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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

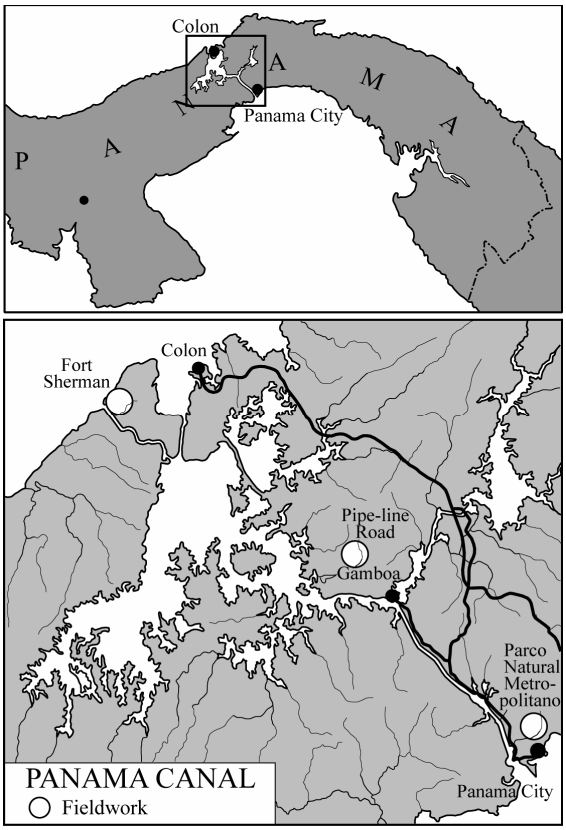


Figure 1: Map of research area.

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.

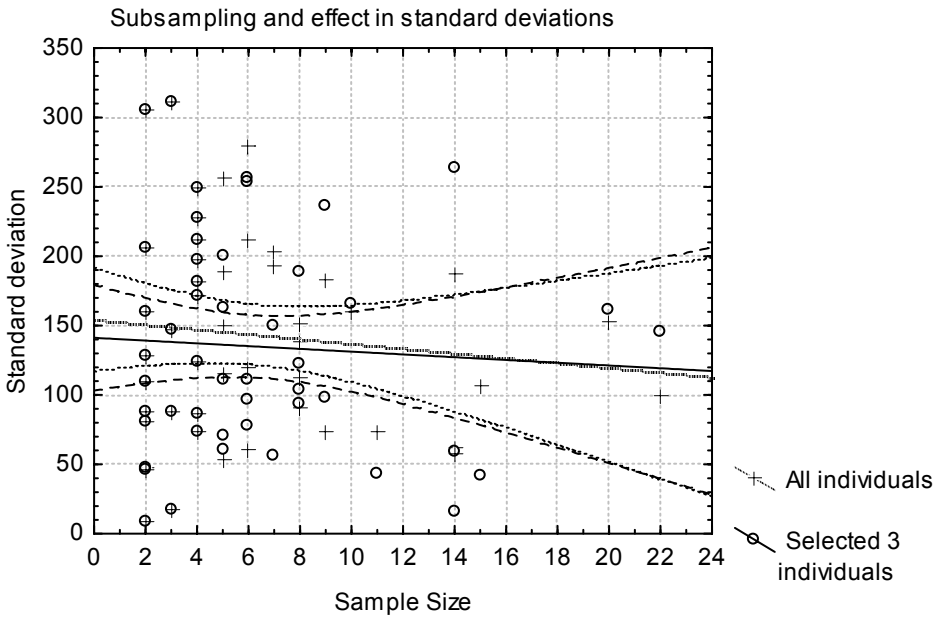


Figure 2: Impact of sub-sampling on the estimated standard deviations for dry weight. Sub-samples were obtained from around the median of the family samples of the *D. malerkotliana* dataset.

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.

