, age) and genetic (allelic variation) composition of single demes will diverge.

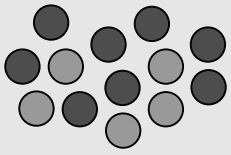
small population size is often referred to as genetic erosion (Bijlsma *et al.* 1994). On the other hand, the levels and distribution of genetic variation and genetic processes such as local adaptation may strongly affect the demographic parameters (*e.g.*, population size, migration rate, extinction probability) and spatial structure of a metapopulation (Mopper & Strauss 1998, Wade & Goodnight 1998, Keller & Waller 2002, Mix *et al.* 2006). Despite their obvious interdependence, the demographic and genetic dynamics of a metapopulation are often treated separately (Vellend & Geber 2005), although an integrated approach is crucial for understanding the dynamics of biodiversity in fragmented habitats (Lande 1988, Vucetich & Waite 1999, Lambin *et al.* 2004), and for adequate conservation management (Alvarez *et al.* 1996, Neel & Cummings 2003, Oostermeijer *et al.* 2003, Cabeza *et al.* 2004b). Consider for example a management program supplementing small populations, where the newly introduced individuals may severely disrupt the dynamics of locally adapted populations resulting in a decline of population fitness rather than the intended increase. In this section, I review the theoretical framework of (population genetic) metapopulation modelling.

ECOLOGICAL MODELS

Ecological metapopulation models are broadly categorised by the level of demographic dynamics and the level of spatial complexity. The lowest-level models focus on the demography within patches and the persistence of single demes. The "metapopulation aspect" of these models is limited to the dynamics of migration, by way of monitoring the numbers of immigrants and emigrants affecting population size. The spatial configuration of other patches where migrants come from or go to is not relevant, hence these models are spatially implicit at the metapopulation level. The higher-level patch-occupancy models do not consider the local demographic dynamics within patches but describe the dynamics of a metapopulation as a stochastic balance of local extinction and

recolonization of empty patches. The classic metapopulation model of Levins (1969, 1970, box 1.2), which is essentially a variation of the model of balanced extinction and migration that is the theoretical basis of island biogeography (MacArthur & Wilson 1967), is the most notorious example of a patch-occupancy model. Patch-occupancy models can be either spatially implicit or spatially explicit. Spatially implicit models do not consider any specific spatial configuration, but assume that the exchange of migrants between two or more demes is independent of distance. Spatially explicit models consider the effect of spatial structure on migration. Spatial structure in these models is commonly limited to a one- or two dimensional regular lattice, but may be more complex when the actual geography is included ("spatially realistic models", Hanski 1994). The most complex ecological models consider both the local demography within the patches, and the dynamics and spatial configuration of the patches within the metapopulation.

Box 1.2 Levins' classic metapopulation model



Levins model

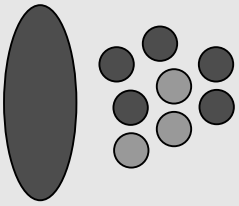
The **Levins model** (Levins 1969, 1970) assumes an infinite number of equally connected patches, and is therefore spatially implicit. The fraction occupied patches P changes over time with extinction rate e and colonization rate c per patch:

$$dP / dt = cP(1 - P) - eP$$

The metapopulation will persist at an equilibrium level of occupancy $\hat{P} = 1 - (e/c)$ when inter-patch colonization takes place at an equal or higher rate as extinction ($c/e > 1$).

The **mainland-island model** (Harrison 1991) is a special case of the Levins model reflecting the original assumption of island biogeography theory that empty patches (islands) are also colonized at rate C by migrants from a very large source population (mainland) insusceptible to extinction:

$$dP / dt = (C + cP)(1 - P) - eP$$

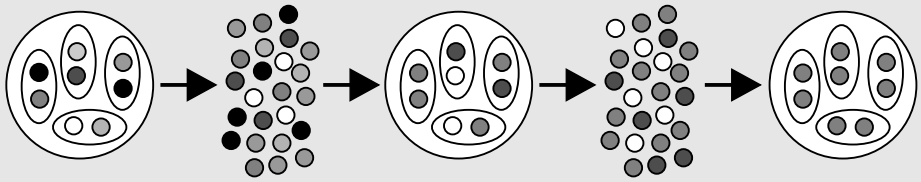


Mainland-island model

Hence, the metapopulation will survive without any inter-patch colonization ($c = 0$) at an equilibrium level of $\hat{P} = C/(C + e)$ because there is always some immigration from the mainland.

Although (simple) analytical models have the advantage of mathematical solvability, the conclusions tend to be relatively abstract because the studied systems are generally defined by a small number of key parameters that are not linked on a one-to-one basis to the biological parameters commonly used in applied studies. This type of models is well suited for the development of general theory, but less suited for generating local rules-of-thumb for the management of specific natural systems. Spatially detailed (simulation) models better meet the needs of practical applications for nature management, but they

Box 1.3 Wright-Fisher model of genetic drift



The ancestral population (left) consists of $N = 4$ individuals having $2N$ different alleles, and produces infinitely many gametes with frequency $p = 1/2N$ of each type to reproduce. For each next generation (middle) $2N$ alleles are randomly chosen from the gamete pool to combine into $N = 4$ new individuals. Each individual gets either two alleles originating from the same ancestor ("autozygosity") with probability F, or two alleles originating from different ancestors ("allozygosity") with probability $(1 - F)$. Due to the sampling procedure from one generation to the next ("genetic drift"), alleles get lost until only one of the ancestral alleles will be left and becomes fixed in the population (right).

The **Wright-Fisher model** describes the effect of binomial sampling on allele frequencies in finite populations of size N over many generations. For two alleles A and a with frequency p and $(1 - p)$, respectively, the variance in allele frequency from one generation to the next equals $p(1 - p)/2N$. Hence, the expected heterozygosity $H = 2p(1 - p)$ will decline over generations according to:

$$H_{t+1} = (1 - 1/2N)H_t$$

If the autozygosity F is approximated by homozygosity (Hartl & Clark 1997), then $F = 1 - H$ and will increase asymptotically to unity due to random genetic drift:

$$F_{t+1} = 1/2N + (1 - 1/2N)F_t$$

have the disadvantage of requiring a lot of specific data regarding location and species for parameterization, making the results case-specific as well. For a detailed review of ecological metapopulation models, their application in a number of case studies, and discussions on the advantages and disadvantages of the different types of models, I refer to Hanski's (1999) monograph on metapopulation ecology and the sections on ecology and modelling in recent edited volumes on metapopulation biology (Hanski & Gilpin 1997, Hanski & Gaggiotti 2004).

POPULATION GENETIC MODELS

This section introduces population genetic models in a metapopulation context. Genetic metapopulation models generally fit one of two main types. The island-type models comprise the largest class and are spatially implicit. The isolation-by-distance models comprise a second large class that are spatially explicit to a greater or lesser extent. Both types of model describe the effects of genetic drift within and gene flow between the demes within a metapopulation on the distribution of genetic variation within and

among demes. Without the exchange of individuals between demes, gene flow is absent and the level of genetic variation within demes is subject to random genetic drift only. Gene flow can be continuous by way of migration between continuously occupied demes, and discontinuous as a result of population turnover by way of recolonizing empty patches after local extinction events. This review presents models of increasing complexity by starting with genetic drift without gene flow, including continuous gene flow through migration in the next subsection, and adding extinction/colonization dynamics in the final subsection.

GENETIC DRIFT

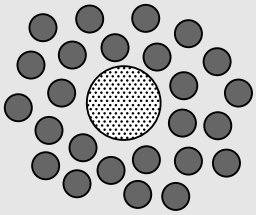
The starting point for population genetic modelling is often a single infinitely large, idealized population without gene flow. In such an idealized population without selection and mutation, the genotype frequencies of a diallelic locus with Mendelian segregation are in Hardy-Weinberg equilibrium and will not change over generations (Hartl & Clark 1997). In contrast with the idealized model population, however, all natural populations are finite, and thus subject to genetic drift. This is essentially a binomial sampling process on all available alleles each generation that increases the autozygosity, *i.e.*, the probability that two randomly chosen alleles are identical by descent (box 1.3). The Wright-Fisher model of random genetic drift (Fisher 1930, Wright 1931) describes the effect of such binomial sampling on the distribution of allele frequencies in finite populations over many generations by assuming an infinite number of equivalent idealized demes of constant size without gene flow (Hartl & Clark 1997, box 1.3).

GENE FLOW THROUGH MIGRATION

Wright's (1931, 1951) island model of migration includes continuous gene flow through migration in a standard Wright-Fisher population (Hartl & Clark 1997). The original model assumes an infinite number of demes populating an infinite migrant pool that in turn provides migrants to all demes at an equal rate (box 1.4). This infinite island model closely resembles the ecological Levins model. Slatkin's (1977) interpretation of the infinite island model as a continent-island model with a finite number of equivalent demes and a very large ("continent") population outside the metapopulation replacing the infinite migrant pool relaxes the unrealistic assumption of infinitely many demes (box 1.4). The continent-island model is very similar to the mainland-island extension of the Levins model. The *n*-island model (Latter 1973, Slatkin 1977) is a finite interpretation of the infinite model where the metapopulation comprises a finite number of equivalent demes equally contributing individuals to the migrant pool, and receiving randomly chosen immigrants from the pool at an equal rate (box 1.4).

The difference between the infinite continent and the finite migrant pool is genetically important because individuals from the continent are neither related to the individuals in the metapopulation nor to each other. In contrast, all individuals in the migrant pool will be increasingly related to each other and to the resident individuals in each deme over time, because the metapopulation is finite. The constant influx of unrelated individuals in the infinite model (box 1.5, left-hand plot) leads to an equilibrium level of

Box 1.4 Island models of migration



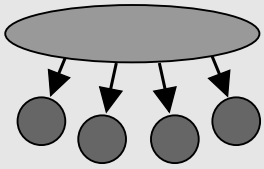
Infinite island model

The **infinite island model** and the **continent-island model** both assume migration into demes of constant size N by unrelated individuals at migration rate m. The migrants are unrelated in the first case because the number of source demes is infinite, and in the second case because they originate from an infinite source outside the metapopulation. Migration affects the change in autozygosity F due to genetic drift over time:

$$F_{t+1} = (1 - m)^2[1/2N + (1 - 1/2N)F_t]$$

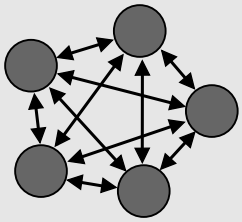
Genetic drift increases both the autozygosity F within demes and the fixation index F_{ST}, *i.e.*, the standardised variance in allele frequencies between generations, which is a measure of differentiation among demes. Migration counters the increase of F_{ST}, and when drift and migration are equally strong F_{ST} approximates the well-known equilibrium:

$$\hat{F}_{ST} = 1/(1 + 4Nm)$$



Continent-island model

Because migrants are not related to residents in the infinite models, migration also counters the increase of F. Hence, the autozygosity F equals the fixation index F_{ST} in this special case (Rousset 2004, see also the appendix in CHAPTER 3).



Finite *n*-island model

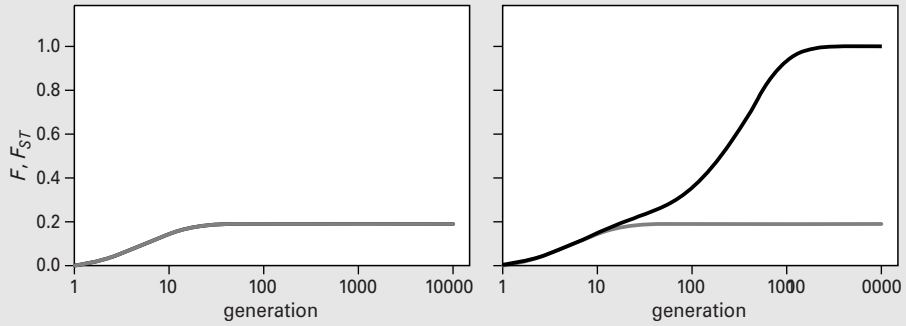
In the finite ***n*-island model**, migrants from all demes within the metapopulation form a migrant pool sending out migrants to all demes at migration rate m. The autozygosities in the focal deme (F_0) and outside the focal deme (F_1) change over time, with parameters a and b summing the probabilities of origin of two alleles sampled in the same deme or in different demes, respectively (Slatkin 1977, details in CHAPTER 3):

$$F_0' = a[1/2N + (1 - 1/2N)F_0] + (1 - a)F_1$$
$$F_1' = b[1/2N + (1 - 1/2N)F_0] + (1 - b)F_1$$

In the finite model, migrants and residents have all ancestral alleles in common because they both originate from the same finite number of demes. Hence, although migration will counter the increase of F_{ST} due to genetic drift resulting in an equilibrium as above, migration can not counter the increase of F to unity because no unrelated alleles are introduced.

autozygosity F in the metapopulation that is equal to the equilibrium level of genetic differentiation F_{ST} (*i.e.*, the standardised variance in allele frequencies between generations) among the demes. In the finite model (box 1.5, right-hand plot), the autozygosity will increase to unity because all individuals in the metapopulation become more related over time, whereas the genetic differentiation among demes will attain a different equilibrium level depending on the migration rate (Rousset 2004, see also CHAPTER 3).

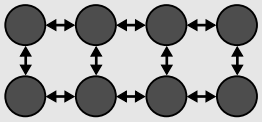
Box 1.5 *F* and F_{ST} in infinite and finite metapopulations



Over time, the fixation index F_{ST} (grey lines) attains similar equilibrium values in both an infinite (left panel) and a finite (right panel) metapopulation with one migrant per generation (*i.e.*, $Nm = 1$). The autozygosity *F* (black lines) equals F_{ST} in the infinite metapopulation on the left, but approaches unity in the finite metapopulation on the right (note that F_{ST} is undefined when $F = 1$). The difference is caused by the unlimited availability of new unrelated alleles replenishing the alleles that are lost due to random genetic drift in the infinite metapopulation. In contrast, the number of unrelated alleles is steadily reduced over time by random genetic drift without replenishment in the finite metapopulation.

Isolation-by-distance and stepping-stone models are the spatially explicit genetic counterparts of the ecological lattice models. Isolation-by-distance models (Wright 1943, Malécot 1948) are continuous models where populations are uniformly distributed in space and gene flow is defined as a probability distribution of the dispersal distances between the locations of parents and offspring. Stepping-stone models (Kimura 1953) consider demes arranged on a lattice in one, two or three dimensions (box 1.6). Migrants move between pairs of adjacent demes taking one or more steps per migration event. Infinite stepping-stone models assume an infinite number of demes, whereas finite one- and two-dimensional models comprise of demes arranged on a row or, for example, in a square. Such a spatial arrangement commonly brings about edge effects due to different behaviour of demes situated on the boundary or ends of a lattice (Maruyama 1970a, 1971, Malécot 1975). A spatial arrangement of many demes in a circle or torus for one or two dimensions, respectively, is commonly assumed to avoid edge effects in finite theoretical models (Slatkin 1985, box 1.6). Since the number of unrelated alleles is limited in finite stepping-stone models when long-distance migration from outside the system and mutation are assumed to be absent, the predictions of these models are comparable with the predictions of the finite *n*-island model in that the fixation index F_{ST} will attain a system-specific drift-migration equilibrium, whereas the autozygosity *F* will eventually increase to unity (Malécot 1975, Slatkin 1985).

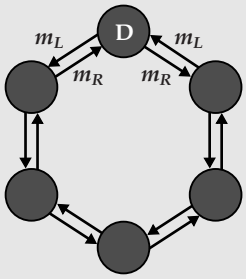
Box 1.6 Stepping-stone models of migration



Finite two-dimensional stepping-stone model

The connectivity of demes in **finite stepping-stone models** depends on their position on the lattice. Different levels of connectivity result in different levels of gene flow and autozygosity in the demes at the boundary of the lattice ("edge effect"). **Infinite stepping-stone models** have no edge effects, and are more commonly applied in theoretical development.

In a **finite circular stepping-stone model** migrants move from the focal deme (D) in both directions at rates $m_L = (1 - r)m$ ("to the left") and $m_R = rm$ ("to the right"). Parameter r indicates the symmetry of migration ranging from symmetrically bidirectional for $r = 0.5$, to unidirectional to the left or to the right for $r = 0$ or $r = 1$, respectively. In a metapopulation with k demes and $m = m_L + m_R$, four recurrence equations describe the autozygosity over time in the focal deme F_0 and in demes 1 to k steps away (F_1 to F_k). Parameters a, b and c indicate the probabilities that two sampled alleles originate from a single migrant or resident, from one migrant and one resident, or from two migrants (Maruyama 1970b, CHAPTER 3):



Circular stepping-stone model with asymmetrical migration

$$F_0' = a[1/2N + (1 - 1/2N)F_0] + 2bF_1 + 2cF_2$$
$$F_1' = aF_1 + b[1/2N + (1 - 1/2N)F_0] + bF_2 + c(F_1 + F_3)$$
$$F_2' = aF_2 + b(F_1 + F_3) + c[1/2N + (1 - 1/2N)F_0] + cF_4$$
$$F_k' = aF_k + b(F_{k+1} + F_{k-1}) + c(F_{k+2} + F_{k-2})$$

$$a = (1 - m)^2 + m_L^2 + m_R^2$$
$$b = m(1 - m)$$
$$c = m_L m_R$$

GENE FLOW THROUGH POPULATION TURNOVER

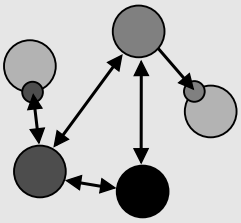
Slatkin (1977) introduced two types of colonization to include gene flow through local extinction and recolonization in the island model of migration (box 1.7). With propagule colonization, colonists occur clustered, such as a capsule with seeds or a clutch of eggs on a host, and hence arrive together in an empty deme. With migrant pool colonization, colonists are recruited from the migrant pool, and may share the same source deme by coincidence upon arrival in an empty deme (Slatkin 1977). Hence, colonists in a propagule will generally be more related than colonists in a migrant pool. Subsequent studies (Wade & McCauley 1988, Whitlock & McCauley 1990, Pannell & Charlesworth 1999) provided a number of predictions for the equilibrium levels of genetic differentiation F_{ST} for both types of colonization in the infinite and finite island models. In case of propagule colonization, the equilibrium level of F_{ST} will generally be similar to or larger than the level in a metapopulation without local extinction, depending on the relative magnitudes of the extinction and migration rates and the relative number of colonists

Box 1.7 Island model with local extinction and recolonization

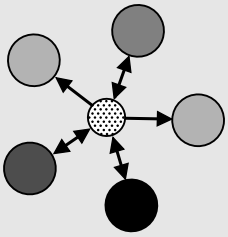
In the **finite *n*-island** model with local extinction at extinction rate e and subsequent colonization of the empty demes at colonization rate $c = e$ by k colonists, the recurrence equations describing the change of autozygosity in the focal deme (F_0) and outside the focal deme (F_1) over time (box 1.4) are extended with the terms E_1, E_2 and E_3 (shown in bold). E_1 to E_3 are the autozygosities among colonists of different origins, and parameters A, B and C are the corresponding probabilities of the different origins (Slatkin 1977, Rousset 2003):

$$F_0' = \mathbf{(1-e)}[a(1/2N + (1 - 1/2N)F_0) + (1-a)F_1] + \mathbf{eE_1}$$
$$F_1' = \mathbf{A}[b(1/2N + (1 - 1/2N)F_0) + (1-b)F_1] + \mathbf{BE_2} + \mathbf{CE_3}$$

E_1 is the autozygosity of two alleles that are sampled in the same colonized deme and depends on the autozygosity F_k among the k colonists, and thus, on whether colonization followed the propagule model or the migrant pool model:



$$\mathbf{E_1} = 1/2k + (1 - 1/2k)\mathbf{F_k}$$



Propagule colonization **Migrant pool colonization**

With **propagule colonization** all k colonists originate from a single source deme, and the autozygosity F_k among them is:

$$\mathbf{F_k} = 1/2N + (1 - 1/2N)F_0$$

With **migrant pool colonization**, the k colonists represent a random sample from the migrant pool that comprises of individuals originating from $n^* = n(1-e)$ extant demes. The autozygosity F_k among them is:

$$\mathbf{F_k} = 1/2Nn^* + (1 - 1/2Nn^*)[(1/n^*)\,F_0 + (1 - 1/n^*)\,F_1]$$

E_2 and E_3 are the autozygosities of two alleles sampled in a colonized and an extant deme, and in two different colonized demes, respectively, and they are equal and independent of the colonization model:

$$\mathbf{E_1} = \mathbf{E_3} = (1/n^*)[1/2N) + (1 - 1/2N)F_0] + (1 - 1/n^*)\,F_1$$

(Wade & McCauley 1988, Pannell & Charlesworth 1999). Migrant pool colonization may either increase or decrease the equilibrium level of F_{ST} compared with a metapopulation without extinction, depending on whether the number of colonists per colonization event is smaller or larger than twice the number of migrants per deme (Wade & McCauley 1988, see also CHAPTER 4).

The theoretical analysis of the effects of gene flow through local extinction and colonization on the genetic differentiation in stepping-stone models is considerably less complete than for the island models. Maruyama & Kimura (1980) analyze the effects of

local extinction and colonization for a finite one-dimensional stepping-stone model without additional gene flow through migration. They conclude that the frequent occurrence of population turnover will substantially increase the equilibrium level of genetic differentiation when migration events are rare or absent (*i.e.*, colonization follows the propagule pool model). This conclusion is not unlike the results for the finite island model under similar conditions (*i.e.*, with propagule-pool colonization and much higher extinction rates than migration rates, Pannell & Charlesworth 1999).
The lack of theoretical studies likely reflects the commonly used approach of describing a stepping-stone model by means of an extended set of recurrence equations that tends to get complicated when including population turnover, and generally allows no easy analytical solutions to predict the expected equilibrium values of genetic differentiation. However, computer simulations provide an alternative approach to study the dynamics of stepping-stone models (Ibrahim *et al.* 1996), or to generate approximations of natural systems (Kitamura *et al.* 2005). In CHAPTER 4, I extend my individual-based simulation model of stepping-stone migration to include stochastically occurring local extinction events. Recolonization takes place whenever a pair of a female and a male, or a single inseminated female arrive in an empty deme as a result of migration. The results from such long-term simulations suggest that systems comparable to the experimental metapopulations are very unstable due to the high stochasticity of population turnover events, and do not attain a stable equilibrium value of F_{ST}.

The island and stepping-stone models are two extremes in a continuum of increasingly more spatially explicit models of gene flow. Island models represent the extreme in long-distance migration, because distance does not play a role and all demes are equally connected. Stepping-stone models, on the other hand, represent the extreme in short-distance migration, because migrants can move between adjacent demes only (Slatkin 1985). Although most natural populations are likely to operate somewhere in between these extremes, the unidirectional circular stepping-stone model (box 1.6) is the point of departure in the migration experiments presented in this thesis (CHAPTER 3). This extreme starting point allows the assessment of the maximum potential discrepancy between inferences on the genetic structure of metapopulations based on the infinite island model and the actual structure. In subsequent experiments I also consider the more natural bidirectional variant of the stepping-stone model, and the *n*-island model allowing random allocation of migrants (CHAPTER 4).

GENETICS IN A METAPOPULATION CONTEXT

Genetics is increasingly used as a tool to elucidate phylogenetic relationships, to unravel the structure and divergence of populations, to determine the mating system or reproductive system, or to monitor the dynamics of an endangered population. To this end, genetic parameters such as allele frequencies, heterozygosities and gene diversity indices are estimated and used to infer demographic parameters such as population sizes, dispersal rates, and level of fragmentation (Weir & Cockerham 1984, Slatkin 1985,

Slatkin & Barton 1989, Wilkinson-Herbots 1998, Williamson & Slatkin 1999, Weir & Hill 2002). These inferences are often implicitly or explicitly based on the standard island models of population subdivision that are derived for systems with continuous gene flow through migration, and assume drift-migration equilibrium of neutral genetic variation. Hence, they can lead to misleading interpretations of the status of a metapopulation where gene flow is not only mediated by migration but also by population turnover. When randomly occurring extinction and founder events play a major role, the resulting pattern and dynamics of genetic diversity may be quite different from predictions considering only population sizes and migration parameters (Slatkin 1977, Wade & McCauley 1988, Whitlock & McCauley 1990, Gilpin 1991, Lande 1992, Pannell & Charlesworth 1999). The occurrence of patch coalescence (*i.e.*, all extant demes in a metapopulation descend from individuals originating from a single deme in the past) may substantially reduce genetic variation in a metapopulation, even though the number of individuals and the amount of gene flow have always been large (Gilpin 1991, Hedrick & Gilpin 1997). Furthermore, the distribution of genetic variation in a metapopulation will to a large extent depend on local demography and migration patterns (Whitlock 1992, Gaggiotti & Smouse 1996, Ingvarsson 2002, Rousset 2004).

MUTATION AND SELECTION

Mutation is the ultimate source of genetic variation, which may be either neutral or adaptive. Neutral variation has very little or no effect on the fitness of individuals, whereas adaptive variation affects fitness, and is targeted by natuaral selection as a consequence. Natural selection favours heritable traits that maintain or increase the reproductive success of organisms, and hence increases the frequency of such traits in a population (Darwin 1859).

Kimura (1968) hypothesized that most genetic variation at the molecular level is neutral or nearly so. Neutral variation is subjected to genetic drift but not to natural selection, hence over time populations will attain an equilibrium level of genetic variation where the loss of alleles due to genetic drift is compensated by the gain of alleles due to mutation. The genetic models in the previous section assume neutral genetic variation in mutation-drift equilibrium in idealized populations, and do not explicitly consider mutation. Including mutation in the finite models, however, would allow for a constant, low influx of new alleles that is comparable to the influx of unrelated long-distance migrants at a low rate in the infinite models, which may considerably affect the equilibrium levels of autozygosity and genetic differentiation (Wilkinson-Herbots 1998). Although experimental metapopulations are finite by default, I apply the genetic models without considering mutation because the intended time-scale of the experiments in this study is very short compared with an average evolutionary time-scale.

The spatial stucture of a metapopulation interacts with natural selection in two ways. Firstly, population subdivision affects the impact of selection even when selection pressures are uniform in all demes (Ohta 1992, Barton 1993, Whitlock 2002, Glemin *et al.* 2003, Roze & Rousset 2003, Rousset 2004). Directional selection tends to be more efficient in metapopulations with continuous gene flow (Whitlock 2002, Glemin *et al.* 2003),

whereas the occurrence of population turnover generally decreases the efficiency of selection (Barton 1993, Cherry 2004). Secondly, spatial heterogeneity may induce differential selection pressures leading to different levels of local adaptation in different demes (*e.g.*, Felsenstein 1976, Hedrick *et al.* 1976, Hedrick 1986, Barton 2001). The focus of this thesis is on the dynamics of neutral genetic variation expressed at a diallelic eye colour marker locus (box 1.14). However, since this presumed neutral marker (Buri 1956) displayed significant adaptive behaviour in practice, I included an optional, simple additive model of viability selection between the zygote and the adult life stages of individuals in my computer simulations to assess the impact of directional selection in our experimental metapopulations (see CHAPTER 2 for details). I also used this selection model to explore some basic expectations of adaptation to changing environmental conditions in my experimental metapopulations (CHAPTER 5).

INDICES OF GENETIC DIVERSITY AND DIFFERENTIATION

The level of genetic diversity in a population is decided by the occurrence of polymorphism (*i.e.*, the number and frequencies of different alleles of a gene) in the population. In all finite, random mating populations genetic drift occurs to some extent, leading to an increase of autozygosity F and the loss of alleles over time, and thus to a gradual decline of genetic diversity. Although autozygosity is thus an obvious indicator of genetic diversity, its applicability is limited because it is not possible to distinguish between autozygosity and homozygosity in practice. Instead, the expected heterozygosity H_E (Hartl & Clark 1997) is used to quantify genetic diversity based on allele frequencies (*e.g.*, Varvio *et al.* 1986, see also box 1.3).

In addition to the effects of random drift in finite populations, the relatedness of individuals will generally increase by population fragmentation. Wright (1951) defined a hierarchical system of *F*-statistics to quantify these effects based on the analysis of the standardized variance of allele frequencies between generations. Since this variance is proportional to the expected heterozygosity (box 1.3), F can be defined as the relative reduction of heterozygosity expected under random mating conditions by comparing the reduction in heterozygosity at any level of population structure to that at a higher, more inclusive level of population structure for a particular generation.

The inbreeding coefficient F_{IS} represents the lowest level of the *F*-statistics and indicates a shortage or a surplus of heterozygotes within a population by comparing the observed heterozygosity H_I with the expected heterozygosity H_S (Hartl & Clark 1997). Hence, F_{IS} is a coefficient of deviation of random mating rather than a coefficient of relatedness. The commonly used term "inbreeding coefficient" suggesting the latter is rather unfortunate, and often leads to confusion in practice (Templeton & Read 1994, Rousset 2002).

The fixation index F_{ST} (box 1.8) representing the next level of the *F*-statistics is the most widely used measure of genetic differentiation among demes within a metapopulation. Wright's (1931) original definition of F_{ST} (*i.e.*, the standardised variance in allele frequencies between generations) is interpreted as the reduction of the average heterozygosity of single demes relative to the heterozygosity of the entire metapopulation.

Box 1.8 Fixation index

Wright (1931) originally defined the fixation index F_{ST} as the standardised variance in allele frequencies between generations. Most equivalent indices for the analysis of molecular data using AMOVA techniques, *e.g.*, θ (Weir & Cockerham 1984), are based on this interpretation of F_{ST} .

$$F_{ST} = \frac{\sigma_{\bar{p}}^2}{\bar{p}\,(1-\bar{p})}$$

The hierarchical *F*-statistic F_{ST} is widely used as a measure of genetic differentiation among demes within a metapopulation. F_{ST} compares the average expected heterozygosity within demes $\bar{H}_S$ to the expected heterozygosity of the entire metapopulation H_T (Wright 1951).

$$F_{ST} = \frac{H_T - \bar{H}_S}{H_T}$$

The more general alternative is based on the ratio of the difference between the probabilities of identity within demes F_0 and among demes F_1 (Rousset 2004).

$$F_{ST} = \frac{F_0 - F_1}{1 - F_1}$$

The above definitions of *F*-statistics are relative to an ancestral random mating population (F_{IS}) and an unstructured population (F_{ST}), respectively. This dependency on a reference population may be applicable under some conditions, but may lead to inconsistencies under different circumstances (Rousset 2002). Rousset (2002, 2004) proposes a generic definition of "inbreeding coefficients" as the ratio of the difference in the probabilities of identity within a structural unit and among two different structural units. Such a structural unit can be an individual within a population, or a deme within a metapopulation (box 1.8, see also CHAPTER 3).

Although the fixation index F_{ST} is widely used to assess the genetic structure of metapopulations, its applicability has been increasingly questioned in recent years (Nagylaki 1998, Whitlock & McCauley 1999, Neigel 2002, Pearse & Crandall 2004). Its definition generally assumes neutral genetic variation and low mutation rates under the infinite-alleles model of mutation (Kimura & Crow 1964). Contemporary studies routinely screen natural populations with the help of molecular genetic markers such as AFLPs, RAPDs, microsatellites or SNPs (Parker *et al.* 1998, Hedrick 1999, Kuhner *et al.* 2000, Sunnucks 2000, Vignal *et al.* 2002). The dynamics of these markers can be quite different already in the absence of population structure because they are often not selectively neutral, mutation rates tend to be high, and mutation more likely follows a stepwise model (Goldstein *et al.* 1995, 1996, Slatkin 1995, Nauta & Weissing 1996). Hence, interpretation of the results strictly in terms of the classic models may yield inaccurate conclusions (Balloux *et al.* 2000, Estoup *et al.* 2002, Slatkin 2005).

In response, one mostly finds two approaches to interpret the results from natural systems: either the modification of the traditional estimators of genetic differentiation, or more recently, the development of new methods to analyze population structure. G_{ST} (Nei 1973, Hanski *et al.* 1996), R_{ST} (Goodman 1997), θ (Weir & Cockerham 1984, Weir & Hill 2002) and Φ_{ST} (Excoffier *et al.* 1992) are some well-known estimators of differentiation based on *F*-statistics adapted to molecular data using AMOVA (Analysis of

MOlecular VAriance) techniques. Newly developed methods include coalescence-based estimators that take account of the demographic history of populations (Wilkinson-Herbots 1998, Beerli & Felsenstein 1999, 2001, Wakeley 2001, Gaggiotti *et al.* 2002, Clegg *et al.* 2003, Anderson 2005), and statistical methods based on maximum-likelihood approaches (Laval *et al.* 2003) or assignment tests that cluster individual genotypes into populations (Waser & Strobeck 1998, Pritchard *et al.* 2000, Paetkau *et al.* 2004, Waples & Gaggiotti 2006). All these methods show a tendency to ever increasing computational demands and complexity, and a number of computer programs has become available to aid in the analysis of (large) datasets, for example FSTAT (Goudet 2000), Genepop (Raymond & Rousset 2003), Arlequin (Schneider *et al.* 2000), Structure (Pritchard *et al.* 2000), BAPS (Corander *et al.* 2003, 2004).

EFFECTIVE POPULATION SIZE AND GENE FLOW

Population size and numbers of migrants indicating the level of gene flow among populations are undoubtedly the most important demographic parameters commonly inferred from genetic parameters in practice, for example to assess stocks in fisheries biology (Hansen *et al.* 2000, Doornik 2002, Turner *et al.* 2002, Ardren & Kapuscinski 2003) or to manage endangered species in conservation biology (Tufto & Hindar 2003, Hedrick 2004). However, such inferences can be problematic since the highly idealized assumptions of the population genetic models are seldom satisfied in natural populations. To allow for deviations between real and model populations, Wright (1931, 1938) introduced the concept of effective population size. The genetic effective size N_e is generally defined as the size of an idealized Wright-Fisher population exhibiting the same dynamics of genetic variation as the natural population in question (Wright 1969, Crow & Kimura 1970, Crow & Denniston 1988). For example, the effective size can be considered as a measure of the decline of genetic variation due to genetic drift in a finite population (Wang & Caballero 1999). In an idealized population where individual contributions to the next generation are Poisson-distributed (*i.e.*, individuals have an equal chance to contribute), the effective size N_e equals the census size N. When all individual contributions are exactly equal (*i.e.*, no variance in reproductive success) N_e is twice the census size N, whereas increasing the variance in reproductive success decreases N_e so that the ratio $N_e / N < 1$ (Wright 1938). Factors affecting reproductive success include for instance sex ratio, age structure, mating system, fecundity or the presence of directional selection (Nunney 1991, 1993, 1996, Caballero 1994, 1995, Santiago & Caballero 1995, Wang 1996, Glemin *et al.* 2003, see also CHAPTER 2).

Unfortunately, effective population size is not an unequivocal concept, since the common definitions of effective size are based on different aspects of a population. The inbreeding effective size, variance effective size, and eigenvalue effective size are defined in terms of autozygosity, variance in allele frequencies and heterozygosity, respectively (box 1.9). All three effective sizes may differ substantially from another depending on whether a population is either growing or declining, or in demographic equilibrium (Crow 1954, Ewens 1979, 1982, Basset *et al.* 2001). These differences can span several orders of magnitude under exceptional conditions, such as extinction events (Ewens 1989).

Box 1.9 Genetic effective population size

The effective population size N_e equals the size of an idealized Wright-Fisher population yielding either the same amount of variance in allele frequency change among the offspring (variance effective size N_e^{σ}), the same level of heterozygosity in the offspring (eigenvalue effective size N_e^{λ}), or the same level of autozygosity in the parents (inbreeding effective size N_e^{i}) as the actual population in any generation:

$$N_e^{\sigma} = \frac{p\,(1-p)}{2\sigma^2_{\delta p}} \qquad N_e^{\lambda} = \frac{H_t}{2(H_t - H_{t+1})} \qquad N_e^{i} = \frac{1-F_t}{2(F_{t+1} - F_t)}$$

If autozygosity is approximated by homozygosity, the inbreeding effective size is equivalent with the eigenvalue effective size, since $H_t = 1 - F_t$. The inbreeding effective size is a measure of the past, as the inbreeding coefficient depends on the number of ancestors in the parent generation. The variance effective size is a measure of the future, as the sampling variance depends on sample size, which is the size of the offspring generation. Thus, in a growing population, N_e^{σ} tends to be larger than N_e^{i}, and in a declining population the reverse is the case. In a stationary population the census size N is constant, so that the average number of offspring per parent is two, and the inbreeding and variance effective sizes are equal (Crow & Kimura 1970, Crow & Denniston 1988).

The estimation of the effective population size (or, alternatively, the effective number of migrants $M_e = N_e m$, *i.e.*, the effective size N_e multiplied by migration rate m) is a crucial step in most genetic screening programmes (*e.g.*, Saccheri *et al.* 1998, Caballero & Toro 2002, Miller & Waits 2003). Hence, one finds a variety of estimators to infer N_e that are developed according to four main approaches (Beaumont 2003). The first approach estimates variance effective sizes based on the (non-genetic) variance in reproductive success among individuals (reviewed in Caballero 1994, box 1.10). This method requires detailed knowledge of the life-history of a population, and may thus be difficult to measure in practice (Frankham 1995, Austerlitz & Heyer 1998, Waples 2002). The second approach esimates the eigenvalue effective size using single genetic samples to measure for example heterozygote excess (Pudovkin *et al.* 1996, Luikart & Cornuet 1999, Balloux 2004) or linkage disequilibrium (Langley *et al.* 1978, Laurie-Ahlberg & Weir 1979, Hill 1981, Hayes *et al.* 2003). However, these estimators tend to have low power because they are affected by many different processes. The third and most widely used approach estimates variance effective sizes based on the difference in allele frequencies between two or more samples of the same population taken at different moments in time. These temporal estimators can be either moment-based (Krimbas & Tsakas 1971, Nei & Tajima 1981, Pollak 1983, Waples 1989, Luikart *et al.* 1999), likelihood-based (Williamson & Slatkin 1999, Anderson *et al.* 2000, Wang 2001, Wang & Whitlock 2003, Tallmon *et al.* 2004, Anderson 2005), or coalescence-based (Beaumont 1999, Berthier *et al.* 2002, Beaumont 2003). In contrast with the above estimators that assume short sampling periods without noticeable effects of mutation, the fourth method estimates inbreeding effective sizes using sequence data from serial samples covering an evolutionary time-scale including mutation (Rodrigo *et al.* 1999, Fu 2001, Drummond *et al.* 2002).

Box 1.10 Effective population size with lottery polygyny and remating

In CHAPTER 2 we use the first approach to estimate the variance effective size for the *Drosophila* lottery polygyny model independantly of genetic data. We assume that females mate only once, or remate with probability ρ, while males attempt to mate as often as possible. We further assume a stationary population with sex ratio 1:1, and use the variance-mean ratio of the number of offspring contributed to the next generation by a single mating α to describe the variance in reproductive success. Hence, we infer N_e:

$$N_e = \left[\frac{1}{1 + \alpha/2 - [\rho/(1+\rho)]^2} \right] N$$

When females mate strictly once ($\rho = 0$) and assuming that the number of offspring per mating follows a Poisson distribution ($\alpha = 1$), the effective population size N_e is $^2/_3$ of the census size N. With the observed variance-mean ratio $\alpha = 1.6$ and remating probability $\rho = 0.2$ for *Drosophila* in our experimental metapopulations, we can predict an effective population size $N_e = 0.56N$ (CHAPTERS 2 and 3).

In CHAPTER 2, I use the first approach based on the variance in reproductive success to predict the effective size independantly of genetic data taking account of two important factors affecting the variance in reproductive success of males in *Drosophila*. The first factor is the lottery polygyny mating system that is often associated with *Drosophila* (Bateman 1948, Nunney 1993). In the basic form of lottery polygyny all females mate exactly once, while males vary in their number of matings because they are randomly chosen as mates by females. The second factor is the probability to remate with a different male for mated females (Bundgaard & Christiansen 1972, Van Vianen & Bijlsma 1993). The *Drosophila* mating system reduces the effective population size considerably depending on ρ, the remating probability, and on α, the variance-mean ratio of the number of offspring contributed to the next generation by a single mating (box 1.10, CHAPTER 2).

In addition, I derive two temporal estimators of N_e based on genetic data to compare with the predictions based on the demographic estimator (box 1.11). Since I collected data each generation, I can use linear regression to infer the variance effective size from the variance in allele frequency change between two successive generations as a function of the allele frequency in the parental generation. I infer the eigenvalue effective size in a similar way through linear regression of the change in heterozygosity between two successive generations as a function of the heterozygosity of the parental generation (box 1.11, CHAPTER 2). Although these estimators yield accurate estimates of the effective population size under laboratory conditions, they are generally not applicable in field studies because they make strict assumptions such as fixed population sizes and strictly neutral genetic markers that are often not satisfied in nature (CHAPTER 2).

From similar considerations as for the contemporary estimators of genetic differentiation, a number of computer programs is available to infer effective population sizes

Box 1.11 Estimators of N_e based on linear regression

Because I sample the allele frequencies each generation, I can infer the variance effective size $\hat{N}_e^{\sigma}$ directly from the allele frequency variation (box 1.9) by applying linear regression to the variance $\sigma^2_{\delta p}$ in allele frequency change δp between two successive generations as a function of the allele frequency p in the parental generation, with p_i the allele frequency of the offspring of a parental population with allele frequency p and i the number of cases that this parental allele frequency p was observed (CHAPTER 2):

$$\sigma^2_{\delta p} = \left[\sum (p_i - p)^2\right] / i = \frac{1}{2\hat{N}_e^{\sigma}} [p(1-p)]$$

Similarly, I can infer the eigenvalue effective size $\hat{N}_e^{\lambda}$ through linear regression of ΔH, the change in heterozygosity between two successive generations t and $t + 1$, as a function of the heterozygosity H_t of the parental generation (CHAPTER 2):

$$\Delta H = H_t - H_{t+1} = \frac{1}{2\hat{N}_e^{\lambda}} H_t$$

and/or migration rates based on molecular genetic datasets, for example MCLEEPS (Anderson *et al.* 2000), MLNE (Wang & Whitlock 2003), TM3 (Berthier *et al.* 2002). The package NeEstimator (Peel *et al.* 2004) provides an interface for the previous three likelihood-based programs, and also includes the moment-based approach following Waples (1989) and two single-sample approaches based on heterozygote excess following Pudovkin *et al.* (1996) and on linkage disequilibrium following Hill (1981). I evaluate the performance of these likelihood-based and moment-based estimators compared to the regression-based estimators that I derived for our experimental data (CHAPTER 2).

In addition to the variation in reproductive success of individuals within a population, subdivision also affects the effective size of a population (Caballero 1994, Wang & Caballero 1999). The effective metapopulation size is defined as the size of an idealized, undivided Wright-Fisher population that would show the same dynamics of variation in allele frequency changes as the actual metapopulation (reviewed in Wang & Caballero 1999). Since the effective size of a metapopulation is a measure of the decline of genetic variation at the metapopulation level, it represents a useful tool to assess for instance the viability of metapopulations for conservation management purposes (Hedrick & Gilpin 1997, Wang & Caballero 1999, Caballero & Toro 2000, 2002). Comparable to the lower level where the reproductive variance of individuals affect the effective size of a single deme, the effective size of the entire metapopulation (box 1.12) depends on the variation among its demes in their contributions to subsequent generations, or the "reproductive success" of demes. In the absence of local extinction and recolonization events, this variation is mainly governed by the dynamics within demes (*i.e.*, differential reproductive success of individuals) leading to fluctuations in deme size and migration rates. More

variation in the productivity of demes will generally decrease the effective population size (Nunney 1999). In the special case where all demes contribute equally to subsequent generations, the effective size is larger than the census size when the migration rate among demes is low (Nei & Takahata 1993). The regular occurrence of extinction events increases the variance of deme productivity enormously, since extinct demes do not contribute at all while a single extant deme may contribute 100% by colonizing an empty patch. As a consequence, the effective metapopulation size may decrease at a dramatic rate (Maruyama & Kimura 1980, Hedrick & Gilpin 1997, Whitlock & Barton 1997, Wang & Caballero 1999, Rousset 2003). Other important factors affecting the effective metapopulation size include the colonization model (*i.e.*, migrant-pool *versus* propagule-pool colonization), the number of demes, and the level of gene flow (Hedrick & Gilpin 1997). Migrant-pool colonization has less adverse effects than propagule-pool colonization (Wade & McCauley 1988, Whitlock & McCauley 1990), since potential colonists in a migrant pool will generally be less related than potential colonists in a propagule (see box 1.7). More demes and low extinction rates affect the effective size positively, as do high levels of gene flow. This last result is opposite to the effect of gene flow in metapopulations without local extinction, because substantial gene flow is required to counter the adverse effects of extinction-colonization dynamics, which may reduce genetic variation within demes to zero (Hedrick & Gilpin 1997. I evaluate the effect of extinction-colonization dynamics on the effective metapopulation size in CHAPTER 4.

Box 1.12 Effective mtapopulation size

The effective metapopulation size N_e^M equals the size of an idealized Wright-Fisher population that would yield the same dynamics of genetic variation as the actual subdivided population. General formulas (*i.e.*, without assumptions about geographical structure or migration model) infer the variance effective size N_e^M in three widely considered special cases (Wang & Caballero 1999):

(i) Deme size N is constant and equal, and all n demes contribute equally to the next generation through migration ($V = 0$):

$$N_e^M = \frac{nN}{1 - F_{ST}}$$

(ii) Deme size N is constant and equal for all n demes, but the demes contribute to the next generation through migration with variance V:

$$N_e^M = \frac{nN}{(1 - F_{ST})(1 + V) + 2NVF_{ST}\, n(n-1)}$$

(iii) With local extinction at extinction rate e and colonization following a migrant-pool model, contribution to the next generation per deme varies between 0 and $N/(1 - e)$ with $V = e/(1 - e)$:

$$N_e^M = \frac{nN(1 - e)}{1 - F_{ST} + 2eNF_{ST}}$$

Without differentiation ($F_{ST} = 0$) N_e^M equals the census size nN, and with maximum differentiation ($F_{ST} = 1$) N_e^M approaches infinity. The expressions (ii) and (iii) reduce to (i) if $V = 0$ and $e = 0$, respectively, and result in N_e^M smaller than (i) otherwise.

BIODIVERSITY AND CONSERVATION GENETICS

When the focus of conservation biology changed from the management of single species to the design and management of nature reserves in the 1970s, this initiated among other things, the well-known debate on the pros and cons of single large *versus* several small populations (SLOSS debate, see *e.g.* Hanski & Simberloff 1997) to preserve biodiversity in the best possible way. The (ecological) metapopulation approach represents a potentially powerful tool to deal with such controversies, for instance by comparing alternative reserve designs (Cabeza *et al.* 2004a) and assessing population viability and minimum viable population size (Reed *et al.* 2002, 2003c). In the long run, however, biodiversity reflects genetic heterogeneity. Hence, the concerns of conservation biology ultimately represent concerns about the loss of genetic diversity. Although disputed at first (Caro & Laurenson 1994, Caughley 1994), nowadays there is a general consensus on the importance of genetics for the persistence and fitness of natural and managed populations (Spielman *et al.* 2004b, Frankham 2005a). The primary genetic threats to population persistence are inbreeding depression, the loss of genetic variation and, to a lesser extent, the accumulation of deleterious mutations (Gaggiotti 2003, Frankham 2005a). The combined occurrence of these threats resulting in the genetic impoverishment of populations is commonly referred to as "genetic erosion" (Bijlsma *et al.* 1994). Once genetically impoverished, a population may no longer be able to track its biotic and abiotic environment and it may lose its potential for adaptation to future environmental challenges (Bürger & Lynch 1997, Reed *et al.* 2003a, Frankham 2005b). The consequences of genetic erosion are not necessarily restricted to the species in question, since the loss of adaptive potential may be of particular importance in the context of species interactions, such as the arms race between hosts and their parasites (Gandon & Michalakis 2002) or the relation between plants and their pollinators (Waser *et al.* 1996). This local co-evolution can be a fast and fine-scaled process (Mopper 1996, Capelle & Neema 2005), and loss of potentially adaptive genetic variation can severely affect the "interaction biodiversity" of a species community (Thompson 1999).

GENETIC EROSION

There is a substantial body of evidence that genetic erosion is an important factor for the persistence of small populations. Firstly, small population size may easily lead to inbreeding depression (Frankham 1998, Hedrick & Kalinowski 2000). Inbreeding depression may in turn significantly enhance the extinction risk of a population, both in captivity (Bijlsma *et al.* 2000, Reed *et al.* 2003b) and in nature (Crnokrak & Roff 1999, Keller & Waller 2002, O'Grady *et al.* 2006). In addition, stressful environmental conditions tend to further increase the extinction risk in many cases (Bijlsma *et al.* 1997, Armbruster & Reed 2005, Bijlsma & Loeschcke 2005). Secondly, small populations are substantially affected by genetic drift causing the loss of potentially favourable genetic variation (Allendorf 1986). Although this is generally a long-term threat to extinction, the loss of allelic variation may represent a more immediate threat when associated with the resistance to disease depending on extremely polymorphic loci (Gaggiotti 2003).

Thirdly, small population size may enhance the process of mutational meltdown (Lynch *et al.* 1993, 1995a, 1995b). To date there is little evidence of the last two factors in natural populations, and their importance for population extinction is unclear, especially in view of opposing processes such as compensatory mutation (Poon & Otto 2000, Whitlock *et al.* 2003).

In a metapopulation context, migration may either mitigate or enhance the effect of genetic erosion on the extinction risk of the metapopulation. On the one hand, migration may lead to positive heterosis since inbreeding depression is likely to involve different sets of deleterious mutations in different demes (Ingvarsson & Whitlock 2000, Whitlock *et al.* 2000), and may restore genetic variation in single (small) demes by (re)introducing new alleles from larger demes (Gaggiotti & Smouse 1996). On the other hand, migration may lead to outbreeding depression by disrupting locally co-adapted gene complexes (Templeton 1986) and to a "migrational meltdown" in heterogeneous habitats by introducing maladapted alleles into locally adapted demes reducing the effective size that in turn reduces the ability to adapt (Ronce & Kirkpatrick 2001). The overall picture of the consequences of genetic erosion in a metapopulation is, however, far from complete, and will require many more theoretical and empirical contributions focusing in particular on the interaction of genetic and demographic processes (Gaggiotti & Hanski 2004).

ADAPTIVE POTENTIAL

A major concern of conservation biology is the loss of flexibility and adaptive potential of small populations due to genetic erosion (Frankham 2005b), particularly in view of the present-day climate change on a global scale (Parmesan & Yohe 2003, Hampe & Petit 2005, Root & Schneider 2006). The ability to respond to changing environmental conditions is defined at two levels (Badyaev 2005, Bijlsma & Loeschcke 2005). As a first step, a population must harbour sufficient genetic variability to ensure its survival during the first manifestation of environmental changes, albeit at a reduced fitness level ("stress tolerance"). Stress tolerance will depend partly on phenotypic plasticity (Bijlsma & Loeschcke 2005) and partly on the available allelic variation (Macnair 1991, Lynch & Lande 1993). In the second step, fitness will be restored to a new optimum by adaptation to the new environment. Since both stress tolerance and adaptation occur on short to intermediate evolutionary time scales, new beneficial mutations will be rare, hence the adaptive response will mainly depend on the standing genetic variation (Bijlsma & Loeschcke 2005). Population fragmentation may represent an additional source of variation to the processes affecting adaptive potential (Laine 2005, CHAPTER 5).

