Testing *Drosophila* life-history in the field

Local adaptation in body size, development time, and starvation resistance

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Local adaptation in body size, development time and starvation resistance.

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Aan mijn vader

Contents

1

General Introduction

In this first section, I will describe chronologically how the idea for my Ph.D. project evolved, without going into details. Within this chronological framework, I will indicate in which section I will discuss the details of the aspect mentioned. In this chapter, I have deliberately chosen not to follow the standards for scientific journals, as that would have made it a highly specialised review only of interest to a select few. When writing the first and last chapters of this thesis, I have kept in mind that they are intended mainly for non-biologists. Therefore, I will be touching on issues of lesser interest to the specialist reader. I am confident that the latter will understand the need for dissemination of scientific results to the larger public. A second reason for deviating from the traditional scientific standard is that it leaves more room for thoughts not directly relevant to the project, but which place this thesis within a larger scope.

Birth of a project

In 1992 and 1993, I worked in the Philippines on a project to measure habitat-related changes in biodiversity (van der Linde 1997, van der Linde & Sevenster 2002). Biodiversity is "The variety of life in all its forms, levels and combinations[1]" (IUCN *et al.* 1991) and I wanted to find out to which degree human activity, for example deforestation or agriculture, had an influence on this biodiversity. As it is impossible to measure all biodiversity, I wanted to use a group of organisms that would be representative for the biodiversity in the area as a whole. I chose to use small *Drosophila* flies for this experiment, because they breed on rotting fruits. These fruits are essential in the tropical forest system as plants use them for dispersing their seeds, and many animals are dependent on them for food (see further under: "*Fruit-breeding* Drosophila *species*). Furthermore, due to their short generation time, these flies can track changes in the fruit availability rapidly. The result was unexpected as the biodiversity, as measured with a whole range of biodiversity indices (Magurran 1988), seemed to be unaffected despite the extreme differences between the collection site habitats. These differences between habitats were as large as that between closed canopy forest and grassland with small scrub patches and even then, human activities did not seem to change the biodiversity. However, when I compared the composition of the *Drosophila* communities collected in the different habitats, I found that these varied enormously and the community overlap was less than 10% between the extreme habitats. From this, I concluded that human activity has a great impact on the community composition. Furthermore, and despite the uniformity of all the biodiversity indices across the different habitats, a complete loss of the forest at a regional scale would result in a significant loss in regional biodiversity, as specialist species would lose their habitat.

As part of this project in the Philippines, I measured development times and starvation resistances of different species of *Drosophila*. Development time is the time between laying the egg and the emergence of the adult individual from the pupae, while starvation resistance is the time an adult individual can live when it

[1] Includes ecosystem diversity, species diversity, and genetic diversity (IUCN *et al.* 1991)

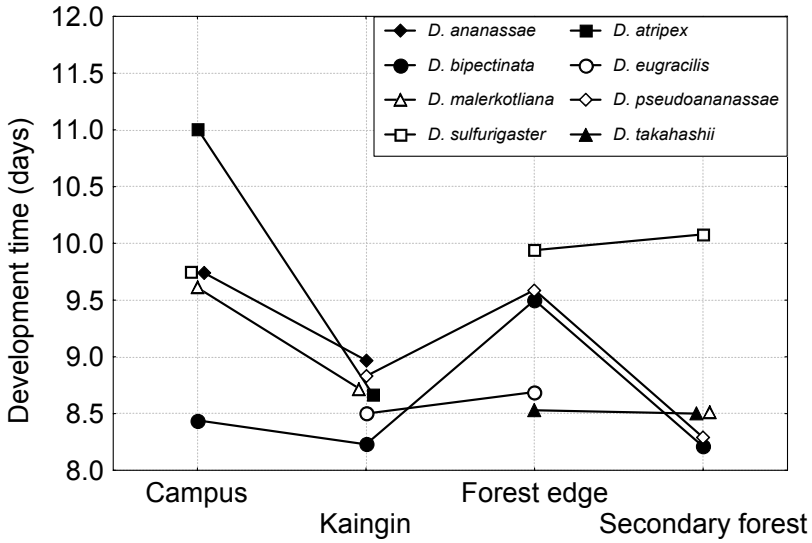


Figure 1: Development time averages (in days) per stock versus habitat. Overlapping points of different species are positioned next to each other to avoid confusion. See further chapter 2.

can not find food (see further under: *"Life-history traits")*. Sevenster & van Alphen (1993a) had found in their study on Panamanian *Drosophila* that across different species, there is a positive relationship between development time and starvation resistance and that this relationship can promote coexistence of those species (see further under "*Coexistence and life-histories*"). The *Drosophila* stocks that I had collected in the Philippines offered an opportunity to test whether this positive correlation between the two traits was also present in another *Drosophila* community. Therefore, I measured the development times and starvation resistances for all the species I had collected, but only after I had returned to the Netherlands in early 1993. The result differed from the results of Sevenster & van Alphen (1993a), as I did not find a positive interspecific relationship between the development time and starvation resistance (K. van der Linde, unpublished results).

Why is this relationship within the Filipino community so different from the Panamanian community? Several explanations could provide the answer. An interesting explanation was provided by Fischer *et al.* (2002) who investigated the relation between body size and egg size in the tropical butterfly *Bicyclus anynana*. Within populations, this relationship between body size and egg size was very shallow, only explaining a mere 1% off all variation. The same relationship including the selection lines for larger and smaller pupae and the control line was already stronger, while the correlation over different species was the strongest (Garcia-Barros 2000). Their idea is that the relation only becomes visible when a large range of differences in body size are considered. In my case, the range in

development times within the Filipino *Drosophila* community (8.2 to 11.0 days (**chapter 2**)) is much narrower then within the Panamanian *Drosophila* community (7.8 to 15.4 days (Sevenster & van Alphen 1993a)). Another option was that my laboratory populations had adapted to the new laboratory environment. The stocks were established in the Philippines several months before my return and maintained for several more months in the laboratory after my return to the Netherlands before I could carry out the experiment (see below "*From field to laboratory*"). Therefore, when I had a new opportunity to collect Filipino flies in 1994, I decided to bring the flies to the laboratory in the Netherlands immediately after collection, in this way eliminating unwanted laboratory selection as much as possible.

During my second stay in the Philippines in 1994, I reflected on the implications of the life-history model of Sevenster & van Alphen (1993b), which I will discuss in detail under the heading "*Implications*". As a consequence of these reflections, I decided to collect flies in four different habitats and to establish separate stocks for each habitat. These stocks were used in a new experiment in which I could determine again whether there is a positive relationship between development time and starvation resistance as this positive correlation is at the heart of the Sevenster & van Alphen (1993a, 1993b). The results for each of the four communities from the four different habitats of that experiment were similar with regard to the relationship between development time and starvation resistance, namely either neutral or negative (K. van der Linde, unpublished results).

Was this the end of the story? On the contrary, this marked the start of my thesis. When I plotted the development times against collection habitat, a remarkable pattern emerged (figure 1). It appeared that all populations within a habitat tended to have shorter or longer average development times compared with populations of the same species in the other habitats. This result was significant, indicating a comparable collection-site effect on the development times of the different species. The results, related to the patterns in the two life-history traits, can be found in **chapter 2** of this thesis.

Based on these results, I wrote a Ph.D. research proposal to investigate the ecological and genetic covariances among three life-history traits: development time, starvation resistance, and adult body size using a combination of field and laboratory work (see further under *"Proposal"*). This proposal is the core of my Ph.D. thesis.

My first aim was to measure life-history traits directly in the field. This has almost never been done before. When flies are brought from the field to the laboratory, many environmental aspects change, and the impact of the change varies with the magnitude of the change (see further under "*From field to laboratory*"). Therefore, in a first experiment, I measured the realised values for the life-history traits, and in a second experiment the impact of differences between the different collection habitats on the realised values for the life-history traits. For these experiments, I went to the Smithsonian Tropical Research Institute (STRI) in Panama to work directly in the field. The excellent research facilities enabled me to carry out the field

experiments as I had envisioned them. For more details on how I carried out the experiments, see under section "*Measuring life-history traits in the field*". The outcome of the experiments is described in detail in **chapter 4**, and in the overall conclusions in **chapter 6**.

My second aim was to determine whether genetic correlations between the different life-history traits exist. A genetic correlation arises when two traits have the same set of underlying genes[2] and therefore, selection on one trait will result in a corresponding change in the other trait. These genetic correlations have the potential to hamper adaptation to a new environment when the selection on one trait, conflicts with the selection of the other traits. Therefore, knowing the sign and magnitude of such correlations is essential to understand the pattern in adaptation. The fieldwork itself could provide some clues about whether genetic correlations exist and, if they exist, whether adaptation is likely to be hampered (see further under "*Genetic correlations*"; **chapter 4**). Nevertheless, additional laboratory experiments to measure the existence of such genetic correlations directly were needed. This laboratory work was carried out in the Netherlands, and is described in detail in **chapter 5**, as well as in the overall conclusions in **chapter 6**.

Life-history traits

"An organism's life history is its lifetime pattern of growth, differentiation, storage and, especially, reproduction" (Begon *et al.* 1996: p 526). In my study as published in this thesis, I have investigated several life-history traits: development time, starvation resistance, and body size. The latter is strictly speaking not a life-history trait, but body size is crucial for the understanding of the evolution of other life-history traits. A larger size may increase fecundity (egg take up space), increase competitive ability, and so on. Body size can be measured in different ways, either by measuring a body part like the length of the thorax, or by weighing the fly on a microbalance.

Coexistence and life-histories

Many mechanisms have been proposed to explain the coexistence of species, and proof has been found for many of these mechanisms in certain circumstances. Biologists still discover more ways that species can coexist. Explaining all possible mechanisms is clearly beyond the scope of this introduction; I will highlight a few relevant mechanisms. (i) Resource partitioning promotes coexistence of species because the species avoid competition as all species have their own specific food resource. (ii) Species can avoid each other in space and time. This applies, for example, to fast growing pioneer species, which occupy new gaps in the forest after an old tree has collapsed, thus creating a gap in the forest. Eventually though, they lose the competition against other, slower species, but by that time, new gaps have

[2] A genetic correlation can also arise from linkage disequilibrium, but break down more easily then genetic correlation arising from pleiotropy.

emerged, and the pioneer species remains in the system. However, not all coexistence of species can be explained in this way, as some species clearly use the same resources, at the same time, at the same place.

Drosophila flies breed on a variety of substrates, fermenting fruits being one of them, hence one of their common names: "fruit flies". Several species of *Drosophila* flies can emerge from a single piece of fruit found on the forest floor. However, if those species are kept together in a population cage, with a single source of food, one species quickly outcompetes the other. Sevenster (1992) investigated several mechanisms that can promote coexistence in *Drosophila*, and several of them indeed contributed to coexistence. In my thesis, I will focus on the implications of one of these mechanisms, namely the coexistence of species in time based on an interspecific ecological trade-off between development time and starvation resistance.

General theoretical studies (Chesson 1985, 1986, Chesson & Huntly 1988, 1989, Comins & Noble 1985, Shigesada *et al.* 1979, Shigesada 1984, Shorrocks *et al.* 1984) predict that species can coexist because they have different life histories. The environment in which the species live varies over the year with the seasons. Food is abundant at some times and scarce at others. Depending on the food availability, different species have a superior fitness. If the food availability were constant (in time and space), one of the species would consistently outcompete the others. However, as food availability varies during the year, none of the species are able to outcompete all other species.

From this observation, Sevenster & van Alphen (1993b) developed a coexistence model for *Drosophila* flies breeding on fermenting fruits, based on the positive ecological correlation between development time and adult life span under starvation. They based this on the observation of Charnov & Berrigan (1990) that 'the ratio of the developmental period to the adult life span appears to be constant within taxa[3] at the class or family level'. Central to the in Sevenster & van Alphen (1993a, 1993b) model is the ecological trade-off of two life-history traits. A fast-developing, short-lived *Drosophila* species is a better larval competitor than a slower species, simply because it is more likely to complete its minimal feeding period before the food is exhausted. Slow-developing, long-lived species have an advantage when breeding substrates are rare, because the probability that they find a new breeding site is higher due to their longer life span. The result is an ecological trade-off between competitive ability and dispersal ability that could promote coexistence because both types of species have periods of time when they

[3] A taxon (pleural: taxa) is a named group of animals/plants/bacteria which are believed to share a common ancestor and are more closely related to each other than to members of any other group. Each group, or taxon, is part of another, more inclusive group which has more members but those individual members have fewer similarities. One or more species are grouped in a genus, one or more genera are grouped into a family, one or more families in an order, one of more orders in a class, one or more classes in a phylum, and one or more phyla in a kingdom.

are superior. Laboratory and fieldwork by Sevenster & van Alphen (1993a) on *Drosophila* species from Barro Colorado Island (BCI), Panama showed the positive correlation between the two traits and the predicted negative correlation between fruit abundance and prevalent life-history strategy in the community. Moreover, Krijger *et al* (2001) showed in their study on the same community that development time was indeed positively correlated with competitive ability.

Toda *et al.* (1999) tested this model in a study on mushroom-breeding *Drosophila* from Japan. At first, they failed to find the positive correlation between development time and starvation resistance. However, they found that relative egg-size (the ratio between egg size and body size) varied a lot between species. A relatively larger egg size results in relatively larger larvae, which gives the larvae a head start compared to its smaller competitors, and thus ultimately increases the survival of the larvae. At an ecological level, it shortens the development time of the larvae without affecting the lifespan under starvation. This implies that species can improve their competitive position when breeding substrates are abundant, without shortening their longer lifespan, which has a competitive advantage when food is scarce. The expected loss of fecundity (eggs are big, so females can carry and produce only a limited number of them, and therefore a relatively larger egg results in a smaller number of eggs) associated with the larger relative egg size may be (more than) compensated by the increase in the larval survival. This shows that coexistence of species can be promoted by other combinations of life-history traits then development time and starvation resistance.

Krijger (2000) examined the role of temporal heterogeneity in maintaining community diversity by also testing the model of Sevenster & van Alphen (1993b). For all six communities of *Drosophila*, the data clearly showed that slower, competitively weaker but longer-lived species are more abundant in periods of resource scarcity. However, the average relative abundances of the faster and slower species were similar among the different communities, despite large differences in average resource abundance. Finally, he found that species diversity was positively related to the degree of temporal heterogeneity in resource abundance. This again confirmed the impact of temporal heterogeneity on the coexistence of the species.

Implications

The model of Sevenster & van Alphen (1993b) predicts that fast developing, but short-lived species can coexist with slow-developing, long-lived species in a temporal heterogeneous environment. Underlying the prediction is an ecological trade-off between dispersal ability and competitive ability at a community level. However, the model is embedded within a whole system. In this section, I will explore some of the implications of the environment on the model and vice versa.

Extinction and invasion are rare events on the broader scale of the entire metapopulation[4] within a specific habitat, but are quite frequent within local communities within such a metapopulation. A change in the local species' composition through extinction or invasion will logically change the dynamics between the species. However, increased interspecific competition between the invading species and some of the resident species, or decreased interspecific competition between the remaining species after a local extinction, could result in character displacement in the life-history traits in order to reduce the increased interspecific competition. The exact outcome of the change depends strongly on the relative position within the ranking of the other species within the community, but also on the dynamics in time. If the local turnover of species in the community is too rapid, then local adaptation is unlikely.

To illustrate this character displacement with an example, consider a community with a reasonable number of species. At one end of the range, there is a generalist species with long development time and related high starvation resistance. This species is, as predicted by the model, most abundant in times of resource scarcity. If this species goes extinct, it leaves a gap that offers opportunities for other species, most likely for the species second in line that is closest in development time and especially in starvation resistance. In time, the population of that species has the opportunity to evolve and improve its starvation resistance with an associated longer development time because there is no competitor that prevents this. This would relax competition with the species now second in line, which in turn can evolve towards the first species also. Eventually, this is expected to result in a new balance within the community.

A different situation arises when communities between neighbouring habitats are compared. Not only is the species composition different, but so are at least some aspects of the environment. The actual species composition can vary greatly between habitats, even over relatively small distances. In a previous study in the Philippines, I showed that the actual *Drosophila* biodiversity does not change between the different habitats, but that the overlap percentages[5] between the grassland and closed canopy forest communities is less than 10% (van der Linde & Sevenster 2002). The distance between these two habitats was less than 15 kilometres (see also Nevo *et al.* 1998).

Habitats differ from each other in many aspects; the species composition is merely a result of those differences. Whilst vegetation differences are the most obvious variable, many other factors are directly related to these differences. When the canopy is opened, the microclimate becomes drier, light intensity at the ground increases and daily temperature patterns and averages change. The latter occurs mainly because of increased midday temperatures, but also due to the

[4] Metapopulation: "a subdivided and patchy population in which the population dynamics operates at two different levels, within patches and between patches" (Begon *et al.* 1996)
[5] The overlap percentage is estimated as the shared proportion of individuals between two communities (Renkonen 1938).

disappearance of the dampening effect of the canopy on extreme fluctuations in the microclimate disappears (Walter 1984).

The change in vegetation often has an effect on the fruit availability during the year (Tabarelli *et al.* 1999). Fruit plantations have a large impact on the fruit availability in terms of species and numbers, as well as patterns of quality and decay. This change in fruit availability could have an impact on the coexistence of the species that show differences in their life-history traits. A high starvation resistance facilitates survival during periods of the year when fruit is scarce. If it becomes less scarce during that period, the relative importance of a long starvation resistance (surviving a long time without food) disappears and selection on this trait will be less intense. In the extreme case that fruit is readily available the whole year round, starvation resistance will not be important anymore for the coexistence of the species and development time becomes the sole factor determining the species composition.

This idea is supported by a study of Krijger *et al.* (2001) who showed that development time is an good indicator for the competitive outcome in tropical *Drosophila*. They conducted pair-wise competition experiments with seven Panamanian *Drosophila* species, in all possible combinations. Within pairs, the effect of the competition on fitness-related parameters (total mass of emerged adults, larval survival and thorax length) was significantly explained by the difference in larval development time. Consequently, a reduction of the difference in development time between species would reduce the interspecific competition within the larval stage. Other mechanisms such as aggregation will then become more important in maintaining the species diversity within the community (Krijger & Sevenster 2001, Sevenster & van Alphen 1996).

Climatic change by itself can have an impact on the life-history traits. Studies on latitudinal clines shows that flies from lower latitudes have a longer development time (James *et al.* 1995, van 't Land *et al.* 1999) and a smaller body size (Coyne & Beecham 1987, David & Bocquet 1975a, Imasheva *et al.* 1994, James *et al.* 1995, Stalker & Carson 1947, van 't Land *et al.* 1999, Watada *et al.* 1986). A more complex picture is apparent when examining starvation resistance. Hoffmann & Harshman (1999) found that tropical populations of several species of *Drosophila* have a longer resistance than temperate populations, at least in all studies on starvation resistance clines available at that time. In more recent studies, Robinson *et al.* (2000) and Hallas *et al.* (2002) did not find such a latitudinal cline in South-America or Australia, respectively. Robinson *et al.* (2000) suggest that the Indian latitudinal cline as found by Karan *et al.* (1998a), is due to the specific Indian climatic situation. Although the exact selective agent is unknown, the repeatability of several of these clines suggests a common cause, and climatic effects could be the key. Temperature-mediated artificial selection in the laboratory results in larger flies at lower temperatures (Anderson 1966, 1973, Cavicchi *et al.* 1985, Neat *et al.* 1995, Partridge *et al.* 1994a, Powell 1974) which have a shorter development time (Anderson 1966, James & Partridge 1995, Partridge *et al.* 1994a, b). When the abiotic environment has an impact on the realised life-history traits, indirectly it can

also influence the coexistence model, but it is the lack of data on this relationship between coexistence of species and abiotic environmental factors that makes predictions difficult.

Genetic correlations

One issue I frequently encountered was the idea that perfect genetic correlations between two traits can pose a barrier to adaptation (Falconer & Mackay 1996, Via & Lande 1985). If two traits share the same genetic variation, selection on one trait will result in a corresponding response in the other trait. If the selection pressures on both traits require opposite changes in the underlying genes, adaptation in one trait is retarded or made more difficult by the requirements of the adaptation in the other trait. Furthermore, it also determines the extent to which genetic correlations can evolve. Therefore, determining the sign and magnitude of the genetic correlations between life-history traits is an essential first step for exploring their role in the whole system and the species potential for adaptation to a new environment. However, there is evidence from practical and theoretical work that the above view does not always hold in more complex multiple trait situations (see for example: Blows *et al.* 2004).

The positive phenotypic correlation between development time and starvation resistance is fundamental for the life-history model of Sevenster & van Alphen (1993a, 1993b). If both traits are free to evolve independently of each other, this could potentially result in a single species that has optimised both traits in such a way that it outcompetes the other species regardless of the availability of the breeding substrate. A genetic correlation within the species could prevent such a species from evolving. Sevenster & van Alphen (1993a, 1993b) based their assumption of such an underlying trade-off on the observation of Charnov & Berrigan (1990) that 'the ratio of the developmental period to the adult lifespan appears to be constant within taxa at the class or family level'. Furthermore, they showed that between species, this positive correlation between the two traits indeed exists.

In two experiments, I investigated this interspecific positive correlation between the two traits in *Drosophila* flies from the Philippines (**chapter 2**; unpublished results). On both occasions, the result was not as expected, as the correlation was either neutral or negative. Furthermore, the pilot experiment clearly showed that there was no relation between the patterns of the two traits (**chapter 2**; unpublished results); something to be expected if such a genetic correlation existed. Therefore, I seriously started to doubt whether this genetic correlation at intraspecific and interspecific level between development time and starvation resistance was present in the field. In this thesis, I will investigate in more detail the relation between development time and starvation resistance, particularly the genetic and environmental aspects, and the potential of this correlation in retarding or limiting adaptation to new environments.

From field to laboratory

When animals or plants are collected in one environment and brought to another environment, e.g. from the field to the laboratory, we change at least some of the parameters of their environment. The stocks that I used for the first experiment in spring 1993 were collected in late summer and early fall 1992 during the fieldwork period, and first maintained for many months in the open-air laboratory in the Philippines and later in a climate room in the Netherlands. The populations were maintained at a sufficiently large size to avoid changes in the genetic composition of the species by random events (known as genetic drift). The individuals that were transferred to the new environment had to cope with the changes, while the new generations will adapt to the environmental differences between the field and the laboratory. Although I had no proof that laboratory selection is so important that it could change the outcome of an experiment measuring life-history traits, I realised that it could be of greater importance than others expected at that time (and also for recent publications on this subject: Hoffmann *et al.* 2001b, Matos *et al.* 2000a, Matos *et al.* 2000b, Matos *et al.* 2002, Partridge *et al.* 1995, but see Rose 1984, Service & Rose 1985, Sgro & Partridge 2000).

So, to exclude laboratory adaptation in the stocks, I collected new material in the Philippines in 1994, to repeat the experiment with fresh flies that had only encountered a minimum of laboratory related selection (**chapter 2**). This second experiment solved the laboratory selection issue, but the experimental environment was still considerably different from the four different collection sites. The differences in abiotic and biotic aspects between the collection sites were also considerable, so the change in environment due to the transfer to the laboratory might have been different for the different populations depending on their collection habitat if genotype-by-environment interactions were abundant (Lynch & Walsh 1998, Rose 1984). Feeling uncomfortable with this, I wanted to measure the life-history traits directly in the field. This would ensure the elimination of all possible impacts of a change in environment.

The change in environment also occurs under natural circumstances, for example when a fly migrates from one habitat to another, or when the forest is logged. Most of these changes are different from the changes encountered by a transfer from field to laboratory, but much more relevant for the flies themselves. For me, this was another reason why I wanted to measure the life-history traits directly in the field using a transplantation approach in which I could measure the life-history traits of flies cross-transferred to the other habitats under investigation.

Fruit-breeding *Drosophila* species

In this study, I used various species of fruit-breeding *Drosophila* flies for the experiments. *Drosophila* flies are frequently used in research studies because they are easy to handle, easy to rear in large numbers on artificial breeding substrates and have a short lifecycle of just several weeks for most species. Furthermore,

many mutations are known (Lindsley & Zimm 1992) and the genome of the best-known species is mapped completely (Adams *et al.* 2000). These advantages result in the frequent use of *Drosophila*'s as a model organism. This is clearly reflected in the large number of publications on this organism.

However, there are also some additional arguments for the use of them especially for ecological field studies. *Drosophila* flies use a variety of substrates to breed on. These include rotting fruits, fermenting sap fluxes, decaying plant materials, flowers, and a whole range of more exotic substrates. Fruits are an important factor in the tropical ecosystem (Clark *et al.* 2001, Riera 1995), and the percentage of fruiting trees are often reduced with the degradation of the habitat (Tabarelli *et al.* 1999). Fruits are an important source for food for many animal species, ranging from primates to insects. Decline in fruit availability often results in a subsequent decline of frugivorious species (Chapman & Onderdonk 1998, Heydon & Bulloh 1997, Loiselle & Blake 1991, 1993, McCarty *et al.* 2002, Peres 1994, Pontes 1997, Poulin *et al.* 1994). Krijger (2000) showed in his comparison, that overall fruit abundance is indeed lower in the disturbed collection sites compared to the undisturbed collection sites, and that the lower fruit availability resulted in a lower *Drosophila* diversity. The similarity in responses to changes in the fruit abundance of fruit feeding birds and mammals on the one hand and fruit-breeding *Drosophila* on the other hand makes the *Drosophila* flies a suitable choice for this kind of experimental study as they are likely to respond quickly to changes in the environment, and results may be directly extrapolated to other species.

There are over twelve hundred *Drosophila* species world wide (Bächli 1999) and these are found in many different habitats. Some species, like *D. melanogaster* and *D. simulans*, are true generalists, in the sense that they occur in every corner of the world, closely following human habitation. Other species are much more specialist and can have very restricted ranges. The lifecycle of all these species is very similar and starts with a fertilised female, laying eggs on a suitable substrate. After some hours up to a few days, a larva emerges from the egg and starts to feed on the yeast, bacteria, and nutrients available in the breeding substrate. After four to more than 8 days depending on the species, the larva will pupate. After four to seven days, an adult fly emerges from the pupae. The whole development time from egg to adult usually takes between seven and 15 days, depending on the species and temperature. The newly emerged flies mate and disperse to find a new suitable breeding substrate.

Measuring life-history traits in the field

The evolution in life-history traits in *Drosophila* is almost exclusively studied in the laboratory (Hoffmann 2000), except for two recent field cage studies on fecundity (Hoffmann *et al.* 2003b, Mitrovski & Hoffmann 2001) and one study involving laboratory measurements on field collected flies (Sgro & Hoffmann 1998), in which the effect of the transfer to the laboratory on the realised fecundities is unknown. Furthermore, some papers are published on aspects such as body size; however

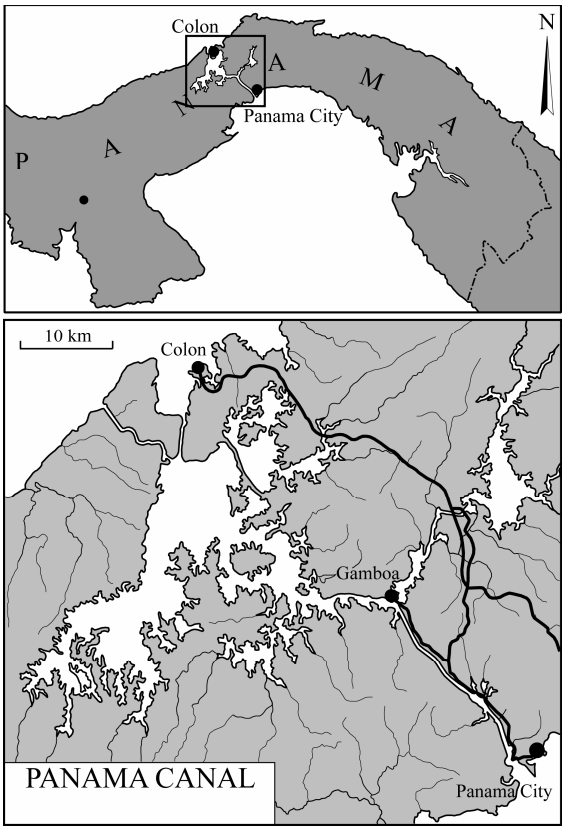


Figure 2: Map of the Canal Zone in Panama with the two field sites indicated as described in the text.

this is strictly speaking not a life-history trait but a morphological trait and needs only collection of the individuals and measuring of the stored (dead) flies. Measuring life-history traits such as development time and starvation resistance in the field directly has never been done before. However, for our understanding of the dynamics in the field, measuring the traits directly in the field is essential. The question that remained was how to do it.

As with many things, it starts somewhere unexpected, with my lightweight tent that I use for trekking through the mountains and other places in this world. I bought it because I was guaranteed that it would keep the midges out, nasty little biting insects abundantly available in northern areas of this world. The netting used in the tent is extremely fine and open enough to have the wind blow freely through the tent when both doors were open. One call to one of the better outdoor stores and a subsequent trip to that store provided me with the key to perform the experiments the way I wanted. With the netting, I made small cages of iron wire for the development time experiment, which were 12 cm high and 10 cm in diameter. These cages were placed in a water lock, so that the insects could not enter or escape. The netting proved during the fieldwork to be fine enough to exclude the smallest parasitoids in Panama from the developing larvae, and simultaneously open enough to provide almost the same conditions inside as outside the cages.

A petridish with moist vermiculite was placed in the cages on which the pieces of banana with the developing larvae were placed. Extracting the emerged flies was easy as disturbed flies fly towards the light. Small petridishes with agar as a water resource and covered with the same netting were used for the starvation resistance experiment. All the cages and petridishes were placed in a large roofed cage with iron gauze of 5 mm mesh. The cage kept the larger animals out as well as protecting the contents against the daily rain showers, as I am not interested in the 'disaster ecology' related to either of them.

Proposal

All the thoughts I described until now materialised into a Ph.D. research proposal of which this thesis is the outcome. What I proposed was to investigate the ecological and genetic covariance's among three life-history traits: development time, starvation resistance, and adult body size using a combination of field and laboratory work. I expected that by linking genetics and ecology, I would be able to provide new insights into the evolution of life histories in natural environments.

In brief, I carried out four experiments: two experiments in Panama directly in the field, and in the laboratory in the Netherlands, a common environment experiment and a half-sib design experiment. I worked with the locally available *Drosophila* species. There are about 30 species of *Drosophila* present at Barro Colorado Island (BCI), Panama (Sevenster & van Alphen 1993a, 1996), but not all can be reared in the laboratory or can be caught in sufficient numbers and habitats to be of interest for my project. I expected to collect in total about 15 to 20 species within the first part of the project, something that indeed worked out. Twelve species were collected in sufficiently large numbers and from at least three sites; the remaining species were excluded.

Field experiments

The field experiments were carried out in the Canal Zone, comprising the variety in habitats I needed (figure 2). I selected six sites for the collection of the flies and the experiments. Each transect of three habitats had one closed canopy forest site, a grassland site, and an intermediate zone site. One transect was located near and in the Botanical Gardens of Summit, the other transect was closer to the town of Maria Eugenia, and all six sites were easy to approach by car.

In 1998, I went to Panama for the first fieldwork period. The first step was to collect *Drosophila* flies in the field and to establish stocks in the open-air laboratory. Banana was used as a standard breeding substrate to maintain the cultures, because none of the natural fruits is available during the whole fieldwork period but bananas are. Besides that, most tropical Drosophila species breed without problems on bananas. The following two field experiments were carried out in the months after the initial collection.

FIELD EXPERIMENT 1: EXPRESSION OF LIFE-HISTORY TRAITS IN THE ORIGINAL HABITAT.

The aim of the first experiment was to measure the expression of the three life-history traits, development time, starvation resistance, and body size, directly in the original environments. This provides us with an initial description of the life-history traits of the populations, as well as the level of variation within and between species, habitats and transects. The results of this experiment are described in **Chapter 4**.

FIELD EXPERIMENT 2: CROSS-TRANSPLANTATION EXPERIMENT, OFFSPRING OF MANY FEMALES.

The aim of the second experiment was to unravel the interaction between the environment and genetics. Therefore, we wanted to measure the expression of the flies in the different habitats. Due to the workload, this was possible for a selection of four species that are representative for the whole *Drosophila* community in the research area. If these species show the same pattern, it is likely that closely related species will also show the same tendencies. The advantage of field measurements is that the response of the species to a new habitat is what we can expect of them when they migrate to such a new habitat. The results of this experiment are also described in **Chapter 4**.

Common environment experiment

The common environment experiment was carried out immediately after I returned to the Netherlands, in early 1999. In this experiment, all species were measured in one standard laboratory environment, and this provides information on the degree of genetic differences between species. The advantage over field experiment 2 is that now we could measure all the species and stocks, covering a broader range of species. The results of this experiment are also described in **Chapter 4**.

Genetic experiments

The aim of the genetic experiment was twofold. First, I wanted to determine the heritabilities[6] of the different traits. Adaptation in a trait can only take place when there is ample genetic variation available for that trait. Second, I wanted to determine whether genetic correlations existed between traits, and if so, how strong they were. As explained above, if selection pressures on two traits require opposite changes in the underlying genes, adaptation in one trait is retarded or made more difficult by the requirements of the adaptation in the other trait. Therefore, I estimated the sign and magnitude of the genetic correlations between the different traits as they are essential to understand the observed pattern in ecological adaptation. The scale of the experiments again required a restriction to three species representative for the whole group. This experiment was conducted too long after the first collections were made, and we, therefore, made new collections in Panama, now over a wider climatic range. The results of this experiment are described in **Chapter 5**.

[6] Oversimplified, the heritability of a trait is the proportion of the all phenotypic variation among individuals in a population that is explained by the underlying genetic variation.

2

Local adaptation of development time and starvation resistance in eight *Drosophila* species of the Philippines

Abstract:

The coexistence model of Sevenster & van Alphen (1993a, 1993b) is based on an ecological trade-off between development time and starvation resistance, acting in a heterogeneous environment. Heterogeneity can result from variation in the vegetation that influences both abiotic (e.g. temperature, humidity) and biotic aspects (e.g. fruit availability during the year) of the environment. In this study, we investigated whether differences between the habitats have led to local adaptation of the two life-history traits underlying the model: development time and starvation resistance. *Drosophila*'s were collected in four habitats, ranging from grassland to secondary forest, along a transect of 15 kilometres. The microclimatic and vegetation differences among these habitats were considerable. For development time, different species showed similar genetic responses to different habitats. The shortest development times were found in the secondary forest populations and the agricultural area populations, the longest in the grassland populations while the forest edge populations were intermediate. However, there was no correlation between the habitat ranking based on disturbance and canopy cover, and the ranking of the development times. Local selection did not seem to have a consistent effect on starvation resistance. Furthermore, the data did not confirm the generality of the positive correlation underlying the coexistence model.

Introduction

Sevenster and van Alphen (1993b) developed a coexistence model for fruit-breeding *Drosophila* flies, which is based on a positive correlation between development time and adult life span under starvation. This model also draws on general theoretical studies (Chesson 1985, 1986, Chesson & Huntly 1988, 1989, Comins & Noble 1985, Shigesada *et al.* 1979, Shigesada 1984, Shorrocks *et al.* 1984). Fast-developing, short-lived *Drosophila* species are better larval competitors than slower species (Krijger *et al.* 2001), while slow-developing, long-lived species have an advantage when breeding substrates are rare, as their longer life-span gives them a better chance to reach a new breeding site. The resulting ecological trade-off between competitive ability and dispersal ability promotes coexistence due to temporal variation, as both types of species have periods of time when they are superior. Laboratory studies and fieldwork on *Drosophila* species from Barro Colorado Island (BCI), Panama demonstrated a positive correlation between the two traits (Sevenster & van Alphen 1993a), together with the predicted negative correlation between fruit abundance and prevalent life-history strategy in the community (Krijger 2000, Sevenster & van Alphen 1993a).

A change in forest environment often has an impact on the fruit availability during the year (Tabarelli *et al.* 1999). This also holds in fruit plantations in terms of species and numbers, as well as in patterns of quality and decay. Besides direct effects on the community composition, this external change in fruit availability could have an impact on the coexistence of the species, when this is based on differences in their life-history traits. A high starvation resistance facilitates survival during periods of the year when fruit is scarce, but when it becomes less scarce during that period, the relative importance of a high starvation resistance decreases and selection on this trait will be less intense. In the extreme case that surplus fruit is readily available throughout the whole year, starvation resistance will not be important for the coexistence of the species. Development time now is expected to becomes the sole factor that determines the species composition, and a reduction in development time due to selection will occur within slower species (Krijger *et al.* 2001).

Besides changes in the biotic environment, changes in the vegetation also lead to changes in the local microclimate. The difference in average air temperature between closed canopy and open vegetations can be several degrees centigrade, mainly due to a higher maximum temperature in open vegetation (Walter 1984). The variation in the actual local temperatures is even higher than the air temperatures as recorded by standard measurement techniques. Vegetation that is more open causes a higher light intensity on the ground. In a closed canopy tropical rainforest, less than 1% of the light reaches the ground (Walter 1984). Both temperature and openness affect humidity and the air is near saturation throughout the day in closed canopy forest but fluctuates greatly in more open vegetations (Walter 1984).

Research on large-scale clines has given some insights in the question whether development time responds to climatic variation. James and Partridge (1995) studied *Drosophila melanogaster* populations collected along a latitudinal cline from Australia and found that larvae from higher latitudes developed faster at intermediate experimental temperatures. However, the correlation depends heavily on one population measured at low latitude (van 't Land 1997). Van 't Land et al. (1999) also found a correlation between latitude and development time on their *D. melanogaster* cline in South- and Central-America, but it explained only 0.1% of all the variation. Laboratory temperature selection on development time shows that lines adapted to low temperature have a relative shorter developmental time compared to those adapted to high temperature, when measured at the same temperature (Anderson 1966, James & Partridge 1995, Partridge *et al.* 1994a, b). The latitudinal cline data predict the same pattern as the temperature selection data, and therefore, we expect that opening the canopy (e.g. higher temperatures) will result in longer development times.

All studies mentioned by Hoffmann and Harshman (1999) on starvation resistance clines, indicate that the tropical populations of the various *Drosophila* species have a better resistance than the temperate populations (Da Lage *et al.* 1990, Karan *et al.* 1998a, Karan & Parkash 1998, Parkash *et al.* 1994, Parkash & Vandna 1994, Shamina *et al.* 1993). In more recent studies, Robinson *et al.* (2000) and Hallas *et al.* (2002) did not find such a latitudinal cline for either *D. melanogaster* in South-America or *D. serrata* in Australia, respectively. Parkash and Munjal (1999) found that for their Indian cline the higher starvation tolerance was positively correlated with the minimum temperatures, higher metabolic stress in relation with smaller body size and higher population density and competition. Taking this into account, we expect a more open canopy (e.g. higher temperature) to result in a higher starvation resistance.

Based on the above, we expect that small-scale variation between habitats with regard to vegetation and derived aspects such as microclimate and (patterns in) fruit abundance is considerable and will select for differences between populations. The persistence of the selection effect will depend on the rate of gene flow counteracting it. We also expect that the differences between the habitats will select for similar responses in different species with approximately the same life history. Furthermore, microclimatic changes fluctuate systematically with the change in canopy cover, and if these factors determine local adaptation, we expect a correlated response between degree habitat ranking (as based on the degree of disturbance (van der Linde & Sevenster 2002)) and realised life histories.

The general existence of a genetic correlation between development time and starvation resistance is still debated. Selection for increased starvation resistance in *Drosophila melanogaster* sometimes led to a corresponding increase in development time (Chippindale *et al.* 1996, Harshman *et al.* 1999). However, Zwaan *et al.* (1991) did not find a phenotypic correlation between development time and starvation resistance in flies 15 or 28 days after eclosion, nor did they (Zwaan *et al.* 1995a) find a correlated response for starvation resistance in their upward or

downward selection lines for development time. Robinson *et al.* (2000) did not find a cline for starvation resistance along the pacific coast of South-America, while the same transect did show a minimal cline for development time (van 't Land *et al.* 1999), suggesting the absence of a genetic correlation between the two traits. At the interspecific level, Sevenster & van Alphen (1993a) found a positive interspecific correlation between development time and starvation resistance for Panamanian *Drosophila*, while Toda & Kimura (1997) found a negative interspecific correlation for mycophagous Drosophilids of Japan.

