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)

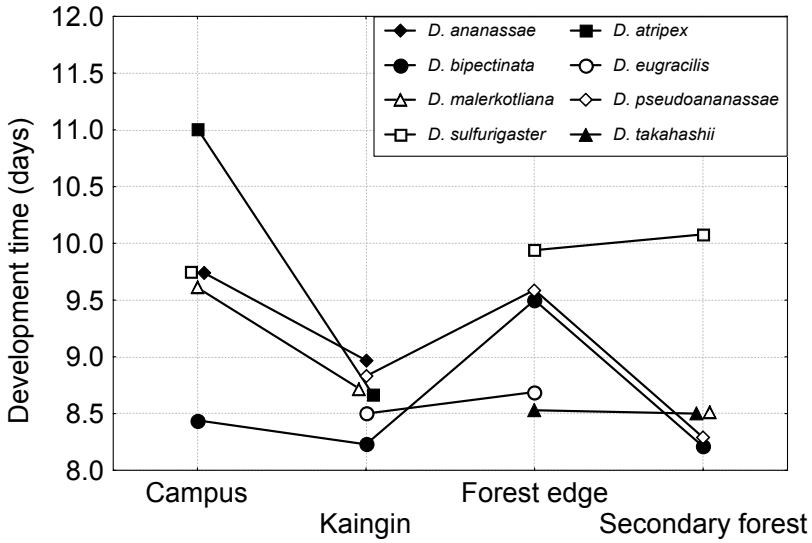

Figure 1: Development time averages (in days) per stock versus habitat. Overlapping points of different species are positioned next to each other to avoid confusion. See further chapter 2.

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

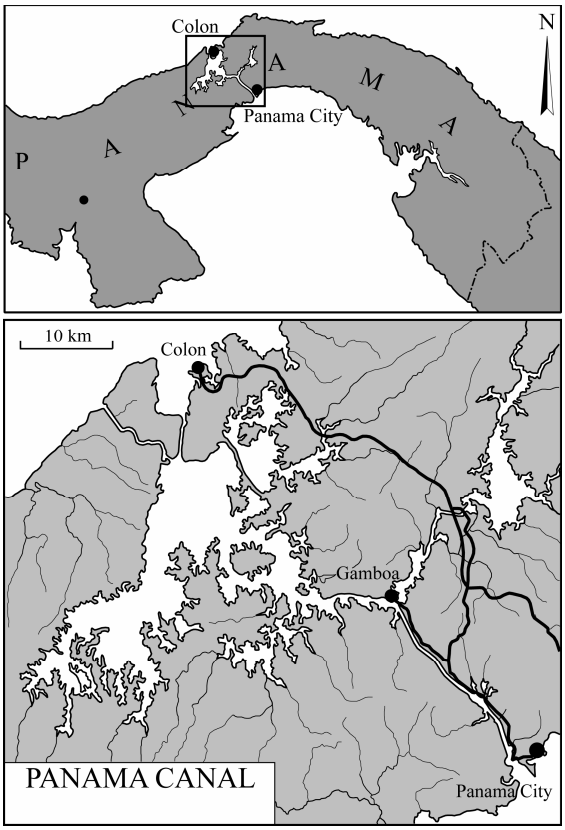

Figure 2: Map of the Canal Zone in Panama with the two field sites indicated as described in the text.

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.