Dynamics of genetic differentiation in experimental *Drosophila* metapopulations

UNRAVELLING THE INTERPLAY OF SELECTION AND MIGRATION

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by

Maria Willemien Kleefsman

born on 22 December 1979
in Veendam

Supervisors
Prof. R. Bijlsma
Prof. F.J. Weissing
Prof. J.P. Bakker

Assessment Committee
Prof. P.J. Palsbøll
Prof. P.H. van Tienderen
Prof. B. Zwaan

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CHAPTER 1

General Introduction

M. W. SMITH-KLEEFSMAN

General background

The structure of genetic and phenotypic differentiation among subpopulations in a metapopulation can be highly dynamic. With the research described in this thesis, I aim to increase our understanding of the complex interactions between processes occurring within and among subpopulations and how this affects genetic and phenotypic variation within metapopulations. The theory of the maintenance and dynamics of genetic variation in population genetics focussed on two different perspectives. According to the neutralist view, alleles are passed to the next generation by the process of genetic drift. This may lead to fixation of certain alleles, while other alleles may disappear from the population. Fixation may be counteracted by the introduction of new alleles in the population; these new alleles are introduced from outside the population by gene flow or appear by mutation (Hu *et al.* 2006). In the contrasting view, natural selection is the main process determining which alleles are passed on to the next generation. Within local populations, genetic variants that increase the fitness of their bearers will increase in frequency, leading to local adaptation and ultimately such alleles may reach fixation. In heterogeneous environments, different genetic variants may produce the fittest individuals in different populations, leading to persistence of genetic diversity. Thus, local adaptation may be an important mechanism maintaining genetic variation (reviewed by Felsenstein 1976, Hedrick *et al.* 1976, Hedrick 1986).

Due to the current global biodiversity crisis, genetic diversity is lost at a large scale. Genetic diversity, however, is extremely important for the existence of species, e.g. to be able to adapt to changing environments or to reduce potential deleterious effects due to inbreeding, and therefore should be conserved (Frankham *et al.* 2002). Population geneticists aim to understand how the spatial and temporal patterns of diversity (alleles and species) are shaped and maintained. In this thesis, I study the dynamics and structure of genetic and phenotypic differentiation within metapopulations, with a main focus on the interplay of gene flow, population turnover and genetic drift as stochastic processes on the one hand, and selective processes resulting in local adaptation on the other hand. I do particularly focus on *i)* validating a commonly used measure for population differentiation, and *ii)* studying the effect of different important metapopulation processes (e.g. differences in the direction of selection among subpopulations, subpopulation turnover, and environmental differences among subpopulations) on population differentiation. In addition, I investigate the role of gene flow in fragmented populations.

Metapopulation dynamics

During the last century, human activities, such as expanding urbanisation, a growing use of resources for consumption and an increased land use for agricultural purposes, started to dominate ecosystems to an increasing extent. This has especially resulted in destruction of natural habitats. As a consequence, for many species the total habitat area available decreased, habitats became more fragmented into patches, and the connectivity between

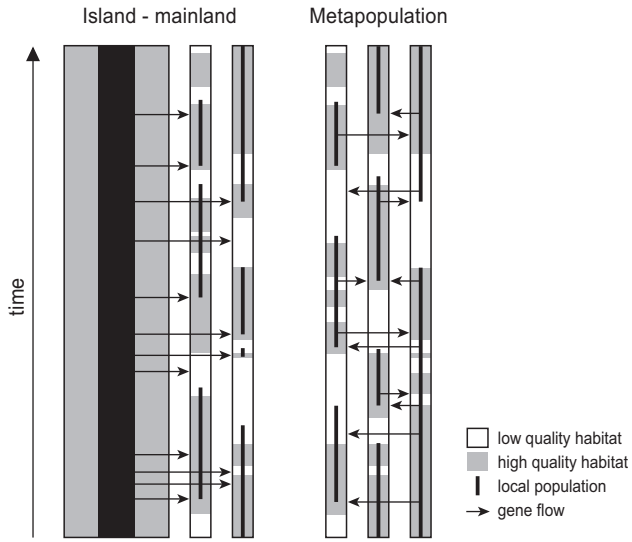


FIGURE 1.1 Schematic representation of a mainland-island model (left) and a metapopulation model (right). In the mainland-island model, gene flow always occurs from the mainland to the islands. Islands can go extinct and be recolonised; the mainland is invulnerable to extinction. In the metapopulation model, gene flow occurs between the different local populations. All populations can go extinct and be recolonised. After Eriksson (1996).

patches decreased. This undoubtedly affects the species living in such disturbed habitats, and significant changes in the distribution of populations, migration rates and the size of local populations have been observed (Harrison and Bruna 1999, Fahrig 2003). In the traditional approach, populations were considered as a large group of individuals living in the same environment and interacting equally with each other. With the on-going fragmentation, new approaches to spatial ecology were needed. In the 1960's, MacArthur and Wilson (1967) developed the equilibrium theory of island biogeography. In their models, there is a large mainland population which is invulnerable to extinction and acts as a source of colonists for surrounding islands (Fig. 1.1, left). This was one of the first steps towards new theory on patchy population structure. At the end of the 1960's, Levins (1969a) introduced the metapopulation concept. He defined a metapopulation as a group of spatially discrete patches in which the species can exist. In time, patches can be occupied with subpopulations or patches can be empty due to extinction of the subpopulation. Subpopulations are to a certain degree connected by migration and empty patches can be (re)colonised (Fig. 1.1, right). A viable metapopulation requires a colonisation rate that is exceeding the extinction rate. Such metapopulation is stable in number of occupied patches, but still can be highly dynamic, involving regular extinctions and (re)colonisation of local patches.

The metapopulation concept of Levins (1969a) has been expanded by others (Hanski 1999, Hanski and Gaggiotti 2004). Assumptions underlying this metapopulation approach are: *(i)* A metapopulation consists of discrete patches. *(ii)* The patches are connected by migration. *(iii)* Empty patches have a certain probability to get colonised and individuals

from every local population have a certain probability to colonise empty patches. *(iv)* Every local population has a certain probability to go extinct, but it can persist for at least some generations. *(v)* Ecological (and genetic) processes take place at two scales: local and regional. And *(vi)* there is little or no correlation between the dynamics of the local populations. Migration is a main characteristic of metapopulations: it makes it possible that empty patches become colonised and it increases the number of individuals in an extant patch, making it less vulnerable for stochastic processes. Especially small isolated populations are vulnerable to environmental and demographic stochasticity. Immigrants (animals or seeds) directly increase the number of individuals in recipient populations, thereby decreasing the effect of environmental and demographic stochasticity and thus increasing persistence probabilities. This process is known as the rescue effect (Brown and Kodricbrown 1977).

GENETIC PROCESSES IN METAPOPULATIONS

Population fragmentation will affect the genetic composition of the individual subpopulations and the metapopulation as a whole. A number of characteristics are important in influencing the genetic variation of a metapopulation: the number and connectivity of patches, subpopulation sizes, extinction and recolonisation rates, the source of the individuals that recolonise empty patches (propagule pool or migrant pool, Fig. 1.2, Slatkin 1977), and the number of founders recolonising a patch (Hedrick 2005b). Mostly, fragmentation leads to reduced gene flow and a reduced overall population size. The same processes that are important in single large populations are also shaping the genetic structure of the subpopulations in the patches. These processes can be divided in the random processes genetic drift and mutation, on the one hand, and natural selection leading to local adapta-

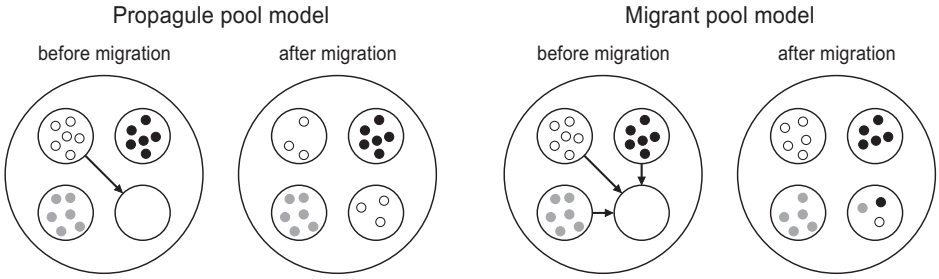


FIGURE 1.2 Schematic illustration of the difference between migration according to the propagule pool (all colonists originate from one subpopulation, left) and the migrant pool model (colonists originate from all subpopulations in the metapopulation, right). In this example, the metapopulations exist of four patches. The propagule pool model assumes colonists to originate from a single subpopulation. These migrants colonising the patch most probably are related and thus may be genetically quite similar. The migrant pool model assumes the colonists founding a new subpopulation to represent a random selection from all subpopulations. In this case the migrants are most probably not related and can be genetically quite distinct.

tion, on the other hand. In addition, gene flow among populations has a homogenising effect opposing genetic divergence through genetic drift and selection. As subpopulations are often small, it may be expected that genetic drift and gene flow become more important, while the impact of selection is reduced compared to large continuous populations (Frankham *et al.* 2002).

Genetic drift

Genetic drift is a change in allele frequencies over generations in a finite population as a result of the random process of sampling selectively neutral variants for the next generation. This change will be negligible in very large populations, but in small populations, genetic drift can lead to substantial fluctuations in allele frequencies and eventually lead to fixation of one allele and loss of the alternative alleles. The extent of genetic drift is proportional to the reciprocal of the population size ($1/2N$): the smaller the population size, the larger the changes in allele frequencies in time and the more important genetic drift becomes. Particularly during bottlenecks, when substantial reductions in population size occur during one or a few generations, the impact of genetic drift is large. The few individuals that are left possess only a small part of the original allelic variation. The population that grows from these individuals may have low genetic variation and by chance a high or low frequency of certain alleles (Frankham *et al.* 2002).

In fragmented populations, subpopulations are mostly relatively small. In addition, subpopulations can be subject to extinction-recolonisation events, drastically reducing population size for one or a few generations. As such, genetic drift may play a substantial role in metapopulations. Because fixation events in different subpopulations are independent, subpopulations are expected to become genetically different. So in time, genetic drift is expected to lead to an increase in genetic differentiation among subpopulations.

Gene flow

Gene flow has a homogenising effect on the genetic composition of different subpopulations (Slatkin 1987). Gene flow restores lost alleles by introducing them through migrants from subpopulations that did not lose that specific allele and as such counteracts genetic drift. When gene flow between adjacent subpopulations is larger than between subpopulations that are more distant, a pattern of isolation-by-distance (IBD) will arise: genetic differentiation among subpopulations is driven by reduced levels of gene flow due to increasing distance (Wright 1943, 1946). So subpopulations that are geographically closer will become more similar than subpopulations that are further apart. IBD patterns are commonly found in nature (Crispo and Hendry 2005, Sexton *et al.* 2014).

Gene flow among subpopulations not only has a homogenising effect on the genetic composition of different subpopulations, but it also may rescue threatened subpopulations. Small isolated populations are not only threatened by environmental and demographic stochasticity causing variation in numbers, but also by threats of inbreeding and loss of genetic diversity (genetic stochasticity). For example, slightly deleterious mutations can

arise and then accumulate, a gradual extinction process called mutational meltdown (Lynch *et al.* 1993, 1995). Due to small population sizes, purifying selection is less effective and genetic drift can lead to fixation of the harmful mutations. Mutational meltdown will lead to loss of fitness and decline of population size in the long-term. At the relatively short-term genetic drift, even under random mating, not only causes loss of genetic diversity, but also deleterious alleles can become more frequent and even become fixed, leading to an increase in homozygosity for deleterious alleles. Homozygosity increases even more rapid in small populations by inbreeding: related individuals are more likely to produce inbred offspring. As many deleterious alleles are recessive, increased homozygosity will cause a decrease in fitness. In summary, the increased expression of the deleterious load present in most populations of diploid organisms leads to a decrease in fitness (inbreeding depression) and an increase in extinction risk (Bijlsma *et al.* 2000, Frankham *et al.* 2002, Bijlsma and Loeschcke 2012). The import of new alleles from elsewhere can slow down or even reverse the fixation of deleterious alleles. In this way, increased homozygosity and loss of genetic diversity that resulted from genetic drift are counteracted, thereby reducing the extinction risk of the subpopulation. This process has been termed genetic rescue (Ingvarsson 2001, Tallmon *et al.* 2004, Bijlsma *et al.* 2010, Hedrick and Frederickson 2010).

Gene flow in plants

Studying gene flow in plants is more complicated than it is in animals. In most higher animals, individuals migrate to other populations and mate, which then can result in gene flow. In plants (except in haploid plant species), two types of gene flow can be distinguished. The first type is zygotic gene flow. In this case, seeds migrate to new populations, leading to plants with a possible deviant genotype from the rest of the plants in that population. In the next generation, these migrant plants can cross with native plants, leading to gene flow between them. Dispersal of seeds is comparable with migration of animals. It affects processes as colonisation probability and extinction risk.

The second type is gametic gene flow, in which pollen disperse to new populations. This pollen fertilises settled plants, leading to "hybrid seeds". Patterns of pollination determine the reproductive neighbourhood size for a plant and the connectivity of the populations in a fragmented population. Pollen grains and seeds are not mobile, but need dispersal vectors to be relocated. These can be either biotic, like insects, birds and mammals, or abiotic, like wind and water (Holderegger *et al.* 2010). Pollen dispersal by insects is strongly dependent on ecological factors influencing the behaviour and occurrence of insects (Richards *et al.* 1999, Velterop 2000). Pollen dispersal by wind is dependent on falling velocity and releasing height of the pollen and on wind characteristics. Not only horizontal speed and direction of the wind are of importance, but also turbulence, in particular thermal turbulence (Tackenberg *et al.* 2003). The study of pollen dispersal has always been challenging, since it is hard to track the physical movement of pollen. A commonly used method is to estimate the movement of pollen that resulted in successful fertilisation through paternity assignment on seeds. For both wind-pollinated and insect-pollinated species, dispersal

regularly takes place over several hundred metres and examples of long-distance pollen dispersal have been found (for a review, see Ashley 2010). Pollen flow cannot lead to recolonisation itself, nor can it affect population size directly. Still, it can reduce the extinction risk of subpopulations, since it counteracts homozygosity and increases genetic diversity (Richards 2000, Ingvarsson 2001). Therefore, pollen flow can play an important role in rescuing local populations (genetic rescue, Tallmon *et al.* 2004, Bossuyt 2007).

Genetic consequences of extinction and recolonisation

Extinction and subsequent colonisation (population turnover) is one of the main characteristics of metapopulations that has a profound effect on the dynamics and level of genetic diversity within such populations. Extinction of a subpopulation may lead to loss of genetic variation in the metapopulation if unique alleles were present in such subpopulation. If the extinction rate exceeds the colonisation rate, the whole metapopulation will go extinct (Fig. 1.3). Recolonisation is closely related to immigration, but the effects are much more drastic, since a whole new subpopulation is established mostly by a few colonists that carry much

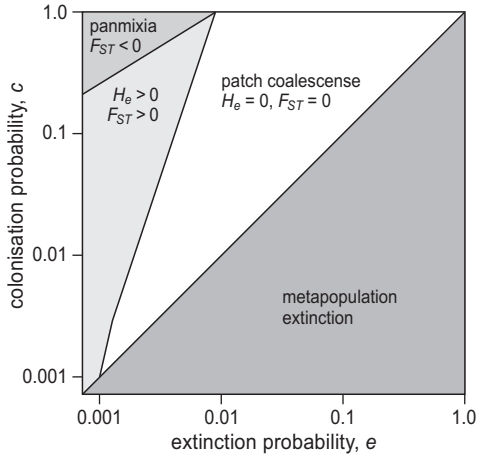


Figure 1.3 Expected levels of genetic diversity in a metapopulation in relation to the extinction (e) and colonisation rate (c). Depending on the relation between the extinction and colonisation probability, we can expect the following situations: *(i)* If the extinction probability exceeds the colonisation probability ($e > c$, right of the diagonal) the metapopulation will go extinct. *(ii)* If the extinction probability is smaller than the migration/colonisation probability, but still of the same order of magnitude, extinctions and (re)colonisations are so frequent that patch coalescence will occur, whereby all individuals are related to only a few ancestors originating for the same subpopulation (Fig. 1.4). In this situation all genetic variation will eventually become lost from the metapopulation. *(iii)* If the extinction probability is low and the migration/colonisation rate not too high, the homogenising effect of migration/colonisation will compensate the loss of variation caused by genetic drift and recurrent bottlenecks whereby genetic variation in the metapopulation will persist ($H_e > 0$), but differentiation among the patches (subpopulations) will also be present ($F_{ST} > 0$). (iv) If the extinction probability is low, but the migration/colonisation rate high, migration among subpopulations become so frequent that the metapopulation can be regarded as a panmictic population, whereby the subpopulations are all genetically similar ($F_{ST} = 0$). Figure after Gilpin (1991).

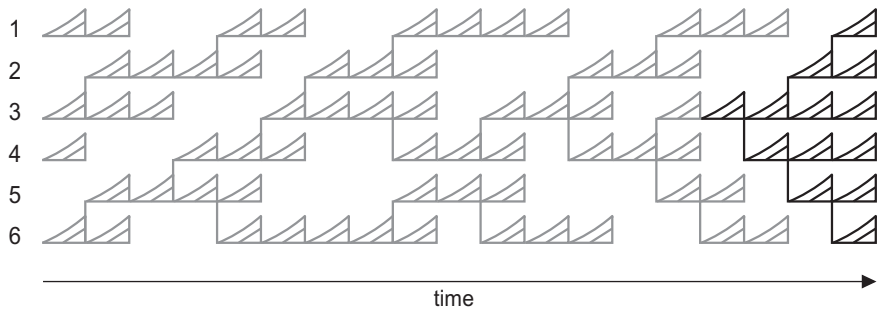


FIGURE 1.4 Schematic of a metapopulation divided in 6 subpopulations that are subject to frequent extinction and recolonisation. The dark-lined subpopulations at the right all trace back to a common recent ancestor population. Modified from Wright (1940).

less genetic variation than the former subpopulation that went extinct. That way, the genetically effective metapopulation size, which is, simply said, the effective number of individuals in a population that contribute offspring to the next generation, is affected by the colonisation rate: If the colonisation rate exceeds the extinction rate and both extinction and recolonisation occur regularly, the genetically effective metapopulation size will get much smaller than expected based on the number of individuals present within the subpopulations, because several individuals may trace back to a recent common ancestor (Fig. 1.4, Wright 1940). The entire metapopulation thus behaves as if it has gone through a series of bottlenecks, which decreases its overall genetic diversity (Lande 1992, Hedrick and Gilpin 1997, Whitlock and Barton 1997, Pannell and Charlesworth 1999, 2000, Hedrick 2001). The diversity within local populations will also tend to be low, if population turnover is frequent, and become similar for all local populations, because all individuals in a subpopulation may trace back to a few colonisers. Ultimately, all variation might become lost from the metapopulation, which is called patch coalescence (Fig. 1.3, Gilpin 1991, Hedrick and Gilpin 1997). With high colonisation and low extinction probabilities ($c \gg e$), all patches will have similar allele frequencies. Since gene flow and colonisation are the same thing in the model of Gilpin (1991), i.e. migrants become colonists as soon as they arrive in an empty patch, not only the colonisation rate becomes high, but also the migration rate. With high migration (and colonisation) rate and low extinction rate, the metapopulation is acting as a random mating population (Fig. 1.3, Gilpin 1991). In a narrow range of colonisation and extinction rate combinations, differentiation in allele frequencies is expected ($F_{ST} > 0$, see Fig. 1.3), due to genetic drift.

In a theoretical study, Wade and McCauley (1988) investigated the effect of extinction and colonisation on the extent of genetic differentiation among local populations comparing the propagule pool and the migrant pool model (Fig. 1.2). They concluded that if new colonies are founded according to the migrant pool model, the genetic effects depend critically upon how colonising groups of individuals are formed, and upon the quantitative relationship between number of colonists (K) and the number of migrants (Nm, Table 1.1). The number of migrants (Nm) is calculated from the number of individuals in the subpopu-

lation (N, each subpopulation containing exactly N individuals) and the migration rate between subpopulations (m). If the number of colonists founding new populations is low compared to the number of migrants exchanged by extant subpopulations ($K < 2Nm$), extinction and colonisation enhance genetic differentiation between subpopulations. However, if the number of colonists exceeds twice the number of migrants ($K > 2Nm$), the genetic diversity of the newly established population is increased, which results in decreased genetic differentiation. Yet, if new colonies are founded by colonists according to the propagule pool model, genetic differentiation is always enhanced (Wade and McCauley 1988, Whitlock and McCauley 1990). Hence, based on their study we can conclude that for metapopulations that are subject to extinction and (re)colonisation, genetic differentiation is generally enhanced. Bakker and colleagues (2008, 2010) experimentally investigated the consequences of subpopulation turnover for fragmented populations, using *Drosophila melanogaster* as a model organism. They found in the presence of recurrent extinction and recolonisation a substantial decrease of genetic diversity and higher levels of differentiation between subpopulations, confirming these theoretical predictions.

Table 1.1 Effect of extinction and recolonisation on the level of genetic differentiation within a metapopulation compared to the situation without population turnover. K is the number of individuals colonising an empty patch. Nm is the number of migrating individuals.

Colonisation mode	Number of colonists	Effect on population differentiation
Migrant pool model	$K < 2Nm$	Increased
	$K > 2Nm$	Decreased
Propagule pool model	$K < 2Nm$	Increased
	$K > 2Nm$	Increased

Local adaptation

Often the geographic range a species lives in encompasses a variety of environmental conditions with which it has to cope and can adapt to. Individuals can adapt to a local environment in different ways. The expression of an individual's genotype can display different phenotypes in response to environmental differences. This is called phenotypic plasticity, a common phenomenon in nature (Bradshaw 1965, Roff 1997, Schlichting and Pigliucci 1998, West-Eberhard 2003). Natural selection can also adapt genotypes to the prevailing local conditions. If gene flow between subpopulations is limited and the selection pressures between subpopulations are in different directions, natural selection can be expected to lead to genetic adaptation of subpopulations to their local conditions (Lenormand 2002). This results in subpopulations existing of individuals that have higher relative fitness in their own habitat than in other habitats (Kawecki and Ebert 2004, Bijlsma and Loeschcke 2005). The presence of diversifying natural selection does not necessarily result in a locally

adapted population. Natural selection can be constrained either by the available genetic variation and underlying genetic architecture, or by regular changes in the local environmental conditions (Kawecki and Ebert 2004). Another important factor influencing the result of natural selection, especially in small populations, is genetic drift (Lowe *et al.* 2004). Genetic drift leads to increasing differentiation between subpopulations, but this differentiation is not the result of adaptation to the local environment, but it is the result of loss of alleles within subpopulations. Since genetic drift is a random process, it will generally result in an increase in the frequency of non-adapted alleles, although by chance it can also lead to an increase in the frequency of adapted alleles (Frankham *et al.* 2002, Kawecki and Ebert 2004). Importantly, natural selection may also be counteracted by migration. Migrants that move to habitats they are not, or only partly, adapted to, may introduce genes that are mal-adaptive to the new environment. With high migration rates, the genetic composition of a local population is rather determined by the genetic variants carried by the immigrants than by local adaptive variants and consequently adaptation will break down (Lenormand 2002). However, migration may not always lead to gene flow (i.e. successful spread of the migrant's genes), since the mal-adapted migrants and their possible (hybrid) offspring have lower fitness than the residents, a process which is also known as 'selection against immigrants' (Rundle and Whitlock 2001, Hendry 2004, Nosil *et al.* 2005). This leads to isolation-by-adaptation (for reviews see Nosil *et al.* 2009, Orsini *et al.* 2013) or isolation-by-environment, although isolation-by-environment is defined broader than isolation-by-adaptation, not only including processes that lead to isolation due to adaption, that is natural and sexual selection against immigrants and reduced hybrid fitness, but also including other processes that lead to isolation between populations due to the environment (Sexton *et al.* 2014, Wang and Bradburd 2014). Isolation-by-adaptation is analogous to isolation-by-distance whereby genetic differentiation among subpopulations is driven by reduced levels of gene flow due to increasing distance (Wright 1943, 1946). With isolation-by-adaptation, effective gene flow is reduced due to migrants being mal-adapted in the new environment.

Measuring genetic population differentiation

The most basic way to quantify genetic differentiation is to consider the variance in allele frequencies across the subpopulations of a metapopulation. For this, the measure F_{ST} is most often used, which corresponds to a normalised variance in allele frequencies. F_{ST} has been generalised in a variety of ways; these more generalised measures are typically called G_{ST}. For many traits of interest, allele frequencies are not readily available. This holds in particular for many quantitative phenotypic traits for which the genetic basis is typically unknown. For such traits, population differentiation is often quantified by the phenotypic measure Q_{ST}.

F_{ST} AND G_{ST}

Wright (1951, 1965) developed a measure that can be used to estimate the amount of genetic variation that can be explained by population structure. He defined F_{ST} as a measure of genetic differentiation based on the variance of allele frequencies among populations (σ_p^2):

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

in which $\bar{p}$ is the average allele frequency for a biallelic locus. If all subpopulations have the same allele frequencies, the variance is 0 and $F_{ST} = 0$. If the subpopulations are fixed for different alleles ($\bar{p} = 0.5$), the variance is at its maximum, and $F_{ST} = 1$.

F_{ST} as defined by Wright can only be used in a two-allele system. Nei (1973) redefined F_{ST} for multiple alleles as

$$G_{ST} = \frac{H_T - H_S}{H_T}$$

Where H_S is the expected average subpopulation heterozygosity and H_T is the expected heterozygosity for the total population. The latter is calculated as $H_T = 1 - \sum \bar{p}_i^2$ in which $\bar{p}_i$ is the average frequency of allele i over subpopulations. Nei (1973) showed that for a two-allele system, F_{ST} is identical to G_{ST}, since at a biallelic locus, $H_T = 2\bar{p}(1-\bar{p})$ and $H_T - H_S = 2\sigma_p^2$. For a locus with more than two alleles, G_{ST} is equal to the weighted average of F_{ST} for all alleles (Nei 1973).

Weir and Cockerham (1984) expanded the variance based method to estimate genetic differentiation among subpopulations. This method is based on an ANOVA of allele frequencies. The genetic differentiation among subpopulations, given by θ, is calculated out of the variance in allele frequency diversity between populations (σ_b^2) compared to the total variance in allele frequencies ($\sigma_b^2 + \sigma_w^2$, in which σ_w^2 is the variance within populations):

$$\theta = \frac{\sigma_b^2}{\sigma_b^2 + \sigma_w^2}$$

The methods described above are the most widely used methods to determine population differentiation based on genetic markers at present.

Q_{ST}

Spitze (1993) defined Q_{ST} by

$$Q_{ST} = \frac{\sigma_b^2}{\sigma_b^2 + 2\sigma_w^2},$$

in which σ_w^2 is the additive genetic variance of a quantitative trait within subpopulations and σ_b^2 the additive genetic variance of this trait between subpopulations. This formula is similar to the formula calculating θ, except for the factor 2 in the denominator, which is due

to the quantitative genetic variance among populations being two times F_{ST} (Whitlock 2008). If the quantitative trait reflects the additive interaction of a large number of identical loci, each with a small effect, and if the allelic variance at all these loci is similar (i.e. all these loci have a similar F_{ST} value), then the variance components correspond to

$$\sigma_w^2 = (1 - F_{ST})\,\sigma_0^2,$$
$$\sigma_b^2 = 2F_{ST}\,\sigma_0^2 \text{ and}$$
$$\sigma_t^2 = (1 + F_{ST})\,\sigma_0^2,$$

in which σ_t^2 is the total variance of the subdivided population and σ_o^2 is the total variance of the trait expected in panmixia (Wright 1951, Spitze 1993). If this is inserted in the equation for Q_{ST}, the result is $Q_{ST} = F_{ST}$. In other words, under the above assumptions the measure Q_{ST} for phenotypic differentiation is expected to be identical to the measure F_{ST} for genetic differentiation.

Comparing Q_{ST} and F_{ST}

Genetic differentiation among subpopulations may be the result of selection leading to local adaptation, but also of neutral processes like gene flow or genetic drift. At present, the role of selection in leading to different phenotypes in nature is an important issue in population biology (Leinonen *et al.* 2008, 2013, Naish and Hard 2008, Räsänen and Hendry 2008). To make inferences about the presence of selection, it has been proposed to compare genetic differentiation for neutral traits like microsatellites, measured as F_{ST}, with genetic differentiation in quantitative traits, measured as Q_{ST} (Merilä and Crnokrak 2001, Spitze 1993), since quantitative traits are subject to the same neutral processes, but can be under selective pressure as well (Leinonen *et al.* 2013). Broadly speaking, we can expect three possible outcomes of a comparison between Q_{ST} and F_{ST} (Merilä and Crnokrak 2001). If Q_{ST} and F_{ST} are more or less equal, this could be explained by genetic drift and gene flow, so there is no evidence for any selection pressure. This does not necessarily mean that there is no selection, but neutral and selective processes are indistinguishable. When Q_{ST} is found to exceed F_{ST}, this is often viewed as evidence for directional selection, leading to the predominance of different genotypes in different subpopulations. If Q_{ST} values are lower than F_{ST} values, there is evidence for selection favouring the same phenotypes in different populations (balancing selection). Results of studies that compare Q_{ST} with the neutral expectation have been reviewed (Merilä and Crnokrak 2001, McKay and Latta 2002, Leinonen *et al.* 2013). Most studies observe that Q_{ST} is exceeding F_{ST}, leading to the conclusion that diversifying selection plays an important role in most natural populations. However, Leinonen and colleagues (2008) argued that this result can partly be a matter of sampling bias or publication bias.

Q_{ST} is calculated from phenotypes, but used as a measure of genetic differentiation. Therefore, a critical assumption underlying Q_{ST} is that the within and between population variance components should be merely determined by additive genetic effects. They should be free of non-additive effects, like dominance (Lopez-Fanjul *et al.* 2003, Goudet and Buchi

2006) and epistasis (Whitlock 1999) and other deviations from the standard assumptions of quantitative genetics like maternal and environmental effects (Merilä and Crnokrak 2001) and linkage disequilibrium (Le Corre and Kremer 2003). It occurs regularly that Q_{ST} is estimated directly from natural populations. However, when measuring quantitative divergence under natural conditions, results are expected to be biased, since not only selection acting on additive genetic variation drives the phenotypic divergence of populations, but also, for example, phenotypic plasticity (Raeymakers *et al.* 2007, Pujol *et al.* 2008). To distinguish between the different ways Q_{ST} can be assessed, it is suggested to use different terms for the different situations: P_{ST} in case differentiation is measured immediately from the natural population, broad sense Q_{ST} in situations in which environmental effects are minimised, but other non-additive effects cannot be excluded, and narrow-sense Q_{ST} in situations in which environmental and non-additive effects are excluded (Leinonen *et al.* 2008). All in all, there are many reasons why Q_{ST} can largely differ from F_{ST}, even in the absence of any (balancing or diversifying) selection. For this reason, inferences based on the comparison of Q_{ST} and F_{ST} only make sense if they are made under conditions where these factors play a minor role.

Besides the difficulties in estimating Q_{ST} accurately, as described above, Q_{ST} also has been shown to have very different properties than often claimed. Miller and colleagues (2008) showed in a theoretical study that it is not necessarily true that the mean Q_{ST} of an additive neutral trait resembles the mean F_{ST} of a neutral trait. They found that Q_{ST} tends to exceed F_{ST} when population subdivision occurred only recently, while F_{ST} tends to exceed Q_{ST} when subpopulations have been isolated for a long period of time. They owed this to the unequal contribution of the loci to the trait: some loci had more effect on the phenotypic trait than others. Whitlock (2008) showed in a theoretical study that both F_{ST} of a neutral locus and Q_{ST} of a neutral trait are expected to show wide distributions. So finding a Q_{ST} and F_{ST} value that differ from each other does not necessarily mean they do not originate from the same distributions. Therefore, Whitlock (2008) recommends to ask whether the Q_{ST} of a trait is expected to be part of the F_{ST} distribution. Notwithstanding, Q_{ST} is thought to be a useful measure to initially explore the role of selection in subdivided populations (Leinonen *et al.* 2008, Miller *et al.* 2008, Whitlock 2008). Further theoretical and empirical investigations are needed to increase our understanding of the precise relationship between Q_{ST} and F_{ST}.

Thesis outline and approach

Experiments

It is still unclear which conclusions can be drawn out of certain patterns of Q_{ST} and F_{ST} values. To investigate this, I studied the dynamics and structure of genetic and phenotypic differentiation among subpopulations while implementing several typical processes specific for metapopulations, such as extinction and recolonisation, diversifying selection and environmental variation. To this end, I performed experiments with *Drosophila*

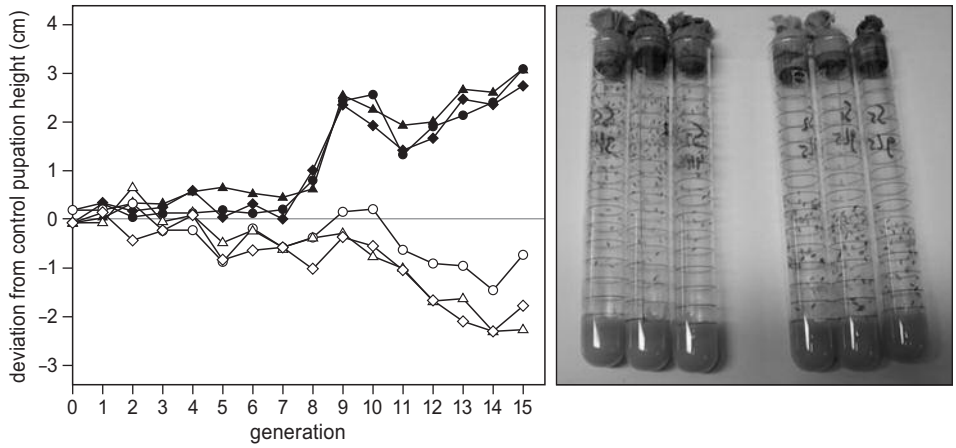


FIGURE 1.5 Information about the high- and low-pupating selection lines used. Left: Directional selection during 15 generations resulted in high- and low-pupating lines. Black symbols indicate the three lines that were selected for high pupation height, open symbols those selected for low pupation height. Unpublished work by M.E.C. van Rijswijk. Right: Vials with high- (left) and low- (right) pupating pupae; the inner walls of the vials are covered with a transparent sheet that is marked with centimetre lines to make it possible to determine the pupation height.

melanogaster, which is a widely used model organism to study population and conservation genetics issues (Frankham 1995, Pertoldi *et al.* 2007, Bijlsma and Loeschcke 2012). I created artificial metapopulations consisting of 3-10 subpopulations. Each subpopulation was reared in a separate vial or bottle. Artificial migration took place between subpopulations, by placing individuals from one vial/bottle into another. To estimate phenotypic differentiation among the subpopulations, I measured two quantitative traits. One of the phenotypic characters I used to assess phenotypic differentiation was pupation height. In the last larval stage, larvae select a suitable pupation site. They typically crawl away from the food, in response to several cues, like larval density (Joshi and Mueller 1993) and abiotic cues, like humidity, light, gravity and texture of the substrate (Godoyherrera *et al.* 1989, Casares *et al.* 1997). Pupation height is a trait that can easily be selected. The selection lines for pupation height that I used were created in 2003 by M. E. C. Van Rijswijk (Fig. 1.5 left, personal communication) and originated from the Groningen 83 (G83) wild population. This strain was founded in 1983 with 403 inseminated females captured at the fruit market in Groningen (The Netherlands), and maintained as a large population since (Zwaan *et al.* 1991, Vermeulen and Bijlsma 2006). The selection procedure resulted in three high and three low lines which differed considerable in pupation height (Fig. 1.5 left). This difference is visualised in Figure 1.5 (right), which shows also how pupation height was assessed. To maintain the selection response, the selection lines were continued under a mild selection pressure after the selection procedure. Every other generation, the highest (H-lines) or lowest (L-lines) half of the pupae was selected to form the next generation.

The other phenotypic character I used to asses phenotypic differentiation was sternopleural bristle number. This trait is governed by many genes with small effect that

largely act additive (Killick 1972, Mackay and Lyman 2005). In addition, sternopleural bristle number is known to be plastic in relation to the developmental temperature and shows a negative correlation with temperature: the higher the developmental temperature the lower the number of bristles and vice versa (Parsons 1961, Thoday and Gibson 1970, Bubliy *et al.* 2000). Therefore, this trait was very suitable to be used in my experiments on the simultaneous effect of phenotypic plasticity and diversifying selection on genetic differentiation. I also calculated genetic differentiation for supposedly neutral markers (microsatellites). The dynamics of the quantitative traits and markers was assessed over a number of generations while implementing several typical processes specific for metapopulations, such as extinction and recolonisation, diversifying selection and environmental variation.

To decrease the experimental variation among replicates, for each subpopulation we determined on forehand the migration, extinction and recolonisation and selection treatments (these include: the sex of the migrants, the number of migrants it donated and the subpopulations these migrants had to migrate into; the number of migrants it received and the subpopulations these migrants originated from; the subpopulation(s) that is/are subject to extinction; the generation an empty patch is recolonised; the subpopulation(s) that is/are subject to selection and the exact pupation height/bristle number that is selected for). The treatments were standardised over the replicate metapopulations, i.e. each replicate metapopulation was treated in exactly the same way.

Simulations

The experiments, generally were short-term, running for a few to maximal 13 generations. Therefore, I added simulations to several of my studies, to investigate what can be expected to happen on the more long-term. The simulation model I used was an extended version of the simulation model of Bakker (2007) and Bakker *et al.* (2010), which allows to study the dynamics of genetic differentiation in a metapopulation under a broad range of conditions. I could vary parameters such as migration rate, migrating sex, mating before or after migration, extinction and recolonisation events and diversifying selection on quantitative traits. In the simulations, pupation height was implemented as a polygenic trait controlled by 20 independent loci with each two alleles that all contributed evenly to the phenotypic trait. Neutral genetic variation was simulated as multiple independent diallelic loci. For each generation, the simulation program calculates important genetic parameters, such as differentiation in quantitative traits (Q_{ST}) and differentiation in neutral markers (F_{ST}).

Chapter overview

The goal of this thesis is to investigate the interplay of different population dynamic and genetic processes on the dynamics of genetic variation in metapopulations, such as population turnover, different direction of selection among subpopulations, migration and genetic drift. In addition, I aim to validate the use of the $Q_{ST} - F_{ST}$ comparison to assess the presence of selection in a metapopulation.

In the absence of selection, extinction and recolonisation are known to lead to an increasing genetic differentiation among subpopulations as frequent extinction and recolonisation events result in lower effective population sizes. In **Chapter 2**, I investigate the impact of subpopulation turnover on genetic differentiation for a polygenic morphological trait (pupation height) in metapopulations in the presence of diversifying selection, both in an experimental and theoretical setting to increase our understanding of the dynamics and persistence of local adaptation for a quantitative trait in metapopulations that are subject to extinction and recolonisation. Furthermore, I address the question whether, and to what extent, the dynamics of Q_{ST} and F_{ST} reflect the presence of diversifying selection in the presence of population turnover.

The $Q_{ST} - F_{ST}$ comparison is based on the assumption that Q_{ST} is affected by the presence of selection and F_{ST} is not, i.e. F_{ST} is calculated from markers that behave selectively neutral. However, it is conceivable that selection for a quantitative trait also affects the dynamics of such neutral loci (background selection). In **Chapter 3**, I investigate whether the dynamics of neutral microsatellite variation is affected by the presence of diversifying selection on the polygenic trait pupation height. By creating experimental *Drosophila* metapopulations that are, or are not, subject to diversifying selection, I will compare Q_{ST} values (based on the polygenic trait pupation height) and F_{ST} values (based on microsatellites) in a replicated and controlled situation. Accompanying simulations allowed me to estimate the generality of the experimental results.

Chapter 4 reports on the same question as addressed in Chapter 3, but in this case I investigate whether neutral polygenic variation is affected by diversifying selection on another polygenic trait. I describe the results of an experiment in which I quantify the change in pupation height (polygenic trait that is target of selection) and bristle number (polygenic trait that is not target of selection) in the same metapopulation and compare the dynamics of genetic differentiation among subpopulations for both traits. In addition, I investigate the effect of different migration regimes by comparing stepping stone versus island migration.

Q_{ST} in a broad sense is thought to be a measure for the level of genetic differentiation among subpopulations, but free of environmental effects. Many traits (if not all), however, do respond to the environment. In **Chapter 5**, I use temperature clines to study the consequence of environmental variation on Q_{ST}. To this end, I establish simple metapopulations for which the two factors genetic variation (measured as bristle number) and environmental temperature co-vary either positively (cogradient variation) or negatively (countergradient variation).

Chapter 6 is a literature study evaluating how gene flow by pollen in relation to distance can be approached. In this chapter, I evaluate what physical and biological components should be included in a mathematical model that aims to estimate the probability of successful gene flow among subpopulations by means of pollen. Since this chapter was accomplished on the authority of the Dutch committee of genetic modification (COGEM), it is oriented on outcrossing probabilities of genetically modified plants, but most processes described are similar for natural plant populations.

In the final chapter (**Chapter 7**), I summarise the main results of the chapters 2-5 and discuss their possible consequences for the use of the $Q_{ST} - F_{ST}$ comparison to infer the presence or absence of selection for morphological and life history traits in natural populations.

CHAPTER 2

Evolution in experimental *Drosophila* metapopulations: The impact of population turnover on local adaptation

M. W. Smith-Kleefsman
F. J. Weissing
J. Bakker
R. Bijlsma

ABSTRACT

The dynamics of genetic variation within metapopulations is determined by the interplay of selection pressures, genetic drift, and gene flow. Here we study the role of subpopulation extinction and recolonisation on the dynamics of neutral (F_{ST}) and quantitative differentiation (Q_{ST}) in a metapopulation that is subject to migration and local adaptation. In replicated experiments on *Drosophila melanogaster* we followed the short-term evolution of a quantitative trait (pupation height). The experiments were complemented by a simulation study allowing predictions on a longer-term perspective. In the absence of extinctions, both Q_{ST} and F_{ST} approached one under diversifying selection. Adding population turnover had a homogenising effect among subpopulations (Q_{ST} and F_{ST} became lower). This can partly be explained by the fact that our experimental design allowed locally mal-adapted individuals to colonise empty patches. High rates of extinction and recolonisation resulted in frequent bottlenecks, in which continually more genetic variation gets lost, eventually leading to the fixation of a single allele at the metapopulation level. Our results demonstrate that differences between Q_{ST} and F_{ST} strongly depend on the degree of population turnover. Hence, in settings with regular extinction and recolonisation the comparison of Q_{ST} and F_{ST} is of limited use for making inferences on the strength and nature of selection.

INTRODUCTION

The distribution of many organisms resembles that of a metapopulation: the population is subdivided in a set of relatively small subpopulations, which show largely independent demographic dynamics but are interconnected by migration (Levins 1969a, Gilpin and Hanski 1991, Hanski and Gilpin 1997, Hanski and Gaggiotti 2004 and references therein). Even species that under natural conditions show a more continuous distribution are nowadays often forced into a metapopulation structure, as a result of destruction and fragmentation of their natural habitats (Hedrick 2001, Frankham *et al.* 2004). Population fragmentation has since long been recognised to have a significant impact on the structure and dynamics of genetic variation within and among (local) populations (Wright 1952, 1978, Slatkin 1985, Nei 1987). Compared to large continuous populations, the main evolutionary forces all have a different effect in metapopulations (Whitlock 2004); ongoing research has revealed that genetic drift, migration (gene flow), natural selection, local adaptation and local population turnover play pivotal roles in the dynamics and maintenance of genetic variation (Slatkin 1977, Wade and McCauley 1988, Pannell and Charlesworth 1999, Whitlock and McCauley 1999, Lenormand 2002, Spichtig and Kawecki 2004, Bakker 2008).

For selectively neutral variation, the dynamics is mainly governed by the local effective population sizes (determining the extent of genetic drift) and the degree of gene flow between local populations. Under idealised conditions, Wright's F_{ST}, the most frequently used measure of genetic differentiation among local populations (Whitlock 2004), is expected to converge to $1/(4Nm + 1)$ with N being the genetically effective number of breeding individuals in local populations and m the rate of gene flow (Wright 1969). An important assumption underlying this expectation is that subpopulation sizes remain more or less constant over generations. However, as local populations have largely independent demographic dynamics and are relatively small, a typical characteristic of metapopulations is that local populations can go extinct and get recolonised again by migration (Levins 1969a, Hanski and Simberloff 1997, Thrall *et al.* 2000, Ovaskainen and Hanski 2004).

Slatkin (1977) was the first to model the consequences of extinction-recolonisation events for genetic differentiation in a metapopulation with a finite number of local populations under two different migration scenarios: a "propagule pool" scenario (all founders colonising an extinct population originate from the same local population) and a "migrant pool" scenario (the founders originate from different local populations). This work was extended by Wade and McCauley (1988) and Whitlock and McCauley (1990). Generally their results show that including population turnover considerably increases genetic differentiation among local populations, the effect being the strongest under the propagule pool scenario. According to these models, genetic differentiation will only decrease under special circumstances (a migrant pool scenario where the number of founders recolonising an empty habitat patch is at least twice as large as the number of migrants exchanged by extant local populations). Moreover, population turnover is also expected to reduce the overall genetic diversity in metapopulations (Lande 1992, Hedrick and Gilpin 1997, Whitlock and Barton 1997, Pannell and Charlesworth 1999, 2000, Hedrick 2001). At high

rates of population turnover, all variation might become lost from the metapopulation because all individuals in local populations trace back to a few founders (patch coalescence; Gilpin 1991, Hedrick and Gilpin 1997). Experimental work by Bakker (2008) and Bakker *et al.* (2010) using *Drosophila* metapopulations confirmed these general expectations. Moreover, several empirical studies have revealed that genetic differentiation among recently colonised local populations is generally larger than for local populations that have been extant for a more extended period. This finding is an indirect confirmation of the expectation that population turnover increases differentiation in a metapopulation (Whitlock 1992, McCauley *et al.* 1995, Giles and Goudet 1997, Ingvarsson *et al.* 1997, Mopper *et al.* 2000, Haag *et al.* 2005, Bay *et al.* 2008, but see Dybdahl 1994).

The foregoing concerned neutral genetic variation, which is often used for inferring demographic patterns and processes. These results do not necessarily apply to selective variation that from an evolutionary perspective may be more relevant (Hedrick 2001, Bijlsma and Loeschcke 2005, 2012, Sgro *et al.* 2011). Habitats often differ considerably from each other, inducing spatially varying selection pressures. Such selection towards local adaptation is expected to lead to an increase in the differentiation of (selectively relevant) genetic variation among local populations (Lenormand 2002). However, migration among local populations may counteract such local adaptation, since it may cause an influx of mal-adapted immigrants ('migration load', Lenormand 2002, Räsänen and Hendry 2008). Conversely, selection against mal-adapted migrants (and their offspring) will affect the amount of effective gene flow and consequently influence the genetic differentiation among subpopulations (Olivieri *et al.* 1995, Olivieri and Gouyon 1997, Hendry 2004, Nosil *et al.* 2005, Räsänen and Hendry 2008). Reduced effective gene flow leads to isolation-by-adaptation, an equivalent of isolation-by-distance, but with gene flow reduced due to selection pressures instead of distance (Nosil *et al.* 2005, 2009).

The amount of differentiation among local populations will therefore depend on the interplay of diversifying selection and gene flow. At low levels of migration local adaptation will persist while at high(er) levels of migration local adaptation may be lost (Lenormand 2002, Blanquart *et al.* 2012). In the latter case, most allelic variation underlying the adaptive differences will be lost as well (Bulmer 1972, Lenormand 2002). That local adaptation can persist notwithstanding gene flow is evidenced by the observation of sharp clines for heavy metal tolerance in many plant species (Bradshaw 1952, MacNair 1997). As such, spatial heterogeneity is thought to promote the maintenance of genetic variation within (meta)populations, though the conditions for protected polymorphism are generally quite stringent and the presence of soft selection seems a necessity (Christiansen 1975, Felsenstein 1976, Hedrick *et al.* 1976, Hedrick 1986). For polygenic traits the situation may be more complex, but different theoretical approaches have indicated that the maintenance of polygenic variance can also be promoted by spatial heterogeneity (Via and Lande 1987, Gillespie and Turelli 1989, Spichtig and Kawecki 2004), although empirical data have yielded contradictory results (Yeaman and Jarvis 2006, Yeaman *et al.* 2010).

Currently, there is considerable interest in assessing the amount of adaptive variation in natural populations and in demonstrating the presence of local adaptation (Hansen *et al.* 2012, Schoville *et al.* 2012, Andrew *et al.* 2013, Leinonen *et al.* 2013). One of the standard

tools for inferring the nature and importance of selection in fragmented populations, is to compare levels of differentiation in neutral genetic markers like microsatellites (typically quantified by Wright's F_{ST}; Holsinger and Weir 2009) with levels of differentiation in quantitative traits that are presumed to be under selection (typically quantified by Q_{ST}; Whitlock 2008). In the absence of selection, Q_{ST} and F_{ST} are expected to be of the same order of magnitude. When Q_{ST} is found to exceed F_{ST}, this is often viewed as evidence for directional selection, leading to the predominance of different genotypes in different subpopulations (Merilä and Crnokrak 2001, Leinonen *et al.* 2013). When Q_{ST} values are found to be lower than F_{ST} values, this is viewed as evidence for stabilising selection, favouring the same phenotypes in different populations (Merilä and Crnokrak 2001). In nature, Q_{ST} is generally observed to exceed F_{ST}, leading to the conclusion that diversifying selection plays an important role in most natural populations (for reviews, see Merilä and Crnokrak 2001, McKay and Latta 2002, Leinonen *et al.* 2013). Apart from statistical issues (Hedrick 2005a, Whitlock 2008, Edelaar and Björklund 2011), there are several factors that can possibly confound this conclusion. In particular, local population turnover may affect the relationship between Q_{ST} and F_{ST}. The effect of population turnover is, however, not well understood. In fact, to the best of our knowledge, no experimental studies on the maintenance of local adaptation have included population turnover.

In this study, we therefore investigation the effect of extinction and recolonisation on the dynamics and persistence of local adaptation for a quantitative trait in metapopulations. We approach this in two ways. First, we use experimental *Drosophila* metapopulations to study the persistence of local adaptation for pupation height in the presence (or absence) of artificial diversifying selection: half of the subpopulations are selected for high pupation height and the other half for low pupation height. We set up two series: one including both gene flow through migration and population turnover through extinction-recolonisation events, and one including only gene flow. Second, the experiments were complemented with an individual-based simulation study that mimicked the experimental conditions to a large extent. This allowed us to compare the experimental results with theoretical predictions, to investigate additional parameter settings, and to assess long-term effects. In addition to the trait under selection, our simulations also include neutral variation, allowing us to compare the dynamics of both selected and non-selected genetic variation. In particular, we are interested in the question whether, and to what extent, differences in the selection regime are reflected by differences in the dynamics of Q_{ST} and F_{ST}.