Few studies have investigated the effects of local selection on a small geographical scale (Capy *et al.* 1987, Karan *et al.* 1999, Nevo *et al.* 1998, Vouidibio *et al.* 1989), although the small-scale variation in microclimate, vegetation, and related biotic factors can be considerable (Walter 1984). Our collection sites, in four different habitats, were located on a transect of about 15 kilometres, thus excluding macroclimatic differences, while the different habitats ensure differences in the microclimate, vegetation and related biotic factors. Our primary goal is to test whether local adaptation in life-history traits occurs, and to try to relate this to variation between habitats in biotic or abiotic factors. We collected flies from different populations and measured the two traits in the F_3 generation in a common laboratory environment. With this set-up, we can show for the two life-history traits whether genetic differences between the populations were present. More specifically, we have drawn up four expectations. First, we expect there to be genetic variation within species between populations from different habitats. Second, we expect that, if there is variation, the patterns within the single species are similar within all species. The third expectation is that the pattern between the habitats follows the habitat ranking based on disturbance and canopy cover, as various microclimatic variables are correlated with canopy cover. The final prediction, based on the assumed underlying positive correlation between the traits, is that we expect the two overall patterns for development time and starvation resistance to be similar, and that this positive correlation is found in all four different habitats.

Material and Methods

COLLECTION AREA

Frugivorious *Drosophila* were collected in the Philippines, in October 1994. The collection site was east of the town of Cabagan, in Isabela province, on the slopes of the Sierra-Madre (17.5 latitude, 122 longitude). This mountain range, in the north-east of Luzon, is bounded to the east by the Pacific and to the west by the Cagayan Valley.

The Sierra-Madre has one of the last remaining larger areas of tropical rainforest in the Philippines; it is the largest piece of the mere five percent of tropical rainforest that remains in the Philippines (Danielsen *et al.* 1993). By now, the Central Valley area is either grassland or agricultural fields and plantations containing rice and

other commercial crops. Towards the mountains, it changes first to kaïngins (see below), then to secondary forest and finally to primary forest.

The transect ran east-west at right angles to the vegetation zones; collections were made in the following four habitats. These are ranked from most to least disturbed, and from west to east:

Campus (C): Grass is the dominant vegetation (±70%) in this most disturbed habitat. Patches of scrub (±20%) are relatively regularly distributed in the grasslands. The remaining area consists of roads and buildings. Canopy cover is not more than 10%. Distance to next site about 10 km.

Kaïngin (K): This is an agricultural system related to slash and burn, but with a more permanent character. Regeneration is scarce; grasslands become established after the soil is denuded. Canopy cover is on average 25%. Distance to next site about 1 km.

Forest Edge (E): This is the intermediate zone between the Kaïngins and the Secondary Forest, and is essentially a mosaic of the two types. Canopy cover is about 35%. Distance to next site about 1 km.

Secondary Forest (S): This is the dipterocarp forest, the least disturbed habitat, with a canopy cover of about 50%. Distance to next site about 1 km.

The collections were made simultaneously in four different habitats, which ranged from grassland to secondary forest. The difference in floral composition between the habitats was large enough to expect effective differences between the habitats (Danielsen *et al.* 1993, Walter 1984).

COLLECTIONS

The *Drosophila* were collected with oviposition traps. Four traps were placed in each of the four different habitats with at least 200 meters distance between consecutive traps. The traps were constructed out of 500-ml transparent containers suspended from a thin nylon cord of about one meter. A hole of Ø 2.5-cm, covered with 1.5-mm mesh, was positioned on one side of the trap. The hole faced slightly downwards to prevent rain from coming in. The mesh allowed *Drosophila* access to the bait inside for oviposition, but prevented larger animals from entering. A "Manila" banana was used as bait.

The traps were exposed in the field for one week. The bananas with the eggs and larvae were taken to the laboratory in the Netherlands immediately after collection

in the field. In the laboratory, the flies were kept in a climate room at 25°C, 70-85% RH and 13:11 light:dark, roughly corresponding with the natural microclimate. The long-term (1994 -1998) macroclimatic temperature averages for Tuguegarao was 26.8 ºC (PAGASA 2001), and this site is comparable with the campus collection site, while the higher canopy cover in the other collection sites will result in lower local temperatures.

Iso-female lines were set up to isolate and identify the different species, as positive identification of the females in certain species subgroups is difficult (Bock 1971, Bock & Wheeler 1972). The iso-female lines of the same species and habitat were then combined in one stock. The number of iso-female lines per stock was not recorded in detail, but varied roughly with the abundance in the field and most stocks comprised more then 10 lines. In total, 25 stocks belonging to 12 species were established (Table 1).

The available fruits differ between the natural habitats and therefore we used banana during all stages of this study as a standard medium. Banana has proven to be a general accepted breeding substrate for many fruit-breeding *Drosophila* species, contrary to standard breeding media used for *Drosophila* (J. G. Sevenster, C. L. Krijger, K. van der Linde, E. Baldal, unpublished results). The use of one standard substrate makes comparison between population possible as it avoids interpretation problems arising from the use of different breeding substrates.

LIFE-HISTORY PARAMETERS

The offspring (F_2) of the stocks (F_1) were used in the experiment. About forty F_2 flies were put on a fresh slice of banana dipped in yeast suspension, which was on a layer of moist vermiculite. The vermiculite is used by some species to lay eggs on, and by most species to pupate in. In some insect species, stored mature eggs start developing before laying, thus decreasing the measured development time; therefore, to prevent stowage of eggs, the flies were put on a slice of fresh banana dipped in a yeast suspension for two days. For the actual experiment, the flies were allowed to lay eggs for one hour (14:00 – 15:00 hours) in order to synchronise the egg laying. Furthermore, this time window eliminated the potential impact of time-of-day specific egg laying preferences between populations (Dahlgaard *et al.* 2001). The newly emerged offspring (F_3) were collected once a day at 14:00 hours. The time of day was chosen based on the observation that emerging flies show clear diurnal rhythms (Bakker & Nelissen 1963, Belcher & Brett 1973, Pavan *et al.* 1950); most individuals emerge during early morning, in the first hours after sunrise. The collection of flies at several times a day did not improve the accuracy of the development time measurements in a previous experiment (K. van der Linde, unpublished data), probably due to these diurnal rhythms.

Developmental time was measured as the time from oviposition until eclosion of the adult. Starvation time was measured as the time that freshly emerged adults lived after eclosion from the pupae under the availability of water but no food (Sevenster & van Alphen 1993a). The newly emerged adults were transferred in batches of no

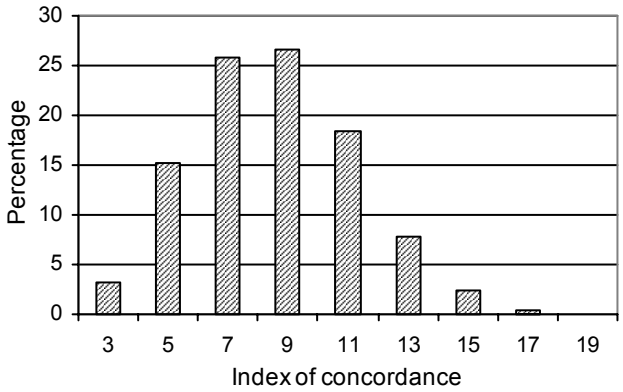


Figure 1: Expected distribution of the overall concordance indices as generated by the randomisation test. Total number of runs was 10.000.

more than 10 flies, to 10-ml tubes with a 2.5-ml layer of plain agar. Dead flies were counted once a day at a fixed time. The whole experiment was carried out with three replicates, starting with the F_2 flies, and in the same climate room in which the stocks were maintained.

The 24-hour period, either between two subsequent collections of the emerged flies or two subsequent counts of the deceased flies, introduces a bias as the flies have emerged and died during the whole 24-hour period. Taking the midpoint between two observations would only give a higher estimate, not a more accurate estimate, and therefore, the data were not corrected in any way, as the bias was the same for all species.

STATISTICAL ANALYSIS

We calculated average development times and starvation times for each individual vial in the experiment. Stock averages were calculated from these three vial averages and therefore, standard deviations could not be estimated. The stock averages were used to test the last three predictions, while the individual data were used to test the first prediction. We used linear regression analysis with the life-history traits as the dependent variables test for a possible influence of density on the life-history traits. As we found no effect of number of individuals per replicate (see under results), no additional corrections for number of individuals were made.

The first question about the extent of genetic variation between populations within the same species was tested using a nested ANOVA design. The dependent variable was the measured development time or starvation resistance of the individuals. The independent variables were population and replicate. The latter was entered as a random variable, and nested within population because the replicates between populations were independent of each other. Due to the large number of tests, we tested whether the number of significant results was higher than could be expected based on type 1 errors, using a binomial test. The 24-hour interval between subsequent scorings of emerged or dead individuals could potentially influence the results of the ANOVA's. However, tests with data collected at a previous experiment, in which we collected freshly emerged flies or deceased

Table 1: Population averages for all species and populations. DT = development time in days; SR = starvation resistance in days. All rearing at 25°C. For a species overview of the Philippines, see Baltazar (1991) and http://www.kimvdlinde.com/professional/biology/drosophila/philippines/ for an updated checklist.

Species	Campus		Kaïngin		Forest Edge		Secondary Forest	
	DT	SR	DT	SR	DT	SR	DT	SR
D. ananassae Doleschall	9.74	1.98	8.97	1.9				
D. atripex Bock & Wheeler	11.01	1.27	8.67	2.45				
D. barbarea Bock & Wheeler					10.01	2.27		
D. bicornuta Bock & Wheeler	10.83	2.5						
D. bipectinata Duda	8.44	2.2	8.23	2.05	9.5	1.5	8.21	2.08
D. eugracilis Bock & Wheeler			8.5	2.25	8.69	2.68		
D. malerkotliana pallens Bock & Wheeler	9.61	1.42	8.72	1.9			8.51	1.84
D. species 1 (1)							8.74	3.34
D. parabipectinata Bock	8.53	2.28						
D. pseudoannanasae pseudoannanasae Bock			8.83	2.56	9.59	1.92	8.29	2.04
D. sulfurigaster albostrigata Wheeler	9.75	2.91			9.94	3.18	10.08	2.79
D. takahashii Sturtevant					8.53	2.29	8.5	1.83

(1) An unidentified species belonging to the *Drosophila nasuta* subgroup of species.

flies three times a day, showed that combing the three daily scoring did not alter the outcome of the tests in a significant way.

With the remaining questions, we ran into the same problem that only one *Drosophila* species was present in all four habitats (Table 1, *D. bipectinata*), leaving open many possible combinations of species and habitats (table 1). We employed randomisation procedures (Gotelli & Graves 1996) in order to test the hypothesis that differences between habitats will select for similar responses in different *Drosophila* species.

The second question, that patterns within different species are similar, implies no a-priori order in the habitats. Therefore, we used an index to test for overall concordance of the within-species patterns for the different species. Our concordance index first counts the number of times a value is highest in each of the two habitats and then takes the absolute value of the subtraction of those two values. The higher this concordance value, the more similar the species reacted. An uneven number of species within a two-habitat comparison results in a minimum value of one. With four habitats, this resulted in six two-habitat comparisons, which are combined to one single value for overall concordance. The second step was to randomise the available populations within each species separately. The concordance index for the randomised combination was calculated and repeated 10,000 times. A theoretical distribution of concordance indexes was created from the calculated values. Due to three (out of the six) two-habitat comparisons with odd numbers of species, the minimum value for our data sets was three and the values ranged between 3 and 19 (with step of 2), with 317, 1512, 2589, 2665, 1846, 790, 231, 47 and 3 hits respectively (Figure 1). The fraction of the 10,000 runs that had the same value as the original value or larger, indicates the probability of finding that value. The one-sided critical (5%) value of the overall concordance index is 15 ($p = 0.0281$).

For the third question, the index should accurately indicate the overall matching between an overall pattern with the *a-priori* habitat ranking. Therefore, we replaced the non-blank values by ranks within every species. For every run and within each run for every species separately, the non-blank cells were randomised. For every possible combination of two non-blank cells within a species, the difference between the ranks was calculated and summed. The total values ranged between -26 and 26 (with step 2), with 0, 3, 9, 27, 76, 127, 220, 361, 517, 624, 747, 880, 894, 951, 904, 856, 782, 659, 494, 392, 222, 140, 74, 30, 10, 1, 0 hits respectively out of 10,000 runs. A result is significant with a score equal or larger/smaller than ±16 (two sided, $p=0.0497$) or ±14 (one sided, $p=0.04695$).

The two traits are expected to covary in response to the local selection if the positive correlation between the two traits is present as predicted. In that case, the two patterns of the development time (Figure 2) and starvation resistance (Figure 3) should be similar or completely opposite. We used again an index with randomisation to test this hypothesis. For the index, we compared each time two populations within a species, and scored whether or not both traits showed either

Table 2: F-values and p-values for the inter-population variation for intercept, habitat and replicate nested in habitat. Bold values indicate significant results.

Species	Development time (days)		
	Intercept	Habitat	Replicate (habitat)
D. ananassae	$F_{1, 429}$ = 5510.56 **p < 0.0001**	$F_{1, 429}$ = 13.03 **p = 0.0056**	$F_{4, 429}$ = 3.4 **p = 0.0094**
D. atripex	$F_{1, 85}$ = 2210.36 **p < 0.0001**	$F_{1, 85}$ = 28.38 **p = 0.0092**	$F_{4, 85}$ = 2.33 p = 0.0623
D. bipectinata	$F_{1, 175}$ = 5608.22 **p = 0**	$F_{3, 175}$ = 3.5 **p = 0.0433**	$F_{7, 175}$ = 0.84 p = 0.5547
D. eugracilis	$F_{1, 96}$ = 5938.14 **p = 0.0002**	$F_{1, 96}$ = 0.53 p = 0.5426	$F_{2, 96}$ = 4.28 **p = 0.0166**
D. malerkotliana	$F_{1, 133}$ = 3536.25 **p < 0.0001**	$F_{2, 133}$ = 7.46 **p = 0.016**	$F_{6, 133}$ = 2.33 **p = 0.0358**
D. pseudoananassae	$F_{1, 247}$ = 24502.42 **p = 0**	$F_{2, 247}$ = 30.15 **p = 0.0004**	$F_{6, 247}$ = 1.52 p = 0.1735
D. sulfurigaster	$F_{1, 762}$ = 7945.85 **p < 0.0001**	$F_{2, 762}$ = 0.58 p = 0.5879	$F_{6, 762}$ = 16.75 **p = 0**
D. takahashii	$F_{1, 103}$ = 93992.02 **p < 0.0001**	$F_{1, 103}$ = 0.2 p = 0.6872	$F_{1, 103}$ = 3.99 **p = 0.0484**

Species	Starvation resistance(days)		
	Intercept	Habitat	Replicate (habitat)
D. ananassae	$F_{1, 429}$ = 416.58 **p < 0.0001**	$F_{1, 429}$ = 3.36 p = 0.0868	$F_{4, 429}$ = 1.82 p = 0.1232
D. atripex	$F_{1, 85}$ = 1532.3 **p < 0.0001**	$F_{1, 85}$ = 164.06 **p = 0.0005**	$F_{4, 85}$ = 2.59 **p = 0.0423**
D. bipectinata	$F_{1, 175}$ = 149.19 **p < 0.0001**	$F_{3, 175}$ = 0.47 p = 0.7134	$F_{7, 175}$ = 4.27 **p = 0.0002**
D. eugracilis	$F_{1, 96}$ = 185.15 **p = 0.0058**	$F_{1, 96}$ = 2.3 p = 0.2698	$F_{2, 96}$ = 6.37 **p = 0.0025**
D. malerkotliana	$F_{1, 133}$ = 452.89 **p < 0.0001**	$F_{2, 133}$ = 4.14 p = 0.0535	$F_{6, 133}$ = 1.35 p = 0.2393
D. pseudoananassae	$F_{1, 247}$ = 256.37 **p < 0.0001**	$F_{2, 247}$ = 2.97 p = 0.1243	$F_{6, 247}$ = 7.02 **p < 0.0001**
D. sulfurigaster	$F_{1, 762}$ = 524.81 **p < 0.0001**	$F_{2, 762}$ = 2.17 p = 0.1948	$F_{6, 762}$ = 16.39 **p = 0**
D. takahashii	$F_{1, 103}$ = 224.39 **p = 0.0041**	$F_{1, 103}$ = 4.61 p = 0.1447	$F_{1, 103}$ = 4.88 **p = 0.0293**

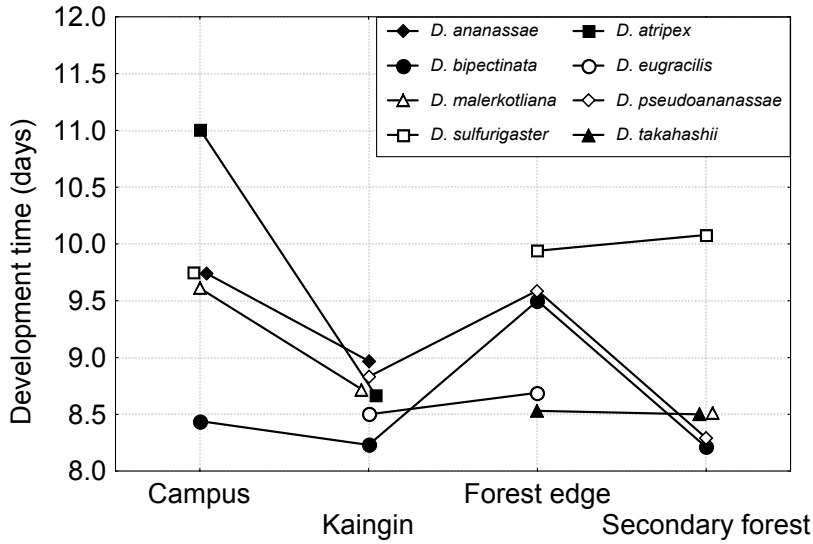


Figure 2: Development time averages (in days) per stock versus habitat. Overlapping points of different species are positioned next to each other to avoid confusion. No error bars are given, see Material and methods.

an increase or decrease in the trait values. This was done for all possible combinations within each species and the overall score was the number of times both traits varied similarly (or dissimilarly). The total number of comparisons was 19, based on 4 species with one comparison (2 populations), 3 species with 3 comparisons (3 populations) and one species with 6 comparisons (4 populations). The theoretical distribution was generated running the model 10,000 times, randomising at every run the non-blank cells within the different species. The values ranged between 0 and 19 with 0, 1, 20, 39, 124, 287, 609, 945, 1341, 1607, 1558, 1305, 1043, 655, 278, 131, 47, 6, 4, 0 hits respectively. The patterns of the two traits are expected to be similar and a one-sided significant result is obtained with a test value equal or larger than 14 (p=0.0471). When the predicted positive interspecific correlation is present, correlations between the two traits across species within habitats are expected to be significantly positive.

Results

Before we could test whether there is genetic variation between the populations of different collection sites, we needed to verify whether density effects played a role in the data. The correlation between development time (residuals of vials averages within species to correct for species effects) versus samples size was non-significant ($r = 0.09$, $p=0.49$); as was that for starvation resistance residuals versus sample size ($r = 0.177$, $p = 0.19$).

VARIATION WITHIN SPECIES

The average development times for the different populations in this experiment varied between 8.21 and 11.01 days, while the values for starvation resistance varied between 1.27 to 3.18 days (Table 1). For development time, five out of eight species showed significant differences between the populations, as did one out of eight species for starvation resistance (Table 2). The number of significant results for development time was higher than the expected type 1 errors using a binomial test ($p = 1.54*10^{-5}$), but lower than expected for the starvation resistance ($p = 0.33$). Replicate was nested within habitat, and showed a significant effect in five and six out of eight species for development time and starvation resistance respectively. Based on this, we concluded that genetic differentiation is present between populations for development time, but not for starvation resistance.

SIMILARITY WITHIN TRAITS

The combined measure of concordance for the development times was 15, thus falling within the 5% probability level of the random model. This result supports our hypothesis that differences between habitats will select for similar responses in different *Drosophila* species. A graphical representation of these data is given in figure 2. It shows that the secondary forest and the kaïngins in particular support fast-developing populations, while the slowest populations were found in the grasslands (Campus site). The forest edge shows intermediate values. This figure also clearly shows that there was no correlation between the ranking of the development times within all species separately and the ranking of the habitats based on disturbance and canopy cover.

Most species belong to the subgenus *Sophophora*, with only one species in the subgenus *Drosophila*. *Drosophila sulfurigaster* was the only species that had an erratic population pattern compared with the other seven species. When the values for *D. sulfurigaster* were excluded, and the randomisation test was applied again for only the *Sophophora* subgenus, the observed overall pattern becomes much stronger. The minimum value in this distribution was four (four comparisons with odd numbers) and the maximum was 16 (with step 2), with 873, 2660, 3255, 2157, 861, 182 and 12 respectively. The overall concordance index for this data set was 16 and is significant ($p = 0.0012$).

The result for the starvation resistance showed a different pattern. The randomisation test for these data indicated no significant overall concordance. This result is contrary to our hypothesis that differences between habitats will select for similar responses in different species (figure 3). Excluding *D. sulfurigaster* in this case does not make any significant difference.

For development time, this leads to the conclusion that all but one of the species respond in a similar way to the differences between the habitats. On the other hand, starvation resistance seems to be unaffected by the differences between the habitat.

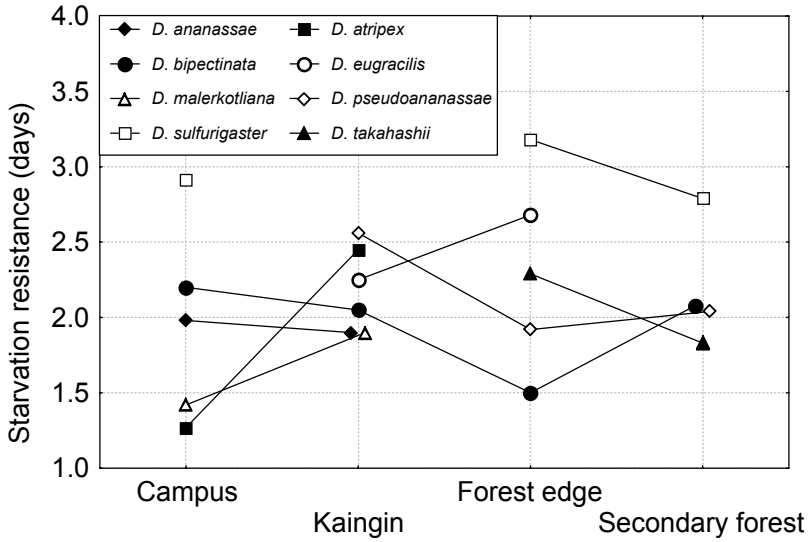


Figure 3: Starvation resistance averages (in days) per stock versus habitat. Overlapping points of different species are positioned next to each other to avoid confusion. No error bars are given, see Material and methods.

HABITAT RANKING - TRAIT COMPARISON

The scores for the habitat rank - development time comparison and the habitat rank- starvation resistance comparison were both minus eight and non-significant ($p = 0.20$). Excluding *D. sulfurigaster* increased the value for the habitat rank - development time comparison to minus twelve ($p = 0.0673$) and decreased the value for the habitat rank- starvation resistance comparison to minus six ($p = 0.2598$), but neither are significant. This leads to the conclusion that the factor that shapes development times is not correlated with any aspect related to habitat ranking such as temperature or humidity.

COMPARISON BETWEEN TRAITS

The patterns for both traits were different from each other. In total, 19 comparisons between two populations could be made, and for each comparison, we scored whether or not both traits showed both an increase or decrease in the trait values. In eight cases, the differences between the two traits were in the same direction, while in eleven cases, they were not. In either case, the results were below the 14 comparisons required for a significant effect ($p = 0.34$ and $p = 0.35$). Excluding *D. sulfurigaster* did not change the conclusion ($p = 0.24$ and 0.25, respectively). The interhabitat correlations across species between development time (DT) and starvation resistance (SR) were determined using vial averages varied with habitat (figure 4). None of the correlations was significant, and only one was positive

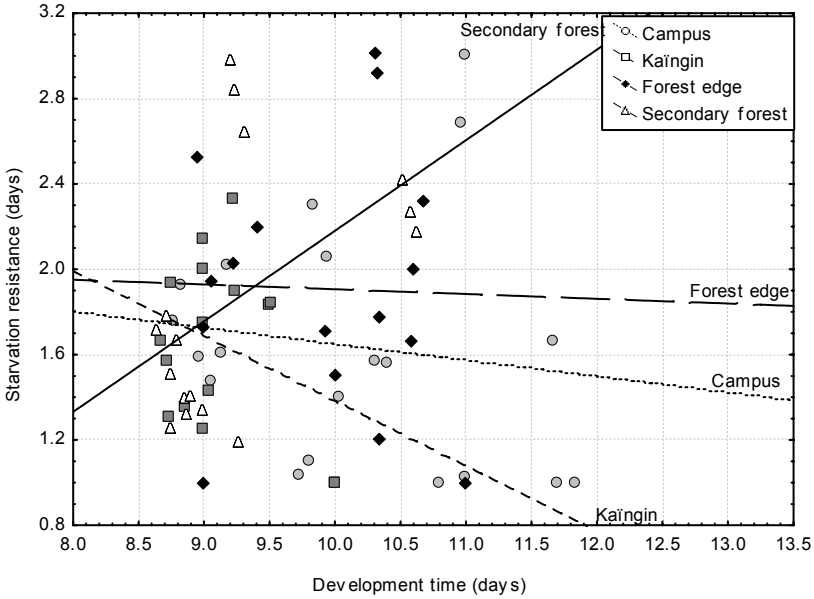


Figure 4: Habitat specific development time - starvation resistance plots, based on vial averages. The different lines represent the correlations between the two traits for the four different habitats, respectively. See text for more details.

(Secondary forest: SR = -2.064 + 0.424*DT, R^2 = 0.24, p = 0.054), the others were negative (Forest edge: SR = 2.13 - 0.022*DT, R^2 < 0.01, p = 0.92; Kaïngin: SR = 4.428 - 0.305*DT, R^2 = 0.11, p = 0.22; Campus: SR = 2.406 - 0.076*DT, R^2 = 0.018, p = 0.58). These results lead to the conclusion that development time and starvation resistance do not show a similar pattern across species and populations. Furthermore, this intraspecific correlation within habitat is not consistently positive.

Conclusion and discussion

The results showed that for development time, five out of eight species had significant differences between the populations, thus indicating that genetic variation for this trait is present in those species (Table 2). The development time patterns within the species were similar for all species (p = 0.028), but excluding the only species not belonging to the *Sophophora* subgenus (*D. sulfurigaster*) increased the overall concordance index substantially (p = 0.0012). The development time patterns within all species were not correlated with the habitat ranking based on disturbance and canopy cover. These results show that the selecting factor or factors for development time have a similar influence on all but one of the *Drosophila* species, but that the selective forces are not related to obvious climatic or ecological variables (see below).

D. sulfurigaster belongs to the subgenus *Drosophila*, while the other species belong to the *Sophophora* subgenus (Baltazar 1991, Grimaldi 1990). Both subgenera

diverged long ago from each other (Beverley & Wilson 1984), while the species of the *Sophophora* subgenus have speciated much more recently (Grimaldi 1990). Therefore, lineage-specific effects due to the early separation of the two subgenera may explain why *D. sulfurigaster* shows a different response than the species of the other subgenus. At the same time, the comparison within the *Sophophora* subgenus is unlikely to be confounded by lineage specific effects and thus appears to reflect more recent selection effects.

For starvation resistance, only one out of eight species showed significant differences indicating genetic variation between populations (Table 2). Furthermore, the pattern appears to be random indicating no consistent influence of habitat on all species alike. Random sampling of a limited number of individuals can result in genetic variation between the different populations, which are unrelated to the actual genetic differentiation between the populations, and would decrease the consistency of a pattern. Most stocks were established using at least 10 gravid females. For starvation resistance, it can not be excluded that sample size effects did play a role, however, the highly consistent pattern within the development times contradicts this, as it would decrease the consistency within the pattern.

Which environmental factor can explain the consistent differences between the habitats as observed for development times? The habitat ranking - trait comparison was non-significant, thus excluding factors that are related to the habitat ranking. Changes in the structure of the canopy result in predictable changes in abiotic factors including temperature and humidity (Walter 1984). This suggests that, in this experiment, neither temperature nor humidity were of primary importance in shaping development times. We were not able to test whether fruit abundance through the year was related to the realised life-history values, as measuring the differences in fruit availability requires a year long sampling to obtain a proper estimate due to habitat specific differences (Krijger 2000, Sevenster & van Alphen 1993a). The use of banana as the breeding substrate could have resulted in the systematic difference between the habitats if local adaptation was driven by variation in the natural available breeding substrates, and this option can not be excluded. However, this does not contradict the conclusion that local adaptation within development time explains the patterns between the populations.

In a previous study, van der Linde & Sevenster (2002) made a ranking based on the degree of disturbance of the habitats. The aim was to test whether this ranking could serve as a predictor for the variation between the habitats with regard to the *Drosophila* diversity. The various biodiversity indexes did not correlate with this ranking, but the overlap percentages between communities closely reflected the difference in disturbance between the habitats. Most species showed a clear preference for disturbed, non-disturbed or intermediate disturbed habitats (van der Linde & Sevenster 2002), which is reflected in the empty cells in our data matrix. The results of this study and the previous one suggest that the factors shaping the community composition and the factors shaping development times within species are of a different nature.

Three of the four habitats were very close to each other, forming a continuous transect of about 2 kilometres. Several studies, both on tropical and temperate species, indicate that daily travel distances up to 100 meters are possible (Burla *et al.* 1950, Taylor *et al.* 1984, van Konijnenburg 1999). Comparing this to our transect length, it suggests either that the differences between habitats form effective barriers for migration, or that there was severe selection against flies migrating to another habitat. Several studies confirm the potential for local adaptation between populations separated by short distances (Capy *et al.* 1987, Harry *et al.* 1999, Karan *et al.* 1999, Nevo *et al.* 1998, Vouidibio *et al.* 1989). However, all but one of these studies was limited to a single species. In contrast, our study showed a consistent pattern for development time for all but one species, making it more likely that we found a real pattern.

The comparison between traits showed that the patterns within the two traits vary independently of each other. Furthermore, only one of the four correlations across species within habitats was positive, but not significant, while the remaining correlations were all negative and non-significant. This result casts doubt about the generality of the expected positive correlation. Fischer *et al.* (2002) found for the relation between egg size and body size in the tropical butterfly *Bicyclus anynana*, that correlations between the two traits may represent an emergent property, visible only when a large range of differences in body size is considered. Comparably, the range in development times in this study is between 8.2 and 11.0 days, which is much narrower than within the Panamanian *Drosophila* community (7.8 to 15.4 days (Krijger *et al.* 2001, Sevenster & van Alphen 1993a). When the Panamanian data set is limited to the same range as the data set of the Philippines, the correlation between the traits is no longer significant.

Our aim was to test whether local adaptation is present in the different *Drosophila* species and if so, whether the patterns between the populations within species were similar. Based on the results presented here, we conclude that genetic differentiation between populations is present in at least five out of eight species for development time and that the patterns within the different species are similar. The observation that the different species show a similar pattern leads to the conclusion that there is a selecting factor or factors that does have a similar influence on the development times of all but one of the *Drosophila* species in this community. However, this factor is not directly correlated with the disturbance / canopy cover ranking of the habitat. Starvation resistance does not show genetic differentiation between populations, nor was the intraspecific pattern similar between species. Our study did not confirm the generality of the positive correlation between development time and starvation resistance. The patterns within the two traits did not correspond with each other, which implies that selection on the two traits occurs independently of each other.

3

Review of body size, development time and starvation resistance in species of *Drosophila*.

Introduction

In the past decades, the life-history research on different *Drosophila* species has generated a wealth of knowledge. In this chapter, I review the relevant *Drosophila* literature concerning the three life-history traits I have investigated: body size, development time, and starvation resistance. I have organised the relevant literature with respect to the type of environmental variation influencing the different traits. These are gradients along latitudinal clines, temperature, and crowding effects.

Geographical variation in life-history traits is often observed, and this variation is sometimes clearly related to latitude (or altitude). These latitudinal clines provide insight into how natural selection shapes these traits, but different aspects of the environment change simultaneously and disentangling them is not always easy. One often mentioned environmental factor that could explain the geographical variation is temperature. Oversimplified, average temperature is highest in the tropics, and becomes lower with increasing latitude. Therefore, temperature could be the explanatory factor and I thus discuss the temperature-related relevant literature separately. The third environmental factor that I discuss is crowding. Although the actual crowding levels in nature are poorly documented, they do play a role in *Drosophila* (Sevenster 1992) and there are some indications that crowding does differ between patches (Atkinson 1979) or habitats and across seasons (Krijger 2000, Krijger & Sevenster 2001, Sevenster & van Alphen 1996). The final aspect I review in this chapter is the genetic correlations between these three traits.

Geographical variation in life-history traits

Geographical variation in life-history traits is commonly observed, and can provide valuable clues about which environmental factors could be responsible for the selection of the traits under natural conditions. An overview is given in Table 1.

BODY SIZE

Bergmann's rule states that "The smaller sized geographical races of a species are found in the warmest parts of the range, the largest races in the cooler districts" (cf. Mayr 1942). Body size within species of *Drosophila* usually show clear latitudinal clines with larger individuals at higher latitudes (Azevedo *et al.* 1996, Capy *et al.* 1993, Coyne & Beecham 1987, David & Bocquet 1975a, David & Bocquet 1975b, David & Kitagawa 1982, Hoffmann *et al.* 2001a, Hyytia *et al.* 1985, Imasheva *et al.* 1994, James *et al.* 1995, 1997, James & Partridge 1998, Karan *et al.* 1998c, Lemeunier *et al.* 1986, Misra & Reeve 1964, Parkash *et al.* 1998, Parkash & Munjal 1999, Parsons 1983, Prevosti 1955, Robinson *et al.* 2000, Stalker & Carson 1947, Tantawy & Mallah 1961, van 't Land *et al.* 1999, Watada *et al.* 1986). The North-American cline reported by Long & Singh (1995) for *Drosophila melanogaster* was non-monotonic in nature, with the largest flies at middle latitudes and smaller flies in the north and south. Detailed analysis of whether temperature might account for this

non-monotonic cline showed that neither temperature, nor related aspects are likely to explain the observed cline. Hallas *et al.* (2002) found a non-linear cline for *D. serrata* with a sharp reduction in body size in the tropics. Clinal variation in *D. melanogaster* collected along the east coast of North America is maintained over a wide range of experimental developmental temperatures (Coyne & Beecham 1987, Tantawy & Mallah 1961) indicating that the differences along the cline is genetic. Hoffmann *et al.* (2001a) established iso-female lines from *D. melanogaster* flies collected at five locations in Queensland (tropical) and three locations in Tasmania (temperate), and scored the thorax length of 8 to 10 individuals from each iso-female line. The nested analysis of variance showed that the variation among iso-female lines within each collection site, and between geographic regions were significant, while the component of variation among the collecting locations within geographic region was not significant. The variation among iso-female lines, collection sites and geographic regions explained 13.5, 1.7 and 3.3 percent of the total variation, respectively. In general, the clinal patterns within the various *Drosophila* species are thus consistent with Bergmann's rule.

DEVELOPMENT TIME

Latitudinal clines in *Drosophila* for development time have been reported for Australia and South America, with egg-to-pupae and egg-to-adult development times (James & Partridge 1995, 1998) and egg-to-adult development times (van 't Land *et al.* 1999) that are longer at lower latitudes. However, the South-American cline is very shallow, explaining only 0.1 percent of the measured variation, while the variation between sites independent of latitude is considerable. The significant effect in the Australian cline depends heavily on one low-latitude population (A.C. James, personal communication in van 't Land *et al.* 1999), and only one of the two correlations between latitude and pupation time remained significant after removal of this single data point out of 13. Van der Linde & Sevenster (chapter 2) measured egg-to-adult development times of *Drosophila* species from the Philippines in a common environment. They found that there were significant differences between populations of five out of eight species on a short transect covering four different habitats ranging from grassland to secondary forest. All but one species displayed a similar pattern in development times among habitats, giving a highly regular response to the underlying differences between the collection sites. However, the pattern was not related to habitat-ranking as based on microclimates and vegetation.

STARVATION RESISTANCE

Hoffmann & Harshman (1999) showed in their review of all available studies, that tropical populations of the various *Drosophila* species had a better starvation resistance than temperate populations (Da Lage *et al.* 1990, Karan *et al.* 1998a, Karan & Parkash 1998, Parkash *et al.* 1994, Parkash & Vandna 1994, Shamina *et al.* 1993). In more recent studies, Robinson *et al.* (2000) and Hallas *et al.* (2002) did not find such a latitudinal cline in South-America or Australia. Robinson *et al.* (2000)

Table 1: Overview of the results as reported in the literature for the three life-history traits. Effects of temperature and crowding are each partitioned into phenotypic and genetic effects. For references, see the text.

Trait	Geographical variation	Temperature variation: selection experiments	Temperature variation: environmental effects	Crowding effect: phenotypic	Crowding effect: genetic
Body size	Lower latitudes -> smaller; Sometimes inverted U-shape with smaller at both ends; iso-female variation much larger than geographical or population	higher -> smaller	higher -> smaller; Sometimes inverted U-shape with smaller at both ends	increased -> smaller, sex difference increased	The two studies contradict each other
Development time	Lower latitudes -> longer	higher -> longer	higher -> shorter	increased -> longer, sex difference often decreased	increased -> shorter
Starvation resistance	Inconsistent, but most tropical>temperate, some no effect; iso-female variation much larger than geographical or population	-	Inverted U-shape, shorter at both ends	15-28 days after emerging: Increased -> longer; 0 days after emerging: Increased -> shorter; sex differences increased	increased: 0 days -> longer

suggest that the Indian latitudinal cline (Karan *et al.* 1998a) is due to the specific Indian climatic situation and exclude the possibility of rapid laboratory selection in the South-American lines because other traits had not undergone laboratory adaptation since collection (Azevedo *et al.* 1996, van 't Land *et al.* 1999). However, Hoffmann *et al.* (2001b) and Matos *et al.* (2002) found rapid loss of starvation resistance in the laboratory within short periods after establishing the stocks. This is supported by the rapid responses to laboratory selection regimes (Borash & Ho 2001, Chippindale *et al.* 1996, Harshman & Schmid 1998, Hoffmann & Parsons 1989, Service *et al.* 1988). Hoffmann *et al.* (2001a) measured the variation in starvation resistance among iso-female strains. The females were collected at several localities in Tasmania and Queensland (Australia). Geographic region had a significant impact on the realised starvation resistance confirming earlier results (Queensland populations having a higher starvation resistance), but it explained only a small fraction of the variation (2.7%). Most variation (over 40%) was attributable to the strain effect. Van der Linde & Sevenster (chapter 2) found that two out of eight species from the Philippines showed significant genetic variation between populations from different habitats, but found no systematic correlation with habitat. Parkash & Munjal (1999) found that higher starvation tolerance was positively correlated with minimum temperatures, higher metabolic stress in relation with smaller body size, and higher population density and competition.

SUMMARY

Development time and body size both show clear and repeatable responses to latitude, while a generalised pattern for starvation resistance is less obvious. Studies show either that tropical populations have a longer starvation resistance, or that there is no relation with latitude. The genetic variation within populations is generally very wide for body size and development time, compared to the geographical variation. This abundant genetic variation could facilitate local adaptation to environmental differences.

The impact of temperature on life-history traits

Temperature is one of the variables that covaries with latitude, but other variables, including additional climatic factors, also covary with latitude. In addition, changes in vegetation alter the daily temperature pattern (Walter 1984). Temperature is often suggested as a key factor explaining latitudinal cline variation in life-history traits. For that reason, the effect of temperature on life-history evolution has been tested in the laboratory. However, it is crucial to keep in mind that those experiments are conducted under constant temperatures while the temperatures in the field fluctuate greatly. Therefore, the laboratory results are not necessarily related to the latitudinal cline patterns. The effects of the temperature are divided into two categories: genetic and environmental effects. An overview is given in Table 1.