THE OUTLINE OF THIS THESIS

In this thesis I use a very broad definition of a metapopulation including three stages of connectivity enabling different levels of gene flow. The simplest stage is a cluster of demes that are completely isolated without any gene flow. The next stage is a cluster of demes that experience continuous gene flow through migration only. The final stage

corresponds to the strict definition of a metapopulation, and includes both continuous gene flow through migration and discontinuous gene flow through population turnover due to local extinction events followed by recolonization in subsequent generations. The increasing complexity of gene flow is mirrored in the experimental setup of this study. CHAPTER 2 deals with genetic drift only, CHAPTER 3 adds continuous gene flow through migration, and CHAPTER 4 includes both migration and population turnover. CHAPTER 5 focuses in more detail on the adaptive potential of a metapopulation.

RESEARCH QUESTIONS AND APPROACH

This study aims at analyzing the consequences of a metapopulation context for the dynamics of genetic diversity and genetic differentiation addressing the following questions:

- What are the consequences of metapopulation structure for the inference of demographic parameters based on classic population genetic models?
- What are the consequences of metapopulation structure for genetic processes such as inbreeding depression, genetic erosion and adaptive potential?
- What are the implications for practical applications in conservation management, *e.g.*, how important are assumptions regarding mating system, migration system or marker neutrality?

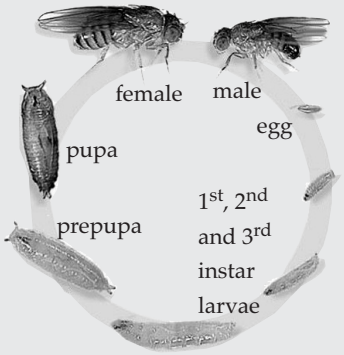
The most obvious approach to deal with these questions would be the analysis of natural, subdivided populations. Studies of natural systems, however, are typically descriptive and/or limited to drawing *a posteriori* inferences because it is hardly possible to carry out controlled experiments on fragmented populations of species that are often rare and endangered. Moreover, many of the processes involved are stochastic, implying that replicated observations are needed to allow generalization of the emerging patterns. Hence, one needs to develop *a priori* predictions and evaluate these based on controlled, replicated observations, for example by confronting the predictions from theoretical models with the outcome of structurally similar experiments, and *vice versa*. I followed this approach by developing simulation models approximating the genetic patterns emerging in a metapopulation, and using a model organism to set up replicated laboratory metapopulations that are easy to manipulate.

MODEL ORGANISM

Drosophila melanogaster is one of several well-established model species for investigating a wide range of issues in population genetics and conservation biology (Miller & Hedrick 1993, Frankham 1995). It is an ideal model organism (box 1.13) because of its short generation interval, ease and low expense of culture, the width and depth of knowledge of its genetics, and the availability of a range of stocks and markers for analysis.

I used a phenotypic marker based on eye colour mutations (box 1.14) that allows easy visual monitoring of genotype frequencies. The alleles bw and bw^{75} at the brown locus in combination with the mutation *scarlet* (*st*) in homozygous condition result in distinct eye colours for the three genotypes at the *bw* locus at 25°C. Homozygous *bw;st*

Box 1.13 Life cycle and breeding of *Drosophila melanogaster*



For this study, *Drosophila* flies are bred on standard medium (26 g dead yeast, 54 g sugar, 17 g agar, and 13 ml nipagine solution (10 g nipagine in 100 ml 96% alcohol) per liter). For easy handling, the flies are commonly anaesthetized with CO_2.

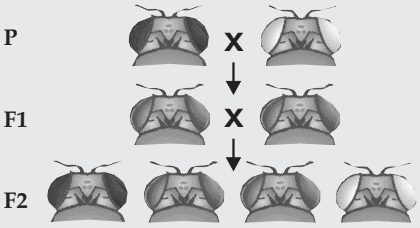
Under standard conditions (25°C, 40-60% RH) it takes 10-12 days to develop from egg to adult fly (picture after Weigmann *et al.* 2003). Males are generally smaller than females. They are identifiable by the black tip of the abdomen, among other things.

flies have white eyes, homozygous bw^{75}*;st* flies have red-brown eyes, and heterozygous bw^{75}*/bw;st* flies have intermediate orange eyes. The eye colour marker at the *brown* locus is similar to the marker used in Buri's (1956) classical experiments quantifying random genetic drift in small *Drosophila melanogaster* populations.

I obtained homozygous bw^{75}*;st* and *bw;st* stocks from the *Drosophila* stock centre in Umeå, Sweden. Since both stocks may have had considerably different genetic backgrounds, I homogenized the genetic background (with exception of the *bw*-marker region) by intercrossing and selecting virgin heterozygotes to found the next generation for six consecutive generations. From this cross, I established new homozygous bw^{75}*;st* and *bw;st* stocks to initiate populations for all experiments.

Box 1.14 Eye colour genetics of *Drosophila melanogaster*

The eye colour of a wild type (WT) fly is determined by red and brown pigments. The mutations *scarlet* (*st*) and *brown* (*bw*) suppress the production of brown and red pigments, respectively. Homozygous *st* or *bw* flies have scarlet (bright red) eyes or brown eyes, respectively, and homozygous *bw;st* flies have neither pigment, and thus, white eyes.



The brown mutation bw^{75} suppresses red pigmentation less efficient than *bw*. Homozygous bw^{75}*;st* flies have reddish brown eyes, and heterozygous bw^{75}*/bw;st* flies are intermediate with orange eyes.

The choice for a single phenotypic marker locus may appear outdated in view of today's common practice of using a (large) number of molecular markers (reviewed in Vignal *et al.* 2002). However, obtaining allele frequencies based on such markers is a very labour-intensive procedure that tends to limit the number of sampled individuals considerably. This is generally not a big problem in applied studies where a small number of demes is sampled in one or a few locations at one moment in time only (*e.g.*, Van de Zande *et al.* 2000). In an experimental study monitoring replicated metapopulations over a number of generations for several different scenarios the required number of samples tends to increase exponentially, which is not feasible logistically for many molecular marker loci. Hence, I chose to use a single visual marker locus, which has the additional advantage of a non-lethal monitoring procedure allowing sampled flies to found successive generations.

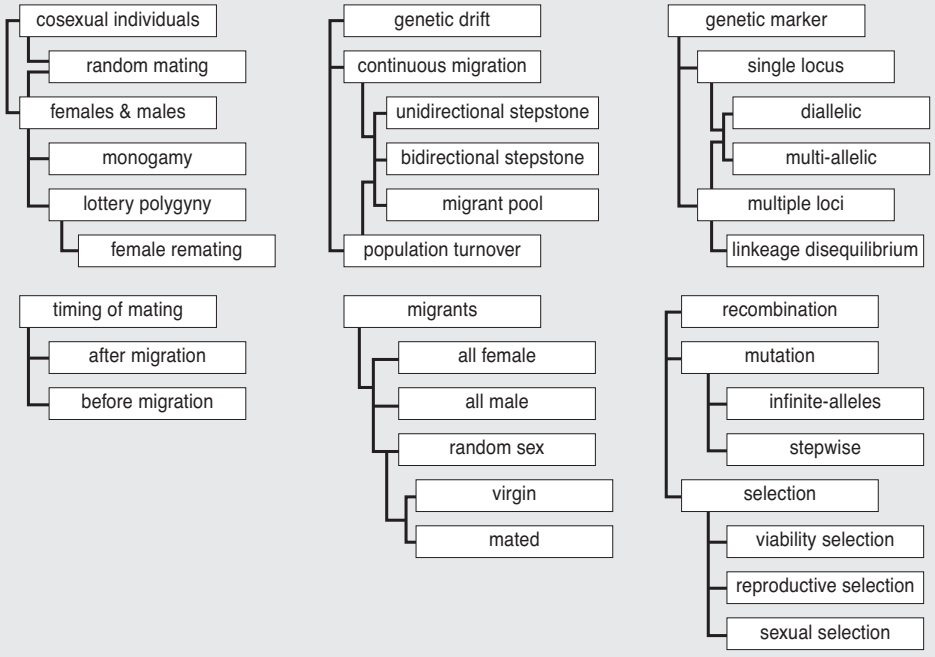
COMPUTER SIMULATIONS

In addition to the experimental metapopulations, I used computer simulations to create comparable *in silico* metapopulations. The purpose of the simulations is threefold:

- To generate approximations for the patterns of genetic diversity and differentiation emerging in experimental metapopulations.
- To extend the experimental setup, for example by increasing the numbers of demes and individuals, the number of marker loci, and the experimental time frame.
- To provide a correct statistical framework for the experiments. Because the results of my experiments mostly take the form of time-series over generations, the individual data points are not independent. Simulations allow for the construction of confidence bands based on a large number of replicate runs that include the interdependence of generations.

I used an object-oriented, individual-based design enabling easy implemention of different aspects of metapopulation genetics (box 1.15). In the initial stage of development, I implemented a standard Wright-Fisher population of cosexual random mating individuals (*i.e.*, allowing selfing, Crow & Kimura 1970) to validate the program structure, and I extended the model to include sex-differentiated, random mating individuals to generate baseline results for future comparison with more complex models. In the next stage I adapted the model to the reproductive system of *Drosophila* (*i.e.*, lottery polygyny with the probability to remate before actual reproduction takes place, see CHAPTER 2), and included options to modify gene flow in a metapopulation (*e.g.*, different migration models mimicking spatial structure and stochastic occurrence of local extinction events, see CHAPTERS 3 & 4). The default genetic structure is a selectively neutral, diallelic single locus system comparable with the eye colour marker. In order to extend the experimental setup, I added options for multiple loci and/or alleles, and for simple additive viability selection. The simulation program calculates a number of genetic measures each generation (*e.g.*, allele frequencies, heterozygosity, autozygosity and fixation index), monitors migration, extinction and colonization events, and writes the data to separate output files that are easy to process in a spreadsheet program.

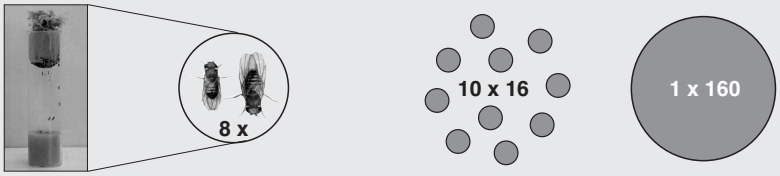
Box 1.15 Individual-based simulation program



EXPERIMENTAL METAPOPULATIONS

In contrast with nature's complexity, experimental systems allow for simplification in a consistent way. The starting point of this thesis is a simple setup of a subdivided population without gene flow comparable with Buri's (1956) classic genetic drift experiments (box 1.16). In CHAPTER 2, I look into the effects of genetic drift on the genetic diversity and the effective population size of these subdivided populations, and I evaluate different estimators of effective population size.

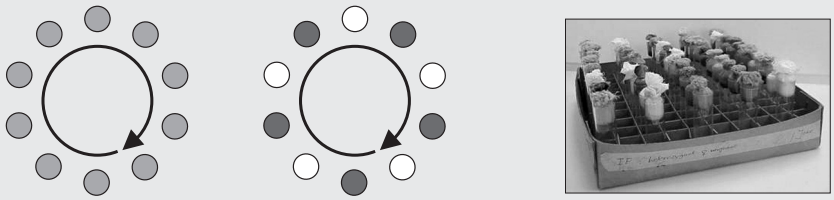
Box 1.16 Experimental metapopulations with genetic drift



Genetic drift experiments. A small population (deme) is founded by eight females and eight males. A metapopulation consists of ten isolated demes. A large undivided population of equal size as a metapopulation is founded by 80 females and 80 males.

In subsequent experiments I add complexity step-by-step by introducing different levels of gene flow. In CHAPTER 3, I continue with the addition of gene flow through migration at different effective migration rates (box 1.17). Since *Drosophila* is a sexually reproducing, diploid organism, I distinguished between female and male dispersers, and between migration taking place before and after mating. The initial level of differentiation (none or maximal) within metapopulations is the third factor that I varied. I analysed the patterns of genetic diversity within demes and genetic differentiation among demes within metapopulations and I evaluated the effects of population size and gene flow on population fitness.

Box 1.17 Experimental metapopulations with unidirectional stepping-stone migration

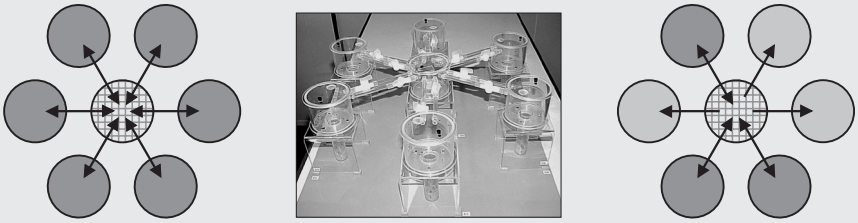


Migration experiments. One migrant is moved into the next deme each generation in a circular pattern among 10 demes of a metapopulation. Three factors are varied: (i) migrants are all females or all males, (ii) flies mate after migration or before migration, and (iii) the initial level of genetic differentiation is null (orange-eyed heterozygotes, left) or maximal (alternating red-eyed and white-eyed homozygotes, middle).

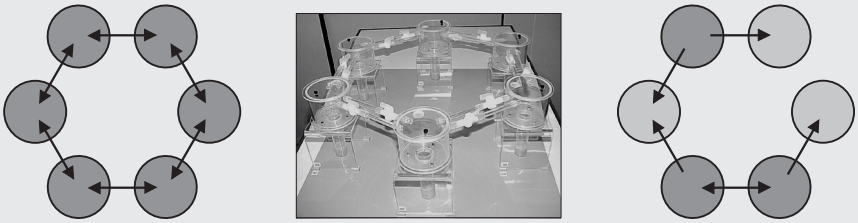
In CHAPTER 4, I added local extinction and recolonization to the metapopulations for two different spatial configurations supporting one-dimensional, bidirectional stepping-stone migration and migrant-pool migration (box 1.18). In the stepping-stone configuration migration was restricted to adjacent demes, resulting in a limited amount of (local) gene flow. In the migrant-pool configuration migrants from all demes were able to roam the entire metapopulation and might immigrate into any of its demes at random, thus maximizing the efficiency of gene flow in the metapopulation. Founder events following local extinction of a deme were subject to the same spatial restrictions, since potential colonists were recruited among the migrants. I analysed the patterns of genetic diversity and differentiation within and among demes and metapopulations, and I evaluated the effects of different demographic histories on population fitness and on tolerance to external stress factors.

In CHAPTER 5, I investigated the consequences of differences in tolerance to external stress factors for the adaptive potential of single demes and entire metapopulations (box 1.19). I subjected a subset of six metapopulations and six large undivided populations to six generations of selection in three stressful environments (high temperature, medium with salt, and medium with a high concentration of ethanol). I assesed the relative change of stress tolerance after selection as a measure of the adaptive potential of the metapopulations.

Box 1.18 Experimental metapopulations with local extinction and recolonization

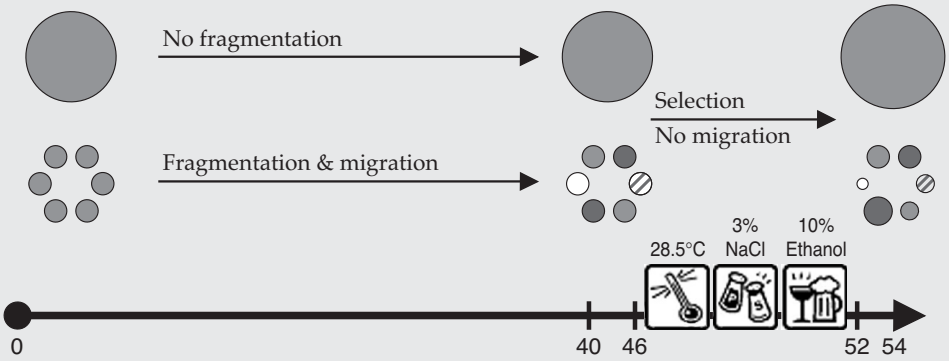


Migrant-pool configuration: all migrants gather in the central compartment ("migrant-pool") before randomly dividing over all available demes. Empty demes (light grey) are colonized from the migrant-pool.



Stepping-stone configuration: migrants move to adjacent demes on both sides of their source deme. Empty demes (light grey) can only be colonized from adjacent extant demes.

Box 1.19 Adaptive potential after 40 generations of population fragmentation



In the final CHAPTER 6, I place the results of my study in a wider context, and I discuss its implcations for the application of population genetic theory in a metapopulation context, for example in conservation biological projects. In addition, I evaluate the *pros* and *cons* of this experimental setup in particular, and the value of experimental metapopulation studies in general.

CHAPTER 2

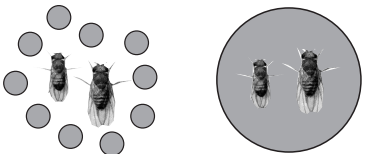
Genetic differentiation in experimental *Drosophila melanogaster* metapopulations I - Random genetic drift

with R. BIJLSMA and F. J. WEISSING

ABSTRACT

Conservation genetic studies routinely apply genetic markers to assess the genetic structure and future prospects of small, fragmented populations. This assessment is based on metapopulation models whose assumptions are often not satisfied in natural systems. In a series of papers we evaluate the implications of such a mismatch by comparing the genetic structure of evolving *Drosophila melanogaster* metapopulations with analytical predictions and individual-based computer simulations. Here, we focus on the effects of isolation by comparing replicate metapopulations of small, unconnected demes with undivided populations of equal total size. We infer the effective population size, the fixation rate, the heterozygosity within demes, and fixation indices within and among metapopulations.

We draw four main conclusions. First, even under standardized conditions the replicates diverged substantially. As a consequence, a sample of ten demes did not provide a representative picture of the entire metapopulation consisting of 50 demes. Second, effective population size was only about half of the census size. Even in a standardized laboratory environment the loss of genetic variation is strongly affected by the mating system and variance in female reproductive success. Third, the phenotypic marker chosen for its presumed neutrality was actually subject to selection. Moreover, the strength of selection depended on experimental details. Fourth, many commonly used "snapshot" estimators of effective population size substantially overestimated N_e, resulting in biased conclusions concerning the state of the metapopulation.



INTRODUCTION

Ever since Wright (1943, 1951), evolutionary biologists have realized that spatial subdivision and restricted gene flow can have profound implications for the genetic structure and the adaptive potential of a population (*e.g.*, Kimura *et al.* 1963, Maruyama 1970, Nei *et al.* 1975, Slatkin 1987, McCauley 1993, Harrison & Hastings 1996). Many present-day studies routinely screen natural populations with the help of molecular genetic markers such as microsatellites, AFLPs or SNPs (Bruford & Wayne 1993, Parker *et al.* 1998, Hedrick 1999, Jorde *et al.* 1999, Robinson & Harris 1999, Kuhner *et al.* 2000, Sunnucks 2000, Jehle & Arntzen 2002, Vignal *et al.* 2002). The resulting pattern of genetic variation is often interpreted in terms of theoretical expectations based on classical population genetic models (Fisher 1930, Wright 1931, 1951), and used to infer demographic parameters as population size or number of migrants.

The founding fathers of population genetics realized already that such inferences can be problematic, since the highly idealized assumptions of population genetic models are seldom satisfied in natural populations. To allow for a comparison between real and model populations, Wright (1931, 1938) introduced the concept of effective population size N_e, defined as the size of an idealized model population exhibiting the same dynamics of genetic variation as the natural population in question (see Caballero 1994 for a discussion of this concept). The estimation of the effective population size (or derived concepts like the effective number of migrants) is a crucial step in every genetic screening programme (*e.g.*, Saccheri *et al.* 1998, Miller & Waits 2003). To infer N_e from genetic data a variety of moment-based or likelihood-based estimators (Waples 1989, Berthier *et al.* 2002, Wang & Whitlock 2003, Balloux 2004) has been developed.

Despite intense theoretical research (*e.g.*, Pannell & Charlesworth 2000, Whitlock 2002, Glemin 2003), the reliability and interpretation of such estimates is still difficult to judge. All estimators are based on a number of assumptions that may not be met in natural systems. In practice it is typically unclear whether, and to what extent, discrepancies between model and empirical systems translate into a misjudgment of parameters as the effective population size. Studies that validate inferences based on genetic data by independent demographic information are missing. This is not surprising, since validation is extremely difficult in a field setting where crucial information may be difficult to obtain and controlled replication is often impossible. In order to bridge the gap between theory and the complexity of the real world and to achieve at least a partial validation of common genetic screening methods, we performed replicated, controlled lab experiments with metapopulations of *Drosophila melanogaster*. In two companion papers, we focus on genetic drift in small isolated populations (this CHAPTER), and on gene flow among small population fragments (CHAPTER 3).

In this chapter, we present the results of the first series of experiments assessing the effects of isolation, *i.e.*, the effects of genetic drift (and perhaps of inbreeding and selection) in small unconnected populations. We use a setup similar to Buri's (1956) classical experiments quantifying random genetic drift in small *D. melanogaster* populations to estimate effective population size, fixation rate and genetic differentiation. The aim of

this part of the study is twofold. First, we want to obtain the baseline values of our system without gene flow enabling us to validate our individual-based simulation model and to distinguish between the effects of drift and migration in subsequent experiments. For this purpose we perform a hierarchical analysis of genetic differentiation to allow comparison with later experiments including migration (CHAPTER 3). Second, we address a number of questions that are important for empirical studies. (i) How big is the discrepancy between theoretical predictions and the behaviour of a highly standardized experimental metapopulation? What is the relative importance of factors such as mating system or weak selection? (ii) How much variation is there between replicate populations? How representative is a small subsample of populations for the metapopulation as a whole? (iii) How reliable are the commonly used estimators of effective population size?

MATERIAL AND METHODS

DROSOPHILA STOCKS

For our study we chose phenotypic markers similar to those used in Buri's (1956) experiments, since eye colour mutations allow easy visual monitoring of genotype frequencies. We used two alleles bw and bw^{75} at the *brown* locus (II-104.5) that, in combination with the mutation *scarlet* (*st*) (III-44) in homozygous condition, result in distinct eye colours for the three genotypes at the *bw* locus at 25°C. Homozygous *bw*//*bw* individuals have white eyes, homozygous bw^{75}//bw^{75} individuals have red-brown eyes, and heterozygous bw^{75}//*bw* individuals have intermediate orange eyes. We obtained homozygous bw^{75}//bw^{75} and *bw*//*bw* stocks from the *Drosophila* stock centre in Umeå, Sweden. Since both stocks may have had fairly different genetic backgrounds, they were intercrossed and maintained for six consecutive generations by selecting only virgin heterozygotes to found the next generation. This ensured reasonable homogenization of the genetic background except around the *bw*-marker region. From this cross, we established new homozygous bw^{75}//bw^{75} and *bw*//*bw* stocks to initiate all experiments henceforth.

In general, we bred the flies in 125 ml bottles on 30 ml of standard medium (26 g dead yeast, 54 g sugar, 17 g agar, and 13 ml nipagine solution (10 g nipagine in 100 ml 96% alcohol) per liter) with antibiotics (250 mg streptomycin per liter) under standard conditions (25°C, 40-60% RH and continuous light). The experimental populations were also raised under standard conditions, either in 40 ml glass vials containing 9 ml of standard medium for the small populations, or in 125 ml bottles containing 30 ml of standard medium for the larger populations. We anaesthetized the flies with CO_2 for handling and counting.

METAPOPULATION EXPERIMENT: SETUP AND PROCEDURE

The experimental setup included both small populations in vials, and large populations in bottles. We initiated 50 replicate vial populations with 16 founding parents (eight females and eight males) in each generation (fig. 2.1A), and five replicate bottle popula-

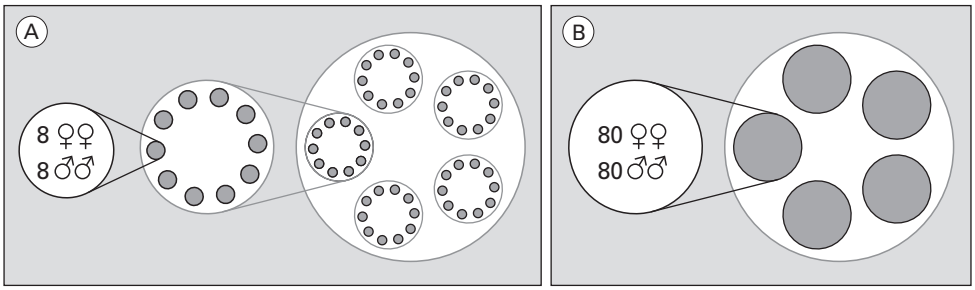


Figure 2.1. Setup of the experimental *Drosophila melanogaster* metapopulations. A: A small vial population (= deme) is founded by 16 breeding individuals (eight pairs) each generation. Ten vial populations represent one subdivided population (= metapopulation with ten demes) with a total size of 160 individuals. All five replicate metapopulations together may be viewed either as a global metapopulation consisting of five vial-metapopulations, or as one large vial-metapopulation with 50 demes, both with a total size of 800 individuals. B: A bottle population is initiated with 160 individuals in each generation. Five replicate bottle populations represent a metapopulation with five large demes and a total size of 800 individuals.

tions with 160 founding individuals (80 females and 80 males) per generation (fig. 2.1B). We grouped the 50 vial populations *a priori* in five series of 10 vials each (fig. 2.1A) corresponding to five replicate metapopulations with 10 demes to serve as controls in the second part of the study, where 10 demes of a metapopulation were connected through migration (CHAPTER 3). This enables us to compare the dynamics of genetic variation in a subdivided metapopulation to that of an undivided population of the same total size (160 founding parents). In addition, the setup allows us to evaluate the variance among replicate metapopulations, the effect of deme number (n = 50 demes in the overall metapopulation compared with n = 10 in the five series), and the effect of population size (N = 16 in the vial populations *versus* N = 160 in the bottle populations).

We initiated all 50 replicate vial populations and five replicate bottle populations with heterozygous individuals obtained from the mixed offspring of the reciprocal crosses $bw^{75}//bw^{75}$ x $bw//bw$ and $bw//bw$ x $bw^{75}//bw^{75}$. We maintained this heterozygous founder population parallel to the experiment to provide baseline values of a very large ("infinite") population consisting of 20 bottles adding up to about 3100 individuals that were mixed each generation. Generations did not overlap and took 14 days to develop and to provide sufficient offspring for sampling. The number of offspring varied from 50 to 90 per vial and from 200 to 350 per bottle. Each generation we collected 15–25 virgin flies of each sex per vial (160–200 per bottle) on the first days of eclosion, and we selected eight individuals of each sex per vial (80 per bottle) at random from these samples to found the next generation. We allowed the parental flies to mate and lay eggs for three days, and then transferred them to fresh containers for two more days to provide an emergency backup. Note that our procedure is different from the more artificial one used by Buri (1956), who first selected flies from each population to score the genotypes for the parents, then mixed the flies collected from all populations to avoid genetic drift at loci

other than the marker locus, and finally founded the new populations according to the previously scored genotype proportions with flies from the mixed batch. In the large founder population, the number of offspring per bottle varied mostly between 90 and 220, and these flies were mixed and then redistributed over fresh bottles in each generation to ensure random mixing of the total population. We randomly selected five of these 20 bottles per generation to monitor genotype frequencies in the total population.

We determined the genotype frequencies of the samples of $N = 16$ ($N = 160$) individuals selected to found the next generation from each vial (bottle) population for each of 20 generations, enabling us to determine allele frequencies, to infer observed and expected heterozygosities and to monitor fixation events. From these data we estimated the average variance and inbreeding effective population sizes, and the levels of genetic differentiation. We made estimates for each of the five replicate vial metapopulations (consisting of 10 demes each) separately, for all 50 vial populations combined into one large metapopulation, and for the five replicate bottle populations combined.

ESTIMATES OF GENETIC DIFFERENTATION

As a measure of genetic differentiation within a metapopulation we calculate the fixation index F_{ST} as defined by Wright's hierarchical F-statistics (Wright 1951, Hartl & Clark 1997):

$$F_{ST} = \frac{H_T - \bar{H}_S}{H_T}. \tag{2.1}$$

H_T indicates the expected heterozygosity at the metapopulation level, and $\bar{H}_S$ is the average expected heterozygosity at the level of individual demes, which can be either vial (fig. 2.1A) or bottle (fig. 2.1B) populations.

To compare between replicate metapopulations, we use the analogous F-statistic F_{TG}:

$$F_{TG} = \frac{H_G - \bar{H}_T}{H_G}. \tag{2.2}$$

$\bar{H}_T$ is here the average expected heterozygosity at the level of individual metapopulations, and H_G is the expected heterozygosity at the (highest) global level. Hence, F_{TG} is a measure of genetic differentiation between the five replicate metapopulations (fig. 2.1A).

ESTIMATES OF EFFECTIVE POPULATION SIZE

Under the assumptions of the Wright-Fisher model of random genetic drift (*i.e.*, many independent subpopulations of cosexual organisms reproducing through random fusion of gametes that are produced in infinite numbers by N breeding individuals each generation; Fisher 1930, Wright 1931) several population characteristics change in a random but predictable way. First, the heterozygosity H that is expected under Hardy-Weinberg conditions decreases from one generation to the next according to:

$$H_{t+1} = \left(1 - \frac{1}{2N}\right) H_t = \lambda H_t, \tag{2.3}$$

where $\lambda = 1 - (1/2N)$ equals the largest non-unit eigenvalue of the Markov chain transition matrix describing the standard Wright-Fisher model (*e.g.*, Hartl & Clark 1997).
Second, the autozygosity F (*i.e.*, the probability that two randomly chosen alleles are identical by descent; Crow & Kimura 1970) increases from one generation to the next according to:

$$F_{t+1} = \frac{1}{2N} + \left(1 - \frac{1}{2N}\right) F_t \,. \tag{2.4}$$

Third, given the allele frequency p, the expected allele frequency change δp from one generation to the next has binomial variance (Crow & Kimura 1970):

$$\sigma^2_{\delta p} = \frac{p(1-p)}{2N} \,. \tag{2.5}$$

The effective population size N_e is commonly defined as the size of an idealized Wright-Fisher population that would lead to either the same level of heterozygosity in the offspring (eigenvalue effective size N_e^{λ}), the same level of autozygosity in the parents (inbreeding effective size N_e^{i}), or the same amount of variance in allele frequency change among the offspring (variance effective size N_e^{σ}) as the actual population in any generation. We can derive expressions for the three effective population sizes from equations (2.3), (2.4) and (2.5), respectively:

$$N_e^{\lambda} = \frac{H_t}{2(H_t - H_{t+1})}, \tag{2.6}$$

$$N_e^{i} = \frac{1 - F_t}{2(F_{t+1} - F_t)}, \tag{2.7}$$

$$N_e^{\sigma} = \frac{p(1-p)}{2\sigma^2_{\delta p}}. \tag{2.8}$$

From equation (2.6), we can write ΔH, the change in heterozygosity between two successive generations t and $t + 1$, as a function of the heterozygosity H_t of the parental generation, and infer the eigenvalue effective size by linear regression (forced through the origin):

$$\Delta H = H_t - H_{t+1} = \frac{1}{2\hat{N}_e^{\lambda}} H_t \,. \tag{2.9}$$

To infer the inbreeding effective size we would need to quantify the increase of autozygosity over generations, which was not possible in our experimental setup. If - as is often done - homozygosity is used as an estimator of autozygosity, then (2.7) is equivalent with (2.6), and inferences of the inbreeding and eigenvalue effective sizes will be equal.

We can infer the variance effective size directly from the allele frequency variation (2.8) by applying linear regression (forced through the origin) to the variance $\sigma^2_{\delta p}$ in allele frequency change δp between two successive generations as a function of the allele frequency p in the parental generation:

$$\sigma^2_{\delta p} = \frac{1}{2\hat{N}_e\sigma}\,[\,p(1-p)]. \qquad (2.10)$$

In the experiments the expected allele frequency of offspring might deviate from the allele frequency of the parental generation due to additional factors such as directional selection. Hence, we use the sample variance $s^2_{\delta p} = [\sum(p_i - \bar{p}_i)^2]/(i-1)$, with p_i the allele frequency of the offspring of a parental population with allele frequency p, i the number of cases that this parental allele frequency p was observed, and $\bar{p}_i = (\sum p_i)/i$. For the results of individual-based simulations (see next section) the expected allele frequency p is equal from one generation to the next, so that we may calculate the population variance $\sigma^2_{\delta p}$ from $[\sum(p_i - p)^2]/i$ without losing a degree of freedom by estimating $\bar{p}_i$.

Since we mostly consider a single locus, we apply regression to the pooled data of all replicates, *i.e.*, either n simulation runs or n experimental populations, for both estimators (2.9) and (2.10). When we consider the simulated data of L loci per population (see the discussion), we apply regression to the pooled data over n replicates per locus to obtain L regression coefficients $b = 1/2N_e$. We then calculate the mean $\bar{b}$ of the regression coefficients, and the variance $\sigma^2_{\bar{b}} = \sigma^2_{pooled}/L$ from the pooled variance σ^2_{pooled} of L regressions (Zar 1974). Finally, we calculate an overall estimate of N_e from $2N_e = (1/\bar{b})[\,1 + (\sigma^2_{\bar{b}}/\bar{b}^2)]$, thereby correcting for the nonlinear relationship between b and N_e.

INDIVIDUAL-BASED SIMULATIONS AND STATISTICAL ANALYSIS

We use individual-based Monte Carlo simulations to assess the expected variation between replicates due to stochastic processes, to evaluate the experimental results statistically, and to analyze the effects of complicating factors (*e.g.*, selection) that occurred during the experiment.

We implemented a standard Wright-Fisher population without sex differentiation consisting of N cosexual (hermaphrodite) diploid individuals. Each generation, the parental population of size N is replaced by an offspring population of the same size. To produce each of the N offspring, we draw two parents at random from the total parental population (*i.e.*, including the possibility of selfing) that each contribute a randomly chosen allele at a given locus to the offspring's diploid genotype.

We also implemented various mating systems for a sex-differentiated population. Here we focus on lottery polygyny, a mating system that resembles the situation in *Drosophila* reasonably well (Bateman 1948, Nunney 1993). In the basic form of lottery polygyny all females mate exactly once, while males vary in their number of matings because they are randomly chosen as mates by females. In our implementation of lottery polygyny each generation started with a round of mating where each of the $^1/_2N$ females got associated with a male that was randomly chosen (with replacement) from the male population of size $^1/_2\ N$. Then $^1/_2N$ female and $^1/_2N$ male offspring were

produced by randomly choosing a mated female and supplying the offspring at each locus with a randomly chosen allele from the mother and the mother's mate, respectively.

Standard simulations of our experimental setup take account of the lottery polygyny mating system of *Drosophila*. Generations are discrete, and population sizes are kept constant at either $N = 16$ or $N = 160$ individuals with an equal sex ratio. In the initial runs the genetic make-up of individuals is one locus with two alleles without mutation or selection. As our experimental results indicated the occurrence of selection, we included selection in some of the simulations (see below) to explore different selection models, and to estimate the selection coefficient. To mimic the *Drosophila* mating system even more closely we also investigated the possibility of female remating (Bundgaard & Christiansen 1972, Van Vianen & Bijlsma 1993). Since the predicted effect of remating appeared to be very limited (appendix, (2A.4) *versus* (2A.5)), we did not implement remating in the simulation model for isolated populations. When isolation is mitigated through migration, some form of remating will be required to enable the reproduction of males that migrate into a population after the residents have mated (see CHAPTER 3).

The statistical analysis of our time series data has to take account of the fact that data obtained in successive generations are not statistically independent (*e.g.*, a population that has become fixed at one point in time stays fixed in the future). We therefore calculated the 2.5 and 97.5 percentiles from 1000 simulation runs to compare between experimental results and predictions from simulations, enabling us to analyse graphically whether our experimental results are within 95% confidence ranges.

RESULTS

HETEROZYGOSITY AND GENETIC DIFFERENTIATION

As expected for populations of small size, the allele frequencies (not shown) and expected heterozygosities (fig. 2.2A) of individual vials fluctuated wildly. In line with theoretical expectations the average expected heterozygosity $\bar{H}_S$ over all 50 vial populations decreased steadily (fig. 2.2A, red circles). As described above, we arbitrarily grouped the 50 vials from the start to form five replicate metapopulations with 10 demes each. The average expected heterozygosities of these metapopulations also declined, but the rate of decline differed considerably (fig. 2.2B). To investigate whether differences of that magnitude are to be expected we repeatedly (1000 times) determined the average expected heterozygosity of sets of 10 demes that were randomly selected from our 50 demes (fig. 2.2B, red line). This bootstrap resampling procedure (Howell 2002) yielded a 95% confidence interval for the average expected heterozygosity of a metapopulation consisting of 10 demes (fig. 2.2B, dashed lines). Figure 2.2B shows that the average expected heterozygosities of our five metapopulations fell just within this 95% confidence interval. We conclude that our *a priori* grouping of vials resulted in metapopulations evolving in a relatively extreme but not atypical way. Hence, a sample of 10 demes may give a rather biased impression of the behaviour of the entire metapopulation of 50 demes.

Figure 2.2C shows the change in expected heterozygosity of the five bottle populations. These populations diverged substantially from each other from generation 10 onwards. In generation 20, the variation in expected heterozygosity was even more pronounced than that among the five vial metapopulations that each had the same size (10 x 16 = 160) as a bottle population. We have no explanation for the strong decrease of heterozygosity from generation 10 onwards, although it might be related to a bacterial infection occurring around that time, possibly in combination with some selection (see below).

We used the fixation indices F_{ST} and F_{TG} (eqns (2.1) and (2.2), respectively) to assess genetic differentiation for the different hierarchical levels resulting from the *a priori* grouping of the vial populations and for the bottle populations. Genetic differentiation among the vial populations was high, both among all 50 demes (fig. 2.2D, filled black circles), and among the 10 demes of each of the vial metapopulations (open black circles). In contrast, genetic differentiation among the five replicate metapopulations (filled red circles) is relatively low, and comparable to the level of differentiation among bottle populations of the same size (open red circles).

In figure 2.3A we compare the experimental results with the predictions from individual-based simulations of cosexual random mating populations set up in accordance with the assumptions of the Wright-Fisher model. It is not too surprising that the fit is rather poor, as the Wright-Fisher model does not properly reflect the mating system of *Drosophila*. When lottery polygyny is included in the simulations (fig. 2.3B) the fit improves markedly for the vial populations where the experimental results fall for the better part within the confidence interval. Still, heterozygosity decreased at a faster rate in the experimental vial populations than is to be expected for lottery polygyny alone (see also the next section). For the bottle populations the fit with either model is very poor, at least in the last 10 generations of the experiment where the bottles diverged from each other (fig. 2.2C). We will discuss potential explanations for the discrepancy between experimental results and theoretical expectations below.

EFFECTIVE POPULATION SIZE

For each of the five vial metapopulations, for all 50 vial populations, and for all five bottle populations, we estimated the eigenvalue (2.9) and the variance (2.10) effective population sizes (table 2.1). The effective size of the vial populations turned out to be about one half of the census size ($N_e^{\lambda} = 9.0$ and $N_e^{\sigma} = 8.4$ while $N = 16$). These effective sizes are even more extreme than the ones reported for Buri's (1956) classical experiments ($N_e^{\sigma} = 8.9$ for series I and $N_e^{\sigma} = 11.5$ for series II). The eigenvalue effective sizes of the replicate vial metapopulations differ substantially (range: 6.4 to 16.3), reflecting the large differences in the rate of decrease of heterozygosity (fig. 2.2B). In general, variance effective sizes tend to be less variable (range: 8.0 to 9.9) than eigenvalue effective sizes.

The low effective sizes can be partly explained by the fact that in *D. melanogaster* (and in many other species) male reproductive success is more variable than female reproductive success. The *Drosophila* mating system approximates lottery polygyny (Bateman 1948, Nunney 1993) where females mate once with a randomly chosen male, while males may vary in their number of matings. For a stationary population with lottery

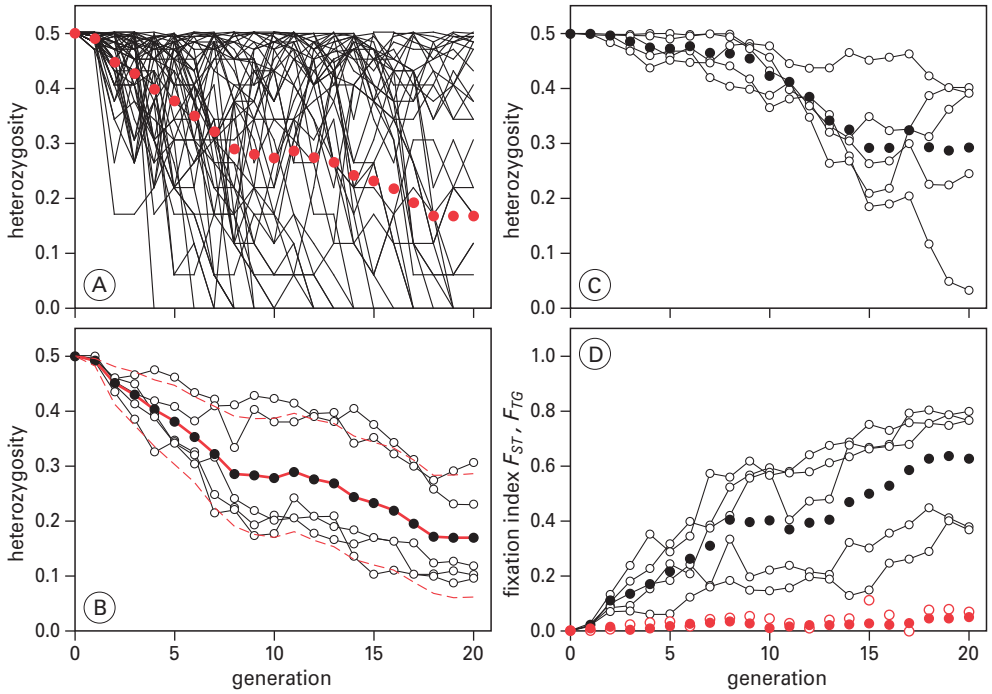


Figure 2.2. Observed variation among experimental *D. melanogaster* populations. A: Change of heterozygosity H_S in individual vial populations (lines), and of average heterozygosity $\bar{H}_S$ of 50 vial populations (red circles). B: Decrease of average heterozygosity $\bar{H}_S$ in five replicate metapopulations consisting of 10 demes each (black open circles), and of the average over all five replicates (black filled circles), which is equal to the average over 50 replicate vials. The red line is the average of 1000 arrangements of 10 vial populations (randomly selected from the 50 vials) into a metapopulation, and the dashed lines indicate the corresponding 95% confidence band. C: Decrease of heterozygosity H_S of individual bottle populations (open circles), and of average heterozygosity $\bar{H}_S$ of five replicates (filled circles). D: Increase of genetic differentiation for two hierarchical levels. (i) Fixation index F_{ST} for five replicate metapopulations of 10 vial populations each (black open circles), for a metapopulation of 50 vial populations (black filled circles), and for a metapopulation consisting of five bottle populations (red open circles). (ii) Fixation index F_{TG} for a global-level metapopulation consisting of the five replicate vial-metapopulations (red filled circles).

polygyny the effective population size can be predicted from

$$N_e = \left(\frac{2}{2+\alpha}\right) N \ , \tag{2.11}$$

where $\alpha = \sigma_k^2 / \mu_k$ is the variance-mean ratio of the number of offspring contributed to the next generation by a single mating (see appendix). Assuming that the number of offspring per mating is Poisson-distributed, the variance equals the mean and $\alpha = 1$. As a consequence, $N_e = 10.7$ for the vial populations and $N_e = 106.7$ for the bottle populations in our experimental system (table 2.1).

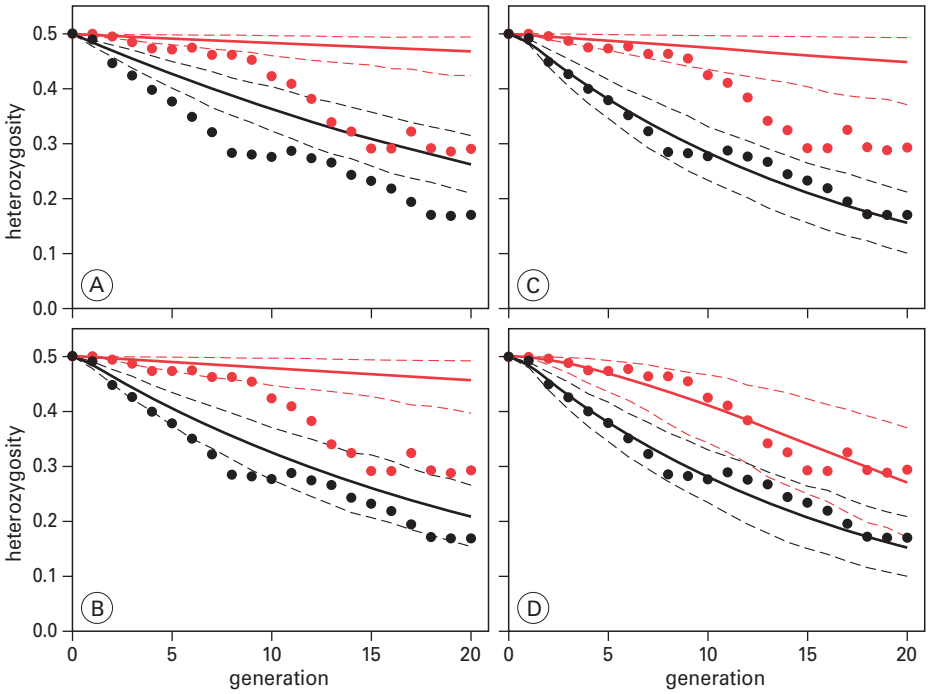


Figure 2.3. Comparison of the experimental heterozygosity with the results from individual-based simulations of different scenarios. Data points correspond to the average heterozygosity $\bar{H}_S$ of 50 vial populations (black circles) and of five bottle populations (red circles) and are the same in all panels. The lines indicate the simulation results (solid) for 50 vial populations (black) and five bottle populations (red) with the corresponding 95% confidence bands (dashed). A: Random mating cosexual populations satisfying the assumptions of the Wright-Fisher model. B: Sex-differentiated populations with lottery polygyny. C: Sex-differentiated populations with lottery polygyny and a variance-mean ratio $\alpha = 1.6$ (see text for details). D: Sex-differentiated populations with lottery polygyny, $\alpha = 1.6$ and viability selection (see text for details).

While the effective population sizes in Buri's series II could be explained by lottery polygyny alone, additional factors must be present in our experiments and in Buri's series I. Buri explained the lower effective population size in his series I by the relatively large variation in female reproductive success due to the high levels of female competition for oviposition space in the smaller type of container used for this series. To check whether similar effects might have played a role in our experiments we counted the number of eggs laid by individual females under similar egg-laying conditions as in our experimental setup (data not shown). Females laid 9.8 eggs on average with a variance (15.8) that is about 1.6 times as large as the mean, while the Wright-Fisher model assumes that the variance in female reproductive success is approximately equal to the mean. Using $\alpha = 1.6$ in equation (2.11) yields an effective population size of 8.9 that roughly corresponds to the values found in our experiments. Results from individual-based simulations including both lottery polygyny and a high variance in female repro-

Table 2.1. Observed and predicted eigenvalue and variance effective sizes for the vial populations with *a priori* grouping (five metapopulations with $n = 10$ demes each) and without ($n = 50$ demes), and for the bottle populations ($n = 5$). For the vial metapopulations the average effective sizes over the five replicates are also given (standard error in brackets). For comparison we included predictions from individual-based simulations (italics, 95% confidence interval in brackets) with lottery polygyny (lp) alone and in combination with variable female reproductive success (vf), as well as the corresponding predicted values from the Wright-Fisher (W-F) model for separate sexes (bold). In case of just lottery polygyny (lp) we assume that the number of offspring contributed to the next generation per mating follows a Poisson distribution ($\alpha = \sigma_k^2/\mu_k = 1$). In case of variable female reproductive success (lp + vf) we assume $\alpha = 1.6$ based on the results of separate experiments.

	Census N	Eigenvalue $\hat{N}_e^{\lambda}$	Variance $\hat{N}_e^{\sigma}$
Vial metapopulation 1 ($n = 10$)	16	7.0	8.0
Vial metapopulation 2 ($n = 10$)	16	6.4	8.1
Vial metapopulation 3 ($n = 10$)	16	7.0	9.9
Vial metapopulation 4 ($n = 10$)	16	13.8	9.8
Vial metapopulation 5 ($n = 10$)	16	16.3	9.1
Metapopulation mean (s.e.)		10.0 (2.1)	9.0 (0.4)
Vial populations ($n = 50$)	16	9.0	8.4
Sim. vials (lp, n = 50)	*16*	*12.8 (10.1 - 17.5)*	*12.3 (11.3 - 13.6)*
Sim. vials (lp, n = 1000)	*16*	*11.8 (11.2 - 12.5)*	*11.6 (11.3 - 11.9)*
W-F model (lp)	**16**	**10.7**	**10.7**
Sim. vials (lp + vf, n = 50)	*16*	*8.5 (7.0 - 11.0)*	*8.9 (8.1 - 9.9)*
Sim. vials (lp + vf, n = 1000)	*16*	*9.2 (8.8 - 9.7)*	*8.8 (8.6 - 9.2)*
W-F model (lp + vf)	**16**	**8.9**	**8.9**
Bottle populations ($n = 5$)	160	23.4	91.6
Sim. bottles (lp, n = 5)	*160*	*352.9 (80.8 - ∞)*	*156.8 (123.2 - 216.3)*
Sim. bottles (lp, n = 1000)	*160*	*105.5 (97.5 - 115.0)*	*105.7 (100.8 - 111.1)*
W-F model (lp)	**160**	**106.7**	**106.7**
Sim. bottles (lp + vf, n = 5)	*160*	*47.8 (24.8 - 606.2)*	*87.2 (63.5 - 140.7)*
Sim. bottles (lp + vf, n = 1000)	*160*	*89.6 (83.0 - 97.4)*	*89.8 (86.2 - 93.8)*
W-F model (lp + vf)	**160**	**88.9**	**88.9**

ductive success corresponding to a value of $\alpha = 1.6$ (fig. 2.3C, black line) fit the experimental results for the vial populations much better than predictions from simulations including only lottery polygyny (fig. 2.3B).

The considerations above can not account for the estimated eigenvalue effective size of the bottle populations that was as low as $1/8$ of the census size. We do not have an explanation for this, although the bacterial infection in generation 10 mentioned above might have played a role. From this generation onwards, the heterozygosity decreased much more rapidly than expected. This change in pattern is reflected in the relation between ΔH and H that is not linear, in contrast to predictions based on the Wright-Fisher model. Additional factors such as selection (see below) will most probably also have been of importance.

ALLELE FREQUENCIES AND FIXATION RATES

Figure 2.4 shows the observed number of fixation events among the 50 vial populations (after 20 generations) and the change in frequency of the bw^{75}-allele in the vial populations, the bottle populations, and the large founder population. Fixation did not occur in any of the five bottle populations within 20 generations.