Materials and Methods: Experiment

Drosophila stocks

For the experiment, flies from the Groningen 83 (G83) wild type population were used. This population was founded in 1983 with 403 inseminated females captured at the fruit market in Groningen (The Netherlands), and maintained as a very large population since (Zwaan *et al.* 1991, Vermeulen and Bijlsma 2006). From this population, several selection lines for

FIGURE 2.1 Pupation height measurement. Left panel: picture of experimental vials with the inside lined with a clear plastic sheet with a centimetre scale at which pupae are attached. Right panel: schematic of the unfolded sheet with pupae showing the interval classes used for scoring pupation height. Pupae exactly on a scaling line were scored as belonging to the lower interval.

pupation height were established by directional selection for high and low pupation height during 15 generations. At the end of the selection procedure these lines differed significantly in pupation height: means 9.8 cm & 4.4 cm, respectively (for scaling, see Fig. 2.1). Thereafter, the lines were kept under a weak selection regime for about 40 generations. At the start of this experiment, the lines still differed in pupation height: means 9.4 cm & 3.8 cm, respectively.

Flies were maintained and cultured under standard conditions (25°C, 50–60% RH) in bottles or vials on standard medium (26 g dead yeast, 54 g sugar, 17 g agar, 13 ml nipagine solution (10 g nipagine in 10 ml 96% alcohol) and 250 mg streptomicine per litre). For handling, flies were anesthetised with CO_2.

EXPERIMENTAL SET-UP AND PROCEDURE

At the start of the experiment metapopulations, comprising 10 subpopulations each, were established. Figure 2.2 shows a diagram of the different experimental steps involved. A subpopulation was initiated with 20 inseminated females that were placed in a 20 cm high glass vial with about 2.5 cm medium. The inner wall of these vials was lined with a piece of transparent plastic sheet with a printed centimetre scale at which larvae adhere during pupation (Fig. 2.1). This sheet, with the pupae adhered, was removed from the vial, to enable easy determination of pupation height and selection of pupae (see below).

The mated females were placed in clean vials in the morning, and were allowed to lay eggs for a maximum of 24 hours. The number of eggs was checked regularly to prevent

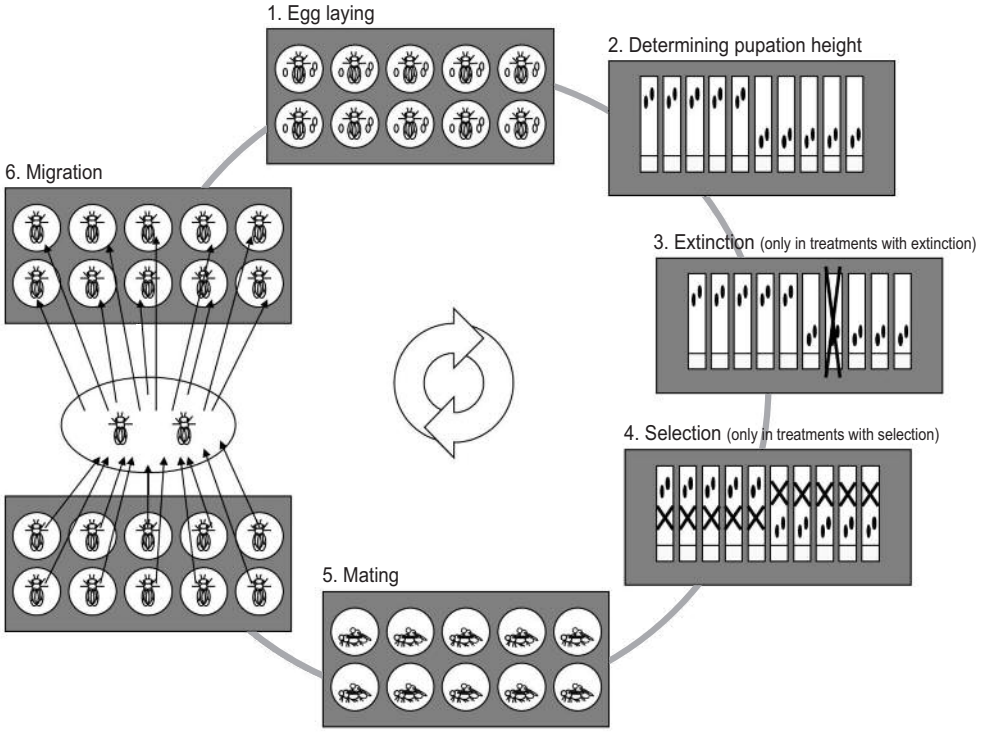


FIGURE 2.2 Schematic overview of the experimental steps for metapopulations established with five high- and five low-pupating subpopulations. First, females were allowed to lay eggs (1). In the pupal stage, pupation height was determined (2), and the appropriate treatments, either extinction (3) and/or selection (4), were applied. After eclosion, flies were allowed to mate within their subpopulation (5) and migration was implemented according to the migrant pool scenario (6). Thereafter the cycle started all over again.

crowding and when sufficient eggs were laid the females were removed. After pupation, the height of all pupae was determined using the centimetre scale on the sheet, as outlined in Figure 2.1. Thereafter, extinction and selection were implemented (see treatments section) and the remaining pupae were transferred to clean vials. After eclosion, adults were allowed to mate and 20 inseminated females were randomly chosen from each subpopulation to establish the next generation. From these 20 females, one was randomly selected to migrate according to the migrant pool model. Migration was done in such way that all subpopulations had one individual emigrating, and one individual immigrating. This migration scheme thus resembles the migrant pool model (Slatkin 1977, Wade and McCauley 1988), except that the migrants were uniformly distributed over the subpopulations. As the migrants were randomly assigned to one of the subpopulations, they could with a probability of 1 out of 10 migrate back to their subpopulation of origin, resulting in an average of nine migrants per generation per metapopulation. The exact migration scheme was pre-set for each generation separately before the start of the experiment and was the same for all

replicate metapopulations to ensure true replication. After migration, the flies were transferred to clean vials and the next generation started. This procedure was continued for 7 generations.

Treatments

For each metapopulation, five of the 10 subpopulations (SP1 to SP5) were each initiated with 20 mated females from the high-pupating line and five (SP6 to SP10) with each 20 mated females from the low-pupating line. As such, the metapopulations started with a high level of differentiation.

We applied four different treatments: *(i)* selection only, *(ii)* extinction only, *(iii)* selection and extinction simultaneously, and, as a control, *(iv)* no selection and no extinction. Selection for pupation height took place in the pupal stage. In the treatments with selection, half of the subpopulations (SP1 to SP5) was selected for high pupation height. After removing the sheet from the vial and determining pupation height, the upper part of the sheet containing the 50 highest individuals was selected and transferred to a clean vial, the rest of the sheet was discarded. The other half of the subpopulations (SP6 to SP10) was selected for low pupation height. In this case, the lower part of the sheet containing the 50 lowest individuals was selected and transferred to a clean vial. In the treatments without selection, after determining pupation height, the whole sheet was transferred to a clean vial.

In the treatments with extinction, every generation one subpopulation of each metapopulation was randomly selected to go extinct by discarding all pupae of that subpopulation, resulting in an extinction rate of $e = 0.1$. The order of extinction was pre-set at the start of the experiment and was the same for all metapopulations. Migration was done in the same way as for the treatment without extinction, except for the subpopulations that went extinct. As the extinction event occurred before migration was implemented, these subpopulations did not contribute an emigrating individual to the migrant pool. Consequently the subpopulation that was destined to receive the migrant from this subpopulation did not receive an immigrant. Due to the uniform way in which the migrants were distributed over the subpopulations, extinct subpopulations were colonised in the same generation as they went extinct, except when the immigrating individual was supposed to come from the subpopulation itself, which occurred for subpopulations 7 in generation 3.

For every treatment, five replicates were run and exactly the same migration scheme and extinction scheme were applied to all treatments and to all replicates within treatments to standardise migration and extinction and diminish variation between the replicates.

Estimates of genetic differentiation

Differentiation of pupation height was quantified by Q_{ST}. This dimensionless measure was developed to quantify the genetic differentiation of quantitative traits. It is defined as

$$Q_{ST} = \frac{\sigma_b^2}{\sigma_b^2 + 2\sigma_w^2}$$

(Spitze 1993), where σ_b^2 is the additive genetic variance attributed to among-subpopulation variation and σ_w^2 is the additive genetic variance attributed to within-subpopulation variation. In our experiments, we only measured phenotypic variances and calculated Q_{ST} from these variances. Since the experiments were performed in a common environment, we assume the components of phenotypic variance to be proportional to the corresponding components of additive genetic variance.

The variance components used for calculating Q_{ST} are estimated by an analysis of variance (ANOVA). One of the assumptions underlying an ANOVA is that the variance of the different groups is unrelated to the group average. This was not the case in our study. We found a linear relationship between the standard deviation in pupation height (s) and the average pupation height ($\bar{x}$):

$s = 0.19\bar{x} + 0.95$, $R^2 = 0.63$.

As recommended in the statistic literature (Miller 1986), we corrected for this by log-transforming our data: $x_i' = \log(x_i + 1)$. After this transformation, there was only a very weak association between standard deviations and averages:

$s = -0.04\bar{x} + 0.19$, $R^2 = 0.06$.

Materials and Methods: Individual-based simulations

We adapted an existing simulation model used by Bakker (2008) and Bakker *et al.* (2010) to reflect the set-up of our experiments: Metapopulations consisted out of 10 subpopulations of which the population size was kept at 40 individuals (20 females and 20 males). After eclosion, mating took place within subpopulations, in line with the mating system of *D. melanogaster* (lottery polygyny; see Bakker (2008) for details). For the treatments with extinction, extinction of subpopulations occurred at random with probability e per subpopulation. We simulated four situations; *(i)* No extinctions, $e = 0$, *(ii)* low extinction rate, $e = 0.01$, *(iii)* intermediate extinction rate, $e = 0.05$ and *(iv)* high extinction rate, $e = 0.10$. After extinction, migration took place. From each extant subpopulation, one mated female was randomly chosen as emigrant and randomly assigned to one of the ten subpopulations. This resembles the migrant pool model of migration (Slatkin 1977, Wade and McCauley 1988) and is thus slightly different from the experimental set-up where a subpopulation never received more than one immigrant.

Hereafter, the next generation was produced: randomly selected mated females produced 20 female and 20 male offspring matching the selection criteria for each subpopulation. We did not take intra-subpopulation demography into account, so even after a recolonisation event by one inseminated female subpopulation size was immediately back to carrying capacity. However, in the generation directly following a recolonisation event, no selection was implemented to account for the fact that in the experimental situation typically no selection pressure was exerted after a recolonisation event, because subpopulations were not yet at carrying capacity and the soft selection scheme was applied (see below).

Pupation height was implemented as a polygenic trait that is controlled by 20 independent loci with two alleles (0 and 1) each that act additively within and across loci. The phenotype was scaled (see Fig. 2.1) from zero cm (all loci fixed for the 0 allele) to 15 cm (all loci fixed for the 1 allele). In addition to pupation height, neutral genetic variation was represented by another set of 20 independent loci, also with two alleles each.

Simulations were initiated either with a low level of differentiation among subpopulations for both sets of genes or with a high initial level of differentiation. In the latter case, five subpopulations were started with high-pupating individuals (average pupation height 10 cm) and the other five with low-pupating individuals (average 4 cm), resulting in an initial Q_{ST} = 0.8. At the same time, for the neutral variation, the first five subpopulations were initiated with all loci fixed for one of the alleles at each locus while the other five were fixed for the alternative allele, resulting in an initial F_{ST} = 1. In the simulations starting with an initially low level of differentiation, all subpopulations were started at an intermediate pupation height (average 7 cm) and an average allele frequency of 0.5 for the neutral genes, thus starting with Q_{ST} = 0 and F_{ST} = 0.

Simulations were run both in the absence and presence of diversifying selection for pupation height, which in the latter case means that five subpopulations were selected for high pupation height (in the range 9–15 cm) and five for low pupation height (range 0–5 cm). The selection scheme applied corresponds to soft selection, since these ranges were adjusted to the phenotypic values of all potential offspring in a subpopulation. To this end the range selected was extended downwards (in case of the 'high' selected subpopulations) or upwards (in case of the 'low' selected subpopulations) when the parents were unable to produce offspring in a particular generation that fitted the selection criteria for that subpopulation. This was particularly the case after an extinction event and when the colonising female(s) came from subpopulations that were selected in the opposite direction of that of the receiving subpopulation.

Results: Experiment

Effect of selection on pupation height

According to theory, ongoing migration will slowly erode the phenotypic differences (e.g. in pupation height) between subpopulations in the absence of selection (Hedrick 2005b). In line with this expectation, the difference in mean pupation height of the 10 subpopulations, compared to the overall population mean, gradually decreased (Fig. 2.3, top left). Mean subpopulation pupation height converged to the overall mean for the metapopulation. This regression to the overall mean was roughly linear, with a highly significant linear regression coefficient (b = 0.26, p < 0.001; linear regression of the average difference of subpopulation pupation height and metapopulation height on generation number, where the deviation of subpopulation pupation height and metapopulation height in "high" subpopulations SP1-SP5 was multiplied by –1, to make them commensurable with the "low" subpopulations SP6-SP10 that started below the metapopulation mean). Figure 2.3 (top

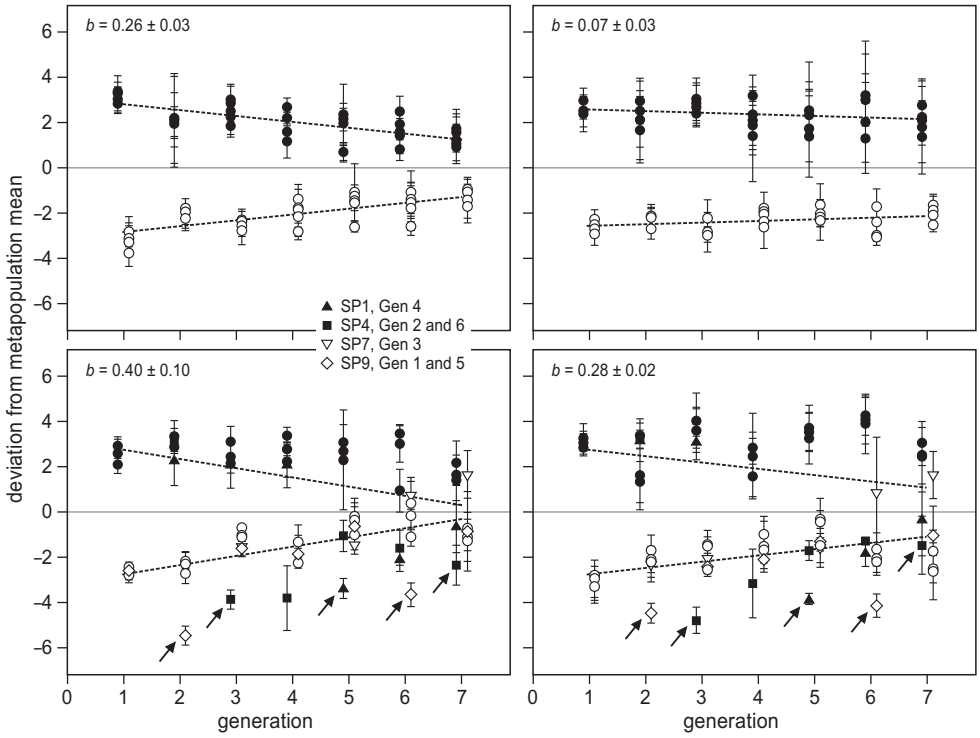


FIGURE 2.3 Change in the difference between subpopulation pupation height (± sd) and mean pupation height in the metapopulation over generations for four different treatments. Top left: control (no selection, no extinction); Top right: diversifying selection for pupation height, no extinction; Bottom left: no selection, population turnover through extinction and recolonisation; Bottom right: selection and population turnover. Filled and open symbols indicate subpopulations that were initiated with flies from the high- and low-pupating line, respectively, which in the appropriate treatments were selected for high and low pupation height, respectively. Circles indicate subpopulations that did not go extinct; Other symbols indicate subpopulations that went extinct and were immediately recolonised in the generation as indicated (SP7 was not recolonised immediately after extinction, but one generation later). The dashed lines show the linear regression lines; Regression coefficients (± s.e.) are presented at the top of each panel.

right) shows corresponding results in the scenario where the homogenising effects of migration were counteracted by antagonistic directional selection for pupation height. We still observed a significant regression of subpopulation pupation height to the mean metapopulation level (b = 0.07, p = 0.014), but the regression coefficient is significantly lower than in the scenario without selection (Fig. 2.3 top left) and the regression to the metapopulation mean is very slow.

The bottom panels of Figure 2.3 show the effect of extinction and recolonisation on the interplay of migration and diversifying selection. Again, homogenisation of pupation height proceeds significantly faster in the absence (bottom left panel) than in the presence (bottom right panel) of diversifying selection. In both cases, homogenisation was considerably faster than in the absence of extinction events. However, the situation is complicated

by the fact that subpopulations that underwent an extinction-recolonisation event (indicated by arrows in Fig. 2.3) had a considerably lower mean pupation height than the other subpopulations that did not undergo such an event.

Effect of extinction-recolonisation events on pupation height

Figure 2.4 shows in more detail the dynamics of pupation height for the four subpopulations (SP) that underwent one or two extinction-recolonisation events. Of these, SP1 and SP4 were initiated with flies from the high-pupating line (and selected for high pupation height), while the other two (SP7 and SP9) were initiated with flies from the low-pupating line (and selected accordingly). SP1 became extinct in generation 4 and was subsequently recolonised by a high-pupating migrant. Nevertheless, the average pupation height was exceptionally low in the next generation and increased gradually in the next generations towards the average pupation height of the corresponding subpopulations that did not go

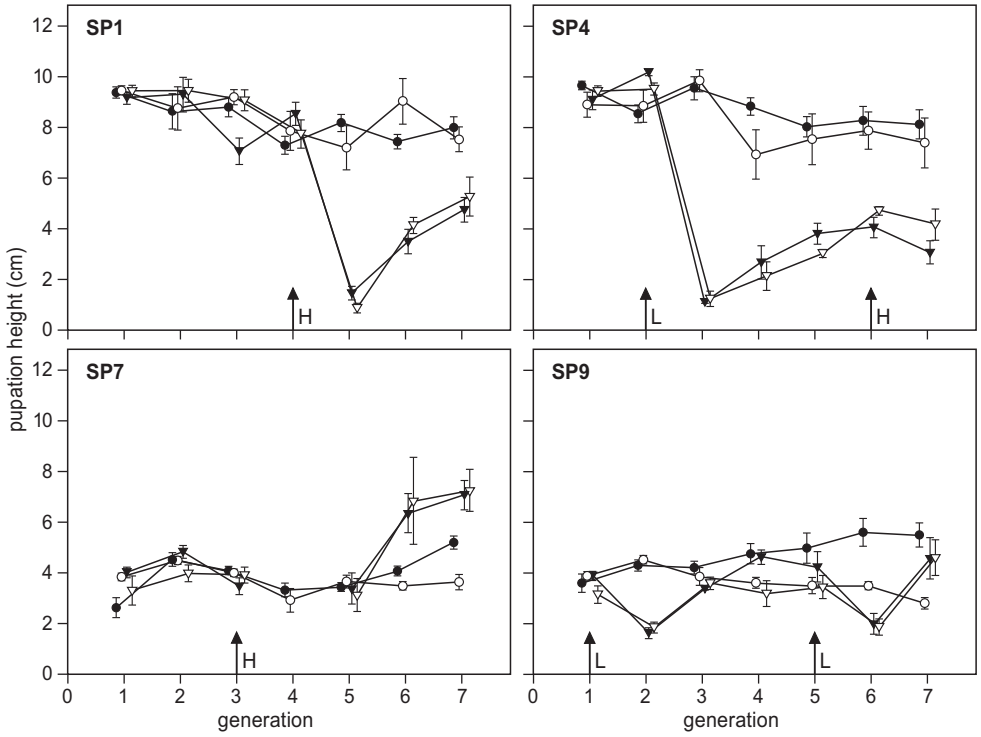


Figure 2.4 Average pupation height (± s.e.) of the four subpopulations that underwent extinction compared to the same subpopulations of the no-extinction treatment (averages over 5 replicates). Circles indicate treatments without extinction, triangles those including extinction and recolonisation; Filled symbols indicate treatments without selection, open symbols those including diversifying selection. Arrows indicate the generation where extinction and immediate recolonisation occurred. H and L indicate that the subpopulation was recolonised by a migrant from one of the subpopulations 1–5 and 6–10, respectively. Subpopulation SP7 was not recolonised immediately after extinction, but one generation later.

extinct. A similar pattern is observed for SP7, which after extinction was also recolonised by a high-pupating migrant. For this subpopulation, average pupation height was still low in the following generation, only to increase gradually thereafter. SP4 (in generation 2) and SP9 (generation 1 and 5) were recolonised by migrants from low-pupating subpopulations and also showed a very low average pupation height directly after colonisation. These findings are in line with the observations in Figure 2.3 (bottom panels) that the average pupation height of subpopulations that underwent an extinction-recolonisation event are clearly lower than the metapopulation mean.

The explanation for this phenomenon is that the average pupation height is strongly dependent on density: the lower the density, the lower the pupation height (Joshi and Mueller 1993). This density dependency occurs in both pupation lines, but is strongest in the high-pupating line (see Supplementary Material S1). After an extinction event, the patch is recolonised by a single female. Since one female can produce only few offspring, density in the generation after extinction and recolonisation is low, resulting in low pupation height, even for the high-pupating line (Fig. S1). The effect of density on pupation height in the generation directly following an extinction overrules the effect of diversifying selection (compare open versus filled triangles in Fig. 2.4).

The effect of selection and extinctions on subpopulation differentiation

Differentiation in pupation height among subpopulations within metapopulations as measured by Q_{ST} is shown in Figure 2.5. In agreement with the observed changes in pupation height, Figure 2.5 shows that Q_{ST} values stayed high in the presence of antagonistic directional selection (open symbols) while Q_{ST} decreased gradually in the absence of selection (filled symbols). The presence or absence of extinction and recolonisation events had very little effect on the changes in Q_{ST}. We used a two-way ANOVA to test if selection and extinction-recolonisation events had a significant effect on subpopulation differentiation in generation 7: The effect of selection was highly significant ($F_{1,16}$ = 29.030; p < 0.001), but extinction-recolonisation events had no significant effect ($F_{1,16}$ = 0.014; p = 0.91), nor had the interaction between selection and extinction and recolonisation ($F_{1,16}$ = 2.688; p = 0.12).

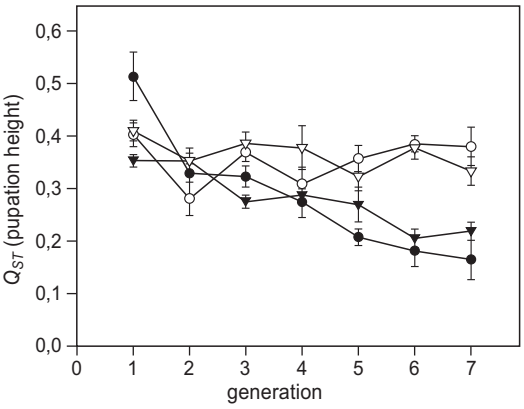


Figure 2.5 Genetic differentiation (Q_{ST} ± s.e.) of pupation height for the different extinction and selection treatments. Filled circles: no extinction and no selection; Open circles: diversifying selection without extinction; Filled triangles: extinction without selection; Open triangles: both extinction and diversifying selection.

Results: Simulations

We used individual-based simulations to explore the long-term consequences of extinction and recolonisation on the interplay of migration and diversifying selection. For comparison, we included neutral loci in the simulations and studied the dynamics of these at the same time.

Evolution of pupation height

Figure 2.6 shows the dynamics of mean pupation height for four extinction rates in both the absence and presence of antagonistic directional selection. The simulations presented here were all initiated with a maximum level of differentiation, but the outcome was essentially similar for simulations initiated with no differentiation among subpopulations (data not shown). When no selection was implemented, all subpopulations converged to a pupation height of around 7 cm (Fig. 2.6, left panel). In the presence of extinction and recolonisation, the same outcome was observed, but convergence was faster: the higher the extinction rate, the faster the equilibrium value of 7 was reached.

The right panel of Figure 2.6 shows the evolutionary dynamics in the presence of diversifying selection. In the absence of extinction (solid lines), the subpopulations selected for high and low pupation height rapidly diverged, leading to equilibrium values of about 10 and 4, respectively. Diversifying selection is apparently strong enough to achieve local adaptation despite considerable gene flow. This is confirmed by an inspection of individual

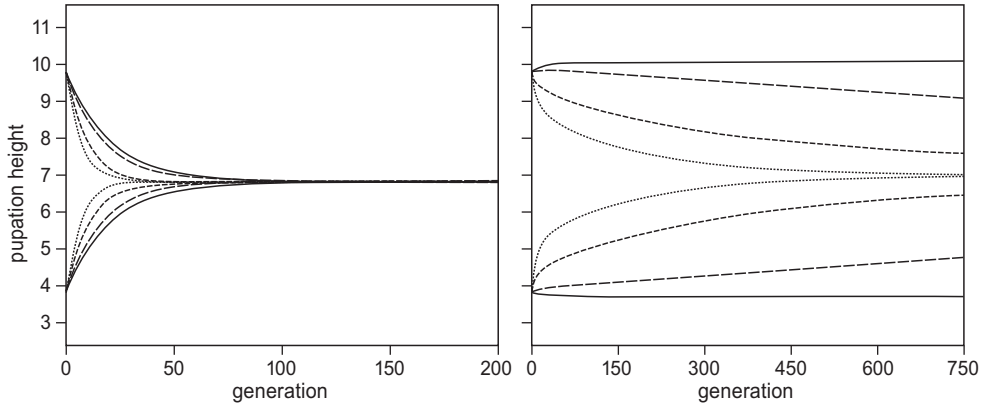


Figure 2.6 Change in mean subpopulation pupation height over generations for the different treatments. Pupation height was averaged over 1000 replicate runs and combined for subpopulations (SPs) 1–5 and SPs 6–10. SPs 1–5 and SPs 6–10 were initiated with the high-pupating and low-pupating line, respectively, and in the appropriate treatments selected for high and low pupation height, respectively. Subpopulations were initiated with high genetic differentiation. Left panel: selection absent (no further changes were observed after generation 200); Right panel: diversifying selection included. Solid lines: no extinction; Long dashed lines: low extinction rate (e = 0.01); Short dashed lines: intermediate extinction rate (e = 0.05); Dotted lines: high extinction rate (e = 0.10).

simulation runs (see Supplementary Material S2, runs 1–4). In the presence of extinction and recolonisation, population differentiation disappeared and all subpopulations converged to the same pupation height in a long-term perspective, although convergence to a homogeneous equilibrium was very slow for low extinction rates. Clearly, extinction and recolonisation promote effective gene flow between subpopulations with selection occurring in opposing directions. More detailed information about the dynamics of this process can be found in the Supplementary Material S2 (runs 5–12).

Dynamics of differentiation

Figure 2.7 shows the dynamics of differentiation for pupation height (Q_{ST}) and neutral loci (F_{ST}) for the four extinction rates both in the presence and absence of diversifying selection. The simulations were initiated with high differentiation between subpopulations. (When simulations were started without any initial differentiation, the results were essentially similar, data not shown.) In the absence of selection (left panels), Q_{ST} and F_{ST} rapidly converged to an equilibrium. Averaged over all runs, the equilibrium values were similar for Q_{ST} and F_{ST}. These equilibrium values show a clear positive relation with extinction rate, indicating that in the absence of selection, extinction and recolonisation increase differentiation for both measures. As we implemented extinction before migration this may, at least in part, be explained by the fact that higher extinction rates automatically lead to a lower on average number of migrants per generation (9.8, 9,2 and 8.3 for e = 0.01, e = 0.05 and e = 0.10, respectively; see Supplementary Material S3).

It has to be noted that the solid lines in Figure 2.7 depict the average calculated only over those metapopulations that were still genetically variable at the time of measurement. However, at the higher extinction rates (e = 0.05 and e = 0.10) an increasing number of metapopulations had lost their genetic variation (Q_{ST} and/or F_{ST} were equal to zero). Including these metapopulations in the calculation resulted in a decreasing overall differentiation (dotted lines).

In the presence of diversifying selection, genetic differentiation for pupation height stayed high or increased when simulations were initiated at high Q_{ST} = 0.8 (Fig. 2.7, top right). In the absence of extinction, Q_{ST} even converged to the maximal value 1. These results confirm that the applied selection pressure was sufficient to counteract the homogenising effect of migration. Implementing extinctions again significantly affects the level of genetic differentiation within metapopulations, but opposite to what we observed in the absence of selection: the higher the extinction rate, the lower the Q_{ST} becomes. This is in line with the convergence observed for mean pupation height (Fig. 2.6)

Both diversifying selection for pupation height and extinction rate also strongly affect genetic differentiation for neutral loci. In the presence of selection, F_{ST} stays near to 1 (Fig. 2.7, bottom right), which greatly contrasts the trajectory observed in the absence of selection (Fig. 7, bottom left). The finding that F_{ST} is near to maximal in the absence of extinction suggests that selection on pupation height also effectively counteracts the homogenising effect that migration is expected to have on neutral loci, leading to the conclusion that migrants exchanged by subpopulations that differ in the direction of selection do not, or

rarely, lead to effective gene flow (see also Chapter 3, Smith-Kleefsman *et al.* 2015b). Extinction-recolonisation events have a pronounced effect on genetic differentiation for neutral loci. A low extinction rate of $e = 0.01$ decreases the (near) equilibrium value already substantially (from $F_{ST} \approx 1$ to $F_{ST} \approx 0.7$, Fig. 2.7, bottom right). This shows that extinction and recolonisation lead to more effective gene flow between subpopulations. The effect becomes stronger when the extinction rate increases, and at the highest rate F_{ST} seems to decrease continuously in the end for those metapopulations that are still genetically variable.

Comparison of Q_{ST} and F_{ST}

Q_{ST} and F_{ST} are often studied in parallel in order to make inferences on the type and strength of selection (Merilä and Crnokrak 2001, Leinonen *et al.* 2013). To investigate whether, and to what extent, a comparison of Q_{ST} and F_{ST} allows inferences on diversifying selection in a metapopulation, we calculated for all our simulations how the difference

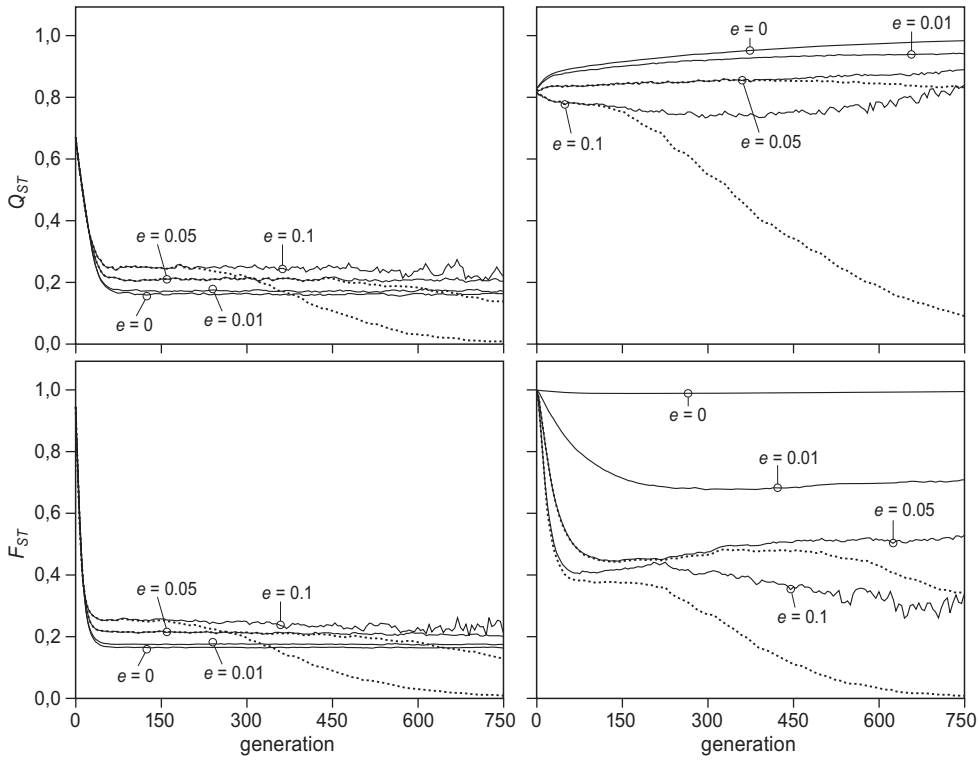


Figure 2.7 Mean level of genetic differentiation for pupation height (Q_{ST}, top panels) and for neutral loci (F_{ST}, bottom panels). The left panels show the results when selection was absent and the right when diversifying selection was present. Means were either calculated over only the metapopulations that were still genetically variable for the trait (solid lines) of including both variable and fixed metapopulations (dotted lines, only for the two higher extinction rates $e = 0.05$ and $e = 0.1$).

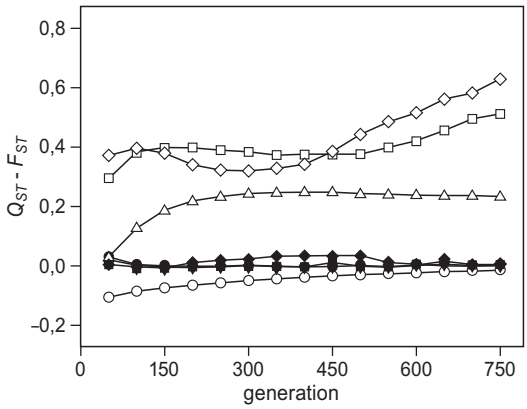


FIGURE 2.8 Mean difference between Q_{ST} and F_{ST} (expressed as Q_{ST} minus F_{ST}) averaged over 1000 metapopulations that were subject to different extinction rates. Selection was either absent (filled symbols) or present (open symbols). Four different extinction rates were implemented: No extinction (circles), Low extinction (e = 0.01, triangles), Intermediate extinction (e = 0.05, squares) and High extinction (e = 0.1, diamonds). NB: genetically fixed metapopulations were omitted from the calculations (see Fig. 2.9 for percentage of metapopulations fixed).

between Q_{ST} and F_{ST} changed over the generations. Figure 2.8 shows the results for the simulations that were initiated with high genetic differentiation among subpopulations.

In the absence of selection, Q_{ST} equals F_{ST}, as expected from theory (Merilä and Crnokrak 2001). In the presence of selection and absence of extinction, Q_{ST} and F_{ST} both converge to 1 (Fig. 2.7 right), implying that the difference on average converges to zero, as was indeed observed (Fig. 2.8). In the presence of both selection and extinction, Q_{ST} and F_{ST} converged to intermediate values, with Q_{ST} exceeding F_{ST} (Fig. 2.7 left). Hence, diversifying selection leads to a positive difference between Q_{ST} and F_{ST}. However, the magnitude of this difference is strongly affected by the extinction rate: the higher the extinction-recolonisation rate the smaller the difference between Q_{ST} and F_{ST}. These results may be important for inferences drawn from a comparison between Q_{ST} and F_{ST}: the magnitude of this difference may more strongly reflect factors like population turnover than the strength of selection.

METAPOPULATION FIXATION

High rates of extinction and recolonisation events may lead to patch coalescence, i.e. the variation in the metapopulation collapses to a single genotype, as all individuals relate to a few colonisers of the past generations (Gilpin 1991, Hedrick and Gilpin 1997). From the simulations, we observed this process only at the higher extinction rates (e = 0.05 and e = 0.1) as evidenced by the dashed lines in Figure 2.7. The rate of metapopulation fixation is shown in Figure 2.9 for metapopulations started with no initial genetic differentiation. After some 100 generations the first metapopulations become genetically fixed for either the pupation height loci (Q_{ST} = 0) or the neutral loci (F_{ST} = 0) and this number increases

steadily over time. The higher the extinction rate the more rapid fixations occur. In the absence of selection (solid lines) the trajectories are, as expected, similar for both loci under selection and neutral loci. Including antagonistic directional selection in the simulation causes fixations to occur earlier in the process (dashed lines), but for the neutral loci the rate of increase in the number of fixed metapopulations is comparable to the one observed in the absence of selection. For the loci under selection, however, the number of fixed metapopulations seems to increase more slowly than in the absence of selection, and the two lines intersect when around 50% of the metapopulations have become fixed for pupation height. If simulations were initiated with a high genetic differentiation, the results were qualitatively similar (data not shown).

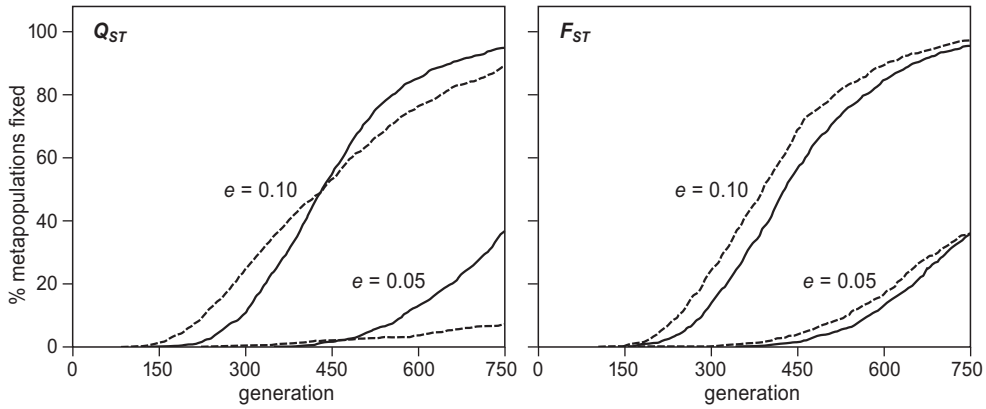


FIGURE 2.9 Simulation results for metapopulation fixation at the two higher extinction rates. Graphs depict the percentage of metapopulations that had become fixed over time for pupation height (Q_{ST} = 0, left panel) and neutral loci (F_{ST} = 0, right panel) for two different extinction rates: e = 0.05 and e = 0.1. Selection was either absent (solid lines) or present (dashed lines).

TABLE 2.1 Mean pupation height in the fixed metapopulations in generation 750. The average pupation height ($\bar{x}$ ± s.e.) and the number of metapopulation that had become fixed (N) out of thousand runs is presented for both selection treatments (antagonistic directional selection present or absent) and for the intermediate and high extinction rates. Means are shown for both simulations that started with a high and that started with low initial genetic differentiation.

	Intermediate extinction rate		High extinction rate	
	High differentiation	Low differentiation	High differentiation	Low differentiation
No selection implemented				
$\bar{x}$ ± s.e.	6.30 ± 0.12	6.61 ± 0.10	6.41 ± 0.08	6.58 ± 0.06
N	345	315	903	903
Selection implemented				
$\bar{x}$ ± s.e.	7.00 ± 0.13	6.96 ± 0.14	7.00 ± 0.03	6.99 ± 0.03
N	58	54	806	791

Pupation height at fixation

As shown in a previous section (Fig. 2.7), antagonistic directional selection leads to high levels of genetic differentiation for pupation height in metapopulations, indicating that the subpopulations consisted of either mainly high- or mainly low-pupating individuals. Patch coalescence than might be expected to result in metapopulation fixation for either one of these extreme phenotypes. However, we observed that on average the phenotype at fixation was mostly intermediate and that variance was pretty low while we did also not find any signs of bimodality. Moreover, the mean differed only slightly from the mean values observed when selection was not present (Table 2.1). This seems due to the fact that selection is absent or weak after an extinction event under low density conditions, which enables mal-adapted migrants and their offspring to settle in subpopulations they are not adapted to. As adapted migrants also can easily migrate into this subpopulation, it may lead to "hybrid" offspring with an intermediate phenotype/genotype. Hereby, the most extreme phenotypes/genotypes may be lost through, for instance, genetic drift, which makes it impossible to attain extreme trait values again, despite the high selection pressure present in later generations. This can ultimately lead to erosion of the extreme trait values in the metapopulation as a whole (For more detail, see Supplementary Material S2).

Discussion

Traditionally, most studies concerning the genetic differentiation among subpopulations within a metapopulation have focused on the interplay between genetic drift and migration (gene flow, Wright 1931, 1952, Slatkin 1985, Nei 1987). More recently, it has been recognised that differences in local selection pressures (Lenormand 2002, Hendry 2004) and population turnover (Wade and McCauley 1988, Whitlock and McCauley 1990) also greatly affect the dynamics of genetic variation within metapopulations. Here we mainly focused on the role of population turnover in the presence of diversifying selection in the dynamics of genetic differentiation in metapopulations using both a short-term experimental and a longer-term simulation approach. Our main conclusions are: *(i)* Antagonistic directional selection, as applied in this study, yields and/or maintains high levels of genetic differentiation between subpopulations, thus not only leading to local adaptation for the polygenic trait (pupation height) under selection, but, based on the simulations, also causing considerable differentiation for neutral loci. The latter can be explained by the fact that migrants exchanged by subpopulations adapted to contrasting habitats are mal-adaptive and rarely lead to effective gene flow among subpopulations differing in the direction of selection, a process referred to as isolation-by-adaptation (Nosil *et al.* 2009, Orsini *et al.* 2013). *(ii)* In the long run, population turnover has a strong homogenising effect among subpopulations even in the presence of diversifying selection. This is largely due to the soft selection applied in our experiments, which allowed mal-adapted migrants to successfully colonise empty subpopulations. *(iii)* Higher rates of population turnover may lead to collapse of the variation in a metapopulation when in the end all subpopulations are descended from a few

migrants in a previous generation, whereby all genetic variation is ultimately lost (patch coalescence; Gilpin 1991).

The effect of diversifying selection and population turnover

To investigate the consequences of diversifying selection and population turnover we first examined the evolution of pupation height and genetic differentiation among subpopulations in the absence of selection and extinction. As expected from standard theory (Wright 1952, Nei 1987), migration results in rapid homogenisation for pupation height in both experiments (Fig. 2.3) and simulations (Fig. 2.6), even when these were initiated with high differentiation among subpopulations. Accordingly, Q_{ST} also decreases rapidly in both cases (Fig. 2.5, Fig. 2.7), attaining an equilibrium value of $Q_{ST} \approx 0.16$ in the simulations. F_{ST} reaches a similar equilibrium value of $F_{ST} \approx 0.16$. This confirms the expectation that in the absence of selection F_{ST} and Q_{ST} are of similar magnitude (Merilä and Crnokrak 2001). The equilibrium value of F_{ST} is higher than might be expected from the number of migrants ($Nm = 2$, as we migrated one inseminated female, resulting in the expectation $F_{ST} = 0.11$). However, the polygynous mating system of *Drosophila* leads to the expectation that effective population size (N_e) is about ⅔ of the census population size ($N = 40$; Nunney 1993, Bakker 2008). This results in $N_e m \approx 1⅓$, and the equilibrium prediction $F_{ST} \approx 0.158$, which agrees well with the observed value.

Extending the model by including diversifying selection has a clear effect on the dynamics of genetic variation in the metapopulation. The applied selection pressure is sufficiently strong to oppose the homogenising effect of migration: both in the experiments and simulations, average subpopulation pupation height did not converge to the metapopulation mean in the treatments with only selection implemented or, when initiated without differentiation among subpopulations, divergence between the five high selected and the five low selected subpopulations developed rapidly (see Fig. S2, runs 1–4). Consequently, Q_{ST} stayed high in the experiment and in the simulations increased gradually, even approaching unity in the end. Note, however, that we did not vary the selection parameters, since we focused mostly on the effect of population turnover. Variation in the strength of selection or the local phenotypic optima will undoubtedly affect the dynamics of genetic variation resulting from the interplay of migration, selection and genetic drift (Wright 1931, Lenormand 2002, Hendry 2004, Yeaman and Otto 2011). More close inspection of individual simulation runs (details not shown) indicated that exchange of migrants between subpopulations of contrasting selective pressures did occur but that they failed to reproduce successfully as their offspring was unable to establish themselves in the next generation, because of the mismatch between their genotype and the selective environment. Thus, while successful gene flow occurs between subpopulations with the same selective environment, the two contrasting sets of subpopulations are effectively reproductively isolated, i.e., they are 'isolated-by-adaptation' (Nosil *et al.* 2005, 2008, 2009). Isolation-by-adaptation tends to be widespread in nature (Nosil *et al.* 2009, Orsini *et al.* 2013, Sexton *et al.* 2014). This reduction in effective gene flow between subpopulations from contrasting environments will also affect neutral genetic variation, as genetic drift

causes the two types of subpopulations to diverge gradually and differentiation to increase over time towards the maximum of one (Fig. 2.7). This indicates that the sets of subpopulations selected for high and low pupation height are genetically completely isolated from one another. At the same time, gene flow among subpopulations of the same selection regime is still effective and prevents strong genetic differentiation among subpopulations within each of these groups (Fig. S2).

Adding recurrent extinction and colonisation to the basic model (excluding selection) is also expected to affect the dynamics of genetic variation in metapopulations. Population turnover typically causes local genetic bottlenecks, which, as a consequence of genetic drift, will lead to lower levels of genetic variability (Nei *et al.* 1975, Pannell and Charlesworth 1999, Gaggiotti and Hanski 2004) and an increase in genetic differentiation among subpopulations compared to the situation without population turnover (Wade and McCauley 1988). Differentiation will only decrease if migration occurs according to the migration pool model and if the number of colonists exceeds twice the number of migrants per subpopulation (Wade and McCauley 1988), a condition not satisfied in our set-up. Our simulations confirm the theoretical models: both Q_{ST} and F_{ST} increase with increasing extinction rates, when excluding the fixed metapopulations from the calculation (Fig. 2.7 left). In the experiment, however, we did not observe an effect of population turnover for Q_{ST}. Most likely, the number of generations was simply too low to detect the effects of extinction-recolonisation on genetic differentiation among subpopulations even though this process clearly affects subpopulation sizes (Fig. 2.3 and Fig. 2.4).

At higher extinction rates, the effect of genetic drift is increasing due to *(i)* a decrease in number of individuals in the metapopulation, since a larger number of subpopulations is not colonised after an extinction event, as is illustrated by Figure S3 and *(ii)* a greatly enhanced variance in reproductive output among individuals: colonists are expected to produce much more offspring than individuals in extant subpopulations (Wang and Caballero 1999). Consequently, genetic drift becomes a dominant force causing the loss of genetic variation from the metapopulation (Hedrick and Gilpin 1997, Whitlock and Barton 1997). Moreover, at a high turnover rate individuals in different subpopulations may all be descendants of a few colonists in a previous generation in the end, leading to the collapse of the metapopulation (patch coalescence, Gilpin 1991). This induces rapid loss of genetic variation from the metapopulation such as we observed for extinction rates of e = 0.05 and e = 0.1 (Fig. 2.7 and Fig. 2.8).

On the other hand, the few individuals that colonise an extinct population have a large impact on the genetic structure of that subpopulation. As such, recurrent extinction-recolonisation can be viewed as an additional stochastic force that can overwhelm the effect of selection and increase the effectiveness of migration (Slatkin 1985, Cherry 2003). This is clearly illustrated in our simulations where, in the absence of selection, the differences in mean subpopulation pupation height between subpopulations initiated with either high- or low-pupating individuals decreases more rapidly at higher extinction rates. Also in the experimental situation there is a tendency that the average subpopulation pupation height converges more rapidly to the metapopulation mean in the presence of population turnover than in the absence of it (Fig. 2.3).

The interaction between population turnover and diversifying selection

In the previous section, we concluded that diversifying selection *(i)* increases the level of genetic differentiation among subpopulations and *(ii)* greatly reduces gene flow among subpopulations from contrasting selective regimes. Extinction and recolonisation, on the other hand, *(i)* increase the strength of genetic drift, which in turn will reduce the effect of selection and *(ii)* increase the impact of migration. Thus, selection and population turnover tend to have opposing effects on the dynamics of genetic variation in a metapopulation and the main question is what happens if both act simultaneously. We observed substantial changes in mean pupation height due to selection, but little effect of population turnover in the short-term experiment. The simulations show that this is to be expected as the effect of selection on mean subpopulation pupation height become much more rapidly visible than the effects of population turnover (Fig. 2.6, middle and bottom panel).

On the one hand, the simulations reveal that population turnover causes convergence of mean subpopulation pupation height to the average metapopulation pupation height despite of selection pressures in opposing directions (Fig. 2.6). The rate at which this happens increases with increasing rate of population turnover. In conclusion, population turnover overrides diversifying selection. This is consistent with the idea that population turnover leads to increased stochasticity, thereby weakening the force of diversifying selection (Cherry 2003, 2004, Porcher *et al.* 2004) and at the same time increasing the effectiveness of gene flow (Slatkin 1985, Hartfield 2012). On the other hand, at the level of genetic differentiation we see that the effect of diversifying selection is still prominent for pupation height (Q_{ST}) and to a lesser extent for neutral loci (F_{ST}). Although the equilibrium values for both measures decrease with increasing population turnover, which indicates an increase in gene flow, they are still substantially higher than in the absence of selection, especially so for Q_{ST} (Fig. 2.7). This apparent contradiction is explained by the fact that many metapopulations have lost their genetic variation and became fixed, especially with high extinction rates (metapopulation collapse). So apparently, the genetic variation within metapopulations becomes eroded through the action of increased genetic drift and patch coalescence resulting from population turnover. The rate of fixation clearly increases with increasing extinction rates both in the presence and absence of diversifying selection (Fig. 2.8). However, with selection present, fixations already start in earlier generations, which might be due to the fact that individual subpopulations are initiated with extreme allele frequencies increasing the probability of fixation due to patch coalescence. For neutral genetic variation the rate of fixation is comparable to when selection is absent. However, for the polygenic trait the rate of fixation seems to be slower than in the absence of selection, most possibly as a result of selection counteracting the homogenising effect of gene flow.

Antagonistic directional selection leads to high levels of genetic differentiation for pupation height in metapopulations. As a result, subpopulations were populated by either mainly high- or mainly low-pupating individuals. Patch coalescence (Gilpin 1991, Hedrick and Gilpin 1997) due to high extinction rates than may result in metapopulation fixation, whereby one of the extreme phenotypes/genotypes swamps local adaptation and takes

over the entire metapopulation (Alleaume-Beharire *et al.* 2005, Bridle and Vines 2007, Yoder *et al.* 2013). However, from the simulations we observed that on average the phenotype at fixation was mostly intermediate and that variance was pretty low. Moreover, we did not find any signs of bimodality. In addition, the mean pupation height in the fixed metapopulations in which selection was implemented differed only slightly from the mean values observed when selection was not present (Table 2.1). To get some insights into the underlying dynamics, we analysed the evolution of mean pupation height over generations for a number of individual metapopulations (see Supplementary material S2). From these data we can infer that two main processes may lead to metapopulation fixation at intermediate pupation height. First, population turnover induces more effective gene flow between subpopulations from contrasting environments, thereby eroding the phenotypic (and genotypic) differences between these environments. This will result in genetically heterogeneous subpopulations where the most extreme genotypes may become lost due to genetic drift and the differences in mean pupation height between the two environments will stabilise again at less extreme values for some time (see e.g. run 8 and 11, Fig. S2). On top of this, frequent extinction-recolonisation events may lead to patch coalescence. The combination of these two processes causes most metapopulations to become fixed in the end at an intermediate pupation height in a stepwise manner, although some become fixed at the extreme value (e.g. Fig. S2, run 6).