BODY SIZE

Temperature-mediated artificial selection results in relatively larger adult body size for lines selected at lower temperatures (Anderson 1966, 1973, Cavicchi *et al.* 1985, Neat *et al.* 1995, Partridge *et al.* 1994a, Powell 1974). Besides shaping the genetics, temperature also has a direct environmental effect on body size resulting in smaller individuals at higher temperatures (Alpatov & Pearl 1929, Atkinson & Sibly 1997, Azevedo *et al.* 1996, Eigenbrodt 1930, Imai, 1933 #908, Imai 1937, James *et al.* 1997, James & Partridge 1998, Karan *et al.* 1998c, Karan *et al.* 1998b, Noach *et al.* 1996, Ray 1960). The response to temperature resembles an inverted U-shaped curve, with a sharp reduction in size at the lower end (David & Clavel 1967, David *et al.* 1983, David *et al.* 1990, David *et al.* 1994, de Moed *et al.* 1997a, Morin *et al.* 1996, Morin *et al.* 1997, de Moed, 1997 #3051).

DEVELOPMENT TIME

Temperature selection on development time shows that low temperatures lines have a shorter larval (Partridge *et al.* 1994b) or egg-to-adult (Anderson 1973, James & Partridge 1995) developmental time compared to the high temperature lines when measured at the same temperature. Pupal development time is less predictable with no difference at lower temperatures but low temperature lines had longer development times at higher rearing temperatures (Partridge *et al.* 1994b). Besides the evolutionary effect, lower temperatures directly result in increased development times (Azevedo *et al.* 1996, James *et al.* 1997, Zwaan *et al.* 1992).

STARVATION RESISTANCE

Several authors (Da Lage *et al.* 1989, Karan & David 2000) have studied the starvation resistance of different *Drosophila* species under different temperatures. All species showed a biphasic response curve at all temperatures measured, with a reduction in resistance to either end of the temperature range. The optimum differed among species, ranging between 4.76 and 14.55 ºC. A comparison of *D. melanogaster* individuals grown at different temperatures showed that flies reared at lower temperatures have a lower optimum than flies reared at higher temperatures (range: 6.2 - 7.5 ºC) but the differences in survival at the higher temperatures are minimal between flies from the different growth temperatures (Karan & David 2000).

SUMMARY

Selection experiments showed that the high temperature selected lines are smaller and have a longer development time. This is in line with the latitudinal cline patterns. The rearing temperature has a similar effect on body size, but development times become shorter with increasing temperatures. Starvation resistance seems to have an optimum temperature, with lower starvation resistances at either end. Habitat change leads to changes in the daily temperature

regime, and this could partially explain the variation between habitats in the realised life-history traits.

The impact of crowding on life-history traits

Crowding can play a role in studies when the experimental densities are not controlled for. Moreover, food abundance varies between habitats, but also within habitats, both at a spatial and temporal scale. These differences in food abundance can result in different levels of crowding. Krijger (2000) found that the mean resource abundance was higher in the three disturbed habitats than the three forest habitats in his study in Panama. Furthermore, the temporal heterogeneity was less in the disturbed habitats. Sevenster (1992, Sevenster & van Alphen 1993a) found that the food availability varied widely during the year, but also at a spatial scale. An overview is given in Table 1.

BODY SIZE

Several authors (Bakker 1961, Borash & Ho 2001, Chiang & Hodson 1950, de Moed *et al.* 1997b, Grimaldi & Jaenike 1984, James & Partridge 1998, Karan *et al.* 1998c, Perez & Garcia 2002, Robinson *et al.* 2000, Santos 1996, Sevenster 1992, Wilkinson 1987, Zwaan *et al.* 1991) have found a phenotypic response in which body size at eclosion is severely reduced with increased crowding. This reduction in body weight at eclosion can be up to 80% of the maximum weight at eclosion. Van 't Land (1997) found that crowding increased the difference between the sexes. *Drosophila* flies from wild populations are generally more variable for body size than their laboratory reared relatives (Coyne & Beecham 1987, David *et al.* 1980, Gibert *et al.* 1998, Imasheva *et al.* 1994, Moreteau *et al.* 1995). This is often explained as an effect of variation in crowding (Prout & Barker 1989, Santos 1996). Perez & Garcia (2002) found that high-density selected lines had smaller body-sizes when measured at the same density in the common garden experiment, while in a similar experiment Borash & Ho (2001) found the opposite, namely that high-density selected flies were heavier. It is unclear how such a difference can be accounted for.

Development time

Flies reared under crowding conditions have a longer egg-to-adult development time than those reared under non-crowding conditions (Borash & Ho 2001, Chiang & Hodson 1950, Perez & Garcia 2002, van 't Land 1997, Zwaan *et al.* 1991). It is also interesting that some studies report that the sex difference disappears with increased crowding because the development time of females increases more rapidly with crowding than males (Santos *et al.* 1994, van 't Land 1997). However, other studies fail to find such a differential response between the sexes (Roper *et al.* 1996). Perez & Garcia (2002) found that the additive genetic variation and the heritabilities for development time varied with the selection density. Selection on

density leads to a decrease in the development time (Borash & Ho 2001, Perez & Garcia 2002).

STARVATION RESISTANCE

Starvation resistance can be measured at different ages of a fly, and the results should be interpreted accordingly. Zwaan *et al.* (1991) measured starvation resistance in non-selected *Drosophila melanogaster* reared at different densities at ages of 15 and 28 days old and found a crowding-related increase in starvation resistance. Baldal *et al.* (in press), using unselected stocks and rearing at different densities, measured flies at eclosion and found that starvation resistance decreased with crowding in *Drosophila melanogaster*, *D. equinoxialis,* and *D. ananassae*. This result matches those of Borash & Ho (2001) for freshly emerged *D. melanogaster* flies of both the uncrowded and crowded selection lines. Several authors (Borash & Ho 2001, Mueller *et al.* 1993) found that selection under high crowding conditions leads to an increase of the starvation resistance. Females reared under crowding conditions generally have a higher starvation resistance, while males reared under crowding conditions seem to have similar starvation resistances or even somewhat lower values (Service *et al.* 1985, Service 1987, van 't Land 1997, Zwaan *et al.* 1991, 1995b).

SUMMARY

Crowding results in smaller adults with longer development times. Starvation resistance is increased at higher age but decreases directly after eclosion. The increased crowding results in increased differences between the sexes in body size and starvation resistance, but those differences decrease for development time. Selection in response to increased crowding results in flies that have shorter development times and increased starvation resistances. The effect of crowding selection on body size is unclear as the two studies contradict each other. Due to the effects of density on the life histories of the flies, it will be important for the data I collect to initially examine the effect of density. Furthermore, resource abundance varies between habitats, but also within habitats, both in time and space. When this leads to habitat specific differences in selection, this could lead to locally adapted populations.

Genetic and phenotypic correlations among traits

Genetic correlations between traits may limit the response to selection when opposite selection pressures work on the two traits. In this section, I not only review the literature for genetic correlations within species, but also explore the interspecific correlations between traits. An overview is given in Table 2.

BODY SIZE AND DEVELOPMENT TIME

Selection experiments for larger body size or longer development time in *D. melanogaster* showed that larger body sizes were associated with longer pre-adult development times (Cortese *et al.* 2002, Gu & Barker 1995, Nunney 1996b, Partridge & Fowler 1993, Partridge *et al.* 1999, Reeve 1954, Robertson 1957, 1960a, b, 1963, Roper *et al.* 1996, Santos *et al.* 1992, 1994, Zwaan *et al.* 1995a). However, in some experiments, the opposite selection for smaller flies did not result in a decrease in development time as expected (Nunney 1996b, Partridge & Fowler 1993, Partridge *et al.* 1999). Betran *et al.* (1998) and Gu & Barker (1995) found positive phenotypic and genetic correlations between the two traits. Van 't Land *et al.* (1999) found a significant negative correlation between latitudes and development time and a significant positive correlation between latitude and wing size. The correlation between the two traits was, however, non-significant. A similar pattern is observed for the Australian cline (James & Partridge 1995). Perez & Garcia (2002) did not find significant genetic or phenotypic correlations between these two traits in their base population, but the sign of the correlations was in all but one case, negative. Their explanation was that the effect was caused by the medium they used. This did not allow yeast growth and may thus have limited the available food versus the situation in which yeast growth offers a continuous fresh supply of food (cf. Bakker 1961). Bakker (1969) found indeed that fast developing individuals were the heaviest. Other authors found that this genetic correlation is dependent on the diet of the flies (Robertson 1963) and that the presumed trade-off between the traits disappeared under non-crowding conditions (Cortese *et al.* 2002), or even changes sign completely with change of the environment (Gebhardt & Stearns 1988).

BODY SIZE AND STARVATION RESISTANCE

Parkash & Munjal (1999) found a negative correlation between body size and starvation resistance within six species of Drosophilids collected along a climatic cline in India. This negative correlation also appears to be present in the data from Hoffmann *et al.* (2001a) who found in the laboratory that Tasmanian populations were larger and had a shorter starvation time than the Queensland populations (estimation from their figure 2: $R^2 = 0.55$, $p = 0.058$). The selection experiment of Hoffmann & Parsons (1993) on both *D. melanogaster* and *D. simulans* did not result in a correlated response for body size, while later experiments showed that lines of *Drosophila melanogaster* selected for higher starvation resistance became larger (Chippindale *et al.* 1996, Harshman *et al.* 1999). Zwaan *et al.* (1991) found no phenotypic correlation between body size and starvation resistance in flies 15 or 28 days after eclosion. Hallas *et al.* (2002) in their study did not find differences in starvation resistance between populations of the cline, but did find a non-linear cline for development time. Toda & Kimura (1997) found a positive interspecific correlation for mycophagous Drosophilids of Japan.

DEVELOPMENT TIME AND STARVATION RESISTANCE

Several authors have shown that lines of *Drosophila melanogaster* selected in the laboratory for starvation resistance had a longer development time (Chippindale *et al.* 1996, Harshman *et al.* 1999). Zwaan *et al.* (1991) found no phenotypic correlation between development time and starvation resistance in flies 15 or 28 days after eclosion, nor did they (Zwaan *et al.* 1995a) find a correlated response for starvation resistance in their upward or downward selection lines for development time. Robinson *et al.* (2000) found no corresponding cline for the South American cline, which does show a slight cline for development time. Sevenster & van Alphen (1993a) have described a positive interspecific correlation between development time and starvation resistance for Panamanian *Drosophila*, while Toda & Kimura (1997) found a negative interspecific correlation for mycophagous Drosophilids of Japan. Van der Linde & Sevenster (chapter 2) in their study on *Drosophila* from the Philippines found that the two traits had completely different patterns and the correlations were either absent or negative, both suggesting that there is no underlying genetic correlation or that differential selection on the two traits is obscuring such a correlation.

SUMMARY

Table 2 is an attempt to provide an overview of the research to date. Despite the large volume of articles on *Drosophila* life-history traits, several cells of the table remain empty, as I could not find any relevant data. Furthermore, many studies report no correlated responses or results varied between similar studies.

Table 2: Overview of estimated correlations among the three traits. First two columns describe genetic correlations, the second pair phenotypic, and the last, interspecific. For references, see text.

	Selection experiments	Other methods	Phenotypic	Latitudinal	Interspecific
Body size - development time	Positive response, sometimes no response in selection for smaller flies	Non-significant negative, variable	Positive	Negative	
Body size - starvation resistance	Absent or positive		Absent	Absent	Positive
Development time - starvation resistance	Absent or positive	Absent		Absent	Positive or absent

Concluding remarks

Since the mid 20th-century, many studies concerning *Drosophila* life-history traits have been published. In this chapter, I reviewed the literature concerning three life-history traits: body size, development time and starvation resistance. Specifically, I reviewed four different topics: latitudinal clines, temperature effects, crowding effect, and genetic correlations among the three traits.

This overview provides a useful starting point to understand how environmental cues can shape life-history traits. In **Chapter 4**, I will present a field-based study in which I examine the effect of habitat differences on the realised life-history traits. In **Chapter 5**, I present a laboratory-based study in which I examine the existence and magnitude of genetic correlations between these three traits. These studies can shed more light on how life-history evolution takes place under field conditions, and whether genetic correlations can slow adaptation to a new environment.

4

Life-history patterns in Panamanian *Drosophila* species from three different habitats

Abstract

In this chapter, I present the results of the field experiments and the common environment experiment as outlined in **Chapter 1**. The results show that local adaptation occurs within all three traits surveyed: body size, development time and starvation resistance. For body size, the genetic variation was not habitat-related, but depended on the particular collection site, while the phenotypic variation showed no consistent pattern. Development time showed clear genetic and phenotypic variation. The phenotypic variation was as predicted from theory, but the genetic differences showed an opposite pattern to that predicted from temperature selection experiments. However, the pattern was consistent with the predictions based on the life-history coexistence model of Sevenster & van Alphen (1993a, 1993b). In starvation resistance, plasticity is very important, and explains most of the variation. Grassland populations have genetically longer starvation resistances than forest populations, and these genetic differences partly compensate for the stress inflicted by the harsher grassland environment. All three traits show considerable amounts of genotype-by-environment interaction. Furthermore, the fit between field and laboratory experiments is often poor, and this, combined with the extensive GxE interactions, prompts for caution when extrapolating laboratory-based results to the field. The interspecific variation for the three traits shows clear interdependence, and a strong signal detected for phylogenetic history suggests that this interdependency follows from a pattern of shared genetic pathways.

Introduction

Normally, the first paragraphs of an article introduce the context for a study, and the relevant literature that is available. However, this type of introduction would merely be a condensed version of **chapter 1**, in which I explained why I carried out this research. I, therefore, refer to that chapter, instead of giving a new condensed version. The literature review can be found in **chapter 3**. The length of the review warranted a chapter on its own, and gives a broad overview of all the relevant literature for this (and the next chapter). Here I will start with the aim and outline of this chapter, followed by my expectations, before continuing with the 'Material & Methods'. In the 'Results' and 'Discussion' sections, each life-history trait is first examined or discussed independently, after which I focus on the interdependencies between the traits. Finally, I will discuss some more general aspects on which these experiments shed some light.

AIM AND OUTLINE

The aim of this study is to investigate the ecological and genetic covariances among three life-history traits in species of *Drosophila*: development time, starvation resistance, and adult body size using a combination of field and laboratory work. Practically, this has resulted in three experiments, two in the field, and one in the laboratory. Flies were collected at six sites in Panama located on two transects, each with a forest, an intermediate and a grassland site. Twelve species were present in at least three collection sites and the stocks were maintained in an open-air laboratory (See Material & Methods).

The aim of the first field experiment (table 1) was to measure the expression of the three life-history traits in the original field environment. I used all twelve species and this experiment will show whether differences between the habitats exist, and if so,

Table 1: Brief summary of the design principles for the three experiments.

First field experiment: original habitat only	Rationale:	Measuring traits for each population in its own habitat. Twelve species, and all populations of each species.
	Aim:	Gain insight into the realised phenotypic values under the original natural conditions the populations have evolved in. Provide insight into the differences among the populations, within and across species.
Second field experiment: transplantation	Rationale:	Measuring traits for each population of four species in their original, and in the two other habitats within the same field transect.
	Aim:	Gain insight into the relative importance of the genetic, environmental and GxE interaction factors.
Common environment experiment	Rationale:	Measuring traits of each population in a common environment in the laboratory. Twelve species, and all populations of each species.
	Aim:	Gain insight into the genetic differences between the different populations.

whether that variation is consistent over all species and is also habitat-related.

The aim of the second field experiment (table 1) was to measure the expression of different populations in all three habitats within a transect, using transplantation of (sub-)populations. The habitats are so close together that the differences between them are within the natural range of differences the flies encounter when they move between habitats. Four species were used for this experiment, which are representative for all the species. With this experiment, I measured the phenotypic plasticity within the different species as expressed in these field experiments and the consistency of this plasticity between the species. Furthermore, the results will also indicate whether genotype-by-environment interactions at the level of populations are present.

The common environment experiment (table 1) was carried out in the laboratory in the Netherlands, with all populations of the twelve species that were still available. This final experiment will give insight into the genetic differences between the different populations, and whether these differences are consistent over all the species.

EXPECTATIONS

Based on the literature review in **chapter 3**, I have drafted some expectations for the individual traits:

Body size: The published data on temperature selection, phenotypic plasticity, and geographical variation taken together predict that the open habitat will result in smaller individuals, both at the genotypic as well as the phenotypic level.

Development time: The latitudinal cline data and the temperature selection data predict that populations from locations with a lower temperature have genetically shorter development times (when measured in a common environment). However, when measured in the field, I expect the grassland populations to develop faster than the forest populations due to the higher environmental temperatures.

Starvation resistance: In the field, I expect that grassland populations have shorter realised starvation times than forest populations. Furthermore, based on latitudinal clines I expect that opening the canopy will result in genetically adapted populations with higher starvation resistances.

Furthermore, based on the life-history model of Sevenster & van Alphen (1993a, 1993b), I expect adaptation towards shorter development times and lower starvation resistances in the more disturbed habitats.

The transplantation (second field) experiment contains in total four main factors: original (or founding) habitat, experimental habitat, transect and sex. These four factors give rise to eleven interaction factors. To ease the interpretation, most of these main and interaction factors can be grouped into three categories: genetic, environmental and Genotype-by-Environment (GxE) interactions. The relative importance of these three categories sheds light to the evolutionary processes underlining the local adaptation. The genetic category (e.g. original or founding habitat related (interaction) factors) sheds light on the underlying genetic variation in the realised trait. The environmental category e.g. experimental habitat related (interaction) factors) underlines the importance of phenotypic plasticity in the realised trait values. Finally, the GxE interaction category incorporates all interaction factors between the original habitat with the experimental habitat. This last category signals whether asymmetry in the response of different populations to the different environments exists.

Material & Methods

FIELD SITE

The fieldwork was carried out in Panama at the Smithsonian Tropical Research Institute (STRI). Here, the closed canopy forest extends right up to the roads within the Canal Zone area. For this study, I established two transects with three habitats along each: closed canopy forest, open grassland with patches of scrub, and an intermediate zone. The distance between the sites within transects was just a few kilometres to ensure limited impact of large-scale factors, such as climate. Each site was of sufficient size so that it could accommodate a large resident population. This increased the likelihood of local adaptation being more important in shaping the life-history traits than immigration from the neighbouring habitats. However, I could not *a priori* exclude the possibility that mass migration between the habitats occurs, which could result in panmixia without local differentiation. The distance between the transects was larger than the length of the transects themselves so that I could test whether the differences within a transect are caused by habitat-related differences and not by local variation covering both transects simultaneously.

The first transect was located near Summit Gardens whilst the second was close to the town of Maria Eugenia (see figure 1). The two transects meet all the criteria mentioned above. The two forest locations are within the same stretch of forest, but the distance between them is around 10 kilometres. The intermediate and grassland locations are separated by this forest and are not connected by the same type of habitat. The forest sites are covered with closed canopy forest. Human activities such as logging, agriculture, settlements, and the Panama Canal have resulted in open areas with grasses as the dominant plants. Scattered in these grassland sites are patches with scrub and small trees, the remaining area is open grassland. The intermediate sites have a higher canopy cover than the grassland sites but lower than that of the forest sites. The intermediate sites differ somewhat

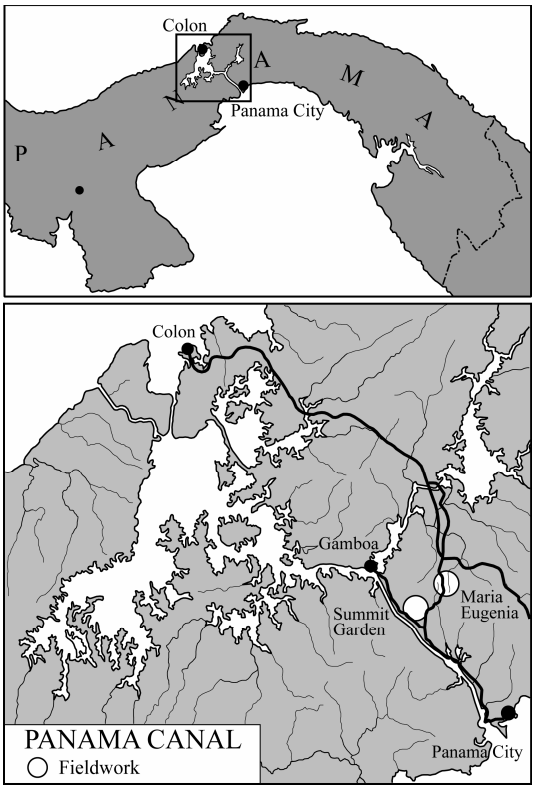


Figure 1: Map of research area. The two circles indicate the two different transects.

from each other in human land use; this might have an influence on the fly populations.

COLLECTIONS AND MAINTENANCE

Drosophila flies were collected using banana-bait traps with up to eight traps per collection site spaced at least 250 meters apart. The traps were constructed from 500-ml transparent containers (7 cm high, and 9-11 cm in diameter) each hanging on a nylon cord of about one-meter in length. A hole of Ø 2.5-cm, covered with 1.5-mm mesh, was positioned on one side of the trap. The hole faced slightly downwards to prevent rain from entering. The mesh allowed *Drosophila* access to the bait inside, but prevented larger animals from entering. The females were identified to the lowest taxonomic level possible (Bock 1980, Val *et al.* 1981) and single females were separately put in small vials with a small piece of banana dipped in yeast suspension. The male offspring were used for definitive identification to the species level. Offspring of iso-female lines of the same species and collection site were combined to give single species stocks. The stocks were maintained in 150-ml containers on pieces of banana dipped in yeast suspension as a breeding substrate and transferred every 10 days to a new vial with fresh breeding substrate.

The open-air laboratory was in Gamboa, under direct influence of the outdoor climate and providing natural variation in ambient temperatures and light regime. Humidity in the closed vials is higher than the ambient humidity.

The experimental space in the field comprised of field cages of approximately 70 x 70 x 120 cm, with a thick wooden floor. The sides were made of gauze (5 mm^2 mesh) and a slightly angled roof extending at the sides for 15 cm. The front was removable for easy access and each cage was on poles of approximately 1.25 meter on which I smeared mineral oil to prevent ants from entering. This set-up kept mammals, birds, and larger insects out. The roof prevented washing away of the experimental set-up (see next paragraph) during heavy rainfall, but also blocked

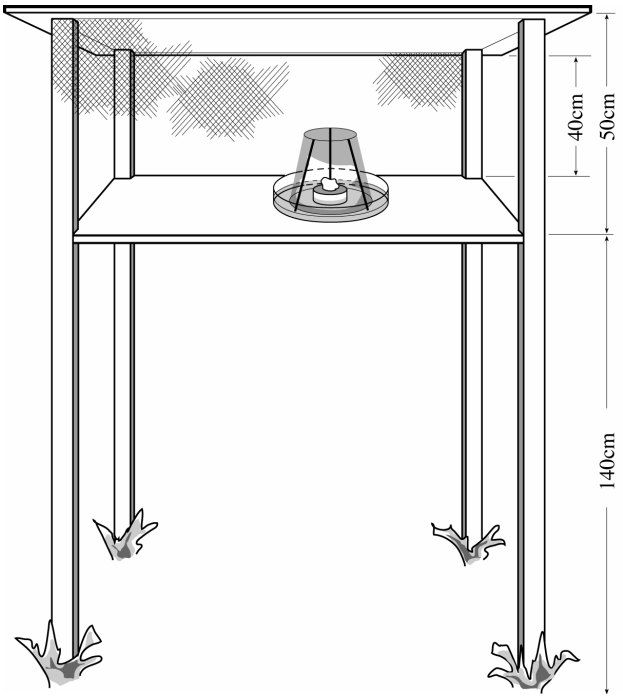


Figure 2: Semi-schematic drawing of the field set-up. The scales of the small cage and the large cage it is located in are not proportional, but modified in order to obtain a clear view of the field set-up.

direct sunshine as insects under natural circumstances can seek shaded places, but could not in these experimental cages (figure 2).

FIELD EXPERIMENTS

Both field experiments had the same basic set-up, in order to reduce differences between them. Each experiment was carried out in three replicas, which were staggered in time, with gaps of three days. The first field experiment was carried out between 21st of August and the end of September 1998, while the second experiment was carried from mid October until mid November.

Field set-up

Instead of vials, I used small cages in the experiments so that the experimental temperature, humidity, and light intensity resembled the ambient temperature more closely. For the development times, I constructed slightly tapered cages of 12 cm high and a diameter of 10 cm made of steel thread and covered with very fine mosquito netting to keep the smallest local parasitic wasps away from the growing larvae. For the egg-laying phase, flies were put on fresh pieces of banana dipped in yeast suspension. This phase was carried out in vials in the open-air laboratory after which the pieces of banana were transported to the experimental cages in the field. The pieces of banana were placed on moist vermiculite in petridishes, over which the small cages were placed. The cages were placed in a thin layer of water, acting as a water-lock preventing entry of insects into the cages. For the starvation resistance, I used petridishes of five cm in diameter covered with the same fine mosquito netting. Five ml of agar was poured inside the petridish to serve as a water source for the flies.

The species for the second field experiment (table 1) were chosen based on their position within the phylogenetic tree and on the relative position within the range of the life-history traits. The four species were representatives for the four main

species groups within this study, *D. melanogaster* (*melanogaster* species group), *D. equinoxialis* (*willistoni* species groups) and *D. sturtevanti* (*saltans* species group), all three of the *Sophophora* subgenus, and *D. cardinoides* (*cardini* species group) from the *Drosophila* subgenus. These species covered the full range in all three traits.

Preparation and egg-laying

Three days before the experiment started, all flies were transferred to a new vial with fresh breeding substrate of banana slices dipped in yeast suspension. This allowed the flies to start producing eggs, and ripe eggs could be laid immediately preventing stowage of eggs. Stowage of eggs can result in shorter development times if the eggs start developing inside the female. On the first day of the actual experiment, groups of flies were transferred to fresh slices of banana dipped in yeast suspension for egg-laying. The parent flies were allowed to lay egg for 6 hours (06:00 - 12:00 hours) in the open-air laboratory after which the pieces of banana were transported to the different field sites.

Development time

Development time was measured as time from egg laying until eclosion as adult. The start of the daily check was as late as possible in the afternoon to have everything checked before sunset, but never before noon. Emerging flies show clear diurnal rhythms (Bakker & Nelissen 1963, Belcher & Brett 1973, Pavan *et al.* 1950): most individuals emerge in the early morning in the first hours after sunrise. Cutting such an emergence peak in two by a random morning visit would result in a random part of the peak counted for that day, while the rest is counted the next day. The daily checking was randomised over transects and sites within the transects to avoid systematic errors as travelling between the sites took considerable time.

Starvation resistance

Starvation resistance was measured as time from adult eclosion until death. In practice, the flies that had emerged between successive checks were transferred to petridishes. Deceased flies were counted once a day simultaneously with the daily check on newly emerged flies. No indications of diurnal rhythms in time of death are known, so randomisation of the counting times was the best way to avoid systematic errors due to variation between counting times and one day in-between periods.

Body size

The flies used in the starvation resistance experiment were stored in vials with 70 % alcohol. Body size was measured using thorax length as a proxy. Both thorax length and wing length are highly correlated with body size (Gu & Barker 1995, Karan *et al.* 1998c, Karan *et al.* 1998b, Karan *et al.* 2000, Parkash *et al.* 1998). However,

wing length shows more interspecific variation than thorax length and was therefore rejected (data not shown). Head width was also rejected, as it, unlike body size, is not affected by temperature (David & Clavel 1967, David *et al.* 1994, Noach *et al.* 1996). Reduction of food levels does not lead to change in the ratio between the characters (Robertson 1987, Thomas 1993).

I measured the length of the thorax between the end of the scutellum and the front rim of the thorax. All measurements were made using a stereomicroscope with drawing mirror and electronic drawing tablet (ACECAD Advanced Digitizer) connected to a computer. Each fly was measured three times and remeasured whenever the variation in the measurements exceeded the 3% tolerance limit. A test run with 25 randomly chosen individuals, measured double-blind two times in random order, showed that the average variation within a series was 0.38 %, while the average variance between the two series was 0.25 % (data not shown). Based on this level of repeatability, one run of three measurements was considered sufficient to obtain reliable data.

Temperature measurements

Variation in temperature is possibly a crucial variable in the explanation of the results. I measured temperatures continuously during the experiments using temperature data-loggers of Onset Computer Corporation (http://www.onsetcomp.com). Data on humidity could not be reliably obtained because condensation short-circuited the measuring element of the data logger.

COMMON ENVIRONMENT EXPERIMENT.

The common environment experiment (table 1) was carried out in the laboratory in Leiden where the flies were kept in a climate room at 25°C, 70-85% RH and 13:11 light:dark. The general set-up was similar to the field experiment, except that I used vials with moist vermiculite for the development time part of the experiment, and tubes with agar for the starvation part of the experiment.

STATISTICS

The "STATISTICA for Windows" software package (versions 5.5 and 6.0) of StatSoft, Inc. (1999, 2004) was used for all statistical calculations unless stated otherwise.

Removing the effect of variation due to sample size

The first step in the analysis was to remove the impact of species and sample size effects on the data. The number of larvae in each piece of banana was uncontrolled and therefore a possible source of errors in the statistics due to crowding (See **Chapter 3**) or Allee effects (Courchamp *et al.* 1999, Hoffmeister & Rohlfs 2001, Rohlfs & Hoffmeister 2003, Stephens & Sutherland 1999, see also Etienne *et al.*

2002, Wertheim *et al.* 2002). I therefore estimated, for each species, a second-degree relationship between the number of flies in the sample and the realised trait values. The residuals of this analysis were used in further analysis. The use of residuals requires a correction in the degrees of freedom for the denominator of the F-distribution. However, when the number of individuals in tests is sufficient large (N > 50), the corresponding impact is small, and therefore the correction can safely be omitted. In case of doubt (results close to the 5% criterion), I tested for the effect, and only cases with significant differences are reported.

Levels of analysis

Collections of flies were made at six locations, three in each transect. In the **basic analysis**, all collection sites were within one single categorical variable: *'collection site'*, while in the **extended analysis**, the six sites were categorised by two variables: *'transect'* and *'habitat'*. The interaction factor is the same as collection site, but without the variation attributable to the main effects. Whenever the two analyses resulted in the same conclusions, the basic analysis was omitted in the results section of that experiment and trait.

There was more than one species in each of the three experiments, which enabled analysis not only at a species level but also at the community level. In general, the analysis commenced at the intraspecific level. For each specific trait, every species was analysed independently for responses to the different factors (**species-specific level**) using the 'Visual General Linear Model' (VGLM) module of STATISTICA with the trait values as the dependent variable, and the different factors and interactions between the factors as the independent variables. The second level of analysis was to combine the species-specific effects, and to test whether all species combined showed a significant response to the factors (**combined-effect level**). This combined effect does not account for the (lack of) similarity between the different species, so opposing species-specific effects could still result in a significant combined effect. The combined effect was estimated using a Fisher-Omnibus test, to examine whether different p-values, as estimated in different tests, show an overall significant effect. These estimates were calculated in a standard spreadsheet program. This test does not consider the direction of the effects, for which the arguments are mentioned above. The rationale behind not using the VGLM module with species as an independent variable is discussed under the next header. The final level of analysis was the overall analysis; to test whether different factors had a similar effect on all species combined (**overall level**). For this analysis, the VGLM module was used in a similar manner to the species-specific analysis, but now with all data from all species.

The variation within an experiment is partitioned into a portion that is explained by the variables in the model (the **explained** variation, which I for clarity will call **'non-error'** variation) and a portion that yields the unexplained variation (**error** component of the model). This error component of the analysis consists of the pure error component, but also all the variation that could have been explained if more variables were added. However, these additional variables are not of interest for the

research questions under investigation, but merely complicate the interpretation of the analysis. One such variable could be the emergence of flies, which takes place over a period of several days and consequently causes a spread in the development times that is both logical and explainable. In addition, body sizes vary in concordance with this spread in development times. Working with averages would have eliminated this aspect in the error component, but also would have decreased the number of data points dramatically and with that, the power of the analysis. I therefore will not indicate the percentage of the total variation that is explained by the different factors, but instead give the proportion of the **'non-error'** variation.

The number of estimated main and interaction factors in a General Linear Model increases exponentially with the inclusion of more independent variables. As valuable as they are, they easily can distort the overall underlying picture. I therefore grouped, when appropriate, the main and interaction factors into broad categories, which are of importance for interpreting the experiment (table 2). For example, in the second field experiment, four main factors are included: sex, transect, original habitat and experimental habitat. This effectively results in 16 estimated main and interaction factors (table 2). For the issue of what proportion of the **'non-error'** variation can be explained by the underlying genetics (e.g. the

Table 2: Grouping of the different main and interaction factors as estimated in the second field experiment analyses, into the broad categories indicative respectively for *'genetic', 'environmental'* and *'GxE interaction*' related factors or *'habitat and collection site'* related factors. See text for a more extensive explanation.

	Grouping into genetic, environmental and GxE interaction related factors	Grouping along habitat and collection site related factors
transect	-	-
original habitat	Genetic	Habitat
experimental habitat	Environment	Habitat
sex	-	-
transect*original habitat	Genetic	Collection site
transect*experimental habitat	Environment	Collection site
original*experimental habitat	GxE	Habitat
transect*sex	-	-
original habitat*sex	Genetic	Habitat
experimental habitat*sex	Environment	Habitat
transect*original*experimental habitat	GxE	Collection site
transect*original habitat*sex	Genetic	Collection site
transect*experimental habitat*sex	Environment	Collection site
original*experimental habitat*sex	GxE	Habitat
transect*original*experimental habitat*sex	GxE	Collection site

adaptive effects of the original habitat), the variation in the '*original habitat'* factor, the '*original habitat*sex'* factor, the '*transect*original habitat'* factor, and the in the *'transect*original habitat*sex'* factor are all of interest. In a similar way, the remaining main and interaction factors can be grouped into an environmental category and a GxE interaction category. Alternatively, grouping can also take place along any other subdivision into categories, such as *'habitat'* (patterns within transect similar) versus *'collection site'* (patterns within transect different).

Troubleshooting

The impacts of different factors on the data were analysed using General Linear Models (GLM), which allows custom-made designs with multiple categorical and/or continuous variables. The analyses are primarily carried out using the Type VI sums-of-squares or 'Effective hypothesis decomposition' (Hocking 1996, StatSoft 1999), and not the often used Type III sum-of-squares. The 'Effective hypothesis decomposition' is based on the philosophy that the estimate should be based only on the variation uniquely attributable to the effect. In an ANOVA design with missing cells, this results in fewer degrees of freedom than in designs without missing cells and for some missing cell designs, the degrees of freedom can drop to zero. Elimination of the higher interaction factors often eliminates at least some of the empty cells in the design and makes estimation of the other variables possible. In those cases, higher interaction factors, which are excluded in the design to obtain useful estimates, are indicated in tables with the word 'Zeroed'. In the case of a nested design, the type VI sum-of-squares cannot be used, and a type III sum-of-squares will be used instead.

The overall analysis is sensitive to disproportional impacts of single species and a jack-knife procedure, excluding one species at the time from the analysis, was used to detect such species. The outcome of the analysis is not robust if the outcome of the analysis was altered by elimination of a single species, e.g. when significant effects became non-significant or vice versa.

Between trait variation

To test whether different traits covaried, I estimated the correlations of all possible two-trait combinations. Homogeneity-of-slopes models (factorial analysis with an interaction factor between collection site and independent continuous variable) were used to test for consistency of the interspecific correlations over the six different habitats.

Inter-experiment comparison

The realised phenotypic life-history trait values are a result of the underlying genotype, the environment, and genotype-by-environment (GxE) interactions. In order to estimate the relative importance of the genetic background on the field values, the population averages of the first-field experiment were plotted against the population averages of the common environment experiment, both for averages

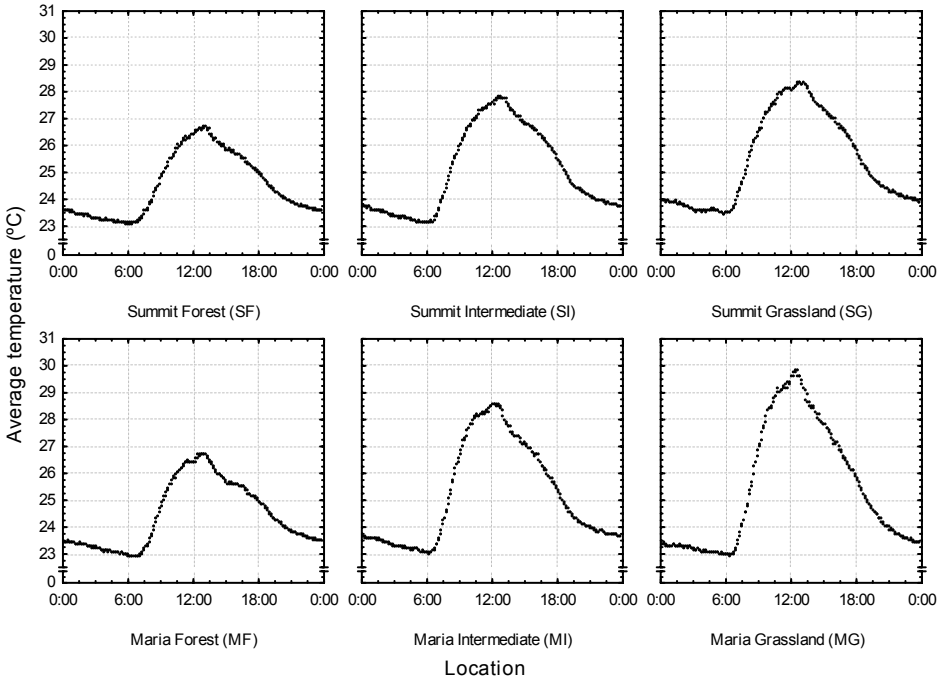


Figure 3: Daily temperature curves for the six habitats during the first field experiment period. The measurements were made between August 21st and September 23rd 1998, with time interval of 6 minutes, and temperatures are averaged per measurement per interval over 33 days. The left two graphs are for the forest locations, the middle two graphs for the intermediate locations and the right two graphs for the grassland populations.

based on the raw data and on the residuals as described before. A change in slope and/or intercept within both types of plots is indicative of the influence of the environmental change on the realised life-history values. When the genetics dominates the realised phenotype, and GxE interactions are absent, the intercept will be zero and the slope of the line will be one, regardless of which averages are used. Positive or negative environmental effects, but also GxE interactions will change the slope and intercept of the fitted line. Both variables are estimated with a degree of error, therefore, Reduced Major Axis regression was used (Bohonak 2002, Bohonak & van der Linde 2004, Kermack & Haldane 1950, Ricker 1973, see also: Sokal & Rohlf 1981).

Results

TEMPERATURES

Temperature measurements were made between August 21st and September 23rd 1998, with time intervals of 6 minutes. Figure 3 shows that the temperatures at the

different collection sites fluctuate widely. Temperature measurements varied between 20 ºC and 35 ºC, but the average midday (between 11:00 and 15:00 hours) and midnight (between 23:00 and 03:00 hours) temperatures are less extreme (table 3). Average day temperature (24 hour period), average midday temperature, day temperature standard deviation, and habitat are all highly correlated with each other. Forest habitats have the lowest day and midday averages, and standard deviations, while grassland has the highest day and midday averages, and standard deviations (habitat - day average: $r = 0.86$, $p = 0.029$; habitat - day SD: $r = 0.83$, $p = 0.042$; habitat - midday average: $r = 0.84$, $p = 0.039$; day average - day SD: $r = 0.89$, $p = 0.017$; day average - midday average: $r = 0.95$, $p = 0.004$; day SD - midday average: $r = 0.99$, $p < 0.001$).