The average frequency of the bw^{75}-allele and the number of populations fixed for bw^{75} in generation 20 are higher than expected on basis of selective neutrality (0.61 *versus* 0.50, and 17 *versus* 13.9) for the vial populations. Although these differences are not statistically significant, we have the impression that allele bw^{75} had a selective advantage over *bw*. Therefore we included selection in our individual-based simulation model. Considering the intermediate expression of the heterozygote $bw^{75}//bw$ with respect to eye colour we implemented viability selection through an additive model with relative viabilities of 1, $1 - s/2$ and $1 - s$ for the three genotypes $bw^{75}//bw^{75}$, $bw^{75}//bw$ and $bw//bw$, respectively. We estimated selection coefficients by simulating each population setup for a range of *s*-values and calculating the goodness-of-fit between simulated and observed allele frequencies for each simulation run. This procedure provided us with least-square estimates for *s* of 0.08, 0.15 and 0.22 for the vial, bottle and founder populations, respectively. Note that for a given value of *s*, the effectiveness of selection would increase with increasing population size as genetic drift becomes less important. However, this only provides a partial explanation for our results. The different *s*-values suggest that not only the effectiveness, but also the strength of selection increases in the larger populations, which may be due to the environmental conditions in the bottles being quite different from those in the vials, *e.g.*, with regard to the availability of mating partners. Accordingly, the different *s*-values presumably reflect differences in selection pressures caused by differences in environmental conditions rather than differences in population size *per se*. We can not exclude the possibility that selection may have been frequency or density dependent, or that sexual selection might have played a role. Investigation of this possibility is, however, beyond the scope of this study.

Figure 2.4D shows the fit of the predicted frequency of the bw^{75}-allele for all three experimental population sizes (black, red and grey lines) based on simulations including selection according to the additive model with estimated selection coefficients as indicated above. The resulting decrease of heterozygosity matches the observed results for both types of larger populations fairly well. Selection appears to have a relatively small effect on the heterozygosity in the small vial populations (compare figs 2.3C and 2.3D) due to both weaker selection (*i.e.*, a smaller *s*) and a lower impact of selection relative to that of genetic drift. The effect of selection on the expected number of fixation events is also small (28.7 *versus* 27.0 without selection), but the distribution of fixation events over alleles changes considerably (fig. 2.4C) and fits the observed distribution much better than the simulation model without selection (fig. 2.4A).

One of the aims of Buri's (1956) original study was to detect selection for either of the *brown* mutants, and his results indicated that selection in favour of the bw^{75}-allele occurred to some extent, but not significantly so. The results of our experiments do not support this conclusion, and suggest that we have to take account of directional selection.

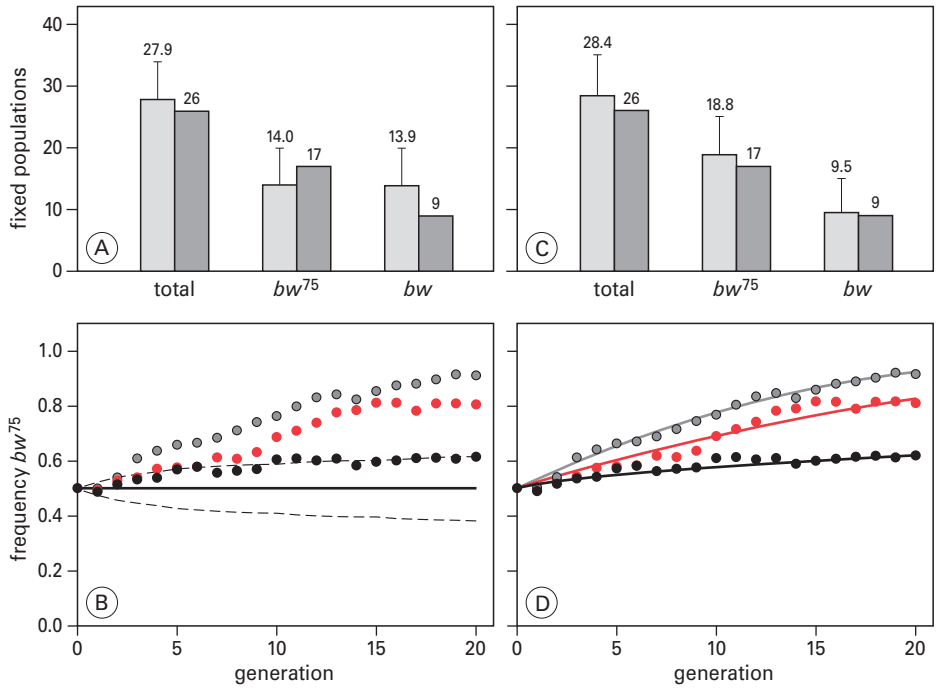


Figure 2.4. Observed and predicted fixation rates and allele frequencies in experimental *D. melanogaster* metapopulations. A: Observed numbers of fixations (dark grey bars) among 50 vial populations after 20 generations for both alleles combined and per allele, and the corresponding predicted numbers (light grey bars) from individual-based simulations with 95% confidence intervals. B: Average frequency of the bw^{75}-allele among offspring of vial populations (n = 50, black circles), bottle populations (n = 5, red circles), and the large founder population (n = 1, grey circles). The black line indicates the results of simulations for all three population sizes with the 95% confidence band for the vial populations (dashed). Confidence bands for both larger population sizes are narrower (not shown). C: Observed (dark grey bars) and predicted (light grey bars) numbers of fixations from simulations including viability selection (see text). D: Fit between the observed allele frequencies and the results from simulations including viability selection for vial populations (black), bottle populations (red) and founder population (grey). All simulations include lottery polygyny and a variance-mean ratio $\alpha = 1.6$.

They indicate in particular that the consequences of selection may depend on environmental conditions, since selection for the bw^{75}-allele occurred in all populations from the earliest generations onwards but the strength of selection differed between populations. The experimental results also contrast with the Wright-Fisher model assuming selection to be absent in an idealized population. This latter assumption is reflected in the use of supposedly selectively neutral markers (*e.g.*, microsatellites) in most field studies. One might argue that our results are thus without general significance, but such markers are not always strictly neutral, *e.g.*, due to hitchhiking (Schug *et al.* 1998, Schlotterer 2000), and our results show that even weak selection may have relatively large effects.

CONCLUSIONS AND IMPLICATIONS

RELIABILITY OF ESTIMATES OF N_e

Even under highly controlled experimental conditions we found that estimates of the effective population size for 10-vial metapopulations deviate considerably, both among replicates and compared with the estimates for the entire 50-vial metapopulation. These results indicate that inferences based on a subset of populations are not very accurate, even if as many as 20% of all populations are sampled.

One might argue that this conclusion reflects the fact that our vial populations were very small and that we focused on genetic variation at a single locus. To investigate this possibility we ran additional simulations with several unlinked loci for deme sizes N =

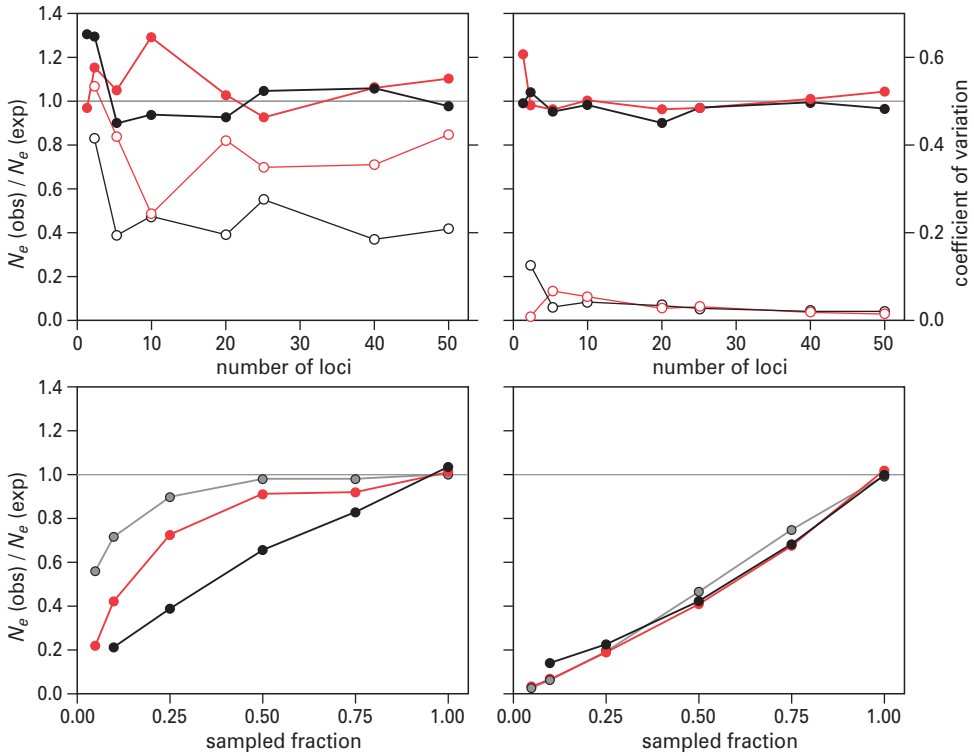


Figure 2.5. Effect of the number of loci and sampling efficiency on the regression-based estimates of N_e. The simulations include lottery polygyny and a variance-mean ratio $\alpha = 1.6$ leading to an effective population size N_e^{exp}, where N is the census deme size (see (2.11)). The left axis shows the ratio N_e^{obs}/N_e^{exp} (filled markers), with the black horizontal line indicating $N_e^{obs}/N_e^{exp} = 1$. Left panels: eigenvalue effective size, right panels: variance effective size. Top panels: Effect of the sampled number of loci L for small ($N = 16$, black) and large ($N = 160$, red) demes, with the coefficient of variation (open markers) on the right axis. Bottom panels: Effect of sampling efficiency (fraction of each deme that is sampled per generation) for 1000 replicate demes of size $N = 16$ (black), $N = 160$ (red) and $N = 1600$ (grey).

16 and $N = 160$. We measured the ratios of the estimated effective sizes $\hat{N}_e^{\lambda}$ and $\hat{N}_e^{\sigma}$ to the theoretical value of $N_e = 8.9$. We estimated the effective size for L loci from the average regression coefficient $1/2N_e$ of L pooled datasets of ten replicates each, and the coefficient of variation to compare the levels of variation among loci.

The simulation results (fig. 2.5 top panels, filled markers) suggest immediately that $\hat{N}_e^{\lambda}$ (left) is a less accurate estimator than $\hat{N}_e^{\sigma}$ (right), in particular for the larger deme size (red) where the variation among loci (open markers) is about twice as high as for the smaller deme size (black). This is likely a consequence of the very small differences in heterozygosity between generations in larger demes that tend to reduce the power of the regression technique. For the small demes the level of variation is more or less constant from five sampled loci onwards, but for the large demes at least 20 loci need to be sampled to keep the variation at a constant level. In case of $\hat{N}_e^{\sigma}$, five sampled loci are sufficient to reduce the variation independently of the deme size.

In addition, we studied the importance of sampling effects (fig. 2.5, bottom panels) by measuring the ratios of $\hat{N}_e^{\lambda}$ and $\hat{N}_e^{\sigma}$ estimated from the pooled data of 1000 replicates with sampled fraction S to the theoretical values of $N_e = 8.9$, $N_e = 88.9$ and $N_e = 888.9$ for deme sizes $N = 16$ (black), $N = 160$ (red) and $N = 1600$ (grey), respectively. The results show that sampling effects will lead to serious underestimates of both $\hat{N}_e^{\lambda}$ (left) and $\hat{N}_e^{\sigma}$ (right). In case of $\hat{N}_e^{\sigma}$, the magnitude of the sampling effect is independent of deme size and the effect is (very) large for all sampled fractions except when the entire population is sampled ($S = 1$). This result is probably due to the extra sampling variance that is added to the variance $\sigma_{\delta p}^2$ of allele frequencies between generations. In case of $\hat{N}_e^{\lambda}$ the sampling effect is similar to the effect of $\hat{N}_e^{\sigma}$ for the smallest deme size (black), but the impact of sampled fraction decreases with increasing deme size (red & grey). Nonetheless, the results suggest that more than half the population should be sampled even when deme sizes are large (~1600) to substantially decrease sampling variation.

The above results strongly suggest that both regression-based estimators are only reliable when the entire population is sampled, which will be generally problematic in field studies. If entire populations can be sampled, $\hat{N}_e^{\sigma}$ tends to be less variable than $\hat{N}_e^{\lambda}$ in general, and needs fewer sampled loci for an accurate estimate when deme sizes are larger.

Performance of snapshot estimators of N_e

In contrast with our experimental setup that allowed us to monitor genotype frequencies of parents and offspring for 20 generations, in practical applications genetic data are typically only available for a few "snapshots" in time. For such situations estimators of N_e^{σ} based on the standardized variance of the change in allele frequencies (F-statistics) of two or few samples over time have been developed that can be either moment-based (Krimbas & Tsakas 1971, Nei & Tajima 1981, Pollak 1983, Waples 1989) or likelihood-based (Williamson & Slatkin 1999, Anderson *et al.* 2000, Wang 2001, Berthier *et al.* 2002). We applied two moment-based estimators $\hat{N}_{eF_c}^{\sigma}$ (Nei & Tajima 1981) and $\hat{N}_{eF_k}^{\sigma}$ (Pollak 1983) and three likelihood-based estimators $\hat{N}_{eML}^{\sigma}$ (maximum-likelihood based on importance sampling calculated with the program MCLEEPS, Anderson *et al.* 2000), $\hat{N}_{eL}^{\sigma}$ (pseudo-like-

lihood calculated with the program MLNE, Wang 2001), and $\hat{N}_{eBL}^{\sigma}$ (Bayesian coalescence-based likelihood calculated with the program TM3, Berthier *et al.* 2002) to our experimental dataset, and compared the results with our own findings in table 2.2. The snapshot estimators expect input from multiple loci rather than from replicate single-locus populations, but since the number of demes is interchangeable with the number of independent loci, we can use them to analyze our datasets. We also included estimates based on individual-based simulations with lottery polygyny and a variance-mean ratio α = 1.6 of 5, 10 and 20 independent loci in a small (N = 16) and a large (N = 160) deme.

All five estimators based on only two points in time tend to overestimate N_e when the rate of allele fixation is lower than predicted (vial metapopulations 4 and 5), and they considerably underestimate N_e in case of the larger bottle populations. For the small vial populations we find the largest bias for Nei and Tajima's F_c, which is consistent with the conclusion from Waples' (1989) study that of the two moment-based F-statistics F_c and F_k, Pollak's F_k performs best for diallelic loci.
The results of the simulated populations show that the moment-based estimators tend to overestimate N_e in small demes regardless of the number of sampled loci, and that the likelihood-based estimators overestimate N_e in most cases when 5 to 10 loci are sampled. All five estimators markedly underestimate N_e in large demes when 5 to 10 loci are sampled but perform equally well as the regression-based estimator with 20 sampled loci, although the confidence intervals tend to be larger for the snapshot estimators. The simulation results confirm the behaviour of the snapshot estimators in our experimental populations. The regression-based approach is more accurate in case of few sampled loci and/or small deme size, but application in practice will likely be difficult because genetic data of many successive generations is usually not available, and because of the expected sampling bias when populations are sampled only partly.

Although we conclude that our experimental results match the theoretical predictions well enough on average, we found some striking deviations. On the one hand, these were caused by obvious differences between the experimental populations and the idealized populations assumed in theoretical models, such as the reproductive success of either sex not following a Poisson-distribution, or other factors than genetic drift, *e.g.*, directional selection, playing a role. On the other hand, a small number of demes may cause considerable deviations from the theoretical predictions, as shown by the results of the 10-deme subsamples compared with the result of the entire 50-deme metapopulation. We presume, however, that this last result is at least partly due to the complete isolation of demes within either metapopulation, so that (limited) migration among the demes might yield more consistent results for small metapopulations. In CHAPTER 3 we investigate this hypothesis.

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Table 2.2. Estimated variance effective sizes and their corresponding 95% confidence intervals (CI). The effective sizes are estimated from regression over 20 generations ($\hat{N}_{eR}^{\sigma}$, *cf.* table 2.1), and from two data points (generations 0 and 20) using two moment-based estimators $\hat{N}_{eFc}^{\sigma}$ and $\hat{N}_{eFk}^{\sigma}$, and three likelihood-based estimators $\hat{N}_{eML}^{\sigma}$, $\hat{N}_{ePL}^{\sigma}$ and $\hat{N}_{eBL}^{\sigma}$. CIs for the regression-based estimator are inferred from the confidence limits of the regression coefficient. The moment-based estimators $\hat{N}_{eFc}^{\sigma}$ (Nei & Tajima 1981) and $\hat{N}_{eFk}^{\sigma}$ (Pollak 1983) and corresponding CIs are calculated following Waples (1989). $\hat{N}_{eML}^{\sigma}$, $\hat{N}_{ePL}^{\sigma}$ and $\hat{N}_{eBL}^{\sigma}$ and corresponding CIs are calculated with the programs MCLEEPS (maximum-likelihood based on importance sampling, Anderson *et al.* 2000), MLNE (pseudo-likelihood, Wang 2001) and TM3 (Bayesian coalescence-based likelihood, Berthier *et al.* 2002) respectively. In the upper part of the table, the estimates are made for the experimental vial metapopulations (n = 10, N = 16), for all experimental populations combined (n = 50, N = 16), and for the bottle populations (n = 5, N = 160). In the lower part of the table we compare data from simulated small (N = 16) and large (N = 160) populations including lottery polygyny and a variance-mean ratio α = 1.6 for L = 5, L = 10 and L = 20 loci. All results are presented relative to the predicted effective size N_e^{σ} (8.9 for the vial populations and 88.9 for the bottle populations). Confidence intervals in bold exclude the predicted effective size N_e^{σ}.

	$\hat{N}_{eR}^{\sigma}$	95% CI	$\hat{N}_{eFc}^{\sigma}$	95% CI	$\hat{N}_{eFk}^{\sigma}$	95% CI	$\hat{N}_{eML}^{\sigma}$	95% CI	$\hat{N}_{ePL}^{\sigma}$	95% CI	$\hat{N}_{eBL}^{\sigma}$	95% CI
Meta 1 (n = 10)	0.90	0.62 - 1.71	1.47	0.69 - 2.54	1.12	0.52 - 1.93	0.79	0.45 - 1.91	0.82	0.43 - 1.51	0.80	0.44 - 1.58
Meta 2 (n = 10)	0.91	0.64 - 1.58	1.41	0.66 - 2.44	1.08	0.51 - 1.87	0.79	0.45 - 1.46	0.76	0.44 - 1.52	0.82	0.46 - 1.53
Meta 3 (n = 10)	1.11	0.86 - 1.60	1.39	0.65 - 2.41	1.05	0.49 - 1.82	0.90	0.56 - 1.57	0.88	0.48 - 1.57	0.74	0.44 - 1.49
Meta 4 (n = 10)	1.10	0.89 - 1.42	2.08	0.97 - 3.59	1.66	0.78 - 2.87	1.80	**1.01 - 3.26**	1.78	0.98 - 3.49	1.72	**1.02 - 3.69**
Meta 5 (n = 10)	1.02	0.81 - 1.40	2.87	**1.35 - 4.96**	2.26	**1.06 - 3.92**	2.25	**1.24 - 5.06**	2.42	**1.21 - 5.06**	2.51	**1.40 - 6.04**
Vials (n = 50)	0.95	0.83 - 1.10	1.70	**1.26 - 2.21**	1.31	0.97 - 1.70	1.24	0.90 - 1.46	1.18	0.89 - 1.55	1.08	0.83 - 1.44
Bottles (n = 5)	1.03	0.68 - 2.22	0.27	**0.08 - 0.57**	0.23	**0.07 - 0.47**	0.31	**0.17 - 0.67**	0.32	**0.17 - 0.68**	0.33	**0.15 - 0.74**
Sim N=16, L=5	0.98	0.59 - 2.28	1.69	0.51 - 3.58	1.29	0.39 - 2.73	1.01	0.45 - 2.36	1.00	0.43 - 2.40	1.17	0.49 - 2.86
Sim N=16, L=10	0.98	0.53 - 4.55	1.83	0.86 - 3.17	1.43	0.67 - 2.47	1.46	0.84 - 2.81	1.50	0.82 - 2.82	1.53	0.81 - 2.85
Sim N=16, L=20	1.02	0.59 - 2.69	1.58	0.96 - 2.36	1.20	0.73 - 1.78	0.90	0.65 - 1.39	0.95	0.61 - 1.44	0.88	0.56 - 1.35
Sim N=160, L=5	1.00	0.60 - 3.08	0.37	**0.11 - 0.78**	0.34	**0.10 - 0.71**	0.45	0.11 - 1.07	0.41	0.14 - 1.05	0.50	0.19 - 1.18
Sim N=160, L=10	0.98	0.62 - 2.37	0.51	**0.24 - 0.89**	0.47	**0.22 - 0.80**	0.51	0.24 - 1.10	0.53	0.24 - 1.10	0.59	0.27 - 1.19
Sim N=160, L=20	1.03	0.59 - 1.20	1.15	0.70 - 1.71	1.01	0.61 - 1.50	1.05	0.54 - 1.88	0.99	0.51 - 1.76	1.11	0.59 - 1.97

APPENDIX: EFFECTIVE POPULATION SIZE

The standard Wright-Fisher model of genetic drift (Fisher 1930, Wright 1931) assumes many independent subpopulations of constant size N and random mating within each subpopulation. The individuals in a subpopulation are cosexual and reproduce by means of random fusion of gametes produced in infinite numbers by N_t breeding individuals in each generation t, thus including the possibility of selfing. Each of the N_t individuals has an equal chance to reproduce and will contribute k gametes to the next generation $t + 1$, where k is binomially distributed with mean μ_k and variance σ_k^2.

The effective population size N_e is defined as the size of an idealized Wright-Fisher population that would lead to either the same amount of variance in allele frequency change among the offspring (variance effective size N_e^{σ}), or the same level of autozygosity in the parents (inbreeding effective size N_e^{i}) as the actual population in any generation. The inbreeding effective size is a measure of the past, as the inbreeding coefficient depends on the number of ancestors in the parent generation. The variance effective size, on the other hand, is a measure of the future, as the sampling variance depends on sample size, *i.e.*, the size of the offspring generation. Thus, in a growing population, N_e^{σ} tends to be larger than N_e^{i}, and in a declining population the reverse will be the case (Crow & Kimura 1970).

In a stationary population, the census size N is constant so that the average number of gametes per parent is two ($\mu_k = 2$), and the inbreeding and variance effective sizes are equal (Crow & Kimura 1970, Crow & Denniston 1988). For a stationary cosexual population with a (constant) proportion of selfing β the effective size can be inferred from

$$N_e = \left[\frac{4 - 2\beta}{\sigma_k^2 + 2(1 - \beta)} \right] N \qquad \text{(2A.1)}$$

(eqn (8) in Wang 1996).

The reproductive system in *Drosophila* is unisexual rather than cosexual (individuals are either female or male instead of hermaphrodite), selfing is excluded, and reproduction takes place by means of random mating rather than random fusion of gametes. The assumption of random fusion of gametes in cosexual populations can be relaxed to account for random mating and sex differentiation (Crow & Kimura 1970, Crow & Denniston 1988, Wang 1996), and the effective population size of a stationary population with separate sexes and a 1:1 sex ratio can be inferred from

$$N_e = \left[\frac{4 - 3\beta}{\sigma_k^2 + 2(1 - \beta)} \right] N \ , \qquad \text{(2A.2)}$$

where σ_k^2 is the variance among offspring per parent, and β is the proportion sib mating (eqn (19) in Wang 1996). In the absence of sib mating ($\beta = 0$) and assuming that the variance among offspring per parent is the average of the variances among offspring per sex, *i.e.*, $\sigma_k^2 = (\sigma_f^2 + \sigma_m^2)/2$, (2A.2) reduces to

$$N_e = \left[\frac{8}{\sigma_f^2 + \sigma_m^2 + 4} \right] N . \qquad (2A.3)$$

In the lottery polygyny model, all males attempt to mate many times, while females mate only once or a few times. Hence, the reproductive success of males is more variable than that of females (Bateman 1948). Let x (y) be the number of matings of a given female (male) with mean μ_x (μ_y) and variance σ_x^2 (σ_y^2). Let k be the number of offspring contributed to the next generation per mating with mean μ_k and variance σ_k^2. From statistical theory (*e.g.*, Mood *et al.* 1988), we may then write the average reproductive success of a female (male) as $S_f = \mu_x \mu_k$ ($S_m = \mu_y \mu_k$) with variance $\sigma_f^2 = \mu_x \sigma_k^2 + \sigma_x^2 \mu_k^2$ ($\sigma_m^2 = \mu_y \sigma_k^2 + \sigma_y^2 \mu_k^2$). We first consider the case where females mate exactly once, i.e., the case without remating. Now $\mu_x = 1$ with variance $\sigma_x^2 = 0$ for females, and the average reproductive success S_f is equal to the average number of offspring contributed to the next generation, i.e., $S_f = \mu_k$ with variance $\sigma_f^2 = \sigma_k^2$. We further assume that the number of matings per male is Poisson-distributed so that $\mu_y = \sigma_y^2 = 1$. For males the average reproductive success is now $S_m = \mu_k$ with variance $\sigma_m^2 = \sigma_k^2 + \mu_k^2$. In a stationary population with a 1:1 sex ratio $\mu_k = 2$, so that $S_f = S_m = 2$, $\sigma_f^2 = 2\alpha$, and $\sigma_m^2 = 2\alpha + 4$, where $\alpha = \sigma_k^2 / \mu_k$ is the variance-mean ratio of the number of offspring contributed to the next generation by a single mating. Using (2A.3) we can infer the effective population size under the lottery polygyny model as

$$N_e = \left[\frac{1}{1 + \alpha/2} \right] N . \qquad (2A.4)$$

Assuming that the number of offspring per mating is Poisson-distributed ($\alpha = 1$), the effective population size is $^2/_3$ of the census size ($N_e = 10.7$ and 106.7 for $N = 16$ and 160), which agrees with the prediction derived by Nunney (1993). With the estimated variance-mean ratio $\alpha = 1.6$ (see main text), the predicted effective population sizes are further reduced to $N_e = 8.9$ for the vials and $N_e = 88.9$ for the bottles.

Next we include the probability of remating. We consider a stationary population with a 1:1 sex ratio where each female mates once, and where a mated female remates with another male with probability ρ. More than two matings do not occur. For each mating a male is randomly chosen from the population of all males (with replacement). The average number of matings per male is then $1 + \rho$, so that assuming a Poisson distribution we get $\mu_y = \sigma_y^2 = 1 + \rho$ for males. Since we assume that all females mate at least once and at most twice, the probability to mate one time is $1 - \rho$, and the probability to mate two times is ρ. As a consequence, $\mu_x = 1 + \rho$ and variance $\sigma_x^2 = \rho(1 - \rho)$ for females. In a stationary population the average reproductive success per sex is $S_f = S_m = 2$, implying that $\mu_k = 2/(1 + \rho)$. Hence, the variances are $\sigma_f^2 = 2\alpha + [4\rho(1-\rho)]/(1 + \rho)^2$ and $\sigma_m^2 = 2\alpha + 4/(1 + \rho)$ for females and males, respectively, and we can infer the effective population size from (2A.3) as

$$N_e = \left[\frac{1}{1 + \alpha/2 - [\rho/(1+\rho)]^2} \right] N. \qquad (2A.5)$$

For our particular experimental setup we estimated the remating probability from the rate of success of homozygous *bw*//*bw* males introduced in populations of mated homozygous bw^{75}//bw^{75} individuals and *vice versa*, so that any heterozygous bw^{75}//*bw* offspring must be the result of remating (see CHAPTER 3 for details). We found heterozygous offspring in 20% of the populations on average, hence the remating probability ρ for a single individual is 0.2. With $\rho = 0.2$ and $\alpha = 1$ the effective population sizes based on (2A.5) are $N_e = 10.9$ and $N_e = 108.7$ for the vials and the bottles, respectively. With $\rho = 0.2$ and $\alpha = 1.6$ the effective sizes are $N_e = 9.0$ for the vial populations and $N_e = 90.3$ for the bottle populations. Since these values do not differ much from the values in the absence of remating on basis of (2A.4), we use (2A.4) in the main text.

CHAPTER 3

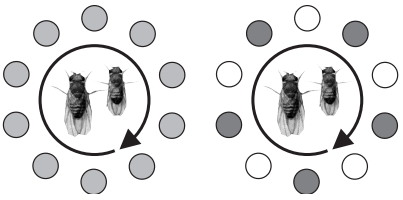
Genetic differentiation in experimental *Drosophila melanogaster* metapopulations II - Stepping stone migration

with R. BIJLSMA and F. J. WEISSING

ABSTRACT

By means of replicated experiments that were run for 20 generations, we study the effects of unidirectional stepping-stone migration on the genetic structure and fitness of *Drosophila melanogaster* metapopulations. We investigated several setups differing with respect to migrant sex, the timing of migration in relation to mating and reproduction, and the initial level of genetic differentiation. Based on the average heterozygosity within demes and fixation indices within and among metapopulations, we assessed the levels of genetic diversity, genetic differentiation and gene flow, and we inferred the effective number of migrants. Throughout, the experimental results were compared with the predictions of general metapopulation models and individual-based simulations tailored to our experimental setup.

We draw three main conclusions. First, despite of the high level of standardization, replicate metapopulations differed considerably from each other, leading to large variation in the estimates of migration rates for the same migration scenario. Second, when individuals mated before migration, the level of gene flow differed substantially between male and female migration. In other words, the effective number of migrants is strongly affected by a sex bias of migration. When compared to the island model of migration, the effective number of migrants was reduced by 50% in our unidirectional stepping-stone setup. Third, even though gene flow was sufficiently large to mitigate genetic differentiation, average fitness declined substantially in the course of the experiment. Our findings illustrate that inferences of demographic parameters from genetic data can be unreliable, and that the evolution of a metapopulation can be strongly affected by migrant sex, the timing of migration, and the spatial configuration of subpopulations.



INTRODUCTION

The present contribution is the second of a series of studies reporting on the effects of genetic drift, gene flow, and local extinction and recolonization on the genetic structure and evolution of metapopulations. We use a replicated experimental metapopulation setup with *Drosophila melanogaster* as a model organism, and combine this approach with individual-based computer simulations in order to explore variations and extensions of the experiments. Our goal is to examine the potential pitfalls of applying theory developed for highly idealized systems to natural metapopulations. It is common practice to use such idealized theory for assessing the viability of metapopulations and to derive recommendations for their conservation (*e.g.*, one-migrant-per-generation rule, Mills & Allendorf 1996, Wang 2004; assessment of connectivity, Hellberg *et al.* 2002, Webster *et al.* 2002; effects of habitat isolation and fragmentation, Segelbacher *et al.* 2003, Banks *et al.* 2005; migration patterns, Hewitt 2000, Chauvet *et al.* 2004).

Empirically deduced patterns of genetic variation in metapopulations are commonly interpreted in terms of Wright's (1931) infinite island model. This model predicts the change of genetic differentiation F_{ST} over time among small, subdivided populations due to genetic drift and migration as a function of population size N and migration rate m. In practice, F_{ST} is often used to infer demographic parameters as effective population size N_e and migration rate m, or more commonly the combined parameter N_em, the (effective) number of migrants per generation. N_em is used in turn as estimator of the (potential) connectivity and level of gene flow between local population fragments (Wright 1951, Slatkin 1985, Slatkin & Barton 1989, Neigel 1997). A metapopulation structure is usually more resilient to adverse conditions than single small, isolated populations, hence connectivity is an indicator of future metapopulation viability (Reed 2004, 2005; Bouchy *et al.* 2005). It is unclear how well theoretical models based on highly idealized Wright-Fisher populations (Fisher 1930, Wright 1931) apply to natural systems in general, and some recent studies indicate that inferences based on these models can be quite misleading in natural metapopulations (Neigel 1997, Bossart & Prowell 1998, Whitlock & McCauley 1999, Balloux *et al.* 2000, Balloux & Goudet 2002, Neigel 2002). A recent study by Pearse and Crandall (2004) reviews the development and use of estimators for the analysis of population genetic data in a conservation genetic context. Up to date, however, there are very few studies that attempt to bridge the gap between model predictions based on idealized populations and natural systems using controlled experiments (but see Warren 1996, Buckling *et al.* 2000, Lavigne *et al.* 2001).

In CHAPTER 2 we presented the results of comparable metapopulations with isolated demes affected by genetic drift only, which provided baseline values of a system without gene flow. Here we address the effects of gene flow (*i.e.*, migration of individuals between demes in a metapopulation) in combination with genetic drift within demes. To this end, we monitor genetic differentiation, and assess fitness in a metapopulation with unidirectional stepping-stone migration. We try to determine how well methods derived from idealized theoretical models are applicable to natural systems, *i.e.* how useful they are for drawing *a posteriori* inferences. For that purpose, we apply these methods to

experimental metapopulations of which the exact demographic structure and history are known, and we investigate how well the demographic parameters can be inferred from the change in genetic structure.

In particular, we will address the following questions: How big is the discrepancy between idealized theory and the behaviour of a highly standardized experimental metapopulation? What is the importance of factors such as migrant sex or the relative timing of events like mating and migration? How much variation is there between replicates? How reliable is the commonly used estimator of the effective numbers of migrants?

Additionally, we intend to obtain the baseline values of a system with continuous gene flow to investigate more complex systems including local extinction and recolonization, and different spatial migration configurations (CHAPTER 4).

MATERIAL AND METHODS

DROSOPHILA STOCKS

For the migration experiments we used the same mutant *D. melanogaster* lines as for the genetic drift experiments (CHAPTER 2), with two alleles *bw* and bw^{75} at the *brown* locus (II-104.5) that in combination with the mutation *scarlet* (*st*) (III-44) in homozygous condition result in distinct eye colours for the three genotypes at the *bw* locus. Homozygous *bw*//*bw* individuals have white eyes, homozygous bw^{75}//bw^{75} individuals have red-brown eyes, and heterozygous bw^{75}//*bw* individuals have intermediate orange eyes at 25°C. A detailed description of these stocks and how they were raised and handled during the experiments is given in CHAPTER 2.

METAPOPULATION EXPERIMENT: SETUP AND PROCEDURE

The experimental setup included metapopulations consisting of small populations in vials (fig. 3.1A), and large populations in bottles (fig. 3.1B). A metapopulation consisted of ten vial populations (= demes) initiated with 16 parents (eight females and eight males) each per generation. Bottle populations were initiated with 160 parents (80 females and 80 males) per generation. We set up five replicates of each metapopulation and of each bottle population.

Migration followed a circular, unidirectional stepping stone pattern with one migrant per generation (figs 3.1C and D), so that $Nm = 1$ and the migration rate for $N = 16$ is given by $m = 1/N = 0.0625$. We selected the migrant at random from the eight founder females or males for the next generation according to the required sex in an experimental series. The three experimental aspects of migration (migrant sex, the sequence of mating and migration, and the initial level of genetic differentiation) provided the following options: migrants are either female (F) or male (M), mating occurs either after (A) or before (B) migration, and the initial level of genetic differentiation is either zero ($F_{ST} = 0$) or maximal ($F_{ST} = 1$). This resulted in eight different combinations of options (migration series AF0, AF1, AM0, AM1, BF0, BF1, BM0, BM1). We used two types of

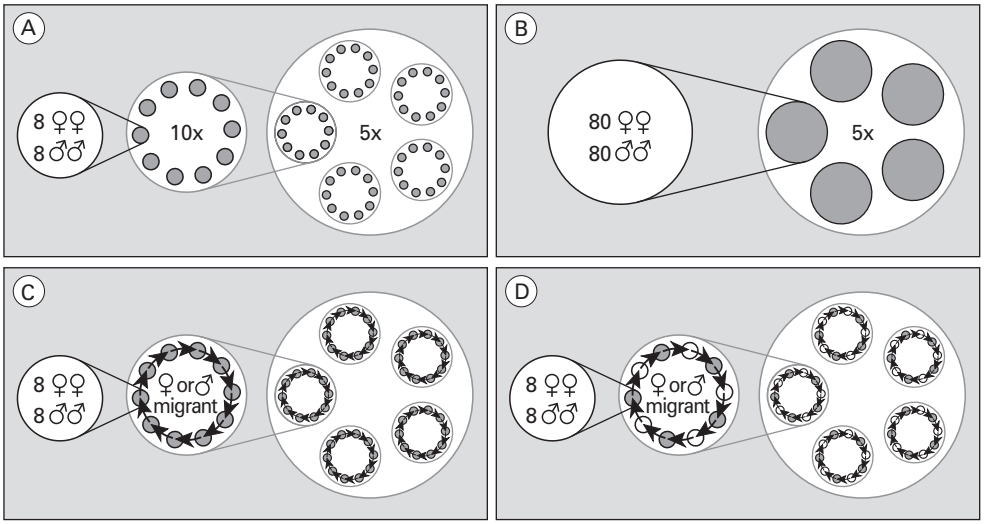


Figure 3.1. Setup of experimental *D. melanogaster* metapopulations. A: One small vial population (= deme) is founded by 16 breeding individuals (eight females and eight males) each generation. Ten of these vial populations represent one subdivided population (= metapopulation with ten demes) with a total size of 160 individuals. All five replicate metapopulations together may be viewed as a higher-level, global metapopulation with a total size of 800 individuals. B: One bottle population is initiated with 160 individuals in each generation. Five replicate bottle populations represent a metapopulation with five large demes and a total size of 800 individuals. C: Migration between the vial populations follows a circular, unidirectional stepping-stone pattern, with one (female or male) migrant per generation. All demes are initialized with heterozygote (light grey) individuals to minimize differentiation. D: Demes are initialized with alternating white-eyed (white) and red-eyed (dark grey) homozygote individuals to maximize differentiation. Migration as in C.

control series (data from CHAPTER 2): vial metapopulations without migration (series C0, fig. 3.1A) and bottle populations (series CH, fig. 3.1B).

We initiated the control series C0 and CH and all demes in the metapopulations starting without genetic differentiation (fig. 3.1C) with heterozygous individuals obtained from the mixed offspring of the reciprocal crosses $bw^{75}//bw^{75}$ x $bw//bw$ and $bw//bw$ x $bw^{75}//bw^{75}$. We continued this heterozygous founder population parallel to the experiment to provide baseline values of a very large ("infinite") random mating population consisting of 20 bottles adding up to *ca.* 3100 individuals that were mixed each generation. The demes in the metapopulations starting with maximal genetic differentiation (fig. 3.1D) were alternately initiated with either homozygous red-eyed or homozygous white-eyed flies from the homozygous $bw^{75}//bw^{75}$ (SR) and $bw//bw$ (SW) stock populations. Hence, although the genetic differentiation among demes differed between experiments, the initial frequencies of both *brown*-alleles were equal (0.5) in all metapopulations and in all migration series. For logistic reasons we carried out the experiments in two successive time periods, with the series implementing mating after migration and the vial controls in the first, and the series implementing mating before migration and

the bottle controls in the second. We started the experiments in each period with a new heterozygous founder population as described above that we will refer to as FH1 and FH2, respectively.

Generations did not overlap and took 14 days to develop and to provide sufficient offspring for sampling. The number of offspring per vial varied between 50 and 90. Each generation we collected 15–25 virgin flies of each sex on the first days of eclosion. After scoring the genotypic composition of this sample of 30–50 flies, we randomly selected eight individuals of each sex from these flies to found the next generation.

For the series with mating occurring after migration we selected and transferred the virgin migrant simultaneously with the transfer of the virgin founders into a fresh breeding vial. We allowed the flies to mate and lay eggs for three days, and then transferred them to fresh vials for two more days to provide an emergency backup. In the series with mating occurring before migration, we placed the virgin founders into a temporary mating vial for 24 hours to mate before selecting a migrant. We then moved both the mated residents and the selected mated immigrant into a fresh breeding vial to lay eggs and remate for two days, and finally we transferred all flies again to fresh vials for two more days to provide the emergency backup. The procedure for both types of control populations and the founder populations is described in detail in CHAPTER 2.

We determined the genotypic composition of the samples from each vial and bottle population for 20 generations to infer allele frequencies and actual and expected heterozygosities, and to monitor fixation events.

ASSESSMENT OF FITNESS AND REMATING PROBABILITY

At the end of the experiment, we assessed the fitness of all experimental populations. As fitness measure we used net fecundity (*i.e.*, the average number of emerging offspring per female), which we calculated from the total number of offspring of a sample of five breeding pairs raised under standard conditions in 23 ml plastic vials on 9 ml of standard food with antibiotics (100 mg ampicillin per liter). The breeding pairs consisted of virgin flies that were allowed to mate and lay eggs for 14 days with transfers to fresh vials every third day, resulting in five consecutive series of offspring that were counted, summed and averaged per remaining female (*i.e.*, corrected for escapees but not for dead females) to obtain one final value per sample. Thus, the measured net fecundity includes female fitness characters as adult survival, fecundity (number of eggs laid) and offspring viability (egg-to-adult survival), and assumes no limiting effects of male virility.

We inferred the net fecundity of a vial population as the average of three replicate samples per vial, and the net fecundity of a metapopulation as the average of 10 x 3 replicate samples, *i.e.*, the three samples from each of its ten demes. In a comparable way, we inferred the average net fecundity of a bottle population from 30 replicate samples per bottle, and the baseline fitness values for the stock and founder populations from 30 replicate samples per population.

We estimated the remating probability ρ (*i.e.*, the probability that females mate with a second male, Bundgaard & Christiansen 1972, Van Vianen & Bijlsma 1993) from the

success rate of homozygous *bw*//*bw* males introduced in populations of mated homozygous bw^{75}//bw^{75} individuals and *vice versa* (experiment series BM1). Any heterozygous bw^{75}//*bw* offspring in such populations must be the result of remating. We monitored the occurrence of heterozygous offspring on 220 of such occasions, resulting in an estimated remating probability $\rho = 0.2$ per individual. Note that this estimate may be inaccurate for two reasons. (1) We could not distinguish between heterozygous offspring of different females. Hence, males that remated with more than one female were considered equal to males that remated once, which tends to make the estimate conservative. (2) Remating occurred about three times more often in case of bw^{75}//bw^{75} males than in case of *bw*//*bw* males on average, which indicates that the three genotypes in our experiments differed with respect to male mating success.

F-STATISTICS

For each generation, we monitored the genotype frequencies of parents and offspring per population and used these to determine allele frequencies, and observed and expected heterozygosities. From these data, we inferred estimates of the level of genetic differentiation within and among the five vial metapopulations (fig. 3.1).

As a measure of genetic differentiation among subpopulations within a metapopulation, we calculate the fixation index F_{ST} as defined by Wright's hierarchical *F*-statistics (Wright 1951, Hartl & Clark 1997) from the expected heterozygosities within and among subpopulations:

$$F_{ST} = \frac{H_T - \bar{H}_S}{H_T} \ . \tag{3.1}$$

In the experimental setup, H_T represents the total expected heterozygosity of the metapopulation, while $\bar{H}_S$ indicates the average expected heterozygosity of the ten vial populations in the metapopulation.

To compare between replicate metapopulations, we use an analogous, higher-level *F*-statistic F_{TG}:

$$F_{TG} = \frac{H_G - \bar{H}_T}{H_G} \ . \tag{3.2}$$

Here, $\bar{H}_T$ is the average expected heterozygosity at the metapopulation level. H_G indicates the total expected heterozygosity in all five replicate metapopulations ("global" heterozygosity), and F_{TG} is then a measure of genetic differentiation ("global fixation index"), and thus an indication of the variation, between these five replicate metapopulations.

The lowest level of *F*-statistics compares the observed heterozygosity within demes H_I to the expected heterozygosity H_S of a population in Hardy-Weinberg equilibrium:

$$F_{IS} = \frac{H_S - H_I}{H_S} \ . \tag{3.3}$$

An F_{IS} value of zero indicates a population in Hardy-Weinberg equilibrium, $F_{IS} > 0$ and $F_{IS} < 0$ indicate a shortage or a surplus of heterozygotes with respect to the Hardy-Weinberg expectation, respectively.

INDIVIDUAL-BASED SIMULATIONS

The individual-based Monte-Carlo simulation model is based on the Drosophila mating system resembling lottery polygyny (all females mate once, while males can mate with more than one female; Bateman 1948, Nunney 1993) with female remating. In case of remating the last male tends to be the most successful due to sperm displacement (*i.e.*, most sperm of a previous male is inactivated). Typically, the fraction offspring sired by the second male is $p_2 > 0.75$ (Prout & Bundgaard 1977, Scott & Williams 1993). For simplicity's sake, we assume that $p_2 = 1$ (*i.e.*, complete instead of partial sperm displacement) in the simulations. We include migration based on the circular unidirectional stepping-stone model that corresponds to the experimental setup.

We implemented the simulation model as follows. Depending on the migration scenario, each generation starts with either migration followed by mating, or mating followed by migration. There are $N_f = N/2$ females and $N_m = N/2$ males per generation. Migration between n demes happens by randomly choosing one female (male) migrant from N_f (N_m) resident females (males) in each deme and moving it into the next deme in sequence. Mating happens within each deme by associating each of the N_f females with a male that is randomly chosen (with replacement) from N_m males. The next step is remating, where each mated female has a probability ρ to be associated with a second male that is randomly chosen from the "new" male population (*i.e.*, one resident male has been replaced by a migrant in case of male migration, or all males have been "replaced" with the male population of the target deme in case of female migration). In case of remating the second male's gametes completely replace the first male's gametes. In the final step reproduction takes place by randomly choosing one mated female per offspring until N_f female and N_m male offspring are produced. Each offspring receives two randomly chosen alleles per locus from the mother and the mother's mate, respectively.

For all simulations the mating system is lottery polygyny with remating, generations are discrete, and population sizes are kept constant at either 16 or 160 individuals, with a sex ratio of 1:1. The remating probability is kept constant at $\rho = 0.2$. The reproductive success of females in our experimental system did not follow a Poisson distribution but had a variance-mean ratio $\alpha = 1.6$. We implemented this by mimicking the experimentally obtained distribution of eggs laid per female (CHAPTER 2). The genetic parameters are kept constant (one locus, two alleles, no mutation). Unless indicated otherwise, the standard simulations do not include selection since we want to focus on the effects of genetic drift, migration and mating system *per se*. When including selection, we use an additive viability selection model (*i.e.*, the relative viability of the genotypes $bw^{75}//bw^{75}$, $bw^{75}//bw$ and $bw//bw$ is 1, $1 - s/2$ and $1 - s$, respectively) with selection coefficient $s = 0.08$ (see CHAPTER 2 for details).

STATISTICAL ANALYSIS

For the statistical analysis of our data, we mostly use the simulation model to construct 95% confidence ranges from the 2.5 and 97.5 percentiles of 1000 simulation runs, with each run representing one metapopulation.

For the statistical evaluation of the population fitness data, we use analysis of variance (ANOVA) with the average net fecundity of a (meta)population as the dependent variable. We test the effect of the different migration scenarios on population fitness in a 2 x 2 x 2 factorial ANOVA. The fixed factors are time of mating (after or before migration), migrant sex (female or male) and initial differentiation (zero or maximal), and there are five replicate metapopulations for each combination of factors. We test the effects of population size and gene flow on population fitness separately in single fixed-factor ANOVAs. The factor population size has three levels: small vial populations (N = 16, 49 (50 minus one outlier) replicates), larger bottle populations ($N = 160$, 15 replicates), and large stock and founder populations ($N \approx 3100$, six replicates). The factor gene flow has five levels: effective numbers of migrants of 0, 0.2, 1.0, 1.8, and ∞ (see the corresponding section for explanation), with respectively 5, 10, 20, 10 and 15 replicates per level. In case of the single factor ANOVAs, we use *post hoc* testing (Tukey's HSD) to pinpoint significant differences.

Each data point in an ANOVA is the average of 30 replicate net fecundity measurements (*i.e.*, three measurements per vial for each of ten vials in a metapopulation, or 30 measurements per bottle, stock and founder population), with the exception of the vials in the analysis of population size, where each data point is the average of three measurements.

RESULTS

GENETIC DIVERSITY AND DIFFERENTATION

In this section we evaluate the changes of the average expected heterozygosity $\bar{H}_S$ as a measure of genetic diversity within demes (Varvio *et al.* 1986), and the fixation index F_{ST} as a measure of genetic differentiation among demes in our experimental metapopulations. Both measures are important in conservation genetic studies to assess the status of populations, and to make recommendations for future management (*e.g.*, Keyghobadi *et al.* 2005). Figure 3.2 shows the expected heterozygosity $\bar{H}_S$ averaged over five metapopulations to depict the global trend for each series, and figure 3.3 shows $\bar{H}_S$ of the individual replicates. Figures 3.4 and 3.5 show the corresponding plots of the fixation index F_{ST}. We will focus on the effect of the timing of mating (top: mating after migration, bottom: mating before migration), and also evaluate the differences between migrant sex (left: female migration, right: male migration) and initial level of differentiation (red: $F_{ST} = 0$, black: $F_{ST} = 1$) at the moment of population fragmentation.