In this study, we chose to apply soft selection, that is, the intensity of selection was density-dependent and minimal at low densities, as contrasted to density-independent (hard) selection (Christiansen 1975, Wallace 1975, Saccheri and Hanski 2006). The outcome of many evolutionary processes greatly depends on whether soft or hard selection is assumed (Van Tienderen 1991, De Meeûs *et al.* 1993, Agrawal 2010, Debarre and Gandon 2011). In the context of this study, hard selection would have prevented migrants from one environment to colonise the other environment (and vice versa) unless we would have relaxed the selection pressures considerably. As such, exchange of migrants would have been greatly impeded and the colonisation rate would have been decreased accordingly. For the higher extinction rates this would have caused the colonisation rate to become much smaller than the extinction rate and led to extinction of metapopulations, as has been observed in several theoretical studies (Gilpin 1991, Björklund *et al.* 2009). In an experimental study using the same *Drosophila* strains as we did, hard selection was observed to cause frequent population extinction, especially with increasing migration rates (M.E.C. van Rijswijk, unpublished data).

$Q_{ST} - F_{ST}$ DIFFERENCE

Comparisons of the differentiation of quantitative traits and neutral markers are increasingly used to infer the presence of selection in natural populations (Leinonen *et al.* 2013). However, we still have to improve our understanding of the dynamics of Q_{ST} (and possibly also of F_{ST}) under different selective conditions (Leinonen *et al.* 2013). Our data contribute to understanding the dynamics of these measures in the presence of population turnover and antagonistic directional selection. In line with earlier studies (Lande 1992, Whitlock

2008) we observed that Q_{ST} and F_{ST} are on average more or less similar in the absence of selection and that this is not affected by population turnover.

In combination with diversifying selection, however, the difference between Q_{ST} and F_{ST} highly depends on the rate of population turnover (Fig. 2.8). Whereas in the absence of population turnover the difference is near to zero, the difference increases when the turnover rate increases. This indicates that in dynamic metapopulations the Q_{ST} - F_{ST} difference can be highly variable even though the selection pressure on the quantitative trait is constant. Moreover, for metapopulations that are not (yet) in equilibrium, the difference can vary considerably over time.

In conclusion, our data show that inferences about the presence of natural selection for quantitative traits become much more complicated if populations regularly experience extinction-recolonisation events.

SUPPLEMENTARY MATERIAL

Section 1 shows the results of a small experiment in which egg density in the experimental vials was varied to determine the relation between egg density and pupation height.

Section 2 presents results from individual simulation runs showing the dynamics of pupation height in individual metapopulations. The results are used to argue why metapopulations that are subject to rapid subpopulation turnover tend to get fixed at an average pupation height and not at extreme values.

In **Section 3**, the average frequency of extinct subpopulations per metapopulation for three extinction rates is compared, in order to infer the decrease in number of migrants in relation to the increasing extinction rate.

S1. DENSITY DEPENDENCE OF PUPATION HEIGHT

We did our best to keep the experimental conditions as constant as possible. However, egg density was hard to control, especially after an extinction-recolonisation event. Extinct patches were colonised with a single female, resulting in lower egg density than in extant patches with normally 20 egg-laying females. To investigate how this lower egg density affects pupation height, we did a separate experiment in which we initiated glass vials with different egg densities (25, 50, 75, 100 and 150 eggs per vial) and determined pupation height for the resulting pupae. Figure S1 shows that for both the high- and low-pupating lines, pupation height significantly increased with density (high line: $b = 0.049 \pm 0.005$, $p < 0.001$, 95% CI: 0.039–0.058; low line: $b = 0.025 \pm 0.002$, $p < 0.001$, 95% CI: 0.021–0.030). Moreover, this relation is significantly steeper for the high-pupating than for the low-pupating line (CI's do not overlap).

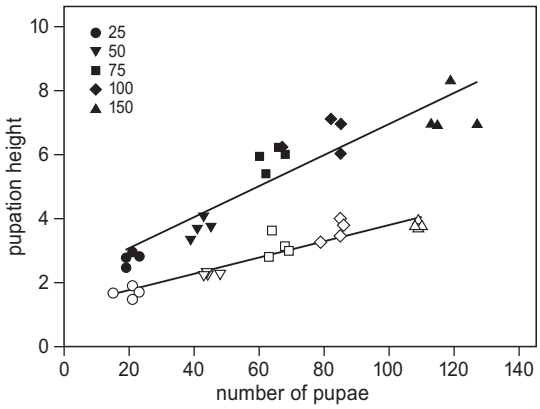


FIGURE S1 Effect of pupal density on pupation height. The five egg densities (ranging from 25 to 150) used for establishing the vials are indicated by different symbols. Filled symbols indicate the high-pupating line; open symbols the low-pupating line.

So not only does average pupation height increase with increasing density, the difference in pupation height between the lines increases with density as well. This relationship between density and pupation height will have affected our metapopulation experiments and explains the significant decrease in average pupation height after an extinction-recolonisation event (see Fig. 2.4, SP1 and SP2).

S2. EVOLUTION OF PUPATION HEIGHT IN INDIVIDUAL METAPOPULATIONS

The simulations we performed showed that genetic variation eventually will be lost in a metapopulation when population turnover rate is high (Fig. 2.8). The mean pupation height of those metapopulations in generation 750 is only slightly different from the mean pupation height observed when selection was not present (Table 2.1). To investigate the underlying dynamics in pupation height leading to this result, we analysed the evolution of pupation height in a number of representative metapopulations. Figure S2 shows 12 individual simulation runs in the absence of (e = 0, runs 1–4) or at a high rate of subpopulation turnover (e = 0.1, runs 5–12). The latter are divided in metapopulations that were initiated without any differentiation (runs 5–8) and with high differentiation (runs 9–12) between subpopulations.

In the absence of population turnover, antagonistic directional selection is capable of inducing and maintaining a substantial level of differentiation in pupation height between subpopulations contrasting in the direction of selection, either a high average pupation height (filled symbols) or a low one (open symbols) depending on the direction of selection (Fig. S2, runs 1–4). Apparently, offspring of high-pupating migrants is successfully selected against in the low-pupating subpopulations and the other way around, so no effective gene flow is occurring between subpopulations with contrasting pupation height, as is particularly clear from run 3.

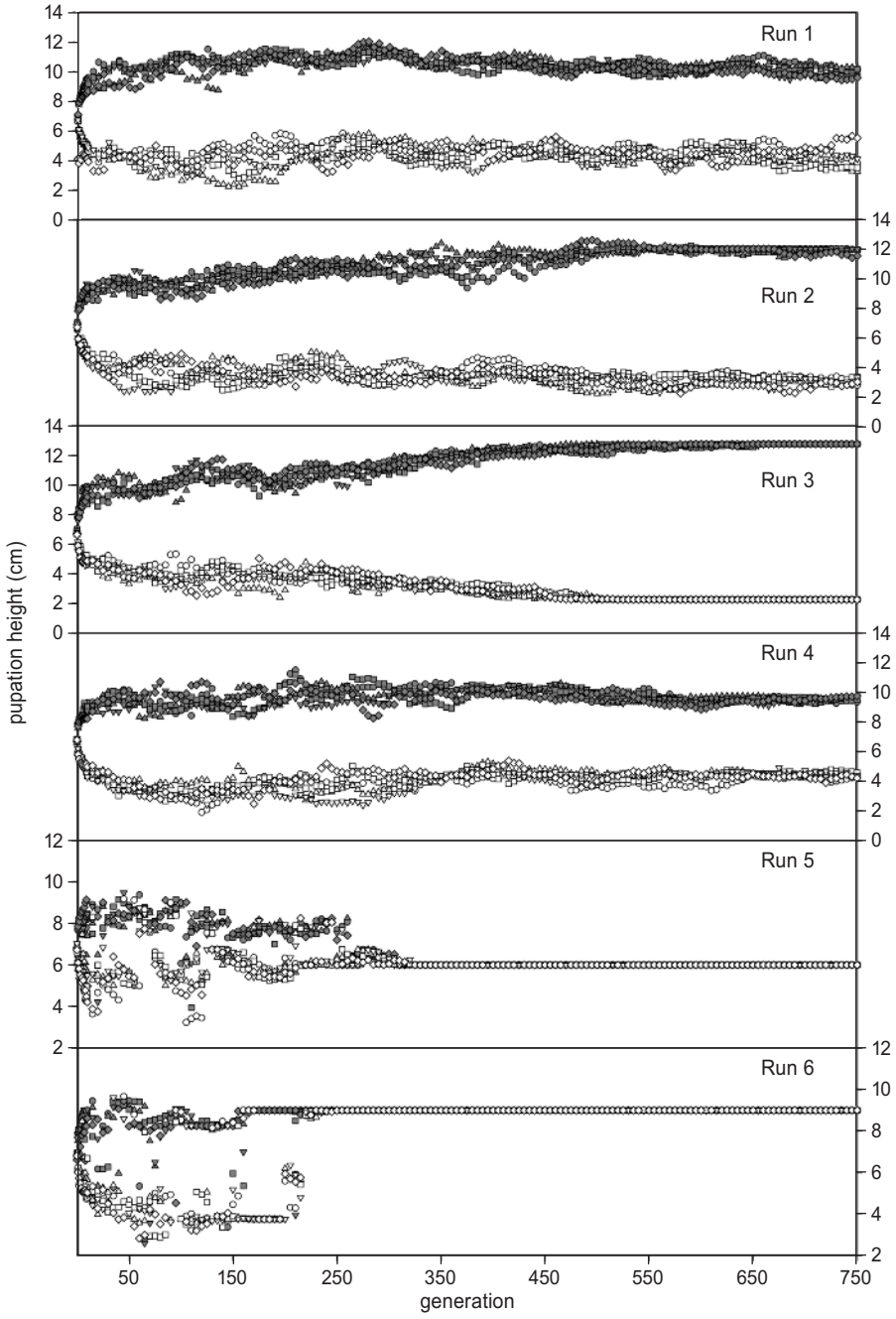
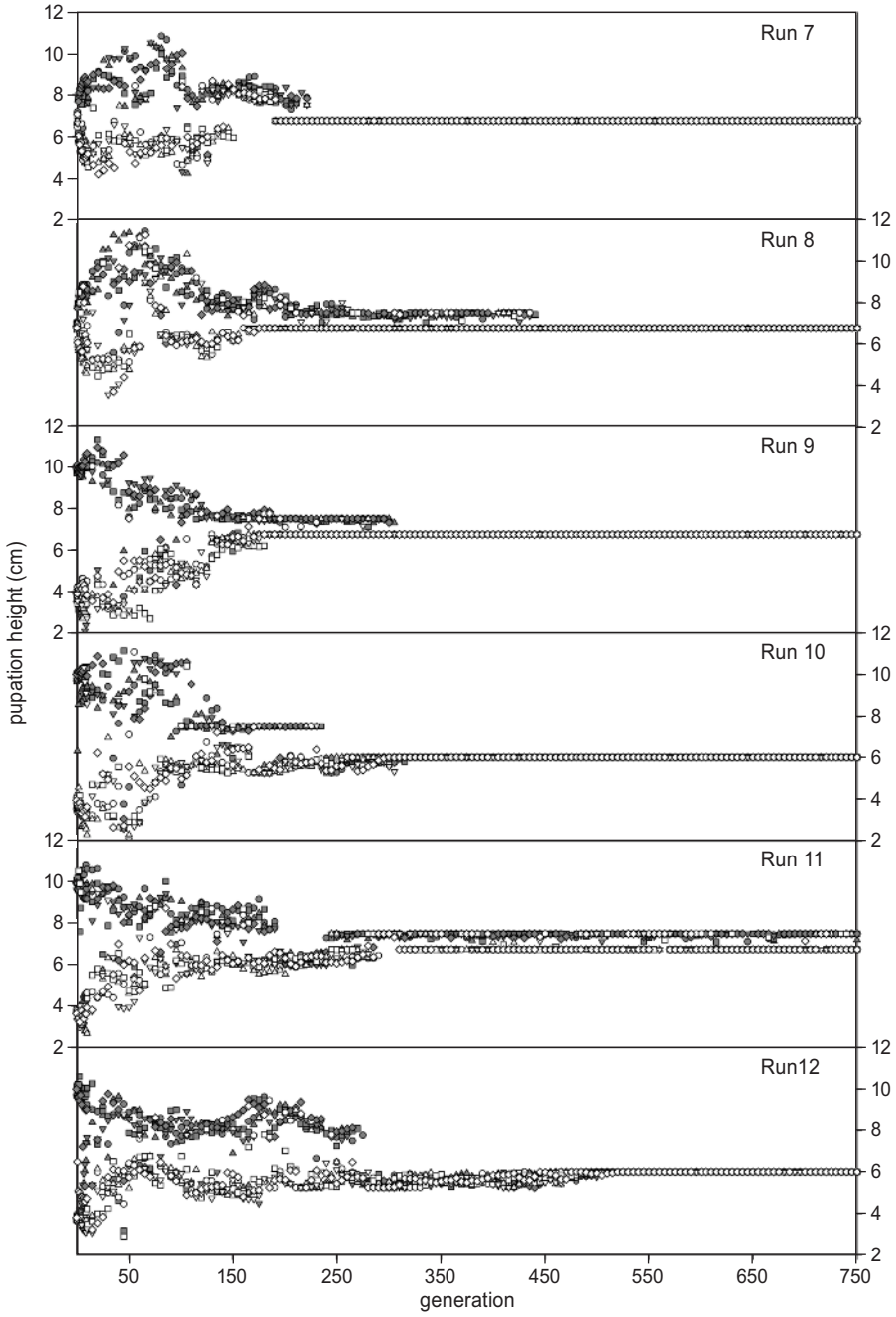


FIGURE S2 Examples of the dynamics of mean pupation height in subpopulations of individual metapopulations for three different treatments. *(i)* Metapopulations started with initially no genetic differentiation among subpopulations in the presence of selection but extinctions absent (runs 1–4). *(ii)* Metapopulations started with initially no genetic differentiation among subpopulations both in the presence of selection and a high extinction rate (runs 5–8). *(iii)* Metapopulations started with initially a high level of genetic



differentiation among subpopulations both in the presence of selection and a high extinction rate (runs 9–12). Grey symbols denote subpopulations selected for a high pupation height and white symbols those selected for low pupation height. Notice that after metapopulation fixation only one white symbol is visible, because all symbols are on top of each other.

When extinctions are implemented, the results show a different pattern. In the first phase of the simulations, antagonistic directional selection is capable of inducing (run 5–8) or maintaining (runs 9–12) a substantial level of differentiation in pupation height between subpopulations contrasting in the direction of selection. However, already early in the process a number of subpopulations selected for high pupation height (filled symbols) show a low mean pupation height and, vice versa, subpopulations selected for low pupation height (open symbols) show a high mean. This means that after an extinction event, those subpopulations have been recolonised by migrants having a "mal-adapted" phenotype. This is possible because in our simulations no selection took place in the generation immediately following an extinction event and selection was weak under the low-density conditions after recolonisation. However, in subsequent generations, locally adapted migrants will arrive and get a foothold, leading to the production of "hybrid" offspring with intermediate phenotypes. In addition, early in the simulation we observe also a number of subpopulations that have an intermediate mean phenotypic value, indicating that some extinct subpopulations have been colonised by high- and low-pupating individuals simultaneously, which also result in subpopulations populated by "hybrid" offspring in the next consecutive generations. Even though the highest, respectively lowest pupating individuals will immediately be selected for in the next generation, the most extreme phenotypes/genotypes might have been lost from the mixed subpopulations by for instance genetic drift, which makes it impossible to reach the most extreme values again.

On top of this, patch coalescence plays a dominant role. This is best illustrated in run 6 showing that around generation 200 the low-pupating subpopulations, after having settled for some generations at more intermediate pupation height, suddenly disappear and collapse with the high-pupating subpopulations. This is explained by the fact that all subpopulations selected for low-pupation height go extinct within a few generations and are being recolonised by high-pupating individuals (data not shown). As such, high-pupating genotypes have taken over all subpopulations, low-pupating genotypes have disappeared from the metapopulations and the metapopulation thereafter goes to fixation at a high phenotypic value. This sudden disappearance of either the on average low- or high-pupating subpopulations is observed in most of the runs where extinction plays a role. Although populations can become fixed at extreme pupation height, as run 6 illustrates, the majority, here 7 out of 8 runs, become fixed at an intermediate level.

Thus, frequent extinction-recolonisation events promote both successful gene flow between the contrasting environments, resulting in phenotypically less extreme 'hybrid' offspring, and patch coalescence. The combined action of these two processes leads to fixation of the metapopulations at the intermediate pupation heights we observed in Table 2.1.

S3. Number of extinct subpopulations

Because we implemented extinction before migration took place, extinct populations do not contribute to the migrant pool and the number of migrants per generation therefore is expected to decrease when extinction rates increase. To get some insight into the average number of extinct subpopulations in relation to the extinction rate, we calculated the

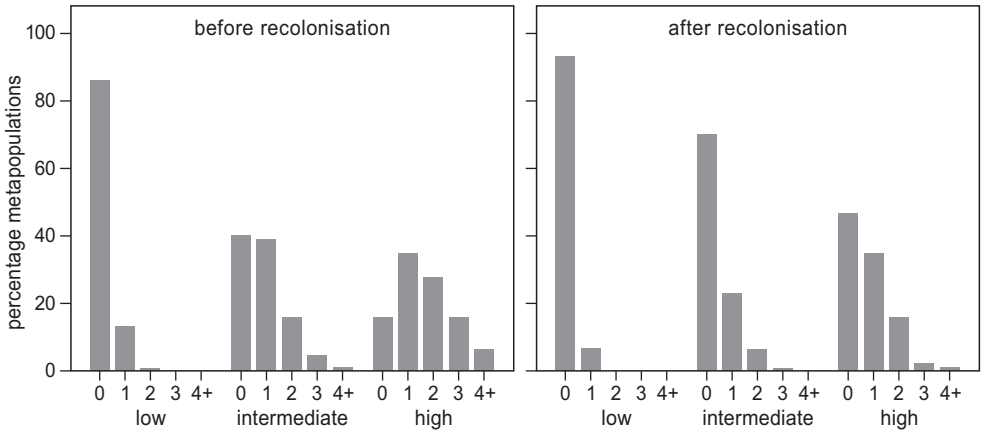


FIGURE S3 Percentage metapopulations that had 0, 1, 2, 3 or more (4+) subpopulations extinct in generation 50 before (left panel) and after (right panel) colonisation. Three different extinction rates are shown: low (e = 0.01), intermediate (e = 0.05) and high (e = 0.1).

number of extinct subpopulations per metapopulation in generation 50 for each of the three extinction rates in order to determine the decrease in number of migrants due to increasing extinction rate. The results are shown in Figure S3. Before migration was implemented (Fig. S3 left panel), the number of extinct subpopulations was quite high, especially for the higher extinction rates. For e = 0.1 nearly 50% of the metapopulations had two or more extinct subpopulation in this generation. Based on these data, we estimate the average number of migrants in this generation to be 9.8, 9.2 and 8.3 for e = 0.01, e = 0.05 and e = 0.1, respectively. Combined with the fact that the migrants were distributed over the subpopulations according to a Poisson distribution, many of these subpopulations do not receive a migrant and stay unpopulated after migration took place (Fig. S3, right panel). For the highest extinction rates this causes some 20% of the metapopulations to have two or more empty subpopulations in generation 50 after migration was implemented.

CHAPTER 3

Evolution in experimental *Drosophila* metapopulations: The dynamics of neutral variation shaped by migration and local selection for a life history trait

M. W. Smith-Kleefsman
F. J. Weissing
J. Bakker
R. Bijlsma

ABSTRACT

We explored the influence of antagonistic diversifying selection for a polygenic trait, pupation height, on genetic differentiation for supposedly neutral microsatellite loci (F_{ST}) in experimental *Drosophila* metapopulations with migrant pool migration. Starting with high initial levels of genetic differentiation, we observe that diversifying selection for pupation height significantly affected the dynamics of F_{ST}, as it strongly opposed the homogenising effect of migration. Based on the spread of private alleles to subpopulations they were initially absent, we argue that this is mainly due to the fact that migrants (and their offspring) that are exchanged between subpopulations differing in the direction of selection are mal-adapted in their new environment and, therefore, are reproductively much less successful than residents. This leads to significantly lower levels of effective gene flow (genetic exchange) between those subpopulations, even though the number of migrants exchanged is essentially the same as for subpopulations that are selected in the same direction. This results in a significant isolation-by-adaptation pattern for the neutral loci. In addition, we observe considerable heterogeneity in dynamics among individual microsatellite loci, which shows that linkage between these loci and genes that are under selection also plays a significant role in the dynamics of neutral genetic variation. These findings illustrate that the assumption that microsatellite loci can be regarded as neutral markers may be significantly violated in metapopulations when heterogeneous selection for ecologically relevant traits is present.

Introduction

To study the dynamics and forces that structure metapopulations, the level of genetic differentiation for genetic markers is often used to infer important ecological and population genetic parameters like migration rates, genetically effective population size, and genetic variation (Wright 1931, Slatkin 1987, Frankham 1995, Palstra and Ruzzante 2008, Luikart *et al.* 2010). Currently, microsatellites are still predominantly used for such studies (DeSalle and Amato 2004, Schlötterer 2004, Oliveira *et al.* 2006, Ouborg *et al.* 2010). It is important to realise, however, that these markers are generally neutral, and that their dynamics is therefore mainly governed by neutral processes like genetic drift and gene flow. As such, these markers are thought to be less suitable to signalise the presence of selective forces like local adaptation (Pemberton 2004, Bijlsma and Loeschcke 2012). The role of selection and adaptation in creating different phenotypes, which can lead to genetic differentiation between populations, is presently targeted as an important issue in population biology, and in particular conservation biology (Leinonen *et al.* 2008, Naish and Hard 2008, Räsänen and Hendry 2008, Leinonen *et al.* 2013).

The difference between genetic differentiation for neutral traits like microsatellites, measured as F_{ST}, and genetic differentiation in quantitative traits, measured as Q_{ST}, has been proposed as an indicator of the presence of selective forces shaping differentiation between populations (Spitze 1993, Merilä and Crnokrak 2001). Quantitative traits are subject to the same neutral processes as neutral traits, but can be under selective pressure as well (Leinonen *et al.* 2013). Broadly speaking, we can expect three possible outcomes of a comparison between Q_{ST} and F_{ST} (Merilä and Crnokrak 2001). If $Q_{ST} > F_{ST}$, antagonistic directional selection favouring different alleles/genotypes in different subpopulations is expected to be present. If Q_{ST} equals F_{ST}, there is little evidence for any selection pressure; this does not necessarily mean that selection is absent, but indicates that neutral and selective processes are undistinguishable. If $Q_{ST} < F_{ST}$, this can be interpreted as evidence for selection favouring the same phenotypes in all subpopulations. Many studies have been performed to investigate the role of selection in natural populations (for reviews, see Merilä and Crnokrak 2001, McKay and Latta 2002, Leinonen *et al.* 2013). From these studies, the main observation is that most often differentiation in quantitative traits is exceeding differentiation in neutral genetic markers, leading to the general conclusion that diversifying selection has a predominant role in natural populations.

There has been quite some discussion about the use of both Q_{ST} and F_{ST}. It is emphasised that Q_{ST} can only be used if the genes involved act purely additive, so maternal, dominance and environmental effects are negligible (Whitlock 2008). More recently, the bias in F_{ST} estimation is under discussion as well: both F_{ST} and Q_{ST} decrease with higher mutation rate. Hence, mutation rates of both should be comparable, especially when gene flow is low compared to mutation rate (Hedrick 2005, Edelaar and Björklund 2011, Edelaar *et al.* 2011). Although Q_{ST} generally exceeds F_{ST} in natural populations, it is still debated if this is due to selective forces in the populations, or just a bias of the $Q_{ST} - F_{ST}$ comparison.

Despite all theoretical studies and the use of the $Q_{ST} - F_{ST}$ comparison for inferences from natural populations, little experimental work has been performed on this subject. In

an experimental study on house mice (Morgan *et al.* 2005), Q_{ST} (measured for wheel running activity) indeed exceeded F_{ST} (calculated from neutral allozyme markers) in a metapopulation context in which diversifying selection took place (although in the absence of migration), supporting the inference that selection affects neutral genetic markers less than it affects a quantitative trait under selection pressure. However, in an experimental study on *Arabidopsis thaliana*, a predominantly selfing plant species, both F_{ST} and Q_{ST} increased under diversifying selection when compared to a situation with uniform selection (Porcher *et al.* 2004, 2006). This study showed that the increase in differentiation for the neutral markers was in part driven by heterogeneous selection for the quantitative trait, indicating that neutral markers not necessarily behave strictly neutral, as their dynamics may be affected by selection at linked loci. Moreover, migration among local populations that differ in the direction of selection may result in mal-adapted immigrants (Ch. 2, Smith-Kleefsman *et al.* 2015a). Selection against these immigrants (and/or their offspring) is expected to decrease effective gene flow rates and, as such, will affect the equilibrium between genetic drift and migration, and thus F_{ST}.

The present study investigates to what extent gene flow can effectively oppose the genetic differentiation present for neutral microsatellite loci in the presence of antagonistic directional selection pressures on a quantitative trait in a metapopulation setting. The results presented here are part of a more extended experiment focusing on the impact of local diversifying selection, migration, extinction and recolonisation on the genetic differentiation for a quantitative genetic trait and published in an accompanying paper (Ch. 2, Smith-Kleefsman *et al.* 2015a). Here, we focus mainly on the dynamics of F_{ST} in metapopulations that experienced six generations of gene flow both in the presence and absence of antagonistic directional selection at the quantitative trait and we compare the changes in F_{ST} under these two treatments. We used *Drosophila melanogaster* lines that were selected for pupation height. This trait is known to be polygenic (M.E.C. van Rijswijk, personal communication, Sokolowski and Hansell 1983) and previous experiments have shown that diversifying selection in a metapopulation context increases the phenotypic differentiation (Q_{ST}) for this character even under moderate migration rates (M. E. C. van Rijswijk, personal communication, Ch. 2, Smith-Kleefsman *et al.* 2015a). Microsatellites that are distributed throughout the genome of *D. melanogaster* are used to study the dynamics of neutral variation in the experimental populations. The experiment is started with high differentiation for both pupation height and microsatellites. Under the assumption of neutrality, theory predicts F_{ST} to decrease at similar rates in either the presence or absence of diversifying selection as the result of migration. However, it is conceivable that even when strictly neutral, microsatellite variation might be affected by the selection on pupation height: either because the microsatellite loci are linked to the loci that govern pupation height, or because local selection pressures affect effective migration patterns (Räsänen and Hendry 2008, Orsini *et al.* 2013). By comparing the dynamics of F_{ST} in the presence and absence of diversifying selection on pupation height, we will provide insights into the impact of the interaction between gene flow and local diversifying selection for the dynamics of neutral genetic markers. Our results clearly show that the dynamics of neutral variation is affected by the presence of diversifying selection for this quantitative trait within the metapopulation.

Materials and Methods

Drosophila stocks

The flies used for the experiments originated from the Groningen 83 (G83) population that was founded in 1983 with 430 inseminated females captured at the fruit market and maintained as a very large random mating population since then (Zwaan *et al.* 1991, Vermeulen and Bijlsma 2004). From this base population, two divergent selection lines were established during 14 generations of directional selection for either high or low pupation height (M.E.C. van Rijswijk, unpublished data). At the time these lines were used, they still differed greatly in pupation height: means 9.8 cm and 4.4 cm, respectively.

Flies were kept under standard conditions at 25°C, 50–60% RH, and 18 ml standard medium containing 26 g dead yeast, 54 g sugar, 17 g agar, 13 ml nipagine solution (10 g nipagine in 10 ml 96% alcohol) and 250 mg streptomicine per litre. For handling, flies were anesthetised with CO_2.

Experimental set-up and procedure

The experiment was started by creating metapopulations, consisting of 10 subpopulations each. Five of these were each initiated with 20 mated females from the high-pupating line (HP1-HP5) and the other five with 20 mated females from the low-pupating line (LP1-LP5). Consequently, the metapopulations were initiated with considerable phenotypic divergence between the subpopulations. Figure 3.1A summarises the different experimental steps involved. At day 1, the mated females were placed in clean glass vials in the morning, and were allowed to lay eggs for a maximum of 24 hours. These vials were 20 cm high glass vials with about 18 ml (± 2.5 cm) food at the bottom of which the inner walls were lined with a clear plastic sheet at which the larvae could pupate. At day 7, when nearly all larvae had pupated, the sheet containing the pupae was removed from the vial. Pupation height of all pupae was determined, using the centimetre scale on the sheet (see Fig. 3.1B). Two different treatments were applied: local selection being absent or present. In the treatment with local selection present, subpopulations HP1 to HP5 were selected for high pupation height by selecting the 50 highest pupating individuals. The other five subpopulations (LP1 to LP5) were selected for low pupation height by selecting the 50 lowest pupating individuals. This treatment represents antagonistic directional selection that in the remainder of the paper will be referred to as diversifying selection. Immediately after determining pupation height, selection was implemented by cutting of the part of the sheet containing the selected individuals for each subpopulation and transferring it to a fresh vial. In the treatments without selection, the whole sheet was transferred to a fresh vial.

After eclosion, adults were allowed to mate at random within their own subpopulation before migration was implemented. Thereafter 20 mated females were randomly chosen from each subpopulation to start the next generation. Migration was implemented randomly selecting one of the 20 selected females per subpopulation and redistributing these 10 migrants at random over the 10 subpopulations, so that each subpopulation

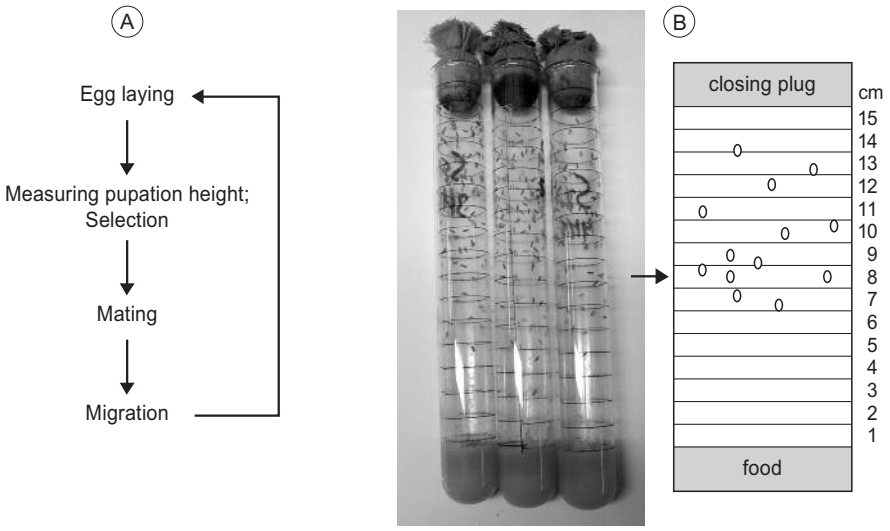


FIGURE 3.1 A. Overview of the different steps that were performed during the experiments. B: Experimental vials showing pupation height (left) and schematically the plastic lining with pupae used to determine pupation height in centimeters (right). All pupae observed between two scaling lines were given the same score; pupae observed on the scaling lines were assigned to the lower class.

received one migrant individual. As such, the migration model resembles the migrant pool model (Slatkin 1977, Wade and McCauley 1988), except that the migrants are uniformly distributed over the subpopulations. As migrants could end up in the subpopulation they originated from, this resulted in 0.9 migrants per generation on average for each subpopulation (Nm = 0.9, with N being the genetically effective number of breeding individuals in local populations and m the rate of gene flow; Wright 1969).

For every treatment, five replicates were run and exactly the same migration scheme was applied to all treatments and to all replicates within treatments to standardise migration and reduce variation between the replicates. After migration, the flies were transferred to clean vials and the next generation started. This procedure was maintained for 6 generations.

MICROSATELLITE ANALYSIS

For estimating genetic differentiation at neutral loci, seven microsatellite loci were analysed (Table 3.1). These were selected from a set of more than 50 loci for which genetic variation was known to exist in the G'83 base population (A. G. J. M. Ayrinhac, personal communication) based on two criteria. *(i)* The presence of contrasting allele frequencies between the high- and low-pupating lines; this ensures that the experimental metapopulations not only were initiated with high phenotypic differentiation, but also with high genetic differentiation for the microsatellite loci. *(ii)* The loci should preferably mark different regions of the genome.

Several of the microsatellite loci we chose showed alleles that were observed in only one of the pupation height lines (private alleles). For both the high- and low-pupating line the allele frequencies were determined based on a sample of 32 females each. For genotyping, DNA was extracted from the flies using the Puregene DNA purification kit (Gentra Systems). PCR products were separated by fragment length using the ABI 3730 DNA analyser (Applied Biosystems, California, USA) and analysed using Peak Scanner Software v1.0 (Applied Biosystems, California, USA).

Estimates of genetic differentiation

Q_{ST} *measure*: Q_{ST} is a dimensionless measure that was especially developed to determine genetic differentiation for quantitative traits, and is an analogue to Wright's F_{ST} (Wright 1951, Spitze 1993). To calculate Q_{ST} for a trait, in this experiment pupation height, two quantities are required: the additive genetic variance within populations (σ_w^2) and the genetic variance among populations (σ_b^2). Q_{ST} is calculated as:

$$Q_{ST} = \frac{\sigma_b^2}{\sigma_b^2 + 2\sigma_w^2} \ ,$$

(Wright 1951, Spitze 1993). As in our experiment all individuals experience the same environmental conditions, we assume that the phenotypic variation we observe for the traits reflects the underlying genetic variation.

To determine the genetic variance within and among populations, an analysis of variance (ANOVA) is used. One of the underlying assumptions of ANOVA is that the variance is

Table 3.1 Overview of the microsatellites used. The different columns designate: Microsatellite name (MicroSat); Location of the locus (Location: chromosome and mapdistance in centimorgan); Number of alleles per locus (# Alleles); Allele length and frequency (in brackets) of the private alleles (Private All (p)); Base line in which this allele was present private: HP = high-pupating line; LP = low-pupating line.

MicroSat	Location	# Alleles	Private All (p)	Private in Line
DmX4†	X, 14	2	-	
Drosev2‡	X, 33	3	140 (0.75)	HP
			154 (0.52)	LP
Odd‡	2L, 11	2	175 (0.78)	HP
Suvar†	2L, 31	2	-	
Dm30‡	3L, 2	3	369 (0.73)	LP
Dm3g†	3L, 15	3	185 (0.83)	HP
			187 (0.09)	HP
Dm3b†	3R, 68	3	146 (0.39)	HP

Primer sequences were taken from: †Charles Aquadro laboratory (Schug *et al.* 1998, Dm30 was named DMCPDR) and ‡Christian Schlötterer laboratory: http://i122server.vu-wien.ac.at/Microsatellite%20Loci/Loci%20Titelpage.html (DmX4, Suvar, Dm3g and Dm3b were named DS00589, su.var, 3L5235154gt and 3R16177365gt, respectively).

unrelated to the mean. This was not the case in our study. We found a linear relationship between the standard deviation in pupation height (s) and the average pupation height ($\bar{x}$):

$s = 0.15\bar{x} + 1.12$, $R^2 = 0.53$.

As recommended in the statistic literature (Miller 1986), we corrected for this by log-transforming our data: $x_i' = \log(x_i + 1)$. Accordingly, the relationship has become weakened:

$s = -0.11\bar{x} + 0.24$, $R^2 = 0.27$.

F_{ST} *measure*: After generation 6, allele frequencies were determined for all microsatellite loci by genotyping the 20 females that produced the next generation for each subpopulation of all experimental metapopulations. For each metapopulation, genetic differentiation was calculated over all loci combined as well as for each locus individually using the estimator θ (Weir and Cockerham 1984). Confidence intervals of the overall F_{ST}'s were calculated using bootstrapping over loci. For these calculations, the FSTAT software (Goudet 1995) was used.

Genetic differentiation in generation 6 was compared with the initial values at the start of the experiment. These initial values were estimated as follows: each subpopulation was assumed to be started with a random sample (sampled with replacement) of 20 females from the 32 that were genotyped of either the high- or the low-pupating line, depending on the type of subpopulation. F_{ST}'s were then calculated as described above. The assignment of subpopulations and calculation of F_{ST}'s was repeated 250 times. The change in F_{ST} comparing generation 0 and 6 was used to infer the dynamics of F_{ST}.

Individual-based simulations

Simulations allowed us to place the experimental results in a theoretical perspective and to assess experimental expectations. The simulation model used was an adapted version of the simulation model of Bakker (2008, Bakker *et al.* 2010). This is an object-oriented, individual based design, enabling easy implementation of different aspects of metapopulation genetics: reproduction and mating, genetic drift and migration among demes and genetic markers. Population size was kept constant at $N = 40$ (simulating the experiment with 20 singly mated females) during the six discrete generations simulated.

Genetic variation for microsatellite loci was implemented resembling the experimental situation: seven loci with two or three alleles each and an initial allele frequency, corresponding to the number of alleles and allele frequency in the original pupation height lines (see Table 3.1). The generated offspring in each subpopulation was allowed to mate after which migration of one inseminated female took place.

To compare the result of different situations, we simulated four different migration schemes: *(i)* No migration (Sim-No), which simulates how six generations of genetic drift within subpopulations affects the level of genetic differentiation. *(ii)* Migration according to the migrant pool model, whereby the 10 migrants were distributed according a Poisson distribution (Sim-Isl). *(iii)* Migration resembling the migrant pool model, but the 10 migrating females were uniformly, but randomly, distributed over the 10 subpopulations (Sim-UniR). *(iv)* Migration according to a fixed scheme exactly as used in the experiment (Sim-UniF).

RESULTS

DYNAMICS OF Q_{ST}

Figure 3.2 shows the observed change in Q_{ST} during the generations both in the presence and absence of diversifying selection on pupation height. Clearly, in the presence of selection there is on average a small non-significant change in Q_{ST} (paired t-test: t = 1.09, df = 4, p = 0.339), while Q_{ST} is significantly decreasing without selection (paired t-test: t = 5.28, df = 4, p = 0.006). Moreover, in generation 6 Q_{ST} was significantly higher in the presence of selection than in the absence of selection (t-test: t = –5.84, df = 8, p < 0.001), while this was not the case in generation 1 (t-test: t = 2.17, df = 8, p = 0.061).

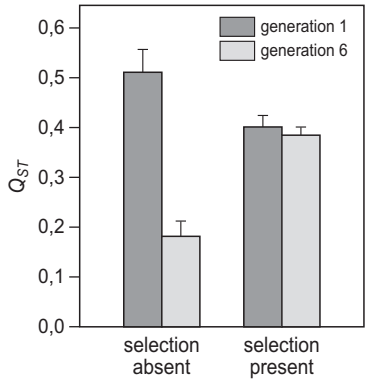


FIGURE 3.2 Mean phenotypic differentiation in pupation height (Q_{ST} ± s.e.) in generation 1 and 6 averaged over five replicate metapopulations without (left) and with diversifying selection on pupation height (right). Based on the data of Smith-Kleefsman *et al.* (2015a).

DYNAMICS OF OVERALL F_{ST}

The simulations show that the initial high level of differentiation for the neutral microsatellite loci at the start of the experiment (Base-Pop: F_{ST} = 0.455 ± 0.002; average ± s.e.) is expected to be maintained during six generations when no migration is implemented (Fig. 3.3, Sim-No: F_{ST} = 0.455 ± 0.002). When migration is allowed, genetic differentiation is expected to decrease significantly in six generations independent of the precise migration pattern implemented (Fig. 3.3, F_{ST} = 0.286 ± 0.002, F_{ST} = 0.285 ± 0.002, F_{ST} = 0.293 ± 0.002 for Sim-Isl, Sim-UniR and Sim-UniF, respectively). The fixed migration model applied in the experiment (Sim-UniF) does not deviate significantly from the migrant pool model (Sim-Isl), at least not for the initial six generations simulated here.

The two right panels in Figure 3.3 show the F_{ST} values observed for the experimental metapopulations in the absence (N1-N5) and the presence (S1-S5) of diversifying selection. We find a highly significant difference in the average F_{ST} over the five replicate metapopulations (t-test: t = –6.43, df = 4, p < 0.001): mean ± s.e.: 0.201 ± 0.023 and 0.387 ± 0.018 for selection absent and present, respectively (dashed lines in Fig. 3.3). In the absence of selection, F_{ST} decreased significantly from the estimated initial values (one sample t-test: t = –9.89, df = 4, p = 0.001), while in the presence of selection no significant difference was

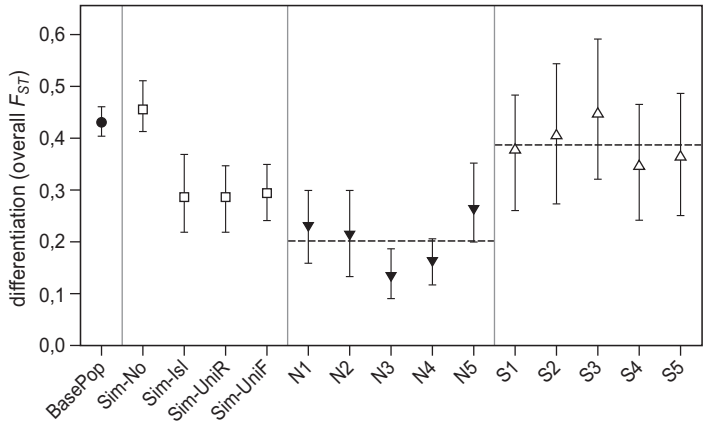


FIGURE 3.3 Observed and expected level of genetic differentiation, calculated as overall F_{ST}, combining all microsatellites. The most left section presents the overall F_{ST} observed in the base population (generation 0). The second section presents the simulation results for the mean expected values of the six generations in the absence of migration (Sim-No) or in the presence of the three migration treatments Sim-Isl, Sim-UniR and Sim-UniF (see text for further explanation on the treatments). For each, the mean (square) and the range in which 95% of the simulation results were found (bars) is shown. The two sections at the right present the experimentally observed F_{ST}'s in generation six for each of the replicated metapopulations when selection was absent (N1-N5) or present (S1-S5). The dashed lines indicate the F_{ST} value averaged over the five replicates. Bars designate the 95% confidence interval based on the bootstrapping procedure implemented in FSTAT (Goudet 1995).

observed (one sample t-test: $t = -2.39$, $df = 4$, $p = 0.075$). These results indicate that the presence of diversifying selection significantly opposes the homogenising effect of migration, which may also explain why on average the observed F_{ST}'s are significantly higher than the F_{ST} value obtained for the simulation of the experimental situation, Sim-UniF (one sample t-test: $t = 5.42$, $df = 4$, $p = 0.006$). On the other hand, the decrease in F_{ST} in the experimental populations in the absence of selection is larger than expected on basis of this simulation ($t = -3.96$, $df = 4$, $p = 0.017$).

COMPARISON OF F_{ST} AND Q_{ST}

To compare the behaviour of F_{ST} with the behaviour of Q_{ST}, the relative change in differentiation ($RC\text{-}F_{ST}$) was calculated as: $RC\text{-}F_{ST} = (F_{ST\text{-}end} - F_{ST\text{-}start}) / F_{ST\text{-}start}$, in which $F_{ST\text{-}end}$ is the F_{ST} in generation 6, and $F_{ST\text{-}start}$ is the F_{ST} in the base population. For the relative change in differentiation of Q_{ST} ($RC\text{-}Q_{ST}$), the same procedure was applied. The results are depicted in Figure 3.4. Clearly, both Q_{ST} and F_{ST} changed considerably more in the absence of diversifying selection than in its presence ($t = -6.30$, $df = 8$, $p < 0.001$ and $t = -6.43$, $df = 8$, $p < 0.001$, for Q_{ST} and F_{ST}, respectively). On the other hand, the difference in relative change in differentiation between both measures was not significantly different for either selection treatment ($t = -1.08$, $df = 8$, $p = 0.313$ and $t = 0.92$, $df = 8$, $p = 0.386$ for the presence and absence of diversifying selection, respectively).

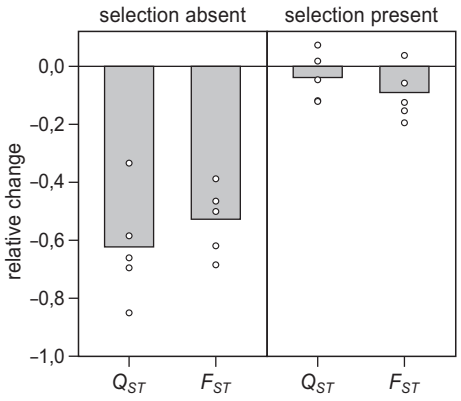


FIGURE 3.4 Mean relative change in differentiation (bars) in the absence (left panel) and presence (right panel) of diversifying selection for pupation height. The open symbols denote the observed values for individual metapopulations.

DYNAMICS OF INDIVIDUAL LOCI

The assumption that microsatellites behave effectively neutral may be violated when they are linked to other loci that are under selection. To investigate whether all our loci behave in a similar fashion, the observed F_{ST}-values for each locus are depicted in Figure 3.5. For comparison, the plot also shows the estimated initial F_{ST} and the expected F_{ST}-value after six generations of migration for the migration scheme actually applied in the experimental metapopulations (Sim-UniF) for each locus separately. With regard to the two selection treatments, we see more or less similar dynamics as observed for the overall F_{ST}: In the

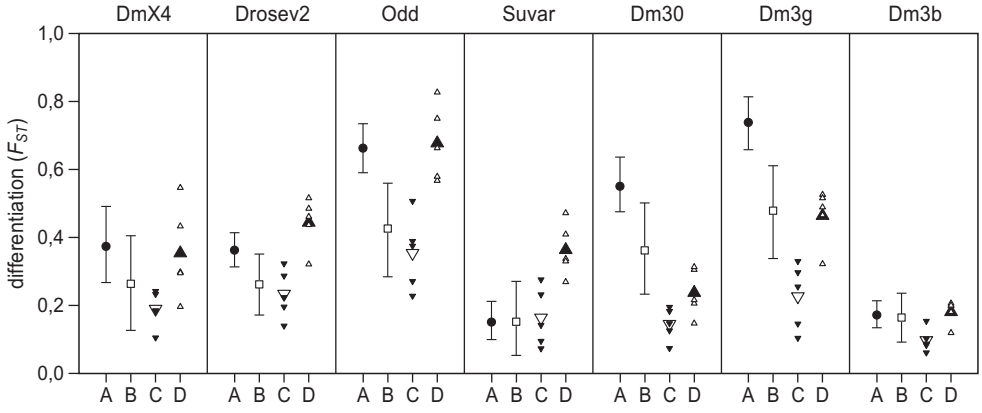


FIGURE 3.5 Mean expected and observed levels of genetic differentiation (F_{ST}) for each microsatellite locus separately. For each microsatellite locus, columns A and B show the observed F_{ST} for the base population (generation 0) and the simulation result after six generations of migration according to the Sim-UniF model, respectively. Bars indicate the range in which 95% of the results were found. The columns C and D represent the observed F_{ST} for the experimental metapopulation in the absence and presence of diversifying selection for pupation height, respectively. The small symbols show the F_{ST} value for each of the five replicates, while the large symbol indicates the average over these five replicates.

absence of diversifying selection there is a significant decrease in F_{ST} during the six generations for each locus except Suvar (Fig. 3.5; Table 3.2, 1st column). In the presence of selection, most loci show a non-significant change in F_{ST} compared to the initial F_{ST}, but three show a significant change in F_{ST} in this situation as well, albeit not in the same direction (Table 3.2, 2nd column). In summary, we find for each locus that F_{ST} over six generations is significantly higher in the presence of selection than in the absence (Fig. 3.5; Table 3.2, 3rd column), similar to the overall F_{ST}.

There are some conspicuous differences between loci, however. Loci Dm3g and Dm30 show a significantly larger decrease in F_{ST} in the absence of selection than expected on basis of the simulations (Fig. 3.5). As the effective migration rate (see next section) would affect all loci similarly, this indicates that these loci are influenced by more forces than only migration and genetic drift. Suvar, on the other hand, shows in the presence of diversifying selection a much higher level of genetic differentiation than initially was present (Fig. 3.5; Table 3.2) for all five replicated metapopulations. This implies that diversifying selection not only affects pupation height, but also the allelic variation at this locus, suggesting linkage between one or more loci determining pupation height and this microsatellite.

TABLE 3.2 T-tests for the change in F_{ST} during six generations for each individual microsatellite locus both in the absence (1st column) and presence (2nd column) of diversifying selection on pupation height. F_{ST}'s in generation six were compared to the estimated initial F_{ST} in generation zero with a one-sample t-test. The 3rd column (Effect of selection) shows the results of t-tests comparing the F_{ST}'s in generation six when selection was absent versus when selection was present.