The general picture shows that the daily temperature fluctuations are the largest in the grassland locations, and the smallest in the forest locations, with the intermediate locations nicely in between. The temperature fluctuations between days show the same picture, with the highest fluctuations between days in the grassland locations and the smallest in the forest locations. In more detail, most fluctuation between days occurs around noon (SD's ranging between 1.5 and 3 °C) while the lowest fluctuations are found during the night (SD's between 0.5 and 0.7 °C). The average midnight temperature is much higher in the Summit Grassland series. This location is located directly next to the Panama Canal, which could dampen the daily temperature fluctuations. Average midnight temperature showed no correlation with any of the other variables, and showed a limited variation of just over 0.5 ºC. The main source of variation between the habitats is the midday temperatures, which are much higher in the grassland, while the midnight temperatures are similar in all habitats (figure 3).

Table 3: Average 24 hour, midday and midnight temperatures, and standard deviation for the average 24 hour temperatures per site (in °C).

Temperature regime (°C)

	Summit Forest	Summit Intermediary	Summit Grassland	Maria Forest	Maria Intermediary	Maria Grassland
Average day (0.00 - 24:00 hours)	24.47	25.01	25.38	24.40	25.15	25.39
SD day	1.47	1.94	2.07	1.54	2.27	2.69
Average midday (11:00 - 15:00 hours)	26.35	27.42	27.97	26.34	27.98	28.97
Average midnight (23:00 - 03:00 hours)	23.48	23.62	23.86	23.39	23.54	23.32

SAMPLE SIZES

Body size, development time and starvation resistance are all influenced by high crowding levels resulting in smaller adults (Bakker 1961, Borash & Ho 2001, Chiang & Hodson 1950, Santos 1996, Zwaan *et al.* 1991). Such adults need a longer time to develop (Borash & Ho 2001, Chiang & Hodson 1950, Zwaan *et al.* 1991), and at eclosion, they have a shorter starvation time (Baldal *et al.* in press, Borash & Ho

2001). Significant interaction effects between sample size and the realised trait value were observed in all experiments for all three traits. Not all species showed a significant impact, but the total number of significant results was higher than expected based on the type 1 errors. I therefore used residuals for the rest of the analysis, except when noted otherwise.

BODY SIZE

First-field experiment

As the basic and extended analyses resulted in the same conclusions, only the extended analysis results are presented. In this extended analysis, with the two main factors *'habitat'* and *'transect'*, *'sex'* was highly significant with all individual species showing significant differentiation between the sexes (table 4). Females were larger than males. The *'habitat*transect'* interaction factor, which is equivalent to *'collection site'*, was significant (table 4), as were the underlying *'habitat'* and *'transect'* factors (table 4). The sensitivity analysis showed that transect had a non-significant effect after removal of *D. melanogaster* (after removal: χ^2= 25.43; df=22; p=0.27682), but that both other factors were robust. The overall estimate of the *'intercept'* was significant (table 4) but not robust to removal of *D. malerkotliana*, which resulted in a non-significant estimate (after removal: χ^2 = 30.42, df = 22, p = 0.11). The remaining interaction factors were non-significant (table 4).

In the overall analysis, the error component explained 37.7% of all variation in both the basic as well as the extended analysis. The sexual dimorphism in body size with larger females was confirmed and explained over 99% of all the non-error variation in each analyses ($F_{1;2704}$ = 4406.55, p < 0.0001). It is unsurprising that this sexual size dimorphism overshadows the other factors in the analysis, as these differences are of a completely different nature from those between sites or habitats. Consequently, the non-error variation that can be explained by the remaining factors is very small, but that does not make them less meaningful.

Both the *'site'* and *'site*sex'* factors were significant in the basic analysis (*site*: $F_{5;2704}$ = 4.82, p = 0.0002, non-error variation explained = 0.54 %; *site*sex*: $F_{5;2704}$ = 2.77, p = 0.017, non-error variation explained = 0.31 %). However, the robustness analysis using the jack-knife method revealed that the *'site'* effect was solely attributable to *D. melanogaster* (remaining species: $F_{5;2243}$ = 2.04, p = 0.07). The *'site*sex'* factor was sensitive to three removals of species in the jack-knife procedure, and the jack-knife procedure showed that excluding *D. melanogaster* resulted in a non-significant estimate for the *'site*sex'* factor. The contrast analysis on *'collection site'* varied with the inclusion or exclusion of *D. melanogaster* and was, therefore, not considered further.

In the extended analysis, only *'transect'* and *'habitat*transect*sex'* showed significant effects (*transect*: $F_{1;2704}$ = 15.75, p = 0.0001, non-error variation explained = 0.35 %; *habitat*transect*sex*: $F_{2;2704}$ = 4.64, p = 0.0097, non-error

Table 4: P-values for the different factors as estimated in the extended analysis, for each species separately and based on body size residuals. The overall p-values were calculated with the Fisher-omnibus test. See Material & Methods for 'zeroed' values. The last column contains the sample sizes.

	intercept	habitat	transect	sex	hab*tran	hab*sex	tran*sex	hab*tran*sex	N
D. cardinoides	p=0.8512	p=0.4165	p=0.9917	**p<0.0001**	p=0.4564	p=0.5783	p=0.5410	p=0.9634	54
D. equinoxialis	**p=0.0065**	**p=0.0162**	**p=0.0306**	**p<0.0001**	p=0.5538	p=0.6520	p=0.0611	p=0.2794	283
D. melanogaster	p=0.0607	p=0.1858	**p<0.0001**	**p<0.0001**	**p<0.0001**	**p=0.0414**	p=0.5330	p=0.3698	468
D. malerkotliana	**p<0.0001**	**p=0.0001**	**p<0.0001**	**p<0.0001**	Zeroed	p=0.3765	p=0.2918	Zeroed	133
D. nebulosa	p=0.7413	p=0.2620	p=0.7669	**p<0.0001**	**p<0.0001**	p=0.5234	p=0.1333	p=0.3414	255
D. neomorpha	p=0.8153	**p<0.0001**	p=0.8598	**p<0.0001**	Zeroed	p=0.2162	p=0.9137	Zeroed	62
D. saltans	p=0.7244	**p=0.0035**	p=0.1096	**p<0.0001**	p=0.1356	p=0.1341	p=0.0617	p=0.9107	259
D. simulans	**p<0.0001**	**p=0.0098**	p=0.1153	**p<0.0001**	**p<0.0001**	p=0.2928	p=0.2584	p=0.2058	601
D. septentriosaltans	**p<0.0001**	p=0.1366	p=0.4501	**p<0.0001**	Zeroed	p=0.4993	p=0.4676	Zeroed	87
D. sturtevanti	p=0.0620	p=0.3417	p=0.5876	**p<0.0001**	p=0.8566	p=0.1836	p=0.4646	p=0.1868	127
D. tropicalis	p=0.4310	**p=0.0022**	p=0.1498	**p<0.0001**	Zeroed	p=0.2120	p=0.5956	Zeroed	227
D. willistoni	p=0.4439	p=0.0503	p=0.9628	**p<0.0001**	Zeroed	p=0.1103	**p=0.0244**	Zeroed	184
Chi-square value	46.29	45.2	51.6	>344.60	42.38	14.5	15	5.85	
Df	24	24	24	24	14	24	24	14	
p-value	**p=0.0041**	**p=0.0055**	**p=0.0009**	**p<0.0001**	**p=0.0001**	p=0.9344	p=0.9206	p=0.9702	

variation explained = 0.21 %). In the robustness analysis, *'transect'* seems to be solely accounted for by *D. melanogaster* (remaining species: $F_{5;2243} = 1.64$, $p = 0.20$). When *D. nebulosa* was removed from the species pool, the *'habitat'* and *'habitat*transect'* factors became significant (remaining species: *transect*: $F_{1;2410} = 4.59$, $p = 0.0102$; *habitat*transect*sex*: $F_{2;2410} = 15.75$, $p < 0.0001$). The robustness analysis on the remaining species showed that *'habitat*transect'* was robust, but *'habitat'* was not. All the other interaction factors did not show robust significant results. The '*habitat*transect'* factor showed the same pattern in the contrast analysis as in the basic analysis. The contrast analysis for *'habitat'* showed as expected no differentiation between the habitats, unless *D. nebulosa* was removed from the data set. After removal, the contrast analysis (with superscripts indicating similarity groups) for *'habitat'* showed the following order: Intermediate [a] < Grassland [a, b] < Forest [b].

Most species showed clear intraspecific variation between the samples collected at different sites and part of that variation was related to the differences in habitat. However, in the combined analyses and the overall analysis, the results were not robust. This leads to the conclusion that neither *'habitat'*, nor *'collection site'* had a consistent impact on the realised body sizes in the field despite clear variation within the different species.

Second-field experiment

The four species in the second-field experiment were present in unequal numbers; 171 individuals for *D. sturtevanti*, 210 for *D. cardinoides*, 1208 for *D. melanogaster* and 1239 for *D. equinoxialis*. Thus, the last two species have a relatively large influence on the overall analysis, and one species could easily dominate the outcome of the whole analysis. Therefore, the different species were first analysed independently.

The analysis of *D. cardinoides* suffered heavily from many empty cells, especially in combination with the 'effective hypothesis decomposition' (Hocking 1996) that I used for the sums-of-squares calculations. Many cells in several interaction factors could not be estimated, and none of those factors, which were estimated, were significant. Removal of the highest interaction factor resulted in four significant factors: *'original habitat'* ($F_{1,207} = 8.14$, $p = 0.0048$), '*original*experimental habitat'* ($F_{2,207} = 6.71$, $p = 0.0015$), '*transect*original*experimental habitat'* ($F_{2,207} = 4.52$, $p = 0.012$) and '*transect*experimental habitat*sex'* ($F_{2,207} = 8.08$, $p = 0.0004$). The use of type III sums-of-squares resulted in the same significant results, but also in some additional results which were significant (*intercept*: $F_{1,206} = 26.13$, $p < 0.0001$; *transect:* $F_{1,206} = 12.78$, $p = 0.0004$; *experimental habitat*: $F_{2,206} = 5.52$, $p = 0.0046$; *sex*: $F_{1,206} = 272.55$, $p < 0.0001$; *transect*experimental habitat*: $F_{2,206} = 3.96$, $p = 0.021$).

The interpretation for *D. equinoxialis* was more straightforward. This species showed a clear sexual dimorphism (table 5). Of the other factors, seven were significant. It is noteworthy that the two transects differed significantly (table 5). Of

Table 5:: F-statistics and p-values for the different main and interaction factors as estimated in the second field experiment, each based on body size residuals and for all four species separately. The use of the 'Effective Hypothesis Decomposition' model causes the reduction to zero of the degrees of freedom (see Material & Methods).

Species	*D. cardinoides*			*D. equinoxialis*			*D. melanogaster*			*D. sturtevanti*		
	DF	F	p	DF	F	p	DF	F	p	DF	F	p
Intercept	0		-	1	2.92	0.0875	1	3.63	0.057	0		-
transect (tran)	0		-	**1**	**7.16**	**0.0076**	**1**	**187.85**	**<0.0001**	0		-
original habitat (or)	0		-	**2**	**17.44**	**<0.0001**	**1**	**26.40**	**<0.0001**	1	1.45	0.2309
Experimental habitat (ex)	0		-	**2**	**29.55**	**<0.0001**	**2**	**10.35**	**<0.0001**	1	0.75	0.3875
Sex	0		-	**1**	**4872.01**	**<0.0001**	**1**	**1060.20**	**<0.0001**	0		-
tran*or	0		-	**2**	**4.37**	**0.0129**	**1**	**22.56**	**<0.0001**	1	1.40	0.238
tran*ex	0		-	**2**	**5.35**	**0.0049**	**2**	**6.04**	**0.0025**	1	0.08	0.7733
or*ex	1	2.98	0.0856	4	1.27	0.2795	**2**	**7.81**	**0.0004**	3	0.40	0.7533
tran*sex	0		-	1	0.13	0.723	**1**	**4.76**	**0.0294**	0		-
or*sex	0		-	2	0.81	0.4431	**1**	**4.61**	**0.0321**	1	0.07	0.7905
ex*sex	0		-	2	0.56	0.5697	2	1.34	0.2633	1	0.03	0.8648
tran*or*ex	1	2.41	0.122	**4**	**5.46**	**0.0002**	**2**	**3.37**	**0.0348**	3	1.68	0.1735
tran*or*sex	0		-	2	0.66	0.5159	1	1.01	0.3145	1	3.73	0.0552
tran*ex*sex	0		-	**2**	**4.24**	**0.0146**	2	2.42	0.0891	1	0.28	0.5948
or*ex*sex	1	0.46	0.4984	4	1.35	0.2511	**2**	**3.78**	**0.0231**	**3**	**3.13**	**0.0274**
tran*or*ex*sex	1	1.35	0.2472	4	0.44	0.7765	2	2.02	0.1334	3	1.30	0.2763
Error	206			1201			1180			151		

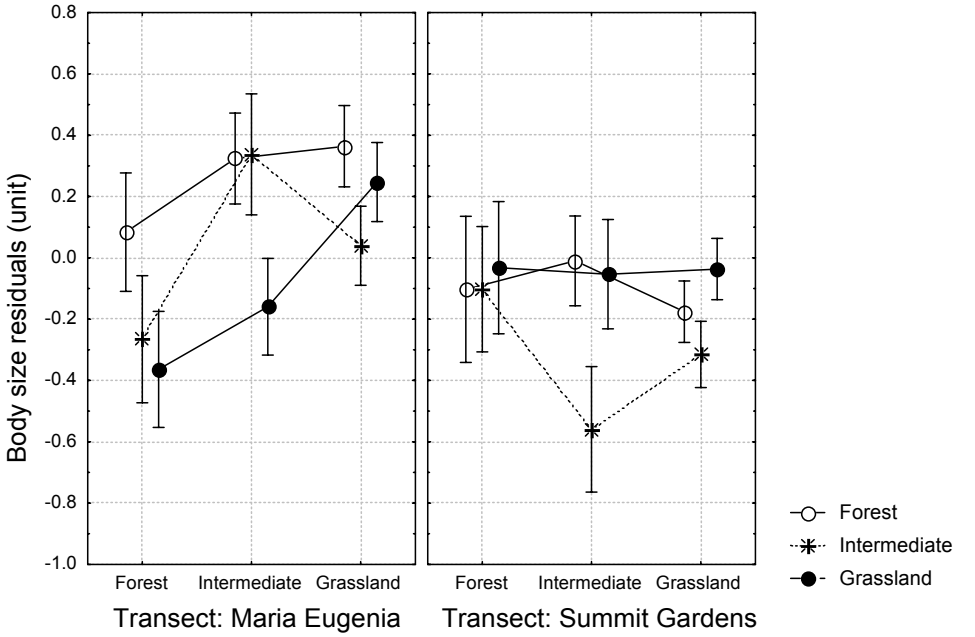


Figure 4: Transect-specific plot for body size residuals (with standard errors) with effects of experimental habitat (lines), original habitat (forest-grassland), and transect (Maria-Summit).

the remaining significant factors, '*experimental habitat', 'transect*experimental habitat'* and *'transect*experimental habitat*sex'* were related to the experimental habitat (table 5), two to the original habitat (table 5: *original habitat; transect*original habitat*) and the last one was related to the interaction between original and experimental habitat (table 5: *transect*original*experimental habitat*).

The analysis of *D. melanogaster* showed the most significant factors. This species also showed a clear sexual dimorphism (table 5), and transect effect (table 5: *transect; transect*sex*). Of the remaining significant factors, two were related to the experimental habitat (table 5: *experimental habitat; transect*experimental habitat*), three to the original habitat (table 5: *original habitat*; *transect*original habitat*; *original habitat*sex*) and the last three to the interaction between original and experimental habitat (table 5: *original*experimental habitat*; *transect*original*experimental habitat*; *original*experimental habitat*sex*).

Finally, *D. sturtevanti* also had some empty cells that made the analysis less straightforward. Removal of the highest interaction factor resulted in a change of the estimated factors: the '*original*experimental habitat*sex'* factor dropped out, while '*transect'* and *'sex'* became significant (table 5). When I analysed the data using the type III sums-of-squares, all three mentioned significant factors remained significant.

The overall analysis for body size suffered somewhat from the low number of species, and the large differences in sample size between the species. The error component explained about 40 % of all variation. Because of this unequal contribution, several factors dropped out in the jack-knife procedure, and only three factors were robust. The species were clearly sexually dimorphic (*sex:* $F_{1,2804}$ = 3961.63, p < 0.0001, non-error variation explained = 95.98 %), leaving a mere 4 % to be explained by the other factors in the analysis. The two other robust factors were *'transect'* ($F_{1,2804}$ = 29.17, p < 0.0001, non-error variation explained = 0.71 %) and '*transect*original*experimental habitat'* ($F_{4,2804}$ = 5.37, p = 0.0003, non-error variation explained = 0.52 %, figure 4). The five factors which were not robust were the *'intercept'* ($F_{1,2804}$ = 4.45, p = 0.035, non-error variation explained = 0.11 %), two factors related to the experimental habitat (*experimental habitat*: $F_{2,2804}$ = 10.24, p < 0.0001, non-error variation explained = 0.50 %; *transect*experimental habitat*: $F_{2,2804}$ = 11.37, p < 0.0001, non-error variation explained = 0.55 %), two to the original habitat (*original habitat*: $F_{2,2804}$ = 4.60, p = 0.01, non-error variation explained = 0.22 %; *transect*original habitat*: $F_{2,2804}$ = 13.08, p < 0.0001, non-error variation explained = .63 %) and the last one to the interaction between experimental and original habitat (*original*experimental habitat*: $F_{4,2804}$ = 2.74, p = 0.027, non-error variation explained = 0.27 %). The contrast analysis (with superscripts indicating similarity groups) showed for *'experimental habitat'* the following order: intermediate ^{a} < grassland ^{b} < forest ^{b} and for the *'origin habitat'*: forest ^{a} < grassland $^{a, b}$ < intermediate ^{b}.

In the overall analysis, almost 41 percent of the variation were attributed to the error component of the model. Of the non-error variation, 95.98 % was explained by the differences between the sexes. Removal of this sex difference by using residuals for the analysis would increase the percentage variation explained by the different categories, but would not change the importance of the different categories relative to each other. About one percent was explained by each of the three categories (table 5): genetic (1.04%), environmental (1.14%) and GxE interactions (1.00%). The remaining variation, which could not be grouped into one of the main categories, was in the *'transect'* (0.71 %) and *'intercept'* (0.11 %) factors. When the factors were grouped according to whether they showed *'collection site'* or *'habitat'* specific differences, 1.97 percent is *'collection site'* related (e.g. showed transect specific variation between the habitats) while 1.21 percent was *'habitat'* related (e.g. habitat related variation which was similar between transects). The remaining variation was in the *'intercept'*, *'sex'* and *'transect'* components. Both *'habitat'* and *'collection site'* related variation is divided among these three categories: genetic, environmental and GxE interactions.

The different species showed clear responses to differences in their habitat. However, they showed hardly any similarity in those responses to the different *'habitats'* or *'collection sites'*. The two transects differed consistently from each other. Females are larger than males, and this was confirmed for all four species. The GxE interaction factor at the *'collection site'* level*,* was the only other factor in this analysis that was robust and significant.

Common environment experiment

The results of the basic and extended analyses are equivalent, so only the results of the extended analysis are presented. The sexual dimorphism in body size for all but one species showed a highly significant effect (table 6), and a strong combined and robust result (table 6). Furthermore, *'habitat'* (table 6: 9 out of 12), *'transect'* (table 6: 8 out of 11) and *'habitat*sex'* (table 6: 4 out of 12) had significant combined estimates, only *'habitat*sex'* was not robust. The remaining interaction factors did not have a significant combined estimate.

The overall analyses showed that 40.9% of the variation was in the error component. Of the non-error variation, nearly 99 % was attributable to the sexual dimorphism present in these species ($F_{1;9010}$ = 12818.6, p < 0.0001, non-error variation explained = 98.61 % (basic), 98.57 % (extended)).

In the basic analysis, all other factors were significant (*site*: $F_{5;9010}$ = 22.23, p < 0.0001, non-error variation explained = 0.85 %; *site*sex*: $F_{5;9010}$ = 5.14, p = 0.0001, non-error variation explained = 0.20 %; *'intercept'*: $F_{1;9010}$ = 44.22, p < 0.0001, non-error variation explained = 0.34 %), and the results were robust for all factors as removal of single species did not change the overall outcome of the test. The contrast analysis showed that Summit-Grassland and Maria-Forest individuals were smaller than the individuals of the remaining sites.

In the extended overall analysis, all but two factors (*'transect'* and *'transect*sex'*) were significant (*transect*: $F_{1;9010}$ = 0.035, p = 0.85, non-error variation explained < 0.01 %; transect*sex: $F_{2;9010}$ = 0.36, p = 0.55, non-error variation explained < 0.01 %). However, the jack-knife procedure showed that *'habitat*sex'* ($F_{2;9010}$ = 7.92, p < 0.0001, non-error variation explained = 0.12 %) and *'habitat*transect*sex'* ($F_{2;9010}$ = 6.41, p = 0.0017, non-error variation explained = 0.10 %) were not robust after removal of respectively *D. saltans* or *D. septentriosaltans*. The remaining two factors, '*habitat'* and *'habitat*transect'* were significant, and robust to jack-knifing the data (*habitat*: $F_{2;9010}$ = 6.21, p = 0.002, non-error variation explained = 0.10 %; *habitat*transect*: $F_{2;9010}$ = 50.27, p < 0.0001, non-error variation explained = 0.77 %). The contrast analysis showed that the grassland individuals were smaller than those from the forest or the intermediate habitat. The contrast analysis for the *'habitat*transect'* factor was consistent with the basic analysis.

The conclusion is that all but one species showed clear differentiation between the different populations. In the overall analysis, both a collection site effect as well as a habitat effect were present. Individuals from Summit-Grassland and Maria-Forest were smaller than individuals from other locations. Individuals collected in the grassland were genetically smaller than those collected in the other two habitats.

Table 6: P-values for the different factors as estimated in the extended analysis, for each species separately and based on body size residuals. The overall p-values were calculated with the Fisher-omnibus test. See Material & Methods for 'zeroed' values. The final column gives the number of individuals per species.

	Intercept	Habitat	Transect	Sex	Site	Hab*sex	Tran*sex	Hab*tran*sex	N
D. cardinoides	**p=0.0335**	**p=0.0002**	**p=0.0250**	**p<0.0001**	Zeroed	p=0.9253	Zeroed	Zeroed	248
D. equinoxialis	**p=0.0017**	**p<0.0001**	**p=0.0019**	**p<0.0001**	**p=0.0171**	p=0.6338	p=0.6898	p=0.1334	1586
D. melanogaster	**p=0.0001**	**p=0.0026**	**p<0.0001**	**p<0.0001**	p=0.5550	p=0.8658	**p=0.0103**	p=0.9155	1081
D. malerkotliana	p=0.4567	**p=0.008**8	p=0.1301	**p<0.0001**	Zeroed	p=0.2447	p=0.9929	Zeroed	136
D. nebulosa	p=0.7917	**p=0.0450**	**p=0.0423**	**p<0.0001**	Zeroed	p=0.3737	p=0.1482	Zeroed	400
D. neomorpha	p=0.7679	p=0.3388	Zeroed	p=0.6124	Zeroed	p=0.1088	Zeroed	Zeroed	10
D. saltans	p=0.2458	**p<0.0001**	p=0.7327	**p<0.0001**	p=0.1342	**p<0.0001**	p=0.2144	Zeroed	958
D. simulans	**p<0.000**1	p=0.5479	p=0.1995	**p<0.0001**	p=0.7157	p=0.8268	p=0.8756	p=0.2345	843
D. septentriosaltans	p=0.2523	**p<0.0001**	**p=0.0073**	**p<0.0001**	Zeroed	**p=0.0082**	**p=0.0404**	Zeroed	707
D. sturtevanti	**p=0.0216**	**p=0.0023**	**p=0.0001**	**p<0.0001**	p=0.4632	**p=0.0324**	**p=0.0064**	**p=0.0415**	395
D. tropicalis	p=0.1695	**p<0.0001**	**p=0.0001**	**p<0.0001**	Zeroed	p=0.0634	p=0.6773	Zeroed	1388
D. willistoni	p=0.2941	p=0.1642	**p<0.0001**	**p<0.0001**	Zeroed	**p=0.0062**	p=0.4744	Zeroed	1270
Chi-square value	36.26	97.31	99.4	352.43	6.75	36.54	15.58	5.85	0
Df	24	24	22	24	10	24	20	8	0
p-value	p=0.0518	**p<0.0001**	**p<0.0001**	**p<0.0001**	p=0.7491	**p=0.0486**	p=0.7426	p=0.6639	0

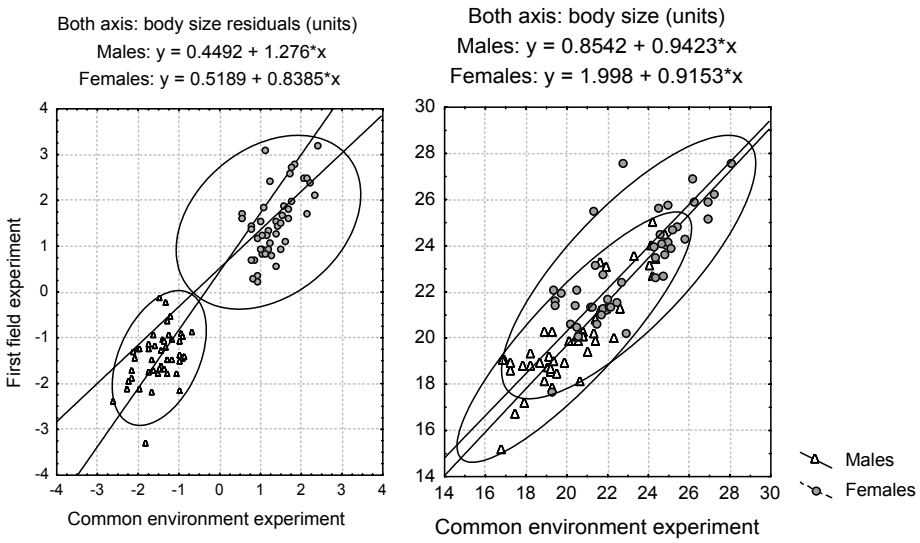


Figure 5: Common environment - first field experiment comparison for body size, full data and residuals (units). Population averages without weight factor, estimated using Reduced Major Axis regression (see Sokal & Rohlf 1981) as both variables are estimated with error. The residuals in the left panel are the same as in the analyses of the specific experiment, estimated over all individuals within a species. Ellipses indicate 95% confidence regions.

Inter-experiment comparison

The correlation across species between the first field experiment and the common environment experiment (figure 5, right panel) is highly significant (males: R^2 = 0.755, n = 47, p < 0.001; females: R^2 = 0.619, n = 47, p < 0.001) and the correlation coefficients do not differ from each other (p = 0.11). The slopes of the Reduced Major Axis (RMA) regression (see Sokal & Rohlf 1981) are close to 1, and are within the bootstrapped 95% confidence intervals (males: slope = 0.9423, range = 0.804 - 1.105; females: slope = 0.9153, range 0.778 - 1.089).

In the inter-experiment comparison using residuals (figure 5, left panel), both sexes showed a significant match between the common environment and first field experiment (males: R^2 = 0.172, n = 46, p = 0.004; females: R^2 = 0.103, n = 47, p = 0.028). The two correlation coefficients did not differ significantly from each other (p = 0.74). The bootstrapped 95% confidence intervals around the slopes of the RMA regression included the x = y line (males: slope = 1.276, range = 0.877 - 1.680; females: slope = 0.8385, range = 0.486 - 1.598).

The comparison between the two experiments shows a high degree of similarity, which suggests that the underlying genetics are more important than the

environment for the realised body sizes. The match between the two experiments is especially striking at the between species comparison, and less so at the within species comparison. This suggests that extrapolation of the common environment experiment to the field situation is possible, especially when patterns across species are considered.

Overall conclusion for body size

Table 7: Overview of body size differences between habitats, regardless of whether the differences between the habitats were significant or not. Superscript indicate groups withn each row.

Experiment 1	Inconsistent
Experiment 2: experimental habitat	Intermediate [a] < Grassland [b] < Forest [b]
Experiment 2: original habitat	Forest [a] < Grassland [a, b] < Intermediate [b]
Common environment	Grassland [a] < Forest [b] < Intermediate [b]

Table 7 shows an overview of the patterns between the different habitats within the different experiments. The results of the second field experiment are split into original and experimental habitat. Within species, genetic and phenotypic variation between the populations was present. At an overall level, the phenotypic consistency between the species was weak and inconsistent. However, at the genotypic level, systematic variation was related to collection site as well as habitat (smaller individuals in the grassland habitat and in Summit-Grassland and Maria-Forest collection sites). This is confirmed by the close match between the realised body sizes in the common environment experiment and the first field experiment. The transplantation (second field) experiment showed that GxE effects played a role, and that this type of effect could explain at least in part the incomplete match in the inter-experiment comparison.

DEVELOPMENT TIME

First-field experiment

The extended analysis resulted in the same conclusions as the basic analysis. In this analysis *'collection site'* was spilt into the underlying *'habitat'* and *'transect'* factors and *'sex'* was significant in eight out of twelve species and in the overall estimate (table 8). Of the remaining factors, only the overall estimate for *'habitat'* was significant (table 8), with four of twelve species showing a significant result. However, this result was not robust, as removal of either *D. malerkotliana* or *D. nebulosa* resulted in a non-significant overall result. The remaining factors were all non-significant and in each case a limited number of species showed significant results.

In the overall analysis, over 91 % of the variation in the data was in the error component for both the basic as well as the extended analysis. Of the variation

Table 8: P-values for the different factors as estimated in the extended analysis, for each species separately and based on development time residuals. The overall p-values were calculated with the Fisher-omnibus test. The last column contains the number of individuals for each species.

	intercept	habitat	transect	sex	hab*tran	hab*sex	tran*sex	hab*tran*sex	N
D. cardinoides	p=0.6414	p=0.6656	p=0.8935	p=0.8355	P=0.0716	p=0.2524	p=0.9713	p=0.2989	97
D. equinoxialis	p=0.1567	p=0.8875	p=0.5891	**p=0.0002**	p=0.8613	p=0.5375	p=0.4522	p=0.2194	638
D. melanogaster	p=0.3696	p=0.1042	p=0.1832	**p<0.0001**	**p=0.0014**	p=0.4080	p=0.2257	p=0.3644	103
D. malerkotliana	**p<0.0001**	**p<0.0001**	**p<0.0001**	p=0.7139	All Zeroed	p=0.4329	p=0.5736	All Zeroed	290
D. nebulosa	p=0.5351	**p<0.0001**	p=0.6290	**p=0.0006**	p=0.1804	p=0.8128	p=0.3265	p=0.4923	621
D. neomorpha	p=0.4869	**p=0.0017**	p=0.6886	p=0.4282	One	p=0.5302	p=0.4144	One Zeroed	112
D. saltans	p=0.3087	p=0.0597	p=0.5002	**p<0.0001**	p=0.0715	p=0.1157	p=0.2576	p=0.3155	516
D. simulans	p=0.8974	p=0.6206	**p=0.0006**	**p<0.0001**	p=0.0804	**p=0.0258**	p=0.1734	**p<0.0001**	139
D. septentriosaltans	p=0.0617	p=0.0608	p=0.1657	p=0.1649	All Zeroed	p=0.6663	p=0.3681	All Zeroed	163
D. sturtevanti	p=0.4296	p=0.4619	**p=0.0345**	**p<0.0001**	p=0.0825	p=0.5048	p=0.8141	p=0.3182	253
D. tropicalis	p=0.0818	**p=0.0075**	p=0.2746	**p<0.0001**	One	p=0.0834	p=0.0819	One Zeroed	456
D. willistoni	p=0.6159	p=0.2843	p=0.2284	**p=0.0002**	All Zeroed	p=0.3614	p=0.3623	All Zeroed	364
Chi-square value	23.99	41.96	34.72	79.84	16.26	13.01	11.03	14.85	
Df	24	24	24	24	14	24	24	14	
p-value	p=0.4620	**p=0.0130**	p=0.0727	**p<0.0001**	p=0.2978	p=0.9660	p=0.9888	p=0.3882	

explained by either model, *'sex'* was the most important component, explaining over two-thirds of the non-error variation (extended: $F_{1;2756}$ = 191.61, p < 0.0001, non-error variation explained = 71.11 %; basic: $F_{1;2756}$ = 191.61, p < 0.0001, non-error variation explained = 71.80 %).

In the basic analysis, *'collection site'* had a significant effect ($F_{5;2756}$ = 7.83, p < 0.0001, non-error variation explained = 14.66 %) as had the interaction factor between *'collection site'* and *'sex'* ($F_{5;2756}$ = 5.77, p < 0.0001, non-error variation explained = 10.82 %) and the *'intercept'* ($F_{1;2756}$ = 7.27, p = 0.007, non-error variation explained = 2.73 %). The jack-knife procedure to examine whether any particular species had a disproportional impact on the basic analysis revealed that the *'intercept'* and the *'collection site*sex'* interaction factors were based on two species (*D. nebulosa* and *D. simulans*) and one species (*D. simulans*), respectively. *D. simulans* was the only species which had a significant result for the interaction factor *'collection site*sex'* (p < 0.0001) in the analysis of the individual species (table 8). A renewed jack-knife analysis with *D. simulans* excluded, showed a significant and consistent effect of *'sex'* ($F_{1;2159}$ = 171.06, p < 0.0001, non-error variation explained = 79.5 %) and *'collection site'* ($F_{5;2159}$ = 6.86, p < 0.0001, non-error variation explained = 15.9 %), while *'collection site*sex'* became non-significant ($F_{5;2159}$ = 1.23, p = 0.29). The *'intercept'* was now not significant (p = 0.056) but was sensitive to removal of four different species. The contrast analysis for *'collection site'* showed that the Maria-Forest populations had significantly longer development times than those from all other collection sites.

Most factors in the extended analysis were significant. After *'sex'*, the interaction factor *'habitat*transect*sex'* explained most of the non-error variation ($F_{2;2756}$ = 8.36, p = 0.0002, non-error variation explained = 6.20 %). The other significant factors explained, respectively, 4.98 % (*habitat*: $F_{2;2756}$ = 6.71, p = 0.0012), 5.61 % (*transect*: $F_{1;2756}$ = 15.13, p = 0.0001), 1.71 % (*habitat*transect*: $F_{2;2756}$ = 6.02, p = 0.0025) and 4.76% (*habitat*sex*: $F_{2;2756}$ = 6.41, p = 0.0017) of the non-error variation, while the non-significant '*transect*sex'* interaction factor explained 0.17 % of the non-error variation ($F_{1;2756}$ = 0.46, p = 0.50). Again, the jack-knife procedure showed a disproportional impact of *D. simulans*. Removal of this species resulted in elimination of all interaction factors with sex. However, the results without *D. simulans* were not robust, and subsequent removal of species resulted in elimination of all factors. The contrast analysis on *'collection site'* confirmed the picture of the basic analysis, in that the Maria-Forest populations had significantly longer development times than those from all other collection sites. The contrast analysis (with superscripts indicating similarity groups) for *'habitat'* revealed the following order: Grassland ^{a} < Intermediate $^{a, b}$ < Forest ^{b}.

This led to the conclusion that the realised development times in the field show a clear relation with *'collection site'*. Partitioning the *'collection site'* component into the underlying *'habitat'* and *'transect'* components did not yield stable results in either the individual analysis, nor in the overall analysis. Removal of *D. simulans* gave much more stable results in the *'collection site'* based analyses, but several species disrupted the overall picture for the extended analyses using *'habitat'* and *'transect'*.

Table 9: F-statistics and p-values for the different main and interaction factors as estimated in the second field experiment, each based on development time residuals and for all four species separately. The use of the 'Effective Hypothesis Decomposition' model causes the reduction to zero of the degrees of freedom (see Material & Methods).

Species	*D. cardinoides*			*D. equinoxialis*			*D. melanogaster*			*D. sturtevanti*		
	DF	F	p	DF	F	p	DF	F	p	DF	F	p
Intercept	0	0	-	1	0.08	0.7816	1	4.16	0.0417	0	0	-
transect (tran)	0	0	-	1	3.37	0.0668	**1**	**15**	**0.0001**	0	0	-
original habitat (or)	0	0	-	2	1.44	0.2381	**1**	**13.94**	**0.0002**	**1**	**3.99**	**0.0477**
Experimental habitat (ex)	0	0	-	**2**	**3.13**	**0.0441**	2	2.61	0.0740	1	0.19	0.6664
Sex	0	0	-	**1**	**114.73**	**<0.0001**	**1**	**56.6**	**<0.0001**	0	0	-
tran*or	0	0	-	2	0.85	0.4279	**1**	**4.64**	**0.0314**	1	0.01	0.9335
tran*ex	0	0	-	2	1.19	0.3033	**2**	**4.88**	**0.0078**	1	0	0.9579
or*ex	1	2.79	0.0966	**4**	**3.18**	**0.0129**	2	1.29	0.2750	3	1.94	0.1254
tran*sex	0	0	-	1	3.48	0.0623	1	0.69	0.4054	0	0	-
or*sex	0	0	-	2	0.23	0.7906	1	2.1	0.1472	1	0.52	0.4733
ex*sex	0	0	-	2	2.31	0.0993	2	2.38	0.0933	1	0.03	0.8534
tran*or*ex	1	0.09	0.7700	**4**	**5.45**	**0.0002**	**2**	**6.22**	**0.0021**	3	0.8	0.4955
tran*or*sex	0	0	-	**2**	**4.01**	**0.0183**	1	0.04	0.8379	**1**	**9.75**	**0.0021**
tran*ex*sex	0	0	-	**2**	**6.13**	**0.0023**	2	1.42	0.2430	1	2.57	0.1112
or*ex*sex	1	0.11	0.7365	4	2.14	0.0733	2	2.71	0.0667	3	0.58	0.6299
tran*or*ex*sex	1	2.08	0.1504	4	2.06	0.0843	2	2.51	0.0815	3	2.19	0.0917
Error	206			1203			1184			151		

Second-field experiment

As for body size, the four species in the second-field experiment were present in unequal numbers, which may influence the overall analysis. Therefore, the different species were first analysed independently.

The analysis of *D. cardinoides* suffered heavily from many empty cells (see Material & Methods), but neither the removal of highest interaction factor nor the use of type III sums-of-squares resulted in significant factors for this species.

The second species, *D. equinoxialis*, showed a clear sexual dimorphism (table 9). Five of the other factors were significant. Two factors were related to the experimental habitat (table 9: *experimental habitat*; *transect*experimental habitat*sex*), one to the original habitat (table 9: *transect*original habitat*sex*) and two for the interaction between original and experimental habitat (table 9: *original*experimental habitat*; *transect*original*experimental habitat*).

D. melanogaster also showed a clear sexual dimorphism (table 9). The impact of the transects was prominent (table 9). The subdivision of the remaining significant factors for this species showed that one factor is related to the experimental habitat (table 9: *transect*experimental habitat*), two factors to the original habitat (table 9: *original habitat*; *transect*original habitat*) and the last to the interaction factor between original and experimental habitat (table 9: *transect*original*experimental habitat*)

The final species, *D. sturtevanti* also suffered from empty cells in the analysis, but less so than *D. cardinoides*. Removal of the highest interaction factor resulted in more estimated factors but in lower estimates for both significant factors, while the factor for *'sex'* could now be estimated and was highly significant. The use of type III sums-of-squares did not give more insight either, other than that this species was also highly sexual dimorphic. The two significant factors were both associated with the original habitat (table 9: *original habitat*; *transect*original habitat*sex*).