Mating after migration

First we consider the scenario where mating occurred after migration (A-series, top panels). As expected, diversity increases rapidly for population fragments without initial

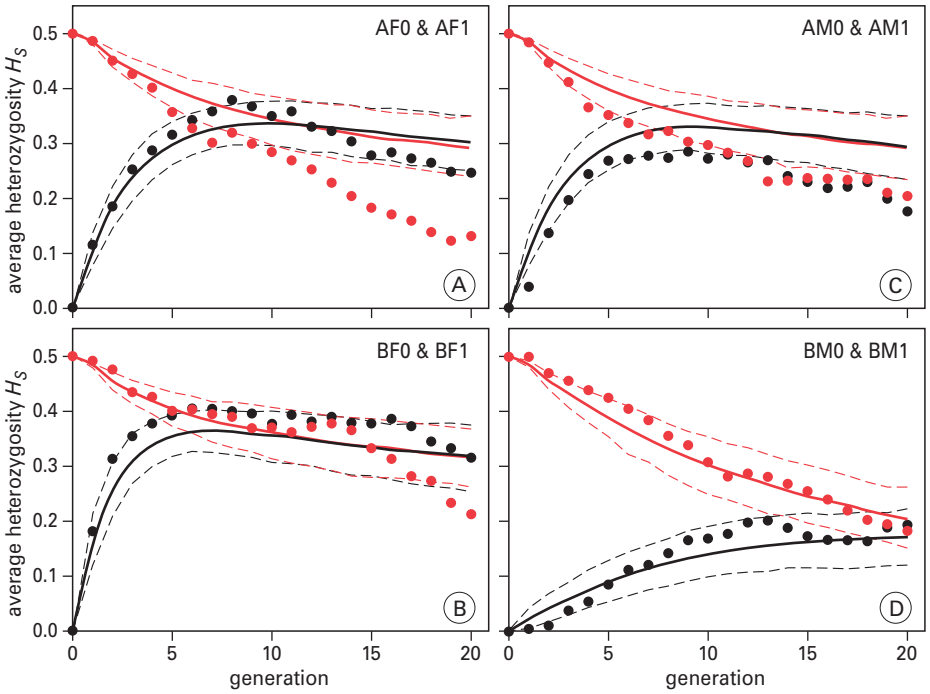


Figure 3.2. Genetic diversity within experimental *D. melanogaster* metapopulations with either female (left) or male (right) migrants that mated after (top) or before (bottom) migration. The plots show the expected heterozygosity $\bar{H}_S$ averaged over five replicate metapopulations (markers), and the prediction from standard individual-based simulations (solid lines) with 95% confidence bands based on samples of five replicates (dotted lines). The red series are the results of initially undifferentiated, heterozygous metapopulations ($\bar{H}_S = 0.5$, $F_{ST} = 0$), and the black series are the results of initially completely differentiated, homozygous metapopulations ($\bar{H}_S = 0$, $F_{ST} = 1$).

diversity that become connected through migration (fig. 3.2, AF1 and AM1). In contrast, we find a decline of genetic diversity starting immediately after the connection of demes in metapopulations with maximal diversity (fig. 3.2, AF0 and AM0). The observed dynamics of diversity is in line with the predictions from simulations (fig. 3.2, solid red and black lines) although over time, the decline tends to become significantly steeper than predicted (*i.e.*, the observed values fall outside the 95% confidence bands indicated by dashed lines). According to the simulations, the higher variance in reproductive success of males should not result in substantial differences in the dynamics of $\bar{H}_S$ in the cases of female and male migration. The experimental results are roughly in line with this prediction, although series AF0 shows a much steeper decline than the other series. When considering the dynamics of $\bar{H}_S$ in individual replicates, figure 3.3 (red markers) shows that all five replicates in series AF0 fall below the predicted decline and that three of the five replicates even fall below the 95% confidence bands. We have no explanation for this discrepancy, since all metapopulations of series AF1 are well in line with the

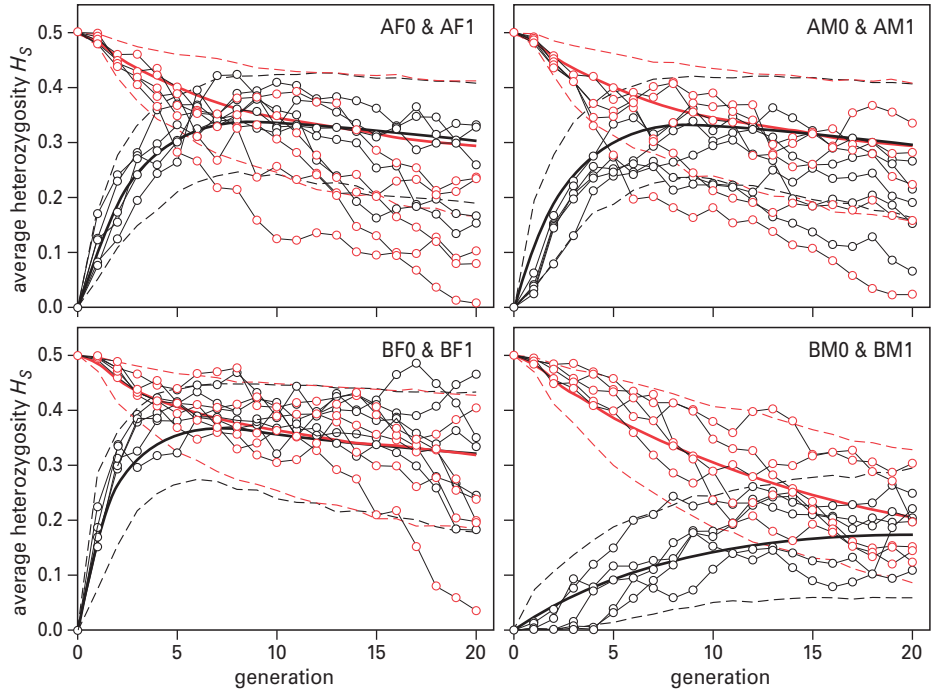


Figure 3.3. Variation of genetic diversity among experimental *D. melanogaster* metapopulations with either female (left) or male (right) migrants that mated after (top) or before (bottom) migration. The plots show the average expected heterozygosity $\bar{H}_S$ of individual replicate metapopulations (connected markers), and the prediction from standard individual-based simulations (solid lines, *cf.* figure 3.2) with 95% confidence bands based on single replicates (dotted lines). Colour codes as in figure 3.2.

predictions. The variation among the other three series is equally large, but mostly within the confidence bands (fig. 3.3, AF1, AM0 and AM1).

Genetic differentiation among demes (fig. 3.4, top panels) appears to approach equilibrium levels that are similar for both initial situations (red *versus* black) and for both migrant sexes (left *versus* right). The dynamics of individual metapopulations (fig. 3.5, top panels) show similar ranges of variation for genetic differentiation as for genetic diversity. The observed levels of differentiation tend to be (significantly) lower than predicted by simulations for all series. The occurrence of directional selection favouring the bw^{75}-allele (CHAPTER 2) suggests an explanation for this pattern, since directional selection will generally reduce the level of differentiation within metapopulations (Crow & Kimura 1970, Glemin *et al.* 2003). Simulations including selection do indeed predict a reduction of F_{ST} (5% on average in generation 20, data not shown), but this reduction is not sufficient to explain the difference between the observed and the simulated patterns. Obviously, selection based on a simple viability model does not fully describe the processes in our experimental metapopulations. Further inspection of our data reveals

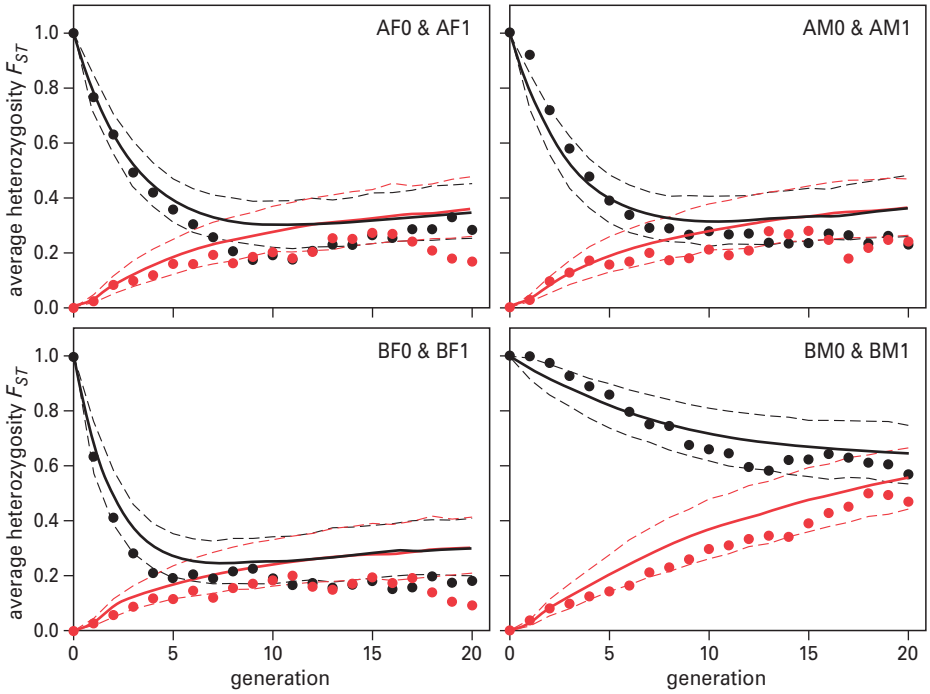


Figure 3.4. Genetic differentiation within experimental *D. melanogaster* metapopulations with either female (left) or male (right) migrants that mated after (top) or before (bottom) migration. The plots show the fixation index F_{ST} averaged over five replicate metapopulations (markers), and the prediction from standard individual-based simulations (solid lines) with 95% confidence bands based on samples of five replicates (dotted lines). The red series are the results of initially undifferentiated, heterozygous metapopulations ($F_{ST} = 0$), and the black series are the results of initially completely differentiated, homozygous metapopulations ($F_{ST} = 1$).

that the number of demes fixed for the bw^{75}-allele is almost twice the number predicted by simulations, suggesting some additional factor favouring the bw^{75}-allele. The experimental regime of 24 hours of light might be such a factor. White-eyed flies tend to be sensitive to high light intensities, which negatively affects their activity (Reed & Reed 1950). Incidental observations of our experimental fly stocks, *e.g.*, the unequal rate of remating between red- and white-eyed males, support this notion. Thus, the experimental procedure limiting egg-laying to three days might have inadvertently favoured the most active flies, suggesting that density-dependent selection is more likely in practice, but validation of more complex selection scenarios is beyond the aims of this study.

Mating before migration

When we next consider the scenario where mating occurred in the resident demes before the migrants were moved into their target demes (B-series), the resulting picture is considerably different (fig. 3.2, bottom panels). In contrast with the previous scenario of mating after migration, we observe a pronounced difference between female (left) and

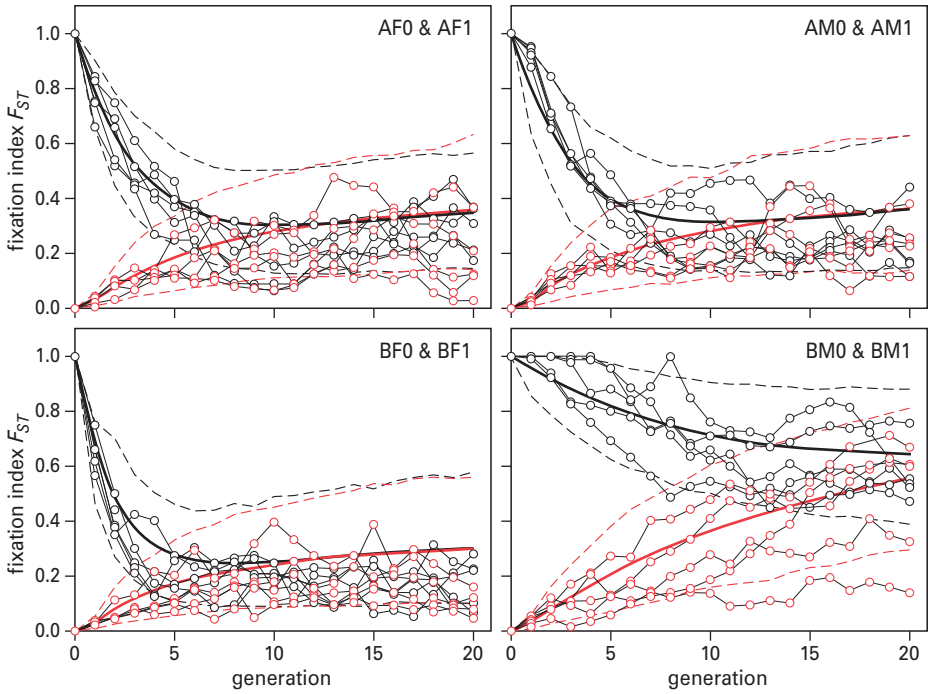


Figure 3.5. Variation of genetic differentiation among experimental *D. melanogaster* metapopulations with either female (left) or male (right) migrants that mated after (top) or before (bottom) migration. The plots show the fixation index F_{ST} of individual replicate metapopulations (connected markers), and the prediction from standard individual-based simulations (solid lines, *cf.* figure 3.2) with 95% confidence bands based on single replicates (dotted lines). Colour codes as in figure 3.4.

male (right) migration that is also predicted by simulations. For both initial situations, the levels of genetic diversity in metapopulations with male migrants (BM0 and BM1) are low relative to the levels in metapopulations with female migrants (BF0 and BF1). The observed results are largely within the 95% confidence bands for all series, and the experimental variation in genetic diversity among replicate metapopulations is also conform the range of variation predicted by simulations (fig. 3.3, bottom panels). We observe similar large differences between female and male migration for the change in genetic differentiation (fig. 3.4, bottom panels). Both the observed and predicted levels of differentiation appear to approach an equilibrium (see appendix) that is similar for both initial situations (red *versus* black), but much lower for female migrants (left) than for male migrants (right). The variation among replicate metapopulations is in line with the predicted range for male migrants, and the variation among metapopulations with female migration tends to be less than predicted (fig. 3.5, bottom panels). These results indicate that migration of males is less efficient than migration of females when individuals have mated before migration.

Effective migration rate

Many theoretical population genetic models make the implicit assumption that mating and reproduction occur after migration, so that both the migrants and the residents in their target demes are virgins. The experimental A-series were set up according to this assumption, with exactly one migrant per deme per generation. The migration rate m is then inversely proportional to the population size N: $m = 1/N$.

In case of the B-series, we allowed the flies to mate in their resident deme before any migration occurred. Now the probability to reproduce successfully after migration is different for both sexes, and the impact of a migrant on the target deme depends on its sex. A mated female reproducing in the target deme without interaction with resident males has twice the impact of a virgin female, since she introduces not only her own gametes but also those of her mate. The effective number of migrants per generation is not one but two in this case.

In the *Drosophila* mating system, a mated female migrant has a probability ρ to remate with one of the resident males before she starts reproducing, so that the resulting effective number of female migrants per generation equals $1 + (1 - \rho) = 2 - \rho$. Male migrants, on the other hand, will only contribute to reproduction if they successfully compete with the resident males for remating opportunities. The effective number of male migrants per generation is thus reduced to ρ, and with $\rho = 0.2$ we can calculate effective migration rates

$$m_{e,f} = (2 - \rho)/N = 0.1125\,, \quad m_{e,m} = \rho/N = 0.0125 \tag{3.4}$$

for mated females and for males arriving in populations where individuals have already mated, respectively. Our results illustrate that knowledge of a species' life-history is indispensable for the correct interpretation of patterns of genetic diversity or genetic differentiation.

EXPECTED PATTERNS OF GENETIC DIFFERENTIATION

We now investigate how well the observed results correspond to predictions from standard theoretical models, of which the island model of migration (Wright 1931) is the most notorious. Wright's model assumes many independent demes of constant size N with random mating within each deme, and an equal migration rate m for all demes (Wright 1951). Slatkin (1977) distinguished the infinite 'continent-island' model (*cf.* Wright's model), and the finite "n-island" model. For both models, the change of autozygosity F (*i.e.*, the probability that two alleles within one individual are identical by descent) over time can be predicted from recurrence equations (appendix, fig. 3A.1). Under the additional assumption that individuals from different demes are unrelated (Rousset 2004), as is the case in the continent-island model where migrants originate from outside the metapopulation, the autozygosity F equals the fixation index F_{ST} that measures genetic differentiation among demes. If m is sufficiently small, F_{ST} converges rapidly to the well-known drift-migration equilibrium:

$$\hat{F}_{ST} = \frac{1}{1 + 4\,N_e m} \tag{3.5}$$

In contrast with the continent-island model, migrants in the *n*-island model originate from demes within the metapopulation. Thus, individuals from different demes will become more related over time due to migration, so that the autozygosity F may not be equated to F_{ST}, and will eventually converge to an equilibrium value $\hat{F} = 1$ (appendix, fig. 3A.1). In such a situation F_{ST} is given by (Rousset 2004):

$$F_{ST} = \frac{F_w - F_b}{1 - F_b} \tag{3.6}$$

where F_w is the probability of identity (either by descent or by state) within demes and F_b is the probability of identity between demes.

In a stepping-stone model of migration gene flow is high between adjacent demes, but it gets lower as demes are further apart. This asymmetry is larger as the model has fewer dimensions (Kimura & Weiss 1964). Maruyama (1970) considered a one-dimensional, bidirectional stepping-stone model with symmetric short-range migration by analyzing a set of recurrence equations predicting the autozygosity F for two alleles that might have originated from any combination of the same or different demes. We use a similar approach to derive a more general set of recurrence equations that allows for asymmetric migration as in the unidirectional model in our experiments (appendix). As for the *n*-island model, individuals in different demes become more related over time due to migration in the stepping-stone model, so that F and F_{ST} are not equal. To predict the change of F_{ST}, we use eqn (3.6) equating F_w to the autozygosity in the focal deme and F_b to the average autozygosity between demes that are one or more steps apart (appendix).

In figure 3.6 we compare the observed F_{ST} with predictions from the stepping-stone (red and grey lines) and island (black lines) models based on eqn (3.6). In the first 20 generations (the duration of the experiment) the patterns expected on basis of the island model (black lines) and the stepping-stone model (red and grey lines) only differ when the metapopulation is started with maximum initial differentiation. The equilibrium values of F_{ST} differ markedly between the two migration scenarios, but these differences would only have become apparent in later generations. At such a later stage inadequate use of the island model might have led to substantially biased predictions (see the next section).

The predictions in fig. 3.6 suggest that none of the experimental series should have reached its equilibrium in generation 20, although the observed values (red and grey circles) appear to level off in the last five generations of the experiment. We presume that this discrepancy is mostly due to the effect of selection in favour of the bw^{75} allele in the experimental series that is not included in the theoretical predictions.

EFFECTIVE NUMBER OF MIGRANTS

In practical applications (*e.g.*, Seppa & Laurila 1999, De Matthaeis *et al.* 2000, Antolin *et al.* 2001, Anderson *et al.* 2004, Roberts *et al.* 2004, Shephard *et al.* 2005), predictions based

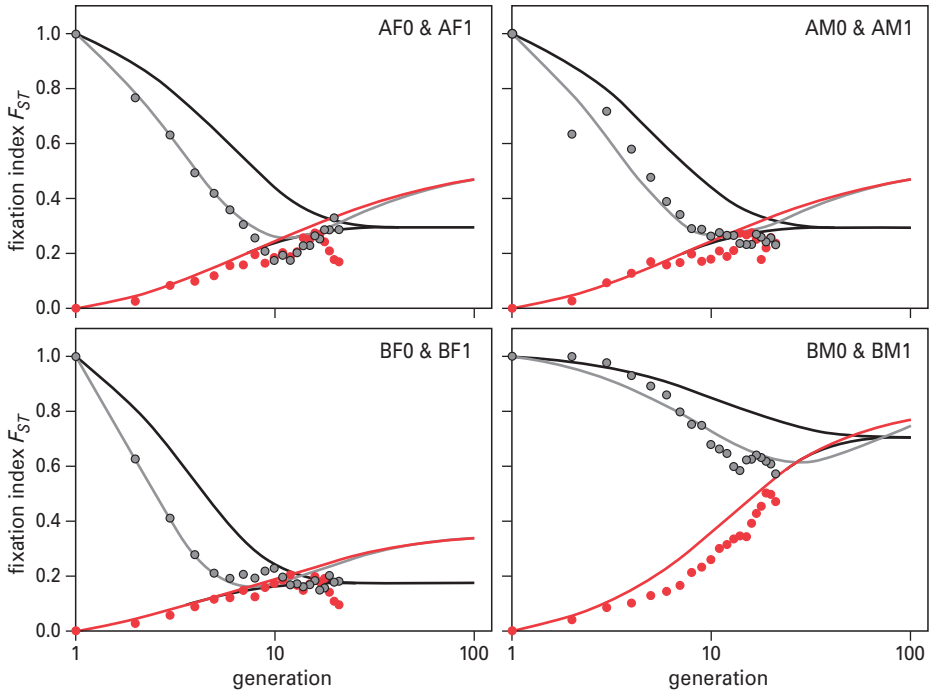


Figure 3.6. Observed and predicted differentiation within *D. melanogaster* metapopulations with female (left) and male (right) migrants that mated after (top) or before (bottom) migration. The plots show fixation indices F_{ST} averaged over five replicate metapopulations (red: initial $F_{ST} = 0$; grey: initial $F_{ST} = 1$). Predictions are based on the analytical stepping-stone (red & grey lines) and n-island (black lines) models and are inferred from the corresponding recurrence equations (see appendix) with $m = 0.0625$ (top) or $m_{e,f} = 0.1125$ (bottom left) and $m_{e,m} = 0.0125$ (bottom right), and using the effective population size $N_e = 0.56N = 9$ to compensate for lottery polygyny and a variance-mean ratio $\alpha = 1.6$ for the reproductive success per mating. Note that the red and black lines coincide for populations with initial $F_{ST} = 0$, and the log-scale of the time-axis.

on Wright's island model of migration are often used to infer estimates of the effective number of migrants per generation $N_e m$ from the measured levels of genetic differentiation F_{ST} among local population fragments. In table 3.1 we apply eqn (3.5) to the observed F_{ST} to estimate the effective number of migrants $N_e m = (1 - F_{ST})/4F_{ST}$ for each replicate metapopulation in generation 20 (M1 to M5). We compare these numbers with predictions from individual-based simulations in generation 20 (SIM20). In line with the observed variation in diversity and differentiation, the estimated $N_e m$ vary considerably among replicate metapopulations and tend to be (significantly) larger than predicted in most cases, even with the confidence intervals (SIM20) spanning a factor ten. Although none of the experimental metapopulations is at equilibrium in generation 20, neither are the simulated metapopulations (fig. 3.6). Hence, the non-equilibrium condition of the populations does not explain the difference between the experimental and simulated results. We presume that this difference is mostly due to the effect of directional selection

Table 3.1. Estimates of the effective number of migrants in *D. melanogaster* metapopulations. N_em estimates are calculated using F_{ST} for the experimental (M1 to M5) and simulated (SIM20 and SIMeq) metapopulations, and using the effective population size $N_e = 9$ and migration rates $m = 0.0625$, $m_{e,f} = 0.1125$ and $m_{e,m} = 0.0125$ for the analytical unidirectional stepping-stone (SSeq) and island (IMeq) models (see text for details). Rows are ordered by increasing expected effective migration rate. Bold numbers indicate experimental results that fall outside the 95% confidence intervals predicted by simulations.

Series	M1	M2	M3	M4	M5	SIM20 (95% CI)	SIMeq (95% CI)	SSeq	IMeq
BM0	0.52	0.16	**1.55**	0.17	0.12	0.20 (0.06-0.60)	0.06 (0.00-0.41)	0.05	0.11
BM1	0.08	0.20	0.23	0.28	0.22	0.14 (0.03-0.39)	0.06 (0.00-0.32)	0.05	0.11
AM0	**1.94**	0.86	0.41	0.81	0.72	0.44 (0.15-1.54)	0.29 (0.07-1.68)	0.27	0.56
AM1	0.32	**1.87**	1.28	1.20	0.73	0.44 (0.15-1.44)	0.28 (0.07-1.71)	0.27	0.56
AF0	**1.78**	**8.61**	0.43	0.96	**1.85**	0.45 (0.14-1.55)	0.28 (0.07-1.53)	0.27	0.56
AF1	0.56	1.19	0.93	0.44	0.42	0.47 (0.19-1.53)	0.28 (0.07-1.75)	0.27	0.56
BF0	1.77	1.31	**5.39**	**3.47**	**3.05**	0.58 (0.20-2.03)	0.45 (0.12-2.41)	0.49	1.01
BF1	0.88	0.64	0.85	**3.47**	**2.28**	0.59 (0.18-2.26)	0.44 (0.11-2.45)	0.49	1.01

that reduces F_{ST}, and hence will increase the estimates of effective migration. The large confidence intervals imply that estimates of N_em from single metapopulations might deviate by a factor three on average, but when we consider the experimental metapopulations the estimates deviate by even higher factors up to 20 (replicate M2 in series AF0).

The simulated metapopulations had approached their equilibrium after 100 generations (300 generations in case of series BM). The values of N_em (tab. 3.1) in simulated non-equilibrium metapopulations (SIM20) are much higher than in simulated metapopulations approximately in equilibrium (SIMeq). This suggests that the number of migrants can easily be overestimated in natural populations, which might often be out of equilibrium due to e.g., environmental disturbance or human intervention (Boileau *et al.* 1992, Whitlock 1992, Ingvarsson *et al.* 1997, Stewart *et al.* 1999, Bohonak & Roderick 2001, Baguette 2004).

When all assumptions of the island model are met, we expect effective numbers of migrants N_em (IMeq) with $N_e = 9$ and migration rates $m = 0.0625$, $m_{e,f} = 0.1125$ and $m_{e,m} = 0.0125$ as defined by eqn (3.4). These numbers are almost twice as high as the equilibrium values based on the analytical stepping-stone model (SSeq), which is in line with the higher equilibrium levels of F_{ST} predicted by this model (fig. 3.6). Although the simulated equilibrium values (SIMeq) match the predictions from the analytical stepping-stone model (SSeq), the wide confidence intervals imply large variation among single metapopulations.

DIFFERENTIATION AMONG METAPOPULATIONS

The relative impact of genetic drift and gene flow determine to what extent replicate metapopulations diverge from each other. We used the global fixation index F_{TG} defined in eqn (3.2) to evaluate the level of genetic differentiation among metapopulations for

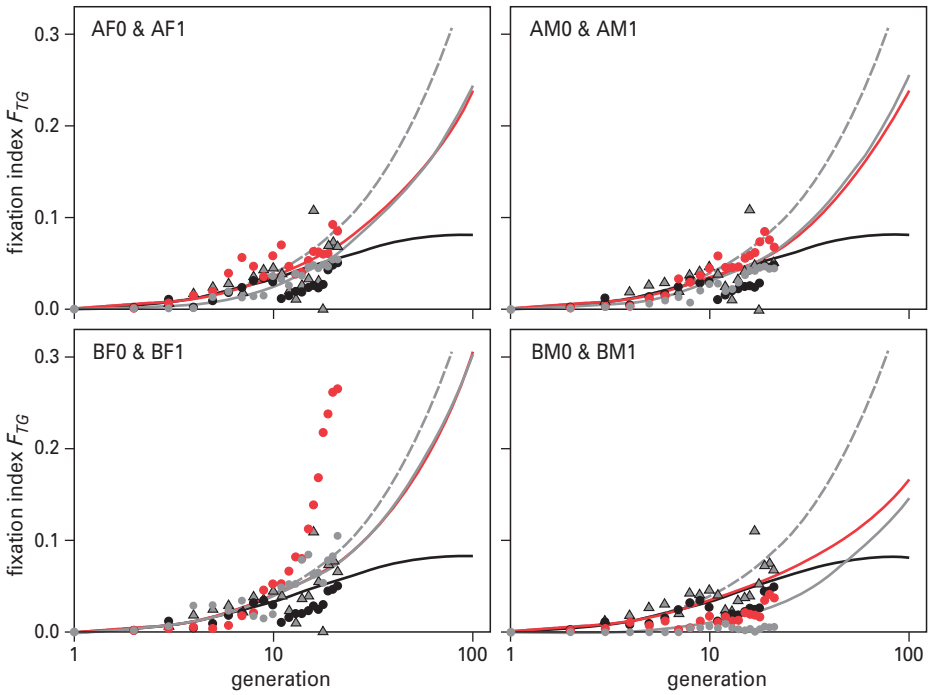


Figure 3.7. Observed and predicted differentiation among *D. melanogaster* metapopulations with female (left) and male (right) migrants that mated after (top) or before (bottom) migration. The plots show global fixation indices F_{TG} calculated from five replicate experimental metapopulations with migration (red: initial $F_{ST} = 0$; grey: initial $F_{ST} = 1$) or without migration (black), and the fixation index F_{ST} of five undivided populations of equivalent size as a metapopulation (triangles). The predicted F_{TG} (lines in corresponding colours) or F_{ST} (dashed line) are inferred from standard individual-based simulations. The very high F_{TG} for series BF0 (bottom left, red circles) is mostly due to a single replicate where the average frequency of the bw^{75}-allele decreased rather than increased over time in spite of selection favouring the bw^{75}-allele. Note the log-scale of the time-axis.

each experimental series (red and grey circles) in figure 3.7. Using data from CHAPTER 2, we included metapopulations without gene flow (C0, black circles), and undivided populations of identical size (bottle populations CH, grey triangles).

We found earlier that high levels of gene flow (*i.e.*, high effective migration rates) result in relatively low levels of differentiation within a metapopulation. The results in figure 3.7 suggest that the opposite is the case for the differentiation among metapopulations. In the absence of migration, we observe a higher level of differentiation among the (larger) bottle populations than among the (smaller) vial metapopulations, which is consistent with predictions from simulations (black *versus* dashed lines). We can readily explain this by allele fixation due to genetic drift in almost all demes in the vial metapopulations without migration relatively early in time. Allele fixation within most demes limits both the effect of directional selection at all levels, and differentiation

within and among metapopulations because the amount and distribution of genetic variation in the system also becomes fixed. A low effective migration rate (BM0 and BM1) results in the smallest change of F_{TG}, a high effective migration rate (BF0 and BF1) results in a relatively large change of F_{TG}, and an intermediate migration rate results in an intermediate change of F_{TG} (A-series). The (predicted) longer-term levels of differentiation for all scenarios with migration (red and grey lines) are intermediate between the levels of differentiation of isolated metapopulations (black line) and of single panmictic populations of identical size (dashed line).

These results indicate that the level of gene flow within a metapopulation affects the distribution of genetic variation within and among metapopulations. When migration rates are relatively low, the genetic variation within demes will shift to differentiation among demes. When migration rates are relatively high, however, the differentiation among demes will shift even further to differentiation among metapopulations.

We conclude that even very low migration rates may mitigate the effect of genetic drift within demes sufficiently to prevent the fixation of variation among demes within a metapopulation (BM0 and BM1 *versus* C0). At relatively high migration rates allele fixation, and thus, the loss of genetic diversity, at the metapopulation level becomes a possibility. Allele fixation within the entire metapopulation will be facilitated when directional selection plays a role, as in our experimental systems.

Deviation from Hardy-Weinberg expectations

To test for potential deviations from Hardy-Weinberg (H-W) proportions within demes, we used the lowest-level *F*-statistic F_{IS} (eqn (3.3)) averaged over 50 demes per migration series (10 demes x 5 replicate metapopulations). In figure 3.8 we present the observed F_{IS} for all experimental migration series (red and grey circles), for vial metapopulations without migration (C0, black circles), and for the (larger) bottle populations (CH, grey triangles). The F_{IS} -values are mostly negative, in particular for the smallest population size (red, grey and black circles). This is also the case for the F_{IS} predicted from standard individual-based simulations (red, grey and black lines). These results all indicate a surplus of heterozygotes as compared to the number of heterozygotes expected in a H-W population, which is larger as population size N is smaller (circles *versus* triangles). In a sex-differentiated population, a (small) excess of heterozygosity will be caused by random differences in allele frequencies between the female and male breeding populations (Falconer 1989, Rousset 2004), resulting in an expected deviation from H-W proportions:

$$F_{IS} = -\frac{1}{2N_e}. \qquad (3.7)$$

From equation (3.7), we can infer the predicted levels of deviation from H-W proportions (fig. 3.8, thin black lines) approximating $F_{IS} = -0.056$ for the vial metapopulations ($N_e = 9$) and $F_{IS} = -0.0056$ for the bottle populations ($N_e = 90$). Both the observed and the simulated F_{IS} are more negative on average than predicted by eqn (3.7). We have no ready explanation for this discrepancy.

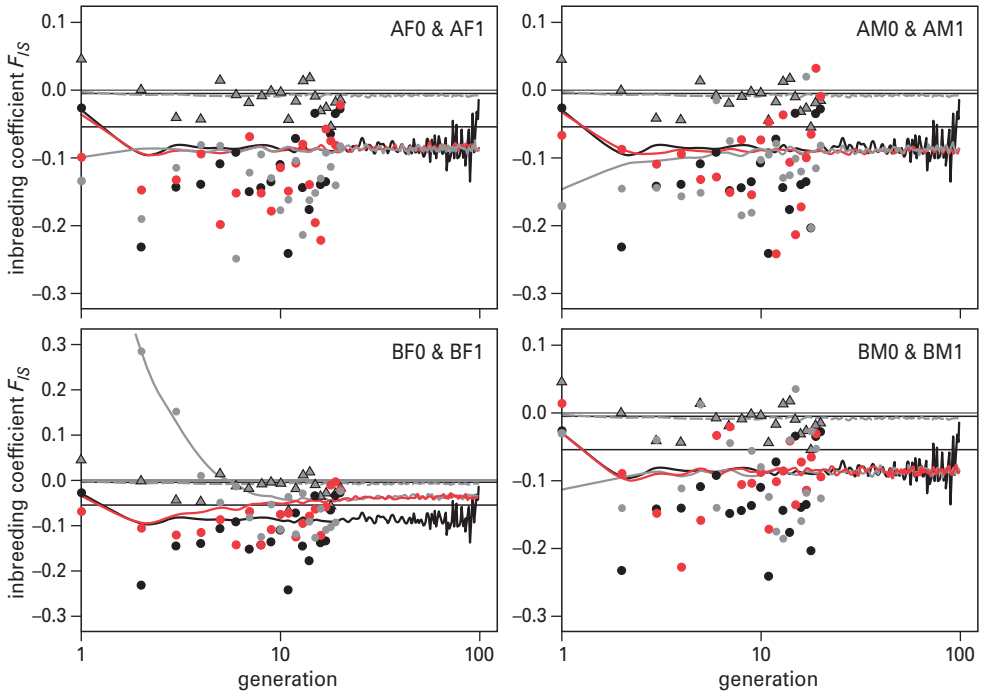


Figure 3.8. Observed and predicted deviation from Hardy-Weinberg proportions within *D. melanogaster* metapopulations with female (left) and male (right) migrants that mated after (top) or before (bottom) migration. The plots show experimental average inbreeding coefficients F_{IS} calculated from 50 demes (*i.e.*, five replicate metapopulations x 10 demes) with migration (red: initial $F_{ST} = 0$; grey: initial $F_{ST} = 1$) or without migration (black), and from five undivided populations of equivalent size as a metapopulation (triangles). The predicted F_{IS} (lines in corresponding colours, dashed for the triangles) are inferred from standard individual-based simulations. Thin black lines indicate the expected deviation from Hardy-Weinberg proportions for the vial ($F_{IS} = –0.056$) and bottle ($F_{IS} = –0.0056$) populations. Note the log-scale of the time-axis, and the different scale for mated female migrants (bottom left).

POPULATION FITNESS

Although we observed an excess of heterozygosity with regard to Hardy-Weinberg expectations (*i.e.*, given the allele frequencies within each generation), homozygosity will increase considerably over time because allele frequencies change due to genetic drift and the relatedness among individuals increases. As a result, the expression of recessive deleterious alleles will also increase leading to fitness reduction (*i.e.*, inbreeding depression, Thornhill 1993, Crnokrak & Roff 1999, Bijlsma *et al.* 2000, Hedrick & Kalinowski 2000, Reed *et al.* 2002), although the observed heterozygote excess may act as a buffer against the increased expression of deleterious alleles to some extent. To test for the possible occurrence of fitness reduction, we estimated the average fitness of each replicate vial population at the end of the experiment by measuring net fecundity, and compared the results of (i) the different migration scenarios (*i.e.*, the factors timing of

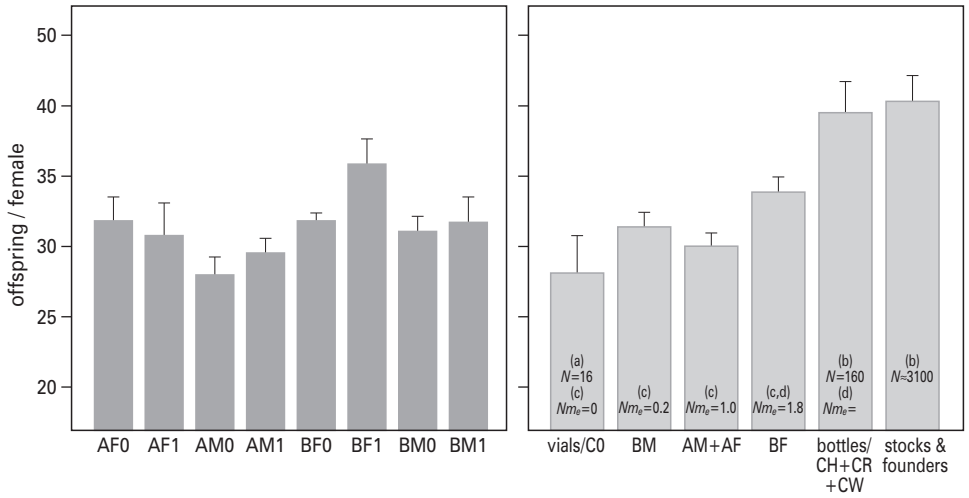


Figure 3.9. Effect of migration scenario, population size and level of gene flow on population fitness. Population fitness is measured as the average net fecundity (number of surviving offspring per female) in generation 20 per (meta)population. Left panel: Migration scenarios are combinations of three factors: timing of mating (A *vs* B), migrant sex (F *vs* M) and initial differentiation (0 *vs* 1). Right panel: Population sizes are $N = 16$, $N = 160$, and $N \approx 3100$. Gene flow is indicated by the effective number of migrants $Nm_e = 0$, $Nm_e = 0.2$, $Nm_e = 1.0$, $Nm_e = 1.8$, and $Nm_e = \infty$. Significant pair-wise differences (Tukey HSD) are indicated by (a), (b) for population size and by (c), (d) for level of gene flow. The bars represent standard errors.

mating, migrant sex, and initial differentiation), (ii) the different population sizes (*i.e.*, vial ($N = 16$), bottle ($N = 160$) and founder ($N \approx 3100$) populations), and (iii) the level of gene flow in (meta)populations of size $N = 160$ (*i.e.*, effective numbers of migrants of respectively 0, 0.2, 1.0, 1.8, and ∞).

In figure 3.9, we present the average net fecundities of the migration series (left) and of different population sizes and levels of gene flow (right). We tested the effect of the migration scenarios on population fitness simultaneously (three-way ANOVA), and we found significant effects of timing of mating and migrant sex, but not of initial differentiation (table 3.2). None of the interaction terms was significant, hence all three factors are likely independent.

The effects of population size and gene flow (single factor ANOVAs) on population fitness are both significant (table 3.2). In this test, the factor gene flow included the scenarios without gene flow and with unlimited gene flow, and the data from all A-series representing the same level of gene flow were combined. *Post hoc* pair-wise comparisons (Tukey HSD) indicate that the fitness in the small vial populations is significantly lower than in both types of larger populations (fig. 3.9 right panel, categories 1, 5 and 6). Gene flow (right panel, categories 1 to 5) can partly counteract such effects if the effective migration rate is sufficiently high, as in the case of the mated female migrants.

Table 3.2. Effect of migration scenario, population size and gene flow on population fitness. The migration scenarios were tested in a three-way ANOVA with fixed factors time of mating (M = after or before migration), migrant sex (S = female or male) and initial differentiation (I = zero or maximal). Since none of the interactions were significant in the full model, we used the restricted model instead. Population size (N = 16, N = 160 or $N \approx 3100$) and gene flow (effective number of migrants $Nm_e = 0$, $Nm_e = 0.2$, $Nm_e = 1.0$, $Nm_e = 1.8$, and $Nm_e = \infty$) were each tested in a one-way ANOVA. The dependant variable in all cases is the average net fecundity of a (meta)population.

Factor	*df*	*MS*	*F*	*P*
Time of mating (M)	1	65.280	5.81	0.0211
Migrant sex (S)	1	63.303	6.00	0.0193
Initial differentiation (I)	1	17.135	1.57	0.2178
error	36	10.889		
Population size	2	986.703	16.67	0.0000
error	67	59.208		
Gene flow	4	238.611	8.91	0.0000
error	55	26.796		

CONCLUSIONS AND IMPLICATIONS

VARIATION AMONG SINGLE METAPOPULATIONS

Despite our relatively simple and highly controlled experimental system, we found considerable variation between replicate metapopulations. Although this high variation will be partly a consequence of the logistic constraints (such as a single marker locus, small deme size and few replicates) of the experimental setup as discussed in CHAPTER 2, we expect even higher levels of variation in natural systems with large fluctuations in environmental and ecological parameters. Most probably, estimates of gene flow for such systems will be even less accurate than those inferred for our experimental metapopulations. We can illustrate this by comparing the ranges of variation in N_em of our experimental system from table 3.1 (0.08-1.55, 0.32-8.61 and 0.64-5.39 for low, intermediate and high gene flow, respectively) with the results from some recent studies that estimated values of N_em. Typical ranges of variation were 3-5, 0.23-0.50 and 0.03-0.80 for three species of sand hoppers (De Matthaeis *et al.* 2000), 1.53 to 5.88 for wild dogs (Girman *et al.* 2001), 2.9 to 30.2 for cougars (Anderson *et al.* 2004) and 3.34 to 9.03 for sea turtles (Roberts *et al.* 2004). In many cases, the range of variation between different (sub)populations considerably overlaps the range of variation among replicates predicted by our experiments. Hence, implied differences in gene flow based on such estimates might be way off target. In line with earlier studies (Whitlock & McCauley 1999, Neigel 2002) we conclude that recommendations for nature management based on such estimates must be used with great care and wide safety margins.

DEVIATIONS FROM STANDARD MODEL ASSUMPTIONS

Not surprisingly, we found a considerable effect of migrant sex on the migration rate m in populations where mating occurred before migration. When migrants mate before migration and are predominantly female, the effective migration rate $m_{e,f}$ is approximately twice as high as the expected rate m. In contrast, $m_{e,m}$ might be close to zero when migrants are predominantly male and arrive in target demes late in the mating season. Although these differences are rather obvious, common estimates of $N_e m$ do often not consider such sex differences in migration (but see Berg *et al.* 1998, Vitalis 2002). The possibility of sex-biased migration underlines the importance of a correct interpretation of the one-migrant-per-generation rule of thumb that is widely used in conservation biology (Mills & Allendorf 1996, Wang 2004).

The unidirectional stepping-stone model of migration in our experimental series represents one end of a range of migration models with decreasing spatial structure. The island model that is basically independent of spatial structure represents the opposite end, and most natural populations will fall in between these extremes (Slatkin 1985). By using the extreme as point of reference we intended to infer the maximum level of discrepancy that can be expected when estimates of $N_e m$ are calculated in a standard way according to the island model. Our analytical and simulation-based analyses of stepping-stone migration predict equilibrium values that are a factor two higher than predictions based on the island model. Among the estimated values of our experimental metapopulations, we found wider error margins than the predicted confidence intervals. Both findings illustrate that such discrepancies between the natural system and the underlying model might easily lead to incorrect estimates of demographic parameters.

The results of this study suggest two important reasons to demonstrate caution when using estimates of $N_e m$ in empirical studies. First, regardless of the average correspondence of our experimental results with theoretical predictions, the variation among single replicates was large. Since replicate metapopulations are generally not available in empirical studies, any estimate of $N_e m$ based on a single replicate will fall into a corresponding wide confidence range, and thus have limited accuracy. Second, in addition to the intentional differences in effective migration rate and migration model, our metapopulations did never attain drift-migration equilibrium and our eye colour marker system was subject to selection favouring one of the marker alleles. This illustrates that unintentional discrepancies of model assumptions can easily occur despite a relatively simple and highly controlled experimental setup. Both the known and the unknown factors have likely added to the estimates way off the target in table 3.1. Since natural systems are much more complex, many other (unknown) factors can be expected to play additional roles, and to confuse estimates of $N_e m$ even more.

ACKNOWLEDGEMENTS We thank M.H. Edelenbos, A.C. Boerema and L. Hoeksema for their technical assistance. This project was financially supported by the Netherlands Foundation for the Advancement of Tropical Research (WOTRO), as part of the Priority Program "Biodiversity in Disturbed Ecosystems" of the Netherlands Organization for Scientific Research (NWO).

APPENDIX: MIGRATION MODELS

In this appendix, we first summarize the relationship between de autozygosity F (*i.e.*, the probability that two alleles within one individual are identical by descent) and the fixation index F_{ST} for the infinite "continent-island" model (*cf.* Wright's (1931) classical island model) and for the finite "n-island" model (Slatkin 1977) that is more appropriate if the number of demes in a metapopulation is small.

Next, we derive a set of recurrence equations that predicts the autozygosity in a stepping-stone model of migration, and we relate the autozygosity to the fixation index for this model in a similar way.

CONTINENT-ISLAND MODEL

Based on coalescent theory, the autozygosity F (*i.e.*, the probability that two alleles within one individual are identical by descent) of a diploid Wright-Fisher population of size N and with mutation rate μ (Malécot 1948) changes according to the recurrence equation:

$$F_{t+1} = (1-\mu)^2\left[\frac{1}{2N} + \left(1-\frac{1}{2N}\right)F_t\right] \tag{3A.1}$$

Under the assumption that individuals in different demes are unrelated (Rousset 2004), the autozygosity F for the continent-island model with migration rate m is given by:

$$F_{t+1} = (1-\mu)^2\,(1-m)^2\left[\frac{1}{2N} + \left(1-\frac{1}{2N}\right)F_t\right] \tag{3A.2}$$

At equilibrium,

$$\hat{F} = \frac{(1-\mu)^2\,(1-m)^2}{(1-\mu)^2\,(1-m)^2 + 2N\left[1-(1-\mu)^2\,(1-m)^2\right]} \approx \frac{1}{1+4N(\mu+m)} \tag{3A.3}$$

where the approximation applies when μ and m are small. With $\mu << m$, the autozygosity F equals the fixation index F_{ST}.

N-ISLAND MODEL WITH MIGRANT-POOL MIGRATION

Following Slatkin (1977), we consider a metapopulation with n demes of size N and an equal migration rate m between all demes. We assume that migrants from all demes assemble into a migrant pool, and redistribute themselves at random over all demes including the source deme. The autozygosity F_i in respectively the focal deme ($i = 0$) and any other deme ($i = 1$) is given by two recurrence equations:

$$F_0' = a\left[\frac{1}{2N} + \left(1 - \frac{1}{2N}\right) F_0\right] + (1-a)F_1 \quad \text{(3A.4.a)}$$

$$F_1' = b\left[\frac{1}{2N} + \left(1 - \frac{1}{2N}\right) F_0\right] + (1-b)F_1 \quad \text{(3A.4.b)}$$

where F_i' and F_i are the autozygosities of deme i in generation (t +1) and generation t, respectively. The parameters a and b are defined as follows: a is the sum of probabilities that two alleles sampled from the same deme originate from two residents, one resident and one migrant, or two migrants, respectively (Slatkin 1977, eqn (11)):

$$a = (1-m)^2 + \frac{1}{n}\, 2m(1-m) + \frac{1}{n}\, m^2 \quad \text{(3A.5)}$$

and b is the sum of probabilities that two alleles sampled from two different demes originate from either two migrants from the same source deme, or from one resident and one migrant from the same deme (Slatkin 1977, eqn (12); Pannell & Charlesworth 1999, eqn (A3)):

$$b = \frac{1}{n}\, m^2 + \frac{1}{n}\, 2m(1-m) \quad \text{(3A.6)}$$

F_{ST} in the n-island model can be predicted based on the relation between the probabilities of identity (either by descent or by state) within demes F_w and between demes F_b (Rousset 2004):

$$F_{ST} = \frac{F_w - F_b}{1 - F_b} \quad \text{(3A.7)}$$

In this model F_w corresponds to F_0 and F_b to F_1.

ONE-DIMENSIONAL STEPPING-STONE MODEL WITH ASYMMETRIC MIGRATION

We consider a metapopulation with n demes of size N each, and migration rate $m = m_R + m_L$ where $m_R = rm$ is the probability of migration in one direction ("to the right") while $m_L = (1-r)m$ is the probability of migration in the other direction ("to the left"). Thus, an individual in deme i may either originate from i, or from any of the other demes [i+1…….i+k], [i–1…….i–k] ($k < n$) at any time t. The autozygosity $F_{i,j}$ indicates the probability that two alleles drawn from deme i and deme j are identical by descent. If we assume that all autozygosities $F_{i,j}$ for any combination of demes that are k steps apart are equal, we can define $F_k = F_{i+k,i} = F_{i-k,i}$. Hence, F_0 indicates the autozygosity for two alleles coming from the same deme (k = 0), *i.e.*, the focal deme. If we further define $a = (1-m)^2 + m_R^2 + m_L^2$ (*i.e.*, the probability that both alleles are either from a resident, or

from a migrant), and $b = m(1 - m)$ (*i.e.*, the probability that one allele is from a resident and one allele is from a migrant), and $c = m_R m_L$ (*i.e.*, the probability that one allele is from a migrant in one direction, and the second allele is from a migrant in the other direction), we can infer the autozygosities in the next generation F'_k from a set of recurrence equations that describe all possible contributions of autozygous alleles from demes that are $k = 0$ to $n - 1$ steps away from the focal deme:

$$F'_0 = a\left[\frac{1}{2N} + \left(1 - \frac{1}{2N}\right) F_0\right] + 2bF_1 + 2cF_2\,, \tag{3A.8.a}$$

$$F'_1 = aF_1 + b\left[\frac{1}{2N} + \left(1 - \frac{1}{2N}\right) F_0\right] + bF_2 + c(F_1 + F_3), \tag{3A.8.b}$$

$$F'_2 = aF_2 + b\,(F_1 + F_3) + c\left[\frac{1}{2N} + \left(1 - \frac{1}{2N}\right) F_0\right] + cF_4\,, \tag{3A.8.c}$$

$$F'_k = aF_k + b\,(F_{k+1} + F_{k-1}) + c\,(F_{k+2} + F_{k-2}). \tag{3A.8.d}$$

The first term in equation (3A.8.a) is the probability that two alleles drawn from the focal deme are identical by descent, the second term is the probability that one allele drawn from the focal deme and one allele drawn from an adjacent deme are identical by descent, and the third term is the probability that two alleles drawn from one of two possible adjacent demes each are identical by descent. The other equations are derived similarly.