Microsat	Selection absent			Selection present			Effect of selection		
	t	*df*	*p*	*t*	*df*	*p*	*t*	*df*	*p*
DmX4	-7.6	4	0.002*	-0.3	4	0.762	-2.5	8	0.037
Drosev2	-3.9	4	0.017*	2.4	4	0.071	-4.5	8	0.002*
Odd	-6.3	4	0.003*	0.3	4	0.783	-4.6	8	0.002*
Suvar	0.3	4	0.752	6.1	4	0.004*	-3.8	8	0.005*
Dm30	-18.7	4	<0.001*	-9.9	4	<0.001*	-2.4	8	0.042
Dm3g	-11.7	4	<0.001*	-7.4	4	0.002*	-4.2	8	0.003*
Dm3b	-4.7	4	0.009*	-0.6	4	0.586	-3.7	8	0.006*

*Significant at α = 0.05 after sequencial Bonferroni correction (Rice 1989)

THE EFFECT OF DIVERSIFYING SELECTION ON MIGRATION RATES

When comparing the changes in F_{ST}-value in the presence and absence of divergent selection for pupation height we observed that the dynamics of F_{ST} was clearly different for the two scenarios (see above). A possible explanation for this finding is that local selection affects the effectiveness of migration. We used the frequency of alleles that were private in either the high- or the low-pupating base populations to study this. Figure 3.6 shows for

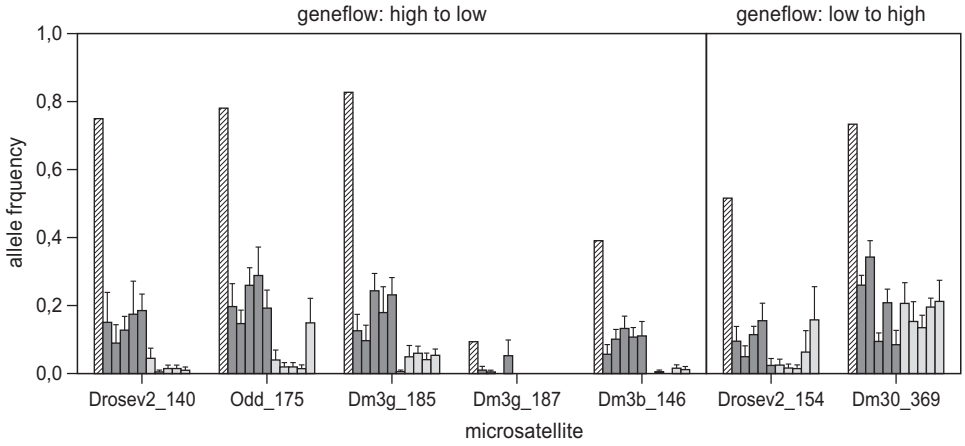


FIGURE 3.6 Introgression of private alleles into subpopulations in which the allele was absent at the start of the experiment. The left panel shows introgression from initially high-pupating subpopulations into initially low-pupating subpopulations, while this is the other way around in the right panel. For each microsatellite locus the hatched bars indicate the initial frequency of the private allele in the donor subpopulations; the dark grey bars show the frequency of this allele in the five subpopulations in which this allele was initially not present in the absence of diversifying selection for pupation height, while the light grey bars indicate the frequency for the same subpopulations when diversifying selection was present. For each individual subpopulation, the allele frequency was averaged over the five replicate metapopulations.

several microsatellite loci the frequency of private alleles after 6 generations of migration for those subpopulations in which the allele was not present at the start of the experiment. As private alleles were observed in nearly all those subpopulations in which they were previously absent, it is clear that migrant females did in fact successfully produce offspring in those subpopulations. This was found even in the presence of strong opposing local selection, i.e. in a situation that the females produced offspring with a phenotype that was selected against. Only for one microsatellite locus (Dm3g, allele 187) no effective gene flow was observed in the presence of diversifying selection. This, however, may be due to the very low frequency of this private allele in the donor subpopulations.

Still, the frequency of the private alleles was clearly higher in the absence of diversifying selection compared to the situation with diversifying selection present (Fig. 3.6). A Mann-Whitney U-test showed that the frequency of the private alleles averaged over the five subpopulations per metapopulation was significantly higher for the five metapopulations without selection than with selection for all loci where the private allele was initially present in the high-pupating base population, except for again locus Dm3g_187 of which the initial frequency was too low (Dm3b: $U = 0$, $p = 0.004$; Dm3g_185: $U = 0$, $p = 0.004$; Dm3g_187: $U = 5$, $p = 0.0754$; Drosev2_140: $U = 0$, $p = 0.004$; Odd: $U = 1$, $p = 0.079$; one-sided probabilities). For the two cases where the private allele was initially present in the low-pupating base population, the average frequency after 6 generations of migration was not significantly different between the two treatments (Mann-Whitney U-test, Dm30: $U =$

11, p = 0.460; Drosev2_154: U = 8, p = 0.210). This suggest that in the presence of diversifying selection migrants from the high-pupating line were less successful as migrants into subpopulations selected for low pupation height than the other way around, although the number of loci assayed is low.

Discussion

We experimentally explored the influence of antagonistic directional selection for a polygenic trait, pupation height, on genetic differentiation for microsatellite loci (F_{ST}) in *Drosophila* metapopulations in the presence of substantial migration. We studied a situation in which the level of genetic differentiation was initially high for both pupation height and microsatellites, as we anticipated that the magnitude of the responses to the interacting forces of local selection and migration would be more rapid and more evident than when we had initiated the experiment with a low level of genetic differentiation. However, we believe that our results would have been qualitatively the same if we had started the experiment in the latter situation. This notion is supported by the work of Porcher *et al.* (2004, 2006), using *Arabidopsis*, who showed that genetic differentiation for both quantitative traits and neutral genetic markers increased readily when diversifying selection for a life history trait was implemented. Moreover, Smith-Kleefsman *et al.* (2015a, Ch. 2), using individual based simulations, did show that the end result was the same whether the simulations were initiated at zero differentiation for both characters or at maximum differentiation.

Our main results show that the different processes implemented in this experiment significantly affect the neutral variation (F_{ST}) in our experimental metapopulations.

Selection versus migration: Isolation-by-adaption

The most conspicuous observation is that diversifying selection for pupation height greatly affected the dynamics of F_{ST}, as it strongly opposed the homogenising effect of migration (Fig. 3.4). Based on the low levels of introgression of private alleles to subpopulations in which they were initially absent (Fig. 3.6), we argue that this is mainly due to the fact that migrants that are exchanged between subpopulations differing in the direction of selection for pupation height are mal-adapted in their new environment because their offspring does not meet the selection criteria of the receiving population. As such, these migrants are reproductively much less successful as they produce considerably less offspring that will reproduce in the next generation compared to when selection on pupation height is absent. This leads to significant lower levels of effective gene flow (genetic exchange) between subpopulations that are selected in the opposite direction for pupation height, even though the number of migrants exchanged among those subpopulations is essentially the same as for subpopulations that are selected in the same direction. In other words, the effective migration rate (sensu Barton and Bengtsson 1986, Kobayashi *et al.* 2008) becomes much smaller in the presence of antagonistic selection than in the absence.

Our previous simulation study, exploring the interplay between migration, local selection and population turnover using a set-up comparable to this experimental study (Ch. 2, Smith-Kleefsman *et al.* 2015a), confirms this conclusion: migrant females are not successfully reproducing in a receiving subpopulation when the direction of selection in that subpopulation opposes the direction of selection in the subpopulation of their origin. Migrant females tended to be only successful when colonising an extinct subpopulation (Ch. 2, Smith-Kleefsman *et al.* 2015a).

This process of local diversifying selection pressures opposing migration is termed isolation-by-adaptation (for reviews see Nosil *et al.* 2009, Orsini *et al.* 2013) or isolation-by-environment (although isolation-by-environment is defined broader than isolation-by-adaptation, Sexton *et al.* 2014, Wang and Bradburd 2014). Isolation-by-adaptation is analogous to isolation-by-distance where genetic differentiation among subpopulations is driven by reduced levels of gene flow due to increasing distance, but in the case of isolation-by-adaptation the level of gene flow is reduced by antagonistic selection pressures among subpopulations. Both gene flow patterns are commonly observed in nature: In 70 studies Sexton *et al.* (2014) found evidence of isolation-by-adaptation in 20% of the studies, 37 % showed isolation-by-distance, while for another 37% both patterns were detected. Under isolation-by-adaptation we can expect differentiation for neutral loci to be correlated to adaptive phenotypic divergence (Nosil *et al.* 2008, Orsini *et al.* 2013), as is confirmed by the data presented in Figure 3.4. This is also true when comparing F_{ST} and Q_{ST} among the 5 subpopulations selected in the same direction (high-selected: F_{ST} = 0.112 ± 0.014, Q_{ST} = 0.140 ± 0.016; low-selected: F_{ST} = 0.083 ± 0.011, Q_{ST} = 0.047 ± 0.019), while these values are significantly higher when high- and low-selected subpopulations are compared (F_{ST} = 0.387 ± 0.018, Q_{ST} = 0.385 ± 0.016).

Isolation-by-adaptation is thus independent of distance and can already occur between adjacent populations. A classic example of isolation-by-adaptation is the evolution of heavy metal tolerance for plant species growing on waste heaps of mines, which allows these plants to grow on metal contaminated soils while such tolerant plants are at a disadvantage on normal soils (see MacNair 1997 for review). The very steep clines in tolerance phenotype observed for a number of wind pollinated species at the edge of the mine waste heap demonstrate that selection acts against gene flow (Hickey and McNeilly 1975, MacNair 1987). Most probably, the hybrid offspring is at a disadvantage in either habitat (MacNair 1987). This situation is referred to as ecological hybrid inviability and has been observed for many species (Nosil *et al.* 2005). As such, hybrid inviability resulting from isolation-by-adaptation can ultimately lead to speciation as it promotes reproductive isolation between populations living in alternate ecological conditions (Rundle 2002, Plath *et al.* 2013). In our experiment the situation is somewhat different, as the mated migrating females produce non-hybrid offspring that is expected to show the 'homozygous' phenotype of the subpopulation of origin, making antagonistic selection even more effective.

Asymmetry of gene flow and selection pressures

Our short term experiment showed that selection for a life history trait hinders genetic exchange between subpopulations that differ in the direction of selection. Consequently, the antagonistic selection pressures do also maintain a high level of genetic differentiation for neutral genetic markers within the metapopulations, as is indicated by the finding that F_{ST} did not significantly decrease during the generations in the presence of selection (Fig. 3.4). However, the rate of introgression of private alleles under these selective conditions implies still a considerable amount of gene flow among subpopulations of contrasting selection regimes. Had the experiment been extended for more generations, we would expect the F_{ST} to decrease significantly, but to a much lesser extent than in the absence of selection. After all, from the data presented in Figures 3.3 and 3.4 and Table 3.2, we can safely conclude that the effective migration rate among subpopulations is significantly smaller in the presence than in the absence of selection.

Interestingly, the introgression of private alleles in the presence of selection for pupation height appears to be asymmetric: the rate of introgression is considerably lower for alleles of the high-pupating subpopulations into the subpopulations selected for low pupation height than the other way around (though the number of samples is somewhat low). This is most likely explained by the fact that pupation height is greatly affected by larval density: the lower the density, the lower the pupation height. Especially the high-pupating line was found to be sensitive to larval density (see Fig. S1 in Ch. 2, Smith-Kleefsman *et al.* 2015a). This caused the difference in average pupation height between the high- and low-pupating selection lines to be much smaller at low densities than at high densities. The consequences of this are illustrated in Figure 3.7, showing the distribution of pupation height for all ten subpopulations of an individual metapopulation in generation four. Clearly, of those subpopulations selected for high pupation height (HP1-HP5) the ones with a low number of individuals, HP1 and HP4, show a much lower maximum pupation height than those with higher density, HP2, HP3 and HP5. In fact, the pupation height distribution of HP1 and HP4 completely overlaps the distribution of the subpopulations selected for low pupation height. As we applied soft selection, these phenotypically low-pupating individuals would nevertheless be selected as parents for the next generation, most likely including the 'mal-adapted' offspring of an immigrant female originating from a subpopulation selected for low pupation height. Although the distribution of the high-pupating subpopulations is skewed towards low pupation height, the overlap with the phenotypic distribution of the individuals selected for low pupation height (LP1-LP5) is only marginal. The net result is that migration from low to high is expected to occur more frequently than the other way around because the selection pressure on low-pupating migrants on average will be lower than the selection pressure for high-pupating mal-adapted migrants.

Thus, as a consequence of the plasticity of pupation height in relation to density, selection against mal-adapted migrant females becomes on average asymmetric and causes the asymmetry in emigration and immigration rates we observe in our experiment. In nature, asymmetric dispersal and gene flow rates are found regularly as a result of different factors, such as differences in habitat quality (Paul *et al.* 2011, Fedorka *et al.* 2012), direc-

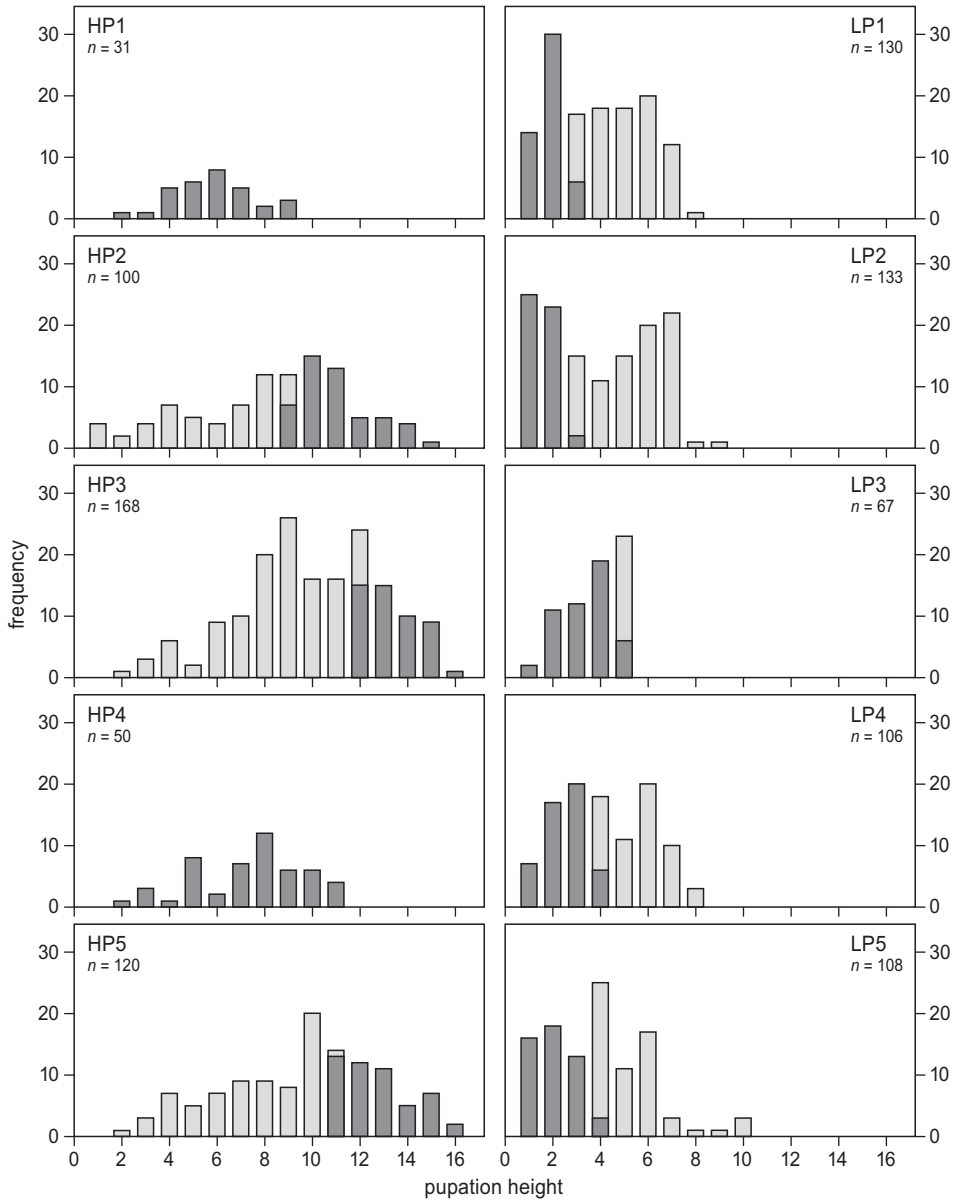


FIGURE 3.7 Example of pupation height distribution for a single metapopulation in generation four. The light grey bars show the number of individuals per pupation height class for each subpopulation, while the dark grey section denote the pupation height of individuals that were selected for the next generation (HP1-HP5 were selected for high pupation height and LP1-LP5 for low pupation height).

tional water flow (Pollux *et al.* 2009, Paz-Vinas *et al.* 2013), prevailing wind direction (Messeguer *et al.* 2001) and altitude (Wilson *et al.* 2012, Muir *et al.* 2014). These systems often result in source-sink dynamics causing gene flow to be asymmetric, where natural selection is likely to be stronger in the source than the sink populations (Kawecki and Holt 2002) similar to our observations. Theoretical models exploring the evolutionary consequences of asymmetric gene flow indicate that depending on the actual migration rates and selection pressures, the levels of genetic diversity and degree of local adaptation are significantly affected by this process; particularly the sink populations will be affected and eventually may end up to be permanently mal-adapted to their local conditions (Kawecki and Holt 2002, Morrissey and De Kerckhove 2009, Sexton *et al.* 2014). Although we observed genetic differentiation for both the selected and the neutral trait to stay high in our experiment, we may not yet have reached the equilibrium conditions, given the experimental limitations (see below).

The considerable level of effective gene flow observed between subpopulations that differ in the direction of selection in the experiment, seemingly contradicts the outcome of computer simulations that mimicked the experimental design (Ch. 2, Smith-Kleefsman *et al.* 2015a). In that simulation study nearly no effective genetic exchange among contrasting subpopulations was found in the absence of population turnover. For one part, this may be explained by the fluctuations in subpopulation density causing reduced selection pressures allowing effective gene flow between contrasting subpopulations, as discussed above. On the other hand, *Drosophila* is polygynous and probably some migrant females do remate with resident males in the receiving subpopulations, as remating occurs regularly in *D. melanogaster* (Bundgaard and Christiansen 1972, Van Vianen and Bijlsma 1993). Given that the genes for pupation height act mostly additively (Bauer and Sokolowski 1988, Singh and Pandey 1993), migrant females that end up in the contrasting environment and remate will produce 'hybrid' offspring that has an intermediate phenotype and would be less maladaptive than 'non-hybrid' offspring. This might also explain why we observed significant levels of effective gene flow between subpopulations that differ in the direction of selection (Fig. 3.6). However, previous simulations using a similar set-up as in the experiment (including remating) predicted effective gene flow to be virtually absent (Ch. 2, Smith-Kleefsman *et al.* 2015a).

Dynamics of individual microsatellite loci: Linkage and selection

In the previous sections, we argued that diversifying selection exerts its influence on neutral loci mainly through changes in the rate of effective migration among subpopulations. Everything else being equal, this is expected to affect the dynamics of all microsatellite loci similarly. By and large, this is reflected in the significantly higher F_{ST}'s for almost all loci in the presence of selection at the end of the experiment (Table 3.2). Though for most loci the observed change in F_{ST} is conform expectation, we also detect conspicuous exceptions (Fig. 3.5). On the one hand, the loci Suvar and Drosev2 (the latter at the border of significance) showed an unexpected increase in F_{ST} in the presence of selection. Although other factors might be involved, this is most likely explained by assuming that these neutral

loci are linked to a gene or genes that are involved in determining pupation height in *Drosophila*. Recently, genome scans have indicated that many putatively neutral microsatellite loci show signals of being linked to adaptive genetic variation (Hansen *et al.* 2010, Kokita *et al.* 2013, Jalvingh *et al.* 2014). A survey by Nosil *et al.* (2009) showed that 5–10% of the supposedly neutral loci show significantly higher levels of divergence than expected under neutrality. That such loci are also encountered in our study is by no means unlikely. After all, the loci we used were selected because they had become divergent among the pupation height lines as a result of 15 generations of artificial directional selection.

On the other hand, the loci Dm30 and Dm3g show a highly significant faster decay of F_{ST} than expected (Table 3.2, Fig. 3.5). Because this finding was observed both in the presence and absence of diversifying selection, it is most probably not related to selection on pupation height. The most plausible explanation in this case would be that the selection lines during their construction and later maintenance had undergone some inbreeding and had become fixed for some deleterious alleles located in the chromosome region containing the microsatellite loci (as these loci are only 15 cM apart it might be the same locus for both). Successful migration between subpopulations that differ in initial pupation height will then result in 'hybrids' between both selection lines that show an increased fitness because they are recovering from inbreeding depression (Keller and Waller 2002, Bijlsma *et al.* 2010). Assuming that 'hybrids' have indeed higher fitness than their parents, this will certainly increase the decline of genetic divergence within the metapopulation over generations, irrespective whether selection is present or absent.

Experimental limitations and future perspectives

This experiment provides several insights into the action of isolation-by-adaptation and how not only the structure of local adaptation for a life history trait, but also that of neutral variation is shaped by local antagonistic selection pressures. The strength of this study is that it directly and causally relates constraints on gene flow to the presence of adaptive divergence within a metapopulation, while in most cases inferences about divergent selection constraining gene flow are inferred indirectly from the correlation of gene flow with environmental differences (Räsänen and Hendry 2008). However, due to several logistic constraints we could study this only for a specific parameter set and in a short term experiment, leaving ample space for further research.

Firstly, we used reasonably strong selection pressures coupled with a fair amount of migration to increase the probability to obtain unambiguous results. It would be interesting to investigate more scenarios using combinations of different selection pressures and/or migration rates including asymmetric scenarios. This would make it possible to entangle the interaction between migration and local adaptation to infer the conditions under which gene flow does constrain adaptation or, the other way around, adaptation constrains gene flow, a question that has not yet been resolved (Räsänen and Hendry 2008, Orsini *et al.* 2013, Sexton *et al.* 2014).

Secondly, the structure of the metapopulation we used in our experiment resembled the island model for which migration rates among (sub)populations are independent of the

distance between these populations. However, many metapopulations show a spatial structure where distance is affecting gene flow patterns among population, as is evidenced by recent reviews (Orsini *et al.* 2013, Sexton *et al.* 2014). A recent experiment of Smith-Kleefsman *et al.* (2015c, Ch. 4) showed that metapopulation structure is also expected to have a significant effect on the balance between local adaptation and gene flow and thus has to be included in studies to understand the dynamics of genetic variation within metapopulations.

Finally, population turnover (extinction-recolonisation events) is thought to be an integral part of metapopulation dynamics (Gilpin and Hanski 1991, Hanski and Gilpin 1997, Hanski and Gaggiotti 2004). Slatkin (1985) showed that recurrent extinction-recolonisation can be seen as a special, highly effective case of gene flow and, as such, is also expected to greatly alter the balance between gene flow and adaptation. Using individual based simulations, Smith-Kleefsman *et al.* (2015a, Ch. 2) observed that population turnover greatly decreased the persistence of local adaptation. This finding was attributed to the fact that they implemented soft-selection, as in this study. It is clear, therefore, that it is also important to study the interaction between gene flow and adaptation under different selection models.

Conclusions

In nature, the interaction between gene flow and local adaptation is observed to be complex and it is often difficult to ascertain whether gene flow constrains adaptation or the other way around (Räsänen and Hendry 2008). However, recent studies correlating genetic differentiation patterns for neutral, ecologically relevant traits with landscape variables suggest that adaptive divergence among populations often constrains gene flow (Orsini *et al.* 2013, Sexton *et al.* 2014). Experimental studies like ours can help to understand and disentangle such complex interaction between the different processes. We showed that local adaptive patterns can persist even in the presence of a substantial level of migration (equivalent to ≈ 2 individuals per generation), through impeding effective gene flow among populations differing in the direction of selection. As such, this study contributes to a better understanding of these complex situations.

CHAPTER 4

Evolution in experimental *Drosophila* metapopulations: Effects of migration on the dynamics of a selected and a non-selected trait

M. W. Smith-Kleefsman
F. J. Weissing
R. Bijlsma

ABSTRACT

Spatially varying selection is expected to result in populations that are adapted to local conditions, but migration can counteract such local adaptation. To study the interplay of spatially varying selection and migration, we performed controlled and replicated experiments on *Drosophila melanogaster*. For 13 generations, we followed the joint evolution of two quantitative traits: pupation height, which was the target of locally diversifying selection, and bristle number, which was selectively neutral. We implemented two migration regimes (stepping stone and island) and four selection gradients (varying from no selection to strong diversifying selection). The metapopulations were initiated with a high degree of differentiation. As expected, migration had a homogenising effect and this effect was more pronounced in case of island migration than in case of stepping-stone migration. A rather steep local selection gradient for pupation height was required to maintain genetic differentiation for this trait. Interestingly, strong selection for spatial differentiation in pupation height also prevented the decay of genetic differentiation in the neutral trait (bristle number). This can be explained by the fact that strong selection on one trait can prevent the establishment and reproduction of immigrants and, hence, reduce the effective gene flow for other traits as well. In our study, the often used parameter Q_{ST} did not always turn out to be an appropriate measure of population differentiation. We argue that the interpretation of Q_{ST}–values can be problematic if the underlying assumptions (homoscedasticity of variances, symmetric distributions of traits around their mean) are not met.

INTRODUCTION

As a result of human activities, many natural habitats have become fragmented, subdividing the populations living in these habitats into (partially) isolated subpopulations. Taken together, these subpopulations comprise a metapopulation (Levins 1969a, Hanski 1999). Conditions between locations often differ, resulting in local variation in the strength and direction of natural selection. Spatially varying selection pressures are expected to result in populations that are adapted to local circumstances, but migration can counteract such local adaptation (Lenormand 2002, Olson-Manning *et al.* 2012). Migrants coming from a different kind of environment may introduce genes that do not fit to their new environment (Wright 1940, Slatkin 1985). However, migration-mediated gene flow may be quite inefficient if mal-adapted migrants and their possible (hybrid) offspring are selected against (Rundle and Whitlock 2001, Hendry 2004, Nosil *et al.* 2005). Accordingly, migration rates may largely overestimate the rate of actual gene flow, allowing the persistence of local adaptation despite the presence of considerable levels of migration. For example, Dionne and co-workers (2008) found that despite similar levels of migration at the local and regional level, the genetic structure of 51 local populations of salmon (*Salmo salar*) closely matched the regional differences in mean temperature, suggesting that local thermal adaptation was not swamped by migration.

To get more insight into the interplay of migration-mediated gene flow and locally varying selection, we performed controlled and replicated experiments. Perhaps surprisingly, rather few such experiments have been performed before. Porcher and co-workers (2004) carried out experiments on *Arabidopsis thaliana* to study if diversifying selection on life span is able to induce genetic differentiation in quantitative traits in the presence of migration. In metapopulations that were initially genetically homogeneous, they observed increased genetic differentiation (quantified by Q_{ST}) between local populations after eight generations of selection (when populations were sufficiently large and genetic drift was limited). While Porcher and colleagues (2004) investigated the build-up of genetic differentiation in the face of migration, Smith-Kleefsman *et al.* (2015a, Ch. 2) studied the persistence of genetic differentiation in pupation height in metapopulations of *Drosophila melanogaster*. Differentiation was achieved by antagonistic directional selection. When selection ceased, differentiation among subpopulations rapidly declined due to migration between the subpopulations. In contrast, the local populations stayed differentiated in the presence of diversifying selection, even when subpopulations were subject to recurrent extinction and recolonisation events. In a *Drosophila* experiment, Van Rijswijk and colleagues (M.E.C. van Rijswijk, personal communication) systematically varied migration rate in order to investigate at which rate genetic differentiation for pupation height still could be maintained. In line with expectations, local adaptation was maintained under the low migration regimes, while it broke down at higher migration rates (unpublished data).

In this study, we aim to generate a more detailed picture of the interplay of diversifying selection and migration in experimental *Drosophila* metapopulations. In contrast to previous studies, we implemented different selection gradients, varying from no selection

to strong diversifying selection. Although we investigated this for only a single rate of migration, we used two different migration schemes (island and stepping stone). A second goal of this study is to investigate the consequence of diversifying selection on one quantitative character for the evolution of a second quantitative trait that itself is not the target of selection. There are two main reasons why a trait not under selection could be affected by selection on a different trait. *(i)* The trait not target of selection might be genetically linked with a trait subject to selection, leading to genetic hitchhiking of neutral variation with selectively relevant variation (Barton 2000). Outlier loci (i.e. loci with higher levels of population differentiation than the genome average) are interpreted as evidence of such hitchhiking. Many examples can be found in the literature (e.g. Beaumont 2005, Storz 2005, Faure *et al.* 2008, Nosil *et al.* 2009). *(ii)* Selection may reduce the gene flow between subpopulations, thus affecting genetic differentiation at neutral loci. If immigrants are selected against (because they are not well adapted to their new environment), they may be not very efficient in spreading their genes (Rundle and Whitlock 2001, Hendry 2004, Nosil *et al.* 2005, Nosil *et al.* 2009). This affects all loci, whether or not subject to selection. As a consequence, genetic differentiation for traits that are not the target of selection will be greater than expected on basis of the migration rate. In a previous study (Smith-Kleefsman *et al.* 2015b, Ch. 3), we observed both abovementioned effects: In metapopulations that were subject to diversifying selection for the quantitative character pupation height, we studied the behaviour of genetic differentiation in neutral markers (quantified by F_{ST}), based on seven microsatellite loci, and we observed that the dynamics of F_{ST} corresponded to that of a population with a significantly lower gene flow than expected on the basis of the migration rate, while one microsatellite locus showed a signature of hitchhiking.

In this study, we compare the dynamics of the trait pupation height that is subject to diversifying local selection to the dynamics of a second quantitative trait that is not subject to selection, sternopleural bristle number, instead of comparing it to independent neutral loci like microsatellites. We performed a replicated experiment, using *Drosophila melanogaster* as model organism. On the subpopulation level, we quantified the change in pupation height (target of selection) and bristle number (not target of selection) throughout the generations by assessing the difference in mean trait value of the local populations and the overall metapopulation mean. On the metapopulation level, we quantified genetic differentiation by Q_{ST}.

Materials and Methods

Drosophila stocks

The flies used for the experiment originated from the Groningen 83 (G83) wild population. This strain was founded in 1983 with 403 inseminated females captured at the fruit market in Groningen (The Netherlands), and maintained as a large population since (Zwaan *et al.* 1991, Vermeulen and Bijlsma 2004). From this strain, several selection lines were established by selecting for high and low pupation height during 15 generations. At the end of

the selection procedure the mean pupation height for the high-selected lines (9.8 cm) was considerably higher than for the low-selected lines (5.9 cm).

The lines were continued under a weak selection regime for about 3½ years. Then, within each selection line for pupation height, two selection lines for a high or a low number of sternopleural bristles were established, eventually resulting in four lines: high-pupating and many bristles (HM), high-pupating and few bristles (HF), low-pupating and many bristles (LM), and low-pupating and few bristles (LF). Selection for bristle number was done as follows: Each of the four lines was initiated by taking the 25 males and 25 virgin females with the highest (resp. lowest) number of bristles in each of the two selection lines for pupation height. To this end, the bristles of 182 males and 326 virgin females were counted in the high-pupating line, while 186 males and 244 virgin females were counted for bristle number in the low-pupating line. Subsequently, for each line, selection for bristle number was continued for eight generations by counting the bristles of about 100 males and 100 virgin females per generation and selecting the 25 males and 25 females with the most extreme bristle numbers. Figure 4.1 gives the results of this selection procedure. Based on the selection response, realised heritabilities (h^2) were established to be: HM-line, $h^2 = 0.16$; HF-line, $h^2 = 0.25$; LM-line, $h^2 = 0.16$; LF line, $h^2 = 0.25$. This is somewhat lower than found in earlier studies, where heritabilities ranged from 0.30 to 0.55 (Gifford and Barker 1991, Bubliy *et al.* 2000, Kristensen *et al.* 2005). Most possibly, this indicates that some loss of genetic variation for sternopleural bristle number occurred when the pupation height lines were established. For unknown reasons (possibly genetic drift, or linkage between pupation height and bristle number), the high-pupating line started with fewer bristles than the low-pupating line. This difference continued to exist during the generations, resulting in a smaller difference between the HMxLF lines (later called metapopulation type A) than between the HFxLM lines (later called metapopulation type B). At the end of the selection for bristle number the mean pupation height of these lines were found to be: HF: 9.2 cm; HM: 9.4 cm; LF: 5.6 cm; and LM: 5.7 cm. This would have

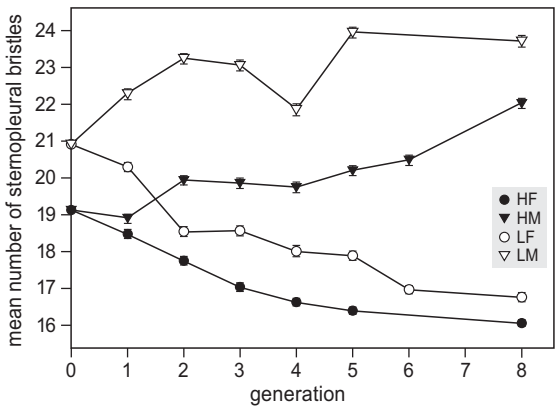


FIGURE 4.1 Response to selection for bristle number within the high and low pupation height lines. The resulting lines are designated: HF: high-pupating and few bristles; HM: high-pupating and many bristles; LF: low-pupating and few bristles; and LM: low-pupating and many bristles.

resulted in a Q_{ST} (defined below) of 0.27 for the HF-LM combination and 0.23 for the HM-LF combination.

During the experiment, flies were kept under standard conditions at 25°C, 50–60% RH, and 18 ml standard medium containing 26 g dead yeast, 54 g sugar, 17 g agar, 13 ml nipagine solution (10 g nipagine in 10 ml 96% alcohol) and 250 mg streptomicine per litre. For handling, flies were anesthetised with CO_2.

General set-up

We aimed to study how dispersal and selection interact to maintain or dilute previously existing genetic and phenotypic differentiation. To this end, we established 32 metapopulations with 6 subpopulations each that were subjected to two migration and four selection treatments in a blocked design, and monitored these for 13 generations. Selection in the six subpopulations varied in a gradient-like manner, where treatments differed in the steepness of the gradient; migration either followed the island model or proceeded in a stepping-stone manner. Each of the eight selection-migration treatments were replicated four times, where two of the replicates were of "type A" and the two others of "type B" metapopulation, where the type of a metapopulation refers to the initial association of pupation height and bristle number (see previous section).

Initialisation of the metapopulations

Subpopulations 1 to 3 of each metapopulation were each initiated with 40 individuals from the high-pupating line, while subpopulations 4 to 6 were initiated with 40 individuals from the low-pupating line. In metapopulations of type A subpopulations 1 to 3 were initiated with the HM line (many sternopleural bristles) and subpopulations 4 to 6 with the LF line (few bristles); in type B metapopulations, subpopulations 1 to 3 were initiated with the HF line (few bristles) and subpopulations 4 to 6 with the LM line (many bristles).

Experimental procedure

Figure 4.2 gives a schematic overview of the experimental procedure. At day 1, flies were placed in clean vials in the morning and were allowed to lay eggs for a maximum of 24 hours. These glass vials (each comprising a single subpopulation) had a height of 20 cm and contained 18 ml (± 2.5 cm) food at the bottom. The inner wall of each vial was lined with a clear plastic sheet on which the larvae could pupate. Each vial contained the individuals of one subpopulation. The number of eggs was checked regularly to prevent overcrowding. After 24 hours (or earlier when egg numbers where sufficiently high) the parents were removed from the vials and discarded. At day 7 or 8, when nearly all larvae had pupated, the sheet containing the pupae was removed from the vial and pupation height of all pupae was determined up to one centimetre of precision (see Fig. 2.1 in Ch. 2, Smith-Kleefsman *et al.* 2015a). Immediately afterwards, the pupae were subjected to selection and migration (see below). The sheets containing the 50 selected pupae were transferred to clean vials.

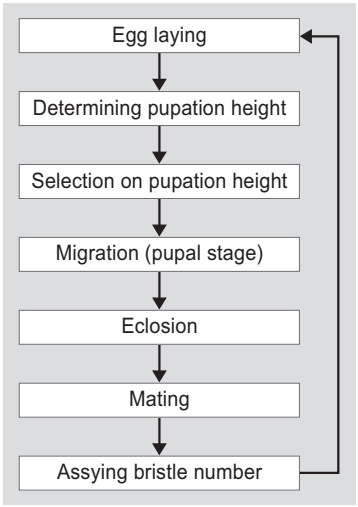


FIGURE 4.2 Schematic overview of the different experimental steps. First, individuals were allowed to lay eggs. In the pupal stage, pupation height was determined, selection for pupation height took place and individuals were migrated according to the appropriate migration treatment. After the pupae had eclosed, individuals were allowed to mate, sternopleural bristles were counted and the cycle started over again for the next generation.

After eclosion, adults were able to mate at random within their own subpopulation. In generations 3, 7 and 13, of each subpopulation sternopleural bristle number of 20 randomly picked individuals (males and females) was determined. Sternopleural bristle number was defined as the sum of the bristles at the left and right side. At day 14, the flies were transferred to clean vials and the next generation started. This was continued for 11 generations. After the eleventh generation, the pupae were transferred into bottles. In the twelfth generation, the flies multiplied, since many subpopulations had low density. In the thirteenth generation, eggs were laid on lids and collected carefully. To obtain equal densities for all subpopulations, each subpopulation was started with 150 eggs. The pupation height of the resulting pupae was determined, and after the pupae had eclosed, sternopleural bristles were counted and the experiment was stopped.

SELECTION TREATMENTS

Selection for pupation height was done in the pupal stage after determining pupation height and before migration took place. Four different selection treatments were used: ST0, ST1, ST2 and ST3 (Fig. 4.3, Table 4.3). *(i)* In the ST0 treatment, no selection for pupation height took place in any of the subpopulations (SP). The 50 pupae closest to the right vertical border of the plastic sheet were chosen to form the next generation. *(ii)* The ST1 treatment corresponds to a shallow selection gradient, where the optimal pupation height gradually decreases from SP 1 to SP 6. To this end, the 50 pupae closest to the subpopulation-specific target value were selected. This target value decreased from 12 cm in SP1 via 10 cm in SP2, 8 cm in SP3, 6 cm in SP4, and 4 cm in SP5 to 2 cm in SP6. *(iii)* The ST2 treatment corresponds to a steeper selection gradient for pupation height: SP1 and SP2 were selected for high pupation height (with a target value of 12 cm); SP5 and SP6 were selected for low pupation height (with a target value of 2 cm), and no selection on pupation height

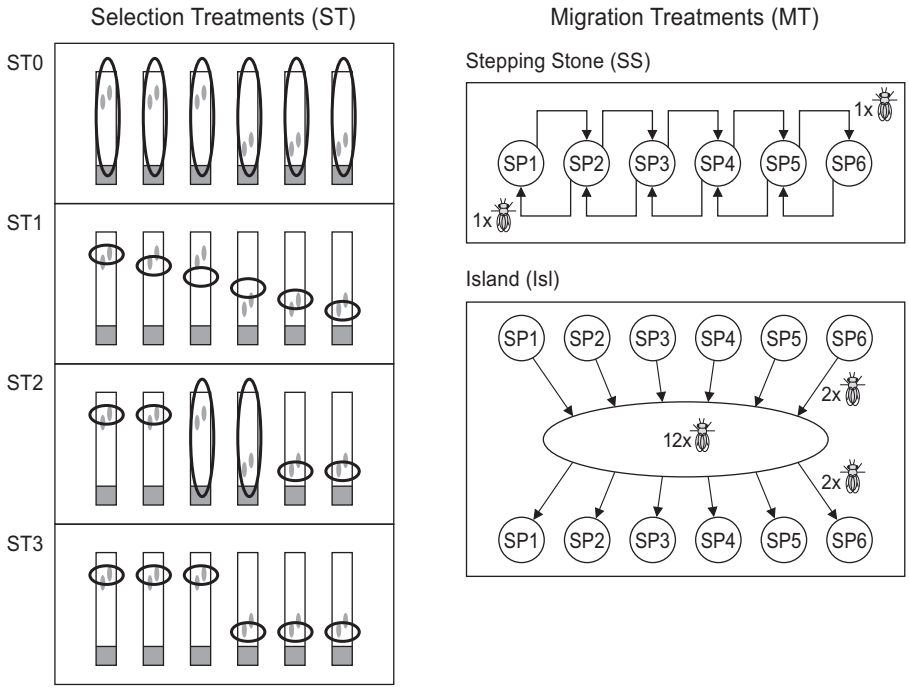


FIGURE 4.3 Schematic diagrams depicting the different selection and migration treatments applied. The four selection treatments for pupation height were: no selection (ST0), gradual clinal selection (ST1), stepwise clinal selection (ST2) and antagonistic directional selection (ST3). In the selection treatment panels, the six vials represent from left to right the six subpopulations SP1-SP6 as depicted for the migration treatments. Migration either resembled a stepping stone (SS) or an island model (Isl). For further details, see the Material and Method section.

TABLE 4.1 ANOVA of Q_{ST} in generation 13 using a general linear model (GLM) procedure with genetic differentiation as the dependent variable and selection treatment (ST), migration treatment (MT) and metapopulation type (MPt) as fixed factors for both traits.

	Pupation height			Bristle number		
	df	MS	F	*df*	MS	F
ST	3	0.340	8.184**	3	0.032	3.543*
MT	1	0.013	3.210	1	0.351	38.369***
MPt	1	0.013	0.093	1	0.196	21.437***
ST * MT	3	0.003	0.675	3	0.017	1.805
ST * MPt	3	0.005	1.161	3	0.003	0.283
MT * MPt	1	0.011	2.576	1	0.031	3.543
ST * MT * MPt	3	0.006	1.476	3	0.006	0.698
Error	16	0.004		16	0.009	

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

was imposed on SP3 and SP4 (like in the ST0 treatment). *(iv)* The ST3 treatment corresponds to a very steep selection gradient for pupation height: now SP1 to SP3 were selected for high pupation height (with a target value of 12 cm), while SP4 to SP6 were selected for low pupation height (with a target value of 2 cm).

Migration treatments

Migration took place in the pupal stage immediately after selection. Out of the 50 selected pupae two were randomly chosen for emigration. This was done irrespective of sex, since gender cannot easily be determined until the late pupal stage. Two different migration treatments were used: stepping stone and island migration (Fig. 4.3). In case of island migration, the emigrating pupae were randomly distributed over the six subpopulations, in such a way that each subpopulation received two immigrant papae. In this scheme, it was possible that an emigrating pupa ended up in the subpopulation of origin. Therefore, this resulted in an average of ten migrating individuals per metapopulation every generation. In the stepping stone migration treatment, the two migrants always migrated to the two neighbouring subpopulations, both receiving one of them (Fig. 4.3). In the edge populations, there was just one emigrating (and one immigrating) individual. This resulted in ten migrating individuals per metapopulation every generation, which is the same number as the expected average in the island migration.

Estimates of genetic differentiation

Wright (1951) introduced F_{ST} as a measure to quantify genetic differentiation within a metapopulation. Since F_{ST} is based on the variation of allele frequencies among populations, this measure can only be applied for traits with known genetic basis. For quantitative characters the underlying genetics is typically unknown. To quantify genetic differentiation for such characters, Spitze (1993) introduced the measure Q_{ST}, which is defined as:

$$Q_{ST} = \frac{\sigma_b^2}{\sigma_b^2 + 2\sigma_w^2},$$

where σ_b^2 is the additive genetic variance attributed to among-subpopulation variation and σ_w^2 is the additive genetic variance attributed to within-subpopulation variation. As in our experiment all individuals experience the same environmental conditions, we assume that the phenotypic variation we observe for the traits reflects the underlying genetic variation.

To determine the genetic variance within and among populations, an analysis of variance (ANOVA) is used. One of the underlying assumptions of ANOVA is that the variance is unrelated to the mean. This was not the case in our study. We found a linear relationship between the standard deviation in pupation height (s) and the average pupation height ($\bar{x}$):

$s = 0.25\bar{x} + 0.67$, $R^2 = 0.57$.

As recommended in the statistic literature (Miller 1986), we corrected for this by log-transforming our data: $x_i' = \log(x_i + 1)$. Consequently, the relationship had mostly disappeared:

$s = 0.02\bar{x} + 0.14$, $R^2 = 0.01$.

Statistical analysis

We were interested in differences between subpopulations; therefore, the data are presented as relative measures (deviations from the metapopulation mean). For pupation height, these deviations were initially positive in subpopulations 1 to 3 (initiated with the high-pupating lines) and negative in subpopulations 4 to 6 (initiated with the low-pupating lines). By the set-up of our experiment, this was also the case for bristle number in metapopulations of type A, while in metapopulations of type B bristle number initially was lower than the metapopulation mean in the first three subpopulations and higher in the last three. To facilitate the comparison between both types of metapopulations, the difference between subpopulation mean and metapopulation mean in bristle number was multiplied by –1 in metapopulations of type B.

To quantify the change in pupation height (or bristle number) throughout the generations, we performed a regression analysis of the mean trait value on time. A decay of local adaptation due to migration should be reflected by a regression to the mean: subpopulations starting at a higher-than-average trait value are expected to have a negative regression coefficient, while those with a lower-than-average trait value are expected to have a positive regression coefficient. The absolute value of the regression coefficient is a measure for the rate of decay of local adaptation. We did not want to present here the regression coefficients per subpopulation, but one overall value for the metapopulation as a whole. To achieve this, the trait values of all populations were pooled, after having multiplied all values (which are deviants from the metapopulation mean) of subpopulations 1 to 3 with –1. In this way, subpopulations with an initial trait value above the metapopulation mean become commensurable with populations below the metapopulation mean. The score thus obtained was negative, reflecting the initially strong local adaptation. A decay of local adaptation corresponds to an increase of this score towards zero and this is quantified by a positive regression coefficient.

The effect of the different selection treatments (ST0, ST1, ST2, ST3), migration treatments (stepping stone or island) and metapopulation types (A or B) on population differentiation was analysed by performing an ANOVA with pupation height or bristle number as dependent variable and selection treatment, migration treatment and metapopulation type as fixed factors.

Results

Pupation height

Figure 4.4 shows how pupation height evolved over 13 generations in 32 metapopulations, each consisting of 6 subpopulations. In a blocked design, those metapopulations were subject to four selection treatments (ST0-ST3) and two migration treatments (stepping stone versus island) and all combinations of treatments were replicated four times. All metapopulations started at a similar level of differentiation in generation 0. The decay of

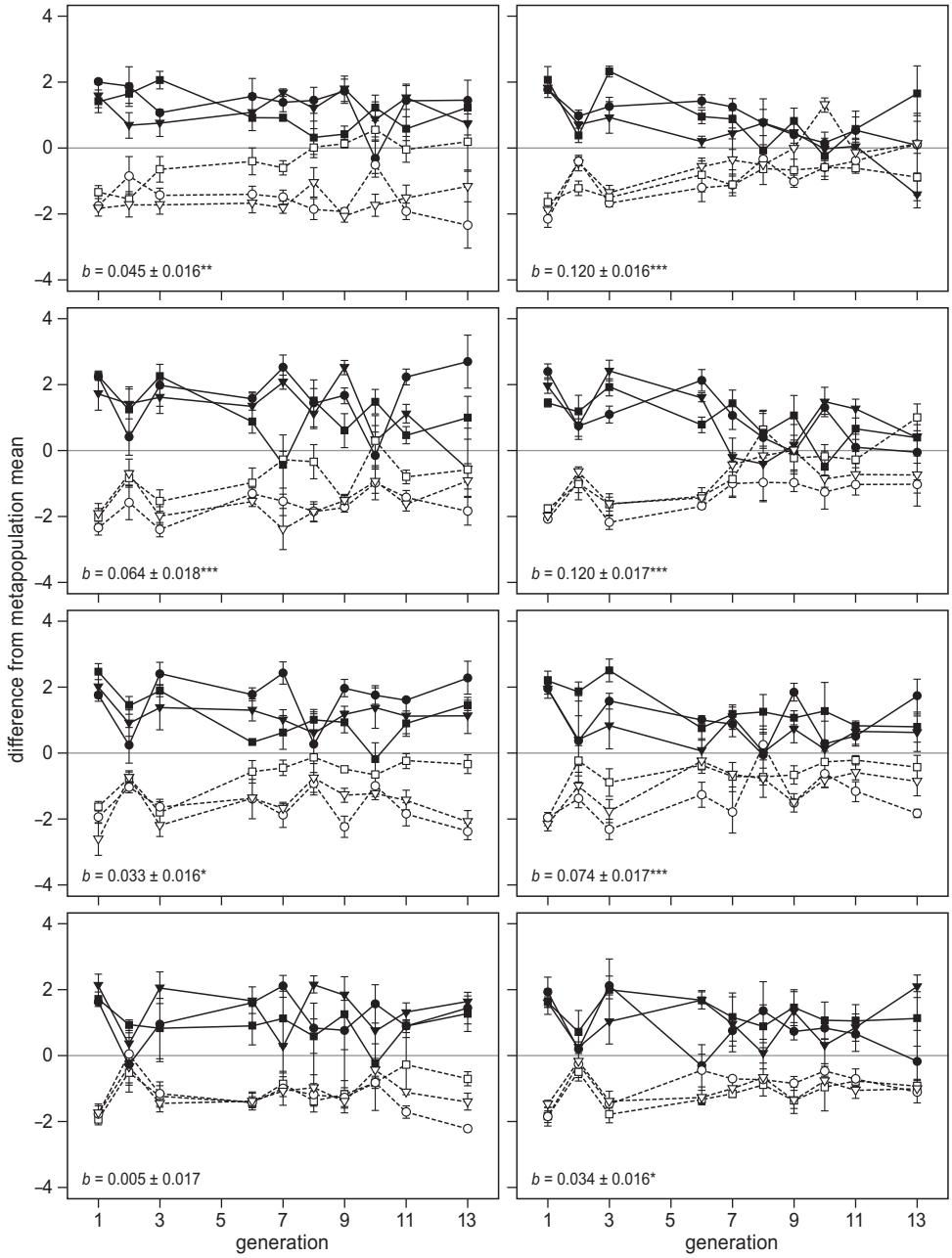


FIGURE 4.4 Deviation of mean pupation height of the different subpopulations from the metapopulation mean (in cm) for Stepping Stone migration (left panels) and Island migration (right panels) for each of the four selection treatments (from top to bottom: ST0, ST1, ST2 and ST3, respectively). For each subpopulation (SP1-SP6) means (± s.e.) were calculated over the four metapopulations. Symbols: SP1: filled circles; SP2: filled triangles; SP3: filled squares; SP4: open squares; SP5: open triangles; SP6 open circles (for subpopulation notation see Fig. 4.3). For the regression coefficients (b): *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (for calculation method see the Material and Methods section).

this differentiation is quantified by the regression coefficient *b*. In all cases, this coefficient was substantially higher in the island treatment than in the stepping stone treatment (Fig. 4.4). This conforms to our expectation, since in the stepping stone treatment only neighbouring subpopulations interchange individuals. When comparing the selection treatments, the regression coefficient *b* tends to decrease from ST0 (no selection) to ST3 (the steepest selection gradient, see Fig. 4.3).

In the stepping stone treatment, the subpopulations that were in the middle of the migration scheme (subpopulations 3 and 4, in Fig. 4.4 symbolised by respectively closed and open squares) tended to converge the most to the metapopulation mean. This is according to the expectation, since only these two subpopulations directly exchange individuals from the two different pupation height lines. In the ST3 treatment, this effect is not so much noticeable.

Generation 2 shows in comparison with generations 1 and 3 in many eases a relatively small deviation from the metapopulation mean. In this generation, pupal density was in general low. About half of the subpopulations contained less than 50 individuals and two third less than 70, while in generations 1 and 3, on average less than 10 percent of the subpopulations contained less than 50 individuals, and about 20 percent less than 70. Pupation height is known to be strongly influence by population size. This is especially true for the high-pupating line (see Fig. S1 in Ch. 2, Smith-Kleefsman *et al.* 2015a). The lower the density, the smaller the difference in pupation height between the high- and low-pupating lines. This effect causes the deviation from the metapopulation mean in these generations to be much smaller.