Several factors in the overall analysis were significant and the robustness analysis left several factors untouched, despite the large and unequal contribution to the dataset by two species. Most variation was within the error component, (90.76 %). Overall, the species showed a clear sexual dimorphism ($F_{1,2830}$ = 170.0, $p < 0.0001$, non-error variation explained = 58.89 %). Two of the three remaining factors were related to the experimental habitat (*experimental habitat*: $F_{2,2830}$ = 8.36, p = 0.0002, non-error variation explained = 5.8 %; *transect*experimental habitat*sex*: $F_{2,2830}$ = 10.0, $p < 0.0001$, non-error variation explained = 6.94 %), the final one to the interaction between original and experimental habitat (*transect*original*experimental habitat*: $F_{4,2830}$ = 6.9, $p < 0.0001$, non-error variation explained = 9.58 %, figure 6). Three factors dropped out of the list as they were not robust, two related to the original habitat (*original habitat*: $F_{2,2830}$ = 4.61, p = 0.01, non-error variation explained = 3.2 %; *transect*original habitat*: $F_{2,2830}$ = 4.73, p = 0.0089, non-error variation explained = 3.28 %) and one related to the experimental

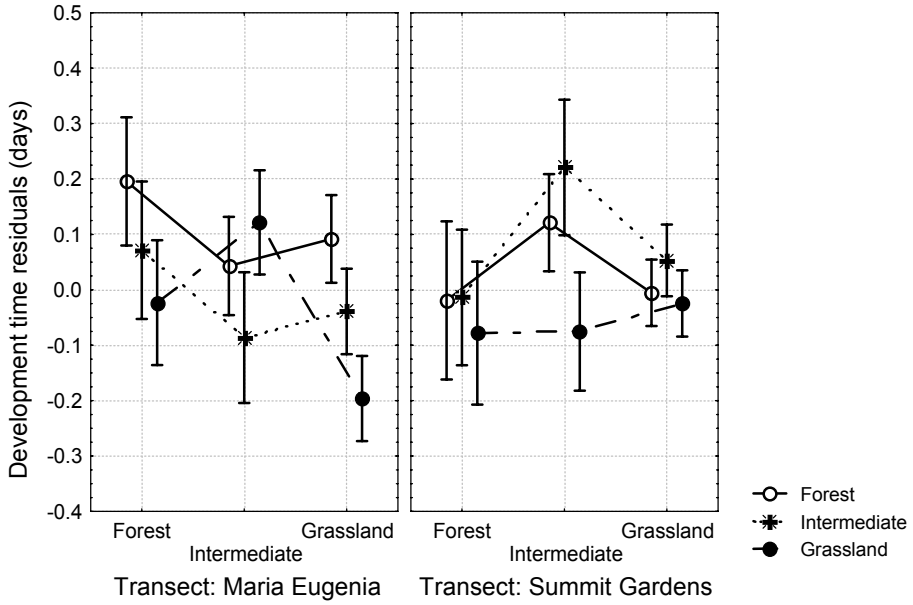


Figure 6: Transect-specific plot for development time residuals (with standard errors) with effects of experimental habitat (lines), original habitat (forest-grassland), and transect (Maria-Summit).

habitat (*transect*experimental habitat*: $F_{2,2830} = 4.70$, $p = 0.0092$, non-error variation explained = 3.26 %). The contrast analysis (with superscripts indicating similarity groups) showed for *'experimental habitat'*: grassland ^{a} < intermediate ^{b} < forest ^{b} and for the *'origin habitat'*: grassland ^{a} < forest $^{a, b}$ < intermediate ^{b}.

All factors except the *'intercept'*, *'transect'* and *'sex'* factors could be attributed to the genetic component, the environmental component, or the GxE interaction component (table 2). Most variation was in the error component, explaining 90.76% of all variation. When the remaining variation was divided over the different categories, 58.89 percent was explained by the sexual dimorphism, 7.91 percent by the genetic component, 16.37 percent by the environmental component and 15.77 percent was in the GxE interaction component. The remaining variation was in the *'transect'* and *'intercept'* factors. When the factors were grouped according to whether they showed differences between the transects or not, 27.56 percent was *'collection site'* related while 12.49 percent was *'habitat'* related (the remaining was in *'intercept'*, *'sex'* and *'transect'* components). Both *'habitat'* and *'collection site'* related variation was divided among all three components: genetic, environmental and GxE interactions.

Overall, it is clear that genetic, environmental and GxE interactions play a role in the expression of the development times between the different habitats. However, there was substantial variation between the different species. The similarity between the

two transects was low as about twice as much variation was related to *'collection site'* than to *'habitat'*.

Common environment experiment

In the extended individual species analysis, which resulted in the same conclusion as the basic analysis, several factors had a significant combined effect. *'Sex'* had the largest combined impact (table 10) and seven out of twelve species showed a significant effect. Half of the species showed a significant effect for *'habitat'*, and the combined estimate was significant and robust (table 10). Six out of eleven species showed a significant *'transect'* effect; the estimate for one species (*D. neomorpha*) was zeroed, as the remaining populations were all from one transect. The combined estimate was robust and significant (table 10). Seven of the twelve estimates for the *'habitat*transect'* factor were zeroed due to missing combinations between habitat and transect. Three out of the remaining five species showed a significant result, as was the robust combined result (table 10). Some of the estimates for the remaining factors were significant, but none of the factors had a significant combined estimate.

Most variation in the basic and extended overall analysis was pure error (basic: 94.79 %; extended: 95.09 %). Both analyses confirmed that females have a shorter development time than males, in both cases explaining 49.26 % of the non-error variation (both: $F_{1,9035} = 229.63$, $p < 0.0001$). *'Collection site'* was the only other significant factor in the basic analysis ($F_{5,9035} = 50.85$, $p < 0.0001$, non-error variation explained = 51.22 %), and the results were robust as none of the species had a disproportional impact on the outcome in the jack-knife procedure. The interaction factor *'site*sex'* and the *'intercept'* were both not significant (*site*sex*: $F_{5,9035} = 1.97$, $p = 0.08$, non-error variation explained: 1.98 %; *intercept*: $F_{1,9035} = 2.71$, $p = 0.10$, non-error variation explained: 0.55 %). The contrast analysis showed three groups as indicated by the superscripts: Maria-Grassland ^{a} < Maria-Intermediate ^{b} < Maria-Forest ^{b} < Summit-Grassland ^{b} < Summit-Intermediate ^{c} < Summit-Forest ^{c}.

In the extended analysis, both the *'habitat'* and *'transect'* factors were significant (*habitat*: $F_{2,9035} = 51.45$, $p < 0.0001$, non-error variation explained: 22.07 %; *transect*: $F_{1,9035} = 120.91$, $p < 0.0001$, non-error variation explained: 25.94 %). Both results were robust using the jack-knife method which showed that none of the species had a disproportional impact on the outcome of the analysis. The remaining (interaction) factors were all non-significant ($p < 0.10$), explaining less than one percent of the non-error variation. Contrast analysis showed that individuals collected in the grassland sites or in the Maria transect had shorter development times and formed a separate group versus the other two types of habitats or the summit transect.

The conclusion is that there is a clear habitat and transect related impact on the development times as measured in the laboratory under a common environment regime. Furthermore, this impact was consistent for all species.

Table 10: P-values for the different factors as estimated in the extended analysis, for each species separately and based on development time residuals. The overall p-values were calculated with the Fisher-omnibus test. Last column contains the number of individuals for each species.

	intercept	habitat	transect	sex	hab*tran	hab*sex	tran*sex	hab*tran*sex	N
D. cardinoides	p=0.7434	p=0.3446	p=0.6413	p=0.3247	Zeroed	p=0.0692	Zeroed	Zeroed	249
D. equinoxialis	p=0.7463	**p<0.0001**	**p=0.0011**	**p<0.0001**	**p=0.0242**	p=0.0850	p=0.1140	p=0.2294	1590
D. melanogaster	**p=0.0010**	**p<0.0001**	**p<0.0001**	**p<0.0001**	p=0.1322	p=0.2292	**p=0.0219**	p=0.8244	1084
D. malerkotliana	p=0.9746	**p=0.0481**	p=0.1197	p=0.4638	Zeroed	p=0.6832	p=0.2894	Zeroed	136
D. nebulosa	p=0.3671	p=0.5976	p=0.8118	**p=0.0396**	Zeroed	p=0.6080	p=0.4458	Zeroed	402
D. neomorpha	p=0.8691	p=0.8691	Zeroed	p=0.2739	Zeroed	p=0.2739	Zeroed	Zeroed	10
D. saltans	**p=0.0163**	**p<0.0001**	**p=0.0353**	p=0.1583	**p<0.0001**	p=0.9266	p=0.8876	Zeroed	959
D. simulans	p=0.7998	**p=0.0027**	p=0.6121	**p<0.0001**	p=0.1000	p=0.5039	p=0.1291	p=0.4216	844
D. septentriosaltans	p=0.3254	p=0.4501	p=0.2010	**p<0.0001**	Zeroed	p=0.9340	p=0.7708	Zeroed	710
D. sturtevanti	p=0.3774	p=0.1120	**p=0.0005**	**p<0.0001**	**p<0.0001**	p=0.7792	p=0.0872	p=0.7071	395
D. tropicalis	p=0.5207	**p=0.0002**	**p<0.0001**	**p<0.0001**	Zeroed	**p=0.0012**	p=0.2744	Zeroed	1393
D. willistoni	p=0.2174	p=0.0532	**p<0.0001**	p=0.2655	Zeroed	p=0.9371	p=0.9670	Zeroed	1275
Chi-square value	14.97	98.31	96.47	112.57	50.1	14.47	12.36	2.5	
Df	24	24	22	23	10	24	20	8	
p-value	p=0.9217	**p<0.0001**	**p<0.0001**	**p<0.0001**	**p<0.0001**	p=0.9352	p=0.9030	p=0.9618	

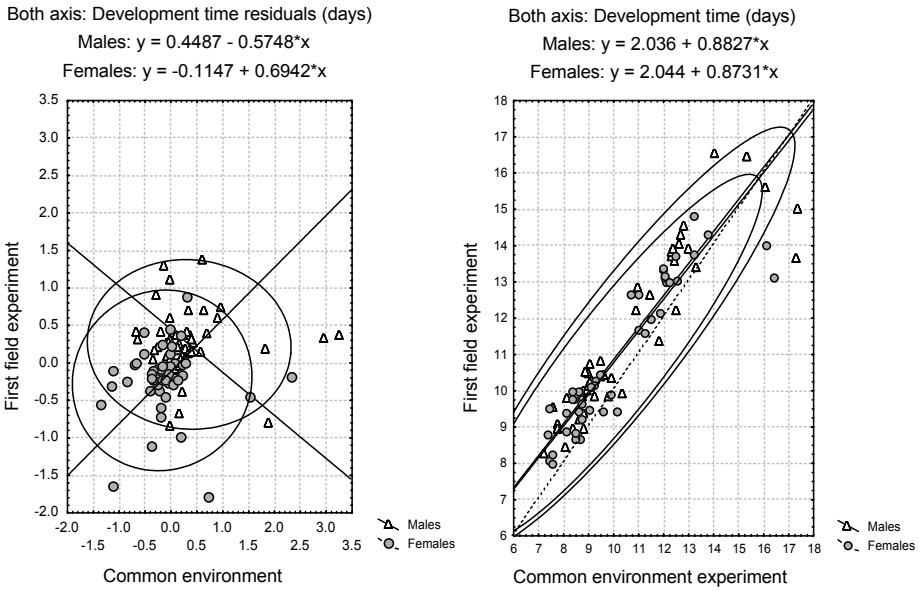


Figure 7: Common environment - first field experiment comparison for development times, full data and residuals (days). Population averages without weight factor, estimated using Reduced Major Axis regression (see Sokal & Rohlf 1981) as both variables are estimated with error. The residuals in the left panel are the same as in the analyses of the specific experiment. Ellipses indicate 95% confidence regions.

Inter-experiment comparison

The inter-experiment comparison on the averages of the raw data (figure 7, right panel) shows a clear correlation between the two experiments. The results of the two experiments were highly correlated, while there were no differences between the sexes (males: $R^2 = 0.831$, n = 47, p <0.0001; females: $R^2 = 0.826$, n = 47, p < 0.0001; comparison for difference: p = 0.47). The bootstrapped 95% confidence intervals of the slopes of the Reduced Major Axis (RMA) regression (see Sokal & Rohlf 1981) include the x = y line (males: slope = 0.8827, range = 0.733 - 1.083; females: slope = 0.8731, range = 0.720 - 1.030).

The same analysis with the averages from the residuals (figure 7, left part) showed a weak and non-significant correlation between the trait values of the common environment experiment and the first field experiment (males: R^2 =0.001, n = 47, p = 0.82; females: $R^2 = 0.002$, n = 47, p = 0.78). The bootstrapped 95% confidence intervals around the slopes of the RMA regression were extremely large, underlining the absence of any correlation between the two experiments.

This result led to the conclusion that the outcome of both experiments is highly correlated at the interspecific level, but not correlated at all at the intraspecific level. The first field experiment was carried out in three different environments, while the

common environment was similar for all populations. This makes the extrapolation of the results obtained in the common environment experiment to the field situation difficult.

Overall conclusion for development time

Table 11: Overview of development time differences between habitats, regardless of whether the differences between the habitats were significant or not.

Experiment 1	Grassland < Intermediate < Forest
Experiment 2: experimental habitat	Grassland < Intermediate < Forest
Experiment 2: original habitat	Grassland < Forest < Intermediate
Common environment	Grassland < Intermediate < Forest

There is evidence that local adaptation has occurred in most of the twelve species in this study. This local selection was *'habitat'* and *'transect'* specific with shorter development times for individuals from the grassland habitats and the Maria transect. However, development times as measured in the field do not show *'habitat'* or *'transect'* specific differentiation, but rather are *'collection site'* related. This difference between the common environment experiment and the first-field experiment suggest that GxE interactions might play a role. These GxE interactions were indeed found in the second-field experiment and explained a larger proportion of the variation than genetic differences alone. The lack-of-fit within species between the first field experiment and the common environment underlines that the realised development times are dependent on all three factors: environment, genetic and GxE interaction.

STARVATION RESISTANCE

First-field experiment

Due to the experimental set-up, it was impossible to match starvation resistance values to development times, body sizes, and sex of the same individual flies. However, the impact of sex on starvation resistance was estimated by regressing the residuals of the sample averages for starvation resistance as the independent variable and the sex ratio of the same sample as the dependent variable. The influence of sex ratio on starvation resistance was non-significant (R^2 = -0.0538, N = 162, p=0.5). Apparently, the variation in the sex ratios among the different samples appeared not to have influenced starvation resistances in the experiment and were not considered further.

The impact of the *'collection site'* was determined using the starvation resistance residuals with *'site'* as a categorical factor. The results in table 12 show that *'site'* was a significant factor in eight out of twelve species. The overall estimate showed that the overall effect was significant and none of the species had a disproportional impact on the combined outcome.

Table 12: P-values for the different factors as estimated in the basic and extended analysis, for each species separately and based on starvation resistance residuals. The final column gives the number of individuals per species. The overall p-values were calculated with the Fisher-omnibus test. (df=24 in each case; log(0) replaced with -16).

	Basic analysis		Extended analysis				
	Intercept	Site	Intercept	Habitat	Transect	Site	N
D. cardinoides	p=0.5413	**p=0.0131**	p=0.5413	**p=0.0117**	p=0.9966	**p=0.0239**	54
D. equinoxialis	p=0.8290	**p=0.0194**	p=0.8290	p=0.0867	p=0.0765	p=0.2983	283
D. melanogaster	p=0.5699	**p=0.0321**	p=0.5699	p=0.0597	p=0.5711	p=0.0921	464
D. malerkotliana	p=0.5552	**p=0.0001**	**p=0.0002**	**p=0.0043**	**p<0.0001**	Zeroed	129
D. nebulosa	p=0.9257	p=0.1128	p=0.9257	p=0.0570	p=0.2490	p=0.6313	297
D. neomorpha	p=0.5900	**p=0.0337**	p=0.5662	p=0.2076	p=0.0960	Zeroed	60
D. saltans	p=0.9057	**p<0.0001**	p=0.9057	**p=0.0002**	p=0.0813	**p=0.0043**	259
D. simulans	p=0.6602	**p=0.0004**	p=0.6602	**p=0.0182**	p=0.0643	**p=0.0317**	597
D. septentriosaltans	p=0.5960	p=0.1167	p=0.7285	p=0.0637	p=0.6509	Zeroed	83
D. sturtevanti	p=0.5205	p=0.0996	p=0.5205	p=0.2217	p=0.1453	**p=0.0220**	127
D. tropicalis	p=0.4735	**p<0.0001**	p=0.3360	**p<0.0001**	p=0.4702	Zeroed	225
D. willistoni	p=0.8851	p=0.2923	p=0.3881	p=0.1193	p=0.2828	Zeroed	178
Chi-square value	4.44	58.81	12.24	48.22	24.23	17.81	
Df	24	24	24	24	24	14	
p-value	p=1.0000	**p=0.0001**	p=0.9771	**p=0.0024**	p=0.4483	p=0.2154	

The extended analysis showed that the starvation resistance of five out of twelve species was significantly affected by *'habitat'*, as was the combined estimate (table 12). However, this combined result was not robust, as removal of *D. tropicalis* yielded a non-significant result (after removal: χ^2 = 33.67, df = 22, p = 0.053). Populations of *D. malerkotliana* showed significant differentiation between the two transects, while four out of the twelve species showed a significant *'habitat*transect'* interaction. However, the combined estimates for '*transect'*, *'habitat*transect'* and intercept were not significant (table 12).

In the overall analysis, most variation in the data was in the error component (basic and extended: 98.69 %). In the basic analysis, *'collection site'* had a significant effect ($F_{5;2921}$ = 7.58, p < 0.0001, non-error variation explained = 97.99 %), while the *'intercept'* was non-significant ($F_{1;2921}$ = 0.78, p = 0.38, non-error variation explained = 2.01 %). The jack-knife procedure showed that none of the species had a disproportional impact on the outcome of the analysis. The contrast analysis revealed three groups (with superscripts indicating different groups): Maria-Grassland ^{a} < Summit-Intermediate $^{a, b}$ < Summit-Grassland $^{b, c}$ < Maria-Intermediate $^{b, c}$ < Summit-Forest $^{b, c}$ < Maria-Forest ^{c}.

In the extended overall analysis, both the *'habitat'* and *'habitat*transect'* factors were significant (habitat: $F_{2;2921}$ = 8.64, p = 0.0002, non-error variation explained = 51.2 %; habitat*transect: $F_{2;2921}$ = 8.60, p = 0.0002, non-error variation explained = 47.56 %). The result was, however, not robust as *D. simulans* had a disproportional impact on the *'habitat*transect'* factor (after removal: $F_{2;2298}$ = 1.57, p = 0.21). The renewed analysis with *D. simulans* omitted showed that *D. tropicalis* now had a disproportional impact on the outcome of the *'habitat'* factor (after removal of both species: $F_{2;2065}$ = 2.81, p = 0.06). The two remaining factors were non-significant (*transect*: $F_{1;2921}$ = 0.001, p = 0.97; *intercept*: $F_{1;2921}$ = 0.78, p = 0.38). The contrast analysis showed two groups (with superscripts indicating different groups): grassland ^{a} < intermediate $^{a, b}$ < forest ^{b}.

The overall conclusion is that flies show clear differences in starvation resistance. Flies from the forest habitats had higher starvation resistance than the intermediate populations, and both had a higher starvation resistance than the grassland populations, although the variation was not consistent at the *'habitat'* level unlike the *'collection site'* level.

Second-field experiment

As with body size and development time, the different species were first analysed independently because of the large variation in numbers of individuals for the different species.

The analysis of *D. cardinoides* suffered from many empty cells (see Material & Methods) and none of the factors that could be estimated was significant. However,

Table 13: F-statistics and p-values for the different main and interaction factors as estimated in the second field experiment, each based on starvation resistance residuals and for all four species separately. The use of the 'Effective Hypothesis Decomposition' model causes the reduction to zero of the degrees of freedom (see Material & Methods).

Species	*D. cardinoides*			*D. equinoxialis*			*D. melanogaster*			*D. sturtevanti*		
	DF	F	p	DF	F	p	DF	F	p	DF	F	p
Intercept	0	0	0	1	1.99	0.1591	1	2.7	0.1007	1	0.03	0.8741
Transect (Tran)	0	0	0	1	66.2	0	1	27.11	0	1	0.2	0.6518
Original habitat (Or)	1	2.09	0.1495	2	1.09	0.3379	1	8.46	0.0037	2	0.86	0.4265
Experimental habitat (Ex)	0	0	0	2	87.7	0	2	19.93	0	2	2.1	0.1253
Tran*Or	1	0.49	0.4837	2	14.6	0	1	0.98	0.3235	2	0.42	0.6592
Tran*Ex	0	0	0	2	11.14	0	2	5.02	0.0067	2	1.33	0.2676
Or*Ex	2	1.54	0.2172	4	7.42	0	2	1.95	0.1423	4	1.48	0.2114
Tran*Or*Ex	2	0.7	0.4964	4	4.72	0.0009	2	11.68	0	4	0.29	0.8855
Error	218	0	0	1247	0	0	1234	0	0	171	0	0

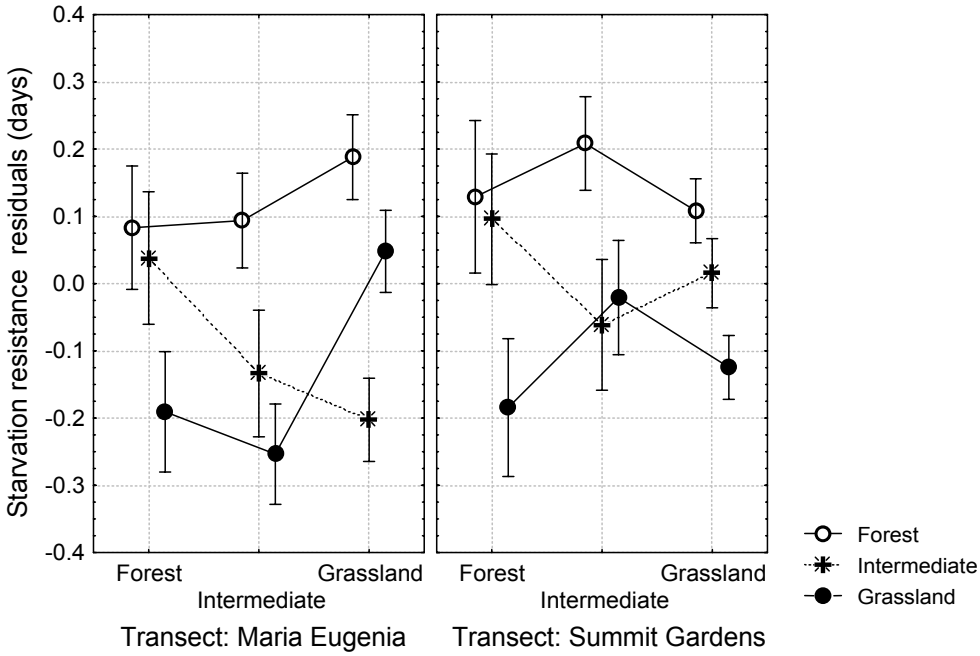


Figure 8: Transect-specific plot for starvation resistance residuals (with standard errors) with effects of experimental habitat (lines), original habitat (forest-grassland), and transect (Maria-Summit).

when the highest interaction factor was removed, *'experimental habitat'* became significant ($F_{2;220}$ = 4.11, p = 0.018). Using type III sum-of-squares gave the similar results. *D. equinoxialis* showed significant effects of all but two factors in the analysis (table 13). The number of significant results for *D. melanogaster* was lower than for the previous species (table 13). For *D. sturtevanti*, none of the factors was significant.

In the overall analysis, 92.25% of the variation was in the error component and five of the factors were significant. After the jack-knife procedure to test whether any of the species had a disproportional impact on the outcome, three factors remained. The most important factor was *'experimental habitat'* ($F_{2;2915}$ = 66.16, p < 0.0001, non-error variation explained: 54.04 %). The other two factors were *'transect'* ($F_{1;2915}$ = 6.73, p =0.01, non-error variation explained: 2.75 %) and *'transect*original*experimental habitat'* ($F_{4;2915}$ = 9.02, p < 0.0001, non-error variation explained: 14.73 %, figure 8). Two interaction factors were not significant after removal of *D. equinoxialis* which is in line with the individual species analysis in which only this species had significant effects for these two factors (*transect*original habitat*: $F_{2;2915}$ = 8.49, p = 0.0002, non-error variation explained: 6.94 % ; *original*experimental habitat*: $F_{4;2915}$ = 10.49, p < 0.0001, non-error variation explained: 17.33 %). The remaining factors were not significant. The

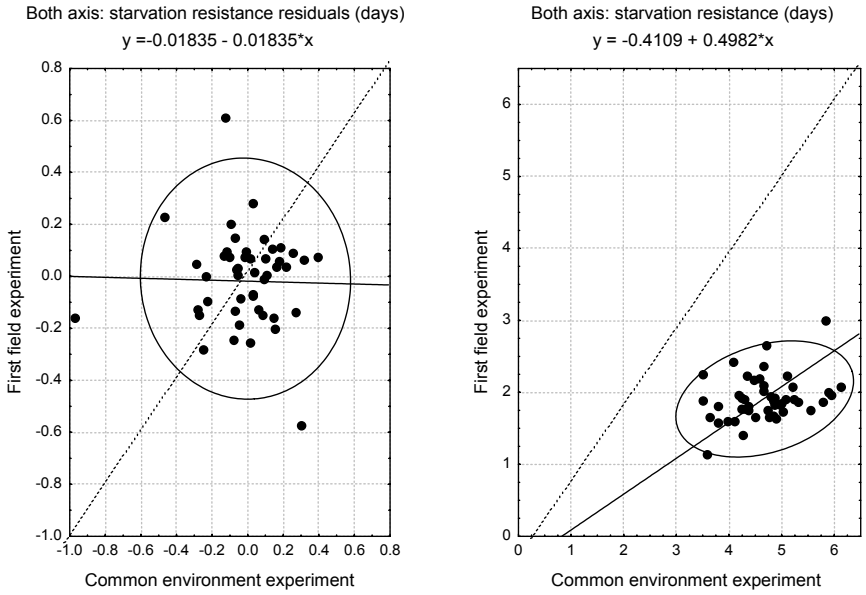


Figure 9: Common environment - first field experiment comparison for starvation resistance, full data and residuals (days). Population averages without weight factor, estimated using Reduced Major Axis regression (solid lines, see Sokal & Rohlf 1981) as both variables are estimated with error. The dotted line indicates when estimated values within both experiments would be equivalent. The residuals in the left panel are the same as in the analyses of the specific experiment. Ellipses indicate 95% confidence regions.

contrast analysis (with superscripts indicating similarity groups) showed for *'experimental habitat'*: grassland ^{a} < intermediate ^{b} < forest ^{c}.

A partitioning of the explained variation into genetic, environment and GxE fractions (table 2) showed that the fractions explained 8.70, 56.36 and 32.06 percent of the variation, respectively. A similar subdivision of the explained variation in a *'transect'*, *'habitat'* and *'collection site'* fraction showed that these fractions explained 73.13, 23.99 and 2.75 percent, respectively.

We can make the following conclusions for starvation resistance. The *'experimental habitat'* had a larger impact on the realised values than the original habitat, as it explained most of the variation in the overall analysis as well within three of the four species. Grassland populations had the lowest starvation resistance, while forest populations survived for the longest periods. The origin of a population seemed to be relatively unimportant in this experiment, but a GxE interaction was clearly present.

Table 14: P-values for the different factors as estimated in the basic and the extended analysis, for each species separately and based on body size residuals. The final column gives the number of individuals per species. The overall p-values were calculated with the Fisher-omnibus test. (df=24 in each case; log(0) replaced with -16).

	Basic analysis		Extended analysis				
	Intercept	Site	Intercept	Habitat	Transect	Site	N
D. cardinoides	p=0.6356	**p=0.0003**	p=0.3898	p=0.1779	p=0.5738	Zeroed	249
D. equinoxialis	p=0.1176	**p<0.0001**	p=0.3967	**p<0.0001**	p=0.8653	**p<0.0001**	1588
D. melanogaster	p=0.3654	**p=0.0001**	p=0.3654	**p=0.0032**	**p=0.0013**	**p=0.0057**	1082
D. malerkotliana	p=0.6750	p=0.4536	p=0.5720	p=0.2340	p=0.2662	Zeroed	136
D. nebulosa	p=0.9861	**p=0.0262**	p=0.5070	p=0.2471	p=0.4743	Zeroed	402
D. neomorpha	p=0.8743	p=0.6938	p=0.8743	p=0.6938	Zeroed	Zeroed	10
D. saltans	**p=0.0214**	**p<0.0001**	**p=0.0098**	**p=0.0285**	p=0.7161	p=0.7269	956
D. simulans	p=0.1777	**p<0.0001**	p=0.1777	**p<0.0001**	p=0.5966	**p=0.0007**	844
D. septentriosaltans	p=0.5216	**p=0.0113**	p=0.8576	p=0.0549	p=0.9379	Zeroed	710
D. sturtevanti	p=0.5328	p=0.7677	p=0.5328	p=0.6902	p=0.5364	p=0.9083	386
D. tropicalis	p=0.3927	**p<0.0001**	p=0.1630	p=0.1758	**p<0.0001**	Zeroed	1393
D. willistoni	p=0.0802	**p=0.0045**	**p=0.0095**	**p=0.0035**	**p=0.0272**	Zeroed	1270
Chi-square value	12.55	97.8	15.5	47.28	35.08	21.52	
Df	24	24	24	24	22	10	
p-value	p=0.9729	**p<0.0001**	p=0.9051	**p=0.0031**	**p=0.0380**	**p=0.0177**	

Common environment experiment

The basic analysis with only *'collection site'* as an explaining variable showed that nine out of the twelve species had clear differences between the populations (table 14) and removal of any of the species did not result in a different overall outcome. The extended analysis showed significant results for five out of twelve species for *'habitat'*, three out of eleven species for *'transect'*, and three out of five species for *'collection site'* (table 14). However, all three factors were not robust. *D. equinoxialis* determined the effect within *'habitat'*, while *D. melanogaster* and *D. tropicalis* had a disproportional impact on *'transect'* and *D. equinoxialis* and *D. simulans* on *'collection site'* .

Both the basic and the extended analysis showed that most variation was present in the error component of the analysis (basic: 99.50 %; extended: 99.43 %). The overall basic analysis showed that *'collection site'* had a significant impact on the realised starvation resistances in the laboratory ($F_{5;9040}$ = 9.0, p < 0.0001), and the jack-knife procedure showed that the outcome was robust. The *'intercept'* was not significant (table 17: $F_{1;9040}$ = 0.004, p = 0.95, non-error variation explained: 0.01 %). The contrast analysis for *'collection site'* showed the following order (with superscripts indicating similarity groups): Maria-Forest ^{a} < Maria-Intermediate ^{b} < Maria-Grassland ^{b} < Summit-Forest ^{b,c} < Summit-Intermediate ^{b,c} < Summit-Grassland ^{c}.

In the extended analysis, *'habitat'* and *'transect'* effect were significant (habitat: $F_{2;9040}$ = 10.9, p < 0.0001, non-error variation explained: 41.9 %; transect: $F_{1;9040}$ = 25.09, p < 0.0001, non-error variation explained: 48.4 %). However, the jack-knife procedure showed that the *'transect'* effect was not robust and solely attributable to *D. tropicalis* (after removal: $F_{1;7642}$ = 0.66, p = 0.41), whilst the effect of *'habitat'* was robust. The interaction between the two factors was not significant ($F_{2;9040}$ = 2.5 p = 0.08, non-error variation explained: 9.68 %) as was the *'intercept'* ($F_{1;9040}$ = 0.004, p = 0.95, non-error variation explained: 0.01 %). The contrast analysis for the interaction factor before removal of *D. tropicalis* showed exactly the same pattern as in the basic analysis, but after removal, the pattern was as follows: Maria-Forest ^{a} < Summit-Forest $^{a, b}$ < Maria-Intermediate ^{a,b,c} < Summit-Intermediate ^{a,b,c} < Summit-Grassland ^{b,c} < Maria-Grassland ^{c}. The clear impact of the different habitats was also found in the contrast analysis after removal of *D. tropicalis* for *'habitat'* alone: Forest ^{a} < Intermediate ^{a} < Grassland ^{b}. Before removal of *D. tropicalis*: Forest ^{a} < Intermediate $^{a, b}$ < Grassland ^{b}.

The overall conclusion is that populations showed clear differentiation between the different habitats. The grassland populations had the longest starvation resistances while the forest populations had the shortest.

Inter-experiment comparison

The inter-experiment comparison using the starvation resistance averages from the raw data (figure 9, right panel) showed a large difference between the two

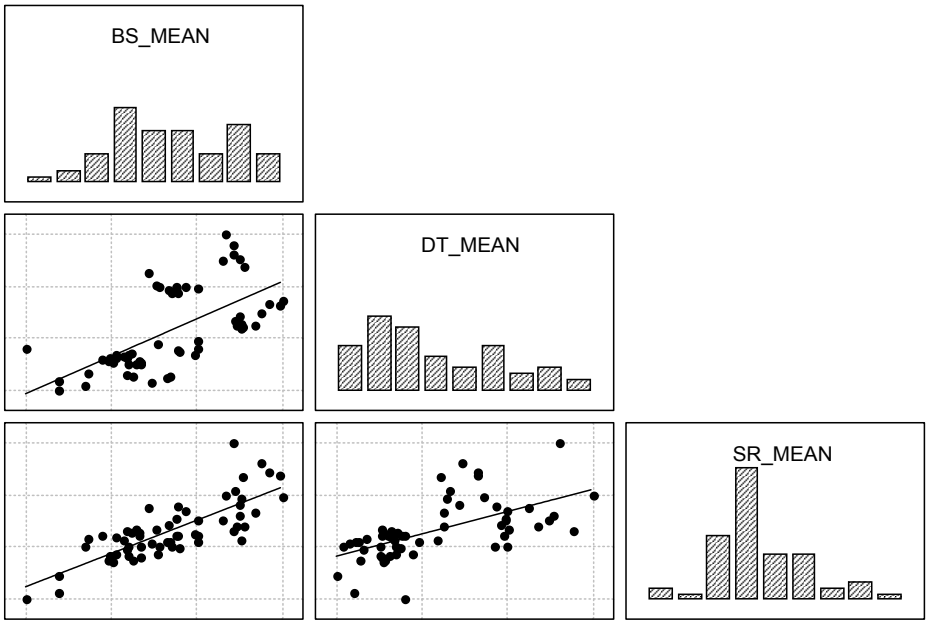


Figure 10: Correlations across species based on population averages from the first field experiment, for body size, development time and starvation resistance. Plots on the complete diagonal axis indicate distribution of the values within a trait, while off-diagonal plots are scatterplots between two traits. Data along the x-axis in each scatterplot correspond to the histogram above the plot, while the data along the y-axis data correspond to the histogram at the right of the plot. BS_MEAN: body size means; DT_MEAN: development time means; SR_MEAN: starvation resistance means.

experiments. The unweighted data showed a barely significant correlation (R^2 = 0.09, n = 48, p = 0.038). The bootstrapped 95% confidence intervals around the estimated Reduce Major Axis (RMA) regression slopes were large, but did not include the unity (x = y) line (slope: 0.4982; range: -0.4041 - 0.6852). The correlation between the two experiments using residuals showed no correlation (R^2 = 0.0004, n = 47, p = 0.89). Fitting a regression line was meaningless as the bootstrapped 95% confidence intervals were extremely large and included both x = y and x = -y lines (figure 9, left panel). The lack of fit between both the intraspecific as well as the interspecific comparison makes it impossible to extrapolate the common environment results to the field situation.

Overall conclusion for starvation resistance

The overall conclusions are straightforward. In the first-field experiment, the differences between the species were clearly related to the habitat, and the populations from the forest had the longest starvation resistances while those from the grassland had the shortest. The *'experimental habitat'* component in the second

field experiment showed the same pattern as in the first field experiment, the grassland population had the shortest, while the forest populations had the longest starvation resistance. In contrast, the *'original habitat'* component showed an opposite trend, indicating that genetically, grassland populations were more highly adapted to starvation resistance than forest populations. The common environment experiment supported this picture as the pattern was similar to the *'original habitat'* component of the second field experiment.

Table 15: Overview of starvation resistance differences between habitats, regardless of whether the differences between the habitats were significant or not.

Experiment 1	Grassland < Intermediate < Forest
Experiment 2: experimental habitat	Grassland < Intermediate < Forest
Experiment 2: original habitat	Forest < Intermediate < Grassland
Common environment	Forest < Intermediate < Grassland

The pattern as described fits neatly with the idea of countergradient variation (Conover & Schultz 1995). The grassland populations have the highest genetically determined starvation resistance, but the starvation resistance values as measured directly in the field are the lowest. This pattern fits the idea well, in that forests represent a more favourable environment for these species, while the grassland is the least favourable environment. This could then explain the increased starvation resistance in the grassland. However, the presumed adaptation is apparently not complete, as the realised starvation resistances in their own habitat are still lower for the grassland populations than for the forest populations. When this mechanism underlies the pattern in starvation resistance, and there is sufficient genetic variation, adaptation is expected to continue towards flies with even higher starvation resistances. The absence of any meaningful correlation on the residuals in the inter-experiment comparison does fit within this pattern. However, the variation between populations is large and the lack of a better fit could be attributable to GxE interactions that explained about one-third of the non-error variation in the second field experiment. The large deviation between the averages based on the raw data is discussed under 'Discussion'.

BETWEEN SPECIES CORRELATIONS

All correlations between two traits were positive and highly significant ($p<0.0001$; $N=59$; R^2 body size-development time: 0.41; R^2 body size-starvation resistance: 0.63; R^2 development time-starvation resistance: 0.36). The results of the corresponding homogeneity-of-slopes model showed that the independent trait was significant in all cases while the interaction factor was never significant. Development time versus body size: intercept: $F_{1,47} = 0.47$, $p = 0.5$; collection site: $F_{5,47} = 0.19$, $p = 0.96$; body size: $F_{1,47} = 33.1$, $p < 0.0001$; collection site * body size: $F_{5,47} = 0.17$, $p = 0.97$. Starvation resistance versus body size: intercept: $F_{1,47} = 4.24$, $p = 0.045$; collection site: $F_{5,47} = 2$, $p = 0.096$; body size: $F_{1,47} = 100.85$, $p < 0.0001$; collection site * body size: $F_{5,47} = 2.29$, $p = 0.061$. Starvation resistance versus development time: intercept: $F_{1,47} = 16.92$, $p = 0.0002$; collection site: $F_{5,47} = 0.37$, p

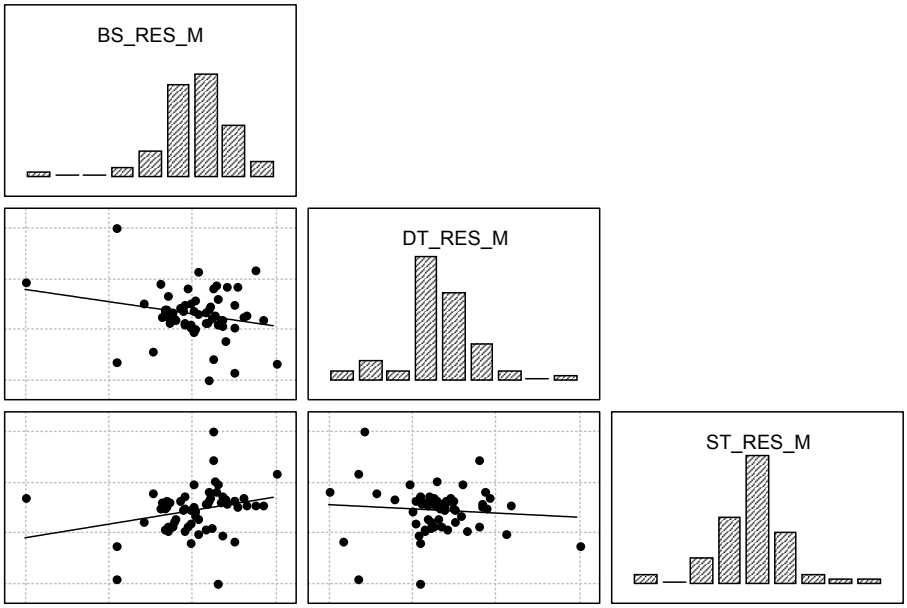


Figure 11: Correlations across species based on population averages from the first field experiment, for residual of body size, development time and starvation resistance. Plots on the complete diagonal axis indicate distribution of the values within a trait, while off-diagonal plots are scatterplots between two traits. Data along the x-axis in each scatterplot correspond to the histogram above the plot, while the data along the y-axis data correspond to the histogram at the right of the plot. Means are based on residuals. BS_RES_M: body size means; DT_RES_M: development time means; SR_RES_M: starvation resistance means.