We use (3A.7) to predict the change in F_{ST} for the stepping-stone models as well, but here we use the autozygosity F_0 within the focal deme to represent F_w and the average autozygosity between demes that are one or more steps apart to represent F_b:

$$F_b = \frac{1}{n-1} \sum_{i-1}^{n-1} F_i\,. \tag{3A.9}$$

In figure 3A.1 we compare the dynamics of autozygosity F (red) and of genetic differentiation F_{ST} (black) for parameters based on our experimental setup ($n = 10$, $N = 16$, $m = 1/N$) over 10,000 generations. The top left panel shows the predictions for the continent-island (open circles) and n-island (solid circles) models, and the top right panel shows the predictions for the bidirectional ($r = 0.5$, solid circles) and unidirectional ($r = 1$, open circles) stepping-stone models. For the stepping-stone models, the two initial situations are simulated by setting all F_k to zero to start without differentiation, and by setting $F_k = 1$ for $k = 0,2,4,6,8$ and $F_k = -1$ for $k = 1,3,5,7,9$ to start with maximal differentiation. According to expectation, the autozygosity F approaches unity in the n-island model

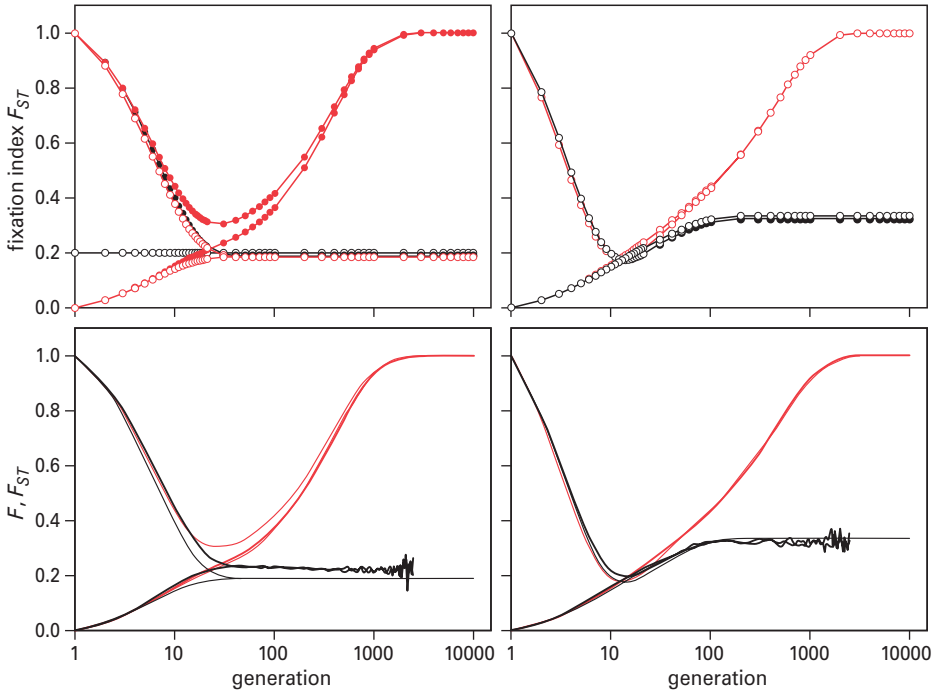


Figure 3A.1. Dynamics of autozygosity F (red) and genetic differentiation F_{ST} (black) in a metapopulation predicted by analytical models and individual-based simulations (see text for details). Top panels: analytical predictions for the continent-island (open circles) and n-island (solid circles) model (left), and for the bidirectional (solid circles) and unidirectional (open circles) stepping-stone model (right). Bottom panels: simulation results (thick lines) and analytical predictions (thin lines) for the n-island model (left) and the unidirectional stepping-stone model (right). The small discrepancy between the simulations and the analytical predictions stems from the difference between the analytical model that assumes cosexual individuals and allows for selfing, and the simulation model that is based on separate sexes. Note that F_{ST} is no longer defined if alleles become fixed within the metapopulation, as indicated by the fluctuations and premature ending of the simulated curves. Note the log-scale of the time-axis.

and both stepping-stone models, whereas it converges to the equilibrium predicted by (3A.3) in the continent-island model. A finite number of demes in combination with the absence of gene flow originating outside the focal system (*i.e,*. long-distance migration or mutation) will eventually lead to total relatedness of all individuals in the metapopulation, and thus to an equilibrium of unity. The results are consistent with predictions from earlier analytical studies (Kimura & Weiss 1964, Maruyama 1970) of finite systems without either long-distance migration or mutation. These conditions are met in our experimental system, since we excluded long-distance migration on purpose, and we look at relatively few generations so that the probability of mutation is negligible. The predicted equilibrium levels of $F_{ST} = 0.32$ for bidirectional (black, solid) and $F_{ST} = 0.34$ for unidirectional (black, open) stepping-stone migration (top right) are considerably

higher than for both island models (top left). The equilibrium F_{ST} = 0.19 for the *n*-island model (black, solid) based on (3A.7) closely approaches the level of F_{ST} = 0.20 for the continent-island model (black, open).

The results of long-term simulations (fig. 3A.1 bottom panels, thick lines) are in line with the analytical predictions (thin lines) for both models. Given the same set of parameters, the approximation of F_{ST} using (3A.7) and (3A.9) (right) yields more accurate predictions for the stepping-stone model than the commonly used approximation (3A.3) based on the continent-island model (left) would have done.

CHAPTER 4

Genetic differentiation in experimental *Drosophila melanogaster* metapopulations III - Local extinction and recolonization

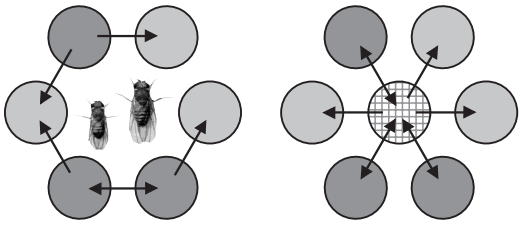
with F. J. WEISSING and R. BIJLSMA

ABSTRACT

In contrast with the large body of theory predicting the dynamics of genetic variation and differentiation in idealized metapopulations, experimental studies aiming to validate these models are mostly limited to unstructured populations. We evaluated experimental *Drosophila melanogaster* metapopulations of increasing complexity by varying the levels and patterns of gene flow. The present study focuses on the consequences of population turnover, *i.e.*, local extinction and recolonization of demes. We examine the difference between migrant-pool and stepping-stone migration, the robustness of results based on replication and the consequences for population fitness and tolerance to external stress factors.

Population turnover increased stochasticity at the metapopulation level, resulting in a more rapid loss of diversity and higher levels of differentiation than without local extinction. Therefore, effective metapopulation sizes decreased much faster in the presence than in the absence of population turnover. Despite striving for constant environmental conditions, migration and colonization rates fluctuated considerably between generations, resulting in high to very high variation among replicates when population turnover occurred regularly. The migration configurations showed several differences resulting mainly from the higher cost of migrant-pool migration, although none were significant due to the high variation among replicates. Assessments of population fitness and stress tolerance both showed substantial interdemic variation, indicating that the distribution of genetic variation in a metapopulation can become very uneven when population turnover occurs regularly.

Although replicated experimental metapopulations provide an excellent means to validate theory because they allow standardization, our study also points out some limitations. Logistic constraints (one marker locus, few demes, few replicates) substantially increase the already considerable variation due to population turnover. Relaxing experimental standardization even a little may result in large deviations from theoretical predictions, which will undoubtedly be larger yet for complex natural systems.



INTRODUCTION

In conservation genetic studies, both the genetic diversity within and the genetic differentiation among demes in a metapopulation are often used to assess its prospects of survival, and to infer recommendations for its future management (*e.g.*, Keyghobadi *et al.* 2005). Local extinction and subsequent recolonization events correspond to genetic bottlenecks for single demes because colonists may be few in number, and may be closely related. Homozygosity may increase rapidly in such newly colonized demes, which will potentially lead to a fitness reduction due to the expression of recessive deleterious alleles (Saccheri *et al.* 1998, Crnokrak & Roff 1999, Bijlsma *et al.* 2000, Hedrick & Kalinowski 2000, Keller & Waller 2002, Reed *et al.* 2003b, Armbruster & Reed 2005, Reed 2005), although increased homozygosity may also stimulate the purging of (highly) deleterious alleles in small populations (Wang *et al.* 1999, Miller & Hedrick 2001, Crnokrak & Barrett 2002, Glemin 2003). Moreover, genetic diversity may be lost due to genetic drift, which may in turn affect the potential of a local population to adapt to changing environmental conditions (Montgomery *et al.* 2000, England *et al.* 2003).

In the last three decades, much theory on the genetic consequences of population fragmentation has been developed. Slatkin (1977) presented the first theoretical study analyzing the effects of local extinction and colonization on the autozygosity (*i.e.*, the probability that two alleles chosen at random within a population are identical by descent) in a metapopulation with gene flow occurring in line with Wright's (1951) classical island model of migration. Slatkin's study introduces two types of island model: the "continent-island" model (model I) assuming that all migrants originate from an infinite source population outside the focal metapopulation of n finite demes, and the "finite-island" model (model II) assuming that all migrants originate from within the metapopulation. A further distinction is made between two models of colonization: the "propagule-pool" model where all colonists founding a particular population originate from a single source and the "migrant-pool" model where the colonists founding a particular population represent a random selection from all migrating individuals in the metapopulation. Neither model includes local demography, but assumes that the constant, maximum deme size N is achieved immediately after colonization (Slatkin 1977). Wade & McCauley (1988) reformulated Slatkin's results by equating autozygosity to F_{ST} (*i.e.*, the standardized variance of allele frequencies) in a system receiving genetically unrelated migrants (Rousset 2004, see also the appendix in CHAPTER 3). They showed that the equilibrium value $\hat{F}_{ST}$ in both models I and II tends to become very similar if the number of demes n is large ($n > 20$). For both models, differentiation increases with extinction rate e under the propagule-pool model of colonization. Under the migrant-pool model, however, differentiation may either increase or decrease depending on the number of colonists k relative to the number of migrants Nm. F_{ST} tends to increase with e when $k < 2Nm$, but to decrease when $k > 2Nm$. These findings were later generalized in a series of theoretical contributions (*e.g.*, by considering various patterns of genetic structure in the group of colonists, Whitlock & McCauley 1990, Pannell & Charlesworth 1999). While theory is fairly well developed for the

various island models of migration, there are relatively few studies on the consequences of stepping-stone migration. Moreover, these studies either exclude local extinction (Maruyama 1970a, b) or migration among extant demes (Maruyama & Kimura 1980).

In contrast to the large body of theory, the number of experimental studies aiming at validating the theoretical models is limited. Recent studies include *e.g.*, the long-term observation of genetic structure in relatively simple natural metapopulations with population turnover (Haag *et al.* 2002, 2005, 2006), or the investigation of the consequences of genetic bottlenecks in a laboratory setting (Reed *et al.* 2002, 2003a). However, a common disadvantage of natural populations is the limited possibility for standardization and replication, whereas the use of experimental populations not embedded in a metapopulation context may not allow for extrapolation to natural metapopulations. Our study aims to extend the replicated experimental approach to a metapopulation context.

We investigate the consequences of population fragmentation and population turnover, with population turnover defined as the local extinction of demes followed by recolonization from the remaining extant demes in either the same or subsequent generations. We monitor the changes of genetic diversity and differentiation based on an eye colour polymorphism in standardized and replicated laboratory *Drosophila* metapopulations, and we compare our findings with expectations based on theoretical models. These experiments closely match and complement our earlier studies focusing on the effects of genetic drift in small isolated populations (CHAPTER 2) and migration between such populations (CHAPTER 3). These studies provided baseline values for metapopulations with constant deme sizes and migration rates in the absence of population turnover. In the current study we allow deme sizes and migration rates to fluctuate, and we include pre-scheduled local extinction events in half of the replicate metapopulations.

To place our experimental results in a more general perspective, we perform individual-based computer simulations that mimic the setup of our experiments. The simulations allow us to predict average patterns of diversity and differentiation and the corresponding levels of variation based on many "*in silico*" replicates, whereas the number of experimental replicates is limited by logistic and time constraints.

We focus on the effect of population turnover on the patterns of genetic diversity and differentiation, addressing three aspects in particular: (i) the effect of spatial configuration (*i.e.*, migration following either a migrant-pool or a stepping-stone pattern), (ii) the consistency of results based on replicate metapopulations, and (iii) the consequences of fragmentation and population turnover for population fitness and tolerance to external stress factors.

Migrant-pool migration where migrants spread globally over the entire metapopulation is generally expected to counteract genetic drift more efficiently than stepping-stone migration where migrants can only make steps of one deme per generation. Hence, we expect that genetic differentiation among demes will happen more slowly in case of migrant-pool migration. In the presence of local extinction, theory predicts that the emerging pattern of genetic differentiation will strongly reflect the relative magnitudes of extinction and migration rates, the number of colonists relative to typical deme sizes,

and the origin of the colonists (Hedrick & Gilpin 1997). All these processes are stochastic in nature, which will likely induce relatively much variation between metapopulations. Local extinction at a high rate may result in many demes being extinct at the same time, which might in turn cause a genetic bottleneck at the metapopulation level. Genetic bottlenecks and a subsequent decrease of the effective metapopulation size might affect population fitness negatively through inbreeding depression and the loss of allelic diversity due to genetic drift, although purging of deleterious alleles may mitigate the decrease of fitness to some extent (Wang & Caballero 1999, Rousset 2003, Gaggiotti & Hanski 2004). Since the effects of purging can be already ambiguous in case of undivided small populations, it is unclear what we might expect in a metapopulation context.

MATERIAL AND METHODS

DROSOPHILA STOCKS

We used the same mutant *Drosophila melanogaster* lines as in our previous study (for a detailed descriptions of these stocks, see CHAPTER 2). The alleles *bw* and bw^{75} at the *brown* locus (II-104.5) in combination with the mutation *scarlet* (III-44) in homozygous condition result in distinct eye colours for the three genotypes at the *bw* locus. Homozygous *bw*//*bw* individuals have white eyes, homozygous bw^{75}//bw^{75} individuals have red-brown eyes, and heterozygous bw^{75}//*bw* individuals have intermediate orange eyes at 25°C. All fly stocks are raised in 125 ml bottles on 30 ml of standard medium (26 g dead yeast, 54 g sugar, 17 g agar, and 13 ml nipagine solution (10 g nipagine in 100 ml 96% alcohol) per liter) with antibiotics (250 mg streptomycin per liter), and under standard conditions at 25°C, 40-60% RH and 24 hours of light. In the experiments, small fly populations were raised on 18 ml of standard medium without antibiotics in 40 ml glass vials under standard conditions. We anaesthetized the flies with CO_2 before handling.

EXPERIMENTAL SETUP

The experimental setup included two different spatial configurations of fragmented populations (metapopulations), each consisting of six demes represented by compartments connected through two plastic 4 mm Ø tubes that could be closed or opened with a small tap (fig. 4.1A). Each compartment held a 40 ml glass vial with 18 ml medium and about 50 flies. The spatial configurations enabled migration according to either an equidistant *n*-island setup, or a circular bidirectional stepping-stone setup. In the *n*-island setup (fig. 4.1B) migrants originating from many small demes ("islands") congregate in a migrant pool and then distribute with equal probabilities to arrive at any deme including their own source population. Hence, in the experimental *n*-island configuration all six demes are connected to a seventh, central compartment ("migrant pool") that all migrants have to pass through before they can move into another deme. The central compartment contained a vial with medium to attract flies in a similar way as populated demes, but it was closed at the top with nylon wire mesh to avoid actual colonization. In

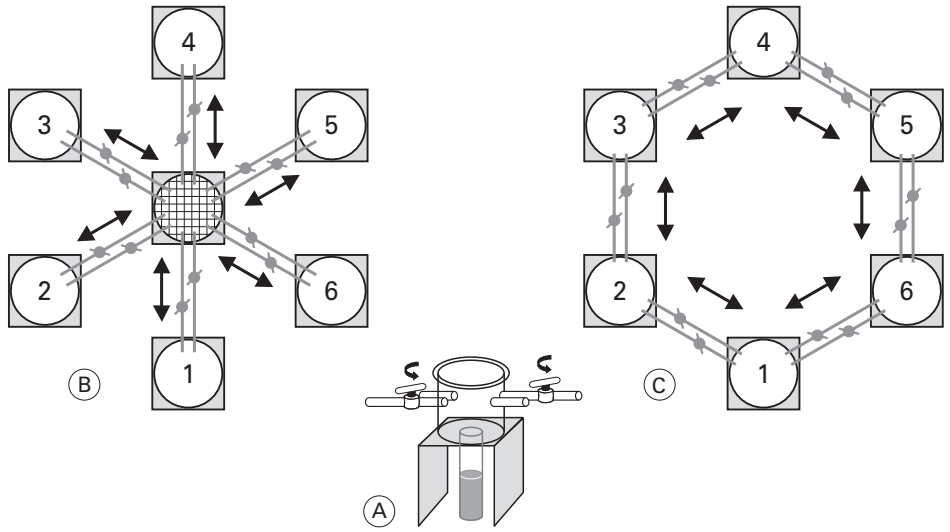


Figure 4.1. Setup of experimental *Drosophila melanogaster* metapopulations in two spatial configurations. A metapopulation consists of six demes represented by compartments (A) connected through tubes that can be opened or closed for migration by taps. Each deme is either connected to all other demes via a central compartment (B) representing a migrant pool, or connected directly to two adjacent demes (C) in a circular stepping-stone pattern. A compartment is populated with a vial containing medium and a fly population, whereas the central compartment in configuration (B) holds a vial containing only medium that is closed on top by a piece of nylon wire mesh. With the taps open, migrating flies can move to and fro through the tubes as indicated by the arrows.

the bidirectional stepping-stone setup (fig. 4.1C) migrants have a large probability to arrive in either one of two adjacent demes, and much smaller probabilities to end up in demes that are more than one step away. In the corresponding stepping-stone configuration the demes are connected to each other on two sides in a circular pattern, so that migrants can both leave and enter in two directions. Due to the presence of the central compartment in the migrant-pool configuration the physical distance between two demes is twice as long as in the stepping-stone configuration and includes an enforced stop in the middle. Hence, the migration rate in the stepping-stone configuration might be higher than in the migrant-pool configuration.

We used six replicate metapopulations for each spatial configuration, and 12 undivided control populations without gene flow. One metapopulation (= replicate) consisted of six demes (= 40 ml glass vials with 18 ml medium) with on average 50 founding individuals per deme in each generation. Ideally, the unfragmented control populations (= 125 ml standard bottles) should have been initiated with 6 x 50 = 300 individuals, but due to experimental limitations they comprised 210 founding individuals per generation on average. Note that the average numbers are harmonic means because the census sizes fluctuated over generations. For each spatial configuration we looked into the effects of local extinction events and subsequent colonizations through

migration. Hence, in three of six replicate metapopulations local extinction of one or more demes occurred according to a pre-defined schedule (see next section), and three metapopulations were used as control populations with migration but without extinction. Thus, this setup resulted in four different experimental series with three replicates each: (i) migration in a migrant-pool configuration with local extinction (replicates MPX1, MPX2 and MPX3), (ii) migration in a migrant-pool configuration without extinction (replicates MP1, MP2 and MP3), (iii) migration in a stepping-stone configuration with local extinction (replicates SSX1, SSX2 and SSX3), and (iv) migration in a stepping-stone configuration without extinction (replicates SS1, SS2 and SS3). The local extinction events for each replicate metapopulation in the extinction series MPX and SSX were assigned *a priori* by randomly allotting extinction events to individual demes with probability $e = 0.1$ per deme for each generation. Note that the three metapopulations within each series are not exact replicates, because the predefined extinction schedules were different for each metapopulation.

EXPERIMENTAL PROCEDURE

All experimental series started without any initial genetic differentiation ($F_{ST} = 0$) with 100% heterozygous orange-eyed individuals collected from the mixed F1 of $bw^{75}//bw^{75}$ x $bw//bw$ parents and the reciprocal crossing $bw//bw$ x $bw^{75}//bw^{75}$. The flies were reared in glass vials that were placed in the matching compartments of the corresponding migration appliances (fig. 4.1) on day 14 of each generation to enable migration. Depending on the activity of the flies that fluctuated considerably from generation to generation, the taps were opened for 12-24 hours aiming at one migrant per deme on average (the duration interval was estimated from different levels of fly activity in previous migration tests for both appliances, data not shown). Since we only controlled duration but no other aspects of migration, migrants might be either females or males, and the majority had likely mated before migration. During migration we placed each appliance under its own circular lamp (Philips Fluotone TL-E Pro 32W/840, dimmed to 45% of its maximum intensity) in a climate room where central lights were turned off to avoid any bias from phototaxis in migration behaviour (Shorrocks 1972). We monitored allele frequencies and heterozygosities (after migration), and the occurrence of extinction and colonization events over 40 generations for each replicate.

Generations did not overlap and took 14 days to develop. One experimental generation consisted of the following six stages. (i) Several hundreds of eggs were laid per vial, and to avoid severe crowding we limited this amount in two successive stages. We cut the layer of food with newly-laid eggs into four pieces and divided these over four new vials. This procedure provided us with one new experimental population and three emergency backup populations. (ii) After pupation we removed any excess offspring, so that about 50 pupae per vial were left. (iii) After eclosion we allowed the flies a few days for maturing and mating, and then we placed the vials in the corresponding appliances and opened the taps for migration. (iv) Extinction happened at this stage just before migration: offspring in a deme destined to go extinct in generation t were scored for that generation, but were not allowed to migrate any more. Instead, we placed vials with

medium of the same quality as in the populated vials but without flies in all empty patches in the appliance to allow for colonization. Thus, extinction and colonization may occur in the same generation. (v) After the allotted migration period (see above) we closed the taps, and we removed all flies from the compartments and transferred them to fresh vials to start the next generation. In this stage we scored the number, sex and genotypes of colonists for all empty patches, and also of any flies that were left in the central compartment of the migrant-pool configuration (fig. 4.1B) after migration. Since these flies were discarded from the experiment, they represent a cost of migration in the migrant-pool configuration. (vi) After egg-laying for 2-3 days all parental flies were frozen and stored to score genotype frequencies at a later time.

ASSESSMENT OF REALIZED MIGRATION RATES

Based on pilot migration tests in both appliances we expected that on average one individual per deme would migrate, corresponding to a migration rate $m = 1/N = 0.02$ for an average deme size $N = 50$. Although we did not monitor the migration process exactly we can estimate the realized migration rates in both appliances retrospectively from the numbers of colonists M_C in n_E empty demes that we did monitor exactly for all metapopulations with local extinction. Most theoretical models do not distinguish between immigration (*i.e.*, the average number of individuals moving into a new deme per generation) and emigration (*i.e.*, the average number of individuals leaving a source deme per generation) because both quantities are equal if all demes are occupied each generation. In our experimental systems, however, extinct demes tended to stay empty for several generations resulting in fewer occupied demes n_O on average than the total number of $n = 6$ demes. Hence, we expect that the emigration rate m_E will be higher than the immigration rate m_I in most cases because fewer demes are available for emigration than for immigration (*i.e.*, $n_O m_E = 6m_I$). To estimate m_I and m_E, we assume that the probability to enter an empty deme is equal to the probability to enter an occupied deme. The average number of colonists per empty deme, $m_C \,/\, n_E$, can then be used to estimate the average number of immigrants per deme $Nm_I = m_C \,/\, n_E$, and to infer the average number of emigrants per deme as $Nm_E = 6M_C \,/\, n_O m_E$.

In the migrant-pool configuration several migrants M_E were usually still in the central compartment when we closed the taps. Hence, the average number of emigrants per deme is given by $Nm_E = (6M_C \,/\, n_E + M_E) \,/\, n_O$. The difference between both expressions of Nm_E indicates the "cost of migration" in the migrant-pool configuration.

ASSESSMENT OF FITNESS AND STRESS TOLERANCE

In generation 35, we evaluated the average fitness and the tolerance to external stress factors of each metapopulation. To infer the average fitness of a metapopulation we measured the net fecundity of females and the egg-to-adult viability under standard conditions. We calculated the net fecundity (*i.e.*, the average number of viable offspring per female) from the number of offspring of five breeding pairs raised under standard conditions in one 23 ml plastic vial on 9 ml of standard medium with antibiotics (100 mg ampicillin per liter). We collected the breeding pairs as virgins and allowed them to mate

and lay eggs for 15 days with transfers to a fresh vial every third day, resulting in five consecutive series of offspring. We counted the total number of offspring in all five series and translated these numbers into an average value per female that was corrected for females escaped during transfer, but not for dead ones. Thus, the net fecundity measure corresponds to the product of female fecundity (= number of eggs laid), offspring viability (= egg-to-adult survival) and adult female survival, and assumes no limiting effects of male fitness traits. In this way, we estimated the net fecundity of a deme as the average of five samples per deme (*i.e.*, 6 x 5 = 30 samples per metapopulation), and the net fecundity of a bottle control population as the average of 30 samples per bottle.

We estimated egg-to-adult viability as the fraction of adult individuals eclosing from 300 eggs that were randomly sampled and were raised either under standard conditions or under stress conditions. We kept females for 4-6 hours in a small container placed upside down with a thin layer of medium on the lid and some fresh yeast to stimulate egg-laying. After removal of the females we carefully picked the eggs from the medium on the lids and placed them in 23 ml plastic vials on 9 ml of the required medium. To avoid crowding we used six vials with 50 eggs per vial for each sample of 300 eggs. We inferred the viability per deme for all metapopulations from samples of 300 eggs per deme, and the viabilities of all bottle control populations from a sample of 300 eggs per bottle.

To assess the stress tolerance in a metapopulation we measured the viability for two environmental stress factors (high temperature, high ethanol concentration in the food medium) and the resistance to starvation of males. We determined the viability of eggs raised on standard medium at 29°C, 40-60% RH and 24 hours of light to assess the effect of high temperature, and the viability of eggs raised on medium with ethanol added in a concentration of 12.5% at standard conditions to assess the effect of high ethanol concentrations. For comparison between treatments, we used stress tolerance $T_S = V_S / V_C$, *i.e.*, the egg-to-adult viability V_S under stress conditions relative to the viability V_C under control conditions.

We estimated starvation resistance from the LT50 (*i.e.*, the median time to death of the tested individuals) of 10 newly eclosed virgin males kept in vials containing 9 ml agar medium only. We checked the vials daily until the first death occurred, and from that moment we scored the numbers of dead males three times per twenty-four hours until all flies had died, using the midpoint of the 8-hour interval where death occurred as an estimate of the time of death. We determined the LT50 of five replicates for each deme in a metapopulation and for each bottle control population. Because we did not have enough flies in all cases to start all five replicates of a deme/bottle at the same time, we used the arithmetic mean of the five replicates to estimate the LT50 of the deme/bottle rather than determining the overall LT50 of 5 x 10 = 50 males.

F-STATISTICS AND EFFECTIVE METAPOPULATION SIZE

For each generation we used the genotype frequencies per deme to infer the allele frequencies and the expected heterozygosity H_S. We then used these heterozygosities to calculate the fixation index $F_{ST} = (H_T - \bar{H}_S)/H_T$ for each metapopulation, where H_T is

the expected heterozygosity of the metapopulation calculated from the average allele frequencies, and $\bar{H}_S$ is the average expected heterozygosity of a deme (Hartl & Clark 1997). We also calculated a higher level, global fixation index $F_{TG} = (H_G - \bar{H}_T)/H_G$ for each series of three replicate metapopulations, with H_G the expected heterozygosity of the cluster of metapopulations, and $\bar{H}_T$ the average expected heterozygosity of a single metapopulation. The index F_{TG} represents the differentiation among metapopulations, *i.e.*, the variation among replicates.

To view our experimental results in light of the general theoretical models, we compared the observed F_{ST} with theoretically predicted equilibrium values, assuming that F_{ST} approximates its equilibrium value in the midrange of the experiment (*i.e.*, generations 15 to 25). Whitlock and McCauley (1990) showed that for a continent-island (model I) metapopulation in equilibrium and with an infinite number of demes, $\hat{F}_{ST}$ can be approximated by:

$$\hat{F}_{ST} = \frac{1 + Ne/k}{1 + 4Nm + 2Ne[1 - \varphi(1 - 1/2k)]} \quad , \tag{4.1}$$

where N is deme size, m is the migration rate, e is the extinction rate, k is the number of colonists, and φ is the probability of common origin, *i.e.*, the probability that two randomly chosen alleles in a group of colonists originate from the same source. In a later study, Pannell and Charlesworth (1999) showed that this approximation also holds for a finite-island (model II) metapopulation with migrant-pool colonization ($\varphi = 0$) when the number of demes n is sufficiently large and e is of the same magnitude as m and k/N. We will discuss the consequences of a small number of demes for this approximation later. In the absence of extinction ($e = 0$), eqn (4.1) reduces to Wright's (1951) well-known approximation $\hat{F}_{ST} = 1/(1 + 4Nm)$.

The effective size of a metapopulation is often used as a measure of the loss of genetic variation. We use the observed F_{ST} to infer the effective size of the experimental metapopulations. The effective metapopulation size is defined as the size of an idealized, undivided Wright-Fisher population that would show the same dynamics of variation in allele frequency changes as the actual metapopulation (reviewed in Wang & Caballero 1999). Whitlock and Barton (1997) proposed a general expression for the effective metapopulation size N_e^M:

$$N_e^M = \frac{nN}{1 - F_{ST} + V[1 + F_{ST}(2N - 1)n/(n - 1)]} \quad , \tag{4.2}$$

where n is the number of demes and V represents the variance in reproductive success among demes. When all demes contribute equally to the next generation through migration, this variance is zero, and eqn (4.2) reduces to $N_e^M = nN/(1 - F_{ST})$. According to Whitlock and Barton (1997), the variance in reproductive success among demes is $V = e/(1 - e)$ for a metapopulation with local extinction at rate e and subsequent recolonization within the same generation.

When applying the above equations, we replace deme size N by the effective deme size $N_e \approx 0.56N$ to compensate for the lottery polygyny mating system of *Drosophila melanogaster* (CHAPTER 2), and we use the actual number of occupied demes n_O instead of n because recolonization occurred generally not within the same generation. We further assume that all demes contribute equally in the absence of local extinction, using the harmonic mean to estimate the average census deme size $N = 50$ over time. Hence, we set $V = 0$ and the effective deme size $N_e = 0.56N = 28$ in equation (4.2). In the presence of population turnover we assume variable contributions of demes with $V = e/(1 - e)$ and $e = 0.1$. Finally, we estimate the effective size of a hypothetical undivided population with a census size equal to the census size of the metapopulations ($N = 6 \times 50 = 300$) as $N_e = 0.56N = 168$.

In addition, we infer the variance and eigenvalue effective metapopulation sizes based on linear regression independent of F_{ST} (CHAPTER 2). The variance effective size is estimated from linear regression of the variance in allele frequency change within the metapopulation between two successive generations as a function of the allele frequency in the parental generation ($\sigma^2_{\delta p} = p(1 - p)/2\hat{N}^M_e$). Similarly, the eigenvalue effective size is estimated from linear regression of the change in heterozygosity between two successive generations as a function of the heterozygosity of the parental generation ($\Delta H = H/2\hat{N}^M_e$).

COMPUTER SIMULATIONS AND PARAMETERS

The aim of using individual-based simulations is twofold. First, the simulations allow us to place the experimental results in a theoretical perspective. They can be used to derive expectations and matching confidence limits that allow detecting significant deviations of the experimental results from various null hypotheses. Second, the simulations allow extrapolating our findings beyond the observed patterns emerging from known variables, *e.g.*, by introducing a multiple locus approach (see the discussion section).

The simulations take account of the lottery polygyny mating system (males can mate with more than one female) that is typical for *Drosophila melanogaster* (Bateman 1948), of differences in fecundity among females, and of directional selection for the (red) bw^{75}-allele that occurred in the experimental fly stocks (CHAPTER 2). Extinction is typically implemented as an event occurring at random with probability e per deme per generation. We also generated *in silico* replicates for each of our metapopulations by imposing extinction schedules identical to those in the experimental metapopulations. However, since the results from these simulations were almost identical to the results of simulations based on the average extinction rate for both configurations, we have not explored this option further. The simulations take no account of intra-deme demography, hence colonized demes grow to size N in one generation similar to the assumption of the theoretical models.

For all simulations, generations are discrete, the mating system is lottery polygyny with remating and the population size and genetic parameters (one locus, two alleles, no mutation) are kept constant. Based on our earlier experiments (CHAPTERS 2 and 3) we assume that the remating probability is $\rho = 0.2$ and that the variance-to-mean ratio of offspring contributed to the next generation is $\alpha = 1.6$. For a given migration rate m, the

number of emigrants per deme is drawn from a Poisson distribution with mean = Nm. The migrant's sex is allotted at random with a probability of 0.5 for each sex, and we assume that migrants have mated before migration. The extra mortality during migration in the migrant-pool configuration is not implemented in the simulations.

Although this study focuses on the interplay of drift, migration and local extinction in the absence of selection, we include selection in the standard simulations because its effect appeared to be substantial in our previous study. Given the bias in favour of the bw^{75}-allele that we found in all previous experiments, we expect that this is the case in our current experiments as well. Hence, we implement viability selection through an additive model with selection coefficient s and relative viabilities of 1, $1 - s/2$ and $1 - s$ for the three genotypes $bw^{75}//bw^{75}$, $bw^{75}//bw$ and $bw//bw$, respectively. Based on the estimates obtained in our previous study where we found that selection coefficients depended on population size (CHAPTER 2), we used selection coefficients $s = 0.12$ for the vial populations and $s = 0.18$ for the bottle populations in the current experiment. Deviations from the standard parameterization indicated above are explained in the appropriate sections.

STATISTICAL ANALYSIS

As in our previous studies, we mostly used simulations for the statistical analysis of the data by constructing 95% confidence ranges from the 2.5 and 97.5 percentiles of 1000 simulation runs, with each run representing one replicate metapopulation. In addition, we calculated the average of three replicates (as for each of our experimental scenarios) that were drawn randomly from the 1000 simulation runs. Repeating this procedure 1000 times resulted in a new dataset of average values that we used to construct the corresponding 95% confidence ranges.

To test the results of the fitness and stress tolerance assessments for differences between groups (*i.e.*, metapopulations with migration and with or without local extinction, and unfragmented control populations) we used a non-parametric Kruskal-Wallis test (Statistix 8.0) with Bonferroni correction for the number of tests.

RESULTS

MIGRATION, EXTINCTION AND COLONIZATION RATES

We intended to set up a small system with relatively few demes, a high extinction rate, and a colonization rate of the same order of magnitude as the extinction rate to ensure sufficient genetic dynamics within relatively few generations. We first evaluate how closely the realized migration, extinction and colonization rates (table 4.1) match the intended setup.

Although it was not possible to quantify migration by direct observation for logistic reasons, we had the impression that migration regularly happened at either higher or lower rates than expected. This variation was probably related to external, (micro-)environmental factors, since migration activity seemed to be correlated among the replicates

Table 4.1. Extinction, migration and colonization in experimental metapopulations with migrant-pool (MPX) and stepping-stone (SSX) migration. The realized extinction rate is the probability that an extant deme did go extinct per generation. The number of extant demes is the harmonic mean over generations. The realized numbers of emigrants and immigrants are the average numbers per deme inferred from the observed numbers of colonists in empty demes. The gross colonization rate is the probability of migrants moving into an empty deme regardless of their subsequent reproductive success, whereas the net colonization rate is the probability of a successful colonization event (*i.e.*, founding a viable population in an empty deme). The last column shows the average number of founders per successful colonization event. Unless indicated otherwise, averages are arithmetic means with standard errors in brackets.

meta population	realized extinction rate e	extant demes n_O	emigrants per extant deme Nm_E	immigrants per deme Nm_I	gross colonization rate c^*	net colonization rate c	number of colonists k
MPX1	0.133	2.54	0.90 (0.34)	0.56 (0.22)	0.133	0.092	2.55 (1.39)
MPX2	0.068	4.62	0.92 (0.27)	0.63 (0.18)	0.347	0.224	2.09 (0.50)
MPX3	0.062	3.36	0.43 (0.24)	0.23 (0.12)	0.050	0.021	3.00 (3.48)
MPX	0.088 (0.023)	3.72	0.75 (0.16)	0.47 (0.12)	0.211 (0.068)	0.127 (0.050)	2.55 (0.26)
SSX1	0.121	2.99	1.79 (0.63)	0.86 (0.31)	0.250	0.111	5.17 (1.94)
SSX2	0.094	4.11	0.95 (0.22)	0.60 (0.15)	0.300	0.217	1.92 (0.38)
SSX3	0.111	4.75	3.14 (0.65)	2.39 (0.55)	0.595	0.500	3.48 (0.57)
SSX	0.109 (0.012)	4.15	1.96 (0.64)	1.28 (0.56)	0.382 (0.108)	0.276 (0.116)	3.52 (0.94)

running in parallel. The consequences of this variation, however, differed between replicates, since at any given time the replicates differed in the number of vacant demes. Accordingly, temporal variation in migration activity resulted in variation between metapopulations.

Extinction in both configurations was planned *a priori* with a probability $e = 0.1$ per deme per generation. However, we found during the experiment that extinct demes did not become colonized for several generations (figs. 4.2 and 4.3), presumably reflecting the large variation in migration activity between generations. The average number of extant demes (tab. 4.1) is therefore considerably lower than the total number of demes $n = 6$, and the realized extinction rates (*i.e.*, the extinction probability of an extant deme per generation) per metapopulation turned out to be more variable than planned (tab. 4.1). In the migrant-pool configuration (fig. 4.2) we cancelled planned extinction events in replicates MPX1 (simultaneous extinction of demes 4 and 5 in generation 14) and MPX3 (simultaneous extinction of demes 1 and 3 in generation 37) to avoid metapopulation extinction (and thus loss of a replicate before the end of the experiment). This reduced the extinction probability for the migrant-pool configuration to $e = 0.09$ on average.

We inferred the migration rates for both experimental configurations retrospectively as indicated in the methods section. The realized numbers of emigrants Nm_E (tab. 4.1) were mostly close to or larger than the planned number of one migrant per deme

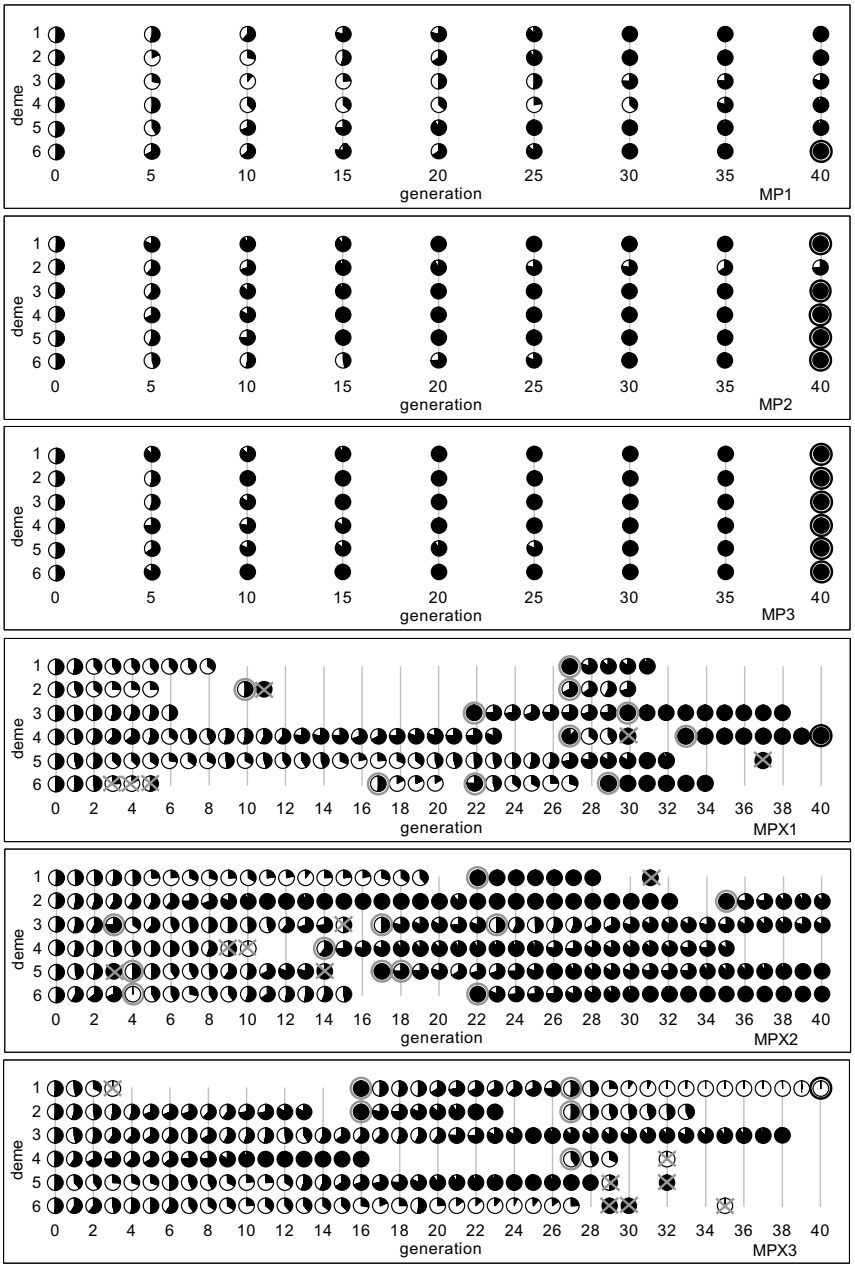


Figure 4.2. Allele frequencies in *D. melanogaster* metapopulations with migrant-pool migration, and in the absence (replicates MP1, 2 and 3) or presence (replicates MPX1, 2 and 3) of local extinction and recolonization. Markers indicate the frequencies of the bw^{75}-allele (black) and the *bw*-allele (white) in each of the six demes in a metapopulation over 40 generations. Empty slots indicate extinct demes. Grey outlines or crosses at a marker indicate successful or unsuccessful (*i.e.*, immigration without founding) colonization, respectively. Black outlines indicate fixation for either eye colour allele in generation 40.

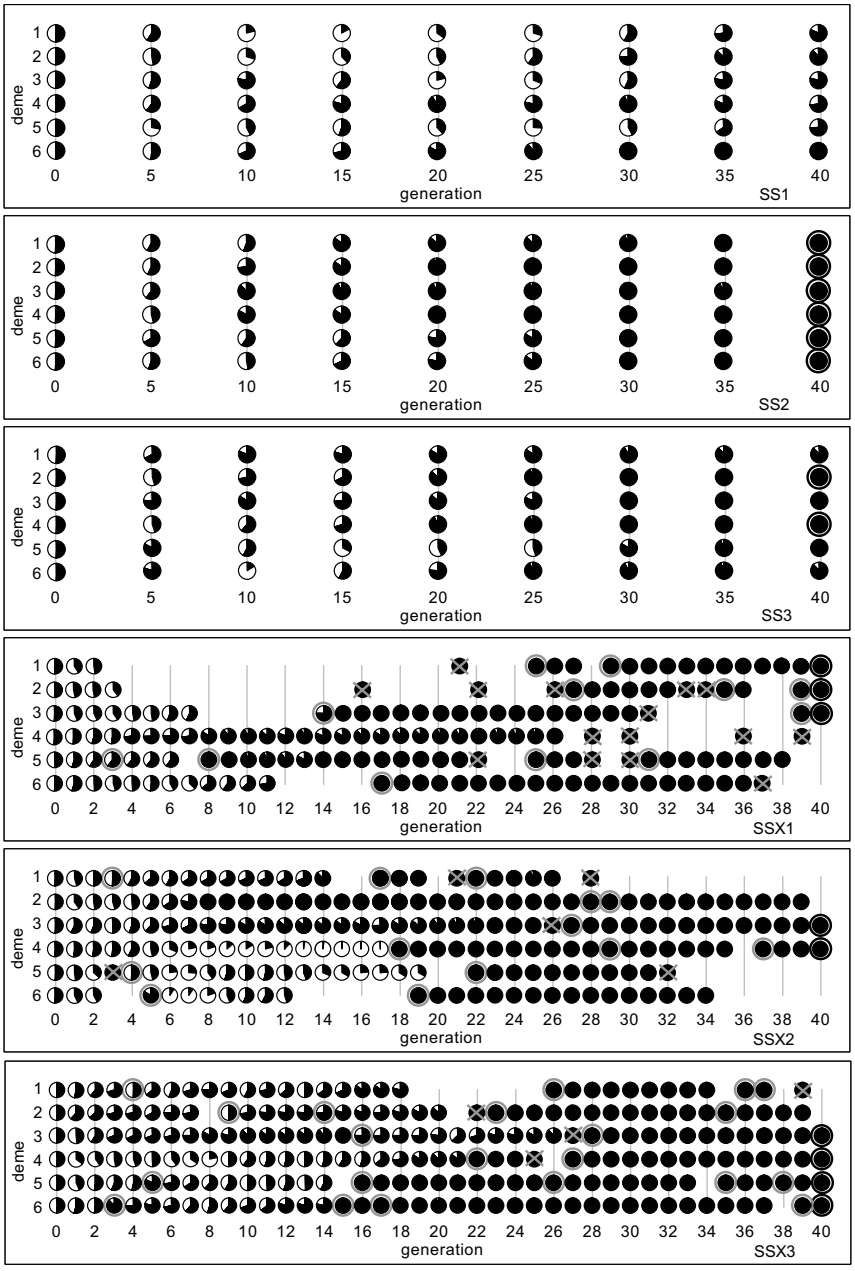


Figure 4.3. Allele frequencies per deme in *D. melanogaster* metapopulations with stepping-stone migration, and in the absence (replicates SS1, 2 and 3) or presence (replicates SSX1, 2 and 3) of local extinction and recolonization. Symbols as in fig. 4.2.

whereas the numbers of immigrants Nm_I are generally lower, although the variation among replicate metapopulations is considerable. Both emigration and immigration tended to be substantially lower in the migrant-pool configuration (MPX) than in the stepping-stone configuration (SSX), but these differences were not significant due to the large variation among the replicates within each configuration.

The gross colonization rates c^* (*i.e.*, all events of migrants arriving in an empty deme regardless of the subsequent founding success, tab. 4.1) indicate that empty demes were populated by migrants at an equal or higher rate than extant demes going extinct on average. However, not every colonization event resulted in a new viable population (figs. 4.2 and 4.3, grey circles *versus* crosses). Hence, although the net colonization rate c (*i.e.*, the probability of colonization followed by the founding of a viable population, tab. 4.1) was considerably higher than the extinction rate on average (tab. 4.1) this was not the case for three individual metapopulations (MPX1, MPX3 and SSX1) due to the large variation in colonization rates. Thus, individual metapopulations may run a high risk of extinction although the condition for metapopulation persistence ($c > e$) is met on average (figs. 4.2 and 4.3).

The average number of individuals per successful colonization event k (tab. 4.1) varied considerably both between and within metapopulations, probably again as a consequence of the large variation in migration activity. In spite of the relatively large numbers of colonists, 63% and 37% of the colonization events were due to single mated females in the migrant-pool and stepping-stone configuration, respectively. In contrast, individual-based simulations predict about 30% colonizations by single mated females on average for both configurations.

For the planned experimental setup, individual-based simulations predict an extinction probability of 7.5% (about 75 of 1000 metapopulations are lost before generation 40) for both configurations. In contrast, 50% (3 out of 6) of our metapopulations would have gone extinct within 40 generations without intervention. The above results suggest that the large variation in migration activity between generations and the resulting low colonization success caused the high observed extinction probability.

GENETIC DIVERSITY AND DIFFERENTIATION

We will now focus on the evolution of genetic diversity within demes and genetic differentiation among demes within metapopulations. Figure 4.2 (migrant-pool migration) and figure 4.3 (stepping-stone migration) show the changes of the allele frequencies at the *bw*-locus within individual demes. In figure 4.4 we compare the average allele frequencies in the metapopulations with the allele frequencies in the undivided bottle populations. Figure 4.5 shows the average expected heterozygosity $\bar{H}_S$ as a measure of the average genetic diversity within demes for each replicate metapopulation, and the average of three metapopulations to picture the global trend for each series. Figure 4.6 shows similar plots of the fixation index F_{ST} as a measure of the genetic differentiation among demes.

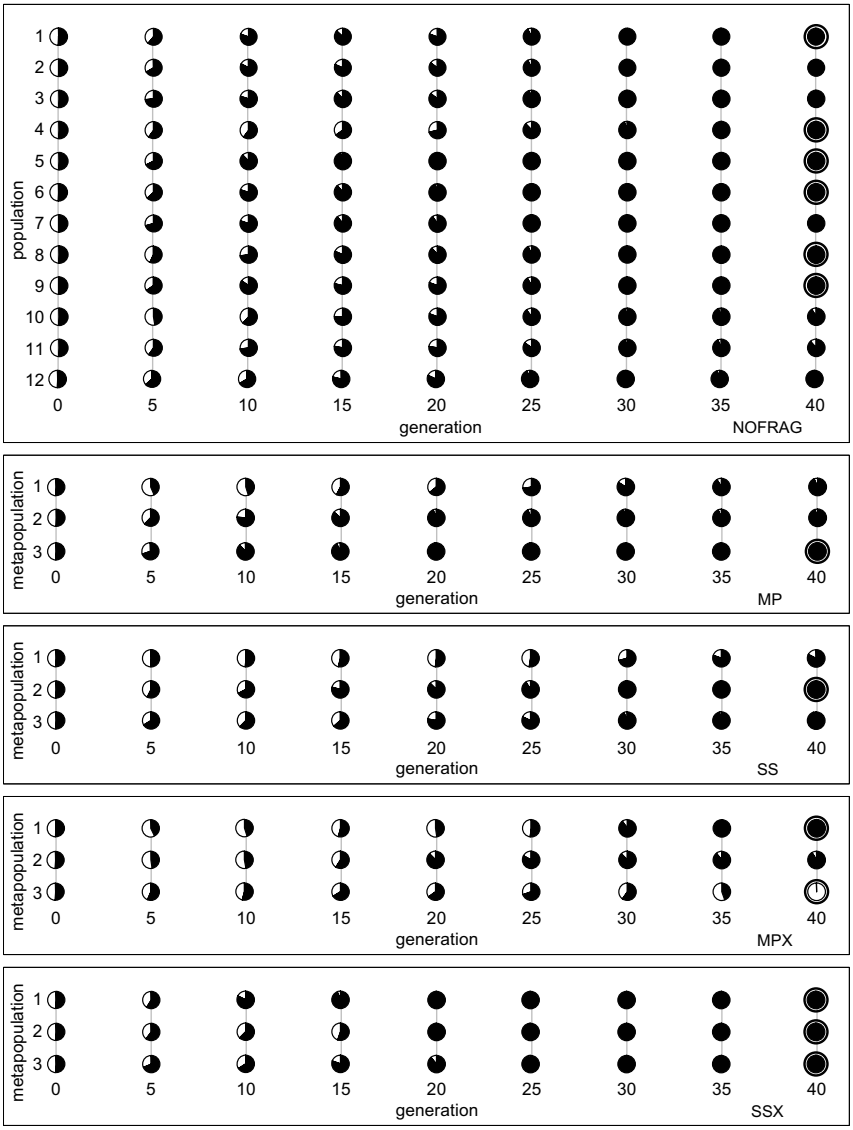


Figure 4.4. Allele frequencies in unfragmented *D. melanogaster* populations (NOFRAG), and average allele frequencies in metapopulations with either migrant-pool or stepping-stone migration, in the absence (MP & SS) or presence (MPX & SSX) of local extinction and recolonization. Symbols as in fig. 4.2.

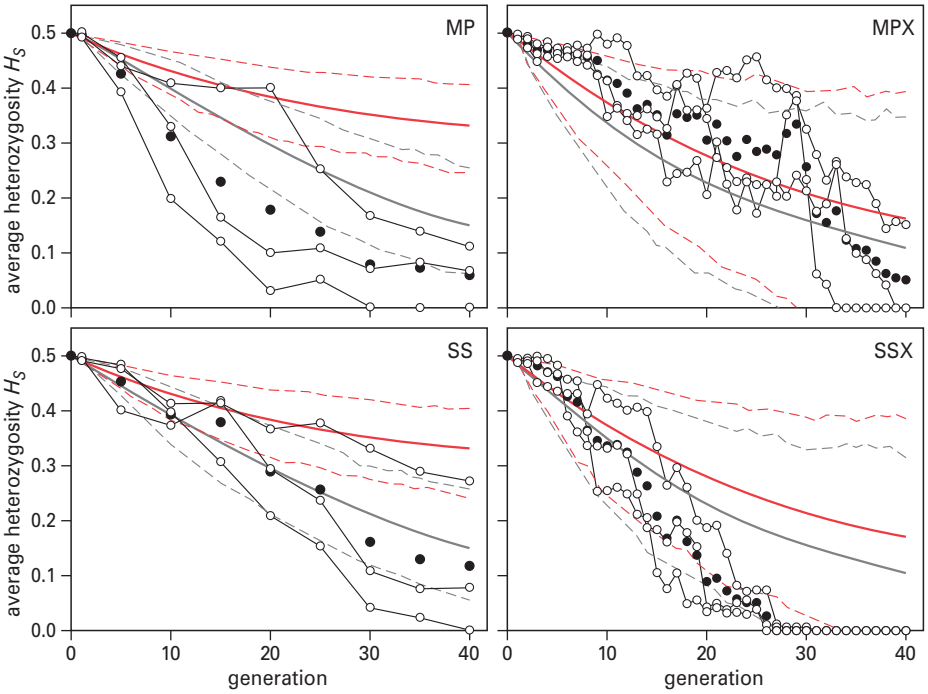


Figure 4.5. Observed and predicted genetic diversity in metapopulations with migrant-pool (top) and stepping-stone (bottom) migration, in the absence (left) or presence (right) of local extinction and recolonization. The plots show the average heterozygosity $\overline{H}_s$ of three replicate metapopulations (white circles) and their average (black circles), and predictions from individual-based simulations without (red lines) and with (grey lines) directional selection ($s = 0.12$). Dotted lines indicate 95% confidence bands for the average of three replicate metapopulations.