Figure 4.5 shows the changes in genetic differentiation within metapopulation (Q_{ST}) for pupation height over generations. Although there are quite some fluctuations in Q_{ST} over time, after generation 6 the island migration treatment results, in general, in a lower differentiation than the stepping stone migration. This is in line with the observation from Figure 4 that subpopulation pupation heights converge faster to the metapopulation mean under island migration than under the stepping stone model. However, ANOVA reveals that the difference between the two migration treatments is not significant in generation 13 (Table 4.1, but see below). For the selection treatments (ST0-ST3), the results are less clear (Fig. 4.5). In generation 13, a statistically significant effect of selection treatment is observed (Table 4.1), which turned out to be due to the ST0 treatment to have a higher Q_{ST} than the other three treatments. Figure 4.5 shows that in this generation this treatment, in the absence of selection for pupation height, reached a conspicuous high Q_{ST} for both migration treatments. This is contrary to our expectation, as we expected migration to be the most effective in opposing differences between local populations in the absence of diversifying selection. However, generation 13 was initiated in a different way than the first 11 generations and after one generation (generation 12) without selection (see Material and Methods). This may have significantly affected the results. Therefore, we statistically analysed the Q_{ST} data also for generation 11. In this generation, the effect of selection treatment was observed to be not significant ($F_{3,16}$ = 0.001, p = 0.663). This is somewhat unexpected, since pupation height was under diversifying selection in three of the treatments.

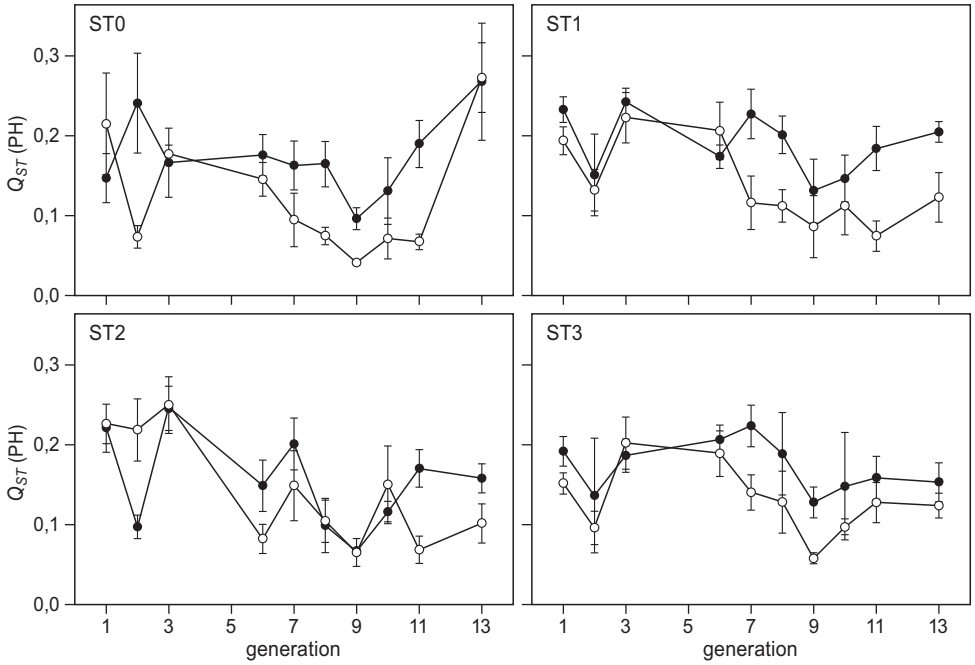


FIGURE 4.5 Mean (± s.e.) population differentiation (Q_{ST}) in pupation height for each of the four selection treatments. The two migration treatments are depicted by different symbols: open symbols, island migration and filled symbols, stepping stone migration.

Notwithstanding, the effect of migration treatment, which may also have been affected by the aberrant data for ST0 in generation 13, was observed to be highly significant ($F_{1,16}$ = 47.437, $p < 0.001$) in this generation. In conclusion, the changes in subpopulation means indicate that both migration treatment and selection treatment affect the dynamics of genetic differentiation within metapopulations. This is supported by the changes in Q_{ST} with respect to migration treatment, but not with respect to selection treatment.

BRISTLE NUMBER

For sternopleural brisle number, we might expect that, like for pupation height, island migration would cause a faster convergence to the mean than stepping stone migration. However, how local selection performed on pupation height would affect differentiation for bristle number is not self-evident. Figure 4.6 shows the deviation of subpopulation bristle number from the metapopulation mean for the two migration treatments and the four selection treatments. As for pupation height, we found for bristle number a significant regression to the mean in all cases. This trend was clearly stronger for bristle number than it was for pupation height (compare regression coefficients). Convergence to the mean is more rapid with island migration than with stepping stone migration for all selection treatments. For the stepping stone treatment, subpopulations that are in the middle of the

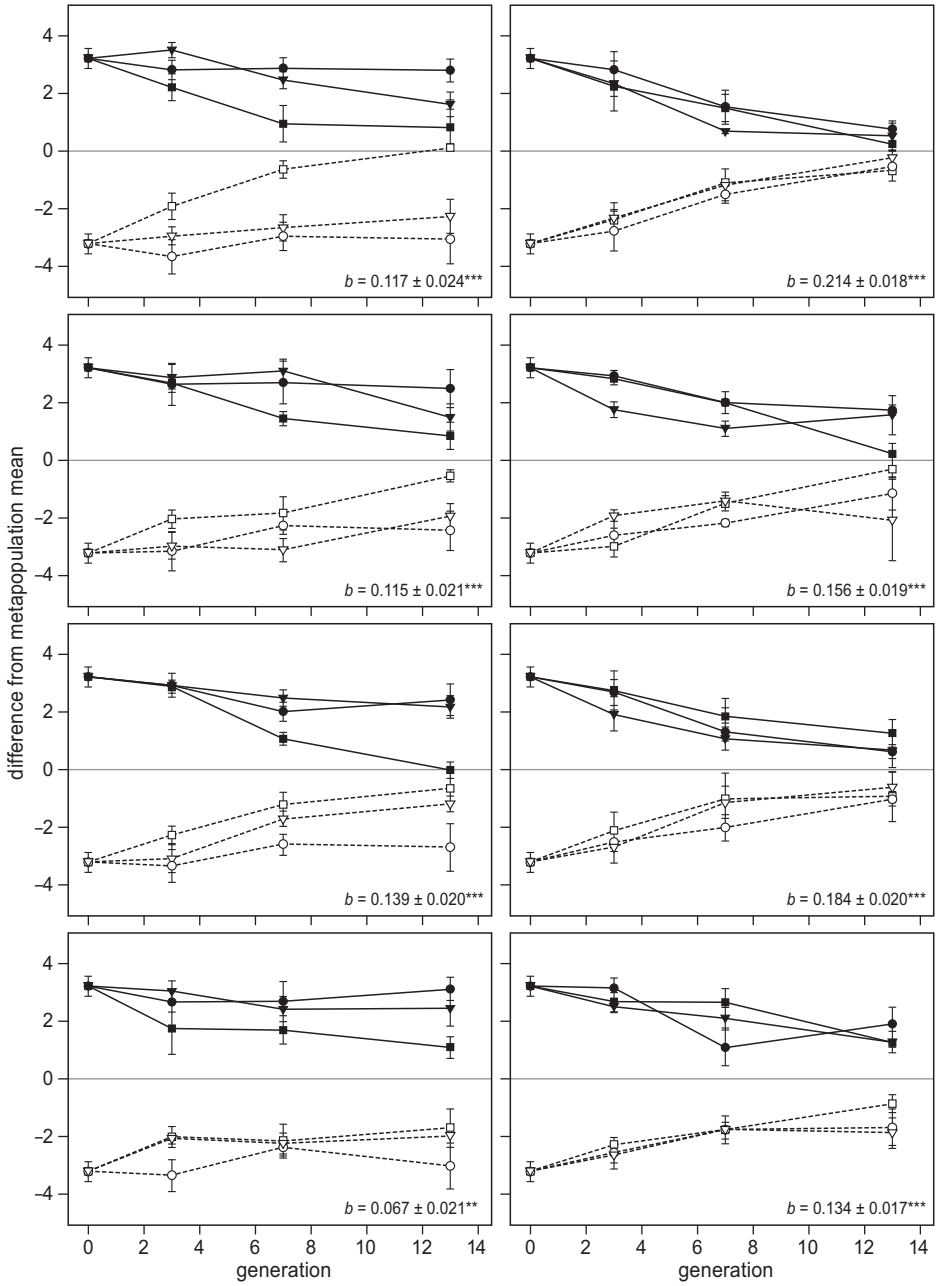


FIGURE 4.6 Deviation of mean sternopleural bristle number of the different subpopulations from the metapopulation mean (in cm) for Stepping Stone migration (left panels) and Island migration (right panels) for each of the four selection treatments (from top to bottom: ST0, ST1, ST2 and ST3, respectively). For each subpopulation (SP1-SP6) means (± s.e.) were calculated over the four metapopulations. Symbols: SP1: filled circles; SP2: filled triangles; SP3: filled squares; SP4: open squares; SP5: open triangles; SP6 open circles (for subpopulation notation see Fig. 4.3). For the regression coefficients (b): $^{**}p < 0.01$, $^{***}p < 0.001$ (for calculation method see the Material and Methods section).

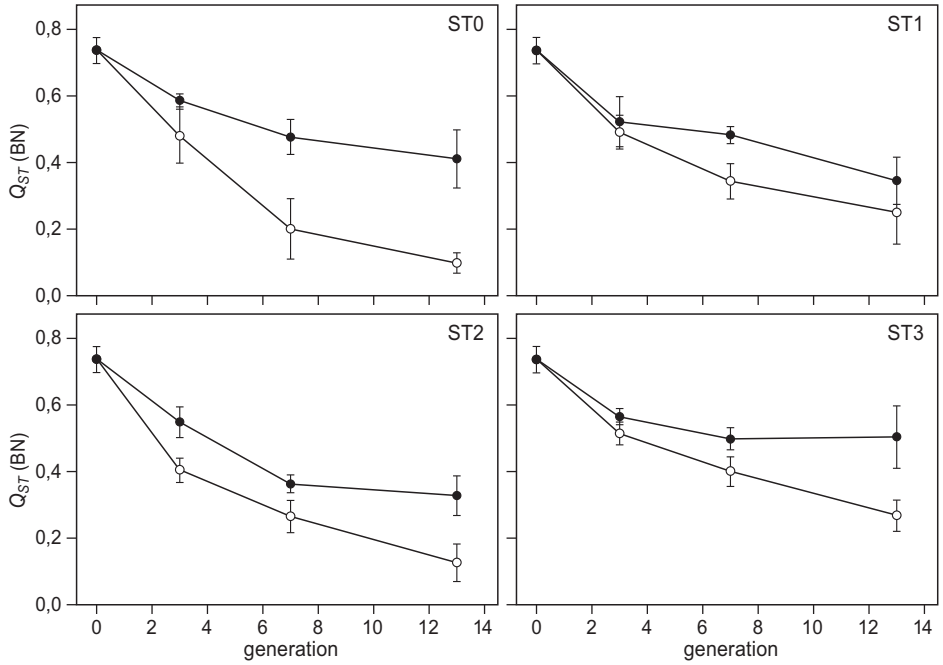


FIGURE 4.7 Mean (± s.e.) population differentiation (Q_{ST}) for sternopleural bristle number for each of the four selection treatments. The two migration treatments are depicted by different symbols: open symbols, island migration and filled symbols, stepping stone migration.

migration scheme (subpopulations 3 and 4, in Fig. 4.6 depicted as closed and open squares respectively) tend to converge faster to the metapopulations mean as the other subpopulations. This effect was especially clear in the ST0, ST1 and ST2 selection treatments, but less so in the ST3 selection treatment. No large differences in regression to the mean were found between the different selection treatments, although the general trend seems that the stronger diversifying selection pressure on pupation height, the slower the convergence to the metapopulation mean. The findings are supported by the results of the analysis of population differentiation for bristle number (Fig. 4.7). For all migration and selection treatments, Q_{ST} is considerably declining during the generations. For all selection treatments, this decline is clearly faster for the island than for the stepping stone migration treatment, resulting in a significant effect of migration treatment in generation 13 (Table 4.1). ANOVA also reveals a significant effect of selection treatment. Particularly for the island migration treatment, it is clear that Q_{ST} is declining the fastest in the absence of selection on pupation height (ST0) and the slowest when strong divergent selection (ST3) is present. This shows that diversifying selection on pupation height significantly affects the dynamics of genetic differentiation for the character that itself is not under selection.

In addition, ANOVA showed a significant effect of the metapopulation type with which the subpopulations were initiated (Table 4.1). The initial difference between the two metapopulation types (Fig. 4.1) was still present at the end of the experiment (see Appendix A).

DISCUSSION

Gene flow is known to constraint adaptive divergence of local populations and, in turn, adaptive divergence is known to constraint gene flow (Räsänen and Hendry 2008). We studied the joint effect of these processes on two quantitative traits, of which one was the target of selection (pupation height) and the other was not (bristle number). Three main results were obtained. First, the decrease in adaptive divergence was stronger when migration was according to the island scheme than when it was according to the stepping-stone scheme. This was the case for both pupation height (target of selection) and bristle number (not target of selection). Second, the decrease in adaptive divergence was weaker with stronger antagonistic directional selection. In contrast to our expectation, this was not supported by the Q_{ST} values. Third, bristle number, that was not target of selection, was significantly affected by antagonistic diversifying selection on pupation height: the stronger the selection pressure on pupation height, the higher the divergence in bristle number. This result is comparable with the results of an earlier experiment (Ch. 3, Smith-Kleefsman *et al.* 2015b), in which we observed microsatellites to behave non-neutral in metapopulations that were subject to antagonistic directional selection on pupation height. Based on the results presented in that chapter, we here conclude that the impact of selection (for pupation height) on bristle number is mostly due to migrants (and their offspring) being less effective in passing their genes to the next generation, i.e. selection against immigrants. So local adaptation for one character (pupation height) caused reduced gene flow, allowing longer existence of the local differentiation of the second character (bristle number).

CONSIDERATIONS UNDERLAYING THE EXPERIMENT / EXPERIMENTAL LIMITATIONS

We performed this study in order to investigate the interplay of migration and selection under high standardised and replicated conditions. Yet, it is important to notice that even under the highly standardised conditions in the lab, the evolving metapopulations showed quite some variation in convergence. The two traits selected for our experiment (pupation height and bristle number) were chosen because they have a number of desirable properties: a clear genetic basis so it can easily be selected (Garcia-Florez *et al.* 1989, M. E. C. van Rijswijk, personal communication), selection can be performed without influencing the generation time and is easy to score. But it is well known that the expression of both traits is affected by local conditions such as temperature, humidity and pupal density (Thoday 1958 quoting Beardmore, Sokal *et al.* 1960, Sokolowski and Hansell 1983, Godoy-Herrera *et al.* 1989, Casares *et al.* 1997, Ch. 2 and Ch. 5, Smith-Kleefsman *et al.* 2015ad). Presumably, fluctuation in these environmental variables at least partly caused the deviation from the general trend (e.g. gen. 2 and gen. 13). If such deviations already occur under highly standardised conditions, even stronger deviations from theoretical expectation are expected in nature.

In our experiment, bristle number represents a trait that is not subject to selection. One might argue that the assumption of selective neutrality is not justified, since the initial variation in bristle number was the outcome of a pre-treatment involving strong directional

selection. It might have been the case that discontinuation of selection on bristle number during our experiment, might have led to the regression of bristle number to its natural mean, which would not be in line with the assumption of neutrality. However, this does not seem to be the case. Control experiments (data not shown) revealed that differentiation in bristle number of the lines used in this experiment was maintained even 50 generations after selection had been ceased.

Q_{ST} AS A MEASURE OF GENETIC DIFFERENTIATION

One of the aims of this study was to investigate the dynamics of Q_{ST} to characterise population differentiation. Our study gives rise to some doubt whether Q_{ST} has a high discriminating ability for this purpose. Figure 4.4 clearly reveals that pupation height is responding to the selection and migration treatments. However, this effect seems not reflected in the dynamics of Q_{ST} (Fig. 4.5). A potential explanation might be that the distribution of pupation heights was rather skewed, in particular in the high line, for which the median was higher than the mean (data not shown). Such skewness may be relevant, since the calculation of Q_{ST} is based on an ANOVA, which presupposes that the data are symmetrically distributed around the mean. It is conceivable that the application of standard statistical techniques to skewed distributions can lead to either inflated or deflated estimates of Q_{ST}. This might be an important consideration for other studies as well, since many traits are not distributed symmetrically in nature (e.g. Zhang 1998, Schmidt-Lebuhn *et al.* 2007, Kitano *et al.* 2008, Grant and Grant 2009). Obviously, an inflation or deflation of Q_{ST}-estimates will have implications for studies that use the ratio of F_{ST} - Q_{ST} to draw inferences on the presence or absence of selection and the kind of selection (e.g. directional, stabilising, or diversifying selection). If one of these two measures is strongly biased, such a comparison may become meaningless. Sometimes a data transformation removing the skewness may resolve the problems addressed above. In our case, this was not possible, because we already had to transform the data in order to remove a positive correlation between mean and variance, which is another prerequisite of an ANOVA.

Even if the data are statistically "well behaved," the interpretation of genetic differentiation data based on the comparison of Q_{ST} and F_{ST} is more problematic than originally anticipated. For example, even in the absence of selection Q_{ST} often differs systematically from F_{ST}. As demonstrated in a simulation study (Miller *et al.* 2008), the mean Q_{ST} for a neutral quantitative trait is expected to exceed the mean F_{ST} when a panmictic population has recently been subdivided into separate demes, while the opposite is the case if the demes have been isolated for a long time. In another theoretical study, Whitlock (2008) points out that F_{ST} and Q_{ST} exhibit huge stochastic fluctuations. So even when the means are expected to be the same by theory, Q_{ST} and F_{ST} can differ substantially for a given locus or trait, in both directions, as shown in Chapter 5 (Smith-Kleefsman *et al.* 2015d).

FUTURE DIRECTIONS

For logistic reasons, we studied only the decline of genetic differentiation due to migration in our experimental set-up, which resembles the situation where local populations that were previously isolated, later became connected by migration. It could also have been interesting to study the build-up of differentiation, when demes in previously well connected populations become more and more isolated. Based on the results obtained here, we would expect the increase of the differentiation to be more slowly in island migration than in stepping stone migration. Likewise, in case of small differences in local selection pressures, we would also expect the increase in differentiation to proceed more slowly. It is more difficult to predict what would happen to a trait that is not the target of selection; experiments complementary to ours would be needed to answer this question. In addition, more research is needed to disentangle the roles of gene flow and adaptation in shaping differentiation between populations. Both gene flow and adaptive divergence can be the cause of differentiation (Räsänen and Hendry 2008). By introducing new alleles, gene flow can constrain local adaptation, and local adaptation in turn can counteract gene flow by selection against immigrants. Even if we leave aside the problem of disentangling the interaction of gene flow and local adaptation, each process on its own can be difficult to quantify (Räsänen and Hendry 2008). One complication is related to the fact that organisms can respond to differences in local conditions in phenotypically plastic manner. Phenotypic plasticity is common in nature (Bradshaw 1965, Roff 1997, Schlichting and Pigliucci 1998, West-Eberhard 2003), but its effect on differentiation may vary. Phenotypic differentiation will be more pronounced than genetic differentiation if the same genotypes express different phenotypes under different conditions; it will be less pronounced, if homeostasis and canalisation will lead to similar phenotypes irrespective of underlying genetic differences. Since Q_{ST} does typically measure the degree of phenotypic (rather than genetic) differentiation (Pujol *et al.* 2008), this gives us another caveat when interpreting Q_{ST} data.

APPENDIX A

At the start of the selection for increased or decreased sternopleural bristle number within the high- and low-pupating lines, an initial difference in mean bristle number already existed between these two lines. In the low-pupating line, flies had on average two bristles more than flies from the high-pupating line. This difference continued to exist during the selection procedure (Fig. 4.1). As a result, metapopulation type A, which combined the HM (subpopulations 1 to 3) and LF (subpopulations 4 to 6) lines started with a lower population differentiation (Q_{ST}) than metapopulation type B, which combined the two other lines. Consequently, the A and B metapopulations already differed at the start of the experiments, Q_{ST}'s being 0.67 and 0.81 for A and B, respectively.

At the end of the experiment, these differences between the two metapopulation types still persisted (Fig. A1): Q_{ST} for type B populations was significantly larger than Q_{ST} for type A (see Table 4.1).

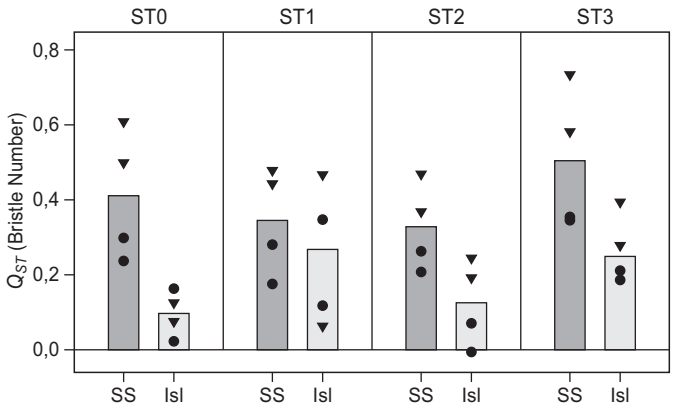


FIGURE A.1 Q_{ST} values in generation 13 for the individual metapopulations of type A (circles) and type B (triangles) for each selection treatment (ST0-ST3) and each migration treatment (stepping stone (SS) and Island (Isl) migration). The bars depict the averages over all four replicated metapopulations.

CHAPTER 5

The impact of cogradient and countergradient environmental variation on Q_{ST} estimation: An experimental approach

M. W. Smith-Kleefsman
F. J. Weissing
R. Bijlsma

ABSTRACT

For many morphological and fitness traits, the phenotypes of species change in a regular way along environmental gradients. Whether such changes reflect genetic variation or/and phenotypic plasticity is still an important ecological and evolutionary question. We used such clinal configurations to investigate the consequences of the interplay between genetic variation and phenotypic plasticity on phenotypic differentiation among populations in an experimental setting for the morphological trait "sternopleural bristle number" in *Drosophila*. To this end, we established simple metapopulations for which the two factors genetic variation and environmental temperature co-varied either positively (cogradient variation) or negatively (countergradient variation). We observed a strong interaction between genetic variation and temperature. In the cogradient alignment both factors acted synergistic, increasing the phenotypic differentiation among subpopulations substantially compared to the situation with only genetic differences present. Conversely, in the countergradient alignment the phenotypic differentiation drops to almost zero, showing that in this situation both factors act highly antagonistic. This signifies that when genetic and environmental variation co-vary, either positively or negatively, the phenotypic differences observed among populations under natural conditions cannot serve as a proxy for genetic differentiation. Separating the genetic and environmental effects is important as it bears significantly on the extent populations are locally adapted, and to their potential to adapt to changing environmental conditions either through evolutionary change or through phenotypic plasticity.

INTRODUCTION

Metapopulations consist of several relatively small subpopulations that are connected by gene flow, but have a largely independent dynamics (Levins 1969b, Gilpin and Hanski 1991, Hanski and Gilpin 1997 and references therein, Hanski and Gaggiotti 2004). Since long, researchers have aimed at understanding the processes that shape the genetic structure of metapopulations. One of the aims is to estimate the presence and role of selection, leading to adaptive phenotypic divergence, in shaping genetic differentiation among subpopulations within a metapopulation. To be able to make inferences about the presence of selection, Q_{ST}, a measure for genetic differentiation in polygenic traits that may be subject to selection, is typically compared to F_{ST}, a measure for differentiation in monogenic markers (Merilä and Crnokrak 2001, McKay and Latta 2002, Leinonen *et al.* 2013). For this com parison, F_{ST} should be based on markers that behave neutral, i.e. are not subject to selection.

A critical assumption underlying the Q_{ST} estimation is that the genes responsible for the phenotypic differences among individuals act purely additive, i.e. dominance and maternal effects, and other non-genetic factors, e.g. environmental variation, should not affect the phenotypic differences (Merilä and Crnokrak 2001, Whitlock 2008). However, adaptive phenotypic divergence is the result of more processes than only selection. One main process affecting adaptive phenotypic divergence is phenotypic plasticity: the capacity of a single genotype to display different phenotypes in response to environmental differences. Phenotypic plasticity seems universal among organisms (Bradshaw 1965, Schlichting and Pigliucci 1998, Agrawal 2001, West-Eberhard 2003). To properly estimate Q_{ST} based on additive genetic variation alone without the confounding effects of phenotypic plasticity, therefore, seems problematic, notably under natural conditions (Sæther *et al.* 2007, Pujol *et al.* 2008). When both genetic and environmental variation are affecting phenotypic differences among populations, not Q_{ST} (genetic divergence), but P_{ST} (phenotypic divergence), a measure based on the total phenotypic differentiation, is assessed (Sæther *et al.* 2007). Pujol *et al.* (2008) evaluated a number of studies that used a pairwise comparison of Q_{ST} and F_{ST} to infer a possible role of selection in natural populations. They argue that in half of the studies genetic and environmental effects were confounded and that, therefore, the conclusion that natural selection affected the trait under study seemed premature in those studies. The few studies that have explicitly investigated this problem show that it is generally of utmost importance to distinguish between P_{ST} and Q_{ST}. (Raeymaekers *et al.* 2007, Alho *et al.* 2010, Defaveri and Merilä 2013).

Clinal environmental variation, such as latitudinal and altitudinal clines, present a special situation for investigating the interplay between genetic and environmental factors. Depending on how these two factors co-vary, we can distinguish two contrasting situations: *(i)* a cogradient alignment, i.e. an individual's genotype and the environment it experiences shift the phenotype in the same direction and *(ii)* a countergradient alignment, i.e. the genotype and the environment shift the phenotype in opposite directions (Levins 1968, 1969b). Both these alignments are regularly encountered in nature (e.g. Kawajiri *et al.* 2011, Iraeta *et al.* 2013, for a review, see Conover *et al.* 2009). In both these situations, the phenotypic

differentiation found in the metapopulation may not be directly indicative for the underlying genetic differentiation. In the case of cogradient variation, the observed phenotypic differentiation could possibly be larger than the genetic variation of the trait under study, i.e. $P_{ST} > Q_{ST}$ (Conover *et al.* 2009). For instance, Byars *et al.* (2007) observed in an alpine grass species (*Poa hiemata*) leaf length to decrease and leaf circumference to increase with altitude. Transplant experiments revealed that these differences were due to both genetic differences between the subpopulations and phenotypic plasticity. In case of countergradient variation, no or little phenotypic differentiation may be observed, while there is in fact underlying genetic differentiation present ($P_{ST} < Q_{ST}$, Conover *et al.* 2009). Ligon and Skelly (2009), for example, found little differences among populations for several growth characteristics of wood frogs (*Rana sylvatica*) in the field situation. However, when raised under standard conditions in the laboratory, they did found ample genetic variation that was phenotypically not expressed in the field. In another study, Laugen *et al.* (2003) assessed developmental rates of common frogs (*Rana temporaria*) along a latitudinal gradient. Laboratory experiments showed that genetic differentiation between the frogs for this trait strongly correlated with the latitudinal gradient. However, in the natural situation these differences were not observed. In their study, environmental effects seem to mask the genetic effects, which led to the conclusion that countergradient variation was present for this cline.

In this study, we experimentally investigate the consequences of an interplay between genetic and environmental variation for both the cogradient and countergradient situation for the level of phenotypic differentiation at a polygenic quantitative trait. To this end, we used *Drosophila* lines that genetically differed in the number of sternopleural bristles as the result of directional selection for either few or many bristles. This trait is governed by many genes with small effect that largely act additive (Killick 1972, Mackay and Lyman 2005). In addition, sternopleural bristle number is known to be plastic in relation to the developmental temperature and shows a negative correlation with temperature: the higher the developmental temperature the lower the number of bristles and vice versa (Parsons 1961, Thoday and Gibson 1970, Bubliy *et al.* 2000). This relation most probably arises as a correlated response to the effect of temperature on body size (Coyne and Beecham 1987). The selection lines were used to establish very simple metapopulations of which the subpopulations varied clinally for mean bristle number and developmental temperature. We investigated both the cogradient alignment (i.e. bristle number correlated positively with temperature) and countergradient alignment (i.e. bristle number correlated negatively with temperature). Our results show that genotype and temperature, depending on the nature of the gradient, act either synergistically or antagonistically thereby greatly affecting the level of phenotypic differentiation for sternopleural bristle number among subpopulations. Consequently, estimates of Q_{ST} without correcting for the environmental differences would be highly misleading in the studied situations.

Materials and Methods

Drosophila stocks

For the experiment, flies from the Groningen 83 (G83) wild type strain were used. This strain was founded in 1983 with 403 inseminated females captured at the fruit market in Groningen (The Netherlands), and maintained as a large population since (Zwaan *et al.* 1991, Vermeulen and Bijlsma 2006). Flies were kept under standard conditions at 18 ml standard medium containing 26 g dead yeast, 54 g sugar, 17 g agar, 13 ml nipagine solution (10 g nipagine in 10 ml 96% alcohol) and 250 mg streptomicine per litre. For handling, flies were anesthetised with CO_2.

Selection lines

We used selection lines that differed significantly in the number of sternopleural bristles and were established by Smith-Kleefsman *et al.* (2015c, CH4) by directional selection on total bristle number, that is the sum of the bristles at both sides, for 8 generations for either increased or decreased bristle number. This resulted in two lines with many bristles (mean ± sd: 22.0 ± 2.0 and 23.7 ± 2.3, respectively) and two lines with few bristles (16.1 ± 1.3 and 16.8 ± 1.7, respectively). These lines were maintained without further selection for about 25 generations before they were used for the experiments reported here. Five generations before starting the experiment, the two lines having Many Bristles were intercrossed resulting in the MB base stock with a mean bristle number of 20.9 ± 2.5. Equally, the two lines having Few Bristles were also intercrossed to establish the FB base stock with a mean bristle number of 16.9 ± 1.5. Also, the original four selection lines were intercrossed resulting in a "Hybrid" base stock (HB) with an intermediate mean number of bristles of 19.1 ± 2.3), but being genetically more variable for bristle number. Offspring of the three resulting base stocks were used in the experiment.

In addition to genetic differences among subpopulations that were mediated using the different base stocks (MB, HB and FB), environmental differences were brought about by implementing three different temperatures during development (egg to adult): 20°C, 25°C and 29°C.

Set-up and procedure

For the experiments, a set of metapopulations were initiated, consisting of three subpopulations each (Fig. 5.1). Each subpopulation consisted of 10 mated females and 10 males. All steps described below were performed for each metapopulation. Females were allowed to lay eggs for a maximum of 24 hours. After emergence, the following four steps were implemented: *(i)* From each subpopulation, 10 mated females and 10 males (not necessarily the males that mated with the females) were randomly selected as parents for the next generation. *(ii)* The sternopleural bristle number of these 20 individuals was determined. *(iii)* Migration was implemented in the following manner: From subpopulation 2 (SP2, Fig. 5.1)

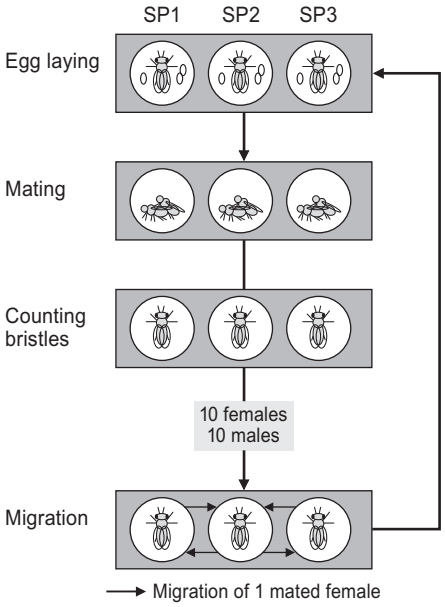


FIGURE 5.1 Schematic overview of the different experimental steps that were performed during the experiment. For more details see Material and Methods section.

one mated female migrated to subpopulation 1 (SP1) and one to subpopulation 3 (SP3). From both SP1 and SP3, one mated female migrated to SP2. This migration scheme is equivalent to a linear stepping stone model (Kimura 1953, Crow and Kimura 1970). *(iv)* The individuals were placed in fresh vials and females were allowed to lay eggs for 24 hours. This procedure was continued for four generations.

Because the difference in breeding temperature results in differences in development time between the subpopulations, short at 29°C and long at 20°C, flies had to be synchronised at the start of each generation. Flies developing at 29°C therefore were stored at 25°C until the flies at 20°C had all emerged.

TREATMENTS AND PARAMETERS ESTIMATED

We used the base stocks and the three developmental temperatures to establish both cogradient (MB, HB and FB subpopulations were cultured at 20°C, 25°C and 29°C, respectively) and countergradient (MB, HB and FB subpopulations were cultured at 29°C, 25°C and 20°C, respectively) situations. At the former, the decrease in bristle number due to genetic differences coincides with the decrease resulting from the environmental gradient (cogradient alignment), while in the latter case both factors oppose each other (countergradient alignment). Both situations are referred to in Table 5.1. For each treatment four replicate metapopulations were established. To estimate the differentiation due to genetic effects in the absence of environmental difference, subpopulations were established using the three base stocks to each start one of the subpopulations which all were further maintained at 25°C (two replicates, Table 5.1). To infer the plasticity of the stocks, i.e. the effect of the clinal temperature gradient separately from the genetic effect, metapopulations were

established using either only flies from the MB stock for the three subpopulations at the different temperatures, or flies from the FB stock (Table 5.1), with two replicates each.

All these metapopulations were maintained for four generations in the manner as described in set-up and procedures. To enable extra controls for parameter estimation, in the fourth generation females from both gradient treatments were transferred to new vials after egg laying to produce an extra set of offspring and these were placed at "control" conditions (see in Table 5.1).

Estimates of differentiation

Total phenotypic differentiation (P_{ST}) for sternopleural bristles when both genetic and environmental differences among subpopulations are present was quantified using the following equation:

$$P_{ST} = \frac{\sigma_b^2}{\sigma_b^2 + 2\sigma_w^2},$$

where σ_b^2 is the phenotypic variance attributed to among-subpopulation variation and σ_w^2 is the phenotypic variance attributed to within-subpopulation variation. This is a dimensionless measure with values between zero, no differentiation, and one, complete differentiation (Wright 1951, Spitze 1993). Because we determined P_{ST} for where genetic and environmental differences among subpopulations co-vary differently, either in the cogradient or the countergradient alignment we use different notations for these contrasting situations: $P_{ST}(Co)$ denotes total phenotypic differentiation for the cogradient situation and $P_{ST}(Cn)$ for the countergradient situation (Table 5.1, D and E, respectively).

Table 5.1 Overview of the different treatments to infer the effect of genetic and/or environmental differences for the genetic/phenotypic differentiation within the experimental metapopulations. The different columns refer to: "Code" is used to indicate a specific treatment in the text; "SP1", "SP2" and "SP3" indicates the base stock that initially was used for this subpopulation at the start of the experiment and the temperature at which the flies of the subpopulation developed; "Estm. of" refers to the differentiation measure that can estimated from this treatment; "Gen" indicates the generation(s) for which this treatment was implemented; "# Reps" indicates the number of replicate metapopulations for a treatment.

Treatment	Code	SP1 ↔ SP2 ↔ SP3	Estm. of	Gen	# Reps
Stocks at 25ºC	A	FB25 ↔ HB25 ↔ MB25	Q_{ST}	1-4	2
Plasticity base stock MB	B	MB20 ↔ MB25 ↔ MB29	E_{ST}	1-4	2
Plasticity base stock FB	C	FB20 ↔ FB25 ↔ FB29	E_{ST}	1-4	2
Cogradient	D	MB20 ↔ HB25 ↔ FB29	$P_{ST}(Co)$	1-4	4
	$D_{Control}$	MB25 ↔ HB25 ↔ FB25	Q_{ST}	4	4
Countergradient	E	FB20 ↔ HB25 ↔ MB29	$P_{ST}(Cn)$	1-4	4
	$E_{Control}$	FB25 ↔ HB25 ↔ MB25	Q_{ST}	4	4

However, when we test genetically different subpopulations in the common environment of 25°C, we assume that environmental differences are negligible and that P_{ST} in this situation is the result of mostly additive genetic differences among subpopulations only and thus an estimate of genetic differentiation, which we denote as Q_{ST} (Table 5.1, A).

The other way around, when genetically similar subpopulations are subjected to different environmental temperatures, we assume that the phenotypic differences among subpopulations in this situation are due to phenotypic plasticity only. This measure we denote as E_{ST} (Table 5.1, B and C).

Results

Testing the genetic differences among stocks and their response to temperature cline

Just before initiating the metapopulations we assayed the genetic differences among the base-stocks that were used to establish the different subpopulations at the common temperature of 25°C. For each stock we counted the bristle number (sum of the right and left side) of 20 individuals of each sex. Figure 5.2 (left panel) shows that there are considerable differences, both among base stocks and among sexes. As expected, males, being the smaller sex, have less bristles then females, while the difference due to selection also clearly show. A 2-way ANOVA with stock and sex as fixed factors revealed that these differences both were highly significant (Stock: $F_{2,114} = 63.5$, $p < 0.001$ and Sex: $F_{1,114} = 16.7$, $p < 0.001$). However, the interaction between both factors was not significant ($F_{2,114} = 1.7$, $p = 0.193$) indicating the genetic differences among base stocks are sex independent. A post-hoc Tukey-test revealed that all three stocks differed significantly in mean bristle number (MB > HB > FB, Table 5.2A). Thus initiating the three subpopulations of a metapopulation each with flies from a different base stock is expected to create significant genetic differences among subpopulations.

In addition, we tested the effect of a temperature cline for the FB and MB base stocks and the results are shown in Figure 5.2 (right panel). We observe a clear difference in mean bristle number among stocks and sexes, confirming the findings at 25°C (Fig. 5.2, left). Using a 3-way ANOVA we tested the effect of the three main factors temperature, stock and sex on bristle number. We found that all three factors significantly affect mean bristle number: Temperature: $F_{2,108} = 50.7$, $p < 0.001$; Stock: $F_{1,108} = 338.7$, $p < 0.001$; and Sex: $F_{1,108} = 32.5$, $p < 0.001$. However, all possible interactions between the factors were observed to be non-significant at the 5% level (data not shown), except for the interaction between Stock and Temperature ($F_{2,108} = 7.48$, $p < 0.001$). The latter indicates that the MB and FB stocks differ significantly in their response to temperature. Conversely, the absence of other significant interactions involving sex shows that, although males and females differ significantly in mean bristle number, their plastic response to temperature does not differ essentially. Analysing FB and MB separately with a post-hoc Tukey-test revealed that for FB mean bristle number differed significantly for all temperatures (20°C > 25°C > 30°C, Table

5.2B) while for the MB stock significantly less bristles were observed at 30°C compared to the other two temperatures (20°C = 25°C > 30°C, Table 5.2C).

During this pilot, we observed that pre-adult mortality was considerable at the highest temperature (30°C). Therefore, we decided to use 29°C as the highest temperature instead of 30°C in the metapopulation experiment.

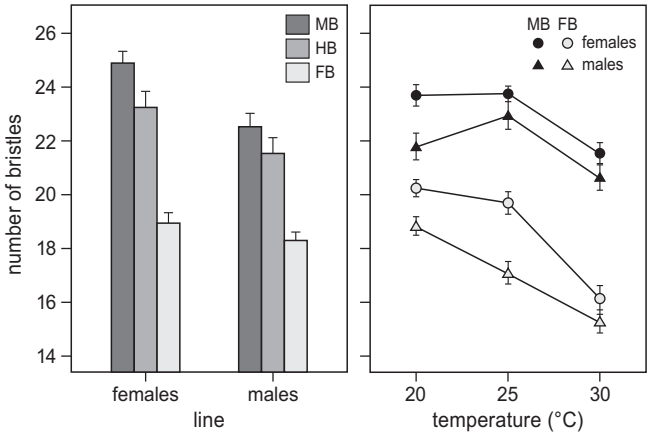


FIGURE 5.2 Mean bristle number (± s.e.) of the different base stocks at a common temperature (25°C, left) and the plastic response of the FB and MB stocks (right). Circles indicate females, triangles males.

TABLE 5.2 Results of the post-hoc Tukey tests for the different comparisons of the data presented in Figure 5.2. A: Comparison of the mean bristle number of the 3 stocks at 25°C. B and C: Comparisons of mean bristle number at the 3 temperatures for the FB and MB base stock, respectively.

	Comparison	Mean difference	*p*
A	MB25 - HB25	1.33	0.015
	MB25 - FB25	5.10	<0.001
	HB25 - FB25	3.78	<0.001
B	FB20 - FB25	1.15	0.018
	FB20 - FB30	3.85	<0.001
	FB25 - FB30	2.70	<0.001
C	MB20 - MB25	0.60	n.s.
	MB20 - MB30	1.65	0.001
	MB25 - MB30	2.25	<0.001

GENETIC AND ENVIRONMENTAL EFFECTS ON BRISTLE NUMBER AND DIFFERENTIATION WITHIN METAPOPULATIONS

Given the absence of significant interactions between sex and the other variables observed in the previous section, we decided not to discriminate between sexes for the metapopulation experiments. As such, we calculated mean bristle number and levels of differentiation by lumping the data for the 10 males and 10 females.

The consequences of the different treatments for the mean number of bristles of the different subpopulations are depicted in Figure 5.3, while Figure 5.4 presents the resulting levels of differentiation (either P_{ST}, Q_{ST} or E_{ST}) within metapopulations. The difference in mean bristle number among the three subpopulations due to the genetic differences among subpopulations alone (Q_{ST}) was assayed at the common temperature of 25°C (Fig. 5.3A). Even though there is some convergence in bristle number, most probably due to effective migration, the three subpopulations show consistent differences over the four generations. The observed differences between subpopulations agree well with the differences between the stocks observed at 25°C in the pilot experiment (Fig. 5.2, left). These differences result in Q_{ST}-values that fluctuate between 0.25 and 0.40. Averaged over the four generations, Q_{ST} was estimated to be 0.35 ± 0.03 (mean ± se).

The consequences of the temperature cline alone (E_{ST}) for mean bristle number was assayed for the MB-stock and FB-stock separately and the results are shown in Figures 5.3B and 5.3C, respectively. Clearly, temperature has a distinct effect on subpopulation mean

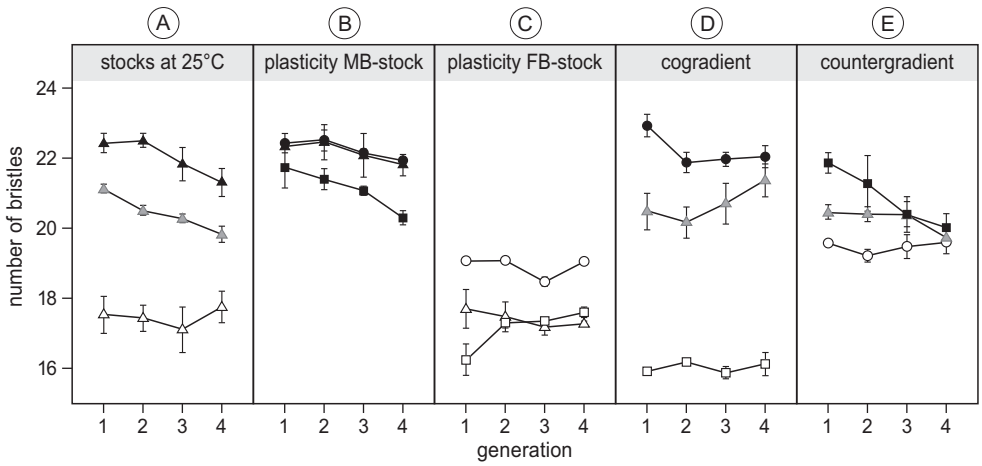


FIGURE 5.3 Mean bristle number of each of the three subpopulations for the different treatments, averaged over the replicate metapopulations. The bars represent the standard error of the replicate means. The different treatments are: A) genetically different subpopulations (FB, HB en MB stock) at 25°C, B) genetically uniform subpopulations (all flies from the MB stock) cultured at different temperatures, C) genetically uniform subpopulations (all flies from the FB stock) cultured at different temperatures, D) cogradient treatment and E) countergradient treatment. For further details see also Table 5.1. Black symbols indicate subpopulations initiated with flies from the MB-stock, the grey symbols those with HB-stock and the white symbols those with FB-stock. Breeding temperatures are: circles 20°C, triangles 25°C and squares 29°C.

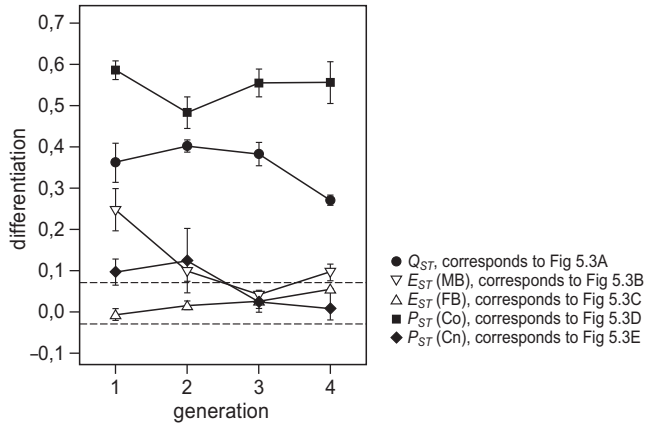


FIGURE 5.4 Mean level of differentiation for sternopleural bristles among the three subpopulations of metapopulations for the different treatments and generations based on the data shown in Figure 5.3. Means are based on either two (E_{ST} and Q_{ST} estimates) or four (P_{ST} estimates) replicate metapopulations. For configuration of the different metapopulations see Table 5.1.

bristle number for both stocks. Interestingly, the effect of temperature seems to differ between the stocks: Whereas for the MB stock it is particularly 29°C that seems to affect bristle number differently, it is 20°C that affects bristle number for the FB stock. Notwithstanding, the differences in bristle number caused by the temperature cline are considerably smaller among subpopulations than those due to genetic differences. This is clearly reflected in the level of differentiation (E_{ST}) for both stocks (Fig. 5.4). Averaged over the four generations, E_{ST} was estimated to be 0.02 ± 0.02 and 0.12 ± 0.09 for the MB and FB stock, respectively.

When genetic and environmental differences both are present, the results depend greatly on how the two factors clinally co-vary. In the cogradient alignment the two factors act synergistically as the FB-subpopulation is experiencing a temperature (29°C) that decreases the number of bristles, while the MB-subpopulation is placed at a temperature (20°C) that increases bristle number. As a result, the difference in mean bristle number among populations increases substantially (Fig. 5.3D), particularly because the FB-subpopulation shows a decreased number of bristles in this situation compared to the situation where all stocks were tested at 25°C (compare Fig. 5.3D with 5.3A). Consequently, the amount of phenotypic differentiation (P_{ST}) is high (averaged over the four generation $P_{ST}(Co)$ = 0.54 ± 0.02) and is significantly higher than the observed Q_{ST} (Fig. 5.4). In the countergradient configuration the factors act antagonistically; the FB-subpopulation is placed in an environment that increases bristle number, whereas the opposite is true for the MB-subpopulation. As a consequence the differences in mean bristle number among subpopulations decreases strongly (Fig. 5.3E). Consequently, the level of phenotypic differentiation at the metapopulation level is decreased (Fig. 5.4) and is not much different from zero despite the underlying genetic differences among subpopulations (averaged over the four generations P_{ST}(Cn) = 0.06 ± 0.06).

In the foregoing we observed that the effect of the temperature cline on phenotypic differentiation alone was quite moderate (E_{ST} being 0.02 and 0.12 for MB and FB, respectively). However, the effect of temperature seems to be much more pronounced when combined with genetic differences in the cogradient and countergradient treatment as P_{ST} and Q_{ST} on average differ considerably: P_{ST} minus Q_{ST} was found to be 0.19 ± 0.04 for the cogradient and –0.29 ± 0.02 for the countergradient configuration. To verify this observation, the parents of the 4th generation of the both configurations produced two batches of offspring to allow a direct comparison between P_{ST} and Q_{ST}. One of the batches was cultured in a clinal environment (either cogradient or countergradient) yielding estimates for P_{ST} (Table 5.1, D and E, respectively), while for the other batch the three subpopulations were cultured at a common temperature of 25°C and thus estimating Q_{ST} (Table 5.1, $D_{Control}$ and $E_{Control}$). The results are shown in Figure 5.5. The Q_{ST} estimates were found to be remarkably similar on average, independent of whether the estimate was obtained for the one or the other clinal configuration (Q_{ST} = 0.242 ± 0.09 and Q_{ST} = 0.236 ± 0.10 for the cogradient and countergradient treatment, respectively). However, the variation among the four replicate metapopulations is considerable. This is most likely due to differences in effective migration rate among metapopulations (not all migrant females successfully leave offspring in the next generation) in combination with genetic drift due to the restrict population size. Compared to these Q_{ST}-estimates the cogradient treatment more than doubles the phenotypic differentiation among the subpopulations (averaged over the four replicates P_{ST} = 0.56 ± 0.05). Given the large variation among replicates for Q_{ST} the difference between the average Q_{ST} and P_{ST} was only just significant (one-sided paired t-test; t = 2.45. p = 0.046). On the other hand, the countergradient treatment decreases the phenotypic differentiation greatly and becomes near to zero (P_{ST} = 0.01 ± 0.00 on average). In this case the difference between the average Q_{ST} and P_{ST} was at the border of significance (one-sided paired t-test; t = 2.30. p = 0.052). Again the difference between Q_{ST} and P_{ST} for both treatments was on average much larger than the estimates for E_{ST} for the MB and FB stocks in the fourth generation (see Fig. 5.4). These results confirm the observation in the previous section. It seems therefore warranted to conclude that the impact of the environmental differences becomes much more pronounced due to the clinal gradients.

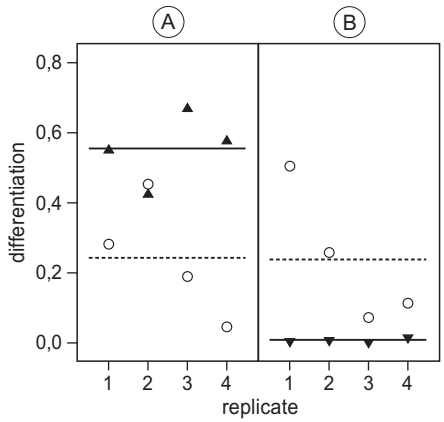


FIGURE 5.5 Direct comparison of total amount of phenotypic differentiation among subpopulations in generation 4 (P_{ST}, black symbols) and the amount of genetic differentiation (Q_{ST}, open symbols) in the same generation for the cogradient (left) and the countergradient (right) treatments. The panels show the level of differentiation for each of the four replicate metapopulations per treatment. The horizontal lines indicates the average over the four replicates per panel for P_{ST} (solid lines) and Q_{ST} (broken lines).