= 0.87; development time: $F_{1,47}$ = 27.88, p < 0.0001; collection site * development time: $F_{5,47}$ = 0.56, p = 0.7298. The analysis for the common environment data gave a similar overall picture. This homogeneity in the slopes of the regressions within the different collection sites leads to the conclusion that the pattern from the correlations is robust and was little changed by differences between the habitats.

WITHIN SPECIES CORRELATIONS

The results of the homogeneity-of-slopes model were very inconsistent between the first-field experiment and the common environment experiment (table 21). However, the number of data points within each collection site is limited and that could obscure any underlying pattern. The correlations between the traits using the whole data set showed that there is a significant positive correlation between body size and starvation resistance, while the other two correlations are negative and non-significant (body size - starvation resistance: R^2 = 0.071, n = 59, p = 0.041; body size - development time: R^2 = 0.052, n = 59, p = 0.081; development time - starvation resistance: R^2 = 0.0074, n = 59, p = 0.52). This suggests that the link

between body size and starvation resistance is stronger than for the other combinations, but also that all relations between traits are weak and variable.

Table 16: Comparison between the first-field experiment and common environment experiment. Bold cells indicate significant effects.

Dependent variable	Factor	Common environment	1st field experiment
Development time	Intercept	$F_{1,37} = 0.31$, $p = 0.5789$	$F_{1,47} = 0.03$, $p = 0.8528$
	Site	$F_{5,37} = 1.34$, $p = 0.2696$	$F_{5,47} = 3.41$, $p = 0.0105$
	Body size	$F_{1,37} = 2.12$, $p = 0.1538$	$F_{1,47} = 4.39$, $p = 0.0416$
	Site*Body size	$F_{5,37} = 1.93$, $p = 0.1121$	$F_{5,47} = 1.64$, $p = 0.1689$
Starvation resistance	Intercept	$F_{1,37} = 0.30$, $p = 0.5842$	$F_{1,47} = 0.03$, $p = 0.8635$
	Site	$F_{5,37} = 0.86$, $p = 0.5167$	$F_{5,47} = 0.93$, $p = 0.4680$
	Body size	$F_{1,37} = 0.04$, $p = 0.8398$	$F_{1,47} = 4.06$, $p = 0.0497$
	Site*Body size	$F_{5,37} = 4.38$, $p = 0.0031$	$F_{5,47} = 1.21$, $p = 0.3213$
Starvation resistance	Intercept	$F_{1,37} = 0.01$, $p = 0.9346$	$F_{1,47} = 0.00$, $p = 0.9823$
	Site	$F_{5,37} = 1.38$, $p = 0.2559$	$F_{5,47} = 2.02$, $p = 0.0936$
	Development time	$F_{1,37} = 8.80$, $p = 0.0052$	$F_{1,47} = 2.53$, $p = 0.1184$
	Site*Development time	$F_{5,37} = 6.91$, $p = 0.0001$	$F_{5,47} = 2.04$, $p = 0.0898$

Discussion

BODY SIZE

The analysis for body size revealed habitat-related phenotypic and/or genotypic variation between populations within most species, but this variation was not consistent over all species at the overall level. At the collection site level, flies from populations collected in the Summit-Grassland and Maria-Forest *'sites'* were generally significantly smaller than individuals from the other *'collection sites'*. However, at a phenotypic level, the variation between the collection sites was inconsistent and varied with the inclusion or exclusion of a particular single species.

The genetic variation (as measured in the common environment experiment) was significantly correlated with the phenotypic variation (as measured in the first-field experiment). However, the correlations did not explain all the variation. The second-field experiment showed that GxE interactions were important in explaining the variation between populations. The common environment as I used in the laboratory roughly matched the natural environment, but large differences remained. For example, the temperature in the laboratory was 25 ºC, which was close to the average temperature in the field, but the daily temperature fluctuations were much greater in the field compared to the laboratory. The sensitivity of the flies for changes in the natural environment suggest that changes in the environment when the flies are transferred from the field to the laboratory could be of great importance and lead to adaptation to the novel laboratory environment (Matos *et al.* 2000b, Matos *et al.* 2002, Schlichting & Smith 2002, Service & Rose 1985).

DEVELOPMENT TIME

Development time showed clear habitat-related variation in the common environment experiment. Grassland individuals had a shorter development time than individuals from the intermediate habitat, which in turn had a shorter development time than the forest individuals. The two transects also differed significantly from each other: individuals from the Maria transect had shorter development times than those from the Summit transect. The first field experiment data showed a similar pattern for the habitats, but the result for the transects was opposite, with Summit individuals having the shorter development times. The same applies to the *'experimental habitat'* factor in the transplantation (second field) experiment, while the order in the *'original habitat'* factor was different, placing the forest habitat between the two others. This concordance, although with exceptions, between the experiments suggests that the genotypic and phenotypic variation was determined by the same single underlying cause, and that this cause was related to the habitats as they had the same order within the two transects.

What could this selective factor be? Temperature is an unlikely candidate. The average temperatures in the grasslands were higher than in the forest and higher environmental temperatures are associated with shorter development times (Azevedo *et al.* 1996, James *et al.* 1997, Zwaan *et al.* 1992). This was indeed observed for the *'experimental habitat'* component of the second field experiment, while the results of the first field experiment were roughly in line with the expected pattern also. However, the common environment experiment and the *'original habitat'* component of the second field experiment, were expected to show the opposite pattern comparable with the low temperature selection lines. These selection lines are comparable to the forest habitat and have a short development time in comparison to high temperature lines (Anderson 1966, James & Partridge 1995, Partridge *et al.* 1994a, b). This is in sharp contrast with the data, in which the forest individuals have the longer development times. The difference in average temperatures in my experiment is limited to one degree Celsius. This difference is much smaller than the difference in the temperatures used in the selection experiments. However, this difference, whilst expected to give less dramatic results, is not likely to result in opposite outcomes.

Relative humidity varied also with habitat and was lowest in the grassland with the lowest humidity around midday. However, there are no published results on the effect of relative humidity for development time for *Drosophila* and results for other species than *Drosophila* contradict each other (Krasnov *et al.* 2001, Smith 1993). The design of the experiment was such that effects of desiccation on the larvae were unlikely to occur, as the pieces of banana were located on a layer of moist vermiculite that was kept moist. However, genetic differentiation due to variation in relative humidity among the habitats can not be excluded.

Krijger (2000) found in his study on *Drosophila* species in Panama that mean resource abundance increased with disturbance of the habitat. This was consistent with the expectation based on the life-history model of Sevenster & van Alphen

(1993a, 1993b) that an increase in mean resource abundance would lead to a decrease in mean community development time. Furthermore, it was expected that this change would be accomplished by a relative change in the community composition, e.g. the replacement of slow species by fast species. Contrary to the expectations, Krijger (2000) did not find this negative correlation between resource abundance and mean community development time. However, the calculations of the mean community development time were based on a single estimate for the species-specific development times, regardless of the habitat.

In the present study, grassland individuals have the shortest development times while forest individuals have the longest. Based on the results of Krijger (2000) for the average resource abundance in relation to disturbance, forest habitats had the lowest mean resource abundance while the intermediate habitats had a higher mean resource abundance. No data on the mean resource abundance of the grassland habitats were available. His data were obtained in the same area as my own. Moreover, the pattern found in my study fits the prediction based on the life-history model of Sevenster & van Alphen (1993a, 1993b) in which mean resource abundance is negatively correlated with mean community development time. The main difference is that the variation in mean-community development time was not achieved by the replacement of the slow species by fast species, but by a community wide adaptation to the changed environment.

STARVATION RESISTANCE

Starvation resistance shows high levels of phenotypic plasticity. The transplantation experiment showed that flies from the same population, but reared in different habitats realise a higher starvation resistance in the forest habitat compared to the grassland habitat. This difference in expression suggests that the grassland environment is harsher than the forest environment. The same experiment also showed that the *'experimental habitat'* factors, i.e. the environmental component, were more important in explaining the observed pattern than were the *'original habitat'* factors, i.e. the genetic component. This dominance of the environment over the genetics was reflected in the pattern in the first field experiment, which was similar to the *'experimental habitat'* related factors of the second field experiment. Furthermore, the pattern in the common environment experiment was similar to the *'original habitat'* related factors.

Thus, the patterns within the different experiments point towards an overall picture in which the environment becomes increasingly harsh when it is degraded from primary forest to grassland. Such a trend would then suggest a need for the grassland populations to adapt to the changed environment, which has indeed happened. That the realised starvation resistances remains lower in the grassland than in the forest, indicates that the adaptation is incomplete and, if selectable genetic variation remains (Blows & Hoffmann 1993, Hoffmann *et al.* 2003a, Roff 2003), the populations would be expected to evolve further and become even better adapted to the changed environment.

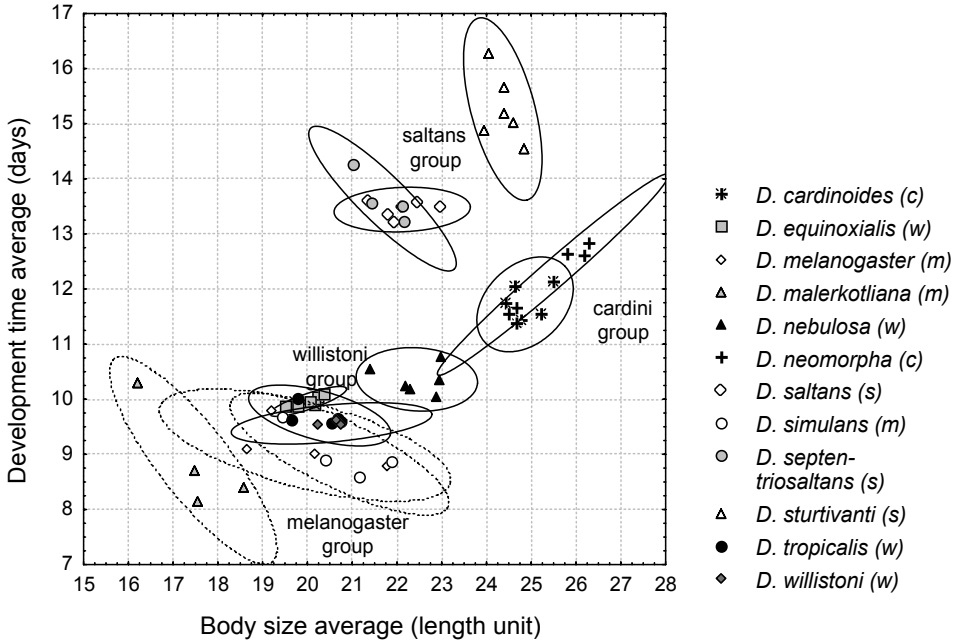


Figure 12: Body size versus development time based on population averages for males and females combined. Ellipses indicate the 95% range for the different species. Line patterns indicate phylogenetic relatedness at the level of species groups. Similar points are only of the same species when they are within the same ellipse.

The differences in the realised starvation values between the first field experiment and the common environment experiment are remarkable (figure 9, right panel). Survival times under desiccation stress are generally much shorter than under starvation stress, and depending on the species, vary from just a few hours for the smaller species like *D. bipectinata* up to 48 hours for the larger species like *D. repleta* (Parkash & Munjal 1999). Estimates for *D. melanogaster* vary between nine (Hoffmann *et al.* 2001b, Hoffmann *et al.* 2001a) and 24 hours (Parkash & Munjal 1999), with most estimates not exceeding 15 hours. The data of the first field experiment on *D. melanogaster* showed an average survival time of 43.7 hours (range 39 - 48 hours) for this species. This is much lower than some laboratory measured starvation resistances on freshly established stocks (105 - 130 hours (Parkash & Munjal 1999)), but within the range reported by other authors (40-80 hours (Hoffmann *et al.* 2001a)). E. Baldal (in preparation) observed in his base line that the variation between generations covered the whole range of reported starvation resistances. This observation underlines the sensitivity of this trait to environmental variation. Furthermore, if repeated measurements of a single stock under constant conditions already result in such a variable outcome, measurements obtained in different environments are likely to be even more variable. This was confirmed in the comparison of the first field experiment with the common environment experiment (figure 9, right panel).

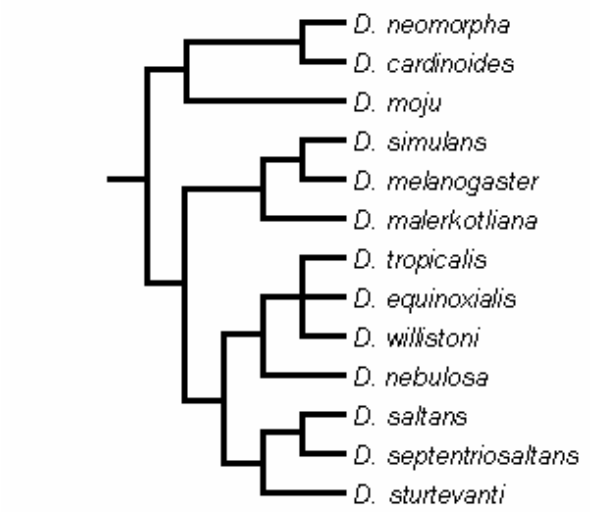
Another reason for why desiccation is unlikely to explain the differences between the experiments, is that the flies were provided with water in the form of water-agar that remained moist for many days and was never visibly dry by the time the last fly in the cohort died. Furthermore, if desiccation had played a role, it would have been likely to affect the grassland populations more severely than the forest populations since the humidity in the forest was always very high and often near saturation. To examine this, I divided the estimate of the first field experiment by the estimate of the common environment experiment and tested whether the ratios differed between the three different habitats. This showed that the ratios did not differ between habitats ($F_{2, 44} = 1.92$, $p = 0.16$), and therefore it is unlikely that desiccation explains the differences between the two experiments.

The differences in average temperature between the laboratory and the field are minimal, but the daily temperature variation in the field is up to 7 ºC, much higher than in the laboratory. Higher temperatures reduce starvation resistances (Da Lage *et al.* 1989, Karan & David 2000), which is thought to be related to an increased metabolism. High temperatures can also induce protection mechanisms (Hoffmann *et al.* 2003c), but starvation resistance might not be increased by this mechanism (Minois 2001) although non-induced flies (e.g. without prior heat-shock treatment) had a longer starvation time than induced flies. Based on the available literature on desiccation resistance, metabolic rates and heat-induced protection mechanisms, no interpretations about the causes of the difference between the experiments can be made.

The life-history model of Sevenster & van Alphen (1993a, 1993b) is based on an ecological trade-off between development time and starvation resistance. Individuals with a long starvation resistance have a better chance of finding a new patch, favouring a higher starvation resistance. The field data of Krijger (2000) showed that the forests with the highest temporal heterogeneity indeed have the lowest mean resource abundance. This favours slow species with long development times and correspondingly longer starvation times. The observed phenotypic pattern for starvation times is consistent with this prediction. However, the genetic pattern is opposite to the expectations. Apparently, the abiotic selection pressure is more important in shaping starvation resistance.

PHYLOGENETIC DEPENDENCE

A component of phylogenetic history is clearly visible within the data (see figure 12 for an example of body size versus development time; members within species groups tend to resemble one another). (Pagel 1999a, b) developed a method for estimating the phylogenetic dependence within such data. The estimated λ ranges between 0 (phylogenetic independence) and 1 (species' traits co-vary in direct proportion to their shared evolutionary history), and it is possible to estimate the phylogenetic dependence for several traits together. For this analysis, I used the data from the first field experiment, for all three traits, all species and all populations. The populations within a species were considered to originate from the same node, so these first nodes correspond with the different species. The higher



species in the study reported here (Bock 1980, Rodriguez-Trelles *et al.* 2000, Val *et al.* 1981, Vilela 1983).

order nodes were based on the phylogenetic classification of Bock (1980), Rodriguez-Trelles *et al.* (2000), Val *et al.* (1981) and Vilela (1983).

This analysis showed that phylogenetic history explained the pattern within the three traits completely ($\lambda = 1$; 95% confidence interval: $0.856 < \lambda <$ larger than one (not estimatable as λ is between 0 and 1)). A similar analysis for each trait separately showed that $\lambda = 1$ for body size (95% confidence interval: $0.837 < \lambda <$ larger than one) and development time (95% confidence interval: $0.833 < \lambda <$ larger than one), and $\lambda = 0.891$ for starvation resistance (95% confidence interval: $0.497 < \lambda <$ larger than one). The clear relation between the phylogenetic history and the interspecific variation shows that a part of the underlying genetic architecture is fundamental. This footprint of the past is neither easily changed, nor related to the current day local adaptation as observed. The λ for starvation resistance is the lowest, which is noteworthy since selection in the field is most obvious for this trait.

INTRASPECIFIC AND INTERSPECIFIC CORRELATIONS

All three interspecific correlations between two traits were positive, and the principal component analysis (data not shown) showed that variation among all three traits could be reduced to a single significant principal component explaining about 75% of the variation among the species. This reduction to one principal component could either indicate that one main cause underlies much of the interspecific variation in these three traits, or that selection on multiple underlying mechanisms has resulted in a consistent simultaneous selection of the traits. In the phylogenetic analysis, as presented above, variation in body size and development time matched perfectly the phylogenetic history of the group indicating that the linkage at the phenotypic level is common, and of ancient origin. Analysis of molecular evolution data sets frequently splits the major groups within the *Drosophila* genus at between 50 and 100 million years ago (Beverley & Wilson 1984). This underlines that the tight linkage between the traits among species is embedded strongly within the *Drosophila* genus. The most likely explanation for my results is that a single set of highly conserved genes and genetic pathways are primarily responsible for the co-variation of all three traits. The alternative explanation, that different selection

pressures independently targeted different traits, is less likely, as that would require the co-occurrence of those selection pressures over at least several millions of years.

For the interspecific correlations, it is likely that underlying genetic correlations were producing the phenotypic correlation, as the phylogenetic history is reflected in all three traits and in the principal component factor. For the intraspecific correlations, the use of phenotypic correlations as a surrogate for genetic correlation is still debated, but review studies on morphological and life-history traits show that for most estimates of two morphological traits, or a morphological and a life-history trait, the sign and magnitude of the phenotypic correlations were similar to the genetic correlations (Cheverud 1988, 1995, Roff 1995, 1996, 1997, 2000). However, exceptions have been reported in which the estimates for the phenotypic and genetic correlations differed in sign (Roff & Mousseau 1987) or magnitude (Hebert *et al.* 1994).

Both the literature and the results presented here were not conclusive about the sign of the different genetic or phenotypic correlations. At the interspecific level, the three correlations were positive, similar to the results from the selection experiments reported in the literature (see **chapter 3** for a literature overview). In contrast, at the intraspecific level, only the correlation between body size and starvation resistance is positive, the other two were negative. This is for the correlation between body size and development time in line with the findings in studies of latitudinal clines, but inconsistent with most selection experiments (Cortese *et al.* 2002, Gu & Barker 1995, Nunney 1996b, Partridge & Fowler 1993, Partridge *et al.* 1999, Reeve 1954, Robertson 1957, 1960a, b, 1963, Roper *et al.* 1996, Santos *et al.* 1992, 1994, Zwaan *et al.* 1995a). The limited published results for the other two correlations were not consistent (see **chapter 3**).

INTER-EXPERIMENT COMPARISONS

The inter-experiment comparisons at the intraspecific level for development time and body size showed a close fit between the common environment experiment and the first field experiment. The differences between the larger and smaller species have increased between the two experiments, while the development times tended to become a little shorter. In contrast, the correspondence between the two experiments for starvation resistance was poor (see discussion on this under starvation resistance). At the intraspecific level, only body size showed a significant correlation between the two experiments. To the contrary, the comparisons for development time and starvation resistance showed no fit between the two experiments.

Potentially, several sources can contribute to this variation between experiments. The genetic variation did not change, but reports of rapid laboratory adaptation suggest that the populations could have changed between the two experiments during the months the stocks were maintained in the open-air laboratory (Hoffmann *et al.* 2001b, Matos *et al.* 2000a, Matos *et al.* 2000b, Matos *et al.* 2002, Partridge *et*

al. 1995, Sgro & Partridge 2000). Furthermore, environmental differences are another potential source for variation. Some aspects of the environment might have changed in a consistent manner, but most of them must have changed to a different degree for populations from different collection sites, as the common environment was the same for all populations. These differences in direction and the extent of the changes are potentially magnified if Genotype-by-Environment interactions exist. The final source of variation is the random variation always present in experiments.

When the data from two experiments, carried out under different environmental conditions, yield closely similar interpretations (i.e. body size), it suggests that the underlying genetics are dominating. It is also an indication that rapid laboratory adaptation is absent. In contrast, a complete lack of fit in such a comparison (i.e. for starvation resistance), underlines that the contribution of the underlying genetics to the realised phenotypes is only small, or that rapid laboratory adaptation has taken place. Based on the inter-experiment comparisons in this chapter, I conclude that the extrapolations of results obtained in a different environment are at least to be interpreted with caution, especially for development time and starvation resistance.

GENOTYPE-BY-ENVIRONMENT INTERACTIONS

Genotype-by-Environment (GxE) interactions arise when different genotypes respond in different ways to variation between environments. In this study, the existence of GxE interactions at the level of populations from different locations was tested using the natural variation between different habitats in the transplantation experiment. The results of the experiment showed that GxE interactions exist at the population level for all three traits and that a part of the GxE interaction variation was consistent over the four species in the experiment. For body size, the GxE interaction component explained 31.4% of the variation explained by genetic, environment, and GxE interactions, while this was 39.4% and 24.5% for development time and starvation resistance, respectively. The consistency of the GxE interaction over the four species may indicate that selection favours similar patterns of GxE interactions across the different species. Furthermore, it showed that GxE interactions are likely to be ubiquitous for those types of key life-history traits in natural populations.

FIELD VERSUS LABORATORY

My primary aim of this study was to measure life-history traits directly in the field to test the extent to which laboratory-based *Drosophila* life-history theory applies to natural conditions. The results presented in this chapter show that measuring life-history traits directly in the field is possible and that it gives additional insight about life-history evolution.

This chapter shows clearly that extrapolating the results obtained in a common environment towards the field situation is not easy. Comparisons across experiments often showed little correspondence, and genotype-by-environment

interactions often explained more of the variation present than the genetic component. Despite this, the patterns within the common environment matched those within the *'original habitat'* component of the transplantation experiment indicating that an accurate prediction of the field pattern is possible based on the common environment experiment.

CONCLUSIONS

This study is the first to measure at the same time the expression of three different life history traits directly in the field. The wealth of information from this approach provides insights into the evolution of the life-history traits in the field. The comparison of the results of the three experiments revealed that the variation within the three traits and the correlations between the traits show different patterns. Both the reported variation between laboratory and field studies and my comparative results stress the ubiquity of GxE interactions.

Starvation resistance shows a pattern in which the adaptation to an environmental stress is not yet completed. Populations from the grassland (high stress) have the shortest starvation times but are genetically more resistant to that same stress. For development time, this direct response to an environmental stress is less clear as the genetic patterns were opposite to those expected pattern based on temperature selection in the laboratory. However, the pattern is consistent with the expectations from the life-history model of Sevenster & van Alphen (1993a, 1993b). Body size seem to be relatively unaffected by the differences among the habitats, or it is less consistently affected than are the other traits.

The comparison between the three traits showed that the interspecific covariance between the three traits was high. At the interspecific level, all correlations between any two traits were positive and the variation between the species shows a clear impact of the phylogenetic history. At the intraspecific level, only the correlation between body size and starvation resistance is positive, the two other correlations of starvation resistance with development time and with body size were both negative. This result contrasts with those found in selection experiments but matches in part the results from other studies such as on latitudinal clines.

The presence of considerable genotype-by-environment interactions at the population level, which is similar across the different species, may indicate that selection favours similar patterns of GxE interactions across the different species. The GxE interactions (for all three traits) and the lack-of-fit between the field experiment and the common environment experiment (for development time and starvation resistance), make the extrapolation of laboratory results to the field challenging. The integration of laboratory work with field-based experiments clearly has an important contribution to make, over and above that of more traditional, laboratory-based studies.

5

Interspecific and intraspecific variation in genetic correlations in *Drosophila*

Introduction

Community ecology and evolutionary genetics are often treated as separate fields of expertise, but community genetics has emerged from the interaction between these two fields. Recently, the debate about community genetics was revived in a special feature in 'Ecology' (Agrawal 2003) with two papers exploring the potential for this integrated field of research (Neuhauser *et al.* 2003, Whitham *et al.* 2003). The original definition of this field came from Antonovics (1992) who 'defined' community genetics as: "The role of genetic variation in influencing species interactions and determining community structure". From a traditional ecological point of view, the underlying genetics of traits and their correlations are unimportant; what matters is the expression of the traits in the field. However, the papers of Neuhauser *et al.* (2003) and Whitham *et al.* (2003) clearly demonstrated that the underlying genetics can play an important role in the community dynamics. Neuhauser *et al.* (2003) illustrated this with four examples of non-equilibrium communities. They showed that including the genetics of the species involved facilitates the understanding of the dynamics of the community. Whitham *et al.* (2003) showed that the effects of a phenotype can reach beyond the level of the population up to the level of the ecosystem processes, and are essential to understanding the higher levels of organisation. Therefore, I will combine quantitative genetic data with the (community) ecological data from the previous chapter, leading to a better understanding of the dynamics within the *Drosophila* communities in the field.

In the previous chapter, I investigated the life-history variation within six Panamanian *Drosophila* communities, two within each of three different habitats: forest, grassland and the intermediate transition zone. The aim of that study was to investigate the phenotypic and genetic variation in three life-history traits - development time, starvation resistance, and body size- and the correlations among them. Human-induced changes in the environment require adaptation to the new environment, and I showed in chapter 4 that local adaptation occurs in the Panamanian *Drosophila* community. The generality of the patterns of local adaptation follows from the fact that similar adaptations occurred in several species simultaneously (**Chapter 4**).

In the previous chapter, I estimated the intraspecific correlations as well as the interspecific correlations based on both sample and population averages for all combinations of the three life-history traits. However, the jury is still out on the question of whether phenotypic correlations are a reliable estimate for the underlying genetic correlations, especially when it concerns life-history traits (Roff 1995). Stearns (1992) defined a (additive) genetic correlation as "The portion of a phenotypic correlation between two traits in a population that can be attributed to (additive) genetic effects". This suggests a match between phenotypic and genetic correlations. However, Bell & Koufopanou (1986) did not find a correlation between the genetic and environmental correlations in their study on *Daphnia*. In contrast, Roff & Mousseau (1987) found for *Drosophila* that the estimates for phenotypic and genetic correlations were positively correlated. The exceptions to this general

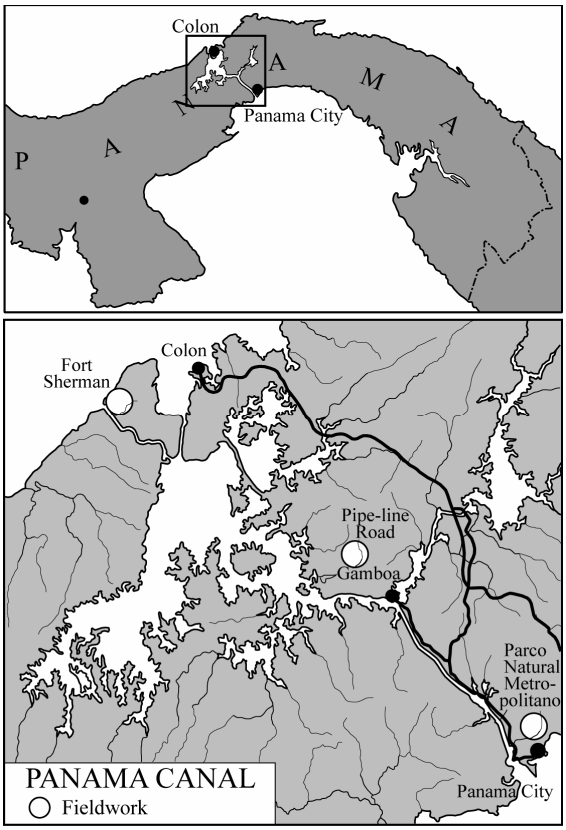


Figure 1: Map of research area.

pattern concerned correlations between two life-history traits (see also (Cheverud 1988)). However, when only studies with sample sizes larger than 40 were included, the patterns of correlation were strikingly similar. Two studies of Roff (1995, 1996) confirmed the suitability of phenotypic correlations as a surrogate of a genetic correlation in the case of two morphological traits or a morphological and a life-history trait. Again though, in the case of two life-history traits, the phenotypic correlation was not a good estimate for the genetic correlation. In a more recent study by Roff (2000) on development time and size at maturity in various species, he showed that estimates from phenotypic correlations are a good estimate for the underlying genetic correlation concerning sign and magnitude.

The interspecific correlations for life-history traits in different species of *Drosophila* that I estimated in the previous chapter were all positive. The principal component analysis underlined the high interdependency of the traits under study. Furthermore, this interdependency correlates with the phylogenetic history of these species. The intraspecific phenotypic correlations did not match the interspecific correlations in two cases as only the phenotypic correlation between body size and starvation resistance was positive. The interspecific correlation between development time and body size, as well as between development time and starvation resistance was negative. These interspecific correlations are similar of sign to the genetic correlations as found in the literature (Chippindale *et al.* 1996, Cortese *et al.* 2002, Gu & Barker 1995, Harshman *et al.* 1999, Nunney 1996b, Partridge & Fowler 1993, Partridge *et al.* 1999, Reeve 1954, Robertson 1957, 1960a, b, 1963, Roper *et al.* 1996, Santos *et al.* 1992, 1994, Tantawy & El-Helw 1970, Zwaan *et al.* 1995a).

The aim of this chapter is to estimate the sign and magnitude of the genetic correlations between body size, development time and starvation resistance. I used three species, *D. malerkotliana*, *D. equinoxialis* and *D. saltans*, which belong to

phylogenetically distant species groups. For each species, two populations from distant locations within the study area were chosen. These data combined with those from the previous chapter can be used to study the relationship between phenotypic and genetic correlations. The findings are discussed in relation to the ecological context.

Material & Methods

COLLECTION SITES

The *Drosophila* stocks were collected in Panama in April 2002. Collections were made across the Isthmus of Panama at three locations, all near the Panama Canal. Fort Sherman (FS) is the northern collection site near the Atlantic Ocean, Pipeline Road (PLR) is in the middle of the isthmus and Parco Natural Metropolitano (PNM) is in the south, basically within the outskirts of Panama City (figure 1). The climatic differences over the Isthmus range between dry and moist (insert rain, sun, and temperature data). The trapping technique and establishing the stocks has been described under Material & Methods in **chapter 4**.

SPECIES & STOCKS

The species were selected based on two criteria. The first criterion was that a species should be easy to rear because the experimental set-up required large numbers of offspring. The second criterion was that the three species were phylogetically distant from each other, so that, when the patterns are similar across those selected species, a generalised intraspecific pattern can be extrapolated to other species within the community under investigation. Based on these criteria, *D. malerkotliana*, *D. equinoxialis* and *D. saltans*, were chosen for this experiment. All are within the *Sophophora* subgenus. *D. malerkotliana* is within the *ananassae* subgroup within the *melanogaster* species group (Bock 1980, Wheeler 1981), *D. equinoxialis* is within the *willistoni* subgroup within the species group of the same name (Val 1982, Wheeler 1981), and *D. saltans* is within the *saltans* subgroup of the equivalently named species group (Val 1982, Wheeler 1981).

Two stocks of each species were used in the experiments. One stock was collected at Fort Sherman for all three species, together with stocks from Parco Natural Metropolitano for *D. malerkotliana* and *D. saltans*, and from Pipeline road for *D. equinoxialis*.

LIFE-HISTORY TRAITS

As far as possible, measurements for various life-history traits were simultaneously collected on the same individuals (see under experimental set-up). *Development time* is defined as the time from egg lying until adult eclosion, while *starvation resistance* is the time from then until death. *Dry weight* was measured on dried flies. After fat extraction, the flies were weighed again to obtain the *fat-free dry weight*.

The *fat weight* is the result of the subtraction of the *fat-free dry weight* from the *dry weight*. The *proportion fat* was obtained by dividing the *fat weight* by the *dry weight*.

CROWDING EFFECTS

The family sizes were uncontrolled in the experiments. These differences in density are a potential source for errors in the statistics due to crowding effects (See **Chapter 3**) or Allee effects (Courchamp *et al.* 1999, Rohlfs & Hoffmeister 2003, Stephens & Sutherland 1999, however, see also: Etienne et al. 2002, Hoffmeister & Rohlfs 2001, Wertheim *et al.* 2002). I therefore estimated, for each species, a second-degree relationship between the number of flies in the family and the realised trait values. The residuals of this analysis were used in the subsequent analysis.

EXPERIMENTAL SET-UP

Two experiments, which differed in several aspects, were carried out (table 1). The first was designed as a full-sib experiment, while the second was a nested half-sib/full-sib experiment. In the first experiment, only one population of each species was measured, while in the second, two populations were measured. Finally, starvation resistance was only measured in the first experiment as the amount of work associated with that trait made simultaneously testing of six populations unfeasible.

Table 1: The essential characteristics of the two experiments. Differences between them are highlighted in bold. Number of families per population is indicated between brackets. One *D. malerkotliana* population in the second experiment failed to produce sufficient offspring.

Experiment	Design	Traits measured	Populations (families) and species
1	Full-sib (1 male: 1 female)	Development time, **starvation resistance**, dry weight, fat-free dry weight, fat weight, fat percentage	**One** population of *D. equinoxialis* (23), **one** of *D. malerkotliana* (16), and **one** of *D. saltans* (26).
2	Nested half-sib /full-sib (1 male: 4 females)	Development time, dry weight, fat-free dry weight, fat weight, fat percentage	**Two** populations of *D. equinoxialis* (50, 50), **one** of *D. malerkotliana* (-, 38), and **two** of *D. saltans* (48, 50)

The experiments were carried out in the same climate room as where the stocks were kept, under 25°C, 70-85% RH and 13:11 light:dark. For the first experiment, 50 pairs of one virgin male and one virgin female were each put together in glass vials; however, not all of them produced offspring (see table 1). The second experiment was essentially the same as the first experiment, except that each male could mate with four females (see table 1). Each glass vial contained moist vermiculite and was closed with a foam stopper. A drop of honey and a drop of yeast were put on the foam stopper as a food source. The flies were given three days to feed on the honey and yeast before being transferred to a fresh vial. For the

second experiment, all five flies in a single vial were transferred to five different vials (the male included, to avoid unnecessary anaesthesia of the flies to sex them).

The new vial contained a small piece of banana dipped in yeast suspension as a breeding substrate on a layer of moist vermiculite. After 24 hours, the parents were removed from the banana. The offspring was collected on a daily basis (e.g. development time data). For the first experiment, one half of the offspring was stored in a plastic eppendorf vial at -5ºC for the various body weight measures, while the other half was transferred to a new vial with 5 millilitres of agar to obtain estimates of the starvation resistance. The agar functions as a source of water. Dead flies were scored daily and removed from the vials. For the second experiment, all offspring were stored in an eppendorf vial at -5ºC. Both experiments were carried out in two replicates with a time lag of three days.

For the various body weight measurements, the first step was to dry the stored flies for three days at 70 ºC after which they were weighed. The weight was measured to 0.0001 mg using a Sartorius Ultramicro balance type 4504MP8. For the next step of the fat extraction, flies were put in 1-2 ml dimethylether for 24 hours. After pouring off the ether and washing them once in ca. 0.25 ml of ether, the flies were dried again for at least 3 days under 70 ºC before being weighed again in the same manner as the first time. The fat-free dry weight was then subtracted from the dry weight to obtain the actual fat weight of the fly. The proportion fat was obtained by dividing the fat weight by the dry weight.

ESTIMATION METHODS

Large experiments such as this one, are a compromise between large number of individuals per trait and the number of traits, stocks, and species. The main objective was to test whether a genetic correlation can pose a barrier to adaptation. Therefore, I wanted to collect comparable data for several species, with at least two stocks from widely different environments. All statistical analyses were performed with STATISTICA (StatSoft 2004) unless noted otherwise such as the CPC analysis.

HERITABILITIES

Broad sense heritabilities could only be estimated for a limited number of traits of which individual-based data for all flies within the experiment were available. These are the data for *D. malerkotliana* in the fat-content experiment (full-sib data), and the development time and starvation resistance data of the same experiment for the other two species. I used standard nested design with Restricted Maximum Likelihood (REML) estimations. The trait value was the dependent variable, and ‘family’ and ‘replica’ were the independent variables for the full-sib designs, with 'Replica' nested within 'family.

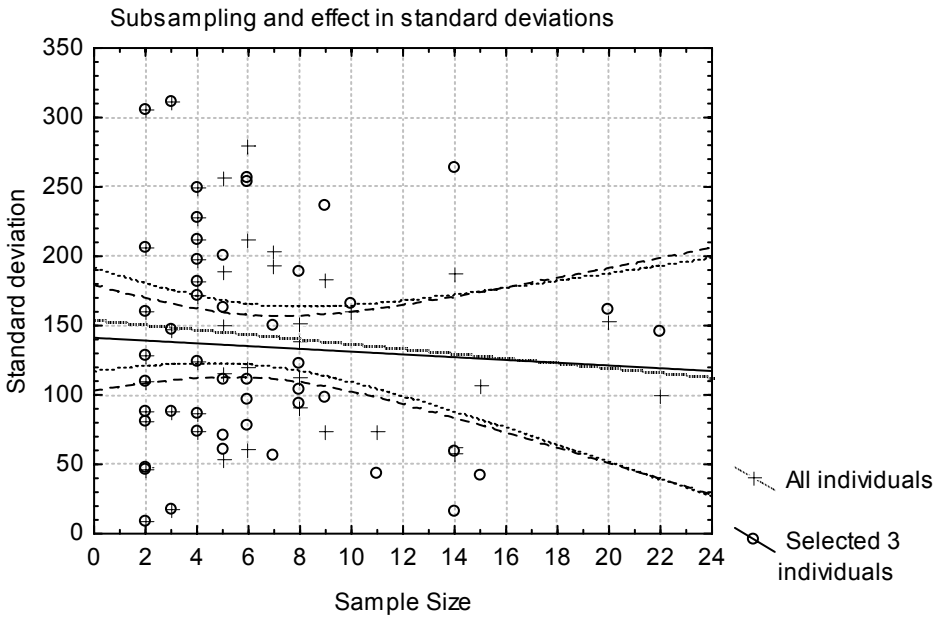


Figure 2: Impact of sub-sampling on the estimated standard deviations for dry weight. Sub-samples were obtained from around the median of the family samples of the *D. malerkotliana* dataset.

GENETIC CORRELATIONS

The Pearson product-moment correlation between family means was first suggested by Via (1984) as an approach to estimate broad sense genetic correlations. This method has the advantage that confidence intervals are easily estimated using linear regression. The estimate is an approximation because the variances and covariances contain a fraction of the within family error term, reciprocal to the average family size. The detailed analysis of this method by Roff & Preziosi (1994) showed that reliable estimates require an average family size of 20 or more individuals, and relative small differences between the genetic and phenotypic correlations. Average family size (males and females) in the fat-content/starvation resistance experiment was 20.7 individuals for *D. equinoxialis*, 20.8 individuals for *D. malerkotliana*, and 22.4 individuals for *D. saltans*. In the half-sib design, the average family sizes were 15.5 and 16.5 individuals for the two *D. equinoxialis* stocks, 20.4 and 23.1 individuals for the *D. saltans* stocks and 13.4 individuals for the *D. malerkotliana* stock.

The family sizes obtained in the two experiments are very variable, ranging from 1 to 87. Using the unweighted mean in the analyses could overvalue outliers based on a single or a small number of individuals. Therefore, each family within a correlation was weighted to the total number of individuals within that family. For the

correlation, the total number of families was kept constant to the original number of families so that the degrees of freedom in the analysis remained unchanged. Besides reducing the influence of outliers on the averages, the relative contribution of the within family variation, as explained above, is reduced, which makes the estimates less biased.