MIGRANT-POOL *VERSUS* STEPPING-STONE MIGRATION WITHOUT POPULATION TURNOVER

First we evaluate the two migration configurations without extinction and recolonization. At the level of individual demes we find that the bw^{75}-allele approaches fixation within most demes (fig 4.2 series MP & fig. 4.3 series SS), confirming our earlier results that bw^{75} is favoured by selection. In generation 40 half of the demes in the stepping-stone metapopulations and two thirds in the migrant-pool metapopulations are fixed (black outlines). At the metapopulation level, two of the six metapopulations (fig. 4.4 series MP & SS) and six of the 12 large undivided populations (series NOFRAG) are fixed in generation 40. In case of neutral variation, theory predicts a more rapid loss of allelic variation in small, relatively isolated demes than in large random-mixing populations, since the effective population size of a deme is generally much smaller than the effective size of a large population. However, this loss of variation will be different for each deme. As a consequence, more variation will remain in a metapopulation than in an undivided population of equal size. The effective size of a metapopulation is larger

than its census size, hence the loss of variation at the metapopulation level will be slower than in an undivided population of equal census size. Directional selection increases the fixation rate of the favoured allele (Crow & Kimura 1970), and will generally be more effective in a structured population than in an undivided population, especially under the assumption of hard selection (Whitlock 2002). Thus, selection favouring the bw^{75}-allele has presumably been responsible for the observed patterns. Individual-based simulations (data not shown) of undivided populations including selection predict 11% allele fixation after 40 generations, whereas simulations of similar undivided populations without selection predict only 0.1% fixation. Hence, the observed high fixation rates in the bottle populations are plausible.

In the case of neutral variation we expect a slower decline of diversity within demes, but higher levels of differentiation among demes than in the presence of directional selection (Crow & Kimura 1970). Simulations of both migration configurations in the absence (figs 4.5 & 4.6, red lines) and presence (grey lines) of selection confirm these expectations. The simulation results also indicate that the dynamics of migrant-pool and stepping-stone metapopulations are similar when migration rates are equal. The results of the experimental metapopulations, however, show clear differences between both migration configurations (figs 4.5 & 4.6, MP *versus* SS). Although we expect only moderate variation among the relatively small number of replicate metapopulations (*i.e.*, the confidence bands in figs 4.5 & 4.6 indicating the expected variation among three replicates), the loss of genetic diversity in both experimental configurations (fig. 4.5 left) differed (much) more than expected based on the simulations. The average increase of genetic differentiation (fig. 4.6 left) is mostly in line with the prediction from simulations for both configurations for the larger part of the experiment. The sharp decline of differentiation at the end of the experiment, particularly in case of stepping-stone migration, is due to allele fixation at the metapopulation level. Further quantification of the variation among replicate metapopulations by means of the global fixation index F_{TG} measuring genetic differentiation among replicates (data not shown) confirms both trends.

The observed differences between the two migration configurations are presumably the result of the lower migration rate in the migrant-pool than in the stepping-stone configuration (tab. 4.1) due to the larger migration distance and the occasional removal of a number of migrants remaining in the central compartment adding to the cost of migration in the former. Additional factors such as sexual or density-dependant selection or differences in activity due to the light regime (CHAPTER 3) favouring the bw^{75}-allele have probably played a role as well.

LOCAL EXTINCTION AND RECOLONIZATION

Next we look into the effects of local extinction and subsequent recolonization for both migration configurations. The large stochastic effects of extinction and founder events on the allele frequencies of individual demes instantly catch the eye. Allele frequencies may become completely reversed within one generation (fig. 4.3, deme 4 in SSX2), which may eventually even allow fixation of the disadvantageous *bw*-allele at the metapopulation level (fig. 4.2, MPX3). In contrast with the scenarios without local extinction, the

levels of allele fixation in the individual demes differ considerably for the two migration configurations. In case of migrant-pool migration (fig. 4.2, series MPX) fixation happens much slower than in case of stepping-stone migration (fig. 4.3, series SSX). Fixation at the metapopulation level happens more rapid than in both the scenarios without local extinction and the undivided populations (fig. 4.4, NOFRAG). All three stepping-stone metapopulations become fixed within 30 generations although each metapopulation comprises at least three extant demes in most generations. Two of the three migrant-pool metapopulations also become fixed eventually, which in one case is due to the extinction of all but one fixed deme. These results suggest that selection is more efficient in the stepping-stone configuration than in the migrant-pool configuration. Individual-based simulations predict a low level of metapopulation fixation (2.5%) in the absence of population turnover that increases substantially (40%) due to extinction and recolonization for both configurations at equal migration rates. Since effective migration is higher in the stepping-stone setup than in the migrant-pool setup, the resulting selection-drift dynamics may be different for both setups. However, even when we consider the extra cost of migration in the migrant-pool configuration in the simulations (data not shown), the actual fixation rate of the bw^{75}-allele is still higher than predicted in all experimental (meta)populations regardless of the relative differences between hierarchical levels, spatial configurations and migration-extinction scenarios. This confirms our earlier conclusion regarding the probable presence of additional factors favouring the bw^{75}-allele.

The differences between both configurations are reflected in the dynamics of genetic diversity (fig. 4.5 right) and differentiation (fig. 4.6 right). As for the scenarios without local extinction, individual-based simulations predict similar dynamics for both configurations in the absence (red lines) and presence (grey lines) of selection at equal migration rates. In contrast to the scenarios without local extinction resulting in a faster than predicted average loss of diversity (fig. 4.5 left) for both configurations, the decline of diversity (fig. 4.5 right) is faster than predicted by simulations in case of stepping-stone migration (series SSX), but slower than predicted in case of migrant-pool migration (series MPX). The sharp decline of differentiation within the stepping-stone metapopulations (fig. 4.6 right) mirrors the rapid loss of diversity and subsequent allele fixation at the metapopulation level. Both patterns suggest an absolute loss rather than a redistribution of genetic variation.

Quantification of the variation among replicate metapopulations by means of the global fixation index F_{TG} (data not shown) is in line with both the observed high variation and the predicted confidence bands among replicate metapopulations. The, in view of selection favouring the bw^{75}-allele, atypical fixation of the *bw*-allele in metapopulation MPX3 provides an explanation for the larger than expected variation among migrant-pool metapopulations (fig. 4.6 right). Individual-based simulations predict maximum differentiation among the metapopulations over time in the presence of local extinction. This suggests that due to the extinction/colonization dynamics, the original variation within demes is redistributed not only to variation among demes, but eventually to variation among metapopulations.

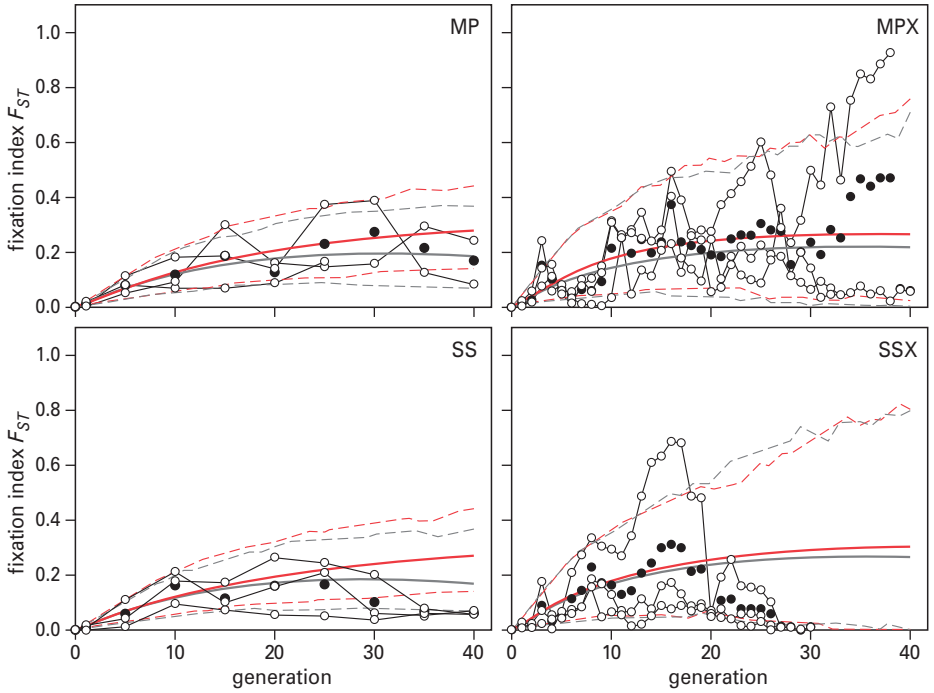


Figure 4.6. Observed and predicted genetic differentiation in metapopulations with migrant-pool (top) and stepping-stone (bottom) migration, in the absence (left) or presence (right) of local extinction and recolonization. The plots show the fixation index F_{ST} with symbols as in fig. 4.5. F_{ST}-series ending before generation 40 are due to allele fixation at the metapopulation level, so that the fixation index is no longer defined.

METAPOPULATION FITNESS AND STRESS TOLERANCE

In this section we investigate the effects of fragmentation and population turnover on the average fitness of our experimental metapopulations and their potential to cope with environmental challenges. In generation 35, we exposed flies from all demes and undivided populations to three environmental stress factors high (29°C) temperature, high (12.5%) ethanol concentration, and starvation. Figure 4.7 shows the results of the fitness assessments, while table 4.2 summarizes the results of the stress tolerance assessments.

Fragmentation in either the presence or absence of population turnover had a markedly different effect on fecundity (fig. 4.7 top) than on viability (middle). The variation in fecundity is relatively large both within (left) and among (right) metapopulations. The average fecundity is significantly lower in the metapopulations (Kruskal-Wallis $H = 11.5$, $df = 17$, $P = 0.0032$) than in the undivided bottle populations (right, white bar). In contrast, the viability at 25°C differs little both within (left) and among (right) metapopulations, and neither is it significantly different from the viability of the bottle populations (right, white bar). These results are most likely a consequence of the difference between vial and bottle populations in the experimental setup rather than of

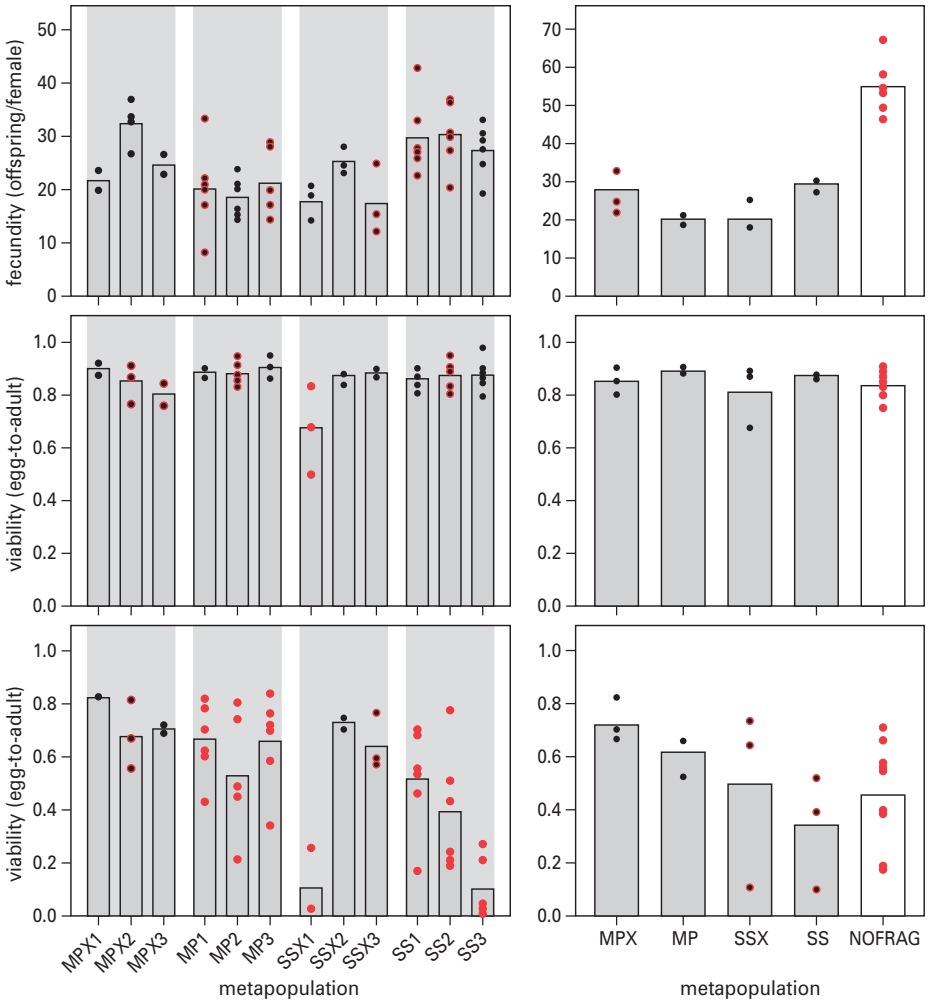


Figure 4.7. Fecundity (top) and viability at 25°C (middle) and 29°C (bottom) of experimental metapopulations with migrant-pool (MPX & MP) and stepping-stone (SSX & SS) migration. Left: average per metapopulation (bars) and values per deme (dots) for migrant-pool and stepping-stone migration in the absence and presence of local extinction. Right: average per migration/extinction scenario (bars) and per replicate metapopulation (dots). Note that the dots in the right-hand plots correspond to the bars in the left-hand plots. The white bars represent the undivided bottle populations. Dot colours indicate the level of significance of ANOVA among demes within metapopulations (left) and among metapopulations within scenarios (right). Black: $p \geq 0.05$, grey with black outline: $0.01 \leq p < 0.05$, and grey: $p < 0.01$.

genetic erosion due to population fragmentation We will discuss this further in the next section. The near maximal and very similar levels of viability of both metapopulations and bottle populations suggest that inbreeding depression plays no important role in any of the migration/extinction scenarios, although single demes might suffer from inbreeding depression to some extent, *e.g.*, in SSX1 (fig. 4.7 middle left).

The bottom plots in figure 4.7 show the egg-to-adult viability at 29°C. Exposure to high temperature reduces the viability in all scenarios, and substantially increases the variation, both within (left) and among (right) metapopulations and bottle populations. Upon visual inspection one might conclude that differences between the scenarios exist. However, none of these differences is statistically significant, and moreover, they do not represent a systematic trend. The tolerance (*i.e.*, relative viability) to high temperature stress (table 4.2) matches the pattern of the viability at 29°C very closely, since the viability under control conditions at 25°C is nearly constant. The results for the tolerance to ethanol stress and starvation resistance show similar, but less pronounced patterns of variation (tab. 4.2). The different tolerance patterns for high temperature and for high ethanol concentrations might partially result from the different genetic bases of both factors (CHAPTER 5). Although tolerance to high temperature is thought to be mostly a polygenic character (Loeschcke *et al.* 1997), conditionally expressed near-lethal alleles occur regularly for this trait (Bijlsma *et al.* 1999, Vermeulen & Bijlsma 2004). Such alleles behave like near-neutral alleles under normal conditions, hence they are merely subject to genetic drift and may show considerable variation in frequency in small populations. This is consistent with the observed high variation in tolerance both within (fig. 4.7 left)

Table 4.2. Stress tolerance in experimental metapopulations with migrant-pool (top, MPX & MP) and stepping-stone (bottom, SSX & SS) migration. Stress tolerance is the egg-to-adult viability under stress conditions relative to the viability under control conditions, and starvation resistance is the median age at death (in hours) of virgin males on agar medium. All averages are arithmetic means with standard errors in brackets.

metapopulation	tolerance to temperature stress	tolerance to ethanol stress	starvation resistance
MPX1	0.918 (0.021)	0.655 (0.001)	115.3 (1.8)
MPX2	0.793 (0.059)	0.782 (0.008)	120.4 (2.6)
MPX3	0.882 (0.065)	0.696 (0.018)	135.3 (10.3)
MP1	0.745 (0.062)	0.818 (0.035)	129.0 (3.2)
MP2	0.600 (0.107)	0.749 (0.036)	129.8 (5.5)
MP3	0.725 (0.076)	0.735 (0.023)	128.2 (2.4)
MPX	0.864 (0.014)	0.711 (0.005)	123.6 (2.7)
MP	0.690 (0.013)	0.767 (0.004)	129.0 (0.9)
SSX1	0.127 (0.090)	0.449 (0.044)	122.0 (0.6)
SSX2	0.841 (0.030)	0.641 (0.060)	119.7 (2.7)
SSX3	0.724 (0.066)	0.780 (0.048)	134.9 (3.8)
SS1	0.605 (0.096)	0.725 (0.057)	123.0 (3.4)
SS2	0.453 (0.112)	0.631 (0.040)	124.4 (2.3)
SS3	0.116 (0.054)	0.573 (0.037)	128.1 (1.4)
SSX	0.564 (0.017)	0.623 (0.005)	125.5 (0.9)
SS	0.392 (0.017)	0.643 (0.006)	125.2 (0.6)
Control bottles[1]	0.540 (0.062)	0.633 (0.032)	132.8 (1.9)

[1]) All measures were calculated for control bottles 1 – 12, but without the data of bottle 7

and among (right) metapopulations. Founder events involving very few individuals may enhance the effect of genetic drift, leading to the near-fixation of either allele at the metapopulation level. In line with this reasoning, we observe less variation among demes in the presence of local extinction (fig. 4.7 left, series MPX and SSX). However, entire metapopulations may become very susceptible to adverse external conditions, as has clearly happened in two of the experimental metapopulations (fig. 4.7 left, SSX1 and SS3). Tolerance to ethanol is mostly determined by a major gene (ADH), with other genes playing additional roles (Chakir *et al.* 1996, Malherbe *et al.* 2005). Analysis of the ADH locus (data not shown) has shown that both alleles ADH-F and ADH-S were initially present at frequencies of 0.44 and 0.56, respectively. During the experiment, selection in favour of the ADH-S allele has occurred in most metapopulations, increasing its frequency up to 1.0, *i.e.*, fixation, on three occasions. These results are somewhat atypical, since the equilibrium frequency of ADH-S in laboratory stocks under standard conditions generally varies between 0.25 and 0.35, whereas high concentrations ethanol induce selection in favour of ADH-F (Van Delden *et al.* 1978). Although one might expect a low tolerance of ethanol stress in populations with a high ADH-S frequency, we observed no correlation at all. We suspect that the ethanol concentrations in the experimental setup were too low to affect viability very much, so that the observed results reflect some other aspect of survival that is probably not connected with tolerance to ethanol at all (see *e.g.*, Bijlsma-Meeles & Bijlsma 1988).

DISCUSSION

EXPERIMENTAL SETUP

We have chosen initial experimental values that allow for ample population turnover with a colonization rate equal to the extinction rate. In such systems we expect the turnover rate of empty and extant demes to maintain viable metapopulations when the number of demes is sufficiently large. The experimental metapopulations, however, did not stabilize and would have gone extinct eventually in most cases. In addition to the cost of migration that negatively affected the turnover rate in the migrant-pool setup, this is probably a consequence of two important deviations between the experimental setup and theoretical assumptions. Firstly, theoretical models generally assume no demography within demes and instant growth to the maximum deme size after colonization, resulting in equal contributions to the next generation for each deme. The observed deme size after colonization ranged from 5 to 108 individuals (38 on average), hence it took two or more generations to attain maximum size on several occasions. We found similar large fluctuations in the size of extant demes, albeit with a higher average, implying that the contribution per generation may have varied considerably for the experimental demes. Secondly, the experimental migration, extinction and colonization rates fluctuated due to large variation in migration activity between generations, whereas these parameters are generally assumed constant in the theoretical models. This applies in particular to the assumption of colonization occurring instantly after extinction,

whereas effective migration, and thus, colonization, was (much) lower than the extinction rate in most experimental metapopulations. In retrospect, migration rates should probably have been planned twice as high, since on average, half of the migrants are males that will commonly contribute to gene flow when they immigrate into extant demes, but not when they colonize empty demes where no females are present. Fluctuations of these parameters have probably substantially increased the variation in the experimental systems, and thus, the risk of stochastic metapopulation extinction. Although we allowed for "natural" migration behaviour within the constraints of the spatial setup and for fluctuating deme sizes on purpose assuming only small differences averaging out within a few generations, these results show that relaxing experimental control to a little extent might easily result in large deviations from predictions based on theoretical models.

We hypothesized earlier that some of the differences between the metapopulations and the bottle populations were probably partly a consequence of the experimental setup. We suspect that even under relatively simple standard conditions, unintentional differences have been more common than expected, *e.g.*, between the vial and bottle populations. Due to the different experimental procedure for both types of containers, the population density in the bottles was generally higher than in the vials. Different densities might *e.g.*, introduce selection on the timing of egg-laying favouring early-laying females in the bottles (see fig. 4.7), resulting in a higher fecundity in the bottles, but not affecting egg-to-adult viability. Such unintentional density-dependant selection effects might provide an explanation for the difference between metapopulations and undivided populations that we found for fecundity, but not for viability. A second example of unforeseen differences is the cost of migration that we encountered in the migrant-pool configuration. The occurrence of such relatively large biases under simple standard conditions strongly suggests that many more unknown deviations of the model assumptions can be expected in natural metapopulations.

We observed (very) high levels of variation among single replicates for all migration and gene flow scenarios. Although such high variation is not unexpected, especially in the presence of population turnover, we suspect that part of the variation is due to the logistic constraints of the experimental setup, *i.e.*, the combination of few replicates, few demes per metapopulation, and using a single marker locus. We used simulations to evaluate the effect of these factors by varying the numbers of demes and marker loci, and averaging over a large number of replicate runs. Figure 4.8 compares the median and the 75%, 90% and 95% confidence intervals for the genetic differentiation in generation 40 based on individual-based simulations of a migrant-pool metapopulation consisting of six (left) or 60 (right) demes for one locus and for eight loci. Increasing either the number of demes or the number of marker loci reduces the width of the confidence intervals, and hence the variation, in both gene flow scenarios. In the presence of local extinction, however, the medians for small and large metapopulations differ considerably for both sets of marker loci, indicating a genuine consequence of metapopulation size rather than merely a sampling effect. Consequently, extrapolation of our experimental results based on metapopulations consisting of only a few demes to larger systems must be considered with care, and has probably limited value.

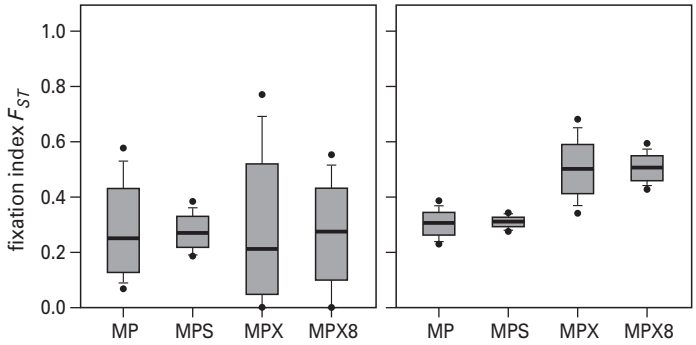


Figure 4.8. Predicted variation in genetic differentiation in a metapopulation consisting of few (left, $n = 6$) and many (right, $n = 60$) demes for a single locus and for multiple loci. The plots show the results of individual-based simulations without selection of 1000 replicate metapopulations with migrant-pool migration in the absence (MP) and presence (MPX) of local extinction and recolonization. Bars, whiskers and points indicate the 75%, 90% and 95% confidence intervals, respectively, for F_{ST} at one locus (MP, MPX) and averaged over eight loci (MP8, MPX8) in generation 40. The black bands in the bars indicate the median.

IMPLICATIONS FOR NATURAL SYSTEMS

We found clear deviations between the experimental results and theoretical predictions. Firstly, individual-based simulation predicted similar dynamics for migrant-pool and stepping-stone migration, whereas the observed patterns were different for both configurations. Secondly, the loss of genetic diversity proceeded generally more rapidly than predicted, resulting in allele fixation at the metapopulation level. Thirdly, the variation between replicate metapopulations was larger than expected in most cases. We can suggest several potential causes that may be responsible for these deviations. Most obviously, the actual migration rates were highly variable over generations, which had different consequences for each of the metapopulations, since the number of empty demes also differed over generations, and a low migration rate will have considerably less impact for extant demes than for empty demes. In addition, the physical dispersal distance was different for the two migration configurations, which unintentionally introduced an extra cost of migration in case of migrant-pool migration. Although variation at the eye colour marker locus was presumed to be neutral (Buri 1956), it was actually subject to selection, probably for subtle reasons such as different activity patterns due to the experimental light regime. In addition, the strength of selection was different in the vials than in the bottles (CHAPTER 2). Small differences in the experimental conditions in both types of container, such as occasional moderate crowding in the bottles, might have resulted in different selection pressures affecting fecundity, and thus the assessment of fitness. Finally, due to the different extinction schedules for each replicate metapopulation chance has played a very important role, which resulted in each replicate being unique and making general inferences difficult. All these factors will to some extent lead to deviations between the experimental results and theoretical predictions. We can

minimize such deviations by further standardization, *e.g.*, by replacing bottle populations with compound vial populations. However, one might argue that fully standardized experiments have limited use, because corresponding results are much easier obtained by simulations. In contrast, less-standardized experiments provide a bridge between theory and the natural world because they draw attention to subtle but important deviations that induce a better understanding of natural populations. Large variation in a relatively standardized setup indicates that even more variation can be expected in natural systems.

Since theoretical models, and also generalized simulation models, are primarily conceptual models providing general insights, it is unclear whether they have predictive value for natural populations that have all specific characteristics. In practice, one might ask how well measures of F_{ST} and n from natural populations are suited to make inferences of *e.g.*, effective metapopulation sizes based on a general model making many restricting assumptions as in equations (4.1) and (4.2). With $N_e = 28$, $m = 0.02$ and $e = 0.1$, we would expect $\hat{F}_{ST}$ (eqn (4.1)) to vary between $\hat{F}_{ST} = 0.27$ for metapopulations with local extinction and migrant-pool colonization ($\varphi = 0$), and $\hat{F}_{ST} = 0.31$ in the absence of extinction. The results in fig. 4.6 suggest that the experimental populations did not attain equilibrium levels of F_{ST} in most cases, since F_{ST} dropped sharply after 20 to 25 generations due to allele fixation in the metapopulations, whereas the simulation results (red lines) indicate that it will generally take longer than 40 generations to attain equilibrium. In addition, the number of $n = 6$ demes in our metapopulations is below the limit of $n = 20$ that is considered sufficiently large to approximate the equilibrium in a model II metapopulation with the model I equation (Pannell & Charlesworth 1999), although simulations (data not shown) indicate that the equilibrium in a model II metapopulation with six demes is only marginally lower than the approximation based on eqn (4.1). Hence, the experimental F_{ST}-values in generation 20 that we used to infer effective metapopulation sizes are probably lower than the actual equilibrium values.

The effective size of a metapopulation is a measure of the loss of genetic variation from the metapopulation. In the experimental metapopulations, the genetic variation within demes decreased, whereas the variation among demes increased initially to drop again as most metapopulations lost all variation due to allele fixation. Estimates of the effective metapopulation size N_e^M based on the generalised model in eqn (4.2) are independent of spatial structure, but depend directly on F_{ST}. Subject to a number of assumptions, table 4.3 shows the estimated N_e^M averaged over the three replicate experimental metapopulations per scenario (col. 2). In the absence of population turnover, the estimated effective size of the metapopulations is larger than the predicted effective size ($N_e = 168$) of an undivided population of equal total size ($N = 6 \times 50 = 300$). Due to local extinction and recolonization, the estimated effective metapopulation size is drastically reduced in comparison with an undivided population. These results are in general accordance with theoretical predictions (Wang & Caballero 1999), but it is unclear whether such estimated values are reliable as absolute quantities.

Equation (4.2) allows for some deviation of its assumptions, for example by using the effective deme size instead of the census deme size to correct for non-random mating within demes. Likewise, using F_{ST} may allow for the presence of directional selection to

Table 4.3. Effective metapopulation size N_e^M for metapopulations with migrant-pool (top) and stepping-stone (bottom) migration, in the absence (MP, SS) or presence (MPX, SSX) of local extinction and recolonization. Column 2 shows the estimated effective size of three replicate metapopulations based on the average F_{ST} in generation 20 (the range of single replicates in brackets). Columns 3 and 4 show the variance and eigenvalue effective metapopulation sizes inferred from linear regression over 20 generations (see text). Values in italics are corresponding results of individual-based simulations of 1000 replicate metapopulations (95% confidence intervals in brackets).

metapopulation	F_{ST}-based N_e^M	variance N_e^M	eigenvalue N_e^M
MPX	62.1 (19.3 – 85.5)	528.7 (386.0 – ∞)	43.3 (18.8 – 422.2)
MP	192.0 (183.6 – 198.9)	33.3 (16.4 –132.2)	14.4 (7.8 – 126.0)
MPX sim	*43.3 (37.0 – 109.9)*	*34.9 (31.7 – 39.0)*	*34.9 (29.0 – 43.8)*
MP sim	*210.8 (185.4 – 250.7)*	*203.3 (194.7 – 212.7)*	*188.7 (175.0 – 204.7)*
SSX	93.6 (67.6 – 89.6)	320.0 (164.9 – ∞)	9.3 (7.0 – 12.4)
SS	199.6 (178.0 – 228.3)	62.7 (24.7 – ∞)	33.8 (17.4 – ∞)
SSX sim	*43.8 (38.6 – 117.7)*	*32.9 (30.1 – 36.1)*	*37.5 (31.3 – 46.9)*
SS sim	*208.0 (184.9 – 246.5)*	*199.5 (190.5 – 209.3)*	*176.3 (156.4 – 202.1)*

some extent, because the effects of selection are reflected in the fixation index. However, other simplifying assumptions, for example regarding the variance of reproductive output among demes, might considerably affect estimates of the effective metapopulation size. We expect that the variance of reproductive output among demes is larger than predicted in the experimental metapopulations, since the observed fluctuations in the migration, colonization and extinction rates, as well as in deme size and in the number of extant demes all affect the reproductive output of demes. Inspection of the observed variance (tab. 4.3, col. 3) and eigenvalue (col. 4) effective sizes attracts immediate attention to the striking discrepancies between both concepts of effective size, pointing out that the theoretical approximation obviously does not hold in the presence of natural selection. Comparison of the estimator based on F_{ST} and the variance and eigenvalue effective sizes based on idealized simulated populations without selection (tab. 4.3) suggests that the F_{ST}-based estimator is fairly robust in the absence of population turnover, but inclined to substantial discrepancies (up to 50%) when local extinction and recolonization events occur frequently. Its value for applied studies where the effective metapopulation size is used as an indicator of for instance the risk of inbreeding depression in a population might therefore be limited in practice.

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CHAPTER 5

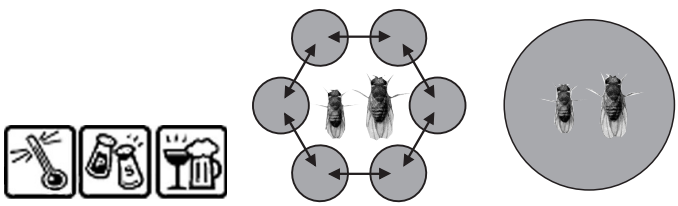
Consequences of fragmentation for the ability to adapt to novel environments in experimental *Drosophila* metapopulations

with M.E.C. VAN RIJSWIJK, F. J. WEISSING and R. BIJLSMA

ABSTRACT

Environmental deterioration and habitat fragmentation threaten the survival of many natural populations, and may reinforce each other as habitat fragmentation causes erosion of genetic variation that is crucial for adaptation to environmental challenges. We studied the consequences of habitat fragmentation for the adaptive response by subjecting populations of *Drosophila melanogaster* that had been either subdivided or undivided for several generations to three novel stress environments (high temperature, medium with ethanol or salt added). We assessed the viability both at first exposure to stress (tolerance) and after some generations of adaptation (adaptive potential). We used individual-based simulations to generate predictions of the adaptive response depending on degree of subdivision, intensity of selection, and genetic architecture of the stress resistance trait.

Population subdivision resulted in substantial inter-deme variation in tolerance due to redistribution of genetic variation from within demes to among demes. Contrary to expectations based on simulations, tolerance to high temperature and ethanol was higher in the subdivided than in the undivided populations, probably as a consequence of experimental variation resulting in some unintentional selection. In line with the simulation results, the adaptive response was generally lower in the subdivided than in the undivided populations. The pronounced differences in tolerance and adaptive response between the three stress factors are probably related to the different genetic architectures involved in resistance to these factors. Fixation of conditionally expressed near-lethal alleles in some demes was mainly responsible for the large variation in adaptive response to high temperature stress. Our results indicate that the adaptive response of single demes may vary substantially, in particular when major genes with conditionally expressed detrimental alleles are involved.



INTRODUCTION

The ongoing increase of human activity has already caused major environmental changes such as climate change, chemical pollution, habitat destruction and habitat fragmentation. (Pimm *et al.* 1995, Parmesan & Yohe 2003). All these changes tend to act in concert, thereby impairing the fitness of individual organisms and threatening the persistence of entire populations.

To meet such environmental challenges, it is crucial that a population is able to cope with the imposed stress at short term, and to evolve adaptive mechanisms on a longer time scale. To ensure the short-term survival of the population, albeit at a reduced fitness level, enough individuals have to be able to tolerate the stressful conditions to a sufficient degree. The ability to tolerate stress is largely determined by two factors: phenotypic plasticity and/or genetic variation (Bijlsma & Loeschcke 2005). Even if a population is stress-tolerant to a certain degree, its fitness will typically be severely reduced. To ensure long-term survival the population needs to adapt to the new environment. Rapid adaptation to novel environments requires the availability of ample genetic variation (Macnair 1991, Lynch & Lande 1993). Since both stress tolerance and adaptation occur on short to intermediate evolutionary time scales, new beneficial mutations will be rare, hence the adaptive response will mainly depend on the standing genetic variation (Bijlsma & Loeschcke 2005). Thus, the availability of genetic variation is crucial for the persistence of populations exposed to deteriorating environmental conditions, and mostly determines the adaptive potential of a population.

The availability of genetic variation may be compromised, however, if environmental deterioration is associated with habitat fragmentation. In fact, subdivision of a formerly large population into small population fragments or demes may have profound consequences for the availability of genetic variation. Genetic drift will become more prominent in small populations, resulting in the loss of genetic variation from single demes and increased differentiation among demes within the entire metapopulation. (Wright 1951). In other words, genetic variation is redistributed from the within-deme level to the among-deme level, substantially reducing the adaptive potential of single demes (Whitlock 2002). Furthermore, small populations are prone to inbreeding and accompanying inbreeding depression that often causes populations to be more sensitive to environmental deterioration (Crnokrak & Roff 1999, Bijlsma *et al.* 2000, Reed *et al.* 2002, 2003), and to inbreeding depression that decreases fitness and increases the risk of population extinction (Saccheri *et al.* 1998, Bijlsma *et al.* 2000, Frankham 2005).

Thus, whereas exposure to deteriorating environmental conditions requires an adequate response, the prerequisites for such a response appear to be severely hampered by the results of habitat fragmentation. As a consequence, research into the adaptive response of subdivided populations to environmental challenges is of major importance for conservation biology. Recent studies on plants, butterflies and *Drosophila* have shown that indeed the adaptive potential generally decreases with increasing severity of genetic bottlenecks (Frankham *et al.* 1999, Whitlock & Fowler 1999, Saccheri *et al.* 2001, Reed *et al.* 2003, Swindell & Bouzat 2005, Briggs & Goldman 2006). However, except for the

studies by Frankham and co-workers most of these studies involve the adaptive response of traits that are not, or only indirectly, related to fitness.

This study aims to investigate the adaptive response to environmental deterioration of subdivided populations using *Drosophila melanogaster* metapopulations as a model system. Resulting from an earlier experiment (CHAPTER 4) we had two types of population at our disposal that mainly differed in their history of fragmentation. Six populations had been subdivided for 40 generations with gene flow through migration at a relatively low level ("metapopulations"), and these were matched by similar-sized, undivided control populations. This provided us with a unique opportunity to investigate the effect of population subdivision on the adaptation to changing environments. We subjected both the subdivided and the undivided populations for six generations to different environmental stress factors, and measured the survival at first exposure to the stress (*i.e.*, stress tolerance), as well as the increase in survival after six generations of exposure and adaptation (*i.e.*, adaptive potential).

Adaptation to stress environments can presumably be regulated by different genetic architectures for different stress factors. Therefore, we used the three stress factors high temperature, salt and ethanol. Tolerance to high temperature is mainly a polygenic character (Loeschcke *et al.* 1997), but a genotype-by-environment interaction may occur in the form of conditional deleterious alleles that are only expressed at high temperatures (Vermeulen & Bijlsma 2004). Tolerance to saline environments is regulated by many genes with small effects (*i.e.*, entirely polygenic, Wallace 1982). In case of tolerance to ethanol, the alcohol dehydrogenase gene *Adh* is involved as a major gene, although several other genes play a role as well (Chakir *et al.* 1996, Malherbe *et al.* 2005).

We focused on the following questions: (i) What is the effect of habitat fragmentation on stress tolerance of a population? (ii) What is the effect of fragmentation on the adaptive potential of a population? (iii) Is the response to stress different if the underlying genetic architectures differ?

In addition, we ran individual-based computer simulations to place our experimental results in a more general perspective. We combined different options of genetic architecture with a range of increasing selection strengths and a range of increasing levels of gene flow to derive quantitative hypotheses of the effects of genetic architecture and population fragmentation on stress tolerance and adaptive potential.

MATERIALS AND METHODS

POPULATION HISTORY

All populations of *Drosophila melanogaster* used for the experiment were founded with flies from two marker stocks with either red-brown eyes (*bw*^{75};*st*) or white eyes (*bw;st*) in homozygous individuals. Prior to all experiments, the two marker stocks had been crossed and maintained as a mixed population for several generations to homogenize the genetic background except for the genomic region around the marker loci. We then

used all heterozygous orange-eyed (bw^{75}/*bw;st*) flies to initiate six subdivided metapopulations with six demes each and six large undivided populations.

Each deme of a metapopulations was maintained in a glass vial containing 18 ml of standard medium (26 g dead yeast, 54 g sugar, 17 g agar, and 13 ml nipagine solution (10 g nipagine in 100 ml 96% alcohol) per litre), whereas the undivided populations were maintained in 125 ml bottles containing 30 ml of the same medium. All populations were kept in the same climate room at 25°C, 40-60% RH and 24 hours of light.

A detailed description of the metapopulation setup and the subsequent fragmentation history is given in CHAPTER 4. The six metapopulations (M1-M6) each comprised of $n = 6$ demes with an approximate size of $N = 50$ individuals (harmonic mean over 40 generations: 57.9, 56.2, 56.1, 51.0, 52.5, 50.7) each (CHAPTER 4). In these metapopulations, adult flies were allowed to migrate between the demes for 12-24 hours, approximately three days after eclosion. In three of the metapopulations (M1-M3) flies migrated according to a circular stepping-stone pattern (*i.e.*, migrants move between adjacent demes only). In the other three metapopulations (M4-M6) flies migrated according to an equidistant *n*-island ("migrant-pool") pattern (*i.e.*, migrants first assemble in a central pool, and then move on into any of six demes). After migration, the individuals from each deme were transferred into fresh vials to lay eggs for the next generation. The average number of immigrants was 0.5 and 1.3 for migrant-pool and stepping-stone migration, respectively. See CHAPTER 4 for a discussion of the realized migration rates in both configurations.

The undivided populations (P1-P6) were each maintained in a single bottle with an approximate population size of $N = 220$ individuals (harmonic mean over 40 generations: 233.8, 208.1, 225.3, 216.0, 199.1, 228.0). Every two weeks the newly emerged adults were transferred into a fresh bottle to lay eggs, and discarded after 1-2 days of egg-laying to maintain discrete, uncrowded generations.

All populations were continued for 40 generations, after which we set up the flies from all demes and all undivided populations as (36 + 6) separate lines maintained in two bottles each (*circa* 250 individuals per bottle, individuals from the two bottles were mixed each generation). We increased the population size to minimize the effects of genetic drift on the genetic variation present after 40 generations of subdivision. We maintained the lines at standard conditions for five generations until the start of the adaptation experiment. Unfortunately, we lost two lines from metapopulation M4 due to a bacterial infection, reducing the total number of lines to (34 + 6).

ADAPTATION EXPERIMENT

To initiate the adaptation experiment, flies from all lines were allowed to lay eggs in two bottles in the different stress environments: high temperature (28-28.5°C), salt medium (3%) and ethanol medium (10%). In addition, we maintained all lines in two bottles at standard conditions as controls. Individuals from the two bottles were again mixed in each new generation. Egg-laying occurred for approximately two days, after which we discarded the parents to maintain discrete generations. Each generation, we initially collected the adults emerging in the stress environments in bottles at standard condi-

tions, and transferred them to the stress environments for the next generation after most of the adults had emerged.

We kept the stress treatments at a moderate level to prevent extinction of lines. For the high temperature treatment, we assessed the viability at 28.5 ± 0.5°C, but we maintained a backup at 28°°C during the adaptation process. Due to small fluctuations in the climate room the temperature sometimes became 29°C, which may induce male sterility (David *et al.* 1983). In these cases, the backup at 28°C, which never suffered from sterility, was used to start the next generation.

After six generations of stress treatment, we maintained the lines at standard conditions for two more generations before assessing viability in order to obtain sufficient individuals, to synchronize the generations and to avoid carry-over effects. This procedure may, however, have resulted in some reverse selection.

To determine the level of adaptation of each line, we tested both the original, unadapted lines and the adapted lines in the stress environments. We allowed *circa* 40 females of each line to lay eggs for 5–10 hours on standard medium, and we then carefully transferred the eggs to vials containing stress medium, or vials that were placed at high temperature. We set up six replicates of 50 eggs each for all lines. After emergence, we counted all flies to calculate the egg-to-adult viability per line.

Normally, one would estimate the viability cost of exposure to stress relative to the viability under benign conditions. This was not necessary in the present study, however, because the viability under benign conditions was very high, *i.e.*, near the maximum possible under the experimental conditions, and not significantly different between lines (average viability = 0.87, range over lines = 0.75-0.98, see CHAPTER 4).

We used Kruskal-Wallis tests to analyze differences in viability, with Dunn-Şidak correction to adjust the significance levels for multiple testing (Sokal & Rohlf 1995). To test for the effects of stress, subdivision and their interaction at the population level (*i.e.*, six metapopulations *versus* six undivided populations), we applied the Scheirer-Ray-Hare extension of the Kruskal-Wallis test that enables a non-parametric two-way ANOVA. All tests were performed with Statistix (version 8.0).

INDIVIDUAL-BASED SIMULATIONS

Simulations allow us to systematically evaluate the effects of subdivision on the adaptive response for a wide range of relevant parameters. The resulting framework creates baseline expectations that facilitate the interpretation of the experimental results. In addition, we used simulations to generate expectations on the variance in fitness before and after adaptation to stress conditions for different levels of gene flow, varying from minimal (*i.e.*, completely isolated demes) to maximal (*i.e.*, an undivided population with the size of a metapopulation) levels and different selection pressures.

The simulation model mimics the experimental setup (*i.e.*, 40 generations of subdivision in a standard environment followed by six generations of exposure to a stress environment) and is parameterized for the mating system of *Drosophila melanogaster* (*i.e.*, lottery polygyny, female remating, variation in female reproductive success, see CHAPTERS 2, 3 and 4 for details). The impact of stress is implemented as viability selection in

the zygote life stage. We assume an additive model with selection coefficient s and per-locus viabilities 1, $1 - s/2$ and $1 - s$ for the genotypes *AA, Aa* and *aa* in case of non-lethal alleles. For a recessive lethal allele, the viabilities are 1, 1 and 0, respectively.

In the initial stage of a simulation run, we let both the metapopulations and the undivided populations evolve for 40 generations in the absence of selection and with gene flow through migration in the metapopulations as in the original experimental setup. Since previous simulations never yielded large differences between migrant-pool and stepping-stone migration (CHAPTER 4), we only looked at migrant-pool migration. We used the experimental average deme size $N = 50$, but in contrast with the experimental average size of undivided populations ($N = 220$) that turned out lower than planned, we used $N = 300$, *i.e.*, the size of an entire metapopulation (6 x 50), for the undivided populations. In the final stage, we increased the size of each deme to $N = 300$ and disabled migration (*cf.* setting up the experimental lines), and we subjected all resulting "lines" to viability selection for six generations as in the adaptation experiment.

We implemented four different genetic architectures comprising either polygenic traits regulated by few (5) or many (20) loci, or traits regulated by one major gene with "normal" additive variation or a recessive lethal allele at a single locus. For the single gene traits, the selection coefficients of $s = 0.1$, $s = 0.4$ and $s = 0.7$ represent additive selection of increasing strength, while recessive lethals correspond to $s = 1$. For the polygenic traits, the k loci are unlinked with equal selection coefficients $s_k = s/k$ per locus. Fitness is calculated multiplicatively across loci (Hedrick 1994), and is similar to the corresponding values of the single gene traits. We ran simulations for a range of migration rates m (0, $0.1/N$, $1/N$, $10/N$ and 1), regarding the undivided populations as subdivided populations with unlimited migration, *i.e.*, $m = 1$. We ran 1000 replicate runs of a single metapopulation adding up to 6000 replicate demes, or 6000 replicate runs of undivided populations.

RESULTS

In figure 5.1 we summarize the average viabilities in all lines before adaptation (top) and after adaptation (bottom). The migrant-pool metapopulations did not differ significantly from the stepping-stone metapopulations with regard to viability (Kruskal-Wallis tests, adjusted $\alpha = 0.0085$) for either of the three stress factors, both before (high temperature: $H = 0.98$, $P = 0.32$, salt: $H = 0.17$, $P = 0.68$, ethanol: $H = 0.45$, $P = 0.50$) and after (high temperature: $H = 0.17$, $P = 0.68$, salt: $H = 5.2$, $P = 0.023$, ethanol: $H = 0.064$, $P = 0.80$) adaptation. The single significant difference for salt stress after adaptation becomes non-significant upon correction for multiple testing. Visual inspection of the data also confirms that the spatial configuration of the metapopulations (island *vs.* stepping-stone) had no systematic effect on the outcome of the experiments. In the rest of this chapter we therefore pool the results of both spatial configurations into the single category "metapopulation" (*i.e.*, subdivided populations as opposed to undivided populations).

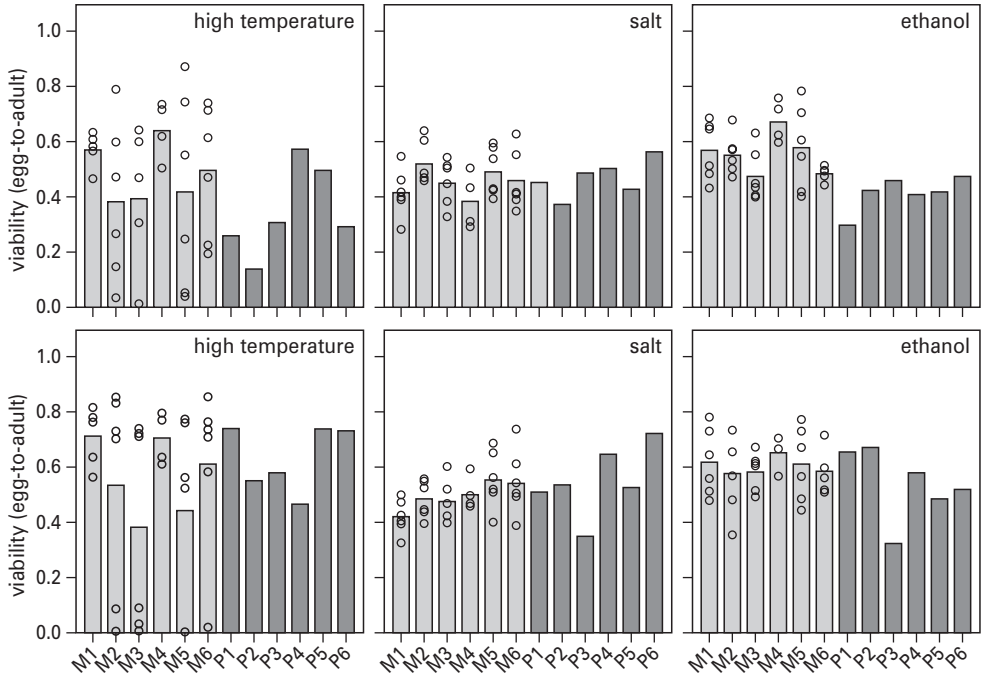


Figure 5.1. Viability in three stress environments before (top) and after (bottom) six generations of adaptation. The bars show the average viability for each metapopulation (M1-M6, light grey) and for each undivided population (P1-P6, dark grey). The circles indicate the viability for the six demes within a metapopulation. M1-M3 are the stepping-stone metapopulations, and M4-M6 are the migrant-pool metapopulations.

VIABILITY BEFORE ADAPTATION

As all lines showed a similar fitness in the control environment, we assessed the viability at first exposure as a measure of the tolerance to a novel environment, which is the prerequisite for an adaptive response to environmental challenge. The viability in the stress environments varied greatly around values of *circa* 0.5 and 0.4 for the subdivided and undivided populations, respectively (fig. 5.1, top panels), a substantial reduction when compared to the value of 0.86 obtained under standard conditions (CHAPTER 4). The large scatter of the open circles shows that the viability in the six demes within a metapopulation differed considerably, particularly for high temperature stress. These differences are mostly significant even after correction for multiple testing (table 5.1). Hence, population subdivision has resulted in significant variation in tolerance to the novel environments among demes. Increased genetic drift in the small demes resulting in genetic differentiation among the demes within a metapopulation might explain this large variation. The variation among demes is highest for the high temperature stress, where some demes show near absolute mortality, whereas others show more than 80% survival. This outcome may reflect the presence of conditionally detrimental alleles

Table 5.1. Average viability (± SD) in the metapopulations (M1-M6) at first exposure (top) and after adaptation (bottom) to three stress environments. * indicates a significant[1)] difference in viability among the demes within a metapopulation. In addition, the average viabilities (± SD) in the six metapopulations (M1-6) and in the six undivided populations (P1-6) are given. # indicates a significant[2)] difference between metapopulations and undivided populations.