DISCUSSION

It has since long been recognised that for many morphological and fitness traits the phenotypes of species change in a regular and predictable way along environmental gradients, like latitudinal, altitudinal or temperature. Whether such changes reflect genetic variation or/and phenotypic plasticity is still an important ecological and evolutionary question (Endler 1986, Conover *et al.* 2009, and references therein). We used such clinal configurations to investigate the interplay between genetic variation and phenotypic plasticity and the consequences this had for the phenotypic differentiation among populations and for Q_{ST} estimations in an experimental setting for the morphological trait "sternopleural bristle number" in *Drosophila*. To this end, we established simple metapopulations (3 subpopulations each) for which the two factors genetic variation and environmental temperature covaried either positively (cogradient variation) or negatively (countergradient variation). The amount of phenotypic variation was compared to situations with only one of these two factors present.

Our results are quite explicit. Notwithstanding the fact that temperature significantly affected the average number of bristles (Fig. 5.2, right panel; Fig. 5.3B,C), its impact on phenotypic differentiation among subpopulations was quite small and non-significant when it is the only force present (Fig. 5.4, E_{ST}). This is most probably due to the fact that our estimates are based on a mixture of the two sexes that differed in mean bristle number and, as a result, inflates the variation within lines and subpopulations. In contrast to temperature, genetic variation alone did cause highly significant phenotypic difference among subpopulations (Fig. 5.4, Q_{ST}).

However, when genetic variation and temperature co-vary, we observe a strong interaction between both factors. In the cogradient alignment, we find that the phenotypic differentiation among subpopulations increases more than 50 to 100% compared to the situation with only genetic differences present, indicating that in the cogradient alignment both factors do act highly synergistic. Conversely, in the countergradient alignment the phenotypic differentiation drops to almost zero, showing that in this situation both factors act highly antagonistic. Consequently, despite the presence of highly significant genetic differences for the trait among subpopulations, the phenotypic differentiation among these is found to be near zero. This signifies that when genetic and environmental variation covary, either positively or negatively, the phenotypic differences observed among populations under natural conditions (P_{ST}) cannot serve as a proxy for Q_{ST}. Because cogradient and countergradient variation are observed frequently in nature, particularly for traits related to development, growth, metabolic rate, etc. (Conover *et al.* 2009), this will limit the usefulness of the comparison of P_{ST} (as a substitute for Q_{ST}) and F_{ST} to infer the presence and role of (adaptive) selection in shaping phenotypic and genetic differences among populations. As such our experimental results confirm the findings and conclusions of Sæther *et al.* (2007) and Pujol *et al.* (2008).

The situation in nature can be even more complex. As stated by Roff (1997, p196, when citing Travis (1994)) "nearly every conceivable trait is known to respond to almost every conceivable environmental factor". This means that many traits may respond plastic to

several environmental factor at the same time (see Roff, 1997, Chapter 6). For instance, sternopleural bristle number not only is affected by environmental temperature, but also by other environmental variables. Although we aimed at keeping other environmental variables during the experiment constant, relative humidity still varied among the different temperature regimes. Humidity has been shown to affect body size and through this possibly bristle number (Coyne and Beecham 1987). Especially in the first generation, we observed that the relative humidity was around 70%, 55% and 45% for 20°C, 25°C and 29°C, respectively, while in the other generations the differences were considerably smaller (data not shown). To what extent this may have influenced our data is unclear, although it might explain the somewhat deviating values for the FB-line at 29°C in Figure 5.3C and the increased E_{ST} (Fig. 5.4) for this line in generation 1.

All in all, our results show that the total phenotypic differentiation (P_{ST}) can be rarely used an indicator for the level of genetic differentiation (Q_{ST}). How then can we estimate Q_{ST} properly? Several approaches have been proposed to disentangle the genetic and environmental contribution to phenotype of a trait more directly (see among others Conover *et al.* 2009, Hansen *et al.* 2012). An often applied approach is to assess the phenotypic differences among populations in a common garden experiment, thus assaying all populations under the same environmental conditions. Assuming that the differences in trait value then observed are due to genetic differences only, this is supposed to provide a good estimate of Q_{ST} (Sæther *et al.* 2007, Edelaar *et al.* 2011). In fact, this is the method we used in our experiment to obtain an estimate for Q_{ST}. However, this method may be less reliable when different genotypes respond differently to the various environmental conditions, in other words when there exists a significant genotype-by-environment interaction (Lynch and Walsh 1998, Conover *et al.* 2009, Rogell *et al.* 2013). In our experiment we found that the two base stocks (FB and MB) responded differently to the applied temperature cline as evidenced by the significant interaction we observed between Stock and Temperature (Fig. 5.2, right panel). Consequently, we might have obtained (slightly) different estimates for Q_{ST} when we would have used 20°C or 29°C as the common garden temperature instead of 25°C to estimate the genetic differences between the three subpopulations of a metapopulation. Such a situation can be mediated by a reciprocal transplant experiment, whereby the phenotypic differences among (sub)populations are assessed under all relevant environmental conditions and the phenotypic variances is partitioned over all the variance components (Lynch and Walsh 1998, Conover *et al.* 2009). Due to logistic limitations this was not possible in our experiment. However, we are confident that our results would have qualitatively been the same had we used this latter approach. Another possible approach would be using molecular markers, such as SNP's, to link existing genetic differences among populations to the observed phenotypic differences. This can provide an estimate of which part of the phenotypic differences is due to underlying genetic differences. Vasemägi and Primmer (2005) reviewed several possible methods to do so. However, the disadvantage of these molecular techniques is that it is often difficult to unambiguously prove that the genetic variation observed is linked to the phenotype (Vasemägi and Primmer 2005).

In conclusion, our experiment clearly demonstrates that covariance between genetic and environmental influences significantly affects the level of phenotypic differentiation

(P_{ST}) observed among populations. Depending on the alignment of the two factors, they do act either strongly synergistic (Cogradient situation) or strongly antagonistic (Countergradient situation), thereby affecting trait means much more strongly than both factors would do in isolation. Consequently, the level of observed phenotypic differentiation observed under natural conditions can not be indicative for the amount of genetic differentiation (Q_{ST}) present among population in those situations. Separating the genetic and environmental effects is important as it bears significantly on the extent populations are locally adapted, and to their potential to adapt to changing environmental conditions either through evolutionary change or through phenotypic plasticity (Bijlsma and Loeschcke 2012, Hansen *et al.* 2012). This is highly relevant for issues related to global climate change and to conservation biology.

CHAPTER 6

Quantifying outcrossing probabilities of genetically modified plants

DEVELOPMENT OF A PREDICTIVE MODEL

M. W. SMITH-KLEEFSMAN
F. J. WEISSING
R. BIJLSMA

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Extended Abstract

Description of the problem

As an advisory body of the Dutch Ministry of Spatial Planning, Housing and the Environment (VROM), the Netherlands Commission on Genetic Modification (COGEM) has to evaluate, among other things, requests for permission to cultivate genetically modified (GM) crops in the natural environment. Many of these crops are able to cross with wild populations of the same species or one that is closely related. To reduce outcrossing probabilities, the modified crops are grown some distance away from possible recipient populations. The COGEM aims to develop a mathematical model that estimates outcrossing probabilities of GM crops with recipient populations in relation to the separation distance, to arrive at scientifically motivated and justified procedural rules. This literature study evaluates which components should be included in a mathematical model that estimates outcrossing probabilities and discusses possible modes to develop such model. In this report, we only focus on dispersal and outcrossing by means of pollen.

Literature survey

The process that leads to outcrossing can be divided into three steps. *(A)* First, viable pollen must reach the stigma of a compatible specimen, which is usually, but not necessarily of the same species as the GM crop. The main processes and mechanisms that affect pollen dispersal are considered. *(i)* Self-fertilising species can be expected to lack specific (long-distance) dispersal mechanisms. Most self-fertilising species, however, show appreciable levels of outcrossing, but they are expected to have lower chances of outcrossing than typically outcrossing species, since pollen of the former species, being at low concentration in target populations, has to compete with much selfing pollen. Therefore, differences between outcrossers and selfers should be a component of the model. *(ii)* Pollen grains are not able to disperse themselves actively, but need dispersal vectors. In our biogeographic region, insects and wind seem to be the predominant dispersal vectors. Pollen flow by insects as well as by wind can be highly variable among species, among plant populations and over time, depending on the weather, on population characteristics and on the environment. Distances travelled by pollen of wind-pollinated species are generally larger than the distances travelled by insect-dispersed pollen, but the shape of wind and insect pollination curves relating pollination probability (or relative pollen density) to dispersal distance is generally highly similar. A large fraction of the

pollen lands close to the donor plant and only a small fraction disperses further, some of which may travel large distances. From a modelling perspective, this similarity between the dispersal curves for wind-and insect-dispersed pollen has the great advantage that the same mathematical approach can be used for both. *(iii)* During the dispersal process, a certain percentage of the dispersing pollen will have lost viability. These pollen do not contribute to the fertilisation process. It is questionable whether, on the basis of current knowledge, pollen viability rates of crops growing in the field can be predicted.

(B) After landing on a suitable stigma, the pollen has to succeed in fertilising an ovule. Therefore, it has to compete with other pollen that has landed on the same stigma. The competitive ability of GM pollen may be different from that of pollen from the target population. Some important mechanisms that influence fertilisation success are reduced viability, time of arrival, the exact place where the pollen lands and the presence of self-incompatibility mechanisms. Since fertilisation is very complicated process that is affected by many mechanisms, the process needs to be incorporated in the model in a simplified mode to be workable.

(C) When hybrid seed is developed successfully, the modified DNA of the GM species must be incorporated into the gene pool of the receiving species (introgression). Whether this will happen, is in the beginning mainly dependent on stochastic processes that determine whether the modified DNA will establish in the receiving population. Later on, when the modified DNA is established in sufficient numbers of plants to render stochastic effects negligible, deterministic processes will determine the persistence of the modified DNA. Introgression is a very complicated process on which little information is available; therefore, modelling of this part is not considered at present.

Modelling approach

An overview of the proposed mathematical model that estimates probabilities of pollen to land in populations of compatible species and to achieve fertilisation in such population is given in Figure 6.4. This proposed model consists of three modules, but only the first two are programmed at present.

(A) The first module addresses the question: how does pollen, originating from a GM source population, disperse over the landscape? In this module, pollen dispersal of a source population is simulated. Different equations are pre-programmed that can be used

to describe the dispersal pattern of a species. These are a negative exponential equation, an inverse power law, and a uniform distribution. The user can also choose to estimate the dispersal process using two equations, one describing the first part of the curve (i.e. describing the dispersal pattern of the pollen that lands close to the dispersing individual), the other one describing the tail of the curve (i.e. describing the dispersal pattern of the pollen that travels farther away). If the dispersal pattern is described by another known curve than the ones that are pre-programmed, the user can enter this equation using the custom function. In this module, data on pollen viability can be added; pollen survival can be described by a negative cumulative normal distribution, or by an equation added by the user.

(B) The second module addresses the question: what is the expected frequency of seeds in a target population that is fertilised by pollen originating from a given GM source population? In this module, the percentage of seeds that is the result of a cross between pollen from the source population and ovules from the target populations is calculated, thus giving an estimation of the contamination level of the target population with DNA from the GM source population. Relative competitive ability of the pollen is included in this module.

Conclusions

The model will be helpful for estimating the separation distances required to reduce contamination levels with modified DNA to acceptably low levels. The outcome of the model will largely depend on the parameter values entered by the user. The user should be aware of two types of uncertainty associated with this. One type of uncertainty is whether the parameter values used have been estimated correctly. The other uncertainty is caused by variation in parameter values due to stochastic processes. The model, however, only handles 'standard' situations: it does not reckon with fluctuations in time and/or space. The process of pollen dispersal, however, appeared to be highly variable.

We recommend that in the future the COGEM aims to extend and refine the present model, for example *(i)* by including more complex situations, like estimation of gene flow at landscape level, and/or *(ii)* by including stochasticity, allowing to estimate possible deviation from the contamination levels found. By adding modules and functions like these, the procedure to estimate outcrossing probabilities of GM populations with cultivated or wild relatives will be continually improved.

1. INTRODUCTION

1.1 DESCRIPTION THE PROBLEM

As an advisory body of the Dutch Ministry of Spatial Planning, Housing and the Environment (VROM), the Netherlands Commission on Genetic Modification (COGEM) has to evaluate, among other things, requests for permission to cultivate genetically modified[1] (GM) crops in the natural environment. By means of an environmental risk analysis (Box 1), the COGEM estimates the risk involved in cultivating GM plants. This risk can be defined as a function of the effect of cultivating GM plants and the likelihood of the effect, i.e. the likelihood to outcross with a wild or cultivated relative. Many GM crops, as well as non-modified crops, do cross with wild populations of the same species or one that is closely related (for a review see Groot *et al.* 2003). This is not surprising, since crops were developed from wild species only a few thousand generations ago and are therefore expected to have relatively high genetic similarity with their wild relatives (Ellstrand *et al.* 1999). Hybridisation is possibly even more likely to occur between two crop species. Groot *et al.* (2003) conclude that crop-to-wild and crop-to-crop gene flow can be expected in nearly all cultivated crops that are grown in their reproductive phase.

To reduce outcrossing probabilities, the modified crops are grown some distance away from possible recipient populations. At present, the choice of such distances is largely based on an ad-hoc procedure, simply doubling the separation distances advised by the Dutch General Inspection Service[2]. To arrive at scientifically motivated and justified procedural rules, the COGEM aims to develop a mathematical model that estimates outcrossing probabilities of GM crops with recipient populations in relation to the separation distance. Such model would clearly not constitute a complete ERA, since a full analysis should include estimation of the impact on the environment of a potential outcrossing event as well as the probability it occurs. This literature study evaluates which components should be included in a mathematical model that estimates outcrossing probabilities and discusses possible modes to develop such model.

1.2 CHAPTER OUTLINE

The process that leads to outcrossing can be divided into three steps (Fig. 6.1). (A) First, viable pollen must reach the stigma of a compatible specimen[3] (Section 2). Two types of potential recipient species can be distinguished, namely non-modified crops cultivated by

[1] Genetic modification can be defined as any change in the genetic constitution of a living organism (here plants) that has been brought about by joining together *in vitro* genes from different sources or genes that have in some way been modified *in vitro*. Genetic engineering and recombinant DNA techniques are synonymous with genetic modification.

[2] The separation distances used by the Dutch General Inspection Service (NAK) are based on reducing gene flow into crops grown for seed production, to keep contamination within the quality demands of the Inspection Service.

[3] This compatible specimen is usually, but not necessarily of the same species as the GM crop.

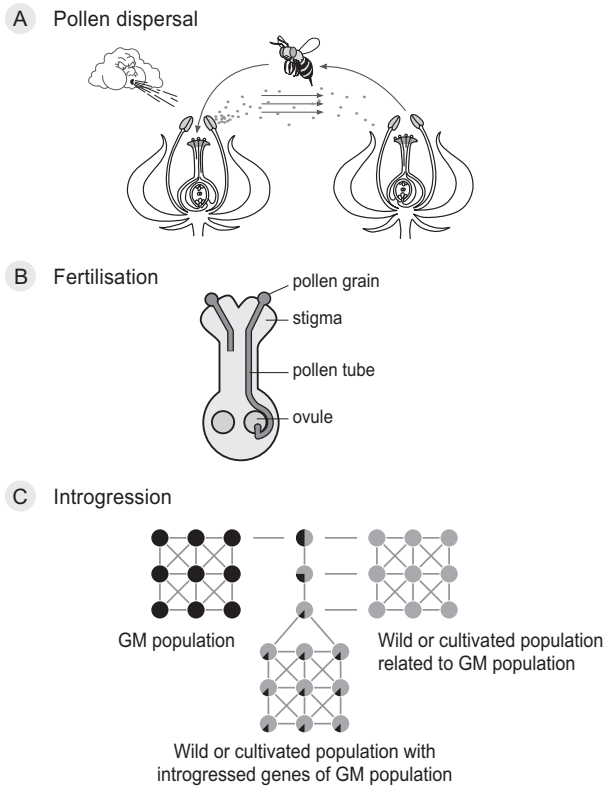

FIGURE 6.1 Schematic depiction of the outcrossing process, which can be divided into three distinct parts (A, B, C). A. Viable pollen grains must disperse over the distance between the GM population and related wild or cultivated populations. B. After reaching such population, the pollen must fertilise an ovule, resulting in hybrid seed. C. The modified DNA present in the hybrid must establish itself and persist (i.e. introgress) in the genome of the receiving population.

farmers and related wild populations. Both will be discussed in this report. The ability to arrive at a suitable stigma depends on species- specific characteristics concerning dispersal. In this report, we only focus on dispersal and outcrossing by means of pollen, since *(i)* pollen exchange, unlike seed exchange, directly results in genetic exchange between the populations involved, and *(ii)* farmers cultivating non-modified crops, especially organic farmers, are interested in contamination levels of their seeds, which is a direct result of pollen exchange. (B) After landing on a suitable stigma, the pollen has to succeed in fertilising an ovule (Section 3). Therefore, it has to compete with other pollen that has landed on the same stigma.

(C) When hybrid seed is developed successfully, the modified DNA of the GM species must be incorporated into the gene pool of the receiving species (introgression, Section 4). Whether this will happen, is firstly dependent on stochastic processes that determine whether the modified DNA will establish in the receiving population or not. Later on, when the modified DNA is established in sufficient numbers of plants to render stochastic effects

Box 1: Environmental risk analysis

At present, the COGEM applies a precautionary principle to assess requests for permission to cultivate genetically modified crops. Which precautions are taken is mainly based upon the amount of information available. When little information is present, only small field experiments may be carried out. When additional information is available about effects on the environment and the transferred elements, larger experiments are permitted, but only when the effects on the environment are expected not to be deleterious. In Table B1.1, the information requirements for different classes of field experiments are given.

If the environmental risk analysis shows that the deliberate release may result in adverse effects, either the request will be refused or, more commonly, risk-management measures will be imposed by demanding (extra) constraints. Mostly, the second option is chosen, which is called risk management. Risk management cannot exclude all possible risks, but it aims to minimise them. One risk management option is to enforce separation distances or increase those that have already been proposed. From the point of view of environmental safety, it is important to know the effectiveness of such a regulation. A mathematical model may give better insight into whether, at the required separation distance, outcrossing probabilities are sufficiently reduced, and may indicate whether adjustments are required.

Table B1.1 Guidelines used by the COGEM for the evaluation of requests concerning field experiments with GM plants. Five different classes are distinguished (COGEM 1999).

Class	Size of field	Max. nr of locations	Spread	Information requirements
1	1 ha per location	1	- Prevented by removal of inflorescence or doubling of the NAK separation distance (see footnote). - The effects of the genetic modification are properly monitored by observing the experimental field(s).	- genetic element involved - donor involved - suspected function(s) of the genetic element after expression
2	1 ha per location	5	As class 1	As class 1 + possible effects of the expression based on former experiments
3	total of 5 ha	10	- No prevention. The genetically modified organisms are kept separated in the field. - The effects of the genetic modification are properly monitored by observing the experimental field(s).	As class 2 + no reasons to suspect deleterious effects of the genetically modified organisms, its offspring or after transfer to other organisms
4	total of 10 ha	10	As class 3	As class 3 + the map of constructs used for the modification, showing the combinations of (regulation) sequences that are expressed and showing other selection elements
5	no max.	no max.	As class 3	As class 4 + - molecular characteristics of the transferred elements - complete performed assessment concerning the safety for the environment, public health and animal feed

negligible, deterministic processes will determine the persistence of the modified DNA. Different approaches can be taken to simulate the outcrossing process (Section 5). In this report we will present what we believe is the best way of modelling the pollen dispersal and fertilisation processes, taking into account the aim for which such a model would be developed; that is, for the use of the COGEM to estimate outcrossing probabilities of GM plants in relation to separation distances. In the end, a description of the model we will propose is given. Introgression is a very complicated process on which little information is available; therefore we will not consider modelling of this part.

2. POLLEN DISPERSAL

2.1 INTRODUCTION

Before a pollen grain has any chance of fertilising an ovule of a compatible species, it must land on the stigma of this species (Fig. 6.1A). In this section, we will consider processes and mechanisms that affect pollen dispersal, thereby affecting the chance of pollen reaching recipient populations growing at different distances. Self-fertilising species can be expected to lack specific (long-distance) dispersal mechanisms. These species will be compared with typically outcrossing species. The two most important pollination vectors, insects and wind, will be considered. During the time between release of the pollen from the GM plant and deposition on a wild or cultivated relative, the pollen may have lost viability, making the pollen grain unable to fertilise. Gene-flow barriers are intended to decrease pollen flow out of GM crops. We will briefly review different types of gene-flow barriers.

2.2 SELF-FERTILISING VERSUS OUTCROSSING

Plant species that are entirely self-fertilising are expected to lack mechanisms that promote pollen dispersal. Most self-fertilising species, however, show appreciable levels of outcrossing, especially animal-pollinated species. Vogler and Kalisz (2001) found most wind-pollinated species to be either highly selfing or highly outcrossing, with intermediate outcrossing rates rare but present. In contrast, animal-pollinated species exhibited a bimodal, but more continuous, distribution of estimates of outcrossing rates (Fig. 6.2). A factor contributing to this difference could be that the presence of wind is relatively constant in natural populations, whereas animal abundance and visitation rates are highly variable. Although most so-called selfers do show low levels of outcrossing, gene flow between different populations is low. Wagner and Allard (1991) estimated gene flow by pollen in barley (*Hordeum vulgare*), a predominantly selfing species with an outcrossing rate of about one percent, by counting the number of 'hybrids' formed (i.e. seeds of crosses between two homozygous parental plants having alternative genotypes). They found two hybrids, one from parents 7 m apart and one from parents 60 m apart. Certainly, more pollen travelled over these distances than only that which resulted in hybrids, but the indication is that pollen flow is low. Golenberg (1987) found that gene flow by pollen in wild

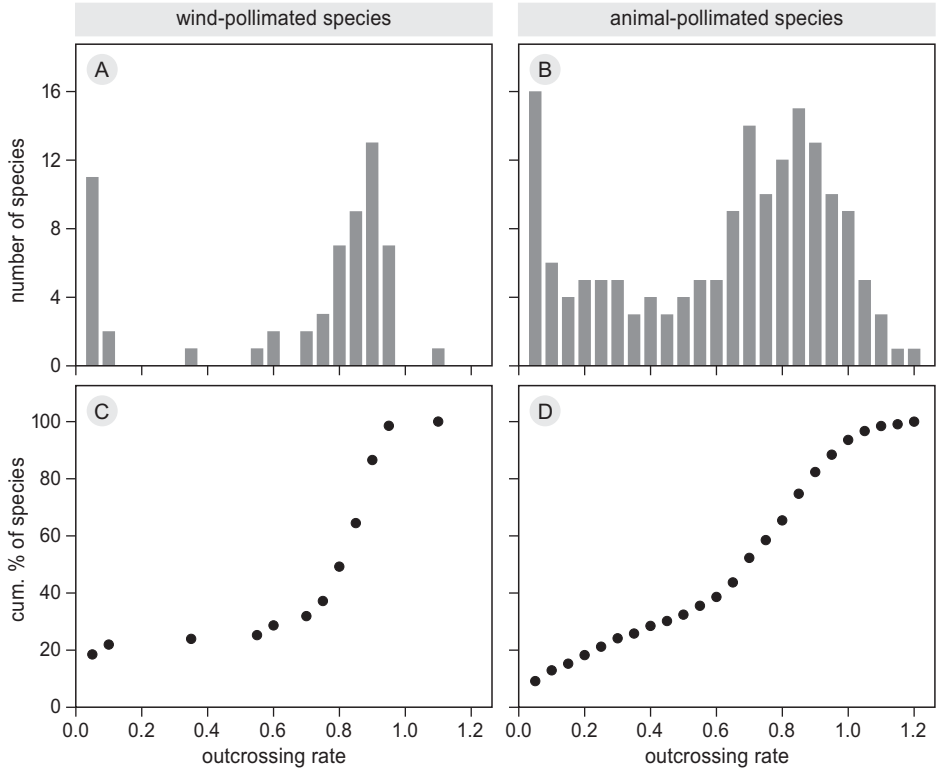

FIGURE 6.2 Distribution of outcrossing rate estimates for wind- (A, C, n = 59) and animal- (B, D, n = 169) pollinated species expressed as number of species (A, B) and cumulative percentage of species (C, D). Outcrossing rates are defined as the proportion of seed produced through outcrossing. Some of the estimated outcrossing rates are higher than one. This is a consequence of the estimation procedure. After Volger and Kalisz (2001).

emmer wheat (*Triticum dicoccoides*, outcrossing rates about 0.5 per cent) to be limited to distances up to 15 m. Other indications of low levels of gene flow can be found looking at the genetic differentiation between subpopulations (Box 2). Berge *et al.* (1998) found high levels of genetic differentiation between, and high levels of inbreeding within, subpopulations of *Arabis thaliana*, a highly selfing species, suggesting low levels of gene flow. This gene flow was accomplished by pollen and seed exchange. Therefore, pollen is expected to have contributed only partly to this already low differentiation.

Although highly selfing species cannot be excluded from our model, differences between outcrossers and selfers should be taken into account, as mainly self-fertilising and mixed-mating species are expected to have lower chances of outcrossing than typically outcrossing species, since pollen of the former species, being at low concentration in target populations, has to compete with much selfing pollen.

Unlike selfers, obligate outcrossing species are not able to fertilise themselves. Especially many animal-pollinated species are obligate outcrossers (Fig. 6.2). Pollen of

these species landing on an own stigma are not competing with outcrossing pollen landing on the same stigma for achieving fertilisation. The main mechanism of obligate outcrossing species to prevent fertilisation is self-incompatibility, which will be discussed in Section 3.2.

BOX 2: GENETIC DIFFERENTIATION

F-statistics are commonly used to measure genetic differentiation, in which F_{ST} is the measure of differentiation between populations. Specifically, F_{ST} is the correlation between random gametes within each subpopulation relative to the gametes of all subpopulations together (Sork *et al.* 1999). It is calculated as follows:

$$F_{ST} = \frac{H_T - H_S}{H_T}$$

where H_S is the expected heterozygosity of an individual per subpopulation, averaged over all subpopulations, and H_T is the expected heterozygosity of an individual in the total population (all subpopulations together). When $F_{ST} = 0$, there is no genetic differentiation, the allele frequencies in all subpopulations are equal, indicating high gene flow among populations. When $F_{ST} = 1$, all subpopulations are fixed for different alleles.

2.3 DISPERSAL VECTORS OF POLLEN

Pollen grains are not able to disperse themselves actively, but need dispersal vectors. These can be biotic, like insects, birds and mammals, or abiotic, like wind and water (Meeuse 1961, Holm 1978). Probably over 90 per cent of the angiosperms is pollinated by animals, and by far the greater part of this by insects (Wilcock and Neiland 2002), while 30 out of 300 plant families contain species that show adaptations for pollen dispersal by wind, for example grasses, sedges and rushes (Knox 1979). In our biogeographic region, insects and wind seem to be the predominant dispersal vectors. In this report we will therefore concentrate on pollen dispersal by insects and wind.

2.3.1 Dispersal by insects

Pollen dispersal by insects is strongly dependent on ecological factors influencing the behaviour and occurrence of insects (Richards *et al.* 1999, Velterop 2000). Characteristics of the pollinating species determine pollen flow patterns and pollination efficiency. Hymenoptera, for example, are characterised by short flights, large pollen loads and high efficiency, while Lepidoptera are characterised by long flights, small loads and medium to low efficiency (Herrera 1987). Insects visiting flowers of the same species without visiting other species are more efficient than those that switch frequently between plant species, since the former deposit higher amounts of conspecific pollen (Velterop 2000). Some insect species visit exclusively certain plant groups (specialists), but most species use a broad

range of different plant species (generalists). Even a generalist species may be able to deposit a large amount of conspecific pollen on plant stigmas, since different individuals may still specialise on only a few or a single species (flower constancy, Chittka *et al.* 1999). Flower constancy is known for several bee species, butterflies and hoverflies (e.g. Waser 1986, Goulson *et al.* 1997, Goulson and Wright 1998).

An important characteristic of insects is their flight distance, which gives an indication of the distance over which pollen flow occurs (Velterop 2000). Butterflies, for example, fly on average larger distances between subsequent flower visits than bumblebees, which fly between neighbouring flowers because of their high energy demands (Handel 1983, Herrera 1987). However, although most pollinators generally keep the flight distance low, many are capable of flying much longer distances. For honeybees was found that they forage till about 10 km from the hive, although especially in agricultural areas, a foraging radius of only a few hundred metres was found (Beekman and Ratnieks 2000). Furthermore, honeybees leaving the hive sometimes carry viable pollen that has remained on the body from an earlier flight trip, so a honeybee may be able to cross-pollinate plants more widely separated than it could visit in a single foraging trip. Another possibility is that pollen may be transferred from one honeybee to another in the hive. There seems to be no reason why some of this pollen should not also be viable (Free and Williams 1972).

Plant population parameters such as size, density and isolation may affect pollen dispersal by insects, because it affects pollinator behaviour. Pollinators forage in such a way that the nectar gain per flower is independent of plant size and the rate of nectar production per flower (Dreisig 1995). Therefore, population size and distance between populations interact with each other, resulting in different patterns of gene flow among populations that are adjacent to each other or far apart (Richards *et al.* 1999). For wild radish (*Raphanus savitus* L.), it has been shown that large populations at larger distances contributed more to pollen import than small populations nearby (Ellstrand *et al.* 1989). In populations with low density, migrant pollen generally constitutes a higher relative fraction of the total pollen amount than in populations with high density (Handel 1983, Richards *et al.* 1999). Other ecological parameters, such as population shape, presence or absence of alternative hosts for the pollinators, plant biomass and rates of flower production, will be of influence as well (Ellstrand *et al.* 1989).

To summarise, pollen flow by insects can be highly variable among species, among plant populations and over time, but the dispersal distances are generally not very high, ranging from 100 to 200 m for short- to medium-distance dispersal, to approximately 10 km for long-distance dispersal. This infrequent long-distance dispersal is of importance, however. For many crops, it's not known how far its pollen is able to spread. Table 6.1 gives large dispersal distances of two important insect-pollinated crop species (oilseed rape and potato).

2.3.2 Dispersal by wind

Pollen dispersal by wind is dependent on falling velocity and releasing height of the pollen and on wind characteristics. Not only horizontal speed and direction of the wind are of

importance, but also turbulence. Two kinds of turbulence can be distinguished (Tackenberg 2003). *(i)* Mechanistic turbulence with high horizontal wind speed. This dominates in stormy weather and is associated with a mean downdraft (i.e. downward air current), although updrafts (i.e. upward air currents) are present. *(ii)* Thermal turbulence. This is caused by an increase in air temperature and is therefore associated with sunny weather. In this case, updraft dominates downdraft. Updrafts are particularly important for seed dispersal, because they lift seeds high in the air, which can explain long-distance dispersal. Therefore, contrary to popular belief, it is not stormy weather but sunny weather that causes seeds to travel over large distances. It is highly likely that the same is true for pollen dispersal.

Wind-dispersed pollen is usually small and light, resulting in low falling velocity and thus promoting pollen dispersal. In most alders, hazels and junipers, average pollen size is about 30 μm. Pollen from wind-pollinated plants, especially heavier ones, often has additional characteristics that support pollen dispersal, such as air sacs or a modified shape (Meeuse 1961).

Pollen grains have very low chances of landing on a compatible stigma, because they land in an arbitrary place. A large amount of pollen is therefore needed to ensure reasonable pollination success. Although some species, like wild oat and brome, release relatively few pollen grains (less than a thousand per spike, Knox 1979), most wind-pollinated species produce vast numbers. For example, the amount of pollen of one catkin can be higher than two million (Meeuse 1961), and some grasses, like ryegrass, cocksfoot, and canary grass, release between two and five million pollen grains from one spike (Knox 1979). To keep wastage of pollen to a minimum, most wind-pollinated species only release their pollen when conditions are favourable, for example in dry weather, and they do not release all of it at once (Meeuse 1961).

Pollination rate by wind depends not only on the characteristics of the pollen and the wind, but also on population size and density (Handel 1983). Raynor *et al.* (1971, 1972) found for ragweed, timothy and maize that relative pollen concentration in the air from a small source decreased more rapidly with distance than from larger sources. For pollen

TABLE 6.1 Overview of the the most important pollination vectors and the largest measured pollen dispersal distance of four important crop species. Notice that for all four species, no measurements at further distances are made than the distances mentioned in the table. Therefore, it is very likely that pollen have travelled further than these distance. Reviewed by Treu and Emberlin (2000).

Species	Main pollination vector	Largest measured distance
Oilseed rape (*Brassica napus*)	Insects	4000 m by insects 3000 m by wind
Potato (*Solanum tuberosum*)	Insects	1000 m
Sugar beet (*Beta vulgaris*)	Wind	800 m
Maize (*Zea mays*)	Wind	800 m

dispersal by wind, topography is of importance too. For example, when a population is growing on a slope, a gust can carry it away from the hillside and thereby increase the releasing height.

To summarise, pollen dispersal by wind can be highly variable, not only depending on the weather, but also on population characteristics. Pollen dispersed by wind is able to travel over large distances (up to over 100 km). Watrud *et al.* (2004) found creeping bentgrass (*Agrostis stolonifera*) to outcross over a maximum distance of 21 km, although most gene flow by pollen occurred within 2 km. Tyldesley (1973) even found pollen of different tree species (e.g. birch, pine, juniper and larch) to travel for at least 250 km over sea. Distances travelled by pollen of wind-pollinated species are generally larger than the distances travelled by insect-dispersed pollen, but the number travelling over such large distances is very small. Table 6.1 gives large dispersal distances of two important wind-pollinated crop species (sugar beet and maize). For many crop species, however, it's not known how far its pollen is able to spread.

2.3.3 Comparing wind and insects as dispersal vectors

Dispersal mechanisms by wind and insects differ considerably (Table 6.2). Despite all these differences, the shape of wind and insect pollination curves relating pollination probability (or pollen frequency) to dispersal distance is generally very much the same, although the distances involved may differ (Fig. 6.3, Bateman 1947c). A large fraction of the pollen lands close to the plant and only a small fraction disperses further, some of which may travel large distances (e.g. Bateman 1947ab, Paterniani and Stort 1974, Klinger *et al.* 1992, Arias and Rieseberg 1994, Lavigne *et al.* 1996, Richards *et al.* 1999, Cresswell *et al.* 2002).

From a modelling perspective, the similarity between the dispersal curves for wind-and insect-dispersed pollen has the great advantage that the same mathematical approach can be used for both.

Table 6.2 Comparison of wind- and insect-mediated pollen dispersal.

Wind	Insects
Moves pollen in large masses and mainly downwind.	Move pollen independently from each other and more or less randomly in all directions, although dependent on the location of nest or hive.
No regard for species. All pollen are taken and deposited at an arbitrary place. Probability of arrival at a compatible stigma is low. Therefore, pollen production is high compared to insect-pollinated species.	Often, distribution is systematically within plant species, due to specialisation or flower constancy. Only pollen of visited species is taken. Probability of arrival at a compatible stigma is high. Therefore, pollen production is low compared to wind-pollinated species.
Unlimited load of pollen.	Limited load of pollen. Only after deposition new pollen can be loaded.

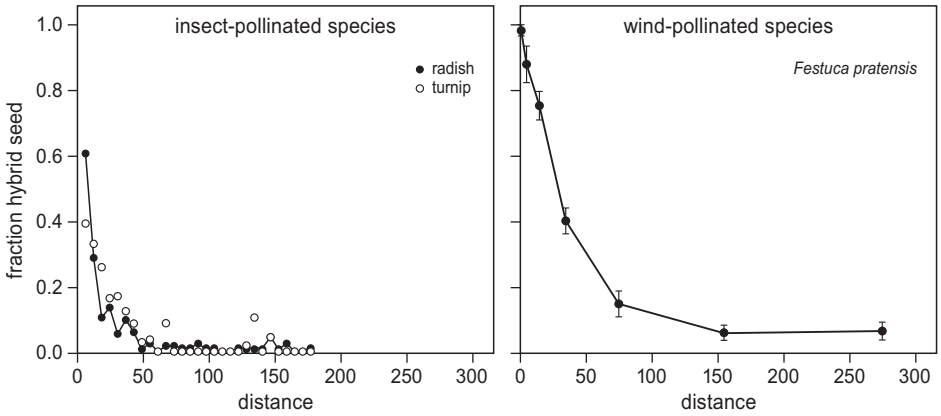

FIGURE 6.3 Examples of pollen dispersal curves for two insect-pollinated species (radish and turnip) and one wind-pollinated species (*Festuca pratensis*). In all cases, two different morphological types were used, one as a source, the other as a target. Pollen dispersal was measured as the fraction of hybrid seed in the target. More pollen grains would certainly have travelled over these distances than those that resulted in hybrids. Data from Bateman 1947ab and Rognli *et al.* 2000.

2.4 POLLEN VIABILITY

During the dispersal process, a certain percentage of the dispersing pollen will have lost viability (i.e. died or lost the capability to germinate and achieve fertilisation). Pollen viability is influenced by three main types of factors: *(i)* internal factors, such as pollen metabolism, *(ii)* morphological factors, such as protected anthers or open flowers, and *(iii)* environmental factors, such as humidity, temperature and UV light (Dowding 1987, Dafni and Firmage 2000).

Dafni and Firmage (2000) reviewed pollen viability for 34 species. For wind-pollinated species, an average longevity of 21.5 ± 27.2 hours was found, whereas for insect-pollinated species it was 8.5 ± 10.4 days, but species differed greatly (respective ranges 0.05–72 hours and 1–40 days). The problem with these data is the great diversity in the methods used to measure pollen viability. The exposure conditions for the pollen were variable (field conditions, exposed to open air, greenhouses, growth chambers), as were the tests used to measure viability (seed set, *in vivo* germination, FCR (fluorescein diacetate reaction) and other tests). These large differences in the methods used can probably partly explain the large ranges. For example, pollen longevity for the species *Oryza sativa* measured in a standard greenhouse was about 20 minutes, while when measured in an open greenhouse it was about four minutes. However, the difference could also be due to the different methods chosen to estimate pollen longevity. It is questionable, whether, on the basis of current knowledge, pollen viability rates of crops growing in the field can be predicted.

With so much information lacking, how can we incorporate pollen viability in a model? One study on pollen storage suggests that pollen longevity follows a normal distribution, with average longevity for most of the pollen grains and decreasing numbers of longer- and shorter-living grains (Hong *et al.* 1999). The same distribution is found for the longevity of

seed and fungal spores. As no other information is currently available, this distribution seems the most sensible one to incorporate in the model.

2.5 Gene-flow barriers

When undertaking cultivation of GM populations in the environment, it is worthwhile considering reducing pollen flow by means of gene-flow barriers. Different gene-flow barriers can be distinguished. One type is a vegetation barrier. Insects can be discouraged from moving between fields by planting a vegetation barrier of a heterospecific species not pollinated by insects around an insect-pollinated crop, thereby limiting pollen flow (Morris *et al.* 1994).

Another kind of gene flow barrier is a trap crop, i.e. a border of plants of the same crop, but not genetically modified. Such traps could "absorb" pollen that disperses out of the GM crop (Morris *et al.* 1994). Indeed, in comparison with bare land, GM pollen flow dispersed by insects outside the trial patch is decreased when a trap crop is present (Morris *et al.* 1994, Reboud 2003). Paterniani and Stort (1974) suggest from an experiment with maize, a typical wind-pollinated species, that the number of plants a pollen grain has to cross is more important than the actual distance. The effectiveness of a trap improves, when the trap-crop area is increased relative to the area of the GM population (Hokanson *et al.* 1997). To limit pollen flow out of agronomic-scale plantings would be extremely difficult, however, because borders are only effective in reducing long-distance dispersal if they are substantially larger than the crop field (Hokanson *et al.* 1997).

The above-mentioned barriers reduce pollen flow between populations, but generally do not prevent it. Genetic isolation mechanisms, such as male sterility, are able to prevent gene flow and would therefore be a more effective barrier (Van de Wiel *et al.* 2003). Nevertheless, when planting small fields for research trial, it is customary to use trap crops or vegetation barriers as an extra precaution measure. In the model, we will not consider the effect of trap crops or vegetation barriers.

2.6 Conclusions

Plant species differ considerably in their pollen-dispersal mechanisms. Some species are mainly self-fertilising and lack mechanisms for pollen dispersal over large distances. These species are expected to have smaller chances of outcrossing than species that are mainly outcrossing and that have mechanisms for long-distance pollen dispersal. The most important pollen-dispersal mechanisms are dispersal by wind and insects. Generally, pollen dispersed by wind travels further than pollen dispersed by insects. The shape of the dispersal curves, however, is similar, such that, from a modelling perspective, the same mathematical approach can be used for both.

Not all pollen grains will be viable when reaching the stigma of a compatible plant, especially not all of the immigrant grains, which include those from GM populations. In our model, we will assume that most pollen has an average lifespan, with the rest spread either side of the mean.

3. FERTILISATION

3.1 INTRODUCTION

In the previous section, we discussed the pollen-dispersal process, resulting in a certain amount of viable pollen that lands on the stigma of a compatible species (Fig. 6.1A). This dispersal is only effective if the pollen that has reached the stigma is able to achieve fertilisation (Fig. 6.1B). Each pollen grain has to compete with other grains present on the same stigma.

This section considers the most important aspects affecting the chances that pollen from GM populations will fertilise plants in wild or cultivated populations.

3.2 POLLEN COMPETITION

Often, more pollen grains are present on a stigma than are needed to fertilise all the ovules (Walsh and Charlesworth 1992). These grains have to compete with each other to achieve fertilisation. The number of competing pollen grains produced by the receiving population can have large effect on the success of incoming pollen (Ingram 2000).

In Section 2, we saw that some pollen may have lost viability by the time it reaches the target population. In fact, viability is not lost from one moment to the next, but declines gradually over time. For example, germination time and time necessary for the pollen tube to reach the ovule both increase with age (Shivanna *et al.* 1991). This reduces the competitive ability of GM pollen compared to resident pollen, since GM pollen has travelled larger distances and is likely to have aged. However, without information on the time it takes to travel certain distances, no inferences can be made about the magnitude of the age differences.

The position of a pollen grain on the stigma affects its speed of germination. Pollen that lands in the fluid filled cleft between two rows of stigmatic papillae germinates relatively quickly, whereas pollen landing on the papillae themselves germinates more slowly. Pollen that is travelled by insects is often sticking together. Pollen being part of such clump have different fertilisation probabilities, depending on the position in the clump (Thomson 1989).

Another factor affecting competitive ability is the relative timing of arrival. A pollen grain that arrives early, when none or relatively few other grains are present on the stigma, is expected to have higher fertilisation success. Indeed, more seed is sired by pollen that arrives first than pollen that arrives later. Nevertheless, part of the seed can be sired by pollen that arrives two hours after the first pollen (Spira *et al.* 1996). The moment of pollen arrival may thus determine which pollen tubes are more likely to be successful (Walsh and Charlesworth 1992).

A further aspect of pollen competition is tube growth rate, which varies between pollen grains. Differences are found between individuals within a population, between selfing and outcrossing pollen, between pollen from different donors and between pollen from different species (e.g. Snow and Spira 1991a, Walsh and Charlesworth 1992, Skogsmyr and

Lankinen 1999). Pollen tubes that grow relatively quickly have an advantage, as they have high chance of reaching the ovule before other pollen tubes, and so have a higher chance of fertilising the ovule (Snow and Spira 1991ab). Direct evidence for the existence of genetic variation in pollen-tube growth rate is lacking (Chasan 1992), but there are indications that it may be heritable (Skogsmyr and Lankinen 1999). Although we know that pollen grains do differ in growth rate, no generalisations can be made, such as a slower growth rate for selfing than for outcrossing species (Snow and Spira 1991a). There is no reason to suspect that pollen containing GM genes has a different growth rate to pollen without such genes, so we will not include pollen-tube growth rate in our model.

A final important aspect of pollen competition is the impossibility of many plant species to self-fertilise. Most flowering plants have hermaphroditic (bisexual) flowers, which greatly increase the efficiency of insect pollination, because deposition of foreign pollen on the stigma and removal of self-pollen from the anthers are accomplished in a single insect visit. However, bisexual flowers have a disadvantage in the increased risk of self-pollination and self-fertilisation, which can result in inbreeding depression. Many flowering plant species, therefore, have evolved mechanisms to prevent self-fertilisation. Some angiosperms have dispersal and reception of pollen separated in time. Other species have unisexual flowers, having either male or female reproductive organs. Dispersal and reception of pollen can also be separated in space within a flower. All these structural barriers prevent selfing to a greater or lesser degree (Fægri and Van der Pijl 1979).

All these barriers do not preclude fertilisation between pollen and ovule of the same plant, but they make it less likely by reducing the chances that dispersing pollen will land on the plant's own stigma. Other plant species have a system in which pollen grains do land on the plant's own stigma (i.e. self-pollination is present), but fertilisation is prevented, because pollen and stigma are incompatible. Such self-incompatibility (Box 3) is a genetically determined pre-zygotic barrier to fertilisation by self or self-related pollen that eliminates any risk of inbreeding and therefore optimises the potential for outbreeding (Hiscock and McInnes 2003). SI systems prevent self-fertilisation and hence decrease the effective number of competing pollen on a stigma. Particularly in populations with low polymorphism, the effective number of competing pollen on a stigma is expected to be reduced, since plants are expected to share genes. In such a situation, pollen from other populations is expected to be favoured, because it has alleles different from those present in the local population. This can have large effects on crop-to-crop fertilisation. Crops that are harvested before seed set can be highly homogeneous, meaning that pollen grains from a cultivated population are almost all incompatible with their own population. Pollen arriving from other (e.g. GM) crop populations, being of a different type to the target population, enjoys an increase in relative effective numbers. In fact, the same holds for crop pollen entering a wild related population: there will be a small chance of overlap of *S*-alleles.

3.3 Conclusions

Fertilisation is a complicated process. Important mechanisms that influence fertilisation success are reduced viability, time of arrival, the exact place where the pollen lands and the

BOX 3: SELF-INCOMPATIBILITY

Self-incompatbility (SI) can be divided in two types, namely heteromorphic and homomorphic. Heteromorphic SI species produce morphologically distinct flowers with regard to their relative style length and anther level, resulting in efficient transfer of intermorph pollen by insects. This mechanistic self-fertilisation barrier augments the biochemical incompatibility that also exists (Ebert et al. 1989). Both morphological and biochemical barriers are governed by the same cluster of genes designated *S* and *s*, which consist of some genes, coding for, among other things, style length, anther length and style-pollen incompatibility (De Nettancourt 1977). The genotype of the parent producing the pollen determines the compatibility of two flowers (i.e. compatibility is sporophytically determined). When the parent is short-styled (*Ss*), its pollen is compatible only with long-styled plants (either when the pollen has genotype *S* or *s*), and vice versa. The genetic control of this cluster ensures that both plant types are present in the same proportion in the population.

Homomorphic SI can be either sporophytic or gametophytic SI. As for heteromorphic SI, rejection of self-pollen in the sporophytic SI is controlled by the diploid genotype of the sporophytic generation (Ebert *et al.* 1989). The control is in the so-called *S*-locus, which is actually a cluster of three tightly-linked loci. Because the plants cannot fertilise themselves, they tend to be heterozygous, carrying two different *S* loci. Pollen will not germinate on the stigma of a flower that contains either of the two alleles in the sporophytic parent that produced the pollen (Fig. B3.1A). This holds true, even though each pollen grain – being haploid – contains only one of the alleles (Ebert *et al.* 1989). This is because the active protein on the exine of the pollen is a product of the internal disomic cell layers in the anthers of the parental plant, which contains the products of both alleles.

The gametophytic SI is controlled by the single S allele in the haploid pollen grain. A pollen grain will grow in any pistil that does not contain the same allele (Fig. B3.1B, Ebert *et al.* 1989). In the gametophytic SI system, as well as in the sporophytic SI system, the *S*-locus is highly polymorphic, containing dozens of different *S*-alleles.

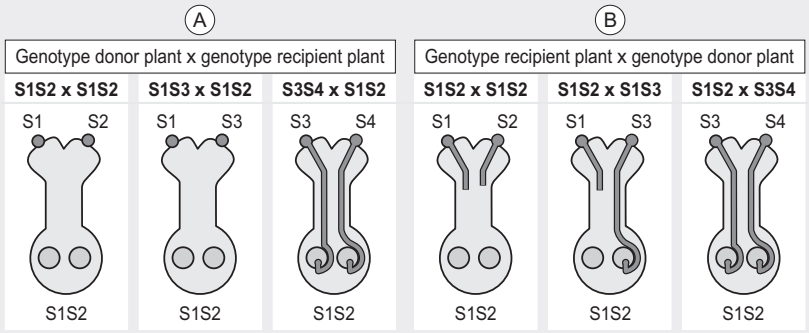

FIGURE B3.1 Schematic depiction of homomorphic self-incompatibility (SI) systems. A. Schematic depiction of a sporophytic SI system. The genotype of the pollen-producing sporophyte (donor plant) determines compatibility of the pollen with the recipient plant. If the recipient plant has at least one allele in common with the donor plant (regardless of whether the pollen grain carries this allele or the other one), fertilisation is prevented. Only when both alleles of the donor plant are different from those of the recipient plant can fertilisation occur. B. Schematic depiction of a gametophytic SI system. If the pollen grain's allele is the same as one of the alleles of the recipient plant, the pollen tube grows for only a few millimetres and fertilisation is unsuccessful. Pollen grains with a different allele, even if the pollen-producing sporophyte did have one of the same alleles, is not inhibited.

presence of self-incompatibility. Some of these effects are too complicated to include in a model. For example, the relative time of arrival not only depends on the distance travelled by the pollen, but also on the timing of its emission, which can differ between individual flowers.

In a model, the process of interest needs to be simplified. Walklate *et al.* (2004) simulated fertilisation probabilities by considering the effective deposition of pollen from the GM population and expressing this as a proportion of the total effective pollen deposition. We propose a similar kind of approach in our model, but envisage that it will be difficult to get reliable data bearing on this.

4. INTROGRESSION

4.1 INTRODUCTION

After the production of a hybrid[4] seed containing modified DNA, several scenarios are possible. One option is that the hybrid is unable to establish, or some plants are able to establish but are not able to backcross or persist. In this case, outcrossing has occurred, but it has no consequences for the wild or cultivated population. A second possibility is that the hybrid does establish and persists as a new species. This can occur in several ways. *(i)* The hybrid can spread vegetatively. In this case, only one successful hybrid needs to establish. *(ii)* Several hybrids develop and cross with each other, producing a new population. *(iii)* A sterile allopolyploid hybrid becomes fertile through chromosome doubling (Box 4). All three processes can affect a wild or cultivated population by competing with them.

In this section, we will not deal with these scenarios, but will instead consider a fourth possibility, namely introgression. The reason for restricting our focus in this way is that introgression is the only process that leads to the incorporation of modified DNA into the genome of wild or cultivated populations.

4.2 INTROGRESSION

Introgression can be defined as the permanent incorporation of one or more genes from the gene pool of one taxon into the gene pool of another taxon (mostly different species), through hybridisation and repeated backcrossing with one of the parental populations. In order to predict introgression chances, it is necessary to decide when you consider a gene to be introgressed. This could be, for example, when the gene is fixed in the population, but such an approach might take too many generations to be workable. A more workable option would be to estimate chances that the modified DNA persists in the population for a certain number of generations by means of backcrossing.