SUBSAMPLING

Based on the observation of Roff & Preziosi (1994), subsampling of the data should only be applied when it does not lead to an increase in the within family variance. Therefore, individual-based data for body weights were obtained for the *D. malerkotliana* stock in the fat content - starvation resistance experiment. We examined whether taking a sub-sample affected the estimated standard deviations (figure 2). To test this, a specific number of individuals that were closest to the median, were selected. The correlation between the standard deviations of the full samples and the subsamples was highly significant, even for subsamples of three individuals ($R^2 = 0.73$, $N = 49$, $p << 0.001$). We concluded that standard deviations obtained from subsamples provide a reliable estimate for the standard deviation of the whole sample. As expected, the largest changes in the standard deviations were in the smaller samples, as the relative impact of a single outlier is then stronger than in larger samples. This also explains why the negative slope decreased with subsampling.

INTERSPECIFIC AND COLLECTION SITE COMPARISONS

A nested ANOVA design was used to test whether species-specific or site-specific variation within the different trait combinations was present. For both experiments, trait combinations were the main factor. Species and sex were nested within the trait combinations for both tests on species effect, while site and sex were the nested factors for the location effect test. Positive effects are in more detail analysed using a Common Principal Component analysis (Flury 1988, Phillips 1998). The variances and covariances of the **G**-matrices were calculated from the averages available for the different species and stocks.

Results

HERITABILITIES

Table 2 gives an overview of the estimated broad-sense heritabilities based on the full-sib design. The heritabilities for the morphological and physiological traits could not be estimated for *D. equinoxialis* or *D. saltans* because the flies were weighed per group, not as individuals. The standard errors are often very large, while the indications of significant effects are based on the REML estimates. The estimated heritabilities for development time and starvation resistance vary between the species and are generally low.

Table 2: Broad sense heritabilities for all traits and their standard errors.

Species	Trait	Heritability	SE
D. malerkotliana	Development time	0.000	0.020
D. malerkotliana	Starvation resistance	0.134	0.077
D. malerkotliana	Dry weight	0.663 *	0.178
D. malerkotliana	Fat free dry weight	0.686 *	0.182
D. malerkotliana	Fat weight	0.026	0.050
D. malerkotliana	Fat percentage	0.007	0.044
D. equinoxialis	Development time	0.000	0.018
D. equinoxialis	Starvation resistance	0.018	0.041
D. saltans	Development time	0.219	0.067
D. saltans	Starvation resistance	0.180 *	0.071

*: $p < 0.05$

GENETIC CORRELATIONS

First experiment

The genetic correlations for all trait combinations were estimated using the family means method of Via (1984). The data were first analysed using all families. The second step was to estimate the genetic correlations having excluded the smallest families, those with fewer than 20 offspring. Finally, the phenotypic correlation was estimated. The results for the genetic correlation are shown in figure 3 (females) and figure 4 (males), while a comparison between the phenotypic and genetic correlations is presented in figure 5. (Matrix plots for the unweighted data, for each species and sex, can be found in Appendix 1.)

The family-mean method is sensitive to large differences between the phenotypic correlation and the actual genetic correlation (see figure 5), due to the inclusion of a fraction of the within family variation in the estimate. Therefore, it is expected that the all family estimates of the genetic correlation are more biased towards the phenotypic correlation than the 20+ families estimates. The elimination of the families with less than 20 individuals increased the difference between the phenotypic and genetic correlations ($F_{1, 59} = 5.1$; $p = 0.028$) indicating the reduced impact of the within family (co-)variance. Furthermore, the effect was species specific ($F_{2, 59} = 9.87$; $p = 0.0002$) with much larger differences for *D. equinoxialis*.

Dry weight and fat-free dry weight were highly correlated in all three species, with values always close to one for both the phenotypic as well as the genetic correlations. Consequently, genetic correlations of either of these two body size traits with another trait are very similar. A similar situation, although to a lesser extent, occurs with the fat weight and fat percentages.

The genetic correlations of development time with any of the five other traits were generally non-significant and variable between the species. Furthermore, this variation was larger among the females than among the males. Only *D. malerkotliana* showed some robust significant effects: both combinations with the body size traits and the females in the combination with fat percentage.

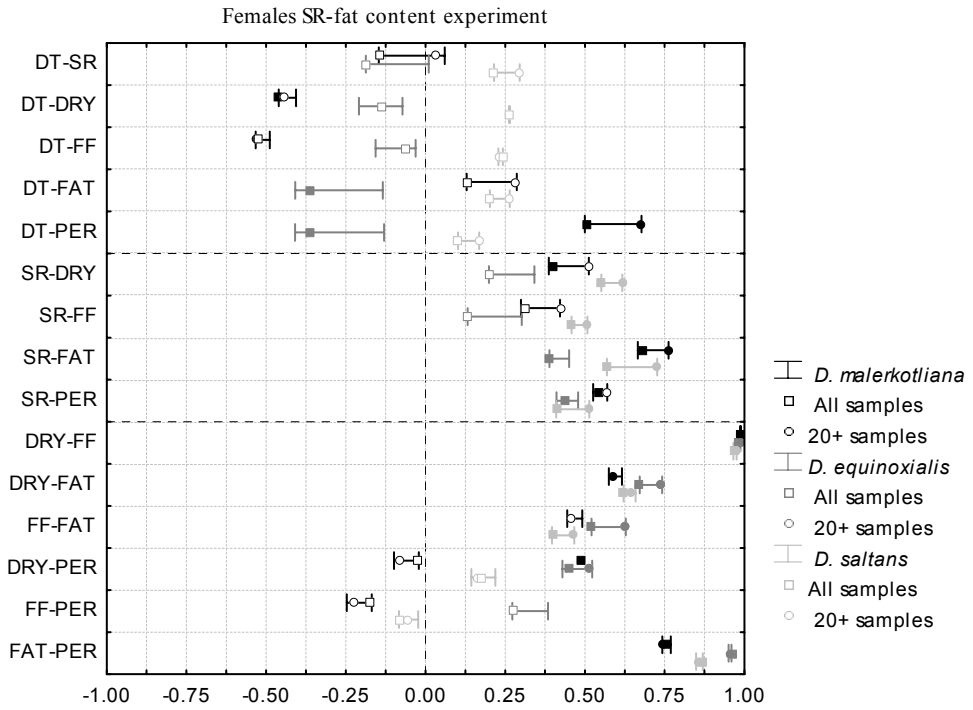


Figure 3: Estimated broad sense genetic correlations for different trait combinations based on family means, for females of three different species. Whiskers give the range in the estimations for the genetic correlations. Range is estimated by exclusion of samples below a certain sample size, ranging from all samples to only samples with 20 or more individuals. Squares indicate estimates using all samples, regardless of the number of individuals in the single samples and circles are estimates based on only samples with 20 or more individuals. Open symbols indicate non-significant results; filled symbols indicate significant results. DT = development time; SR = starvation resistance; DRY = total dry weight; FF = fat-free dry weight; FAT = fat weight; PER = percentage fat relative to total dry weight.

Furthermore, *D. equinoxialis* females showed a significant effect with the two fat-related traits when all families were used. However, the negative phenotypic correlations are very strong and consequently, the genetic correlations with all families could be biased. The exclusion of the smaller families indeed resulted in weaker genetic correlations, which were not significant. Development time and starvation resistance did not show any significant correlation, which suggests that they are independent of each other, regardless of the species.

All correlations between starvation resistance and any of the four morphological and physiological traits are positive. For the females, 75 % of them are significant, and about 40 % in the males. Dry weight and fat-free dry weight showed a significant positive correlation with the absolute fat weight, but only in a limited number of

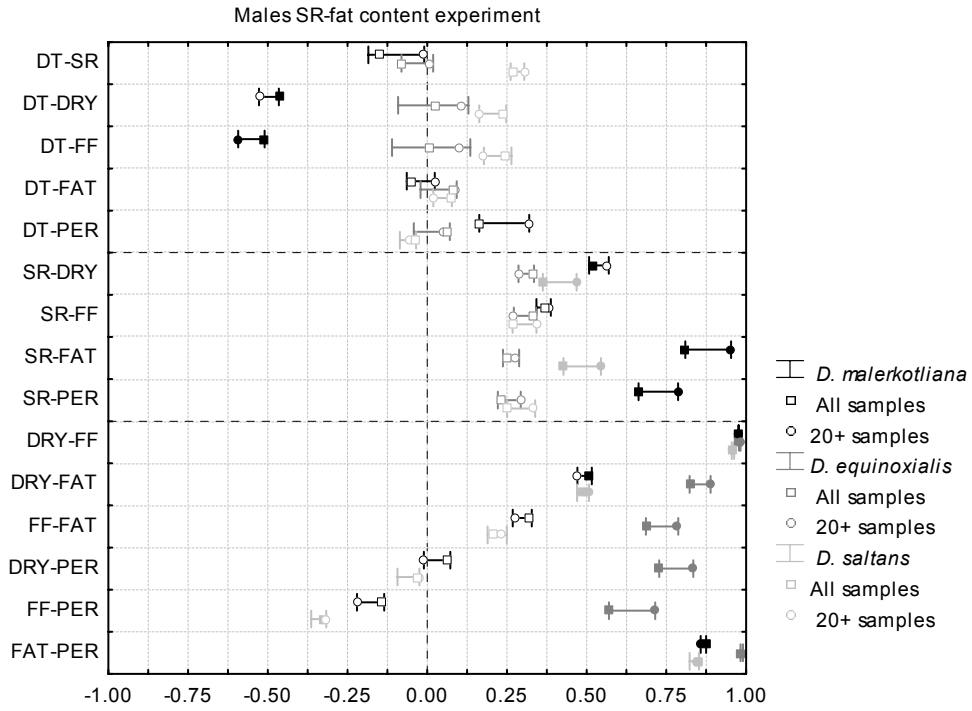


Figure 4: Estimated genetic correlations for different trait combinations based on family means, for males of three different species. For the meaning of the symbols and abbreviations, see legend of figure 3.

cases with the fat percentage (*D. equinoxialis*). Generally, the estimates for the 20+ families were larger than those for all families. The two body size traits (dry weight and fat-free dry weight) had positive genetic correlations with fat weight. The variation between species is limited in the females, but larger in the males. The correlations with fat percentage are variable, and only for *D. equinoxialis* significant in 3 out of 4 estimates, but all four are positive. The genetic correlations between starvation resistance and fat weight were generally stronger than those between starvation resistance and fat percentage (figures 3 and 4).

Second experiment

The second experiment contained not only the three species, but also two populations of each species. In figure 6, the estimates for the different species and populations can be compared for each trait combination. Furthermore, for the comparison, the data of the first experiment are added as well. Generally, the picture is the same as in the first experiment.

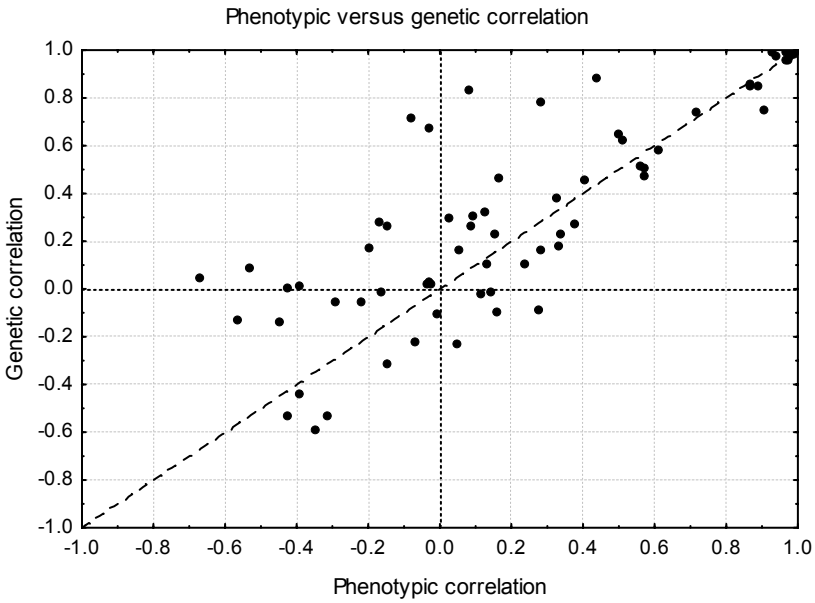


Figure 5: Comparison between phenotypic and genetic correlations. For the genetic correlations, the estimates for only those sample containing 20 or more individuals have been used. The diagonal dashed line indicates the expected location of the dots in case the genetic and phenotypic correlations matched perfect.

The genetic correlations between dry weight and fat-free dry weight are for all species and populations very high. A similar strong and high correlation is observed for the fat weight and fat percentage traits. Most of the estimates for trait combinations with development time and a morphological or physiological trait are non-significant. For the morphological and physiological traits among each other, fat weight showed clear correlations with the overall body size, but fat percentage was usually not correlated. Only four trait combinations showed an overall significant genetic correlation: dry weight - fat-free dry weight; dry weight - fat weight; fat-free dry weight - fat weight; and fat weight - percentage fat.

Interspecific and collection site variation

A nested ANOVA design to test whether species-specific variation or site-specific variation was present showed that these differences were present. The tests on both experiments showed that species had a significant effect on the estimated genetic correlations (experiment 1: $F_{30, 30} = 6.98$, $p < 0.001$, figure 7; experiment 2: $F_{20, 10} = 11.2$, $p < 0.001$). Site also had a significant effect on the realised genetic correlation (experiment 2: $F_{10, 10} = 19.9$, $p < 0.001$, figure 8), but sex did not (experiment 1: $F_{15, 30} = 0.31$, $p = 0.99$).

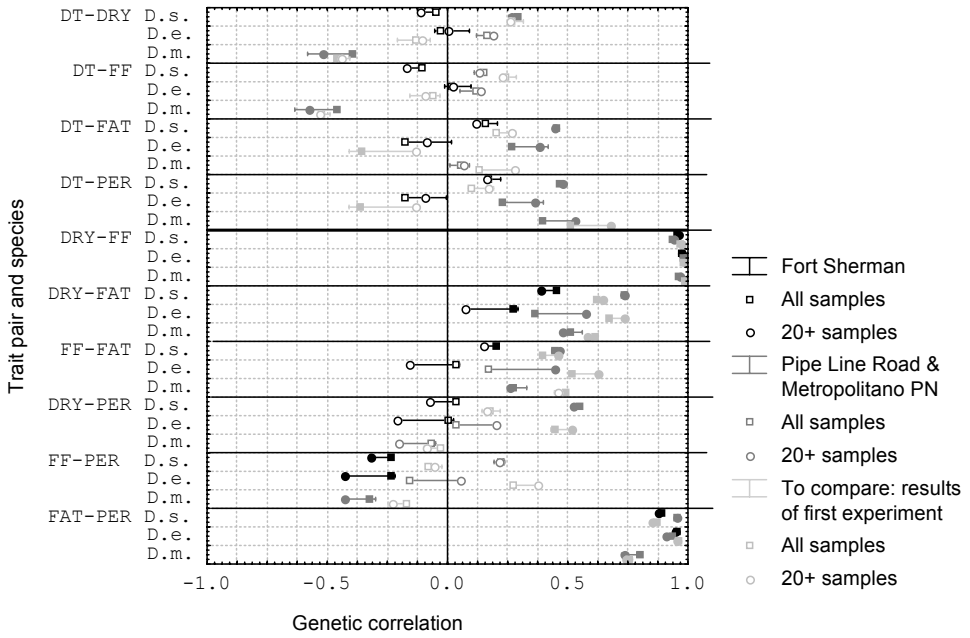


Figure 6: Estimated genetic correlations for different trait combinations based on family means, for females of two different populations of three different species. On the x-axis are indicated the trait combination with the species. D. s. = *D. saltans*; D. e. = *D. equinoxialis*; D. m. = *D. malerkotliana*. The black marks are estimates for the populations collected at Fort Sherman (D. s. and D. e.), while the lighter marks are for the populations collected at Parco Natural Metropolitano (D. s. and D. m.) or Pipe Line Road (D. e.). For the meaning of the symbols and the remaining abbreviations, see legend of figure 3.

The Common Principal Component (CPC) analysis on the **G**-matrices encountered some problems with the calculations, but eliminating the fat-free dry weight variable solved these. The genetic correlation between this variable and dry weight is close to unity, this may have caused the problems (Flury 1988). The results of both experiments showed that the three species do not share a common underlying variance-covariance matrix. This finding was in line with the CPC analysis on the phenotype matrices of all species in the first field experiment (see chapter 4), which showed that these matrices were unrelated (Kim van der Linde, unpublished results). The CPC analyses, in which the **G**-matrix similarity of the populations within a species was tested, showed that, for both *D. equinoxialis* and *D. saltans*, the **G**-matrices differed significantly between the two populations. The **G**-matrices of the two populations of *D. saltans* were unrelated, while those of the two populations of *D. equinoxialis* shared a single principal component. Furthermore, a CPC analysis on males and females for each species showed that the **G**-matrices of the sexes were equal in *D. equinoxialis* and *D. malerkotliana*, and shared all principal components in *D. saltans*.

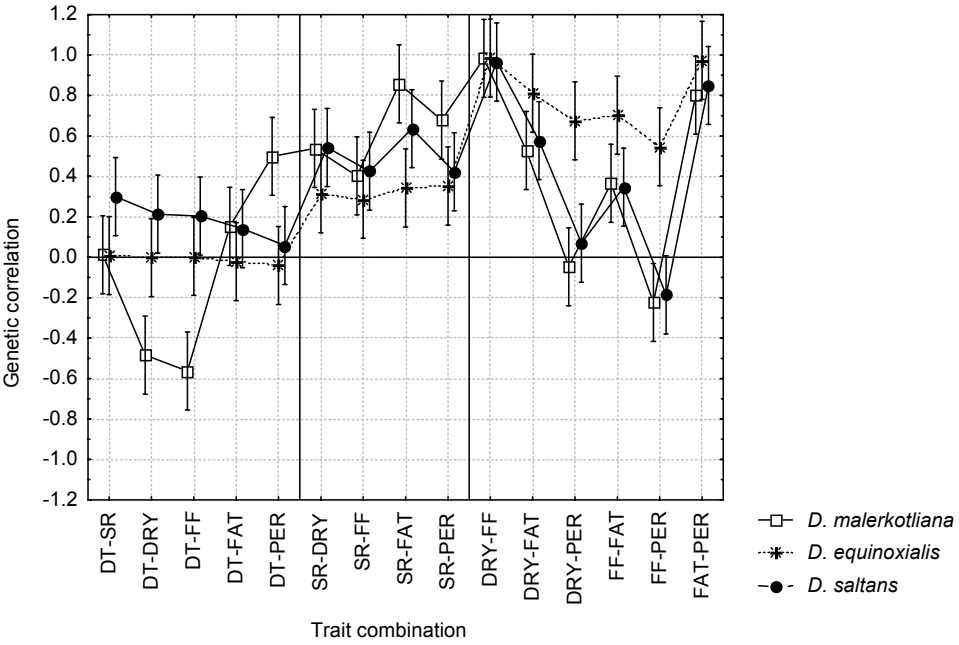


Figure 7: Species specific variation in estimated genetic correlations. Trait combinations are at the x-axis; genetic correlations are at the y-axis. Bars indicate standard errors. DT = development time; DRY = total dry weight; FF = fat-free dry weight; FAT = aft weight; PER = percentage fat relative to total dry weight.

The results presented here show that **G**-matrices obtained for different populations and species can differ significantly. This implies that extrapolating results across species or from one population to another population in a different environment is not advisable. As the populations are collected in different environments, these differences may be the cause of the different G-matrices. Furthermore, the differences between the populations are similar for both species, which suggests that a single common cause underlies these differences.

Discussion

The aim of this study was to investigate whether genetic correlations could pose a barrier to local adaptation. This occurs when two traits are under the (partial) control of the same genes, while selection requires the traits to evolve antagonistically to this underlying genetic coupling. In this study, the presence and magnitude of these genetic correlations between body sizes, development times and starvation resistances were estimated for three different species and two populations of each species.

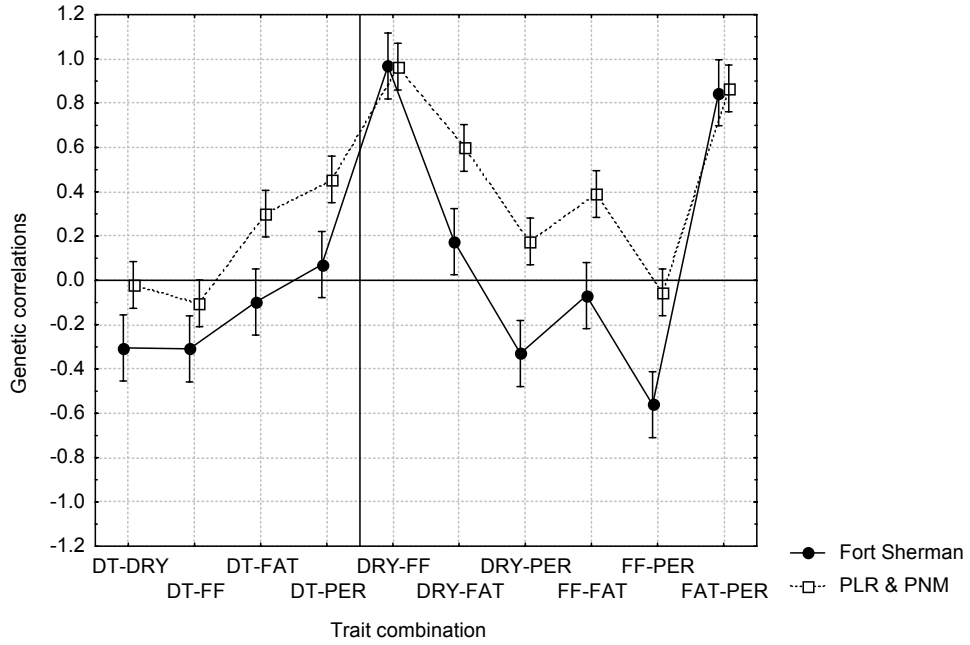


Figure 8: Location specific variation in estimated genetic correlations. Trait combinations are at the x-axis; genetic correlations are at the y-axis. Bars indicate standard errors. DT = development time; DRY = total dry weight; FF = fat-free dry weight; FAT = aft weight; PER = percentage fat relative to total dry weight. PLR = Pipe Line Road; PNM = Parco Natural Metropolitano

The general picture shows that there is a positive genetic correlation between body size and starvation resistance, and no genetic correlation between development time and starvation resistance or between development time and body size. However, the variation between species and populations is large and not all estimates within a trait combination are significant. Furthermore, there are significant differences between species that are independent of collection site, and there are significant differences between the collection sites that are independent of the species. The differences among species were confirmed by the **G**-matrix comparison, which showed that the **G**-matrices of the different species were unrelated. A similar analysis of the populations within a species showed that the **G**-matrices of the two populations of *D. saltans* were unrelated, while those of the two populations of *D. equinoxialis* shared only one of the principal components.

The estimated heritabilities are generally quite low, especially when one takes into consideration that these are broad-sense heritabilities, and thus also include the dominance genetic variation. This might be a side effect of the experimental design, in which we did not fully control the number of offspring per female, and that could have introduced additional environmental variation. Similarly, this maybe can also

explain the absence of consistent genetic correlations such as between development time and the body size measurements.

One potential cause for differences between populations is differences in allele frequencies due to sampling effects. This could lead to differences in the estimated genetic correlations, when such a sampling effect would lead to a difference in the overall pleiotropic effect of the genes responsible for a specific genetic correlation (**Chapter 6**). However, it is unlikely that such a sampling effect would occur in three different species simultaneously, leading to the conclusion that the consistent differences in the genetic correlations across different species is indeed the result of the differences in the collection sites.

The estimated genetic correlations between body size and starvation resistance are much lower than unity. This means that the genetic coupling between these two traits is unlikely to represent a strong barrier to local adaptation when selection pressures from the environment require evolution away from the underlying genetic coupling. However, it is still the best predictor for the potential speed of future evolution (Beldade *et al.* 2002, Zijlstra *et al.* 2003, Zijlstra *et al.* 2004).

The estimation method used in this study is not the most sophisticated option, as some of the traits could not be measured simultaneously on the same individuals, resulting in estimates of the broad sense genetic correlations. Consequently, the within family variation will influence the estimated genetic correlations. This was clearly demonstrated by eliminating the smaller samples, which are more sensitive to this source of variation. Their exclusion simultaneously resulted in a loss of statistical power. Sometimes, when the full dataset produced a significant result, the reduced dataset yielded a higher genetic correlation, which was, however, non-significant. Overall, the estimates based on the data set including all families and those based on the data set excluding families with less than 20 individuals, are highly correlated (both data sets: $R^2 > 0.95$, $p = 0$), underlining the robustness of the different estimates despite the differences in significance. Consequently, this implies that the results can be used to answer the questions as posed in the introduction of this chapter.

Sevenster & van Alphen (1993b) developed a model based on an ecological trade-off between development time and starvation resistance. This was based on the observation of Charnov & Berrigan (1990) that within a class or family level, the ratio between development period and adult life span appears to be constant. Furthermore, they suggested that the underlying reason might be found in the common dependence of the two traits on metabolic rate and/or body size. However, the results presented here showed that such a general genetic correlation does not exist at the species level. This is in line with the observation in **chapter 4** that local adaptation in these two traits appears to be independent of each other. Apparently, the interspecific pattern is not necessarily a close reflection of the underlying genetic architecture shaping these traits.

The results of this study also showed that there is significant variability among the three different species. It depends on the exact trait combination whether they are different or not and if so, how large the differences are. These interspecific differences in the estimates make extrapolation of the results in one species towards another species difficult. In some trait combinations (e.g. development time - dry weight) the estimated genetic correlations range from significantly negative to significantly positive. Both the significant estimates were for two different species but collected at the same location. The CPC analysis on the three species showed that the underlying variance-covariance matrices are unrelated. This implies that the underlying genetic architecture of the three different species differs considerably, and explains effectively the differences between the species.

A similar pattern can be observed along the line of the collection site. Here it depends less on the exact combination. When the estimated correlations are plotted on a range -1 and +1, those for the Fort Sherman populations are generally towards the negative end of that range. This means that they have lower, or even negative estimates for the positive correlations, and more negative estimates for the negative correlations. Here again, extrapolation of results obtained on populations collected at one site can be difficult. However, they do not contradict the general pattern.

In **chapter 4**, the interspecific comparison showed that all three traits, body size, development time and starvation resistance, were positively correlated. The results presented in this chapter clearly demonstrate that this is not due to a simple underlying genetic correlation at the species level. The genetic correlations between development time and body size, and between development time and starvation resistance, were generally absent, and even ranged from significantly negative to significantly positive. The phenotypic correlations based on the data of the previous chapter showed that the correlation between body size and starvation resistance is positive, while the correlations between development time and starvation resistance, and between development time and body size were negative.

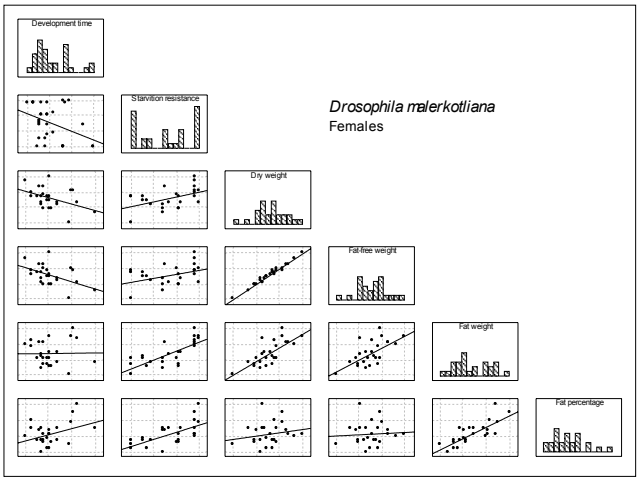
In **chapter 6**, in which I will present a synthesis of the whole thesis, I will briefly present an idea that might shed some light on the underlying genetic mechanism that could explain the results in the variation between and among species and populations. This idea is based on the notion that pleiotropic effects differ between genes (Cheverud 1984, Falconer & Mackay 1996, Lande 1980, Lynch & Walsh 1998, Roff 1997, Wagner 1984, 1989). When the pleiotropic genes are attributed to two different classes of genes, with different pleiotropic effects, the relative importance of the two classes is essential to understand the realised genetic correlation. Such a change in the relative importance of the different gene-classes can be the result of differential gene-expression (Dutta *et al.* 2003, Larribe *et al.* 1997, Lin *et al.* 2002, Ma *et al.* 2001, Phillips & Strauch 2002, Schenk *et al.* 2000, Seki *et al.* 2001, Tepperman *et al.* 2001). Only genes that are expressed contribute to the phenotype of an individual and selection on genes is limited to those genes that contribute to the phenotype. Therefore, this differential gene expression could

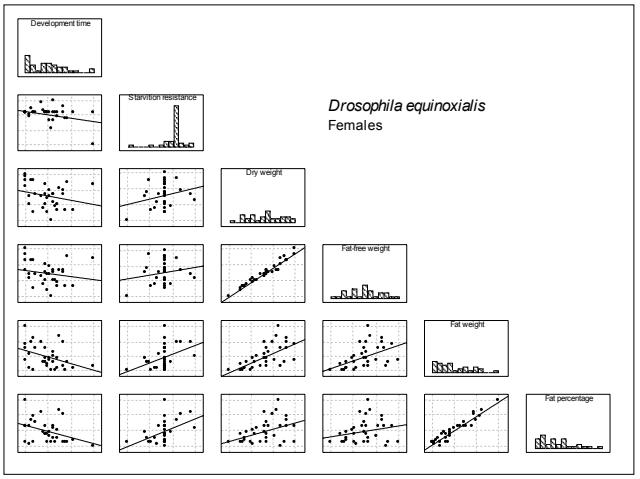
lead to directional selection resulting in changes in the estimated genetic correlations.

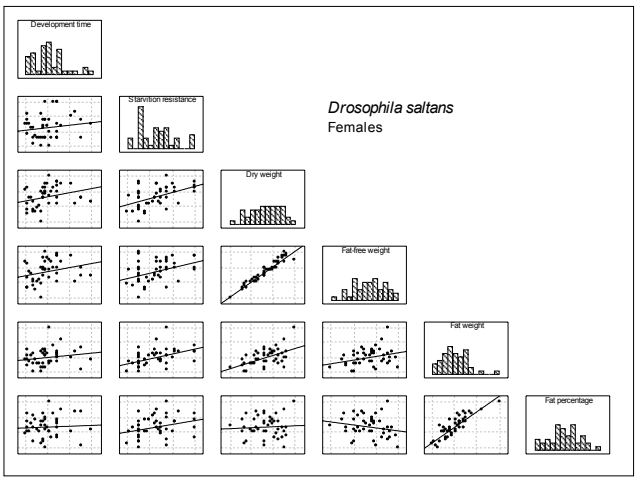
This study showed that genetic correlations between important life-history traits in species of *Drosophila* are unlikely to pose a strong barrier for local adaptation because they are not close to unity. They do however predict the speed at which changes can occur. Overall, these results are in line with the findings in **chapter 4**, and I therefore conclude that the underlying genetic correlations do not hamper local selection, but can slow them down.

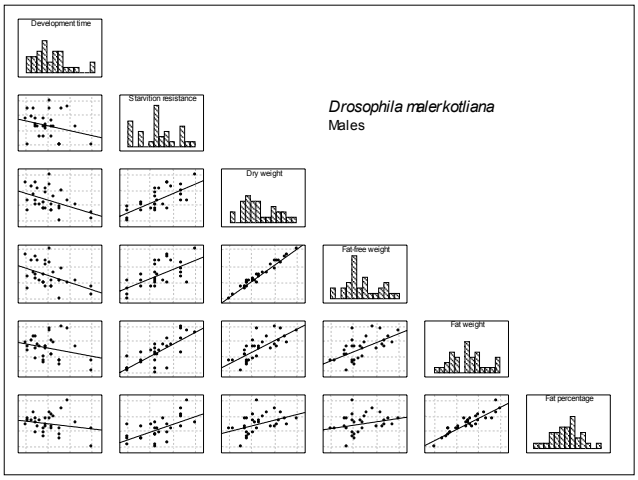
Appendix 1

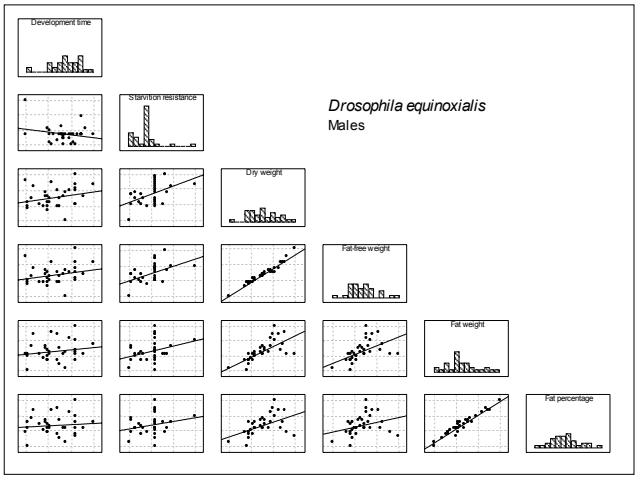
Matrix plots (see next page) for the unweighted data, for each species and sex. Plots on the complete diagonal axis indicate distribution of the values within a trait, while off-diagonal plots are scatterplots between two traits. Data along the x-axis in each scatterplot correspond to the histogram above the plot, while the data along the y-axis data correspond to the histogram at the right of the plot.

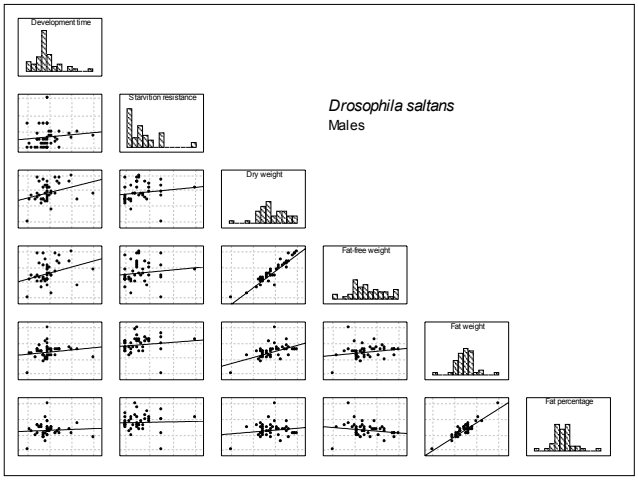

Development time
Starvation resistance
Dry weight
Fat-free weight
Fat weight
Fat percentage
Drosophila melerkotliana
Females


Development time
Starvation resistance
Dry weight
Fat-free weight
Fat weight
Fat percentage
Drosophila equinoxialis
Females


Development time
Starvation resistance
Dry weight
Fat-free weight
Fat weight
Fat percentage
Drosophila saltans
Females


Development time
Starvition resistance
Dry weight
Fat-free weight
Fat weight
Fat percentage
Drosophila malerkotliana
Males


Development time
Starvition resistance
Dry weight
Fat-free weight
Fat weight
Fat percentage
Drosophila equinoxialis
Males


Development time
Starvition resistance
Dry weight
Fat-free weight
Fat weight
Fat percentage
Drosophila saltans
Males

6

Summary and synthesis

In this last chapter, I will summarise the results as presented in the previous chapters. After that, I will discuss two subjects that are central to the thesis. In a similar vein to the first chapter, I will not follow the standards for scientific journals, but rather keep in mind that the content of this chapter is also of interest to the non-biologists who have only read the first chapter.

The central theme of this thesis is local adaptation, with which I mean any genetic differentiation between populations in response to environmental factors. The results in the chapters 2, 4, and 5 provide ample evidence that local adaptation occurs in both the *Drosophila* communities of Panama and the Philippines. Furthermore, I demonstrated that measuring life-history traits, such as development time and starvation resistance, can be carried out in the field. In one of these field experiments, I transplanted flies from one collection site to another site within the same transect, and this showed that this type of field experiment can provide valuable insights into the importance of various sources of variation: genetic, environmental and the interaction between these two, known as the genotype-by-environment (GxE) interaction. This latter is an insight that, by definition, could not be obtained in the laboratory, as the change in environment related to the transfer to the laboratory would obscure the effect of the natural environment and any GxE interactions. Finally, the comparison between the field and laboratory measurements, showed that extrapolation of laboratory data is only possible for body size, not for development time or starvation resistance.

After the summary, I will focus on two aspects that are central to this thesis. First, I will discuss the response to environmental variation. This can take various forms. Second, I will focus on the apparent differences between correlations found at different levels, including species, families, and individuals.

Summary

In **chapter 2**, I presented the results of the pilot experiment, which I carried out in the Philippines several years before the start of my Ph.D. research. The results showed that populations of neighbouring habitats differed significantly in development time, but not in starvation resistance. This pattern was similar within all but one species, suggesting a comparable impact of the environment on all species. Furthermore, the generality of the interspecific correlation between development time and starvation resistance as found by Sevenster & van Alphen (1993b, b) could not be confirmed.

To exclude potential confounding effects of the difference between the field environment and the laboratory environment, I repeated the experiment with Panamanian *Drosophila* species, but this time working directly in the field. The results of this experiment (**chapter 4**) showed that (local) adaptation has also occurred in the Panamanian *Drosophila* community, for all three traits under investigation: body size, development time and starvation resistance. The

intraspecific variation was consistent across species, although the body size variation was not habitat-specific but collection site-specific.

In the second field experiment, flies from a single habitat were reared in all three habitats within the same transect. The aim was to disentangle the different aspects (environmental, genetic and interaction between these two (GxE)) that can affect the realised life-history values. For body size, the genetic variation was not habitat-related, but depended on the particular collection site, while the phenotypic[1] variation showed no consistent pattern. Development time showed clear genetic and phenotypic variation. The phenotypic variation was as predicted from the theory (higher temperature leads to shorter development times), but the genetic differences showed an opposite pattern to that predicted from temperature selection experiments. However, the genetic pattern was consistent with the predictions based on the life-history coexistence model of Sevenster & van Alphen (1993b, b). For starvation resistance, phenotypic plasticity[2] is very important and explained most of the variation. Grassland populations have genetically higher starvation resistances than forest populations, and these genetic differences partly compensate for the stress inflicted by the harsher grassland environment. All three traits show considerable amounts of genotype-by-environment interaction, and this was similar for the different species.

After my return from Panama, I measured the life-history traits in a common laboratory environment, i.e. one that was the same for all species and populations. This experiment confirmed the genetic pattern as found in the field. Furthermore, I was able to compare the data collected in this experiment with those from the first field experiment, as they were collected for the same stocks. Similar field and laboratory estimates indicate that the underlying genetics dominates the estimated phenotypic values[1], but also that extrapolation of the laboratory data to the field situation can be carried out without problems. The results showed that the fit was good for body size, both at the interspecific (across species) as well as the intraspecific (within species) level. However, the same comparisons but for starvation resistance showed that the differences between the two experiments were so large that extrapolation of the laboratory results to the field was not possible for this trait. For development time, the fit was good across species, but absent within species. These results, in combination with the extensive GxE interactions, prompt for caution when extrapolating laboratory-based results for life-history traits to the field.

[1] The phenotypic value is the actual estimate, which is the result of the underlying genetics and the interaction with the environmental effects. The phenotypic variation describes the variation in the estimated values within a population.

[2] Formally defined as: “a change in the average phenotype expressed by a genotype in different macro-environments” (Via 1987, p. 47). The result is a systematic change in the phenotypic values between groups as a result of differences in the environment between the groups, despite that the underlying genetics of the different groups is similar.

A different way of analysing the data is to investigate whether closely related species are more similar to each other than are more distantly related species. In *Drosophila*, some splits between major groups had already taken place 50-100 million years ago (Beverley & Wilson 1984), which implies that, if the pattern is still visible across the various species, the differences observed are probably the result of evolutionary history rather than the present change in the environment. Pagel (1999a, b) developed a method for estimating the phylogenetic dependence within such data. The estimated λ ranges between 0 (phylogenetic independence) and 1 (species' traits co-vary in direct proportion to their shared evolutionary history). These phylogenetic history analyses showed that the patterns in body size and development time closely matched the phylogenetic history ($\lambda = 1$), while the pattern in starvation resistance deviated more ($\lambda = 0.891$). This confirms the idea that the genes for body size are rather insensitive to environmental cues, while those underlying the starvation resistance are more plastic in their response.

