Testing Drosophila life-history in the field: local adaptation in body size, development time and starvation resistance

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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.