[4] With hybrid is meant offspring produced by crossbreeding between a pollen originating from a GM source population and an ovule originating from a non-GM target population.

Box 4: Recovery of an allopolyploid

Many crops are polyploid while their wild relatives are diploid. Crosses between species with different ploidy levels give rise to allopolyploid hybrids, i.e. hybrids that contain a number of chromosomes intermediate to those of the parental species (Fig. B4.1). When these hybrids reproduce, problems with pairing of homologous chromosomes typically occur in meiosis, making them sterile. Sometimes, however, crosses between species with different ploidy levels can be successful, as in *Spartina* (Ellstrand 2003). The hybrid *S.* x *townsendii* is the result of a cross between *S. maritima*, which has 30 chromosome pairs (2n = 60), and *S. alterniflora*, which has 31 chromosome pairs (2n = 62). *S.* x *townsendii* had a chromosome number intermediate to the two parental species (2n = 61), and therefore was not able to reproduce sexually, but it was able to spread vegetatively. Out of this sterile hybrid the new species *S. anglica* evolved by chromosome doubling (2n = 122).

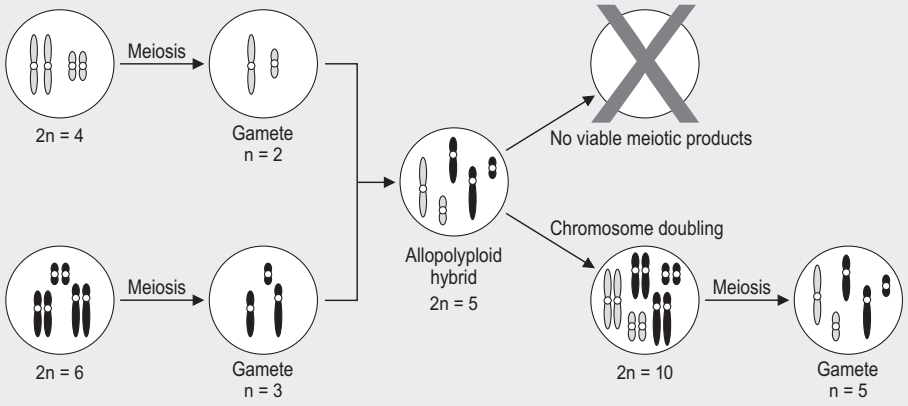

Figure B4.1 Schematic depiction of the recovery of a sterile hybrid from a cross between two species with different numbers of chromosomes. After spontaneous chromosome doubling, chromosomes are able to pair and create gametes.

To become introgressed, first the modified DNA should become established[5], something that is highly dependent on stochastic processes. In the intial stages, while the modified DNA is present in only very low frequencies, there is only a small chance that it will become established in the recipient population. To have a reasonable chance to establish, hybridisation must occur regularly. The cumulative probability of individual hybrids becoming established determines the final chance of establishment, so the more hybrids that develop, the higher the overall establishment chances.

After successful establishment, the modified DNA must be able to persist. Here, deterministic factors and processes become important. One of these is the fitness of hybrids and

[5] The modified DNA is considered established in a population, when the chances that it will persist in the populations are not dependent on merely stochastic processes, but mainly on the fitness of the individual plants carrying the modified DNA.

backcrosses. Hybrids can have either a higher fitness (hybrid vigour, e.g. in crosses within *Raphanus sativus*, within *Oryza sativa* and between *Brassica napus* and *B. rapa*), lower fitness (outbreeding depression, e.g. in crosses between *Brassica napus* and *Hirschfeldia incana*, between *Raphanus sativus* and *R. raphanistrum* and within *Helianthus anuus*), or the same fitness as their parents (e.g. in crosses between *Cucurbia pepo* and *C. texana* and between *Sorghum bicolor* and *S. halepense*, Ellstrand 2003). A complicating factor is that the effect of the modified DNA on the persistence of the plant is not always known beforehand, which makes it hard to estimate introgression probabilities. The fitness effect depends not only on phenotypic characteristics caused by the gene (such as herbicide resistance), but also on other features, such as dominance, association with deleterious crop alleles or traits and location on the chromosome (Stewart *et al.* 2003). For example, certain characteristics of domesticated species may behave recessively in a cross with a wild species. These characteristics do not become expressed in first-generation hybrids. Therefore, first-generation hybrids having a high fitness does not always imply high fitness for later-generation hybrids (Groot *et al.* 2004).

Besides the effect of the modified DNA on the persistence of hybrids and backcrosses, the environmental conditions are important. If, for example, fields with hybrids carrying an herbicide-resistance gene were sprayed with herbicide, this would create a strong selection pressure favouring the gene which would not have existed had the fields not been treated.

Introgression into cultivated populations is a different kind of problem to introgression into wild populations. Most crops are harvested each year. Farmers are then concerned about contamination levels of the seed. Crops cultivated to be used as food or intended for seed production are allowed to be contaminated to a certain level, which height is continuous under discussion. Introgression will only occur, when contaminated seed is repeatedly harvested and plants grown from this will be fertilised next year, so in fact when the farmer collects his own seed for next year's sowing.

4.3 Modelling approach

When hybridisation between a GM plant and its wild or cultivated relatives has occurred, the modified DNA will be present in very low frequencies in the population. Whether the modified DNA will establish is initially a mainly stochastic process. Therefore, a model should use initial establishment probabilities that depend on stochastic processes.

When the modified DNA is established, it should be able to persist. To estimate this part, different modelling approaches can be taken. One is a population genetic approach (e.g. Van Raamsdonk and Schouten 1997, Haygood *et al.* 2003). Such models are based on changes in allele frequencies from one generation to the next depending on the fitness of the different genotypes and on the number of alleles received from the GM population every generation.

Another approach is to divide species into categories with high or low chances of introgression. Stewart *et al.* (2003) based different categories on experimental knowledge of hybridisation and introgression. Species for which no molecular evidence of introgression has been found were considered very low-risk crops, while species that hybridise with wild relatives and for which there is good molecular evidence for introgression were considered

to be high-risk crops. Hancock (2003) based the categories on fitness characteristics of the modified DNA combined with characteristics determining invasiveness. Decisions about invasiveness were based on the number of weediness traits carried by the GM crop and the recipient population (traits such as broad germination requirements, high seed longevity, rapid growth to flowering, seed production in variable environments and vigorous vegetative reproduction). The potential impact of the modified DNA could be ranked by its likely effect on reproductive success, ranging from advantageous to neutral to detrimental. Gressel and Rotteveel (2000) developed a detailed decision-tree-based risk-assessment categorisation methodology for GM herbicide-resistant crops.

4.4 Conclusions

Groot *et al.* (2004) recently reviewed the current knowledge of hybridisation and introgression between GM or conventional crops and their wild relatives. They conclude their report with a long list of knowledge gaps, showing that much experimental work needs to be done before introgression probabilities can be estimated reliably. Therefore, we did not include introgression in our model.

Nevertheless, we would recommend that future models incorporate introgression as soon as these knowledge gaps can be filled, because "the general conclusion with respect to the phenomenon of gene flow between crops and wild relatives is that although chances may vary, in many crop-wild relative complexes sooner or later gene flow will occur. Incorporation of crop genes into recipient taxa will occur through further introgression processes after initial hybrid formation" (Groot *et al.* 2004).

5. Modelling approaches

5.1 Introduction

Before starting with a modelling endeavour, one has to realise that all models have their inherent limitations. The user might want a model that is at the same time simple, robust, realistic, precise, reliable and discriminating. However, some of these desirable properties are inherently incompatible. For example, the outcome of a robust model is not much affected by a small change in parameters. A discriminating model, however, is expected to reflect precisely such differences. Accordingly, a model cannot be robust and discriminating at the same time. Depending on the purpose of a model, the developer has to decide which properties are more important and which less. In Box 5, a short overview is given of different types of models and their properties.

Box 5: Types of models and their properties

Roughly, models can be classified in three types: conceptual, mechanistic and statistical (e.g. Lavigne *et al.* 2004). A short description of these types will be given.

Conceptual models would probably strive to describe pollen dispersal in a relatively simple way, appealing to intuition and without putting too much emphasis on the details of the process. Such an approach would most probably be based on a negative exponential distribution of pollen away from its source, for two reasons. First, this approach is mathematically simple and elegant. Second, it has a simple statistical interpretation: if pollen is moving at a given speed and in a given horizontal direction, an exponential distribution is generated if the probability of landing is constant and independent of the distance from the source.

A **Mechanistic** model of pollen dispersal by wind would most probably be based on the physical principles of transportation by air. Such a model would probably take into account factors such as wind direction, horizontal wind speed, thermal turbulence and several weather and landscape parameters having influence on the process. Given information on all these processes, a mechanistic model could then derive a pollen dispersal curve. It is by no means sure that this curve would be an exponential one. A mechanistic model of pollen dispersal by insects would mostly be based on insect behaviour, taking into account such factors as flight distance and direction of the insect involved, pollen load and pollen carry-over. For examples of mechanistic models of seed dispersal by wind see Tackenberg (2001, 2003) and Tackenberg *et al.* (2003) and for those of dispersal by insects see Morris (1993), Cresswell *et al.* (1995, 2002) and Cresswell (2003).

With a **statistical** description of the process, a given data set is used, e.g. an experimentally determined distribution of pollen around a source. The statistical approach then fits some curves with simple and well-known statistical properties through the data and chooses that statistical model that provides the best balance between goodness-of-fit and number of parameters that have to be estimated from that data (Myung *et al.* 2000).

This rough distinction does not mean that any given model fits perfectly to any of the three categories described above. For example, no model can take all mechanisms into account (otherwise the model would be as complex as reality). Hence, a mechanistic model is only mechanistic to a certain degree. Similarly, a conceptual model may incorporate elements that are based on statistical analysis. For example, it would be easy to replace a negative exponential pollen distribution by an inverse power law if it turns out that power functions give a better description of the process.

Figure B5.1 illustrates how the type of model relates to the properties it typically has. Conceptual models are mainly intended for giving qualitative insights guiding intuition, and are therefore less suitable for making quantitative predictions and providing guidance to management decisions. Statistical models can easily lead to a description which fits well to the data, but for unknown reasons. These models therefore contribute little to a better understanding of the processes involved. This may have important implications for management decision, since these decisions often involve considering situations for which reliable data are not yet available.

In such situations one has to extrapolate from the given data to unknown situations and such an extrapolation can be risky if it is based on a (statistical) model whose mechanistic foundation is not known. In the context of a mechanistic model, extrapolation from a known

situation to unknown ones is much less risky, at least in those situations where the underlying mechanisms are well understood. The problem with a mechanistic approach is that typically a large number of mechanisms are responsible for a given process and these processes may interact in a rather complicated way. Fully mechanistic models therefore tend to be highly complicated. Moreover, these models are often dependent on many parameters on which information is not readily available in a given situation.

Concerning the question of genetic exchange, the model we have developed is mainly statistical, since otherwise reliable quantitative predictions are hard to obtain. However, as indicated above, one has to be aware of the extrapolation problem. It might therefore be useful to develop in parallel a suite of "mechanistic models of intermediate complexity" (models incorporating a few mechanisms) in order to judge the reliability of the conclusions derived from the statistical approach.

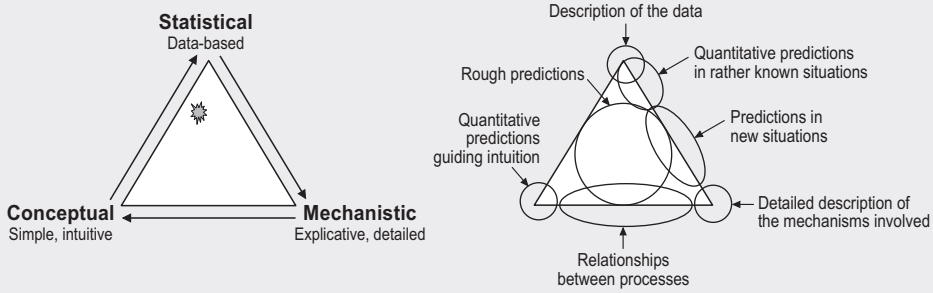

FIGURE B5.1 *Left triangle*: Distinction of three types of models which have different purposes and properties. The rough distinction into conceptual, mechanistic and statistical models does not mean that a given model fits exactly into one of these categories. Most models fall in between, represented by the symbol in the triangle. *Right triangle*: Illustration of some important aspects related to a proper choice of model. After Lavigne *et al.* (2004).

5.2 MODELLING APPROACH

Figure 6.4 gives an overview of the mathematical model we propose, and have partly developed, for estimating the probabilities that GM pollen will land in populations of compatible species and achieve fertilisation in such a population. The model consists of three modules. The first module addresses the question: how does pollen, originating from a GM source population, disperse over the landscape? In this module, pollen dispersal of a source population will be simulated. The second module addresses the question: what is the expected frequency of seeds in a target population that is fertilised by pollen originating from a given GM source population? In this module, the percentage of seeds that would originate from a cross between pollen from the source population and ovules from the target populations is calculated, thus giving an estimation of the contamination level of the target population with DNA from the GM source population. The third module addresses the question: which

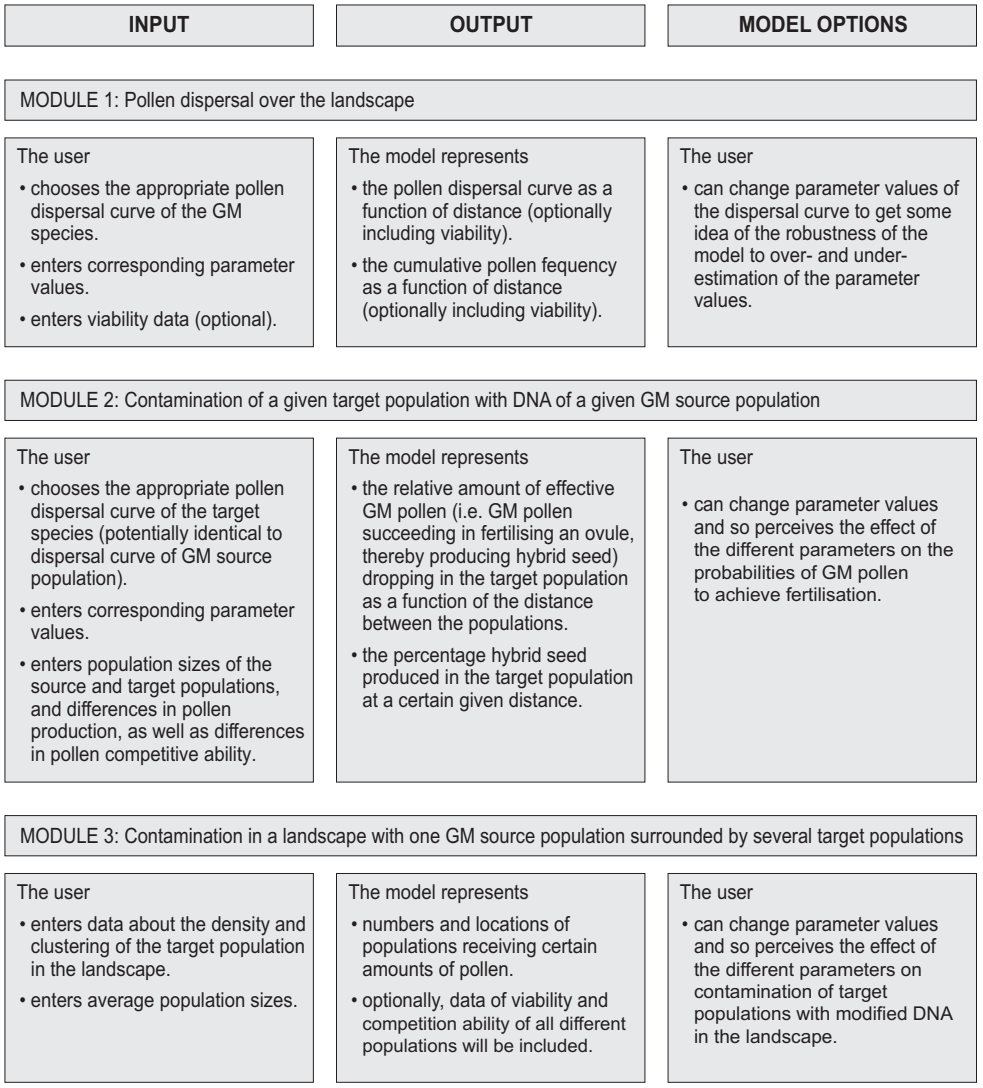

FIGURE 6.4 Schematic depiction of the mathematical model that is proposed. In the first column (user), the parameter values that have to be entered by the user are given. In the second column (output), the model calculations are represented. In the third column (model options), the possibility of the user to change parameters is given.

percentage of seed of a collection of target populations is fertilised by pollen originating from a given GM source population? In this module, many target populations are situated in a landscape with one GM source population. Depending on population size, proximity to other target populations and to the GM source population, different target populations are expected to be contaminated to a greater or lesser extent. In the following paragraphs, we will go into the different modules of the model in more detail.

Box 6: Equations used to discribe pollen dispersal curves

Many different equations can be used to describe the dispersal pattern of pollen through the landscape. Here, different equations are discussed. The equations are defined as a probability density. The mathematical definition of a continuous probability densitmy function, *f(D)*, is a function that satisfies the following properties:

1. The probability P that the distance D is between two points D_1 and D_2 is
$$P(D_1 \leq D \leq D_2) = \int_{D_1}^{D_2} f(D)dD$$
2. The integral of the probability function is one, that is
$$\int_{-\infty}^{+\infty} f(D)dD = 1$$

What does this actually mean? Since continuous probability functions are defined for an infinite number of points over a continuous interval, the probability at a single point is always zero. Probabilities are measured over intervals, not single points. That is, the area under the curve between two distinct points defines the probability for that interval.
Below, we consider some of the different types of equations used for pollen dispersal curves.

1. One-parameter families of dispersal curves

Negative exponential distribution

The standard negative exponential distribution is described by
$$f(D) = \alpha \exp(-\lambda D)$$

where λ is a shape parameter and α is a scale parameter. If it is used to describe the two-dimensional distribution of pollen around a point source, the parameter α is determined by λ and given by

$\alpha = \frac{\lambda^2}{2\pi}$ (see appendix A1). Hence, $f(D) = \frac{\lambda^2}{2\pi}\exp(-\lambda D)$

The parameter λ, characterising the shape of the dispersal curve, has a simple relationship to the mean dispersal distance and the variance in dispersal distance:

Mean dispersal: $\bar{D} = \frac{2}{\lambda}$ Variance: $\mathrm{var}(D) = \frac{6}{\lambda^2} - \bar{D}^2$

Inverse power law

The standard inverse power law is given by
$$f(D) = \alpha D^{-\lambda}$$

where λ is a shape parameter and α is a scale parameter. Since the inverse power law becomes unreliable towards $D = 0$, $D + 1$ is used instead of D. If the inverse power law is used to describe the two-dimensional distribution of pollen around a point source, the

parameter α is determined by λ and given by

$$\alpha = \frac{(1-\lambda)(2-\lambda)}{2\pi}$$ (see appendix A2). Hence,

$$f(D) = \frac{(\lambda-2)(\lambda-1)}{2\pi}(D+1)^{-\lambda} \quad \text{for } \lambda > 2$$

The parameter λ, characterising the shape of the dispersal curve, has a simple relationship to the mean dispersal distance $\bar{D}$ and the variance in dispersal distance:

Mean dispersal: $\bar{D} = \frac{2}{\lambda-3}$ for $\lambda > 3$ Variance: $\text{var}(D) = \frac{6}{(\lambda-3)(\lambda-4)} - \bar{D}^2$ for $\lambda > 4$

Uniform distribution

The standard uniform distribution is given by

$$f(D) = \frac{\alpha}{D_{\max}}$$

where α is a scale parameter and $D_{\max}$ the maximum dispersal distance. If this equation is used to describe the two-dimensional distribution of pollen around a point source, the parameter α is given by

$$\alpha = \frac{1}{\pi D_{\max}}$$ (see appendix A3). Hence, $f(D) = \frac{1}{\pi {D_{\max}}^2}$

The mean dispersal distance $\bar{D}$ and the variance in dispersal distance are given by

Mean dispersal: $\bar{D} = \frac{2}{3} D_{\max}$ Variance: $\text{var}(D) = \frac{1}{2} D_{\max} - \bar{D}^2$

2. Two-parameter families of dispersal curves

Equation from the exponential power family

The standard equation from the exponential power family is given by

$$f(D) = \alpha \exp\left(-(\lambda D)^b\right)$$

If it is used to describe the two-dimensional distribution of pollen around a point source, the parameter α is determined by λ and given by

$$\alpha = \frac{\lambda^2 b}{2\pi\,\Gamma(2/b)}.$$ Hence, $f(D) = \frac{\lambda^2 b}{2\pi\,\Gamma(2/b)} \exp\left(-(\lambda D)^b\right)$

The parameter λ characterises the shape of the curve. $\Gamma(n)$ is the gamma function, in which $\Gamma(n+1) = n!$ (for $n = 1, 2 \ldots \infty$). By adding the extra parameter b, the equation has adjustable kurtosis (Fig. 6.7). This parameter has following properties:

When $b = 1$, the curve follows a negative exponential distribution,
When $b < 1$, the tail of the curve is fat-tailed compared to a negative exponential distribution,
When $b > 1$, the tail of the curve is thin-tailed compared to a negative exponential distribution

The parameters λ and b have following relationship to the mean dispersal distance $\bar{D}$, the variance in dispersal distance var(D) and the kurtosis κ:

Mean dispersal:	Variance:	Kurtosis:
$\bar{D} = \frac{\Gamma(3/b)}{\lambda\,\Gamma(2/b)}$	$\mathrm{var}(D) = \frac{\Gamma(4/b)}{\lambda^2\,\Gamma(2/b)}$	$\kappa = \frac{\Gamma(6/b)\Gamma(2/b)}{\Gamma^2(4/b)}$

Many other dispersal curves defined by two parameters are used to estimate pollen dispersal patterns. Below, three of them are listed. See Austerlitz *et al.* (2004) for detailed information about these equations.

Equation from the Weibull family $f(D) = \frac{b\,(\lambda\, D^{(b-2)})}{2\pi\,\lambda^{(-b)}}\, e^{-(\lambda D)^b}$

Equation from the bivariate Student's t family $f(D) = \frac{\lambda^2(b-1)}{\pi}\,(1 + \lambda^2 D^2)^{-b}$

Equation from the geometric family $f(D) = \frac{\lambda(b-2)(b-1)}{2\pi}\,(1 + \lambda D)^{-b}$

3. Dispersal curves defined by more than two parameters

Summing two functions

Sometimes, experimentally determined dispersal curves can best be viewed as a weighted average of two dispersal curves f_1 and f_2 (e.g. the exponential and power curve):

$$f(D) = \alpha\, f_1(D) + (1 - \alpha)\, f_2(D)$$

This equation is described by three parameters: the weight factor α, the average of equation 1, $\bar{D}_1$, and the average of equation 2, $\bar{D}_2$. The mean dispersal distance and variance can be calculated as follows:

Mean dispersal: $\bar{D} = \alpha\bar{D}_1 + (1 - \alpha)\bar{D}_2$
Variance: $\mathrm{var}(D) = \alpha\ \mathrm{var}(D_1) + (1 - \alpha)\ \mathrm{var}(D_2) + \alpha(1 - \alpha)(\bar{D}_1 - \bar{D}_2)^2$

5.2.1 Module 1: Pollen dispersal over the landscape

In Section 2, we saw that the shape of wind and insect pollination curves relating pollination probability (or pollen frequency) to dispersal distance is very much the same. A large fraction of the pollen lands close to the source plant and only a small fraction disperses further, of which some travels over large distances (Fig. 6.3).

Many different equations are used to describe pollen dispersal curves. The most promising ones are the negative exponential distribution (NED) and the inverse power law (IPL, Box 6). Having the same average dispersal distance, the NED predicts higher pollen frequencies close to the donor plant and lower frequencies at larger distances compared with the IPL (Fig. 6.5AB). Although commonly used, both curves seem to underestimate pollen frequencies at large distances; pollen dispersal curves are generally more leptokurtic (i.e. more fat-tailed) than predicted by the NED (Nurminiemi *et al.* 1998, Austerlitz *et al.* 2004).

The NED and IPL are both determined by one parameter and therefore restricted. More realistic dispersal curves are defined by two parameters (Box 6). One of these comes from the exponential power family. In this equation, the 'fatness' of the tail of the dispersal distribution is determined by the kurtosis parameter b. When $b < 1$, the tail of the curve is fat-tailed, when $b > 1$, the tail of the curve is thin-tailed compared to the exponential distribution (Fig. 6.6). Austerlitz *et al.* (2004) used this exponential power curve to estimate pollen dispersal curves using genetic markers. They were able to estimate correctly the general trend of the curve, i.e. fat-tailed or thin-tailed. The same equation was used by Clark (1998) and Clark *et al.* (1998) to estimate seed dispersal curves. Other functions that are defined by two parameters are functions of the Weibull family and the geometric and 2Dt families (Box 6).

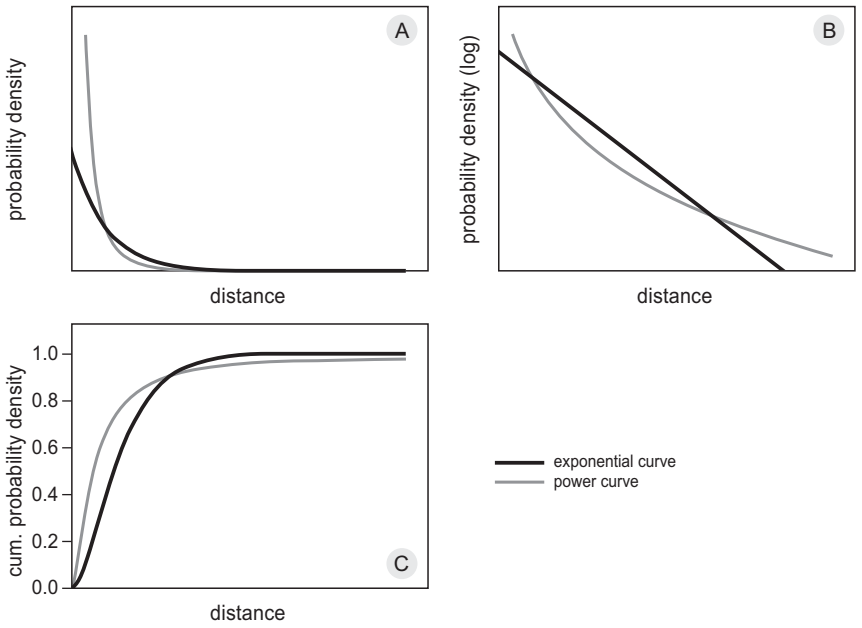

FIGURE 6.5 Graphic representation of the negative exponential curve and inverse power law with the same average dispersal distance on A. a linear scale, B. a semi-log scale and C. represented as the cumulative probability density. The power curve is more leptokurtic than the exponential curve, predicting higher pollen densities close to the donor plant (very short dispersal distances) and at large dispersal distances and lower pollen densities at intermediate dispersal distances.

A different approach is not to estimate the whole dispersal curve at once, but to cut the curve into two parts and estimate each part separately. Lavigne *et al.* (1998) applied this method. They fitted dispersal curves to experimental data from oilseed rape (*Brassica napus*) and found that 55 per cent of the pollen dropped within a few metres, the other 45 per cent landing at larger distances. This latter part of this distribution could best be described by a negative exponential function. Other possibilities are to describe the latter part by a power function or a uniform distribution (Fig. 6.7). The mathematically correct way to use this method is to take the weighted average of the two curves involved (e.g. the exponential and power curve, Bullock and Clarke 2000). Three parameters are needed to do so, namely the average dispersal distances for both equations and a weighting factor for the curves (Box 6). The weighting factor corresponds to the proportion of pollen governed by the curve in question. This method can only be used when it is clear that two different equations are involved, as was the case in the experimental study by Lavigne *et al.* (1998).

How should one choose among these competing models of the same phenomenon? Here we enter the realm of model selection. The model that fits observed data sufficiently well (i.e. is descriptively adequate) in the least complex way (i.e. using fewest parameters) should be preferred (Myung *et al.* 2000). A complex model with many parameters and highly flexible form can often fit data better than a simple model with few parameters; however, beyond a certain point, the improved fit from including extra parameters does not outweigh the increased complexity of the model. Most of the distributions mentioned above are estimated by a few parameters, which makes them relatively simple, but it is questionable that they will describe the data sufficiently well. For more information about model selection see Burnham and Anderson (2002), Pitt and Myung (2002), and Johnson and Omland (2004).

For many species the pollen distribution is not known and therefore must be estimated. In the model, the uniform distribution is included to make a kind of worst-case estimation. This distribution assumes that a constant frequency of pollen lands at every distance. The uniform distribution can only be used as a truncated distribution, since the amount of pollen is limited; at a certain distance all pollen will have landed. Therefore, a maximum dispersal distance should be set. The pollen will be distributed evenly over the area below

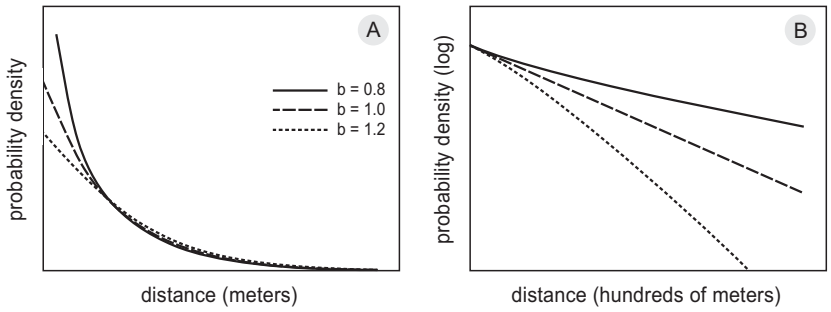

FIGURE 6.6 Different exponential power curves with the same average dispersal distances on A. a linear scales and B. a semi-log scale. When b > 1 the curve is thin-tailed, and when b < 1 it is fat-tailed compared to the standard exponential curve (b = 1).

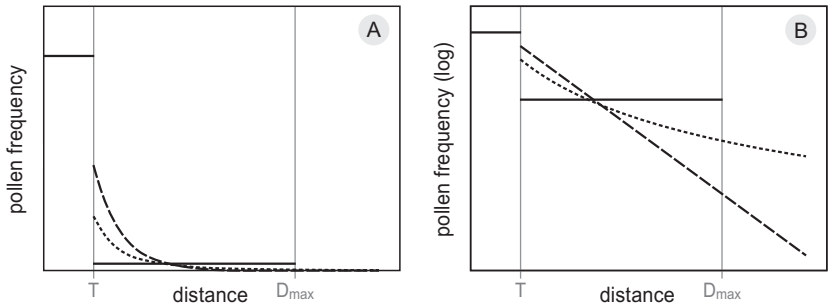

FIGURE 6.7 Schematic representation of how the pollen distribution pattern is estimated with two different equations. Here, the first part of the pollen dispersal curve is estimated with a uniform distribution (solid line), the second part of the curve (the tail) is estimated by a negative exponential curve (dashed line) an inverse power law (dotted line) and a uniform distribution (solid line). To be able to estimate the pollen distribution pattern in this way, there should be a clear 'cutting point', i.e. it should be clear where to end the first equation and to start the second one (T in this Figure). Furthermore, it should be known what fraction of the pollen is described by the first equation and what fraction by the second equation.

this distance. Choosing a large maximum dispersal distance implies little pollen per unit area (since the total area is large); choosing a small maximum dispersal distance implies a larger amount of pollen per unit area. Using the uniform distribution, an overestimation of the pollen frequency is made in the tail of the curve and an underestimation at the short dispersal distances. The uniform distribution can also be used to describe only the tail of the curve. Some authors (e.g. Paterniani and Stort 1974) suggest that the latter scenario is most realistic.

How does this work in the model? The negative exponential distribution (NED), the inverse power law (IPL) and the uniform distribution are included in the model as possible alternatives. The user can also choose to enter an equation that is described by one parameter. In some cases, it may be better not to describe the whole curve, but to estimate only the tail of the curve (Fig. 6.7). For all curves, the parameter values have to be entered. This means the lambda for the NED and the IPL (Box 6) and the maximum dispersal distance for the uniform distribution. If the user chooses to estimate only the tail of the curve, the weight factor should be entered (i.e. the proportion of pollen that lands in this part of the curve). When the appropriate parameters have been entered, the dispersal pattern can be shown graphically, e.g. as a 'standard' pollen dispersal curve or as a cumulative pollen dispersal curve, both as a function of distance. The standard pollen dispersal curve plots the pollen frequency as a function of the distance. The cumulative pollen dispersal curve plots the frequency of all pollen that has dropped up to and including that distance (Fig. 6.5C).

5.2.2 Module 1: Viability of the pollen

It takes some time for pollen to travel from the releasing plant to a recipient population. During this time, part of the pollen is expected to have lost viability. Does a common distribution exist that describes the loss of viable pollen in time? Hong *et al.* (1999) found that,

over time, the changing fraction of surviving stored pollen of *Typha latifolia* followed a negative cumulative normal distribution (Box 7). To our knowledge, this is the only study so far that has tried to find a distribution describing the loss of viable pollen. Other studies analysing pollen *in vivo* (e.g. James and Knox 1993, Fernando and Cass 1997, Aylor *et al.* 2003) seem consistent with the results of Hong and colleagues.

In the model developed, we used this negative cumulative normal distribution to describe the frequency of viable pollen. This curve is defined by two parameters, mean viability and the variation around the mean (Box 7). As knowledge is lacking about the time it takes a pollen grain to arrive at a compatible stigma, we define this equation as a function of the distance.

Besides the negative cumulative normal distribution, it is possible for the user to enter another function that is described by one parameter. The viability data are taken into account in the first module of the program (Fig. 6.4).

5.2.3 Module 2: Fertilisation

In the second module, the model estimates contamination levels of a given target population with DNA from the GM source population. In a target population, two types of pollen can land: pollen from the target species itself and pollen from the GM source population. The higher the relative number of GM pollen grains landing on a stigma compared to the pollen grains of the resident population, the higher the fertilisation chances of these grains. The amount of pollen containing modified DNA that reaches the target population depends on the distance between source and target population, as well as on population sizes and the number of pollen grains produced by the different populations. With this information, we can estimate the relative amount of pollen containing modified DNA that lands on a stigma in the target population.

Perhaps resident pollen will have a higher competitive ability than GM pollen; for example, because GM pollen, although closely related to the target species, is heterospecific, lowering its compatibility, or because the GM pollen will have aged more than resident pollen by the time it reaches the stigma. In other situations, resident pollen might have a lower compatibility than GM pollen, for example due to self-incompatibility systems active within the target population. Resident pollen can be subdivided in several types: self-pollen, pollen from other flowers within the target population and pollen from populations of the same species as the target population growing nearby. Table 6.3 lists the effects of different mechanisms on the relative compatibility of different types of resident pollen.

Now, consider a plant with a certain amount D_{gm} of pollen derived from GM plants and an amount D_{res} derived from resident plants. If the compatibility of the GM pollen is set to one, the effective pollen number of the resident pollen is reduced or increased with a factor η_{res}. The proportion of GM pollen (P_{gm}) is then given by

$$P_{gm} = \frac{D_{gm}}{D_{gm} + \eta_{res} D_{res}}$$

The user of the model should estimate η_{res}, which is the relative fertilisation probability

Box 7: Negative cumulative normal distribution

The **normal (or Gaussian) distribution** is an extremely important probability distribution in many fields. It is actually a family of distributions of the same general form, differing only in their *location* and *scale* parameters: the mean (μ) and standard deviation (σ^2). The distribution is symmetric. The probability density function $P(D)$, with D being distance, is:

$$P(D) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left(\frac{-(D-\mu)^2}{2\sigma^2}\right)$$

Considering the mortality of dispersing pollen grains, this distribution gives the frequency of individual deaths per distance.

In the model we developed, we used the negative cumulative normal distribution as a function of distance to calculate the probability of pollen surviving over the corresponding distance. The cumulative distribution is a special way to represent the normal distribution. Generally speaking, cumulative distribution functions give the probability that the variable takes a value less than or equal to x. In our situation, the variable is the probability of death (or survival) and x is the distance. The cumulative distribution function of the normal distribution does not exist in a simple closed formula. It is computed numerically.

The cumulative **positive** normal distribution gives the probability of a pollen grain to have died before or at the corresponding distance. This curve is called the **mortality** curve. The negative cumulative normal distribution is the opposite of the mortality curve (1 minus the mortality curve) and is called the **survival** curve, as it gives the probability of surviving till the corresponding distance (Fig. B7.1A). The location and scale parameter define the exact shape of the survival curve (Fig. B7.1B).

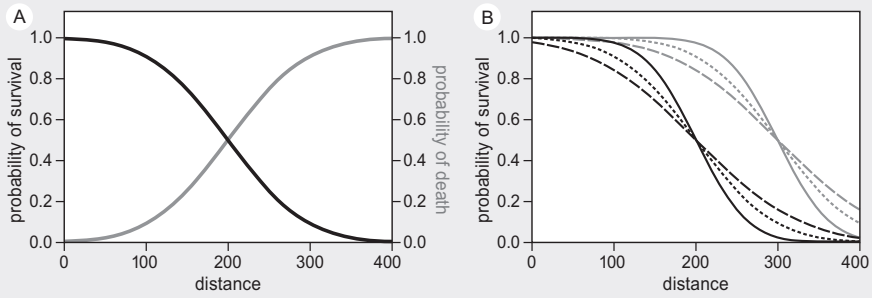

Figure B7.1 A. The mortality curve (grey) gives the probability that a pollen grain will be dead by the time it reaches the given distance. The survival curve (black) gives the probability that the pollen grain will still be alive by the time it reaches the given distance. Since the vertical axis is a probability, it must fall between zero and one. The horizontal axis is the allowable domain for the given probability function. B. The location and scale parameters define the exact shape of the survival curve. An increase in mean viability, i.e. an increase in the distance at which half of the pollen is viable and half of the pollen dead, results in the same frequency of pollen surviving to higher distances (compare the black lines, with a mean of 200, with the grey lines, with a mean of 300). The standard deviation (sd) around the mean determines the rate of loss of viability. A higher standard deviation results in a lower rate (compare the solid line (sd = 50), dotted line (sd = 75) and dashed line (sd = 100) within each colour).

of the resident pollen compared to the GM pollen. This parameter can be split up in a factor estimating the relative fertilisation probability of self-pollen η_{sel} and the relative fertilisation of pollen originating from other plants in the target population η_{in}:

$$\eta_{res} D_{res} = \eta_{sel} D_{sel} + \eta_{in} D_{in}$$

in which D_{sel} is the amount of pollen that lands on the own stigma and D_{in} is the amount of pollen originating from other plants in the population. $\eta_{sel} D_{sel}$ corresponds to the selfing rate of a species. $\eta_{in} D_{in}$ corresponds to successful outcross fertilisations within the target population. Of many species, the selfing rate (or the range in which the selfing rate is) is known, but the amount of pollen that lands on the own stigma or on another stigma within a population is mostly unknown. Therefore, $\eta_{in} D_{in}$ is not easy to estimate. η_{in} covers many different processes that together lead to a certain fertilisation probability relative to the fertilisation probability of outcrossing pollen originating from the GM source population.

With these data, the model can estimate the fertilisation chances of pollen originating from a given GM source population in a given target population for a given distance.

TABLE 6.3 The effects of different mechanisms on the competitive ability of GM pollen (D_{gm}), self-pollen (D_{sel}), pollen from other flowers within the target population (D_{in}) and pollen from populations of the same species as the target populations growing nearby (D_{out}). The competitive ability of the GM pollen is set to one, with that of other pollen given relative to this.

Pollen types	D_{gm}	D_{self}	D_{in}	D_{out}
Effect on compatibility factor η	η set to 1	η relative to GM pollen	η relative to GM pollen	η relative to GM pollen
Mechanism				
Reduced compatibility due to being different species	1	> 1	> 1	> 1
Rejection of pollen that differ too much from own genotype	1	> 1	> 1	(>) 1 (possibly slightly larger than one, due to overlap in genotype as a result of regular gene flow with target population)
Self-sterile or containing barriers preventing self-fertilisation	1	0	1	1
Partly self-sterile	1	<1	1	1
Heteromorphic incompatibility system	1	0 (self-sterile)	1 (compatible with half of the plants (distyly), but so is GM pollen)	1 (compatible with half of the plants (distyly), but so is GM pollen)
Homomorphic incompatibility system (sporophytic as well as gametophytic)	1	0 (self-sterile)	< 1 (dependent on number of different alleles in population; if variation is low, then << 1)	1 (but when exchange with target population is high, then probably <1)

5.2.4 Module 3: Contamination in a landscape

The third module, which will, due to time constraints, not be programmed at present, concerns more complex situations. In this module, a landscape is simulated that contains one or more GM source populations surrounded by several target populations. The contamination levels of these target populations, resulting in seed containing modified DNA, will be estimated. Since pollen originating from every individual population disperses over the entire landscape, every target population has certain chance of being reached by pollen originating from the GM source populations and from the other target populations. This makes the situation much more complex. Pollen originating from every population now has certain probability of reaching a given target population, depending on the distance between the populations and on population characteristics like size and pollen production. Three types of pollen can be present in a given target population: pollen originating from a GM source population, pollen originating from the target population itself and pollen originating from one of the other target populations. This makes that the relative fertilisation chances have to be estimated not only for the GM source and the target population, but as well for pollen of the other target populations arriving at the focal target population (Table 6.3). This estimation should be performed for all target populations.

To simulate a landscape in the model, the user should enter, in addition to the earlier-mentioned dispersal and fertilisation characteristics, the density and clustering of the populations in the landscape, as well as the average population sizes (Fig. 6.4). The model then calculates the number of populations that will have a higher contamination level than the threshold specified by the user or it gives an overview of classes with different levels of contamination.

6. Conclusions

The COGEM uses environmental risk analysis (ERA) to evaluate proposals for the cultivation of GM plants. Estimating the probability of outcrossing is only one step in the ERA, the other being estimation of the consequences of such outcrossing. In this report, we were concerned solely with the first part: our aim was to evaluate the main processes that should be included in a model for outcrossing probabilities. For outcrossing to occur, a pollen grain originating from the GM source population must reach a given target population, fertilise a plant in that target population and then the resulting hybrid seed must establish. Together, these processes determine the probability of modified DNA introgressing in the DNA of the target population.

The process of pollen dispersal is highly complicated, not only differing between species, but also within a species, depending on characteristics such as insect abundance, weather and population characteristics. However, the main dispersal pattern seems to be ubiquitous. Most pollen lands close to the dispersing plant, but the small fraction that travels further may cover large distances. In the model we develop, we include several different equations for pollen dispersal, so that the most appropriate curve can be chosen

for every species. However, every user of the model should keep in mind that even within a species the dispersal curve can differ considerably.

At present, pollen viability is poorly understood. There are some indications that pollen survival follows a negative cumulative normal distribution, but the available information is too preliminary to depend on. Nonetheless, we include loss of viability as a component of our model and describe it using just such a distribution, for two main reasons. First, the same distribution is known to fit well for the survival of seeds and spores. Second, the negative normal distribution is intuitively the obvious choice: most pollen will survive for a certain time period, but a few grains will be able to survive for much longer. In the model, we use loss of viability as a function of distance instead of time, since it is unknown if and how the two are correlated.

After reaching the target population, the next step is fertilisation. In the model, fertilisation chances are based not only on pollen numbers present on a stigma, but also on compatibility data between source and target species. Even when a great deal of information is available regarding the compatibility differences between pollen from the GM source population and pollen from the target population, it may still be difficult to give an exact estimate of this compatibility.

Introgression will not be considered in this first version of our model. Too much information is lacking to know how to simulate this process realistically. Our model can therefore be used to estimate probabilities of outcrossing up to the stage of hybrid seed formation, but no further.

The outcome of the model will largely depend on the parameter values entered by the user. The user should be aware of two types of uncertainty associated with this. One type of uncertainty is whether the parameter values used have been estimated correctly. The other uncertainty is caused by variation in parameter values due to stochastic processes, such as the effect of weather. For the evaluation procedure, it is important to give an exact estimation of the contamination level of a given target population with modified DNA. Including confidence intervals in the program would give an idea about possible deviation from the contamination levels found. One possible way of calculating confidence intervals to account for incorrectly estimated parameter values would be to take a number of random samples around the estimated value. This feature is not included in the model at present, but we recommend that it is added to future versions.

The model will be helpful for estimating the separation distances required to reduce contamination levels with modified DNA to acceptably low levels. With additional time to develop the model further, we could consider several more complex situations. The most obvious step for further development concerns the estimation of gene flow at the landscape level, with multiple target populations surrounding one or more GM source populations. We recommend that in the future the COGEM aims to extend and refine the present model, to continually improve our estimates of the outcrossing probabilities of GM populations with cultivated or wild relatives.

Appendix A. Derivation of Pollen Dispersal Curves

A1. Exponential distribution

A negative exponential function is given by

$$f(D) = \alpha \exp(-\lambda D)$$

in which λ is a shape parameter and α is a scale parameter.

For a given λ, α can be determined by the requirement that the integral of a probability density function over the whole space of possible events has to be equal to 1. In two dimensions, this consistency requirement corresponds to

$$\int_0^{\infty}\int_0^{2\pi} f(D)\, d\varphi dD = 1$$

where D is the distance to a posit source of pollen and φ is the angular direction.
For a given distance D,

$$\int_0^{2\pi} d\varphi = 2\pi D$$

implying

$$1 = \int_0^{\infty} 2\pi\, Da \exp(-\lambda D) dD = \frac{2\pi a}{\lambda^2}$$

As a consequence

$$\alpha = \frac{\lambda^2}{2\pi}$$

For a continuous distribution function, the arithmetic mean $\bar{D}$ is given by

The average dispersal distance $\bar{D}$ of the negative exponential function is inversely proportionate to λ and given by

$$\bar{D} = \int_0^{\infty}\int_0^{2\pi} D\, f(D)\, d\varphi\, dD = \lambda^2 \int_0^{\infty} D^2 \exp(-\lambda D) = \frac{2}{\lambda}$$

For a continuous distribution function, the variance $\mathrm{var}(D)$ is given by

$$\mathrm{var}(D) = \int (D)\, f(D)\, dD = \int D^2 f(D)\, dD - D^2$$

The variance of the negative exponential function is given by

$$\mathrm{var}(D) = \int_0^{\infty}\int_0^{2\pi} D^2 f(D)\, d\varphi dD - \bar{D}^2 = \lambda^2 \int_0^{\infty} D^3 \exp(-\lambda D)\, dD - \bar{D}^2 = \frac{6}{\lambda^2} - \bar{D}^2$$

A2. Inverse power law

An inverse power law is given by

$$f(D) = \alpha D^{-\lambda}$$

where λ is a shape parameter and α is a scale parameter. A power function has the undesirable property that $f(D)$ tends to infinity for D approaching zero. We therfore use the modified version

$$f(D) = \alpha (D + 1)^{-\lambda}$$

For a given λ, α can be determined by the requirement that the integral of a probability density function over the whole space of possible events has to be equal to 1. In two dimensions, this consistency requirement corresponds to

$$\int_0^{\infty}\int_0^{2\pi} f(D)\, d\varphi dD = 1\,,$$

implying

$$1 = \int_0^{\infty} 2\pi\, Da\, (D + 1)^{-\lambda}\, dD = \frac{2\pi\, a}{(\lambda - 2)(\lambda - 1)}\,.$$

As a consequence

$$\alpha = \frac{(\lambda - 2)(\lambda - 1)}{2\pi} \quad \text{for } \lambda > 2.$$

The average dispersal distance $\bar{D}$ is inversely proportionate to λ:

$$\bar{D} = \int_0^{\infty}\int_0^{2\pi} D\, f(D)\, d\varphi\, dD = (\lambda - 2)(\lambda - 1) \int_0^{\infty} D^2\, (D + 1)^{-\lambda} = \frac{2}{(\lambda - 3)} \quad \text{for } \lambda > 3.$$

The variance is given by

$$\int_0^{\infty}\int_0^{2\pi} D^2 f(D)\, d\varphi dD - \bar{D}^2 = (1 - \lambda)(2 - \lambda) \int_0^{\infty} D^3\, (D + 1)^{-\lambda}\, dD - \bar{D}^2 = \frac{6}{(\lambda - 3)(\lambda - 4)} - \bar{D}^2$$

for $\lambda > 4$.

A3. Uniform distribution

A uniform distribution is given by

$$f(D) = \frac{\alpha}{D_{\max}} ,$$

in which α is a scale parameter.

The parameter α can be determined by the requirement that the integral of a probability density function over the whole space of possible events has to be equal to 1. In two dimensions, this consistency requirement corresponds to

$$\int_0^{D_{\max}} \int_0^{2\pi} f(D)\, d\varphi dD = 1$$

implying

$$1 = \int_0^{D_{\max}} \frac{2\pi\, a}{D_{\max}}\, D\, dD = \pi\, \alpha\, D_{\max} .$$

As a consequence

$$f(D) = \frac{1}{\pi\, D_{\max}} .$$

The average dispersal distance $\bar{D}$ is given by

$$\bar{D} = \int_0^{D_{\max}} \int_0^{2\pi} D\, f(D)\, d\varphi dD = \frac{2}{(D_{\max})^2} \int_0^{D_{\max}} D^2 = \frac{2}{3} D_{\max}$$

The variance is given by

$$\int_0^{D_{\max}} \int_0^{2\pi} D^2 f(D)\, d\varphi dD - \bar{D}^2 = \frac{2}{(D_{\max})^2} \int D^3\, dD - \bar{D}^2 = \frac{1}{2} D_{\max} - \bar{D}^2 .$$

CHAPTER 7

Summarising conclusions and implications

M. W. Smith-Kleefsman

BACKGROUND

In nature many species are structured in so-called metapopulations: a number of relatively small local populations that are spatially separated and that are connected by migration. Importantly, many species that previously were living in large continuous populations currently also resemble a metapopulation structure due to the ongoing destruction and fragmentation of their natural habitat by human activities during the last centuries (Frankham *et al.* 2004). Fragmentation has since long been recognised to significantly affect the dynamics and distribution of genetic variation in such a system (Wright 1952, Wright 1978, Slatkin 1985, Nei 1987). To describe the distribution of genetic variation within and among local populations, Wright (1951, 1978) developed the *F*-statistics, with the measures F_{IS}, F_{ST} and F_{IT}, of which F_{ST} indicates the level of genetic differentiation among populations for a monogenic trait. F_{ST} results from the equilibrium between genetic drift (increasing the differentiation) and gene flow (a homogenising force). Typically, selectively neutral molecular markers like microsatellites are used to estimate F_{ST}. For quantitative (=polygenic) traits, a different measure, Q_{ST}, is often used to quantify the degree of differentiation among populations (Spitze 1993).