Metapopulation	High temperature		Salt		Ethanol	
M1	0.56 (± 0.06)	n.s.	0.41 (± 0.09)	*	0.56 (± 0.11)	*
M2	0.38 (± 0.28)	*	0.52 (± 0.08)	*	0.55 (± 0.07)	n.s.
M3	0.39 (± 0.23)	*	0.45 (± 0.08)	*	0.47 (± 0.09)	n.s.
M4	0.64 (± 0.10)	n.s.	0.38 (± 0.10)	n.s.	0.67 (± 0.08)	*
M5	0.41 (± 0.36)	*	0.49 (± 0.09)	*	0.57 (± 0.15)	*
M6	0.49 (± 0.24)	*	0.45 (± 0.11)	*	0.48 (± 0.02)	n.s.
M1-6	0.48 (± 0.11)	n.s.	0.45 (± 0.05)	n.s.	0.55 (± 0.07)	#
P1-6	0.34 (± 0.16)		0.46 (± 0.07)		0.41 (± 0.06)	
M1	0.70 (± 0.12)	*	0.42 (± 0.06)	n.s.	0.61 (± 0.12)	*
M2	0.53 (± 0.38)	*	0.48 (± 0.07)	n.s.	0.59 (± 0.16)	*
M3	0.38 (± 0.37)	*	0.47 (± 0.07)	n.s.	0.58 (± 0.07)	n.s.
M4	0.70 (± 0.09)	*	0.50 (± 0.06)	n.s.	0.65 (± 0.06)	n.s.
M5	0.43 (± 0.35)	*	0.55 (± 0.10)	*	0.61 (± 0.13)	*
M6	0.61 (± 0.30)	*	0.54 (± 0.12)	*	0.57 (± 0.08)	n.s.
M1-6	0.56 (± 0.13)	n.s.	0.49 (± 0.05)	n.s.	0.60 (± 0.03)	n.s.
P1-6	0.63 (± 0.11)		0.54 (± 0.13)		0.54 (± 0.13)	

[1)] Kruskal-Wallis tests, adjusted significance level $\alpha = 0.0028$.
[2)] Kruskal-Wallis tests, adjusted significance level $\alpha = 0.017$.

approaching fixation in some demes while the same alleles may have disappeared from other demes due to genetic drift.

As the possible presence of a highly detrimental allele being only expressed at 29°C was totally unexpected, we recently tested the original marker stocks that were at the basis of all experimental populations for the detrimental effect. The viability of the two marker stocks *bw*^{75}*;st* and *bw;st* was determined at both 25°C and 29°C (five replicates of 100 eggs for each stock and each treatment). The results (mean viability ± SE) for *bw*^{75}*;st* were 0.67 ± 0.15 and 0.51 ± 0.03 for 25°C and 29°C, respectively. For *bw;st* viability was 0.71 ± 0.03 at 25°C, but this dropped to 0.09 ± 0.07 at 29°C. This clearly shows that the *bw;st* stock carries one or more detrimental alleles that are only expressed at 29°C. Based on previous results (CHAPTER 4) we infer that this character is (mostly) recessive.

We use the average of the six demes within a metapopulation to analyze the tolerance at the level of entire metapopulations *versus* that of undivided populations. The difference is only significant for ethanol (Kruskal-Wallis tests, adjusted $\alpha = 0.017$, high temperature: $H = 2.1$, $P = 0.15$, salt: $H = 0.10$, $P = 0.75$, ethanol: $H = 8.3$, $P = 0.004$). Interestingly, the tolerance to ethanol is higher in the subdivided than in the undivided populations. Comparable with the among-deme level of variation, the variation among populations is by far the highest for high temperature stress.

VIABILITY AFTER ADAPTION

After six generations of exposure to the stress environments, we assessed the viability again to detect potential changes due to adaptation. On average, the viability has increased (fig. 5.1, bottom panels), but the variation among demes is still considerable (open circles). The variation among demes in tolerance to high temperature has not decreased but seems to have become even more pronounced, with viabilities that either have increased to near maximum, or are found to be near zero. This latter outcome suggests that most probably a detrimental allele had become fixed in demes where the viability is low. These demes barely survived six generations of exposure to high temperature, and we often had to rely on the backups kept at 28°C to prevent extinction. Their viability did not only not increase, but occasionally even decreased, which may be simply due to experimental variation.

At the metapopulation level, the differences between subdivided and undivided populations have become smaller than those observed before adaptation, and they are not significant for any of the stress environments (Kruskal-Wallis tests, adjusted $\alpha = 0.017$, high temperature: $H = 1.6$, $P = 0.20$, salt: $H = 0.92$, $P = 0.34$, ethanol: $H = 0.42$, $P = 0.64$).

CHANGE IN VIABILITY

Figure 5.2 (top panels) shows the extent of adaptation to the stress environments during the six generations of exposure. We observe substantial differences among the demes within the metapopulations with respect to the change in viability, again particularly for the high temperature stress. In some demes the viability readily improved, whereas in others no improvement occurred at all.

At the metapopulation level (fig. 5.2, bottom panel), the increase in viability was always more pronounced in the undivided than in the subdivided populations. However, given the large variation observed, these differences are not significant when testing each stress environment separately (Kruskal-Wallis tests, adjusted $\alpha = 0.017$, high temperature: $H = 3.7$, $P = 0.055$, salt: $H = 1.6$, $P = 0.20$, ethanol: $H = 1.3$, $P = 0.26$). Testing the three environments combined in a Kruskal-Wallis ANOVA indicates that a significant part of the variation between metapopulations and undivided populations may be due to subdivision ($H = 5.87$, $df = 1$, $P = 0.02$), whereas environment ($H = 2.97$, $df = 2$, $P = 0.23$) and the interaction ($H = 0.31$, $df = 2$, $P = 0.86$) were not significant.

INDIVIDUAL-BASED SIMULATIONS

Figure 5.3 shows the expected effect of population subdivision on stress tolerance (top panels) and adaptation to a stress environment (middle panels), while the bottom panels show the impact of degree of subdivision ($m = 0$, $0.1/N$, $1/N$, $10/N$ and 1), strength of selection ($s = 0.1$, 0.4, 0.7) and genetic architecture (one major gene, polygenic traits regulated by five and 20 loci). The results for 20 loci (not shown) are very similar to the results for five loci, but the quantitative differences in viability are even smaller. Figure 5.4 shows the expected effect of subdivision on tolerance (left) and adaptation (middle) for the special case of selection against a recessive lethal. We can distinguish three main

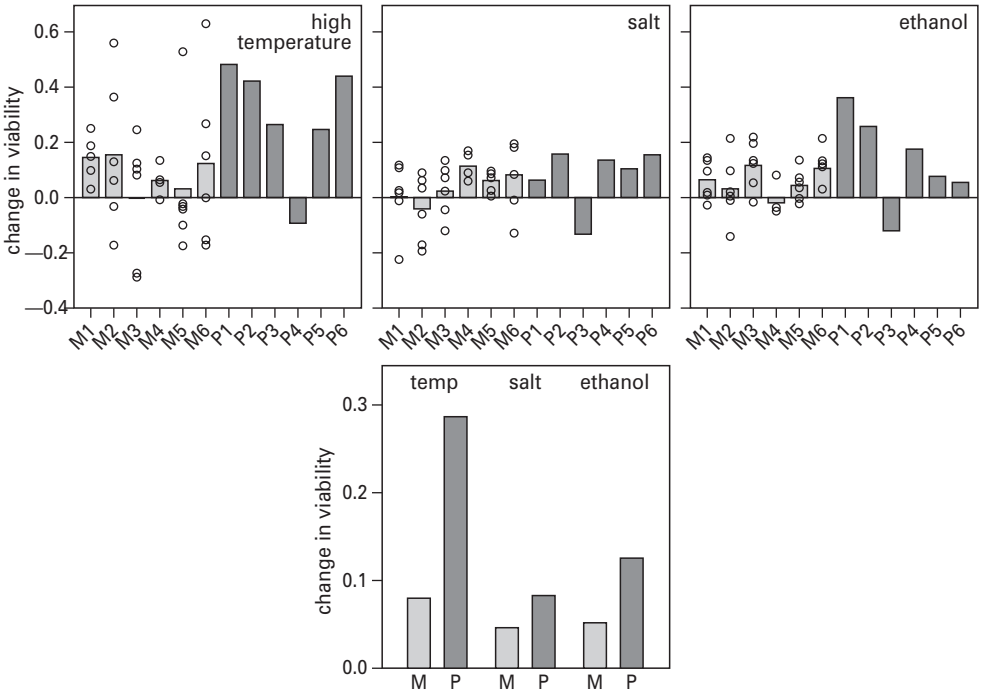


Figure 5.2. Effect of six generations of adaptation on the viability in three stress environments. Top panels: the bars show the average increase or decrease of viability for each metapopulation (M1-M6, light grey) and for each undivided population (P1-P6, dark grey). The circles indicate the change in viability per deme. The bottom panel shows the average change in viability for the metapopulations (light grey) and undivided (dark grey) populations for each environment (note the differently scaled y-axis).

trends. First, the average viability does not change, but the variation in viability among demes decreases substantially when migration rates increase (fig. 5.3, top panels), demonstrating that high migration rates are more efficient in mitigating the effect of local genetic drift on the differentiation among demes within a metapopulation. Second, the efficiency of the adaptation process increases considerably with increasing strength of selection, as indicated by the increase of the median values (fig. 5.3, middle *versus* top panels). Third, the variation becomes lower as the number of loci regulating a trait increases, since the extreme phenotypes are rare when large numbers of unlinked loci with small effects per locus affect a trait (Macnair 1991). In the special case of the recessive lethal (fig. 5.4, left hand plots), a considerable number of demes obviously became extinct at the start of the adaptation process in populations with little or no migration. In these demes, the conditionally lethal allele has become fixed, hence the tolerance to the stress factor is zero. Excluding the extinct demes from the analyses (right hand plots) suggests that almost all populations will rapidly attain maximum fitness due to very strong selection resulting in near complete removal of the deleterious allele.

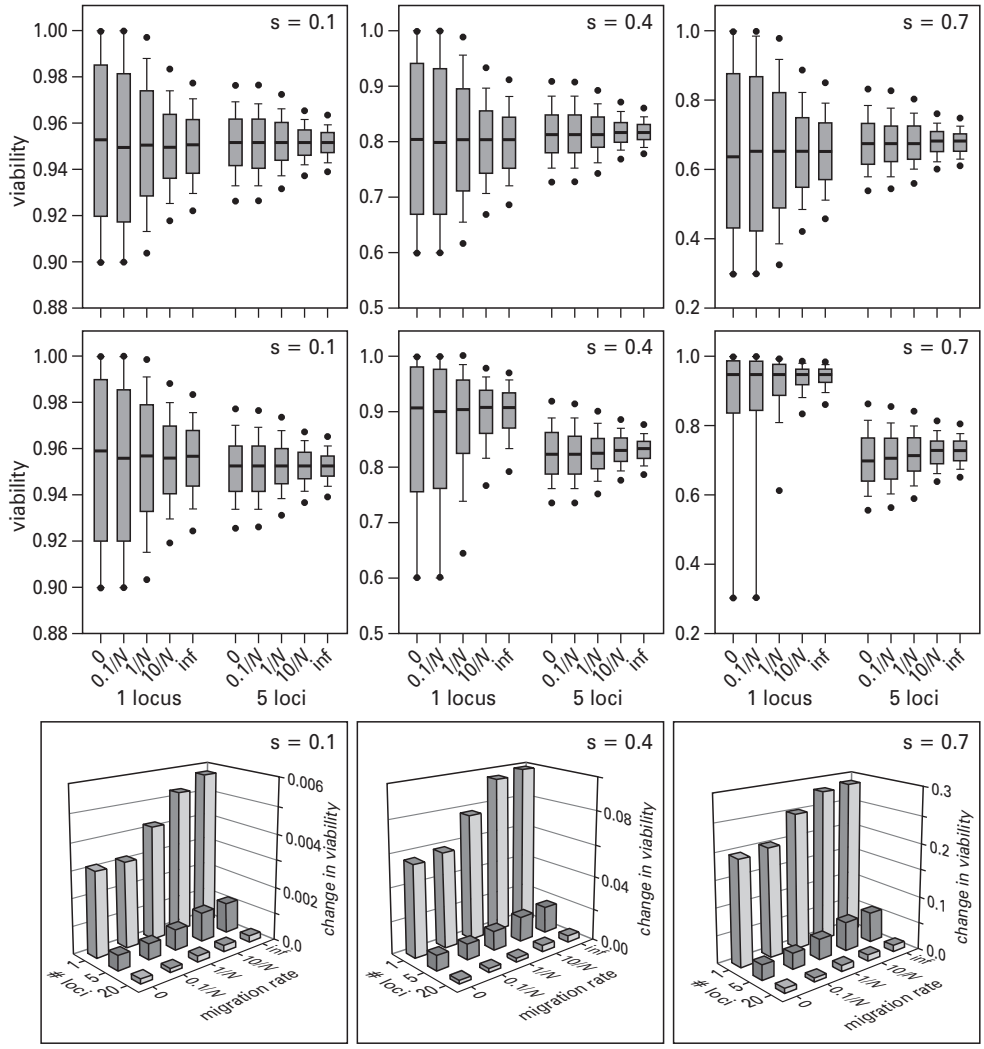


Figure 5.3. Computer simulations indicating the expected effect of population subdivision on initial tolerance to stress (top) and to tolerance after six generations of adaptation to a stress environment (middle). Selection strength (s = 0.1, 0.4, 0.7) increases from left to right. Each panel shows the results of decreasing levels of subdivision (m = 0, $0.1/N$, $1/N$, $10/N$ and 1) for two genetic architectures (major gene and polygenic trait). Bars: interquartile range with median, whiskers: 75% range, dots: 90% range. The bottom panels show the expected effects of subdivision on the adaptive response (*i.e.*, the change in viability) after six generations exposure to a stress environment for increasing selection strength for all three genetic architectures (major gene, polygenic traits regulated by 5 and 20 loci, respectively).

The bottom panels of figure 5.3 summarize the expected effects of six generations of adaptation on viability with regard to population subdivision, strength of selection and genetic architecture. The resulting increase in viability is qualitatively similar for all

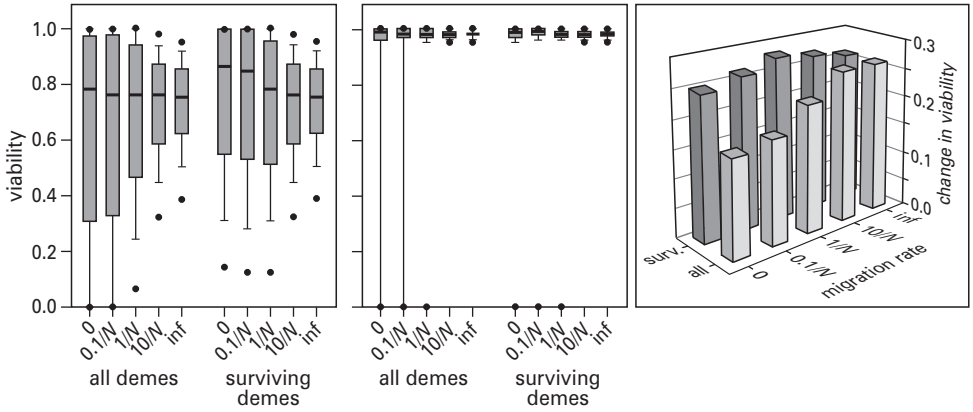


Figure 5.4. Computer simulations indicating the expected effect of population subdivision on initial tolerance (left), and tolerance (middle) and adaptive response (right) after six generations of exposure to a stress environment for strong selection against a recessive lethal allele. The results are analysed for all demes (light grey), and excluding demes with zero initial tolerance (dark grey, see text for details). Bars: interquartile range with median, whiskers: 75% range, dots: 90% range.

three selection strengths, but the magnitude of the change increases with increasing selection intensity. For polygenic traits the selection intensity per locus is low, hence the more loci are affecting the trait, the longer it will take to substantially increase viability, and thus, to properly adapt to a new environment. For a single locus, limited gene flow within a population may result in allele fixation, and thus, in the lack of adaptive potential, in a considerable number of demes, explaining the relatively large effect of the degree of subdivision on the increase of viability. The results for the single-locus eye-colour marker used in the experimental populations (CHAPTER 4) confirm that allele fixation within demes commonly happens when migration rates are low. The right hand panel of figure 5.4 shows similar results for the recessive lethal as for the single locus trait, in particular when all demes are considered (light grey bars).

DISCUSSION

Our study investigates the effects of population subdivision on the adaptive response for traits with various genetic architectures. Most studies testing the effects of genetic bottlenecks and a subsequent loss of genetic variation on the potential to adapt to new environments have used traits that are not or only indirectly related to fitness. Studies on plants, butterflies and *Drosophila* using morphological traits showed that the adaptive potential decreases with increasing bottleneck severity (Whitlock & Fowler 1999, Saccheri *et al.* 2001, Swindell & Bouzat 2005, Briggs & Goldman 2006). Fitness-related traits often suffer from inbreeding depression, which may seriously complicate the results as it may be difficult to separate extrinsic (environmental stress) and intrinsic

(genetic stress) causes. Frankham and co-workers (Frankham *et al.* 1999, England *et al.* 2003) used time to extinction to study the adaptive potential of bottlenecked populations of *Drosophila* exposed to increasing salt concentrations. They found a negative correlation between the severity of the bottleneck and the time to extinction under increasing stress levels. However, they did not attempt to separate the effects of the loss of fitness due to inbreeding (*i.e.*, the survival upon first exposure to stress) from the loss of adaptive potential due to the lack of genetic variation. Nevertheless, it is clear from all these studies that the loss of genetic variation due to genetic bottlenecks can be a significant limiting factor for adaptation under changing conditions. Hence, we would expect lower levels of tolerance and adaptive potential for the metapopulations than for the undivided populations in our experimental setup, since population fragmentation might easily have resulted in genetic bottlenecks for single demes.

Based on our simulation results, we also expect the variation in viability to be lower among the undivided populations (unlimited migration, $m = 1$) than among the subdivided populations (low migration, $0.5/N < m < 1.3/N$), and to increase from salt stress (polygenic) to ethanol stress (major gene) to high temperature stress (recessive lethal) for our experimental setup. After six generations of adaptation, we would expect a high but variable response to high temperature stress and a high response to ethanol stress, but a low response to salt stress because of its polygenic character.

INITIAL TOLERANCE

Surprisingly, the tolerance to ethanol and high temperature was on average lower for the undivided populations than for the metapopulations, although the difference was not significant in the latter case. This is unexpected, since the simulation results (fig. 5.3, top panels) indicate that different levels of gene flow affect only the variation in viability, but not the average viability at first exposure. Although the experimental conditions differed between the metapopulations and the undivided populations (demes were kept in vials and undivided populations were kept in bottles), the uniformly high viability (0.87 ± 0.01, SE over line means) under standard conditions (CHAPTER 4) indicates that both types of population were optimally adapted to the standard laboratory environment prior to the current experiment. However, different sample sizes (36 vials *versus* 6 bottles) may have resulted in different initial allele frequency distributions that are not expressed under standard conditions. In practice, other factors than the genetic make-up, which were not included in the simulations, may affect the variation at a locus, for example crowding or development time affecting variation at the *Adh*-locus (Van Delden & Kamping 1979).

First exposure to the stress environments resulted in substantial variation in tolerance among the demes. In line with the expectations based on the simulations, the variation is smallest for tolerance to salt, which is a polygenic trait. The variation in tolerance to ethanol is less than might be expected for a trait regulated by a single major gene. However, although the *Adh* gene is involved with tolerance to ethanol as a major gene, other genes play a role as well (Chakir *et al.* 1996, Malherbe *et al.* 2005), hence ethanol resistance may behave more like a polygenic trait regulated by a small number of loci.

The simulations predict lower levels of variation for such a system. Although resistance to high temperature is generally regarded to be a polygenic trait (Loeschcke *et al.* 1997), temperature-sensitive, highly detrimental alleles are well known in *Drosophila* (Lindsley & Grell 1968, Suzuki 1970). Such recessive, highly detrimental alleles that are only expressed at high temperatures represent an extreme case of this genotype-by-environment interaction, and are relatively frequent (Oudman *et al.* 1991, Bijlsma *et al.* 1999, Vermeulen & Bijlsma 2004). The high variation in tolerance to high temperature results from the presence of the conditionally expressed detrimental allele that was observed in the *bw;st* marker stock, and is in line with the expectations based on the simulations.

The results for stress tolerance imply in general that substantial amounts of genetic diversity may be preserved in subdivided populations, although the distribution among demes, and hence the tolerance level per deme, will vary considerably. In other words, the available genetic variation within demes became redistributed to variation among demes within a metapopulation due to population subdivision, resulting in greatly increased variation in initial tolerance among demes.

ADAPTIVE RESPONSE

The change in viability after six generations of adaptation to environmental stress conditions is not significantly different between the metapopulations and the undivided populations for the individual stresses, although the simulations predict a higher adaptive response for higher levels of gene flow, *i.e.*, when populations are more connected. For the salt and ethanol environments, the response in either type of population is small (5-10%) considering the relatively large selection intensity that initially allowed only 50% of the individuals to survive. In line with these observations, the simulation results (fig. 5.3, bottom panels) also indicate that the adaptive response is generally very small for polygenic traits. The low response in the ethanol treatment may be partly explained by the fact that 10% ethanol may exert only little selection when the flies lay eggs directly on the food (Bijlsma-Meeles 1979). As such, the system for ethanol might have reacted as a polygenic system rather than a single locus system.

The adaptive response varied substantially for all populations, ranging from no response at all to almost maximal improvement. There are several possible explanations for such a lack of adaptation, of which the most likely are either the lack of sufficient genetic variation in a population to adapt, or the selection pressure being too low to affect a population where the tolerance level is already high. Since the intensity of the stress was kept constant during the adaptation process, the resulting selection pressure may have been different for each population because the initial tolerance also varied considerably. Thus, populations with low initial tolerance will have experienced much stronger selection than populations with moderate to high initial tolerance levels. On the other hand, populations with a high level of initial tolerance have already attained near maximal fitness and can hardly show any adaptive response.

The fact that differences in initial tolerance both affect the selection pressures and limit the maximal adaptive response presents a general problem for this kind of experiments. To provide more insight into these effects, figure 5.5 shows the relationship

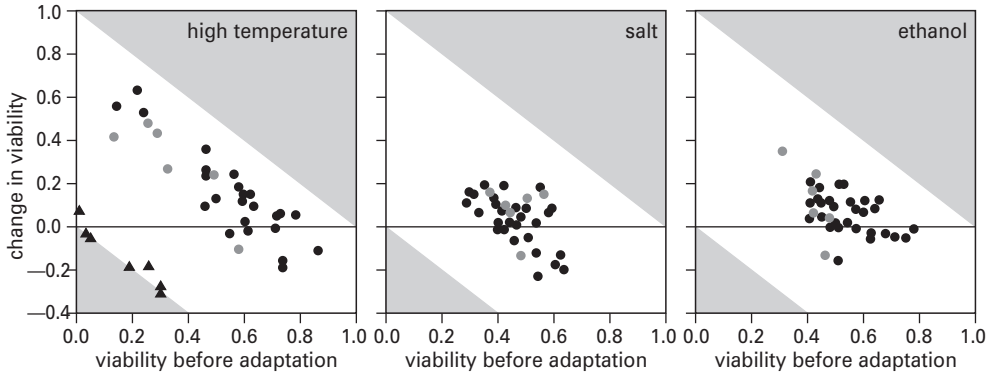


Figure 5.5. Relationship between initial tolerance and adaptive response to three stress environments. The white area comprises all possible outcomes of the relationship, and the horizontal lines indicate zero change. Black circles indicate demes and grey circles indicate undivided populations. Triangles indicate the demes with very low tolerance to high temperature.

between initial tolerance and adaptive response for all populations. For the salt and ethanol treatments, most populations are clustered in the centre of the "adaptation space", indicating moderate to high initial tolerance and the presence of weak to moderate selection pressures. Although the initial tolerance as such leaves some room for fitness improvement, the two traits nevertheless show little adaptive increase of viability. This is consistent with a polygenic architecture underlying these traits, and in line with the simulation results. As selection pressures on individual genes are expected to be small in case of a polygenic architecture, six generations of adaptation might be too short to obtain distinctive results. In this context one should realise that we set stress levels such that the populations were tolerant enough initially to maintain themselves without any adaptation. The high temperature treatment shows a completely different pattern. A small number of populations (triangles) with a very low initial tolerance near to the lower boundary of the adaptation space did not improve viability at all, although the selection pressure must have been high for these populations. Even though we have not done the formal genetics, there is little doubt that these populations had become fixed for the detrimental observed at 29°C in the *bw;st* marker stock. All other populations are more or less aligned along the upper boundary of the adaptation space, indicating that they had all attained near maximal viability despite considerable variation in initial tolerance. Again, this is most likely explained by assuming that the populations with low initial tolerance had attained high frequencies of the detrimental but had not become fixed for it. In this situation natural selection at high temperature can be very effective and rapidly decrease the frequency of this conditionally expressed detrimental, thereby greatly improving viability. The observed pattern aligns well with the results of the simulations as depicted in figure 5.4.

GENERAL IMPLICATIONS

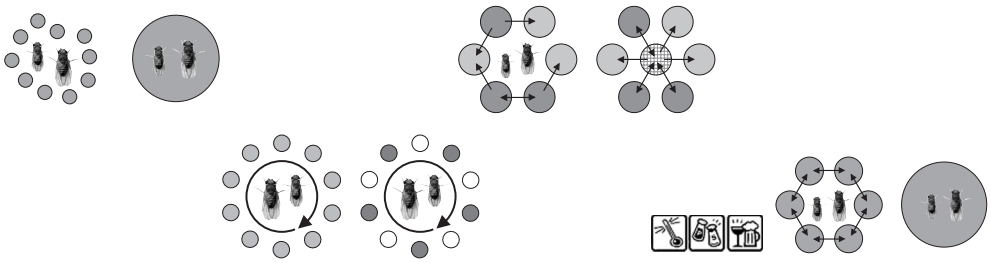
Our findings with regard to high temperature resistance confirm and extend the conclusion of Reed and co-workers (2002) that lethal and highly detrimental alleles of large effect, *i.e.*, acting as major genes, are the likely cause of the large lineage effects they observed. We found similar large variation among our experimental demes, but we were also able to distinguish between initial zero tolerance due to fixation of near-lethal deleterious alleles preventing population survival at first exposure to stress, and the adaptive response of the remaining tolerant populations.

We found a clear effect of subdivision on tolerance resulting in large variation among demes instead of within demes due to increased genetic differentiation (*i.e.*, redistribution of genetic variation), which is supported by the results from simulations. In practice, such large variation among demes implies that many demes within a metapopulation are potentially at risk from a lack of adaptive potential, or more importantly, from having become fixed for the wrong alleles, whereas none of the undivided populations did become fixed for these alleles in the same time frame. The results for high temperature stress where fixation of conditionally expressed detrimental alleles might lead to near-zero tolerance, and thus, population extinction at first exposure, illustrate the importance of the genetic architecture of a trait for its potential adaptive response. Unlike normally expressed detrimental alleles of large effect that are generally efficiently purged from small populations (Wang *et al.* 1999, Glemin 2003), conditionally expressed alleles of large effect may have unexpected, negative effects in small populations (Ross-Gillespie *et al.* 2007). The simulation results indicate that this is of particular importance when migration rates are too low to mitigate the loss of genetic diversity due to allele fixation.

The simulation results indicated that the adaptive response is generally larger on average for undivided than for subdivided populations because there is no genetic differentiation in the former, although we did not always observe this in the experimental situation where undivided populations occasionally showed lower initial tolerance and adaptive response. These predictions are in accordance with the expectations from the literature on bottlenecked populations (Whitlock & Fowler 1999, Saccheri *et al.* 2001, Swindell & Bouzat 2005, Briggs & Goldman 2006). In short, our results indicate that habitat fragmentation may cause the adaptive response of single demes to vary substantially because the genetic variation needed to adapt becomes distributed among rather than within the demes in a metapopulation. This becomes particularly important when major genes are involved that deviate from standard assumptions, for instance by the presence of conditionally expressed detrimental alleles.

CHAPTER 6

Summarizing discussion



In the present-day times where natural environments are heavily manipulated, metapopulations will inevitably arise as a consequence of habitat fragmentation. Such metapopulations comprise of small-sized, partially isolated demes suffering from temporary extinctions but connected to the collective through migration. A considerable body of theory concerning the dynamics of genetic variation and evolution of metapopulations has been developed (*e.g.*, reviews in Barton & Whitlock 1997, Wang & Caballero 1999, Pannell & Charlesworth 2000, Rousset 2004, chapters in Hanski & Gaggiotti 2004). However, most models are highly abstract and based on many simplifying and often unrealistic assumptions. The validity of predictions based on these models have rarely been evaluated under experimental, let alone natural, conditions. Nevertheless, in conservation practice such models are increasingly applied to analyse the demography and dynamics of natural populations and to estimate crucial population parameters that are subsequently used to develop management measures (*e.g.*, management of fish stocks, Hansen *et al.* 2002, Tufto & Hindar 2003, or management of endangered species, Gao & Zhang 2005, Jamieson *et al.* 2006, Bohme *et al.* 2007).

The purpose of this thesis is to provide experimental validation of population genetic models in a metapopulation context. To this end, I used the classic work of Buri (1956) on the Wright-Fisher model of genetic drift as a starting point to set up experimental metapopulations of increasing complexity. I examined the consequences of metapopulation structure for the dynamics of genetic diversity and differentiation in general, and for genetic processes such as genetic erosion and local adaptation in particular. I focused on the implications of metapopulation dynamics for the inference of demographic parameters such as effective population size and migration rate. In the next section, I summarize my findings and discuss the results in the context of my study. In the final section, I present the final conclusions and discuss the contribution of my results in a wider perspective, such as the application in conservation biology.

MAIN RESULTS FROM THIS STUDY

The main approach of this study involved the comparison of results from experimental metapopulations with results from individual-based computer simulations. The simplest experimental metapopulation configuration comprised five replicated sets of ten isolated demes without gene flow between the demes. The experiments based on this setup focused on the effects of genetic drift, and provided baseline values for all subsequent experiments (CHAPTER 2). In a subsequent step I looked into the combined effects of genetic drift and gene flow by adding unidirectional circular stepping-stone migration to the ten-deme metapopulations (CHAPTER 3). In the next series of experiments I focused on the combined effects of genetic drift, migration and population turnover through local extinction and subsequent founder events in six-deme metapopulations. These metapopulations were arranged in two spatial configurations that enabled bidirectional stepping-stone migration and migrant-pool migration, respectively (CHAPTER 4). Although migration will follow a stepping-stone rather than a migrant-pool model in

Box 6.1 The main findings of this thesis summarized per chapter

CHAPTER 2 – RANDOM GENETIC DRIFT

- Under standardized conditions the replicate metapopulations diverged substantially. As a consequence, even a sample of ten demes did not provide a representative picture of the entire metapopulation consisting of 50 demes.
- The effective population size was only about half of the census size. Even in a standardized laboratory environment the loss of genetic variation is strongly affected by the mating system and variance in female reproductive success.
- Many commonly used "snapshot" estimators of effective population size substantially overestimated Ne.
- The phenotypic eye colour marker chosen for its presumed neutrality was actually subject to selection, of which the strength depended on experimental details.

CHAPTER 3 – GENE FLOW: UNIDIRECTIONAL STEPPING-STONE MIGRATION

- Despite the high level of standardization, replicate metapopulations differed considerably from each other, leading to a large variation in the estimates of migration rates for the same migration scenario.
- When individuals mated before migration, the level of gene flow differed substantially between male and female migration. The effective number of migrants was reduced by 50% in the unidirectional stepping-stone setup in comparison with the island model of migration.
- Even though gene flow was sufficient to mitigate genetic differentiation, average fitness declined substantially in the course of the experiment.

CHAPTER 4 – POPULATION TURNOVER: LOCAL EXTINCTION AND RECOLONAZATION

- Population turnover increased stochasticity at the metapopulation level, resulting in a more rapid loss of diversity and higher levels of differentiation than without local extinction. Therefore, the effective metapopulation size decreased much faster in the presence than in the absence of population turnover.
- Despite striving for constant environmental conditions, migration and colonization rates fluctuated considerably between generations, resulting in very high variation among replicates in the presence of population turnover.
- The cost of migration was considerably higher in the migrant-pool than in the stepping-stone configuration, resulting in some notable differences in the dynamics of genetic variation. However, none of these were statistically significant, presumably due to the high variation among replicates.
- Population fitness and stress tolerance showed substantial interdemic variation, indicating that the genetic variation in a metapopulation can get very unevenly partitioned over the demes in the presence of population turnover.

CHAPTER 5 - LOCAL ADAPTATION TO ADVERSE ENVIRONMENTAL CONDITIONS

- Population fragmentation resulted in substantial inter-deme variation in stress tolerance and adaptive responses. Contrary to expectation, stress tolerance and adaptive response were higher in the fragmented populations than in large undivided populations on some occasions .
- The consequences of fragmentation for the adaptive response to adverse environmental conditions depended strongly on the genetic architecture of the traits involved in the stress response.
- Fixation of conditionally expressed near-lethal alleles in some demes was mainly responsible for the large variation in adaptive response to high temperature stress.

many natural habitats with a linear character, such as river banks or road verges, migrant-pool migration is the default assumption in many theoretical models that derive from Wright's (1931, 1951) island model of migration. I explored whether, and to what extent these different migration models affected the dynamics of genetic variation. In the final experiment I looked into the consequences of population fragmentation for the potential of a population to adapt to adverse environmental conditions (CHAPTER 5). The main findings from all experiments are summarized in box 6.1, and will be discussed further in the next sections.

DRIFT *VERSUS* MIGRATION

The findings in the first series of experiments (CHAPTERS 2 & 3) illustrate that inferences of demographic parameters from genetic data can be unreliable, in particular when presumed neutral variation is not neutral. The evolution of a metapopulation is strongly affected by mating system, variation in reproductive success, migrant sex, the timing of migration, and the spatial configuration of subpopulations.

The results of Buri's (1956) classical study on the evolutionary dynamics of eye colour mutants in small populations are often cited as the text-book example illustrating the effects of genetic drift on neutral variation (*e.g.*, Hartl & Clark 1997, Hedrick 2000). Therefore, I decided on purpose to use a similar experimental setup for my metapopulation experiments. In order to avoid differences between lines due to additional fitness effects that occur commonly in mutants, I used two different mutant lines of flies instead of one mutant and one wild type line to avoid. Despite these precautions, however, I found evidence of significant directional selection in favour of the bw^{75}-allele, possibly caused by differences in activity between the bw^{75}*;st* and *bw;st* lines (CHAPTER 2). The lower activity of white-eyed flies is possibly related to the 24-hour exposure to light in my experiments, since flies lacking eye colour pigmentation are known to be more sensitive to bright light (Reed & Reed 1950). As a consequence of the selection advantage of the bw^{75}-allele, the patterns of genetic diversity and differentiation deviated substantially from neutral predictions. The effect of selection was not straightforward, since selection turned out to be frequency- and density-dependent. Moreover, the strength of selection depended on the context, such as the type of container (vial *versus* bottle) and the absence or presence of migration. The standardized setup of the experiments and the close monitoring of the populations allowed me to correct for selection and to obtain reliable estimates of key parameters like effective poplation size. In natural systems this would be much more difficult, if not impossible, since the required information is hard to obtain. The possibility of directional selection should not be neglected in practical applications. The genetic markers used in such applications are presumed to be selectively neutral, but proof of neutrality is usually lacking. It is well-known that markers like microsatellites may be the target of selection (Charlesworth *et al.* 1997, Vitalis *et al.* 2001). Even if this is not the case, a marker may be affected by selection if it is closely linked to a selectively advantageous or a selectively deleterious allele (hitchhiking; *e.g.*, Schug *et al.* 1998, Schlotterer 2000).

In many applied studies (see box 6.2) the effective population size N_e and the effective number of migrants N_em are estimated to infer the level of gene flow between

subpopulations, and to predict the risk of genetic erosion when the exchange of migrants is insufficient. Most estimators of N_e are temporal estimators based on only a few samples in time ("snapshot-estimators", *e.g.*, Waples 1989, Anderson *et al.* 2000, Wang 2001, Berthier *et al.* 2002), since estimates based on the actual per-generation change of, for example, heterozygosity (eigenvalue effective size) or allele frequencies (variance effective size) are generally not possible for natural populations. In contrast, my experimental setup allowed for per-generation monitoring of these changes and subsequent estimates of the eigenvalue and variance effective population sizes based on linear regression (CHAPTER 2). Compared with these variance effective sizes, many of the commonly used snapshot-estimators overestimated N_e, which is likely to result in biased interpretations of the status of a metapopulation. My simulation studies revealed that the eigenvalue effective size is very sensitive to the presence of directional selection. This suggests that the concept of eigenvalue effective size only applies to neutral variation.

The effective number of migrants N_em is commonly estimated from F_{ST} or related measures of genetic differentiation (*e.g.*, Nei 1973, Weir & Cockerham 1984, Excoffier *et al.* 1992) based on classic population genetic models such as Wright's island model of migration. The large variation among replicate metapopulations generally resulted in wide ranges of estimates of N_em for the same migration scenario (CHAPTER 3). The unidirectional stepping-stone model of migration yielded estimates of N_em that differed substantially from estimates based on the island model. In addition, sex-biased migration taking place after mating resulted in large differences between estimates of N_em. These findings indicate that discrepancies between the natural system and the assumed theoretical model can easily result in biased estimates of demographic parameters. Such biased estimates will not present a real problem as long as they are considered a measure of gene flow rather than exact estimates of the number of migrants, and are used in a comparative way within the same species. However, extrapolation to related species with different migration behaviour may be problematic, since similar estimates of N_em may no longer indicate similar levels of gene flow in these cases.

In my experiments, the large variation among replicates resulted in a wide range of estimates of N_em, even for the same migration scenario and under highly standardized conditions. Extrapolating this to natural systems implies that such estimates will have low accuracy. In box 6.2, I compare estimated numbers of migrants from several studies of natural systems with the results in CHAPTER 3. In many of these applied studies, conclusions on gene flow are drawn based on differences between estimated N_em that are of the same order of magnitude as the error margins in my laboratory metapopulations. Mateus and Sene (2007), for example, draw conclusions on the relative importance of gene flow *versus* genetic drift for nine cactophilic *Drosophila* populations by comparing the estimated value of N_em with theoretically predicted (Wright 1931, Kimura & Weiss 1964) limit values signifying almost total isolation ($N_em << 1$) and a panmictic population ($N_em > 4$), respectively. Walker and colleagues (2001) use pairwise N_em estimates to assess the relative isolation of four Scandinavian wolverine populations that translates into cautionary advise regarding harvest quota. In both cases, the range of observed N_em lies within the error margins of a single estimate, which is also the case for

Box 6.2 Effective number of migrants inferred from genetic data in natural systems

Study	Species	Estmate of N_em
CH. 3	*Drosophila melanogaster* (a)	N_em (10 pops): range of 20 replicates 0.32 – 8.61
	D. melanogaster (b)	N_em (10 pops): range of 20 replicates 0.08 – 5.39
1	*Drosophila antonietae*	N_em (9 pops): 3.21 (CI = 1.93 – 6.03)
2	*Thaumetopoea pityocampa*	N_em (7 pops): 0.47 (CI = 0.39 – 0.60)
	T. pityocampa (f)	N_em (7 pops): 0.75 (CI = 0.07 – 0.15)
3	*Talitrus saltator*	N_em (6-22 pops): range of 7 regions 0.03 – 0.80
	Orchestia montagui	N_em (17 pops): 3.00, N_em (6 pops): 4.99
	O. stephenseni	N_em (5 pops): 0.23, N_em (8 pops): 0.50
	Platorchestia platensis	N_em (4 pops): 6.85
4	*Abies guatemalensis*	N_em (10 pops): 1.80 (CI = 1.34 – 3.72)
	A. hickeli	N_em (6 pops): 3.17 (CI = 1.83 – 11.65)
	A. flinckii	N_em (6 pops): 0.67 (CI = 0.46 – 1.43)
	A. religiosa	N_em (11 pops): 0.13 (CI = 0.43 – 1.57)
5	*Inachus dorsettensis*	N_em (2 pops): 4.6
	Hyas coarctatus	N_em (2 pops): 12.2
6	*Argiope trifasciata*	N_em (7 pops): 31.3; pairwise N_em: range 9.2 – 118.6
7	*Anticarsia gemmatalis*	Pairwise N_em (5 pops): range 2.06 – 15.26
8	*Callinectes danae*	Pairwise N_em (4 pops): range 2.32 – 7.64
9	*Chelonia mydas*	Pairwise N_em (16 pops): range 2.55 – 9.03
10	*Canis familiaris*	Pairwise N_em (11 pops): range 0.43 – 10.83
11	*Puma concolor*	Pairwise N_em (6 pops): range 2.9 – 30.2
12	*Gulo gulo*	Pairwise N_em (4 pops): range 1.51 – 10.46
13	*Lycaon pictus*	Pairwise N_em (6 pops): range 1.53 – 5.88
	L. pictus (f)	Pairwise N_em (6 pops): range 0.04 – 2.67
14	*Rana cascadae*	Pairwise N_em (11 pops): range 0.6 – 12.9
		Pairwise N_em (11 pops): range 0.5 – 19.7 (coalescent)
15	*Rana catesbeiana*	Pairwise N_em (11 pops): range 2.4 – 92.3
		Pairwise N_em (11 pops): range 0.11 – 3.81 (likelihood)
16	*Callichirus islagrande*	Pairwise N_em (5 pops): range 3.34 – 11.42 (likelihood W-E)
		Pairwise N_em (5 pops): range 1.71 – 10.68 (likelihood E-W)

Ranges of estimated numbers of migrants N_em inferred from overall or pairwise F_{ST} = $1/(4N_em + 1)$. The results from CHAPTER 3 are pooled for metapopulations with mating taking place after (a) and before (b) migration. Studies 15 and 16 used estimators based on the coalescent and maximum likelihood, respectively. (f) = females only (estimate based on mtDNA); CI = confidence interval.

References: 1 = Mateus & Sene 2007, 2 = Salvato *et al.* 2002, 3 = De Matthaeis *et al.* 2000, 4 = Aguirre-Planter *et al.* 2000, 5 = Weber *et al.* 2000, 6 = Ramirez & Haakonsen 1999, 7 = Sosa-Gomez 2004, 8 = Weber & Levy 2000, 9 = Roberts *et al.* 2004, 10 = Kim *et al.* 2001, 11 = Anderson *et al.* 2004, 12 = Walker *et al.* 2001, 13 = Girman *et al.* 2001, 14 = Monsen & Blouin 2004, 15 = Austin *et al.* 2004, 16 = Bilodeau *et al.* 2005

most of the pairwise N_em estimates. Estimates based on different approaches than the classic Wright formula $F_{ST} = 1/(4N_em + 1)$, such as coalescent-based (Monsen & Blouin 2004) or likelihood-based methods (Austin *et al.* 2004, Bilodeau *et al.* 2005) tend to yield ranges of similar size as estimates based on the classic F_{ST}-approach (but see Austin *et al.* 2004). Notice, however, that these applied studies all use multiple marker loci ranging from four (Roberts *et al.* 2004) to fifteen or more (Weber *et al.* 2000, Sosa-Gomez 2004) loci, whereas my estimates are based on a single locus. Simulations of my experimental system (box 6.3) using a single locus yield a range of estimates of 0.03 – 4.20, which is comparable with the observed range (b) in box 6.2. Similar simulations using eight independent loci (box 6.3) resulted in a range of 0.27 – 0.66, which is considerably narrower than for most studies in box 6.2. These simulation results suggest that using multiple marker loci may substantially increase the accuracy of estimates of N_em in both experimental and natural populations. In the section on computer simulations, I elaborate on this topic in more detail.

In general, estimates of the effective number of migrants appear to have mostly comparative value, for example to compare the levels of gene flow among similar species (Aguirre-Planter *et al.* 2000, De Matthaeis *et al.* 2000, Weber *et al.* 2000), or to detect sex-biased migration (Girman *et al.* 2001, Salvato *et al.* 2002). Large variation in estimated levels of gene flow may result from genuine biological causes, such as the presence of barriers between some (sub)populations but not between others. However, my experimental results suggest that a similar degree of variation may also arise under relatively constant laboratory conditions when only one or few marker loci are used. Moreover, the one-migrant-per-generation rule that is widely used as a rule of thumb in conservation management (Mills & Allendorf 1996, Wang 2004), tends to fall within these error margins in many cases. Thus, the interpretation of such variation will be more complicated than commonly expected, and the correct translation to conservation management measures will be difficult and requires much knowledge of the actual system under consideration.

LOCAL EXTINCTION & RECOLONAZATION

The results of the second series of experiments (CHAPTER 4) indicate that, as expected, population turnover due to local extinction and recolonization substantially increases the variation between replicate metapopulatios. In contrast with the assumptions of most genetic metapopulation models, extinction was often not directly followed by recolonization. As a consequence, the pattern and dynamics of genetic variation differed considerably from the predictions of these models. Even after 40 generations, the metapopulations maintained substantial genetic variation. Much of this variation, however, was between rather than within demes. Accordingly, the demes differed often considerably in their tolerance to novel stress factors and in their adaptive potential.

The introduction of population turnover through local extinction and subsequent founder events (CHAPTER 4) resulted in very high levels of variation among replicate metapopulations. In addition to the dynamics of local extinction and recolonization, I relaxed the level of experimental control compared with the previous experiments by allowing variable deme sizes and migration rates, and I reduced the number of demes in

a metapopulation to six due to logistic constraints. Population turnover generally increased the stochasticity at the level of the metapopulation, resulting in a higher loss of genetic diversity and a higher level of genetic differentiation than in the absence of population turnover. Although small populations are prone to loss of genetic variation reducing the adaptive potential (Whitlock 2002) and to inbreeding depression decreasing fitness and increasing the risk of extinction (Bijlsma *et al.* 2000, Frankham 2005a), the assessment of population fitness and stress tolerance showed no evidence of inbreeding depression under standard conditions. Population turnover partitioned the genetic variation in a metapopulation very unevenly, however, resulting in the loss of all variation at a single locus from individual demes in a number of cases. The effects of population fragmentation on fecundity differed strikingly from the effects on viability. Fecundity was almost twice as high in the undivided populations as in the fragmented populations, whereas the viability was equally high in both the fragmented and the undivided populations. Presumably, these differences are related to the fact that the demes of the fragmented populations had been kept in small vials, while the undivided populations were kept in much larger bottles. The micro-environmental differences between both types of containers can easily have resulted in unintended selection on the reproductive strategies of the flies. For example, the population density in the bottles was generally higher than that in the vials. This may have led to stronger fecundity selection in the bottles. Hence, the higher fecundity found in bottle populations may reflect differences in the environment rather than fragmentation *per se*. It should be noted that such (micro-)environmental differences between fragmented and undivided populations will be of importance in natural populations as well.

My simulations revealed that commonly used estimators of the effective sice of a metapopulation (Whitlock & McCauley 1990, Whitlock & Barton 1997, Pannell & Charlesworth 1999) become unreliable when the assumptions standardly made in theoretical models are not satisfied. In my experiments, it turned out that the extant demes differed strongly in their contribution to subsequent generations. Moreover, despite of the high degree of standardization, migration varied strongly in time and across metapopulations, In principle, such variation can be incorporated in the theoretical models, but in practice the relevant information is generally lacking. Furthermore, most theoretical predictions are based on equilibrium considerations, while real-world metapopulations will often be far from equilibrium. For these reasons, the estimation of metapopulation effective size may be problematic, particularly in the presence of population turnover.

ADAPTIVE POTENTIAL

After 40 generations of fragmentation, the demes of a metapopulation differed substantially in stress tolerance and in their adaptive potential (CHAPTER 5). These differences were most pronounced when major genes with conditionally expressed detrimental alleles are involved. The consequences of metapopulation structure for the adaptive response to adverse environmental conditions depend strongly on the genetic architecture of the traits involved in the stress response.

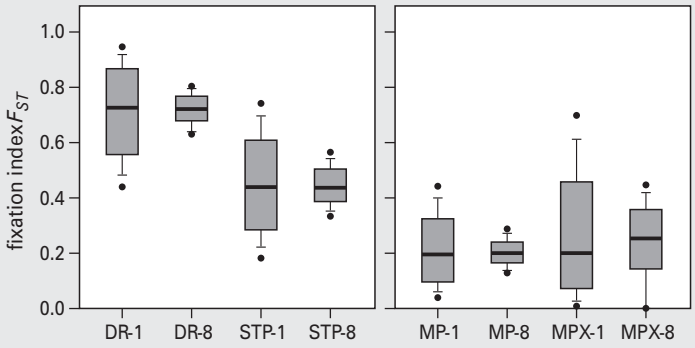
In the final experiment I focused on the adaptive response to adverse environmental conditions (CHAPTER 5) by assessing the initial tolerance (*i.e.*, viability at first exposure to a stress factor) and the adaptive potential (*i.e.*, the change in viability after six consecutive generations of exposure without gene flow between the demes) of the six replicate metapopulations without a history of population turnover and of six undivided populations. Adaptation to novel environments requires ample genetic variation (Macnair 1991, Lynch & Lande 1993), and since both initial tolerance and adaptation occur on short evolutionary time scales leaving little opportunity for beneficial mutations to arise, the adaptive response will mainly depend on the standing genetic variation (Bijlsma & Loeschcke 2005). Contrary to expectations based on simulations, the initial tolerance was higher in the subdivided than in the undivided populations for two of three stress factors. In line with the outcome of individual-based simulations the adaptive response was generally lower in the subdivided than in the undivided populations, although not significantly so. The three stress factors used (development at high temperature, or in medium with salt or with ethanol) lead to pronounced differences in tolerance and adaptive response, presumably due to the different genetic architectures of the involved stress resistance traits. The adaptive response of single demes may vary substantially because the required adaptive genetic variation becomes distributed among rather than within the demes in a metapopulation. This may become particularly important when major genes are involved that deviate from the standard assumptions, for example by the presence of conditionally expressed detrimental alleles.

COMPUTER SIMULATIONS

In both series of experiments, logistic considerations led me to use a single-locus marker system whereas multiple molecular markers are common practice in most applied studies (Vignal *et al.* 2002). Monitoring replicated metapopulations over a number of generations and for several scenarios tends to increase the required number of samples exponentially, making it a labour- and time-consuming task to process large numbers of multiple marker samples. In addition, the monitoring of a visual marker allowed sampled flies to survive and to found successive generations. Obviously, the choice for a single marker locus has contributed to the high level of variation between replicates encountered in the experiments. In such a case, computer simulations provide an easy way to assess the effects of using multiple markers, for example the effect on the variation between replicates. The simulations in CHAPTER 4 show that an increase of the number of loci will, not surprisingly, reduce the variation among replicates. However, a substantial level of variation between replicates remains due to other causes, such as population turnover. In box 6.3, I present similar simulation results comparing the single-locus approach as used at all stages of the experimental setup with a hypothetical multiple-locus approach based on eight independent loci.

