Summary

The key word of this thesis is variation. Variation among individuals, together with differences in chances of survival and reproduction, and in combination with the assumption that the variation involved has a genetical component, may lead to natural selection. Genetical variation can vary on a geographical scale. One of the main problems in population genetics is the question how this geographical variation is produced and how it is maintained.

If this variation correlates with latitude, it is called clinal variation. This is also found in the common fruitfly *Drosophila melanogaster*. Intriguing examples are variation in body size and variation in allozyme and inversion frequency. Flies from tropical areas are generally smaller than flies from temperate regions. Examples of latitudinal variation for allozyme and inversion frequency are the polymorphisms for the enzymes *Adh* (alcohol dehydrogenase), *αGpdh* (α-glycerophosphate dehydrogenase) and the inversion *In(2L)t*. Almost all natural populations are known to contain the two most common alleles of each enzyme: Adh^S, Adh^F and $\alpha Gpdh^S$, $\alpha Gpdh^F$. The frequency of the Adh^S allele increases from 5-10% in populations from temperate regions to 90-100% in populations near the equator. The $\alpha Gpdh^F$ frequency varies from 40-60% in temperate areas to 90-100% in the tropics. *In(2L)t* frequencies are observed to be 0-5% in populations from temperate regions, and increase to ≈50% in the tropics.

There is still no consensus on the exact nature of the selection pressure causing this variation. Likely candidates are climatic factors like temperature and humidity, and environmental stress. It is also not clear to what degree these polymorphisms are linked. Even less evident is the relation between these polymorphisms, on the one hand, and morphological characters like wing length, on the other. It is the intention of the research described in this thesis to find causal explanations for the maintenance of the *Adh*, *αGpdh* and *In(2L)t* polymorphisms in wild populations of *Drosophila melanogaster*.

This thesis reports how we sampled wild populations along the Westcoast of Latin America (Chile, Ecuador and Panama) and measured frequencies of the alleles for *Adh*, *αGpdh* and of the inversion *In(2L)t*. For the first time a latitudinal cline has now been shown to exist on the Latin American continent. A strong correlation has been observed between the Adh^S allele and the $\alpha Gpdh^F$ allele, increasing with latitude, and, especially in the tropics, independent of the inversion *In(2L)t*. Wing length and wing area correlated positively with latitude in wild populations as well as in populations derived from laboratory-raised descendants. Large population differences were observed for the heritability and the coefficient of variation of these two traits, whereas relatively small population differences were found for development time, viability, pupal mortality, sex ratio and their norms of reaction to four developmental temperatures. No clear-cut latitudinal clines were observed for these life-history characters.

For one tropical (from Panama) and one temperate population (from The Netherlands),

using iso-female lines derived from these populations, we constructed lines of homozygote flies, which were intercrossed to obtain all possible genotype combinations for *Adh*, *αGpdh* and *In(2L)t*. We subjected these lines to stressful levels of ethanol and acetic acid, and measured larval and adult resistance to these substances. Tropical larvae and flies appeared to be more sensitive to both ethanol and acetic acid than individuals from the temperate area. In general, Adh^{FF} and $\alpha Gpdh^{SS}$ individuals within both populations are more resistant to the stressors than the other genotypes. Adh^{SS} larvae develop significantly slower when ethanol is added to the medium. Larvae and flies carrying *In(2L)t* show less tolerance to ethanol and acetic acid. The linked responses to ethanol and acetic acid suggest a correlated metabolic flux for the two substances.

These same lines of homo- and heterozygote genotype combinations were used to test the effects of desiccation resistance during the pupal stage at two rearing temperatures (20°C and 29°C). The tropical population was more susceptible to desiccation than the population derived from an area with a temperate climate. $\alpha Gpdh^{FF}$ pupae from the latter population show a lower viability at 20°C. Inversion heterozygotes develop relatively fastest when reared in a desiccated environment at 29°C.

Lastly, all genotype-combinations were subjected to a combined stress treatment: larval food competition (i.e. crowding) followed by adult starvation. Again, the temperate individuals were found to be more stress resistant than the tropical flies. Differences among the genotypes were also observed: the Adh^{FF} genotype was more resistant to crowding and starvation than the Adh^{SF} and Adh^{SS} genotypes, although this was less clear in the tropical population. Larvae homokaryous for the inversion *In(2L)t* developed slower and these flies tended to show a lower starvation resistance than the other two karyotypes, but this difference was not significantly increased by crowding. Starvation resistance appeared to correlate strongly with fat content, which is determined during the larval stage.

We can conclude that, although differences on population level are most prominent, the genotype and inversion effects on stress resistance are substantial. An important issue is that in almost all conditions, effects were measured of genotype *combinations*. Table 1 clearly shows that those genotype combinations which are found in high frequencies in the original wild populations, are the very same combinations which are performing better during stress. It is very likely that in the wild too, selection against certain genotype combinations occur especially in stressful conditions.

Table 1. Overview of the results concerning the effects of certain genotype-**combinations** on the stress resistance of an individual. Depicted are only the combinations which performed **significantly** better than the other combinations.

Stressor	Panama	The Netherlands
Ethanol/Acetic acid (Chapter 4)	no significant interaction-effect found	$Adh^{SS}/\alpha Gpdh^{SF}$ (acetic acid) $Adh^{FF}/\alpha Gpdh^{SS}$ (acetic acid)
Desiccation (Chapter 5)	$Adh^{SF}/\alpha Gpdh^{FF}$	$Adh^{FF}/\alpha Gpdh^{SS}$
Larval and adult food competition (Chapter 6)	$Adh^{SS}/\alpha Gpdh^{FF}$ (adult) $Adh^{SS}/\alpha Gpdh^{SF}$ (larval)	$Adh^{SF}/\alpha Gpdh^{SS}$ (adult) $Adh^{FF}/\alpha Gpdh^{FF}$ (adult) $Adh^{FF}/\alpha Gpdh^{SF}$ (larval)
Wild populations (Chapter 2)	± 85% of the chromosomes contains a $Adh^{S}/\alpha Gpdh^{F}$ combination	± 10% of the chromosomes contains a $Adh^{S}/\alpha Gpdh^{F}$ combination

It is important for a follow-up study to examine what exactly is the magnitude of the effect of each of the above mentioned factors on the maintenance of the allozyme and inversion polymorphisms in the wild. For example, when and where precisely does temperature as an independent factor determine the frequency of the Adh^{S} allele within a wild population, and when (and where) is this major position of temperature taken over by selection because of e.g. starvation stress against the inversion *In(2L)t*, as a result of which the frequency of the Adh^{S} allele may change too? Physiological research could provide us with some answers to the question *how*, for example, the Adh^{S} allele influences the starvation resistance of a fly. The data-set obtained through field work in Panama, Chile and Ecuador (Chapter 2 and 3) should be extended with field data from Peru and the Eastcoast of South America, to fill the gaps in our clinal data-set, and to provide us with better tools to test some unanswered hypotheses about different factors determining the allozyme and inversion frequency, and body size.