Q_{ST} is defined in such a way that, in the absence of selection, Q_{ST} and F_{ST} have the same expected value ($Q_{ST} = F_{ST}$). If for a given quantitative trait Q_{ST} is larger than the F_{ST} of a supposedly neutral marker, this is viewed as evidence for diversifying processes (like disruptive selection) acting on the quantitative trait. Conversely, if Q_{ST} is smaller than F_{ST}, this is viewed as evidence for the action of homogenising factors (such as balancing or directional selection acting in the same manner in all subpopulations) on the quantitative trait (Merilä and Crnokrak 2001). Such comparisons are nowadays routinely used to infer the presence and nature of selection acting on quantitative traits in natural populations (for a recent review, see Leinonen *et al.* 2013).

The goal of this thesis was to investigate the interplay of different population dynamic and genetic processes (such as extinction-recolonisation, migration, selection and genetic drift) for the dynamics of genetic variation in metapopulations and to increase our understanding of the complex interactions between these processes within and among subpopulations. In addition, I aimed to validate whether and to what extent the relationship between the Q_{ST} value of quantitative traits and the F_{ST} value of neutral markers in experimental metapopulations is indeed indicative for the action of selection. To this end, I set up *Drosophila* metapopulations consisting of 3–10 subpopulations that were connected by gene flow and subjected to various degrees of local extinction and recolonisation. Using pupation height and sternopleural bristle number as quantitative traits, I assessed the effects of various types of selection, including selection for local adaptation. The results from my experiments and simulations clearly demonstrate that comparing Q_{ST} and F_{ST} in order to draw conclusions about the presence of selection in a metapopulation is far more complicated than originally thought, since this comparison is affected by many more processes than selection alone.

Main Results and Conclusions

Selection and genetic differentiation

In Chapter 2 and 3, I varied the presence or absence of directional antagonistic selection (further indicated by the term diversifying selection) for pupation height and investigated whether or not supposedly neutral genetic variation at microsatellite loci is also affected by this selection pressure. The metapopulations in my experiments were initiated with a high level of differentiation, both for the neutral and the quantitative traits. Based on this, one would expect Q_{ST} for pupation height to stay high in the presence of diversifying selection and to decline in the absence of selection. These expectations were confirmed by the computer simulations as well as by the experimental results (Fig. 2.3 in Ch. 2 and Fig. 3.2 in Ch. 3). Likewise, one might have expected F_{ST} for the microsatellite loci to decrease over time, because of the homogenising effect of gene flow among subpopulations and the absence of diversifying selection on the microsatellite loci. This intuitive expectation was neither confirmed by the simulations nor by the experiments. The simulations in Chapter 2 clearly show that diversifying selection on a quantitative trait has a 'spill-over' effect on the dynamics of genetic differentiation at a neutral marker locus. F_{ST} stays near to 1 in the presence of selection, which greatly contrasts the trajectory observed in the absence of selection (Fig. 2.7B in Ch. 2). The finding that F_{ST} is near to maximal suggests that selection on pupation height also effectively counteracts the homogenising effect of migration for neutral loci, leading to the conclusion that migrants exchanged by subpopulations that differ in the direction of selection do not, or rarely, lead to effective gene flow. In line with these theoretical expectations, the experiments in Chapter 3 revealed a highly significant effect of diversifying selection on pupation height on the dynamics of F_{ST} of microsatellites (Fig. 3.4 in Ch. 3). When selection differs across subpopulations, immigrants from other subpopulations will often not be locally adapted to the new conditions, causing them to leave few descendants. Hence, diversifying selection on one part of the genome leads to a reduction in effective gene flow which in turn affects genetic differentiation at other parts of the genome, a process called isolation by adaptation (Nosil *et al.* 2009).

To place the above findings in a wider perspective, I also conducted a similar experiment whereby I selected on pupation height in *Drosophila* and inferred its effect on another quantitative trait, sternopleural bristle number, which was not selected. In line with the previous findings, differentiation among subpopulations for bristle number was again significantly affected by antagonistic diversifying selection on pupation height. In fact, the stronger the selection pressure on the latter trait, the higher the divergence in bristle number (Fig. 4.6 and 4.7 in Ch. 4).

The results described above clearly demonstrate that diversifying selection for a polygenic trait not only affects the distribution of genetic variation for the trait under selection, but also for other traits, whether polygenic or monogenic, that are not under selection. This has important implications for the conclusions that can be drawn on the basis of $Q_{ST} - F_{ST}$ comparisons. In line with earlier studies (Lande 1992, Whitlock 2008), we observed that Q_{ST} and F_{ST} are on average more or less similar in the absence of selection. However, in the

presence of diversifying selection, the difference between Q_{ST} and F_{ST} is also near to zero. Following the generally accepted theory, this would lead to the wrong conclusion that no selection is present. So, these findings illustrate that the assumption that microsatellite loci can be regarded as neutral markers may be significantly violated in metapopulations when heterogeneous selection for ecologically relevant traits is present.

Population turnover and genetic differentiation

The fact that the subpopulations of a metapopulation can go extinct, but later in time can become recolonised through migrants, also termed population turnover, plays a pivotal role in metapopulation theory (Levins 1969a, Hanski 1991). Wade and McCauley (1988) showed theoretically that, in the absence of selection, this generally will lead to increased genetic differentiation among subpopulations as frequent extinction and recolonisation events result in lower effective population sizes. My simulation study confirms these findings, showing that genetic differentiation is expected to increase with extinction rate, both for a monogenic and a polygenic character (Fig. 2.7A,B left in Ch. 2). However, my simulation study also addresses the effect of population turnover on population differentiation in the presence of diversifying selection. As expected, a higher population turnover had a negative effect on Q_{ST} (Fig. 2.7A right in Ch. 2). However, even for relatively large extinction rates diversifying selection of similar intensity as in my *Drosophila* experiments was strong enough to maintain Q_{ST} at relatively high levels. The simulations reveal that genetic differentiation concerning a neutral genetic marker (measured by F_{ST}) is also reduced by population turnover, but the drop in F_{ST}, due to extinction and recolonisation, was substantially larger than the corresponding drop in Q_{ST} (Fig. 2.7B right in Ch. 2). This immediately affects the $Q_{ST} - F_{ST}$ comparison. Whereas in the absence of population turnover the difference between those measures is near to zero, the difference increases considerably when the turnover rate increases (Fig. 2.7C in Ch. 2). Hence, in the presence of similar selection pressures, the difference between Q_{ST} and F_{ST} is not stable, due to a different response of Q_{ST} and F_{ST} to population turnover. Apparently, the reduction of effective migration in the presence of diversifying selection plays a minor role in the presence of extinction and recolonisation, leading to the larger drop in F_{ST}. Most probably, this reflects the specific set-up of my experiments (and simulations): at low densities, selection was very weak, thus resembling density dependent (soft) selection, making it possible for colonists to get established even if they were locally not well-adapted. 'Hard' selection might lead to a very different dynamics of genetic differentiation than observed in our simulations.

Migration patterns and genetic differentiation

The effectiveness of migration is determined by more than selection against locally maladapted immigrants alone: *(i)* In Chapter 4, I experimentally compared two different migration schemes: island versus stepping-stone migration. I initiated the populations with a high degree of differentiation and found that differentiation was reduced more efficiently by island migration than by stepping-stone migration. This was the case for both pupation

height (in this experiment the target of selection) and bristle number. *(ii)* In another experiment (Ch. 3), effective gene flow appeared to be asymmetric: the rate of introgression seemed considerably lower for alleles of the high-pupating subpopulations into the subpopulations selected for low pupation height than *vice versa* (Fig. 3.6 in Ch. 3), so the low-pupating subpopulations mostly supplied migrants, while the high-pupating mostly received migrants. Theoretical models indicate that such asymmetries can have important implications. In particular, the populations that mainly receive migrants will achieve a lower degree of local adaptation and become genetically similar to the source populations of the migrants (Kawecki and Holt 2002, Morrissey and De Kerckhove 2009, Sexton *et al.* 2014). As a consequence, genetic differentiation may stay at low levels, even in the presence of strong diversifying selection, i.e. Q_{ST} will be lower than expected based on the selection pressure present in the subpopulations. So, based on the comparison between Q_{ST} and F_{ST}, the presence of diversifying selection will be underestimated in metapopulations with asymmetric gene flow among the subpopulations.

Environmental differences and genetic differentiation

Heretofore, I discussed situations in which phenotypic differences have a clear genetic basis. However, phenotypic differences among individuals can also result from environmental differences that modify the genotype. Most traits have found to be phenotypic plastic, i.e. a single genotype can display different phenotypes in response to environmental differences, a phenomenon referred to as phenotypic plasticity. Clinal environmental variation, such as latitudinal and altitudinal clines, present a special situation for investigating the interplay between genetic and environmental factors. Depending on how these two factors co-vary, we can distinguish two contrasting situations: *(i)* a cogradient alignment, i.e. an individual's genotype and the environment it experiences shift the phenotype in the same direction and *(ii)* a countergradient alignment, i.e. the genotype and the environment shift the phenotype in opposite directions (Levins 1968, 1969b). In both situations, the phenotypic differentiation found in the metapopulation may not be directly indicative for the amount of genetic differentiation being present. In Chapter 5, I used such clinal configurations to investigate the interplay between genetic variation and environmental variation. I specifically focussed on the consequences this had for the phenotypic differentiation among populations. To this end, I performed experiments with *Drosophila* using "sternopleural bristle number" as phenotypic trait. The phenotype of this trait is strongly affected by the developmental temperature: the higher the developmental temperature the smaller the number of bristles. The results revealed a strong significant interaction between genetic variation and temperature for this trait when these two factors co-varied. In the cogradient alignment, in which genotypic and environmental differences co-varied positively, phenotypic differentiation among subpopulations was clearly increased compared to the situation with only genetic differences present, thus both factors acted highly synergistic. On the other hand, in the countergradient alignment, in which genotypic and environmental differences co-varied negatively, the phenotypic differentiation drops to almost zero, showing that in this situation both factors acted highly antagonistic (Fig. 5.4 in Ch. 5).

This signifies that when genetic and environmental variation co-vary, either positively or negatively, the phenotypic differences observed among populations under natural conditions cannot serve as a proxy for Q_{ST}. In order to be able to make the right inferences from the $Q_{ST} - F_{ST}$ comparison, environmental differences between subpopulations should be minimised, for example by using common garden (assuming no environmental differences) or reciprocal transplant experiments (all genotypes are assayed in all environments). Neglecting environmental differences will lead to incorrect conclusions about the presence and strength of selection pressures in a metapopulation.

Final Conclusions

Although I explored only a number of specific situations, the results of the experiments clearly show that genetic differentiation in metapopulations is shaped by the complex interplay of ecological and population genetic processes that all affect the $Q_{ST} - F_{ST}$ comparison. *(i)* Divergent selection among subpopulations for a trait does not only affect the genetic structure of the trait under selection, but also changes effective migration among subpopulations. As such, it can cause isolation by adaptation among subpopulation, thereby affecting both Q_{ST} and F_{ST}. *(ii)* Frequent extinction-recolonisation events also can affect migration patterns among subpopulations and thus also affect both measures. In more extreme cases it can lead to high effective migration rates whereby genetic variation among subpoplations becomes homogenised causing both Q_{ST} and F_{ST} to approach zero despite the presence of strong divergent selection. *(iii)* The results of my cogradient and countergradient experiment clearly show the importance of environmental differences and phenotypic plasticity thereby emphasising the importance of reciprocal transplant experiments for proper the $Q_{ST} - F_{ST}$ comparisons. Overall, my work shows that, even apart from conceptual and statistical problems, the comparison of Q_{ST} and F_{ST} is not a reliable measure to assess the presence of divergent local selection for a trait.

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NEDERLANDSE SAMENVATTING

De dynamiek van genetische differentiatie in experimentele *Drosophila* metapopulaties

BEGRIPPENLIJST

Divergente selectie
Vorm van natuurlijke selectie waarbij in bepaalde omgevingen het ene fenotype een selectievoordeel heeft en in andere omgevingen het tegengestelde fenotype.

Fenotypische plasticiteit
De mogelijkheid van een genotype om in reactie op verschillende omgevingsomstandigheden verschillende fenotypes te produceren.

Gene flow
De overdracht van genen via migrerende individuen van de ene naar de andere populatie.

Genetische differentiatie
Verschil in genetische variatie tussen populaties. Er bestaan verschillende maten om genetische differentiatie te kwantificeren. F_{ST} is een maat die gebaseerd is op de variantie van allelfrequenties tussen populaties. Deze maat is vooral geschikt om de genetische differentiatie van moleculaire merkers in kaart te brengen, waarvan de allelfrequenties gekwantificeerd kunnen worden. Q_{ST} is een maat die gebaseerd is op de vergelijking van fenotypische verschillen binnen en tussen populaties. Deze maat is vooral geschikt om de genetische differentiatie ten opzichte van kwantitatieve kenmerken te bepalen.

Genetische drift
Stochastische verandering van allelfrequenties. Een toevallig verschil in het doorgeven van allelen bij voortplanting veroorzaakt fluctuaties in allelfrequentie binnen populaties en kan leiden tot verschillen in allelfrequentie tussen verschillende populaties. Het effect is het grootst in kleine populaties. Kan uiteindelijk leiden tot fixatie en verlies van genetische variatie in de populatie.

Isolatie-door-adaptatie
Sterke afname van gene flow door lokale adaptatie. Als populaties in ecologisch zeer verschillende habitats voorkomen en individuen alleen kunnen overleven als zij nauwkeurig aangepast zijn aan de lokale omstandigheden, dan zullen migranten tussen deze populaties nauwelijks kunnen overleven en reproduceren. Migratie heeft dus weinig gene flow tot gevolg, en de populaties zijn dus geheel of gedeeltelijk genetisch geïsoleerd.

Lokale adaptatie
Genetische aanpassing van organismen aan hun lokale leefomgeving. Als populaties sterk verschillen in hun leefomgeving, dan leidt lokale adaptatie tot genetische differentiatie.

Metapopulatie
Populatiestructuur waarbij de populatie verdeeld is over een aantal kleine subpopulaties; elke subpopulatie heeft een eigen demografische dynamiek, maar de subpopulaties staan wel met elkaar in verbinding door migratie.

Microsatellieten
Korte stukjes DNA (meestal tot 150 baseparen lang) die bestaan uit 'repeats' van twee tot vier nucleotiden die een aantal keren herhaald worden. Het aantal repeats is variabel. Deze variatie kan worden gebruikt om de genetische structuur van een populatie en de genetische differentiatie tussen populaties in kaart te brengen.

Migratie
Uitwisseling van individuen tussen populaties. Er bestaan verschillende modellen voor migratie: bij **eilandmigratie** kunnen individuen naar elke andere populatie migreren; bij **stepping-stone-migratie** kunnen individuen alleen naar buurpopulaties migreren.

Sternopleurale borstels
Borstels (of haren) op de linker en rechter kant van het borststuk van de fruitvlieg, ongeveer tussen de voorste twee poten.

Achtergrond

Veel dier- en plantensoorten leven niet in grote aaneengesloten populaties, maar in relatief kleine stukken geschikt habitat die ruimtelijk van elkaar gescheiden zijn door minder geschikte gebieden. Dergelijke soorten leven in relatief kleine lokale populaties (verder ook wel subpopulaties genoemd), die demografisch en genetisch gezien een grotendeels onafhankelijke dynamiek hebben, maar door meer of minder *migratie* met elkaar verbonden zijn. Zo'n samenhangend geheel heet een *metapopulatie*: een populatie van populaties.

De genetische samenstelling van een metapopulatie wordt bepaald door verschillende demografische en genetische processen. Omdat de subpopulaties veelal klein zijn, speelt *genetische drift* op lokaal niveau een belangrijke rol. Door genetische drift veranderen allelfrequenties binnen subpopulaties op een willekeurige manier. Hierbij gaat genetische variatie verloren en uiteindelijk kan dit leiden tot fixatie van één van de aanwezige allelen binnen subpopulaties. Omdat in elke subpopulatie andere genetische varianten verloren kunnen gaan en/of fixeren, leidt dit tot *genetische differentiatie* tussen de subpopulaties. *Gene flow* gaat het verlies van genetische variatie juist tegen. Door gene flow worden genetische varianten uitgewisseld tussen subpopulaties en over de metapopulatie verspreid. Gene flow is dus een belangrijk proces om genetische diversiteit binnen subpopulaties in stand te houden en gaat tegelijkertijd de divergentie tussen subpopulaties tegen. Door de tegengestelde werking van genetische drift en gene flow zal er hiertussen een evenwicht ontstaan met betrekking tot de mate van genetische differentiatie tussen de subpopulaties. Omdat subpopulaties veelal klein zijn, is ook het risico op uitsterven van zo'n subpopulatie reëel. Doordat subpopulaties met elkaar in verbinding staan door migratie, zullen uitgestorven subpopulaties geherkoloniseerd kunnen worden. Hierdoor komen er nieuwe genetische varianten binnen, maar omdat het aantal kolonisten veelal beperkt is, is de diversiteit lager dan voorheen.

Gene flow kan echter ook een minder positief effect hebben als populaties sterk aan hun eigen specifieke lokale milieuomstandigheden zijn aangepast als gevolg van *natuurlijke selectie*. Door deze *lokale adaptatie* zullen individuen die aan hun lokale milieu aangepast zijn zich beter voortplanten dan individuen van andere subpopulaties die aan een andere omgeving zijn aangepast. Wanneer selectie in verschillende subpopulaties in tegengestelde richting werkt (*divergente selectie*), kan gene flow tussen zulke populaties de mate van lokale adaptatie verlagen door het introduceren van 'niet-aangepaste' genetische varianten in de subpopulatie. Omgekeerd kan lokale selectie gene flow tegengaan, doordat de migranten minder aangepast zijn dan de individuen die al aanwezig waren in de populatie. Deze migranten hebben minder kans te overleven en zich voort te planten met als gevolg dat er effectief weinig tot geen genetische uitwisseling plaatsvindt tussen subpopulaties die aan een ander milieu aangepast zijn. Deze *isolatie-door-adaptatie* leidt tot een verhoging van de genetische differentiatie tussen subpopulaties, omdat er minder gene flow is om de effecten van genetische drift tegen te gaan. Het is op dit moment nog niet duidelijk onder welke omstandigheden gene flow adaptatie beperkt of, precies andersom, wanneer adaptatie gene flow beperkt.

Omdat het samenspel tussen de hiervoor beschreven processen grote invloed heeft op de hoeveelheid en structuur van de genetische diversiteit in metapopulaties, is dit een belangrijk onderwerp van onderzoek binnen de populatiebiologie. Genetische diversiteit is immers noodzakelijk voor populaties om zich te kunnen aanpassen aan veranderende omstandigheden en met de huidige opwarming van de aarde verandert de biotische en abiotische omgeving in hoog tempo. Om de invloed van de verschillende processen te analyseren, proberen we te schatten hoe groot de *genetische differentiatie* is tussen de verschillende subpopulaties van een metapopulatie. Genetische differentiatie kan op twee verschillende manieren worden gekwantificeerd. Voor afzonderlijke loci, waarvan de verschillende allelen te scoren zijn, zoals bijvoorbeeld voor *microsatellieten*, gebruikt men F_{ST}, een maat die gebaseerd is op de variantie in allelfrequenties tussen subpopulaties. Voor fenotypische eigenschappen die door veel genen met elk een klein effect beïnvloed worden, maar waarvan de genetische achtergrond niet bekend is, zoals in dit proefschrift verpoppingshoogte en aantal *sternopleurale borstels*, gebruiken we Q_{ST} als een maat voor de fenotypische verschillen tussen subpopulaties. Beide maten variëren tussen 0 en 1 en hoe hoger de waarde des te groter de genetische verschillen tussen de subpopulaties. Als er geen selectie plaatsvindt in een metapopulatie, dan is de theoretische verwachting dat Q_{ST} en F_{ST} voor verschillende kenmerken min of meer dezelfde grootte hebben. Wanneer echter in een metapopulatie divergente selectie inwerkt op een morfologisch kenmerk, zoals verpoppingshoogte in dit proefschrift, zal de genetische differentiatie van dit kenmerk, en dus de bijbehorende Q_{ST}–waarde, toenemen, terwijl dit niet geldt voor kenmerken die niet onderhevig zijn aan divergente selectie. Onder meer zou de F_{ST} voor microsatellieten niet beïnvloed moeten worden, want microsatellieten worden geacht niet onderhevig te zijn aan selectie. Met andere woorden: als een kenmerk als verpoppingshoogte onderhevig is aan divergente selectie, dan zou men verwachten dat de Q_{ST} voor verpoppingshoogte groter is dan de F_{ST} van microsatellieten. Omgekeerd wordt verondersteld dat het feit dat de Q_{ST} van een kenmerk groter is dan de F_{ST} van genetische merkers een indicatie is dat er divergente selectie plaatsvindt in de metapopulatie. Het is ook mogelijk dat de selectie op verpoppingshoogte in alle subpopulaties dezelfde kant op werkt. In dat geval is de verwachting dat Q_{ST} kleiner is dan F_{ST}. Daarom wordt vaak verondersteld dat het feit dat de Q_{ST} van een kenmerk kleiner is dan de F_{ST} van genetische merkers een indicatie is voor zulke convergente selectie. Onderzoek dat tot nu toe is gedaan aan natuurlijk populaties laat zien dat Q_{ST} vaak groter is dan F_{ST}. Volgens de bovenstaande redenering betekent dit dat in veel natuurlijke populaties divergente selectie plaatsvindt. Er is echter recent veel discussie of een vergelijking van Q_{ST} en F_{ST} inderdaad veel informatie verschaft over de richting en intensiteit van selectie.

In dit proefschrift bestudeer ik de wisselwerking van de verschillende hiervoor besproken populatie-genetische processen, zoals divergente selectie, migratie, genetische drift en extinctie en herkolonisatie. Het hoofddoel is om te analyseren hoe het samenspel van deze processen de structuur en dynamiek van genetische variatie in metapopulaties beïnvloedt, met de nadruk op de rol van divergente selectie in relatie tot migratie. Daarnaast wil ik het gebruik van de vergelijking van Q_{ST} en F_{ST} om conclusies te trekken over de aan- dan wel afwezigheid van divergente selectie in een metapopulatie valideren.

Experimentele aanpak

Ik heb bovenstaande problematiek op twee manieren benaderd. Aan de ene kant heb ik kortlopende experimenten uitgevoerd om natuurlijke situaties in een gecontroleerde setting na te bootsen. Dit maakt het mogelijk de invloed van de verschillende processen zowel in isolatie als in combinatie te bestuderen. Met behulp van de fruitvlieg *Drosophila melanogaster* creëerde ik simpele metapopulaties (zie Fig. 1A). Elke subpopulatie bestond uit een groepje vliegen (meestal zo'n 20–40 ouders per generatie) die elk in een aparte buis waren gehuisvest. Omdat de subpopulaties klein zijn vindt er altijd een zekere mate van genetische drift plaats. De verschillende processen werden als volgt geïmplementeerd. Migratie vond aan het begin van elke generatie plaats door 1 of 2 individuen te verplaatsen van de ene naar de andere subpopulatie. In de meeste experimenten werd dit gedaan volgens het eilandmodel (*eilandmigratie*, zie Fig. 1B links), maar in andere ook volgens het stepping-stone-model (*stepping-stone-migratie*, zie Fig. 1B rechts).

Extinctie-herkolonisatie-gebeurtenissen werden geïmplementeerd door aan het eind van een generatie alle vliegen uit één van de subpopulaties te verwijderen (zie Fig. 1A, rechts), waarna aan het begin van de nieuwe generatie deze weer door één of twee bevrucht(e) vrouwtje(s) werd gekoloniseerd.

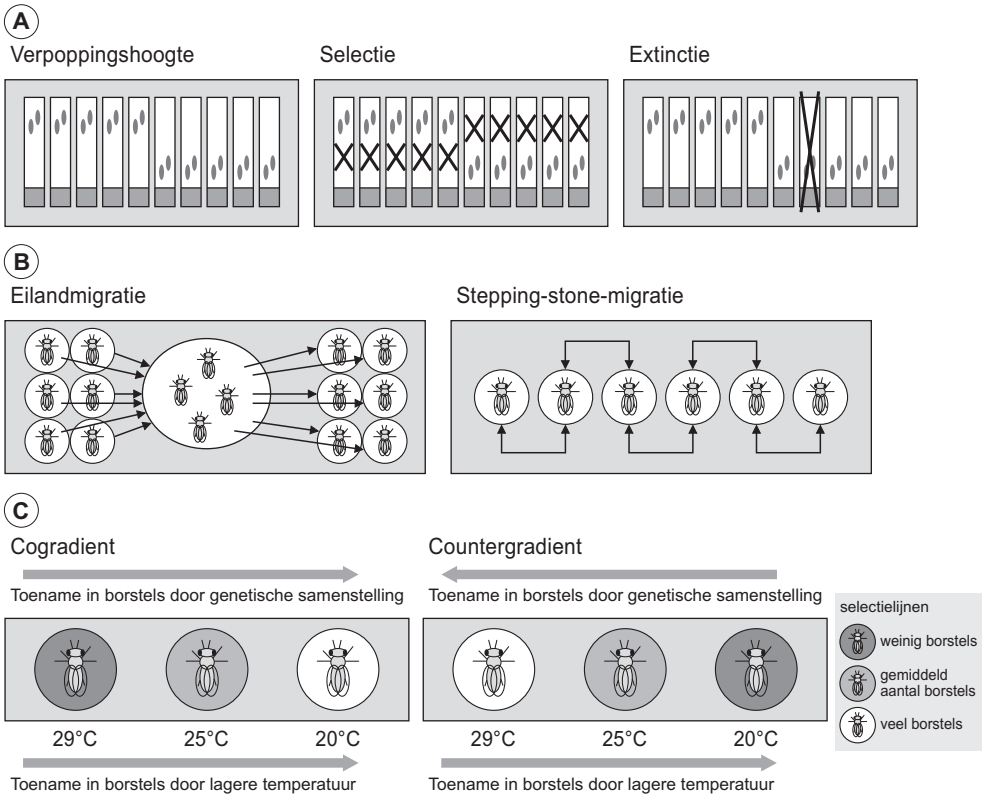
Divergente selectie vond in de meeste experimenten plaats en werd geïmplementeerd door te selecteren op het kwantitatieve kenmerk verpoppingshoogte. De hoogte waarop een larve verpopt is genetisch bepaald en voor de experimenten gebruikte ik vliegen van twee selectielijnen die genetisch sterk verschilden in verpoppingshoogte: de ene lijn verpopt hoog in de buis, dus ver van het voer, en de andere juist laag. Door in een metapopulatie sommige subpopulaties met vliegen van de hoog verpoppende lijn te starten en andere met vliegen van de laag verpoppende lijn (zie Fig. 1A links) begon ik de experimenten met maximale genetisch differentiatie voor verpoppingshoogte in de metapopulatie. Divergente selectie vond plaats door individuen die het hoogst verpopten te gebruiken als ouders voor de volgende generatie in de subpopulaties die gestart werden met vliegen van de genetisch hoog-verpoppende lijn, terwijl in de subpopulaties gestart met vliegen van de genetisch laag-verpoppende lijn juist de laagst verpoppende individuen werden geselecteerd als ouders. Op deze manier versterkt de selectie de genetische verschillen tussen de subpopulaties en kan als zodanig mogelijk de lokale verschillen in verpoppingshoogte in stand houden. Wanneer geen selectie geïmplementeerd werd, werd een random steekproef van de aanwezige vliegen, dus ongeacht hun verpoppingshoogte, gebruikt als ouders voor de volgende generatie.

Aan de andere kant heb ik computersimulaties uitgevoerd die de experimentele situatie zo exact mogelijk nabootsten. Met de simulaties kon ik veel meer generaties en meer replica's onderzoeken. Dit gaf mij de gelegenheid mijn resultaten in een breder kader te plaatsen en de gevolgen op de lange termijn te analyseren. Ook heb ik dergelijke simulaties gebruikt om theoretisch verwachtte waarden voor sommige experimenten te genereren.

DROSOPHILA EXPERIMENTEN

In **Hoofdstuk 2** bestudeerde ik de effecten van zowel divergente selectie als extincties en herkolonisaties op verpoppingshoogte en genetische differentiatie. Daartoe heb ik een kortdurend (6 generaties) *Drososphila* experiment uitgevoerd waarin ik de veranderingen in verpoppingshoogte en de mate van genetische differentiatie in verpoppingshoogte heb gevolgd in metapopulaties bestaande 10 subpopulaties zoals geïllustreerd in Figuur 1A, waarbij uit elke subpopulatie per generatie één vlieg emigreerde en daarnaast elke subpopulatie één migrant ontving (migratie volgens het eilandmodel; zie Fig. 1B links). Allereerst heb ik gekeken of de divergente selectiedruk groot genoeg was om de lokale verschillen in verpoppingshoogte in stand te houden. Ik vond dat in de afwezigheid van selectie de verschillen in gemiddelde verpoppingshoogte tussen de subpopulaties snel afnamen en dus de Q_{ST}, die in het begin hoog was, significant lager werd. In de aanwezigheid van selectie bleven de verschillen echter bestaan en ook de Q_{ST} bleef hoog ondanks de uitwisseling van migranten tussen de subpopulaties. De computersimulaties lieten zien dat ook op de lange termijn dit beeld hetzelfde blijft en dat Q_{ST} uiteindelijk bijna maximaal (Q_{ST} = 1) werd. Ik kan dus concluderen dat de divergente selectie de nivellerende effecten van gene flow domineerde en dat lokale adaptatie in deze situatie dus in stand blijft.

Extinctie-herkolonisatie-gebeurtenissen hadden in de experimenten daarentegen een tegengesteld effect. Zowel in de situatie waarbij selectie afwezig was als waarbij selectie aanwezig was, namen de verschillen in gemiddelde verpoppingshoogte tussen de subpopulaties sneller af dan wanneer extincties afwezig waren. Dus extincties-herkolonisaties lijken de homogeniserende effecten van gene flow te bevorderen. Echter, dit leidde in beide situaties niet tot een significante verandering in de mate van genetische differentiatie (Q_{ST}). Waarschijnlijk was het aantal generaties te laag om effect van extincties-herkolonisaties op genetische differentiatie te vinden. De resultaten uit het experiment werden bevestigd door de langlopende (750 generaties) simulaties. In de afwezigheid van selectie hadden extinctie-herkolonisatie-gebeurtenissen een sterk nivellerend effect op de verschillen in verpoppingshoogte tussen de subpopulaties en dit proces ging sneller naarmate de extinctiefrequentie toenam. Als gevolg hiervan werd de Q_{ST} ook snel lager om uiteindelijk op een evenwichtswaarde te eindigen die enigszins hoger was dan men zou verwachten als alleen gene flow en genetische drift een rol speelden. Echter, in aanwezigheid van divergente selectie bleven de verschillen in verpoppingshoogte tussen subpopulaties bestaan en bleef Q_{ST} significant hoger dan in de afwezigheid van selectie. De evenwichtswaarden die bereikt werden waren echter wel lager naarmate de extinctiefrequentie toenam. Daarbij dient aangetekend te worden dat bij de hoogste extinctiefrequenties bij veel metapopulaties alle genetische variatie verloren ging, omdat, als gevolg van de frequente extincties en herkolonisaties, alle individuen in de metapopulatie afstammen van een paar kolonisten in eerdere generaties (patch coalescence). In die gevallen verdwenen dus de genetische verschillen tussen subpopulaties. Uit het voorgaande kan ik concluderen dat extincties en herkolonisaties, waardoor in zekere zin de genetische uitwisseling tussen subpopulaties wordt bevorderd, de effectiviteit van divergente selectie verminderen en een negatief effect hebben op de sterkte van de lokale adaptatie.



FIGUUR 1 Schematische weergave van methoden die gebruikt zijn in de verschillende experimenten. A. Tien subpopulaties vormen samen een metapopulatie. Links geeft weer hoe het experiment is opgezet, namelijk de helft van de subpopulaties met individuen van de hoog-verpoppende lijn en de andere helft met individuen van de laag-verpoppende lijn. Metapopulaties werden dus geïnitieerd met hoge graad van genetische differentiatie voor verpoppingshoogte. Midden: Selectie werd uitgevoerd door hoge individuen te selecteren in de hoog-verpoppende en lage in de laag-verpoppende lijn. Rechts: Extincties werden geïmplementeerd door een subpopulatie te vervangen door een buis zonder individuen (Hoofdstuk 2 en 3). B. Verschillende typen migratie. Links: Eilandmigratie: alle migranten komen in een pool terecht en worden dan weer verdeeld. Rechts: Stepping-stone-migratie: migratie vindt alleen plaats tussen naast elkaar gelegen subpopulaties (Hoofdstuk 4). C. Cogradient (links) en countergradient (rechts) opstelling. In de cogradient opstelling zijn de lijnen zo bij de verschillende temperaturen geplaatst, dat de subpopulatie met veel borstels opgroeit bij een temperatuur die voor nog meer borstels zorgt, terwijl de subpopulatie met weinig borstels opgroeit is bij een temperatuur die voor nog minder borstels zorgt. In de countergradient opstelling is dit juist andersom: de subpopulatie met veel borstels groeit op bij een temperatuur die zorgt voor minder borstels en de subpopulatie met weinig borstels groeit op bij een temperatuur die zorgt voor meer borstels (Hoofdstuk 5).

Wat zijn de verwachte effecten van divergente selectie en extincties-herkolonisaties voor de genetische differentiatie voor neutrale merkers zoals microsatellieten? Om dit te onderzoeken heb ik in de simulaties ook dit soort merkers meegenomen en naar de verandering in F_{ST} gekeken. De resultaten laten zien dat F_{ST} sterk beïnvloed wordt door divergente selectie op verpoppingshoogte: In de afwezigheid van extincties-herkolonisaties

naderde F_{ST} uiteindelijk de maximale waarde. Als naast selectie ook extincties en herkolonisaties aanwezig waren, werd F_{ST} significant lager en deze reductie was groter naarmate de frequentie van extincties en herkolonisaties hoger was. Dit is vergelijkbaar met het effect van extincties-herkolonisaties op Q_{ST}. Echter, het effect van extincties-herkolonisaties was significant groter voor F_{ST} dan voor Q_{ST} in dezelfde situatie. Dus in aanwezigheid van extinctie-herkolonisaties leidt divergente selectie, zoals verwacht volgens de theorie, tot een positief verschil tussen Q_{ST} en F_{ST}. Echter, de grootte van dit verschil wordt sterk beïnvloed door de extinctie- en herkolonisatiefrequentie. Het is dus in metapopulaties waarin extincties en herkolonisaties plaatsvinden niet mogelijk om de vergelijking tussen Q_{ST} en F_{ST} te gebruiken om conclusies te trekken over de grootte van de selectiedruk. Belangrijker, wanneer alleen divergente selectie aanwezig is worden Q_{ST} en F_{ST} praktisch gelijk ondanks de aanwezigheid van een sterke divergente selectiedruk op verpoppingshoogte in de metapopulatie. In deze situaties is dus de verhouding van Q_{ST} en F_{ST} niet een betrouwbare maat om de aanwezigheid en de sterkte van de divergente selectiedruk te schatten.

Hoofdstuk 2 liet zien dat de aanwezigheid van divergente selectie niet alleen grote invloed heeft op genetische differentiatie van de kenmerken waarop geselecteerd wordt, maar in de simulaties ook de differentiatie voor neutrale merkers beïnvloedde. In **Hoofdstuk 3** heb ik onderzocht of dit ook gold voor de experimentele *Drosophila* metapopulaties die ik in hoofdstuk 2 voor verpoppingshoogte geanalyseerd heb. Omdat ik weinig effect vond van extincties-herkolonisaties op de genetische differentiatie voor verpoppingshoogte in dat experiment, heb ik hier alleen het effect van divergente selectie op de dynamiek van neutrale kenmerken bestudeerd. Op basis van de microsatellietvariatie die in de oorspronkelijke selectielijnen voor verpoppingshoogte aanwezig was, heb ik 7 microsatellietloci uitgezocht waarvoor de allelfrequenties sterk verschilden tussen de hoog- en laag-verpoppende lijn. Bij de start van het experiment was de genetische differentiatie dus zowel voor verpoppingshoogte als voor de microsatellietmerkers hoog. Voor de twee typen metapopulaties, divergente selectie aanwezig dan wel afwezig, werd in generatie 6 de allelfrequenties bepaald voor deze 7 microsatellieten en op grond daarvan heb ik de F_{ST}'s berekend en deze vergeleken met de F_{ST}'s bij de start van het experiment.

In de aanwezigheid van divergente selectie op verpoppingshoogte bleef de genetische differentiatie voor microsatellieten net als voor verpoppingshoogte, het kenmerk waarop wel geselecteerd werd, hoog, terwijl in de situatie dat selectie afwezig was zowel de Q_{ST} als de F_{ST} in gelijke mate daalden. Een mogelijke verklaring hiervoor is dat de divergente selectie op verpoppingshoogte de effectieve genetische uitwisseling tussen de subpopulaties die verschillen in de richting van de selectiedruk sterk vermindert. Om dit te onderzoeken heb ik gekeken naar de verspreiding van allelen die uniek waren voor de hoog- dan wel laag-verpoppende subpopulaties naar subpopulaties waarin ze niet voorkwamen (dus met een tegengestelde verpoppingshoogte), zowel in de aan- als afwezigheid van selectie. In de situatie waarbij divergente selectie plaatsvond, verspreidden deze unieke allelen zich inderdaad significant minder frequent naar subpopulaties waarin ze niet voorkwamen dan wanneer er geen selectie aanwezig was, ondanks het feit dat er evenveel migranten tussen de subpopulaties uitgewisseld werden in beide situaties. Dit geeft aan dat de migranten

geen of weinig nageslacht nalieten in populaties waaraan ze niet geadapteerd waren. De simulaties uit Hoofdstuk 2 ondersteunen deze conclusie. Ook daar vond wel migratie plaats, maar de migranten reproduceerden minder vaak in subpopulaties die in de tegengestelde richting werden geselecteerd dan in die waaraan de migrant geadapteerd was. Hieruit kan ik de conclusie trekken dat migratie niet altijd synoniem is aan gene flow. Eerder zagen we dat een hoge frequentie van extincties-herkolonisaties de mate van lokale adaptatie beperkt, maar in dit geval beperkt divergente selectie juist de mate van gene flow. Hierdoor wordt er een significant hogere graad van genetische differentiatie gevonden voor neutrale merkers dan verwacht op grond van de hoeveelheid migratie. Dit fenomeen wordt isolatie-door-adaptatie genoemd.

In **Hoofdstuk 4** bestudeer ik hoe de dynamiek van een kwantitatieve eigenschap, sternopleurale borstels, waarop niet geselecteerd wordt, wordt beïnvloed door divergente selectie op een andere kwantitatieve eigenschap, verpoppingshoogte. De experimentele opzet van de metapopulaties (bestaande uit 6 subpopulaties) was vergelijkbaar met die in Hoofdstuk 2, maar ik vergeleek drie verschillende selectieregimes, variërend van sterke divergente selectie tot selectie in een geleidelijke gradiënt. In grote lijnen waren de resultaten vergelijkbaar met wat ik in de voorgaande hoofdstukken heb gevonden. Het gemiddelde aantal borstelharen van de subpopulaties bleek ook in dit experiment sterk beïnvloed te worden door selectie op verpoppingshoogte: zonder divergente selectie op verpoppingshoogte namen de genetische verschillen tussen de subpopulaties zoals verwacht af voor beide kenmerken, maar in de aanwezigheid van selectie op verpoppingshoogte bleven de verschillen voor beide kenmerken groter, dus ook voor het 'neutrale' kenmerk sternopleurale borstels. Dit ondersteunt de resultaten van de voorgaande experimenten, namelijk dat de genetische uitwisseling tussen subpopulaties die in tegengestelde richting worden geselecteerd verlaagd is. Ook vond ik dat hoe sterker de selectiedruk, des te groter het verschil in het gemiddelde aantal borstels tussen de subpopulaties die in tegengestelde richting werden geselecteerd.

Omdat de mate van lokale adaptatie wordt bepaald door de wisselwerking tussen migratie en selectie, heb ik in Hoofdstuk 4 niet alleen verschillende selectieregimes, maar ook twee verschillende migratiemodellen bestudeerd: stepping-stone-migratie en eilandmigratie (zie Fig. 1B). De wijze van migratie beïnvloedde duidelijk de mate van uitwisseling tussen subpopulaties: zowel met als zonder selectie vond ik met eilandmigratie kleinere verschillen in verpoppingshoogte tussen subpopulaties dan met stepping-stone-migratie. Met name voor borstelharen, de eigenschap die niet onder selectie stond, was het effect van migratiemodel duidelijk zichtbaar: de afname in genetische differentiatie was significant groter bij eilandmigratie dan bij stepping-stone-migratie. Dus ook de manier waarop migratie tussen subpopulaties plaatsvindt heeft sterke invloed op de genetische differentiatie (Q_{ST}) in metapopulaties.

Voor het krijgen van een betrouwbare schatting van de genetische differentiatie voor fenotypische kenmerken (Q_{ST}) is het een vereiste dat de waargenomen verschillen in fenotype alleen veroorzaakt worden door genetisch verschillen tussen individuen. Echter, de meeste eigenschappen vertonen fenotypisch plasticiteit, dat wil zeggen dat het fenotype ook sterk beïnvloed wordt door verschillen in het lokale milieu. Vaak zijn omgevingsver-

schillen, zoals bijvoorbeeld temperatuur, gecorreleerd met latitude of altitude en vormen een gradiënt. In **Hoofdstuk 5** bestudeer ik hoe een temperatuurgradiënt de schatting van Q_{ST} voor sternopleurale borstels in experimentele *Drosophila* metapopulaties kan beïnvloeden, om de mogelijk storende invloed van *fenotypische plasticiteit* te onderzoeken. Ik gebruikte hiervoor weer sternopleurale borstels, omdat bekend is dat deze eigenschap negatief correleert met de opgroeitemperatuur van de larven: hoe lager de temperatuur is gedurende de ontwikkeling, hoe meer borstels de vliegen krijgen en vice versa. Hiervoor werden vliegen van de selectielijnen gebruikt die genetisch veel, gemiddeld of weinig borstels hadden. Deze lijnen werden gebruikt om simpele metapopulaties te creëren met 3 subpopulaties die elk gestart werden met één van de drie selectielijnen. Deze rangschikte ik op zo'n manier in een temperatuurgradiënt dat de invloed van de temperatuur de genetische verschillen in borstelharen mogelijkerwijs versterkte (cogradient, Fig. 1C links) of juist verzwakte (countergradient, Fig. 1C rechts) en bepaalde de OST voor borstels. Deze werd vergeleken met situaties waarbij de 3 subpopulaties alleen genetisch verschilden of alleen verschilden voor wat betreft opgroeitemperatuur.

De resultaten waren als volgt: Een temperatuurgradiënt alleen, dus zonder dat er genetische verschillen aanwezig waren, leidde tot een lage Q_{ST} die in de meeste gevallen niet significant van nul verschilde. Dit in tegenstelling tot de genetische verschillen die, in afwezigheid van een temperatuurgradiënt al in een hoge Q_{ST} resulteerden. Wanneer de temperatuurgradiënt echter met een genetische gradiënt werd gecombineerd zagen we een sterke interactie tussen de twee factoren. In de cogradient situatie versterkten de genetische variatie en de temperatuur elkaar sterk: de differentiatie tussen de subpopulaties was duidelijk verhoogd ten opzichte van de situatie zonder temperatuurverschillen en de waargenomen Q_{ST} verdubbelde bijna. In de countergradient situatie werkten temperatuur en genetische variatie elkaar zo sterk tegen, dat er bijna geen fenotypische differentiatie meer gemeten kon worden en de Q_{ST} was niet significant verschillend van nul. Dus in de countergradient opstelling werden de grote genetische verschillen tussen de subpopulaties genivelleerd door de temperatuurgradiënt en niet meer waarneembaar. De plaatsing van subpopulaties ten opzichte van een milieuvariabele beïnvloedt dus de schatting van de genetische differentiatie tussen de subpopulaties hoogst significant. De conclusie uit dit experiment is dan ook dat voor een betrouwbare schatting van Q_{ST} het noodzakelijk is te corrigeren voor omgevingsverschillen, bijvoorbeeld door de fenotypische variatie van alle subpopulaties te meten in één en hetzelfde milieu.

Pollen flow bij planten

Gene flow wordt doorgaans gezien als positief, omdat dit resulteert in verspreiding van genetische variatie. Gene flow kan echter ook minder positieve gevolgen hebben, bijvoorbeeld wanneer het resulteert in afbraak van lokale adaptatie (hoofdstuk 2 en 3). Ook praktisch gezien kan uitwisseling onwenselijk zijn, bijvoorbeeld bij uitwisseling tussen genetisch gemodificeerde organismen en hun wilde verwanten. Omdat genetisch gemodificeerd DNA niet natuurlijk is, streeft men ernaar om te voorkomen dat het gemodificeerde

DNA in de wilde verwanten terecht komt. Een belangrijke vraag is dan ook hoe voorkomen kan worden dat deze uitwisseling toch plaatsvindt. Wanneer het gemodificeerde planten betreft, die veelal sessiel zijn, wordt de vraag specifieker: hoe groot moet de afstand zijn tussen genetisch gemodificeerde planten en hun wilde verwanten zijn om kruisingen tussen beide te voorkomen? Voor de commissie genetische modificatie (COGEM) heb ik door middel van een literatuurstudie bestudeerd welke componenten van belang zijn om de kans op uitkruising van planten door middel van stuifmeel in te schatten. De resultaten van de literatuurstudie beschrijf ik in **Hoofdstuk 6**.

Het proces dat tot uitkruisen via pollen leidt, kan worden onderverdeeld in drie stappen: (*i*) Eerst moet levensvatbaar stuifmeel op de stamper van een wilde plant terecht komen. Voor verspreiding heeft stuifmeel een vector nodig en dat zijn in onze biogeografische regio met name wind of insecten. De verspreiding door wind zowel als door insecten kan zeer variabel zijn, afhankelijk van bijvoorbeeld het weer, omgevingskarakteristieken en/of de insectensoort die voor verspreiding zorgt. Over het algemeen verplaatst stuifmeel van soorten die door de wind worden verspreid zich verder dan van soorten die door insecten worden verspreid, maar de vorm van de verspreidingscurve is voor beide vergelijkbaar. (*ii*) Nadat stuifmeel op een stamper is geland, moet er bevruchting plaatsvinden. Hiervoor moet een stuifmeelkorrel concurreren met stuifmeel van de wilde verwant dat op dezelfde stamper terecht is gekomen. Of dit wel of niet leidt tot bevruchting wordt beïnvloed door een aantal componenten: de levensvatbaarheid van het stuifmeel, het tijdstip waarop het op de stamper geland is, de plaats op de stamper waar het stuifmeel terecht is gekomen en de aanwezigheid van mechanismen om zelfbevruchting tegen te gaan. Het proces dat tot bevruchting leidt is gecompliceerd en veel parameters zijn onbekend. (*iii*) Wanneer stuifmeel tot bevruchting is gekomen en zaad is gevormd, zal het gemodificeerde DNA in de loop van de generaties moeten toenemen in frequentie binnen de populatie.

De hierboven beschreven processen zijn alle zeer complex en er zijn momenteel nog te weinig gegevens bekend om de consequenties goed te kunnen inschatten. Er is duidelijk nog veel meer onderzoek nodig om te komen tot accurate inschattingen van de verschillende componenten (pollenverspreiding, met name over langere afstanden, pollen overleving en bevruchtingskans, en de mogelijkheden tot introgressie).

SLOTOPMERKINGEN

Uit de experimenten blijkt duidelijk dat de structuur en dynamiek van genetische variatie in metapopulaties door veel processen beïnvloed wordt. De wisselwerking tussen de verschillende processen die ik in dit proefschrift heb bestudeerd, zoals gene flow, genetische drift, divergente selectie, extincties-herkolonisaties en milieuvariatie is zelfs in een laboratoriumopstelling al heel complex. Divergente selectie kan leiden tot significante genetische verschillen tussen subpopulaties die verschillen in de richting van de selectiedruk (lokale adaptatie) ondanks een substantiële hoeveelheid migratie tussen die subpopulaties. Dit komt doordat migranten en hun nageslacht niet zijn aangepast aan hun nieuwe omgeving waardoor effectieve gene flow sterk beperkt wordt. Deze isolatie-door-adaptatie

beïnvloedt niet alleen de genetische structuur van het kenmerk waarop geselecteerd wordt, maar ook die van genen of fenotypische kenmerken waarop niet geselecteerd wordt. Dit heeft ook weer gevolgen voor de verwachte verhouding tussen Q_{ST} en F_{ST}. Wanneer divergente selectie voor een kenmerk gecombineerd wordt met extincties-herkolonisaties blijft deze lokale adaptatie min of meer intact, tenminste als de frequentie van extincties-herkolonisaties niet al te hoog is. Wel verlagen deze extincties-herkolonisaties de genetische differentiatie tussen subpopulaties, maar dit effect is veel kleiner voor het kenmerk waarop geselecteerd wordt dan voor neutrale kenmerken of genen. Ook dit heeft weer consequenties voor de verwachte verhouding tussen Q_{ST} en F_{ST}. Bij een hoge frequentie van extincties-herkolonisaties, waardoor heel frequent bottlenecks in populatiegrootte ontstaan, domineren gene flow en genetisch drift en is de divergerende selectiedruk niet meer in staat de lokale adaptatie in stand te houden. Uiteindelijk zal dit leiden tot verlies van alle genetische variatie uit de metapopulatie voor zowel kenmerken waarop geselecteerd wordt als kenmerken waarop niet geselecteerd wordt.

Processen als divergente selectie en extincties-herkolonisaties hebben, zoals uit de resultaten bleek, een significante invloed op de verhouding tussen Q_{ST} en F_{ST}. Voor kwantitatieve kenmerken die fenotypisch plastisch zijn is de schatting van Q_{ST} ook nog eens sterk afhankelijk van hoe de genetische variatie voor dit kenmerk correleert met de aanwezige omgevingsvariatie, wat ook weer de vergelijking van Q_{ST} en F_{ST} beïnvloedt. Dus het gebruik van de vergelijking van Q_{ST} en F_{ST} voor het bepalen of een kenmerk aan lokale selectie onderhevig is lijkt in theorie bruikbaar, maar is hoogst discutabel voor natuurlijke situaties. Tot slot dient opgemerkt te worden, dat ik maar een zeer beperkte set van situaties kon onderzoeken. Wat er gebeurt als we bijvoorbeeld de sterkte van de divergente selectie variëren of de grootte van de subpopulaties of het aantal subpopulaties veranderen is nog onbekend. Het mag dan ook duidelijk dat er nog veel meer onderzoek nodig is om de wisselwerking van alle processen met betrekking tot de genetisch structuur van metapopulaties goed in kaart te brengen. Dit is noodzakelijk gezien het belang van de hoeveelheid en structuur van genetisch variatie voor het aanpassen van organismen aan de snel en sterk veranderende natuurlijke omstandigheden die samenhangen met menselijke activiteiten.

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