In comparison to a single marker, the use of eight marker loci reduces the coefficient of variation (CV) by a factor 2.9 in case of a metapopulation without gene flow (DR; from 0.19 to 0.06), by a factor 2.4 in case of stepping-stone migration (STP; from 0.33 to 0.13), by a factor 2.6 in case of migrant-pool migration (MP; from 0.52 to 0.20), and by a

Box 6.3 Effect of the number of marker loci on genetic variation in a metapopulation



Predicted variation in the fixation index F_{ST} for ten-deme metapopulations with genetic drift and stepping-stone migration (left, CHAPTERS 2 & 3) and for six-deme metapopulations with migrant-pool migration and population turnover (right, CHAPTER 4) based on a single locus and on multiple loci. The plots show the results of individual-based simulations of 1000 replicate metapopulations for genetic drift separately (DR) and in combination with unidirectional stepping-stone migration (STP), and with migrant-pool migration in the absence (MP) and presence (MPX) of local extinction and recolonization. Bars, whiskers and points indicate the 75%, 90% and 95% confidence intervals, respectively, for F_{ST} at one locus (DR-1, STP-1, MP-1, MPX-1) and averaged over eight independant loci (DR-8, STP-8, MP-8, MPX-8) in generation 20. The black bands in the bars indicate the median.

factor 1.6 in case of migrant-pool migration and local extinction (MPX; from 0.76 to 0.47). In the last scenario in particular, substantial variation among replicates remains, indicating that the variation observed in the experiments does not just reflect the use of a single marker. However, it is evident that multiple marker loci should be used when possible, since this will reduce the error margins in such different applications as estimating effective population sizes (CHAPTER 2) or the levels of genetic differentiation (CHAPTER 4). The simulation results tentatively suggest that using five to ten independent loci already reduces the error margins considerably, whereas using more than 20 loci will generally not contribute to a further reduction of variation any more. Similar evaluations based on simulations (CHAPTER 4) indicate that increasing the number of sampled demes in a metapopulation is an alternative option to reduce the error margins. Thus, from cost-benefit considerations sampling five to ten independent loci in many demes is a better strategy than sampling larger numbers of loci in only a few demes.

GENERAL CONCLUSIONS AND PERSPECTIVES

In this thesis I focused on the consequences of metapopulation structure for the inference of demographic parameters based on classic population genetic models (Caballero & Toro 2002, Tufto & Hindar 2003, Hedrick 2004) and for genetic processes such as genetic erosion and adaptive potential (Gaggiotti 2003, Reed *et al.* 2003, Spielman *et al.* 2004, Frankham 2005a, b). Both aspects are important for practical applications in conservation management. In this section I discuss the implications of my results for such applications, and the perspectives of the experimental approach I used.

WHAT HAVE I LEARNED?

I found that the average over several metapopulations in my experiments matched the theoretical predictions fairly well, whether they were based on general population genetic theory or on repeated individual-based computer simulation. At the same time, the single replicates often diverged strongly from this average. In other words, a single small system is often not typical of an average theoretical population. Computer simulations may provide confidence intervals for a well-specified and standardized situation in the lab, but in natural systems it will generally be difficult if not impossible to accurately predict the dynamics of a single metapopulation.

This has also consequences for the inference of demographic parameters, such as effective population size or the effective number of migrants per generation. Although these parameters conveniently describe the dynamics of genetic variation in a metapopulation, similar values of N_e or N_em may result from different census sizes or migration behaviour in practice. For questions regarding the actual demography, a thorough understanding of the underlying model assumptions (*e.g.*, with regard to mating system or migration behaviour), is required for each particular population, since deviations of the model assumptions might result in substantially biased estimates of demographic parameters. Deviations such as non-random mating and the presence of natural selection affecting the effective size of single demes (CHAPTERS 2 & 3) occur independent of metapopulation structure, whereas deviations such as stepping-stone migration affecting the number of migrants (CHAPTER 3) and population turnover affecting the effective size of the metapopulation (CHAPTER 4) are the result of metapopulation structure and dynamics. In all cases, however, the range of parameter estimates was considerable, which led to the conclusion that such estimates of demographic parameters will generally have more value as a relative measure (*e.g.*, to compare gene flow among populations) than as an absolute measure (*e.g.*, to predict the exact number of migrants that will arrive in a deme each generation). Although this conclusion is hardly unexpected in view of several critical evaluations (Whitlock & McCauley 1999, Neigel 2002, Austin *et al.* 2004, Pearse & Crandall 2004, Rousset 2004), it is surprising that many studies (box 6.2) continue to apply an approach based on general population genetic theory in spite of obvious discrepancies between the model assumptions and the system under consideration. In many cases where concrete management recommendations are required, alternative approaches that combine

genetic data with ecological and historical data (*e.g.*, Gaggiotti *et al.* 2002, Clegg *et al.* 2003) might be more appropriate.

Many studies (reviews in Armbruster & Reed 2005, Bijlsma & Loeschcke 2005, O'Grady *et al.* 2006) have documented negative effects of inbreeding depression and genetic erosion leading to subsequent negative effects for the adaptive potential of metapopulations. The observed decline of metapopulation fitness in the first experimental series (CHAPTER 3) where deme sizes were very small (16 individuals) and migration rates relatively low might be a consequence of relatively high levels of inbreeding depression within single demes, and supports previous findings. I found little evidence for similar effects on metapopulation fitness in the second series of experiments (CHAPTER 4) where deme sizes were considerably larger (50 individuals on average), although inbreeding depression might have occurred at the level of individual demes to some extent. This genetic erosion became particularly visible in the adaptation experiments (CHAPTER 5), and affected the adaptive potential at the metapopulation level in case of major genes deviating from the standard assumptions, for example by the presence of conditionally expressed detrimental alleles. However, in contrast with my laboratory populations, the occurrence of such genes in natural populations is probably limited, since natural selection tends to effectively purge detrimental alleles when the occurrence of adverse conditions is common (*e.g.*, the MHC-gene in seals during outbreaks of the phocine distemper virus, Hoelzel *et al.* 1999, Lehman *et al.* 2004).

SIMULATED & EXPERIMENTAL METAPOPULATIONS

Experimental metapopulations seek to provide an "interface" between theory and nature by comparing theoretical predictions with experimental results, and extrapolating the experimental findings for application in natural metapopulations. As such, they belong to a class of experimental approaches that are commonly referred to as micro- or mesocosm experiments in ecological studies. The value of such experimental approaches has been disputed (*e.g.*, Kennedy 1995, Carpenter 1996, Schindler 1998, Huston 1999), mainly because of scaling problems yielding inaccurate results when extrapolating experimental results to natural ecosystems. More recently, however, microcosm experiments have been rediscovered as a valuable tool to study evolutionary questions (*e.g.*, Petersen & Hastings 2001, Yedid & Bell 2001, Lilley *et al.* 2003, Jessup *et al.* 2004) and the impact of large-scale environmental changes such as global warming (Benton *et al.* 2007, Van Doorslaer *et al.* 2007). As part of this development, "natural" microcosm experiments combining the advantages of standardization and replication with the realism of field experiments have also been gaining support (Srivastava *et al.* 2004, Steele & Forrester 2005).

In general, research approaches can be arranged from highly abstract to very concrete: (i) general analytical models, (ii) computer simulations, (iii) microcosm experiments and (iv) field observations. Each of these approaches has its specific advantages and disadvantages. Analytical models enable the analysis of parameters based on general formulas that show immediately which assumptions are needed and which factors are relevant. These models are, however, substantially simplified and the

assumptions are often unrealistic. The results typically refer to equilibrium populations and to expected values without much reference to the degree of stochastic variation. Computer simulations do not have these limitations, and they are generally based on more complex and often also more realistic assumptions. Most importantly, running many replicate simulations allows for quantifying the expected degree of variation. However, implementing simulation models generally requires the specification of many parameters, of which only few will be known in practice. Even in case of few parameters, the number of "reasonable" parameter combinations is very large, making it an impossible task to get a complete overview of the model behaviour. Hence, extrapolation of the results to different species will be more difficult than in case of generalized analytical models. Replicated microcosm experiments under controlled, standardized conditions are particularly suited to provide insight into causal relations, and allow experimentations that would not be feasible within the spatial and temporal frames of natural systems. At the same time, the spatial and temporal downscaling reduces the level of realism, as does standardization that excludes much of the complexity of the natural system. Moreover, microcosm experiments tend to use a few well-known model organisms selected for logistic reasons that makes extrapolation to other species difficult. In contrast with simulation models, microcosms often deviate from theoretical assumptions to some extent, for example with regard to selective neutrality or constant migration rates as in my experimental metapopulations. However, such deviations may provide important insights into the potential discrepancies between the presumed and actual behaviour of natural systems. Thus, microcosm experiments have an obvious surplus value because they are much closer to natural systems than simulation models. Natural systems themselves represent the ultimate experimental setup with regard to completeness and realism. However, experiments using natural systems are often not feasible due to logistic (*e.g.*, long generation times or large home ranges) or ethical (*e.g.*, very rare species or severely threatened populations) constraints. Moreover, replication is generally not possible in such experiments, which may considerably reduce the applicability of the results. "Natural" microcosms as advocated by Srivastava and colleagues (2004) might then provide a promising intermediate approach that has the advantage of replication and control without the limitation of model organisms and overly simplified environmental conditions.

The results of mathematical models are generally not directly applicable to natural systems. To bridge the gap between abstract theory and field applications, the use of computer simulations and microcosm experiments as in this study provides an obvious intermediate approach. Computer simulations have similar advantages with regard to microcosm experiments as microcosms have with regard to natural systems. I used computer simulations to generate baseline values for the experimental metapopulations, including estimates of the experimental variation to be expected, and to enable extrapolation of the experimental results. Although both the experimental setup and the simulation model reflect the general theoretical models and include most key assumptions initially, the results of the *in silico* metapopulations and the experimental metapopulations showed numerous discrepancies due to unintentional deviations from the initial

assumptions in the experiments. It turned out that "experimental details" that were not specified in the models, such as differences in microclimate in the vials and bottles, had considerable implications for the experimental outcome. This exemplifies the importance of using both experiments and simulations and contradicts the notion that computer simulations can completely replace an experimental approach. Simulations provide an excellent way to improve an experimental setup by evaluating the effect of variables that are not specified in theoretical models or that can not meet the theoretical assumptions due to logistic or other practical reasons. However, simulations are unable to detect the subtle micro-environmental variation that may substantially affect the results of experimental systems, whereas such discrepancies between theoretical predictions and a controlled laboratory setup will provide valuable insights into the complexity of natural systems.

In my experiments, I generally found that, on average, the results of the replicate metapopulations matched the predictions generated by the simulation model pretty well. The individual metapopulations, however, showed substantial deviations from these averages despite the strict standardization of the experimental setup. In other words, it is generally not possible to predict the dynamics of an individual (meta-)population with confidence and precision. Simulations enable to predict the degree of variation to be expected and they provide an excellent way to evaluate and optimize the effects of parameters such as sample size, the number of sampled demes and the number of genetic markers. However, care is required when extrapolating the simulation results to natural systems, since no real-world system will match the characteristics of either the microcosm experiments or the simulations.

In spite of these restrictions, I am convinced that microcosm experiments, both with *Drosophila* and other model organisms, in combination with computer simulations have the prospective of becoming a very important tool for the study of complex natural systems. This study has provided some important insights with regard to the control and replicability of such experiments. On second thought, for example, I would not use different extinction schedules for each replicate metapopulation, nor would I allow for highly flexible migration rates. Most importantly, I recommend using computer simulations in advance to optimize the experimental design before actually deciding for a specific setup.

FINAL REMARKS

Although the metapopulation concept has become both familiar and popular, the resulting metapopulation approach may not always be the most suitable and sensible way to study a particular natural population. Population genetic theory in particular commonly assumes populations to be in equilibrium. In practice, population fragmentation and habitat deterioration may be ongoing processes that prevent attaining equilibrium (Hutchison & Templeton 1999), or fragmented populations may not behave according to any metapopulation model at all (Anderson *et al.* 2004). In such cases, the metapopulation approach is inadequate to develop management strategies for the conservation of these populations because the underlying models do not match the

actual system (Hanski & Simberloff 1997). My results partially agree with this reservation, in particular for obvious non-equilibrium conditions as encountered during population turnover. Neither the experimental results, nor long-term simulation results of similar populations with regularly occurring local extinction and recolonization events attained equilibrium levels of genetic differentiation (CHAPTER 4), whereas most theoretical models assume such a hypothetical equilibrium (Whitlock & McCauley 1990, Pannell & Charlesworth 1999). These and similar deviations of model assumptions tended to cause discrepancies between model predictions and the actual situation in a single experimental metapopulation, although the match between the predictions and the average result of many replicates was generally quite good in my study, especially when taking the specific life history characteristics of *Drosophila* into account. The observed discrepancies between the results of single replicates and the predicted average, however, illustrate that application of analytical models to natural systems without careful consideration is generally not advisable, since natural populations tend to be unique ("single replicate") and much more complex than experimental systems. Applied studies of natural systems require a comprehensive understanding of the underlying theory, and I plead a thorough cooperation between theoretical and empirical biologists when developing analytical and predictive tools for the assessment of endangered populations and subsequent conservation strategies. In line with this argument, an alternative approach of analysing population structure based on both genetic, ecological and other, historical data sources as advocated by Gaggiotti (2004) appears to be promising.

As a final conclusion, I argue that general theory may reasonably predict the dynamics in experimental and, perhaps, in natural populations in a qualitative way, whereas quantitative inferences are generally not possible, unless one understands the biology and dynamics of the species under consideration in detail, and uses such inferences in a comparative way within a species or at most among closely related species. However, since such estimates may still deviate substantially from actual parameter values, I advise prudence and wide safety margins whenever they are used in practice, for example to develop a management strategy for the conservation of specific natural systems.

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Samenvatting

BEGRIPPENLIJST

- **Heterozygotie, homozygotie & autozygotie**: heterozygote individuen hebben twee verschillende "**allelen**" (varianten van een gen waarvan er één van de moeder en één van de vader afkomstig is). Homozygote individuen hebben twee dezelfde allelen, en autozygote individuen hebben twee identieke allelen; in het laatste geval hebben de allelen niet alleen dezelfde functie, maar zijn ze ook afkomstig van de dezelfde voorouder. De heterozygotie van een populatie is het aandeel heterozygote individuen in de populatie; een hoge heterozygotie betekent dat er veel "**genetische variatie**" (veel verschillende allelen) aanwezig is in een populatie, ofwel dat de "**genetische diversiteit**" van de populatie groot is.
- **Genetische drift**: toevalsproces waardoor sommige allelen uit een populatie verdwijnen en anderen "**gefixeerd worden**" (als enige overblijven). Iedere generatie geven ouders slechts een willekeurige selectie van alle voorkomende allelen door aan hun kinderen. Hoe kleiner de populatie, des te minder kinderen, en des te groter de kans dat een bepaald allel niet wordt doorgegeven en dus uit de populatie verdwijnt.
- **Inteelt**: toename van de "**autozygotie**" (kans op twee identieke allelen in een individu) doordat de individuen in een populatie allemaal tot in een bepaalde graad verwant zijn. Hoe kleiner een populatie, des te groter de kans dat individuen dezelfde (voor)ouders hebben, en des te hoger het inteeltniveau.
- **Inteeltdepressie**: afname van de "**fitness**" (overlevingswaarde) van een individu of populatie doordat veel recessief schadelijke allelen tot uiting kunnen komen als gevolg van inteelt.
- **Genetische erosie**: verslechtering van de genetische kwaliteit van een populatie vanwege een kleine populatiegrootte. Kleine populaties raken makkelijk genetische variatie kwijt door genetische drift waardoor de autozygotie toeneemt (inteelt). Dit kan weer leiden tot inteeltdepressie, en uiteindelijk tot een grotere kans op uitsterven.
- **Metapopulatie**: verzameling subpopulaties die met elkaar verbonden zijn via de uitwisseling van migranten, en die tijdelijk en plaatselijk uit kunnen sterven maar uiteindelijk weer opnieuw gekoloniseerd kunnen worden door migranten.
- **Migratie**: uitwisseling van individuen (en dus van genetisch materiaal) tussen subpopulaties in een metapopulatie, waardoor de genetische differentiatie afgeremd wordt. Migranten kunnen bijvoorbeeld allelen terugbrengen in een subpopulatie waaruit ze door genetische drift waren verdwenen. Bij "**stapsteen-migratie**" kunnen individuen alleen naar de buurpopulaties van hun thuispopulatie migreren; bij "**migrantpoel-migratie**" verzamelen alle migranten zich eerst in een gemeenschappelijke "poel", van waaruit ze vervolgens willekeurig naar elke subpopulatie kunnen migreren.
- **Genetische differentiatie**: het verschil in aanwezige genetische variatie tussen de subpopulaties in een metapopulatie. De "**fixatie-index**" (F_{ST}) vergelijkt de genetische variatie in de subpopulaties met de totale genetische variatie in de metapopulatie. Hetzelfde niveau van variatie in de subpopulaties en in de metapopulatie geeft aan dat de variatie gelijkmatig verdeeld is over de metapopulatie: elke subpopulatie heeft ongeveer dezelfde variatie zodat de genetische differentiatie klein is. Als de variatie ongelijk verdeeld is, doordat bijvoorbeeld in verschillende subpopulaties verschillende allelen gefixeerd zijn, is de genetische differentiatie hoog. In het algemeen zal genetische drift differentiatie verhogen, en zal migratie differentiatie verlagen; als beide processen even sterk zijn, zal de metapopulatie een evenwichtsniveau van differentiatie bereiken.

Het huidige niveau van menselijke activiteit tast in toenemende mate de biodiversiteit van onze planeet aan. Als gevolg van de kwalitatieve achteruitgang en versnippering van hun natuurlijke leefgebied, zijn veel soorten teruggebracht tot kleine, vaak geïsoleerde populaties met een verhoogde kans op uitsterven door toevallige demografische, genetische of omgevingsfactoren. Zulke kleinschalige, gedeeltelijk geïsoleerde subpopulaties die via migratie zijn verbonden tot een groter geheel van samenhangende populaties, worden meestal beschouwd als een metapopulatie. Individuele subpopulaties kunnen soms uitsterven, maar worden dan na verloop van tijd opnieuw gekoloniseerd door migranten uit de andere subpopulaties. Bij het beheren van natuurgebieden wordt in toenemende mate gebruikt gemaakt van theoretische modellen om de demografie en dynamiek van natuurlijke populaties te analyseren en belangrijke populatieparameters te schatten. Zulke parameterwaarden worden vervolgens gebruikt om beleidsmaatregelen te ontwikkelen, bijvoorbeeld voor het beheer van vispopulaties of van bedreigde soorten. De meeste modellen die de dynamiek van de genetische variatie en de evolutie van metapopulaties beschrijven zijn echter zeer abstract, en gebaseerd op een groot aantal vereenvoudigde en vaak onrealistische aannames. De praktische waarde van voorspellingen gebaseerd op deze modellen wordt slechts zelden bepaald, noch in experimentele, noch in natuurlijke omstandigheden.

Voor dit proefschrift heb ik een combinatie van laboratoriumopstellingen met *Drosophila melanogaster* metapopulaties en individu-gerichte computersimulaties gebruikt om experimenteel de waarde van populatiegenetische modellen te bepalen voor toepassingen in de context van een metapopulatie. Ik heb gekeken naar de gevolgen van de metapopulatiestructuur voor de dynamiek van de genetische diversiteit en de genetische differentiatie, met speciale aandacht voor processen zoals genetische erosie en lokale adaptatie. Met het oog op de toepassingen voor natuurbeheer en -behoud heb ik me vooral gericht op de consequenties van de metapopulatiedynamiek voor het bepalen van demografische parameters zoals effectieve populatiegrootte en migratiesnelheid die vaak gebruikt worden als uitgangspunt voor beheersstrategieën.

De aanpak in deze studie is vooral gebaseerd op de vergelijking van de resultaten van experimentele metapopulaties met de resultaten van individugerichte computersimulaties. De eenvoudigste experimentele metapopulatie bestond uit een vijfmaal herhaalde set van tien geïsoleerde subpopulaties zonder “genetische uitwisseling” (de uitwisseling van genetisch materiaal door bijv. migratie) tussen deze subpopulaties. De experimenten in deze opzet waren gericht op de analyse van de effecten van genetische drift, en leverden de uitgangswaarden op voor alle volgende experimenten (HOOFDSTUK 2). Vervolgens heb ik gekeken naar de gecombineerde effecten van drift en genetische uitwisseling door rondgaande stapsteen-migratie in één richting te introduceren in deze metapopulaties van tien subpopulaties (HOOFDSTUK 3). In de volgende serie experimenten heb ik gekeken naar de gecombineerde effecten van genetische drift, migratie en “populatie-turnover”, het plaatselijk uitsterven van subpopulaties gevolgd door herkolonisatie, in metapopulaties bestaande uit zes subpopulaties. Deze metapopulaties waren volgens twee ruimtelijke patronen gerangschikt die stapsteen-migratie in twee richtingen en migrantpoel-migratie mogelijk maakten (HOOFDSTUK 4). Hoewel migratie

vooral volgens een stapsteenmodel zal verlopen in veel natuurlijke habitats met een lineair karakter, zoals rivieroevers of wegbermen, is migrantpoel-migratie de standaard aanname in veel theoretische modellen die afgeleid zijn van Wright's (1931, 1951) eilandmodel voor migratie. Ik heb bekeken of, en in hoeverre deze verschillende migratiemodellen invloed hadden op de dynamiek van de genetische variatie. In het laatste experiment heb ik gekeken naar de gevolgen van populatieversnippering voor het vermogen van een populatie om zich aan te passen aan ongunstige milieuomstandigheden (HOOFDSTUK 5). In de volgende paragrafen vat ik de resultaten kort samen, en bespreek ik de conclusies die eruit getrokken kunnen worden.

GENETISCHE DRIFT *VERSUS* MIGRATIE

De resultaten van de eerste serie experimenten (HOOFDSTUK 2 & 3) laten zien dat de bepaling van demografische parameters zoals de effectieve populatiegrootte of het effectieve aantal migranten per generatie uit genetische gegevens onbetrouwbaar kan zijn. Dit geldt met name wanneer neutraal veronderstelde genetische variatie toch niet neutraal blijkt te zijn. De evolutie van een metapopulatie wordt sterk beïnvloed door de manier van partnerkeuze bij Drosophila, de variatie in voortplantingssucces bij de vrouwen, de sexe van de migranten, het tijdstip van migratie en de ruimtelijke configuratie van de subpopulaties.

Het resultaat van Buri's (1956) klassieke onderzoek naar de evolutionaire dynamiek van oogkleurmutanten in kleine populaties wordt vaak aangehaald als *het* schoolvoorbeeld van het effect van genetische drift op neutrale genetische variatie (bijv. Hartl & Clark 1997, Hedrick 2000). Ik heb daarom bewust besloten om eenzelfde experimentele opzet te gebruiken voor mijn metapopulatie-experimenten. Om verschillen door mutantspecifieke effecten op de fitness tussen de *Drosophila*-lijnen te voorkomen heb ik gekozen voor twee verschillende mutante lijnen in plaats van één mutante en één wild-type lijn. De vliegen uit deze mutante lijnen hadden roodbruine (*bw*^{75}*;st*) ogen of witte (*bw;st*) ogen, en een kruising tussen de twee lijnen leverde vliegen met oranje ogen op. Ondanks deze voorzorg vond ik toch aanwijzingen voor significante, gerichte selectie ten gunste van het (roodbruine) *bw*^{75}-allel, die mogelijk veroorzaakt werd door verschillen in activiteit tussen de *bw*^{75}*;st* en *bw;st* lijnen (HOOFDSTUK 2). De lagere activiteit van de vliegen met witte ogen zou te maken kunnen hebben met de continue blootstelling aan licht in mijn experimenten, aangezien wel bekend is dat vliegen zonder oogkleurpigmentatie (dus met "witte" ogen) gevoeliger zijn voor fel licht (Reed & Reed 1950). Als gevolg van het selectieve voordeel van het *bw*^{75}-allel weken de patronen van genetische diversiteit en genetische differentiatie behoorlijk af van de voorspellingen gebaseerd op neutrale allelen. Het effect van de selectie was geenszins eenduidig, aangezien de gevonden selectie zowel dichtheids- als frequentieafhankelijk bleek te zijn. Bovendien was de sterkte van de selectie afhankelijk van de context, zoals het soort container (buisje of fles) waarin de vliegen werden gekweekt, en het wel of niet optreden van migratie. De gestandaardiseerde opzet van de experimenten en de voortdurende controle van de populaties stelde mij in staat om te corrigeren voor selectie, en

om betrouwbare schattingen van sleutelparameters zoals de effectieve populatiegrootte te verkrijgen. In natuurlijke systemen zou dit veel moeilijker, zo niet onmogelijk zijn, omdat het zeer lastig is om daar aan de benodigde gegevens te komen. De mogelijkheid van gerichte selectie moet echter niet over het hoofd worden gezien in praktische toepassingen. Van de genetische merkers die in zulke toepassingen gebruikt worden, wordt doorgaans aangenomen dat ze selectief neutraal zijn, maar daarvoor bestaat meestal geen expliciet bewijs. Het is algemeen bekend dat merkers zoals microsatellieten aan selectie onderworpen kunnen zijn (Charlesworth *et al.* 1997, Vitalis *et al.* 2001), en zelfs als dit niet direct het geval is, kan een merker beïnvloed worden door selectie als hij heel dicht bij een allel met een selectief voor- of nadeel ligt ("meeliften", bijv. Schug *et al.* 1998, Schlotterer 2000).

In veel toegepast onderzoek (voorbeelden in box 6.2 in HOOFDSTUK 6) wordt de effectieve populatiegrootte N_e en het effectieve aantal migranten N_em geschat om het niveau van genetische uitwisseling tussen subpopulaties te bepalen, en om de kans op genetische erosie te voorspellen als de uitwisseling van migranten onvoldoende is. De meeste schatters van N_e zijn tijdsafhankelijke schatters gebaseerd op slechts enkele meetpunten in de tijd ("momentopname-schatters", bijv. Waples 1989, Anderson *et al.* 2000, Wang 2001, Berthier *et al.* 2002), omdat schattingen gebaseerd op de echte verandering per generatie van bijv. heterozygotie (eigenwaarde-populatiegrootte) of allelfrequenties (variantie-populatiegrootte) doorgaans niet mogelijk zijn voor natuurlijke populaties. In mijn experimentele opzet, daarentegen, was het wel mogelijk om deze veranderingen per generatie te meten, en om vervolgens de eigenwaarde- en variantie-populatiegroottes te schatten door middel van lineaire regressie (HOOFDSTUK 2). Vergeleken met deze variantie-populatiegroottes bleek een groot aantal van de algemeen gebruikte momentopname-schatters de effectieve populatiegrootte te overschatten, wat in veel gevallen zal leiden tot een verkeerde interpretatie van de status van een metapopulatie. Mijn computersimulaties lieten zien dat de eigenwaarde-populatiegrootte zeer gevoelig is voor de aanwezigheid van gerichte selectie. Dit wijst erop dat het concept van eigenwaarde-populatiegrootte alleen van toepassing is in het geval van neutrale genetische variatie.

Het effectieve aantal migranten wordt meestal geschat uit de fixatie-index F_{ST} of soortgelijke maten voor genetische differentiatie (bijv. Nei 1973, Weir & Cockerham 1984, Excoffier *et al.* 1992) gebaseerd op de klassieke populatiegenetische modellen, zoals Wright's eilandmodel voor migratie. De grote variatie tussen de replica's leverde meestal een brede reeks aan geschatte waarden van N_em op voor hetzelfde migratiescenario (HOOFDSTUK 3). Het eenrichtings-stapsteenmodel voor migratie gaf schattingen van N_em die behoorlijk afweken van schattingen gebaseerd op het eilandmodel. Daarnaast resulteerde sexe-specifieke migratie na paring in de thuispopulatie in grote verschillen tussen schattingen van N_em. Al deze resultaten wijzen erop dat afwijkingen tussen het natuurlijke systeem en het veronderstelde theoretische model gemakkelijk kunnen leiden tot scheve schattingen van demografische parameters. Zulke scheve schattingen zijn meestal niet echt problematisch zolang ze geïnterpreteerd worden als een maat voor de genetische uitwisseling in plaats van een exacte schatting van het aantal migranten, en gebruikt worden in vergelijkende zin binnen dezelfde soort.

Extrapolatie naar verwante soorten met ander migratiegedrag kan echter wel problematisch zijn, omdat overeenkomstige schattingen van N_em in zulke gevallen niet meer hoeven te wijzen op overeenkomstige niveaus van genetische uitwisseling.

Grote variatie in de geschatte niveaus van genetische uitwisseling kan natuurlijk gewoon een echte biologische oorzaak hebben, zoals barrières die alleen maar tussen sommige (sub)populaties voorkomen en tussen andere niet. De resultaten van mijn experimenten wijzen er echter op dat een soortgelijke mate van variatie ook kan ontstaan tijdens relatief constante laboratoriumomstandigheden als er maar één of enkele markerloci gebruikt worden. Bovendien blijkt de vuistregel van één-migrant-per-generatie die veel wordt gebruikt in het natuurbeschermingsbeleid (Mills & Allendorf 1996, Wang 2004) regelmatig waarden op te leveren die binnen deze foutenmarge vallen. De interpretatie van zulke variatie zal dus meestal ingewikkelder zijn dan verwacht, en de juiste vertaling naar maatregelen ten behoeve van het natuurbehoud is niet eenvoudig en vereist veel inzicht in het te beheren systeem.

LOKALE EXTINCTIE & HERKOLONISATIE

De resultaten van de tweede serie experimenten (HOOFDSTUK 4) laten zien dat zoals verwacht, populatie-turnover door subpopulaties die plaatselijk uitsterven en opnieuw gekoloniseerd worden de variatie tussen metapopulatiereplica's behoorlijk vergroot. In tegenstelling tot de verwachting in de meeste genetische metapopulatiemodellen, werd extinctie vaak niet meteen gevolgd door herkolonisatie. Als gevolg hiervan verschilden het patroon en de dynamiek van de genetische variatie in hoge mate van de voorspellingen gebaseerd op deze modellen. Zelfs na 40 generaties was er nog behoorlijk wat variatie over in de metapopulaties. Een groot deel van deze variatie manifesteerde zich echter vooral tussen, en niet binnen de subpopulaties. De subpopulaties verschilden dan ook behoorlijk in hun tolerantie van stressfactoren en in hun mogelijkheden tot aanpassing aan ongunstige milieus.

De introductie van populatie-turnover door het lokaal uitsterven en daarop volgende nieuwe kolonisaties van subpopulaties (HOOFDSTUK 4) had zeer hoge niveaus van variatie tussen gerepliceerde metapopulaties als resultaat. Als aanvulling op deze dynamiek als gevolg van lokale extinctie en herkolonisatie, was ook het niveau van experimentele controle lager in vergelijking met de voorgaande experimenten doordat de grootte van de subpopulaties en de migratiesnelheid mochten variëren, en was het aantal subpopulaties in een metapopulatie kleiner (zes in plaats van tien) vanwege allerlei logistieke beperkingen. In het algemeen verhoogde populatie-turnover de rol van het toeval op metapopulatieniveau, wat leidde tot een groter verlies van genetische diversiteit en een hoger niveau van genetische differentiatie dan in metapopulaties zonder populatie-turnover. Hoewel kleine populaties gevoelig zijn voor het verlies van genetische variatie waardoor hun aanpassingsvermogen kleiner wordt (Whitlock 2002), en voor inteeltdepressie waardoor hun fitness lager wordt en hun kans op uitsterven toeneemt (Bijlsma *et al.* 2000, Frankham 2005), wezen de bepalingen van de populatiefitness en de stresstolerantie niet op het voorkomen van inteeltdepressie onder standaardomstandigheden. Als

gevolg van de populatie-turnover was de genetische variatie echter zeer ongelijk verdeeld in een metapopulatie, waardoor in een aantal gevallen alle genetische variatie op een enkel locus was verdwenen uit individuele subpopulaties. Het effect van populatie-turnover op de vruchtbaarheid (het gemiddelde aantal levende nakomelingen per vrouwtje) verschilde opvallend van het effect op de overleving (het percentage eitjes dat overleeft tot volwassen vliegen). De vruchtbaarheid was bijna twee keer zo hoog in de grote, ongedeelde populaties als in de versnipperde metapopulaties, terwijl de overleving in beide soorten populaties even hoog was. Dit verschil heeft waarschijnlijk te maken met het feit dat de subpopulaties in de versnipperde populaties werden gehouden in kleine buisjes, terwijl de ongedeelde populaties werden gehouden in veel grotere flessen. De micro-milieuverschillen tussen de twee soorten containers kunnen gemakkelijk onbedoelde selectie op de voortplantingsstrategieën van de vliegen tot gevolg hebben. De populatiedichtheid was bijvoorbeeld over het algemeen hoger in de flessen dan in de buisjes. Dit zou hebben kunnen geleid tot een sterkere selectie op vruchtbaarheid in de flessen. De hogere vruchtbaarheid die we hebben gevonden voor de flessenpopulaties is dan dus vooral een gevolg van omgevingsverschillen, en niet *per se* van populatieversnippering. Hierbij moet aangetekend worden dat zulke (micro-)milieuverschillen tussen versnipperde en ongedeelde populaties ook in natuurlijk populaties van belang zullen zijn.

Mijn computersimulaties brachten aan het licht dat veelgebruikte schatters van de effectieve grootte van een metapopulatie (Whitlock & McCauley 1990, Whitlock & Barton 1997, Pannell & Charlesworth 1999) onbetrouwbaar worden als de aannames die algemeen gemaakt worden in theoretische modellen niet uitkomen. In mijn experimenten bleken de bewoonde subpopulaties sterk te verschillen in hun bijdrage aan volgende generaties. Bovendien was de migratie zeer variabel in de tijd en per metapopulatie ondanks de hoge mate van standaardisatie. Zulke variatie kan in principe wel ingebouwd worden in de theoretische modellen, maar in de praktijk ontbreekt de relevante informatie meestal. Daarnaast gaan de meeste theoretische voorspellingen uit van evenwichtsomstandigheden, terwijl echte metapopulaties doorgaans helemaal niet in evenwicht verkeren. Al deze factoren zullen de schatting van de effectieve metapopulatiegrootte meestal erg moeilijk maken, vooral als er ook populatie-turnover optreedt.

AANPASSING AAN NIEUWE MILIEUS

Na 40 generaties populatieversnippering verschilden de subpopulaties in een metapopulatie aanzienlijk in hun tolerantie van stressmilieus en in hun mogelijkheden om zich aan zulke milieus aan te passen (HOOFDSTUK 5). Deze verschillen waren het meest uitgesproken wanneer er "conditionele lethalen" (genen waarvan de schadelijke effecten alleen maar tot uiting komen onder specifieke milieuomstandigheden, bijv. bij hoge temperatuur) in het spel zijn. De gevolgen van de metapopulatiestructuur voor het aanpassingsvermogen aan ongunstige milieuomstandigheden zijn in hoge mate afhankelijk van het genetische bouwplan van de eigenschappen die betrokken zijn bij de stressweerstand.

In het laatste experiment heb ik gekeken naar het aanpassingsvermogen aan ongunstige milieuomstandigheden (HOOFDSTUK 5) door de begintolerantie (de overleving van de populaties bij de eerste blootstelling aan de stressfactor) en het aanpassingsvermogen (de verandering van de overleving na zes opeenvolgende generaties van blootstelling zonder verdere genetische uitwisseling) te bepalen van de zes metapopulaties zonder een geschiedenis van populatie-turnover en van zes ongedeelde populaties. Aanpassing aan nieuwe omstandigheden vereist de aanwezigheid van voldoende genetische variatie (Macnair 1991, Lynch & Lande 1993), en aangezien zowel de begintolerantie als het aanpassingsproces plaatsvinden op korte evolutionaire tijdschalen waardoor de kans op het ontstaan van nieuwe, gunstige mutaties klein is, zal de uiteindelijke mate van aanpassing vooral afhangen van de reeds aanwezige genetische variatie (Bijlsma & Loeschcke 2005). In tegenstelling tot de verwachting gebaseerd op inidvidu-gerichte computersimulaties, was de begintolerantie hoger in de versnipperde populaties dan in de ongedeelde populaties voor twee van de drie stressfactoren. In overeenstemming met de voorspellingen van de simulaties, echter, was het aanpassingsvermogen in het algemeen lager in de versnipperde populaties dan in de ongedeelde populaties, hoewel dit niet statistisch significant bleek. De drie stressfactoren (ontwikkeling bij hoge temperatuur, of in voedselmedium met zout of met alcohol) hadden grote verschillen in tolerantie en aanpassingsvermogen tot resultaat, waarschijnlijk vanwege de verschillen in de genetisch bouwplannen van de betrokken anti-stresseigenschappen. Het aanpassingsvermogen van een enkele subpopulatie kan erg verschillen omdat de voor aanpassing benodigde genetische variatie tussen in plaats van binnen de subpopulaties in een metapopulatie verdeeld wordt. Dit kan met name van belang zijn als er sleutelgenen in het spel zijn die afwijken van de standaard aannames, zoals bijvoorbeeld conditionele lethalen.

CONCLUSIES VOOR HET NATUURBEHEER

In mijn experimenten bleek het gemiddelde resultaat van een aantal metapopulaties in de meeste gevallen redelijk overeen te komen met de theoretische verwachting, of deze nu gebaseerd was op de algemeen geldende populatiegenetische theorie, of op herhaalde, individugerichte computersimulaties. Tegelijkertijd verschilden de resultaten van individuele replica's soms aanzienlijk van dit gemiddelde. Met andere woorden, een enkel, klein systeem is vaak niet representatief voor een gemiddelde theoretische populatie. Computersimulaties kunnen betrouwbaarheidsintervallen genereren voor een goedgespecificeerde en gestandaardiseerde laboratoriumopzet, maar in natuurlijke systemen zal het in het algemeen moeilijk, zo niet onmogelijk zijn om de dynamiek van een enkele metapopulatie nauwkeurig te voorspellen.

Dit heeft ook gevolgen voor het bepalen van demografische parameters, zoals de effectieve populatiegrootte of het effectieve aantal migranten per generatie. Hoewel met deze parameters gemakkelijk de dynamiek van de genetische variatie in een metapopulatie beschreven kan worden, kunnen dezelfde waarden N_e of N_em in de praktijk het

resultaat zijn van verschillende censuspopulaties of verschillend migratiegedrag. Voor vragen met betrekking tot de actuele demografie is een grondig inzicht in de onderliggende modelaannames (bijv. wat betreft partnerkeuze of migratiegedrag) nodig voor iedere populatie afzonderlijk, aangezien afwijkingen van de modelaannames tot grote fouten in schattingen van demografische parameters kunnen leiden. Afwijkingen zoals niet-willekeurig paren en de aanwezigheid van natuurlijke selectie die de effectieve grootte van de afzonderlijke subpopulaties beïnvloedt (HOOFDSTUK 2 & 3), komen onafhankelijk van de metapopulatiestructuur voor. Afwijkingen zoals stapsteen-migratie die het effectieve aantal migranten beïnvloedt (HOOFDSTUK 3) en populatie-turnover die de effectieve grootte van de metapopulatie beïnvloedt (HOOFDSTUK 4) zijn daarentegen het gevolg van de metapopulatiestructuur en -dynamiek. In alle gevallen was de range van parameterschattingen echter aanzienlijk, wat leidde tot de conclusie dat zulke schattingen van demografische parameters in het algemeen meer waarde hebben als relatieve maat (bijv. om de genetische uitwisseling tussen populaties te vergelijken) dan als absolute maat (bijv. om het exacte aantal migranten per subpopulatie te voorspellen). Hoewel deze conclusie niet echt als een verrassing komt in het licht van een aantal eerdere, kritische evaluaties (Whitlock & McCauley 1999; Neigel 2002, Austin *et al.* 2004, Pearse & Crandall 2004, Rousset 2004), is het wel verrassend dat veel onderzoek nog steeds zonder meer gebaseerd is op de algemene populatiegenetische theorie, ondanks duidelijke verschillen tussen de modelaannames en het bestudeerde systeem. Voor veel projecten gericht op concrete beheersmaatregelen zou een alternatieve aanpak waarbij genetische gegevens gecombineerd worden met ecologische en historische gegevens (bijv. Gaggiotti *et al.* 2002, Clegg *et al.* 2003) waarschijnlijk beter geschikt zijn.

In veel onderzoeken (overzichten in Armbruster & Reed 2005, Bijlsma & Loeschcke 2005, O'Grady *et al.* 2006) is melding gemaakt van negatieve effecten van inteeltdepressie en genetische erosie die op hun beurt het aanpassingsvermogen van metapopulaties negatief beïnvloeden. De waargenomen afname van de fitness op metapopulatieniveau in de eerste serie experimenten (HOOFDSTUK 3) waarin de subpopulaties erg klein waren (16 individuen) en de migratiesnelheid relatief laag was, zou een gevolg kunnen zijn van vrij hoge niveaus van inteeltdepressie in de afzonderlijke subpopulaties, en komt overeen met de resultaten van eerder onderzoek. Ik heb weinig aanwijzingen gevonden voor soortgelijke effecten op de fitness van de metapopulaties in de tweede serie experimenten (HOOFDSTUK 4) waarin de subpopulaties veel groter waren (gemiddeld 50 individuen), hoewel hier wel wat inteeltdepressie kan zijn voorgekomen op subpopulatieniveau. Dit soort genetische erosie werd met name zichtbaar in de adaptatie-experimenten (HOOFDSTUK 5), en had vooral invloed op het aanpassingsvermogen op metapopulatieniveau in het geval van sleutelgenen die afwijken van de standaard aannames, zoals conditioneel lethalen. In tegenstelling tot in mijn laboratoriumpopulaties, komen zulke specifieke genen in natuurlijke populaties waarschijnlijk slechts zeer beperkt voor, aangezien natuurlijke selectie de schadelijke allelen doorgaans effectief kan verwijderen uit populaties wanneer de ongunstige omstandigheden redelijk vaak optreden (bijv. het MHC-gen in zeehonden bij epidemieën van het phocine distemper virus, Hoelzel *et al.* 1999, Lehman *et al.* 2004).

TERUGBLIK OP DE EXPERIMENTELE AANPAK

Wetenschappelijke studies kunnen in grote lijnen gerangschikt worden van zeer abstract tot zeer concreet: (i) algemene analytische modellen, (ii) computersimulaties, (iii) laboratoriumexperimenten and (iv) veldwaarnemingen. De uitkomsten van wiskundige modellen zijn over het algemeen niet direct toepasbaar in natuurlijke populaties. Om deze kloof tussen de abstracte theorie en toepassingen in het veld te dichten is de combinatie van computersimulaties en labexperimenten zoals in deze studie, een logische aanpak. De experimentele metapopulaties fungeren als een soort "interface" tussen de theorie en de natuur, door de theoretische voorspellingen te vergelijken met de resultaten van experimenten, en de experimentele resultaten te extrapoleren naar toepassingen in natuurlijke metapopulaties. De computersimulaties hebben dezelfde voordelen van herhaalbaarheid, standaardisatie en controle ten opzichte van labexperimenten, als de experimenten hebben ten opzichte van natuurlijke systemen.

In mijn studie heb ik computersimulaties gebruikt om standaard beginwaarden voor de experimentele metapopulaties te genereren inclusief schattingen van de te verwachten experimentele variatie, en om extrapolatie van de experimentele resultaten mogelijk te maken. Hoewel de experimentele opzet en het simulatiemodel allebei uitgaan van de algemene theoretische modellen en met grotendeels dezelfde sleutelaannames beginnen, vertoonden de resultaten van de *in silico* metapopulaties en de experimentele metapopulaties talrijke verschillen als gevolg van onbedoelde afwijkingen van de beginaannames in de experimenten. Experimentele details die niet gespecificeerd waren in de modellen, zoals verschillen in microklimaat tussen de buisjes en de flessen, bleken flinke gevolgen te hebben voor de uitkomst van de experimenten. Dit illustreert hoe belangrijk het is om zowel experimenten als simulaties te gebruiken, en weerspreekt het argument dat een experimentele aanpak helemaal kan worden vervangen door computersimulaties. Simulatie is een uitstekende manier om een experimentele opzet te verbeteren door de evaluatie van het effect van variabelen die niet gespecificeerd worden in de theoretische modellen, of die niet (kunnen) voldoen aan de theoretische aannames vanwege logistieke of andere praktische oorzaken. Simulaties zijn echter niet in staat om de subtiele microvariatie in omgevingsfactoren te bespeuren die de resultaten van experimentele systemen behoorlijk kan beïnvloeden, terwijl zulke verschillen tussen theoretische voorspellingen en een gecontroleerde laboratorium opzet juist belangrijke inzichten in de complexiteit van natuurlijke systemen kunnen opleveren.

Uit mijn experimenten bleek dat de resultaten van de metapopulatiereplica's gemiddeld vrij goed overeen kwamen met de voorspellingen op basis van het simulatiemodel. De individuele metapopulaties vertoonden echter flinke afwijkingen van deze gemiddelden ondanks de strikte standaardisatie van de experimentele opzet. Het is, met andere woorden, over het algemeen niet mogelijk om de dynamiek van een enkele (meta)populatie te voorspellen met enige betrouwbaarheid en precisie. Met behulp van simulaties is het wel mogelijk om de verwachte mate van variatie te voorspellen, en simulaties zijn zeer geschikt om de effecten van parameters als monstergrootte, het aantal gemonsterde subpopulaties, en het aantal genetische merkers te evalueren en te

optimaliseren. Bij een eventuele vertaling van de resultaten van simulaties naar natuurlijke systemen is echter terughoudendheid vereist, omdat geen enkel "natuurlijk" systeem dezelfde eigenschappen heeft als de experimentele metapopulaties of de computersimulaties.

Ondanks de beperkingen ben ik ervan overtuigd dat dit soort "microkosmos-experimenten", zowel met *Drosophila* als met andere modelsoorten, in combinatie met computersimulaties de potentie hebben om een zeer belangrijk hulpmiddel te worden bij het onderzoek aan complexe natuurlijke systemen. Deze studie heeft een aantal belangrijke inzichten opgeleverd met betrekking tot de controle en repliceerbaarheid van zulke experimenten. Bij nader inzien, bijvoorbeeld, zou ik niet een verschillend extinctieschema voor elke metapopulatiereplica gebruiken, en zou ik niet zeer variabele migratiesnelheden toelaten. Mijn belangrijkste aanbeveling is echter om eerst computersimulaties te gebruiken voor de optimalisatie van het experimentele ontwerp, voordat definitief gekozen wordt voor een bepaalde opzet.

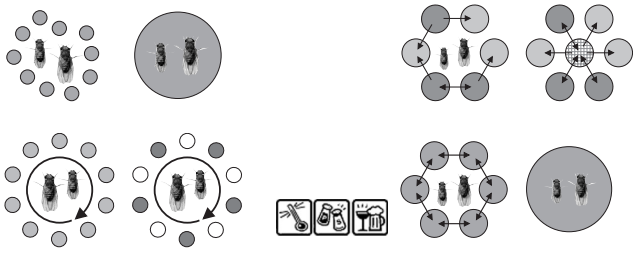
SLOTOPMERKINGEN

Hoewel het concept van de metapopulatie bekend en populair is geworden, hoeft de daaruit volgende metapopulatiebenadering niet altijd de meest geschikte en zinnige manier te zijn om een specifieke natuurlijke populatie te onderzoeken. Vooral de populatiegenetische theorie maakt meestal de aanname dat populaties in evenwicht zijn. In de praktijk zouden populatieversnippering en de achteruitgang van leefgebieden wel eens doorlopende processen kunnen zijn die het bereiken van een evenwicht verhinderen (Hutchison & Templeton 1999), of zouden versnipperde populaties zich helemaal niet volgens welk metapopulatiemodel dan ook kunnen gedragen (Anderson *et al.* 2004). In zulke gevallen is een metapopulatiebenadering ongeschikt om maatregelen voor het beheer en behoud van deze populaties op te stellen omdat de onderliggende modellen niet overeenkomen met het echte systeem (Hanski & Simberloff 1997). Mijn resultaten zijn gedeeltelijk in overeenstemming met dit voorbehoud, vooral wat betreft duidelijke niet-evenwichtssituaties zoals die kunnen voorkomen tijdens populatie-turnover. Noch de experimentele resultaten, noch de resultaten van overeenkomstige simulaties met regelmatig voorkomende lokale extinctie en herkolonisatie over lange periodes bereikten ooit een evenwicht voor de genetische differentiatie (HOOFDSTUK 4), terwijl de meeste theoretische modellen wel uitgaan van zo'n hypothetisch evenwicht (Whitlock & McCauley 1990, Pannell & Charlesworth 1999). Deze en vergelijkbare afwijkingen van de modelaannames veroorzaakten regelmatig verschillen tussen de modelvoorspellingen en de echte situatie in een enkele experimentele metapopulatie, hoewel de overeenkomst tussen de voorspellingen en het gemiddelde resultaat van meerdere replica's over het algemeen best goed was in mijn studie, zeker als je de specifieke eigenschappen van *Drosophila* in aanmerking neemt. De waargenomen verschillen tussen de resultaten van individuele replica's en het voorspelde gemiddelde laten echter zien dat de toepassing van analytische modellen op natuurlijke systemen niet zonder meer aan te raden is,

aangezien natuurlijke populaties doorgaans uniek (een enkel replica) zijn, en bovendien veel complexer zijn dan experimentele systemen. Toegepast onderzoek van natuurlijke systemen vereist een volledig begrip van de onderliggende theorie, en ik pleit voor een uitgebreide samenwerking tussen theoretische en veldbiologen bij de ontwikkeling van analytische en voorspellende hulpmiddelen voor het beoordelen van bedreigde populaties en voorgestelde beheersmaatregelen. In overeenstemming met dit argument, lijkt een alternatieve aanpak om de structuur van een populatie te analyseren met behulp van genetische, ecologische en andere, historische gegevens, zoals aanbevolen door Gaggiotti (2004), ook veelbelovend.

Als eindconclusie zou ik willen betogen dat de algemene theorie de dynamiek in experimentele, en misschien ook in natuurlijke populaties, redelijk kan voorspellen op een kwalitatieve manier, maar dat kwantitatieve bepalingen over het algemeen niet mogelijk zijn, tenzij men de biologie en dynamiek van de betreffende soort in detail kent, en zulke bepalingen vergelijkenderwijs gebruikt binnen een soort, of heel misschien tussen nauwverwante soorten. Aangezien zulke schattingen echter nog steeds flink af kunnen wijken van de “echte” parameterwaarden, raad ik aan om voorzichtig te zijn en brede veiligheidsmarges in acht te nemen bij elke mogelijke praktijktoepassing, zoals bijvoorbeeld de ontwikkeling van beheersmaatregelen voor het behoud van specifieke natuurlijke populaties.

NL



Nawoord

Het heeft iets langer geduurd dan gepland, maar eindelijk ligt hier dan toch mijn proefschrift als het tastbare resultaat van acht jaar "Groningen". Een hoogtepunt in mijn wetenschappelijke carrière, die begon met mijn studie biologie in Leiden, en via een Wagenings onderzoeksinstituut en enkele uitstapjes naar het bedrijfsleven leidde naar de Rijksuniversiteit Groningen. Een flinke stap, van de Randstad naar het "Hoge Noorden". Gelukkig bleek Groningen gastvrij, en ben ik me hier in die acht jaar erg thuis gaan voelen. Ik wil iedereen die daaraan heeft bijgedragen heel erg bedanken.

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Joke