When I combine all the results for the three traits, it appears that body size is the least affected by changes in the environment, while local adaptation in starvation resistance is easily obtained. Furthermore, the interspecific variation for the three traits, as measured in both the first field experiment as well as in the common environment experiment, were clearly related to each other as the patterns of variation across species for the different traits showed clear interdependence. The phylogenetic history suggests that this interdependency follows from a pattern of shared genetic pathways. On top of this, genes independent to these shared genetic pathways are likely to be responsible for the deviations from this interspecific pattern.

In the last chapter, I investigated whether the traits shared common aspects of the genetic architecture as measured by the genetic correlations between traits. Therefore, I estimated the sign and magnitude of the underlying genetic correlations between the various traits for three different species (**chapter 5**), as the correlation can affect the speed at which (local) adaptation takes place (cf. Beldade *et al.* 2002, Zijlstra *et al.* 2003). The species were selected such that a wide phylogenetic range was covered. The results showed that body size and starvation resistance do have a partially shared genetic background. In contrast, the genetic correlations between development time and the various body size measurements, as well as with starvation resistance, seemed to be absent. Furthermore, the estimated genetic correlations were species and collection area specific.

Response to environmental variation

One of the two central themes in the thesis is the response to environmental variation. This not only includes variation between collection sites, but also environmental differences between the field and the laboratory. This response can take different forms such as genetic changes, phenotypic plasticity, and genotype-by-environment (GxE) interactions. In most situations, all three responses are present at the same time, which can make the interpretation of the data difficult.

The ubiquitous presence of local adaptation within the Panamanian *Drosophila* community is striking, especially when the closeness of the collection sites is taken into consideration. These sites were within several kilometres of each other in **chapter 2 & 4**, while those in **chapter 5** were located across the whole Isthmus of Panama. Furthermore, the local adaptation showed great similarity across the various species, at least for development time and starvation resistance. This implies that the ecological context is very important in shaping the various traits, and that panmixia[3] within a larger collection area cannot be assumed *a priori*.

When all the results are taken into consideration, it is clear that the three traits have much in common. All three traits respond to changes in the environment resulting in local adaptation in the populations. Furthermore, they each show considerable amounts of phenotypic plasticity and genotype-by-environment interactions. Finally, most of the responses within traits are similar across species, indicating that the differences related to the habitats or collection sites are responsible for the patterns found.

The most striking difference between the three traits is the extent to which results obtained in one environment can be extrapolated to another habitat. For body size, results obtained in the laboratory give a good indication of the situation in the field and *visa versa*. This can be even valid for different populations within the same species. In contrast, extrapolation of the results for starvation resistance to another habitat gives no match at the interspecific (across species) level, or even at the intraspecific (within species) level. For development time, the results can be extrapolated to other environments at the level of species, but not within a single species. These results at the interspecific level were to a degree also reflected in the phylogenetic analysis of the data (see before).

Correlations between traits

For some trait combinations, the estimated correlations varied in a way that was largely dependent on the 'organisational' level, such as species, populations, families, or individuals (table 1). This is most apparent when the patterns for development time and body size are compared. Both traits covary strongly across species and are positively correlated. Furthermore, these patterns are fully explained by the phylogenetic relatedness of the species. In contrast, a genetic correlation between these two traits within species appears to be absent. This suggests that different components of the genetic architecture can be at their most pronounced at different taxonomic levels. At the phenotypic level, the correlation between development time and body size is negative, perhaps due to the effects of density.

[3] When the rate of exchange of individuals between different areas is high, the effect of selection on individuals with certain traits is overwhelmed by the mixing with the individuals from other areas (and environments). The result is a genetically highly homogeneous population covering a large area, even when there us differentiation at the habitat level.

Falconer & Mackay (1996) note in their key textbook that "A large difference, and particularly a difference of sign, shows that genetic and environmental sources of variation affect the characters through different physiological mechanisms" (p. 315). Of course, these physiological mechanisms themselves have a genetic basis. This is in line with the idea that pleiotropic effect may differ among genes and that strong pleiotropy[4] will not necessarily result in a strong genetic correlation but that pleiotropic effects can cancel each other out (Cheverud 1984, Falconer & Mackay 1996, Lande 1980, Lynch & Walsh 1998, Roff 1997, Wagner 1984, 1989).

Several authors have developed quantitative genetic models, in which two (or more) groups of genes with different pleiotropic effects, have been used (de Jong & van Noordwijk 1992, Houle 1991, Mezey & Houle 2003, Wagner 1984, 1989, Wagner & Altenberg 1996). Most of these models were developed for specific situations, but all have in common that the relative importance of the different groups of genes is essential in explaining the variation at the phenotypic and/or genetic level. This idea with various different groups of genes, each with specific effects on the two traits, could explain why we find for life-history traits in *Drosophila* a positive correlation among species, while the same trait combinations do not show a genetic correlation within species. Table 1 gives an overview of the correlations at different levels: individuals (phenotypic), families (genetic), and species (interspecific).

Table 1: Overview of the phenotypic, genetic, and interspecific correlations. Phenotypic correlations are based on the data in chapter 4 (not shown there), the genetic correlation data are presented in chapter 5, and the interspecific correlation data are presented in chapter 4.

Trait combination	Phenotypic correlation	Genetic correlation	Interspecific correlation
Body size - Development time	negative	absent	positive
Body size - Starvation resistance	positive	positive	positive
Development time - Starvation resistance	negative	absent	positive

The correlations as presented in table 1 suggest that at least two different groups of genes, with different pleiotropic effects, are present within the genetic architecture of the *Drosophila* species (although it can not be excluded that the pleiotropic effects of all the genes form a continuum from positive to negative). The strongest indication for this is that the phenotypic correlation and interspecific correlations, between development time and starvation resistance or body size, respectively, are opposite of sign. With the idea of Falconer & Mackay (1996) in mind, this suggests

[4] The phenomenon that a single gene affects two or more traits. When the first gene has a positive effect on the first trait and a negative effect on the second trait, while the second gene has a negative effect on first trait and a positive effect on the second trait, the estimated genetic correlation between the two traits could be absent as the effects of both genes can cancel each other out.

that at least two different physiological mechanisms are present within the organism.

The first group consists of genes with clear positive pleiotropic effects. These genes are relatively conserved and seem to influence all three traits more or less simultaneously. This idea is supported by the interspecific variation, which is completely explained by the phylogenetic history (**chapter 4**: $\lambda = 1$, see before). Furthermore, the split between the major groups within this phylogenetic analysis occurred between 50 and 100 million years ago (Beverley & Wilson 1984), which underlines that this linkage between the traits is embedded deeply within the *Drosophila* genus. My impression is that this group of genes either determines body size primarily and through that, the other traits, or that these are regulatory genes that affect all three traits.

The action of the genes of the second gene-group results in a negative pleiotropic effect between development time and body size. These genes are more sensitive to the environmental differences between the habitats, which act on a relatively short time scale. The expression of these genes is highly environment-dependent, resulting in highly plastic responses to environmental cues (table 1). These genes are more likely to be found among the so-called orphan genes (Schmid & Aquadro 2001) than under structural genes. Orphan genes are protein-coding regions that have no recognisable homologue in distantly-related species, and are often involved in specific ecological adaptations that change over time (Domazet-Loso & Tautz 2003). As such, they are likely to be very important for local adaptation.

The idea that two groups of genes act at different 'organisation' levels is supported by the results in **chapter 5** that include the genetic correlations estimated between the different traits using a family mean approach.[5] This approach is sensitive to the within family variation, and the contribution is reciprocal to the actual family size. Therefore, the elimination of the smaller samples should result in a shift in the importance of the two gene-groups, resulting in an increased difference between the genetic and phenotypic correlation. This pattern was indeed observed. This added some weight to the idea that two different gene-groups are important in the pattern of correlations from species down to individuals.

[5] The idea behind this approach is that closely related individuals, such as offspring of a single female, are more related to each other than are unrelated individuals. This implies that the variation between families is an indication for the genetic variance of that trait, but only when the different families are reared under identical environmental circumstances. When two traits are genetically linked because the underlying genes are (in part) similar, a change in the underlying genetics will simultaneously affect both traits. The correlation between the family means of the first trait with the family means of the second trait is a measure for the genetic correlation between the two traits. However, the smaller the number of individuals within a family, the larger the effect of random variation on the means, which can introduce a bias in the estimated genetic correlation (Via 1984).

The correlation between body size and starvation resistance is positive, regardless of the "organisation" level. This suggests that one group of pleiotropic genes with positive effects is dominant and therefore responsible for the correlation between the two traits at all levels. However, the influence of the other gene-groups is not absent, which leaves sufficient potential for the starvation resistance to respond to environmental changes. Several authors have reported on the dependence of starvation resistance on fat and/or glycogen (Djawdan *et al.* 1998, Graves *et al.* 1992, Marron *et al.* 2003, Zwaan *et al.* 1991). These products need to be stored within the individual, and the absolute fat content depends in part on the absolute body size (Chapter 5, see also: Eijs & van Alphen 1999, Ellers *et al.* 1998). Hence, a reduction in body size results in a reduction in the stored reserves, and through that, in the starvation resistance.

The negative phenotypic correlation between development time and body size can be explained by competition among larvae. The pupation time is set when an individual larva reaches a critical body mass, soon after the second larval moult. A reduction in food before this critical stage leads to an increase in development time, while a reduction in food after this critical stage results in smaller body sizes (Bakker 1959, Robertson 1963), a feature that is often used to obtain small flies for experiments. Under natural conditions, a reduction in food before the critical stage will be accompanied by one after this critical stage. Therefore, slight variations in feeding rate will result in less food for that larva, and through that, in variation in the second moult, after which the pupation time is set. However, those slow larvae will encounter stronger food limitations than the early moulting larvae. Consequently, they have an increased development time and a decreased body size. This mechanism has been found in some other Diptera species, such as *Toxothynchites brevipalpis* (Lounibos 1979) and *Sarcophaga bullata* (Zdarek 1983), while some other species, such as the yellow dung fly *Scathophaga stercoraria* (Blanckenhorn 1998) have a different mechanism in which these traits covary positively with each other .

The negative phenotypic correlation that I observed between development time and starvation resistance is merely a result of the interaction between development time and body size. When the mechanism as described above leads to a negative correlation between development time and body size, starvation resistance is also negatively correlated with development time due to the positive relation between body size and fat content.

The interspecific correlation as found for the Panamanian *Drosophila* community (chapter 4, Sevenster & van Alphen 1993a) was not confirmed by two other studies on Asian *Drosophila* communities (chapter 2, unpublished data K. van der Linde, Toda & Kimura 1997). Does this suggest that the correlation is different in the Panamanian and Asian communities? The main difference between the Panamanian Drosophila community and the two other communities is the range in development times across the species. This range is more than twice as wide in the Panamanian community (7.8-15.4 days (Sevenster & van Alphen 1993a)) than in the communities from the Philippines (8.2-11.0 days (**chapter 2**, unpublished data

K. van der Linde)) and Japan (10.3-13.8 days (Toda & Kimura 1997)). When I took subsamples from the Panamanian data with the smaller ranges in development time comparable to the Asian communities, the positive correlation rapidly disappeared, or even became negative depending on the exact range considered (data not shown). A similar result can also be obtained by excluding a species or related group of species (data not shown). Apparently, the correlation depends heavily on the ranges used within the dataset (cf Fischer *et al.* 2002).

Overall conclusions

The original aim of this thesis was to "to investigate the ecological and genetic covariances among three life-history traits using a combination of field and laboratory work." As expected, this provided new insights into the evolution of life histories in natural environments.

First, this thesis has demonstrated the benefit of obtaining measurements of life-history traits in the field. Furthermore, it enabled me to begin to unravel the importance of the genetics, the environment, and the interaction between these two (GxE interactions). The results showed that GxE interactions are very important, explaining about one third of the variation not explained by factors such as sex and species. Finally, the large differences between the different habitats were such that extreme care is needed in extrapolating laboratory results to the field as the differences between field and laboratory are often larger than those between habitats (**chapter 4**).

Second, (local) adaptation appears to be ubiquitously present within the Panamanian *Drosophila* community, at least for all three traits under investigation in this thesis (**chapter 4**). The variation in body size was not similar across species, in contrast to the pattern for the two other traits. Furthermore, the genetic correlations differ between collection sites (**chapter 5**). In the *Drosophila* community from the Philippines, only development time appeared to be locally differentiated (**chapter 2**).

Third, genetic correlations exist between body size and starvation resistance, but not between development time and body size or starvation resistance (**chapter 5**). The genetic correlation between body size and starvation resistance is far from unity, and this might have slowed the local adaptation in the starvation resistance (**chapter 4**), but apparently did not prevent it. Furthermore, I provided a hypothesis that can explain the apparent differences between correlations when measured at different 'organisation' levels.

Nederlandse samenvatting

In deze Nederlandse samenvatting zal ik proberen mijn promotie onderzoek uit te leggen aan mensen die geen bioloog zijn. Om dat te doen zal ik enerzijds basale begrippen uitleggen die essentieel zijn voor mijn onderzoek, terwijl ik anderzijds veel van de mitsen en maren zal weg laten en me concentreren op de grote lijn van het verhaal. Voor mensen die een gedetailleerder verhaal willen lezen verwijs ik naar de Engelse inleiding in Hoofdstuk 1 in combinatie met de algemene conclusies en discussie in Hoofdstuk 6.

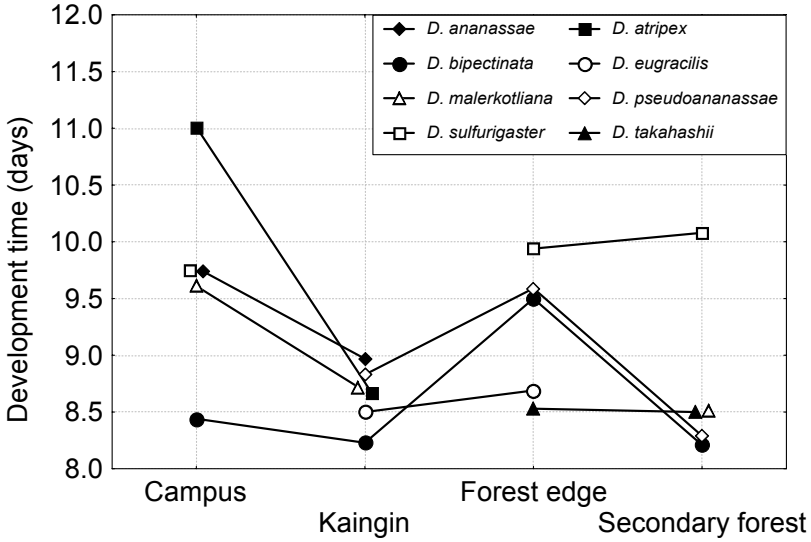
Inleiding

De mens is een zeer succesvolle diersoort en is doorgedrongen in bijna elk ecosysteem van deze aarde. Het neemt daardoor een zeer belangrijke plaats in de natuur van onze aarde. Daarom is het niet meer dan logisch om te kijken wat het effect is van deze uitbreiding expansie op de rest van de natuur. Er is al veel onderzoek gedaan naar de invloed van de mens op de natuur. Voor veel groepen dieren en planten weten we redelijk goed welke soorten problemen hebben met de door ons aangebrachte veranderingen, terwijl we weten dat andere soorten gebruik maken van de antropomorfische (door de mens gemaakte) veranderingen en zich hebben gevestigd in steden, op akkers en langs wegen.

In 1992 heb ik in de Filippijnen een onderzoekje gedaan waarbij ik *Drosophila*`s (fruitvliegjes) verzamelde in verschillende gebieden. Sommige gebieden waren relatief onverstoord door mensen (tropisch oerwoud) terwijl andere gebieden geheel door de mens waren aangepast (grasland voor koeien). Vervolgens heb ik een rangorde gemaakt van onverstoord tot verstoord. Uit het resultaat van mijn onderzoek bleek dat de biodiversiteit (de verscheidenheid aan levensvormen, planten, dieren, schimmels etc) in alle onderzochte gebieden ongeveer gelijk was, of te wel, het aantal soorten *Drosophila* was ongeveer gelijk ongeacht de mate van verstoring. Echter, dit waren niet altijd dezelfde soorten. De overeenkomst in soorten tussen de twee gebieden die het minst op elkaar leken (tropisch oerwoud versus grasland) was slechts 10%. Of te wel, 90% van de *Drosophila*`s uit die gebieden werd niet gevonden in het andere gebied (van der Linde 1997, van der Linde & Sevenster 2002).

Sommige *Drosophila* soorten vond ik alleen in verstoorde gebieden, zoals grasland, terwijl andere soorten enkel en alleen in het tropische oerwoud voorkwamen. Maar er waren ook soorten die in zowel het minst verstoorde en het meest verstoorde gebied voorkwamen. De vraag die daar logisch uit volgde was: “Hoeveel verschil is er tussen individuen van dezelfde soort tussen verschillende gebieden?”

In 1994 ben ik opnieuw naar de Filippijnen geweest, dit keer om *Drosophila*`s te verzamelen in 4 verschillende gebieden, weer van tropische bos tot grasland. Echter, die keer heb ik de vliegjes meegenomen naar Nederland en hier naar het laboratorium gebracht. Van acht soorten had ik vliegjes uit meer dan één gebied. Vervolgens heb ik voor al de populaties twee belangrijke kenmerken gemeten: de ontwikkelingstijd en de hongerresistentie. Deze twee kenmerken zijn belangrijk voor



Figuur 1: Gemiddelde ontwikkelingstijd (in dagen) per soort en verzamellocatie. Overlappende punten van verschillende soorten zijn naast elkaar weer gegeven.

Drosophila`s, waarom leg ik verderop in deze samenvatting uit (zie: *Coëxistentie van soorten)*.

Drosophila`s beginnen hun leven als eitje, dat hun moeder op bijvoorbeeld een stuk rottend fruit legt. Uit het eitje komt een larf die zich te goed doet aan gisten en bacteriën die op het stuk rottend fruit leven. Na enkele dagen, en dit aantal is afhankelijk van de soort, verpopt de larf zich en nog weer enkele dagen later kruipt de volwassen vlieg uit de pop. De ontwikkelingstijd is de tijd tussen het leggen van het eitje en het moment dat de volwassen vlieg uit de pop kruipt. Hongerresistentie is de tijd dat een volwassen vlieg zonder voedsel kan.

Maar nu eerst terug naar het experiment. Het resultaat liet zien dat er grote verschillen waren tussen de verschillende populaties van dezelfde soort (Figuur 1). In figuur één staan langs de onderste horizontale lijn (X-as) de verschillende gebieden. Lang de linker verticale lijn (Y-as) staan getallen die de ontwikkelingstijd aangeven in dagen. De verschillende symbolen in de grafiek geven de verschillende soorten aan. Wat duidelijk wordt is dat de populaties uit het grasland (campus) altijd een langere ontwikkelingstijd hebben dan uit het landbouw gebied (kaingin). Hetzelfde plaatje, maar dan voor honger resistentie (zie hoofdstuk 2, figuur 2) maakt duidelijk dat hongerresistentie slechts een klein beetje verschilde tussen populaties, maar geen consistente patronen liet zien (van der Linde & Sevenster submitted).

Coëxistentie van soorten

De basis idee van competitie is dat de soort die het beste is aangepast aan een specifieke omgeving zal overwinnen en dat de andere soorten zullen verdwijnen (survival of the fittest = overleving van de best aangepaste: Darwin 1859). Echter, vaak leven vele soorten naast elkaar in dezelfde omgeving terwijl ze gebruik maken van de zelfde voedselbronnen. Om dit te verklaren zijn er een heel scala aan coëxistentie modellen ontwikkeld die dit naast elkaar bestaan kunnen verklaren.

Eén zo'n model is ontwikkeld door één van mijn begeleiders (Sevenster & van Alphen 1993a, b). Dit model voorspeld dat twee soorten naast elkaar kunnen bestaan als ze allebei, voor tenminste een deel van het jaar, een relatief voordeel hebben ten opzichte van de andere soort. In dit model is dat gebaseerd op verschillen in levensloopkenmerken[1] en de ecologische relevantie daarvan. In dit model gaat het om kenmerken zoals bijvoorbeeld de ontwikkelingstijd en de hongerresistentie van een vlieg die ik hieronder uitleg.

Fruitbroedende *Drosophila* soorten leggen hun eitjes op rottend fruit en vrouwtjes van meerdere soorten leggen hun eitjes op hetzelfde stuk fruit. Dit heeft als gevolg dat de larven van verschillende soorten *Drosophila* concurreren om hetzelfde voedsel. Hoe sneller je eet, hoe meer kans je hebt om te kunnen verpoppen. Eet je te langzaam, dan heb je pech (eten op) en ga je dood voordat je kunt verpoppen. Of te wel, een korte ontwikkelingstijd is gunstig want je hebt grotere kans om uiteindelijk een volwassen vlieg te worden Wat verder belangrijk is hier, is te weten dat een kortere ontwikkelingstijd vaak gepaard gaat met een kleinere lichaamsgrootte.

Zodra de volwassen vlieg uit de pop kruipt moet hij of zij een nieuwe plek zien te vinden waar hij kan paren en zij haar eitjes kan leggen. Als er veel fruit aanwezig is, dan zijn de afstanden tussen twee plekjes klein en hebben ze geen probleem een nieuw plekje te vinden. Dit is echter niet het gehele jaar het geval en in sommige periodes is er maar weinig fruit aanwezig. Gedurende die periodes moet een net uitgekomen vlieg een lang stuk vliegen en daarvoor moet de vlieg voldoende energie hebben. Grote vliegen hebben meer vet en daardoor een betere hongerresistentie en kunnen daardoor verder vliegen. Ook blijkt dat grotere vliegen sneller vliegen en dus in dezelfde tijd langere afstanden kunnen afleggen. Kleine vliegen daarentegen redden het niet en gaan dood voordat ze een nieuw plekje kunnen vinden.

Als we nu beide mechanismen met elkaar verbinden, dan zien we dat in een periode met veel fruit om eitjes op te leggen, de kleine vliegjes met een korte

[1] Als je de levensloop van een mens beschrijft, dan wordt die geboren na een zwangerschap van ongeveer negen maanden. Meestal wordt er één kind geboren. Hij of zij doet er dan 12 tot 14 jaar over om de reproductieve leeftijd te bereiken. Gedurende het leven kan een mens een x aantal nakomelingen krijgen. Enzovoort. Kenmerken zoals de 9 maanden zwangerschap, 12-14 jaar tot reproductief worden etc zijn dus levensloopkenmerken.

ontwikkelingstijd en slechte hongerresistentie het 't beste doen. In de periode met weinig fruit is dat net andersom. En op deze manier kunnen de twee soorten naast elkaar bestaan, omdat ze allebei een periode hebben dat ze het 't beste doen.

Een aanname in dit verhaal is dat ontwikkelingstijd en hongerresistentie, de levensloopkenmerken, genetisch met elkaar verbonden zijn. Als dit niet zo is, dan kan een soort zowel een snelle ontwikkelingstijd en een goede hongerresistentie kunnen hebben, en zou die soort onder alle omstandigheden kunnen winnen.

Wat we nu hebben is een systeem waarin twee processen een belangrijke rol spelen. Enerzijds zijn de levensloopkenmerken belangrijk voor de coëxistentie van de soorten. Anderzijds is dit model afhankelijk is van de dynamiek in de leefomgeving, namelijk de hoeveelheid fruit die beschikbaar is gedurende het jaar, en met name het patroon daarin gedurende het jaar. Stel nou dat, doordat de mens de omgeving veranderd, dat het patroon in aanwezig fruit gedurende het jaar veranderd, bijvoorbeeld dat het fruitaanbod het gehele jaar door zeer constant is, en dat er altijd veel fruit aanwezig is. Dan is alleen nog de ontwikkelingstijd van belang in de concurrentie tussen de soorten en zal de snelste soort winnen.

Nieuwe vragen

Mijn onderzoek met *Drosophila* soorten van de Filippijnen leidde tot nieuwe vragen. We hadden de vliegjes in het wild verzameld, en vervolgens hebben we in het laboratorium gekeken of ze verschillend waren. De vraag die dat opriep was: "Is dit verschil een gevolg van de verschillen in de genen, of een gevolg van de verandering in de omgeving (van veld naar laboratorium)?". En in hoeverre spelen interacties tussen de genen en de omgeving een rol. Verder wisten we niet of de ontwikkelingstijd en de hongerresistentie genetisch met elkaar verbonden waren. En of deze genetische interactie hetzelfde was in alle soorten waar we gegevens over hadden.

Promotie onderzoek

In mijn promotie onderzoek wilde ik twee vragen beantwoorden:

1. Passen *Drosophila* zich genetisch aan, aan hun veranderende omgeving. En zo ja, op welke manier. Gerelateerd hieraan is de vraag welke invloed de omgeving heeft op bijvoorbeeld de ontwikkelingstijd.

2. Zijn ontwikkelingstijd en hongerresistentie genetisch verbonden met elkaar?

Omdat we de experimenten direct in het veld wilden uitvoeren hadden we een gebied nodig waar goede faciliteiten aanwezig waren. Dat bracht me naar Panama, waar het Smithsonian Institute een goed bereikbaar veldstation midden in het oerwoud heeft.

Drosophila zijn, net als alle insecten, koudbloedig. Dat heeft als gevolg dat de omgevingstemperatuur voor een belangrijke mate bepaalt hoe snel ze zich kunnen ontwikkelen. Als een bos wordt gekapt, dan komt al het zonlicht meteen op de grond in plaats van op het bladerdak. Dit zorgt ervoor dat de temperatuur in een rottend stuk fruit hoger zal zijn in het grasland dan onder het bladerdak van het oerwoud. Een verschil in ontwikkelingstijd van vliegjes in het oerwoud en in het grasland kan dus worden veroorzaakt door de verschillen in de omgeving maar kan ook worden veroorzaakt door een verschil in de genen. De vraag is hoe je deze twee kunt onderscheiden.

Eén manier is om vliegjes te verzamelen in zowel het bos als het grasland en ze vervolgens op te kweken in een laboratorium. Ze ervaren dan dezelfde omgeving en verschillen zijn dus een gevolg van het verschil in genen. Maar zo simpel is het helaas niet altijd. Want de genen kunnen geselecteerd zijn om het juist heel goed te doen onder omstandigheden in het oerwoud, maar niet zo goed onder de omstandigheden uit het grasland. Deze interactie tussen genen en omgeving wordt genotype-met-omgeving interactie genoemd (In engels: Genotype-by-Environment interactions: GxE interactions).

Een betere manier om alle drie de effecten, genetisch, omgeving en de interactie te onderzoeken is om *Drosophila*`s te verzamelen in verschillende gebieden. De ouders van elke populatie laat je vervolgens eitjes leggen op kleine stukjes banaan, welke je vervolgens uitkweekt in de verschillende gebieden waar de verschillende populaties waren verzameld. Zo krijg je gegevens over hoe de bos populatie het doet in het bos, het intermediaire gebied en in het grasland. Maar ook hoe de grasland populatie het doet in die zelfde drie gebieden. En natuurlijk ook voor de populaties van de intermediaire gebieden. Met een hoop statistiek kun je dan vervolgens de verschillende componenten, genetisch, omgeving en GxE, uit elkaar halen en krijg je een veel beter inzicht in wat er gebeurt. Om uit te sluiten dat een verschil tussen bos en grasland toevallig was, heb ik het experiment op twee locaties uitgevoerd, op een redelijke afstand (ongeveer 10 kilometer) van elkaar verwijderd. Binnen die twee locaties heb ik een bos gebied, een grasland gebied en een overgangsgebied gekozen, die op slechts enkele kilometers van elkaar weg lagen. Als de beide locaties hetzelfde resultaat opleveren, dan is het veel waarschijnlijker dat het een echt resultaat is en niet toevallig een gelukstreffer.

Omdat dit soort experimenten erg veel werk is, kon het experiment slechts met een beperkt aantal soorten worden uitgevoerd. Daarom heb ik eerst een experiment gedaan waarbij ik de ontwikkelingstijd, lichaamgrootte en de honger resistentie van alle soorten (12) en alle populaties (4-6 (maximaal 3 populaties per onderzoekslocatie)) in hun eigen omgeving heb gemeten. Vervolgens heb ik een keuze gemaakt voor 4 soorten en die gebruikt in het tweede experiment. Nadat ik in Nederland terug was, heb ik de ontwikkelingstijden, lichaamsgroottes en de hongerresistenties ook nog gemeten in het laboratorium, onder gecontroleerde omstandigheden.

De tweede vraag, of de verschillende kenmerken genetisch met elkaar zijn verbonden vroeg om een heel ander experiment. In dit experiment ben ik alleen geïnteresseerd in de onderliggende genetica van de soorten en daarom heb ik dit onderzoek in het laboratorium uitgevoerd, onder constante omstandigheden zodat de omgeving geen rol speelt. Dit experiment is gebaseerd op het principe dat verwante individuen meer op elkaar lijken dan minder verwante individuen. In dat experiment heb ik individuele vrouwtjes op een klein stukje banaan eitjes laten leggen. Alle vliegjes die dus uit dat stukje banaan komen zijn dus broertjes en zusjes van elkaar. En dus meer met elkaar verwant dan met vliegjes uit andere stukjes banaan waar een ander vrouwtje eitjes heeft gelegd. Omdat de omgeving precies hetzelfde is voor alle families, is het mogelijk om met een hoop statistiek berekenen hoe sterk de verbanden tussen twee kenmerken werden bepaald door de onderliggende genen en welk deel veroorzaakt werd door de variatie in de omgeving.

Resultaten

De resultaten voor de lichaamsgrootte toonden aan dat er geen duidelijk patroon te herkennen in de variatie tussen verschillende omgevingen. Er waren wel verschillen tussen populaties, maar die waren verschillend tussen de verschillende soorten. Het was niet mogelijk een goede verklaring te geven voor de resultaten en het enige dat ik kan zeggen is dat lichaamsgrootte niet in sterke mate lijkt te worden beïnvloed door de verschillen in de omgeving.

Ontwikkelingstijd vertoonde wel een duidelijk patroon, waarin grasland populaties zich sneller ontwikkelden dan bos populaties. En dat patroon was min-of-meer hetzelfde voor alle soorten. Dit was verwacht voor het eerste experiment waarin ik de ontwikkelingstijd had bepaald in hun eigen omgeving. De temperatuur in het grasland is hoger, en dus zullen populaties zich sneller ontwikkelen. Op een genetisch niveau bleek hetzelfde patroon (tussen populaties en tussen soorten) aanwezig te zijn. Ook in het laboratorium ontwikkelden de grasland populaties zich sneller dan de bos populaties. Het coëxistentie model van mijn begeleider (Sevenster & van Alphen 1993a, b) voorspelt dat in verstoorde gebieden het gunstiger is om een korte ontwikkelingstijd te hebben. Dit komt omdat er in het verstoorde grasland gedurende het jaar kleinere verschillen zijn in het fruitaanbod dan in het onverstoorde bos. Dat betekend dat de schaarste periodes minder extreem zijn en dus dat hongerresistentie minder belangrijk wordt. En dat betekend dat de verschillen in ontwikkelingstijd relatief belangrijker worden. En mijn resultaat past dus precies in de voorspelling van dat model.

In het veld hadden de grasland populaties de slechtste (=kortste) hongerresistentie in het grasland, terwijl ze de beste (=langste) hongerresistentie hadden in het bos. De resultaten voor het laboratorium experiment waren precies andersom. De slechtste hongerresistentie vond ik in de bos populaties, en de beste voor de grasland populaties. En dit patroon was ongeveer hetzelfde voor alle soorten.

Dit patroon is als volgt te verklaren. Het grasland is een onaangenamere plek om te leven dan het bos, met name omdat het er droger en warmer is. *Drosophila* zijn koudbloedig en een warmere omgeving heeft tot gevolg dat hun stofwisseling sneller werkt. Als je stofwisseling sneller is, dan verbruik je meer van je vet reserves in een kortere tijd. En dus kun je korter leven op de vetreserves waarmee je uit het de pop bent gekropen. Het gevolg is een slechtere hongerresistentie. Vliegjes met een relatief goede hongerresistentie zullen dus gemakkelijker overleven in het grasland dan vliegjes met een relatief slechte hongerresistentie. En dus is er selectie voor een betere hongerresistentie in de grasland populaties. In het bos is de selectiedruk minder groot, met als gevolg dat daar ook individuen met een relatief slechte hongerresistentie kunnen overleven. Het feit dat de grasland populaties, die genetisch beter zijn aangepast aan hongerresistentie, het nog steeds slechter doen in het grasland, dan de bos populaties in het bos, geeft wel aan hoeveel slechter het grasland is om in te leven. Blijkbaar heeft de selectie voor beter aangepaste vliegjes in het grasland nog niet alle nadelen van dat zelfde grasland kunnen compenseren.

De resultaten van het genetisch experiment laten zien dat alleen lichaamsgrootte en hongerresistentie genetisch verbonden zijn met elkaar. En dit was consistent voor alle drie de soorten. De andere twee combinaties, namelijk ontwikkelingstijd met hongerresistentie en ontwikkelingstijd met lichaamsgrootte, waren soms wel en soms niet genetisch met elkaar verbonden. Dit verschilde tussen soorten en tussen populaties van verschillende locaties maar binnen dezelfde soort. De aanname in het coëxistentie model klopt blijkbaar dus niet (zie: Conclusies en Discussie). Maar zelfs als ze genetisch met elkaar verbonden zijn, dan is het op een zwakke manier. Dat betekend dus dat selectie zonder problemen op beide kenmerken tegelijkertijd kan inwerken, zonder dat de genetische correlatie een belemmering betekend voor de richting waarin de kenmerken kunnen veranderen.

Conclusies en Discussie

In mijn promotie onderzoek had ik twee vragen gesteld. De eerste vraag was of *Drosophila*`s genetisch aanpast zijn aan hun veranderende omgeving. Het antwoord daarop is duidelijk positief, al zijn er grote verschillen tussen de verschillende levensloopkenmerken waarnaar ik heb gekeken. Lichaamsgrootte lijkt niet consistent te veranderen, terwijl ontwikkelingstijd en hongerresistentie dat wel doen. De tweede vraag was of deze kenmerken genetisch aan elkaar verbonden zijn. Het antwoord daarop is, dat alleen lichaamsgrootte en hongerresistentie genetisch verbonden zijn met elkaar, de andere twee dus niet, althans niet consistent voor alle soorten.

En hoe zit het dan met de aanname in het coëxistentie model dat ontwikkelingstijd en hongerresistentie aan elkaar verbonden moeten zijn om het model te laten werken. Dat kan als volgt verklaard worden, althans voor Panama. Het coëxistentie model wordt gesproken over verschillende soorten. Terwijl we hier hebben gekeken naar het verband binnen soorten. En die twee sluiten elkaar dus niet uit. Langzame

soorten hebben nog steeds een betere hongerresistentie vergeleken met snelle soorten. Het blijkt dat als je wat preciezer naar hoe de verschillende kenmerken aan elkaar verbonden zijn, dat de genen die zorgen voor de verschillen tussen soorten behoren tot een andere groep genen dan die genen die zorgen voor de verschillen tussen de verschillende families uit het genetische experiment.

Nawoord

De vraag die niet-wetenschappers vaak aan me stellen is: “En wat is het maatschappelijke nut hiervan?” Eerlijk gezegd heb ik dit onderzoek in eerste instantie gedaan omdat ik het interessant vond, en omdat er leuke wetenschappelijke vragen aan verbonden waren. Maar ik denk dat mijn onderzoek ook maatschappelijk nuttig is. De mens is in hoog tempo bezig de omgeving van veel dier- en plantensoorten te veranderen. Het gevolg is dat soorten uitsterven, maar andere soorten overleven. En die zullen zich moeten aanpassen aan hun nieuwe omgeving. Als we meer inzicht hebben in hoe goed of slecht dieren en planten zich kunnen aanpassen, dan kunnen we ook beter inschatten wat de gevolgen zijn van ons handelen op die natuur.

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Nawoord

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Curriculum vitea of Kim van der Linde

I was born on April 19, 1966 in Meppel, a medium sized town in the northern part of the Netherlands. I do not have any memories of that place, nor do I have any memories of Staphorst, the town were I lived until I was three years old. My first memories are of Voorburg, a town bordering Den Haag, where I lived with my parents until I was 7 years old. By that time, we moved to Haaksbergen, which is in the east, very close to the border of Germany, where I have lived until I left home at the age of 21.

In Haaksbergen, I went to primary and secondary school. I spent my first 5 years in a general secondary school (HAVO), and added another two year in grammar school (Atheneum). It was during that time already that I took an interest in the nature surrounding me. Haaksbergen is surrounded by beautiful nature and especially some of the larger remaining stretches of raised peat bog had my interest. Besides this, parrots and parakeets became another expression of my interest in nature. I am still very thankful to my parents for accepting so much from me with those birds.

By the end of the high school, I had a bird-oriented mindset and I wanted to work with endangered species and to protect them from extinction by breeding them in captivity. With a bit of luck, I got a job working with birds in "Burgers dierenpark" in Arnhem, but I did not like the job and the atmosphere at the job so I quitted. It was then that I decided to study biology.

So I moved to Leiden, were I started my University study in biology in 1986. After a trial at biochemistry, which I did not like, I chose to specialise in population biology and that I liked much better.

My first practical subject was at the evolutionary biology group, with Peter de Jong and Paul Brakefield as my supervisors. We did research on the effects of sunlight on the activity levels of different colour morphs of ladybirds. The underlying aim was to explain the frequency variation of these morphs across the Netherlands. There are two groups of colour morphs with the two-spot ladybirds, primary red and black individuals. The distribution of the two colour morphs is clearly correlated with the amount of sunshine during the year, with blacker ladybird at places with less hours of sunshine. The idea is that the black ladybirds heat up faster in the sunlight then the red morph. Therefore, we tested under controlled conditions whether there were differences in activity between normal and melanic two-spot ladybirds (*Adalia bipunctata*) under artificial sunlight. We concluded that thermal melanism is indeed one of the keys behind the differences in distribution of the two colour morphs. These results were presented in an article (de Jong *et al.* 1996).

In the meantime, I had started a second study, cultural anthropology and sociology of non-western societies. This was an old interest, but was also inspired by my interest in environmental sciences, especially of the third world. I never finished my

bachelors of the cultural anthropology study, as I got the chance to go to the Philippines to work on biodiversity issues within the context of a larger environmental science program (van der Linde 1997, van der Linde & Sevenster 2002). This kind of research is what I liked and I found my niche within the community ecology and related genetics.

In 1994, I returned to the Philippines to collect fresh *Drosophila* flies for a follow up study, in which I tested whether populations of different habitats had differentiated from each other (van der Linde & Sevenster submitted, chapter 2). This proved a good choice and gave me plenty of ideas for a Ph.D. study. During that second stay, I was able to prove that the deforestation on the Philippines had resulted in a new breeding species for that country, the Pied Harrier (*Circus melanoleucos*) (van der Linde 1996a). Furthermore, I observed a presumed extinct species, the Isabela Oriole (*Oriolus isabellae*) (van der Linde 1996b). This latter species is now regularly observed as the preferred habitat was different from expected from the old literature.

After that, I took an involuntary break from science, as I was unable to find an interesting Ph.D.-position. In the meanwhile, I worked as a tour guide for SNP and as a programmer for S.W.I.F.T. c.s. and West Consulting. However, science remained my primary focus, and after 3 years, I decided to write my own PhD research proposal. Dr. Jan Sevenster and Prof. Paul Brakefield successfully submitted the proposal to WOTRO, a section of NWO (Dutch Science Foundation). This research proposal focussed on the effect of habitat change on life-history evolution, and the results are presented in this thesis.

My PhD research brought me to Panama twice, the first time for 6 months in which I colleted the data which are presented in chapter 4. The second time I went for 6 weeks, to collect fresh stocks, as we did not trust the laboratory stocks anymore, which had been maintained for so long in the laboratory. Those stocks were used for the experiments described in chapter 5 of this thesis.

In April 2004, I accepted a post-doc position at Florida State University with Dr. David Houle and in June of 2004, I moved to Tallahassee, Florida. I still work with *Drosophila*, but no longer on life-history evolution or community ecology. I now work on the evolution of complex phenotypes and use *Drosophila* wings and their venation pattern as a model system to investigate how genetics and environment of those complex patterns interact and result in the variation among wings of *Drosophila* species.

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