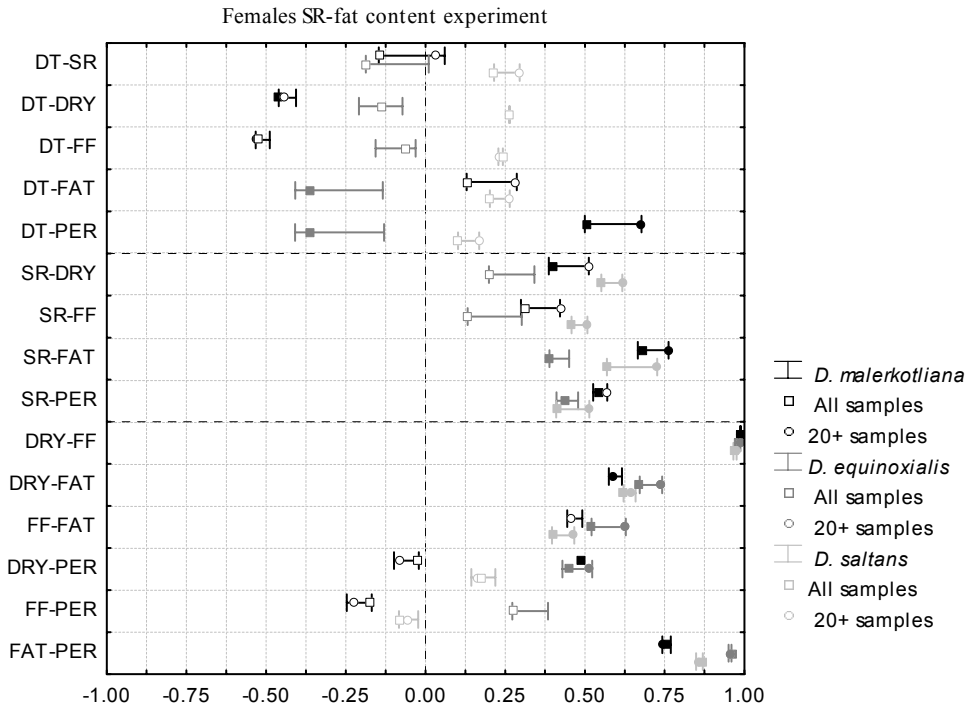


Figure 3: Estimated broad sense genetic correlations for different trait combinations based on family means, for females of three different species. Whiskers give the range in the estimations for the genetic correlations. Range is estimated by exclusion of samples below a certain sample size, ranging 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

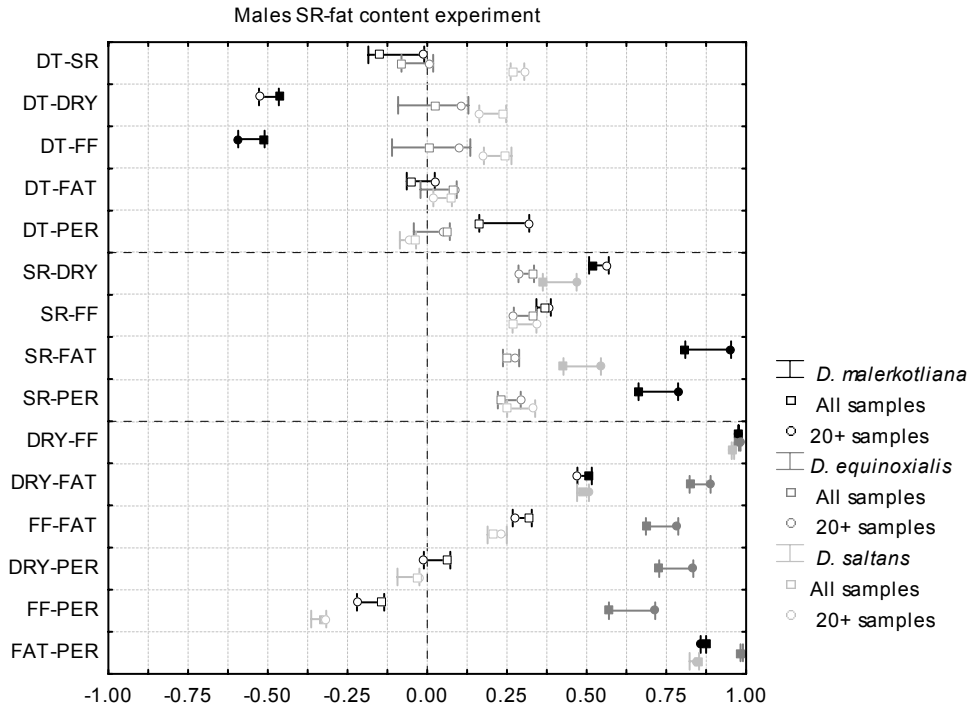


Figure 4: Estimated genetic correlations for different trait combinations based on family means, for males of three different species. For the meaning of the symbols and abbreviations, see legend of figure 3.

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.

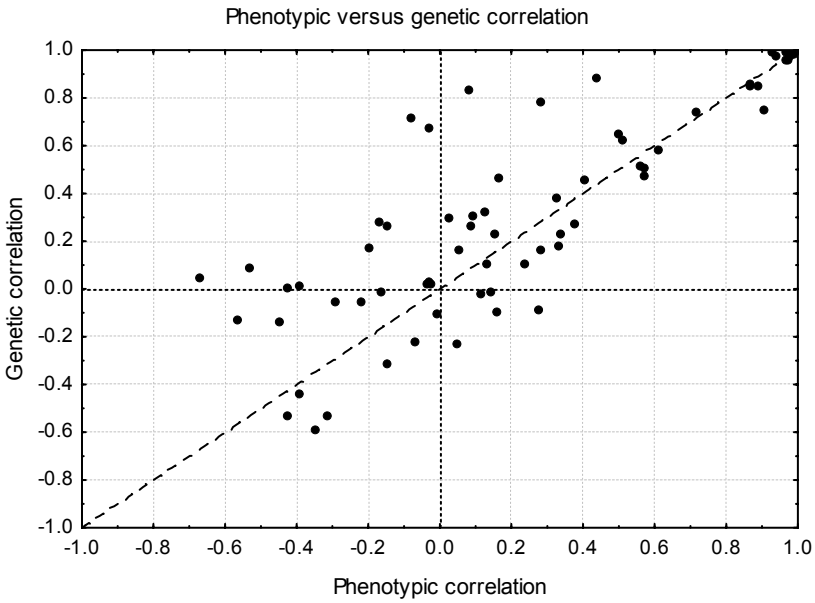


Figure 5: Comparison between phenotypic and genetic correlations. For the genetic correlations, the estimates for only those sample containing 20 or more individuals have been used. The diagonal dashed line indicates the expected location of the dots in case the genetic and phenotypic correlations matched 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: $F_{15, 30} = 0.31$, $p = 0.99$).

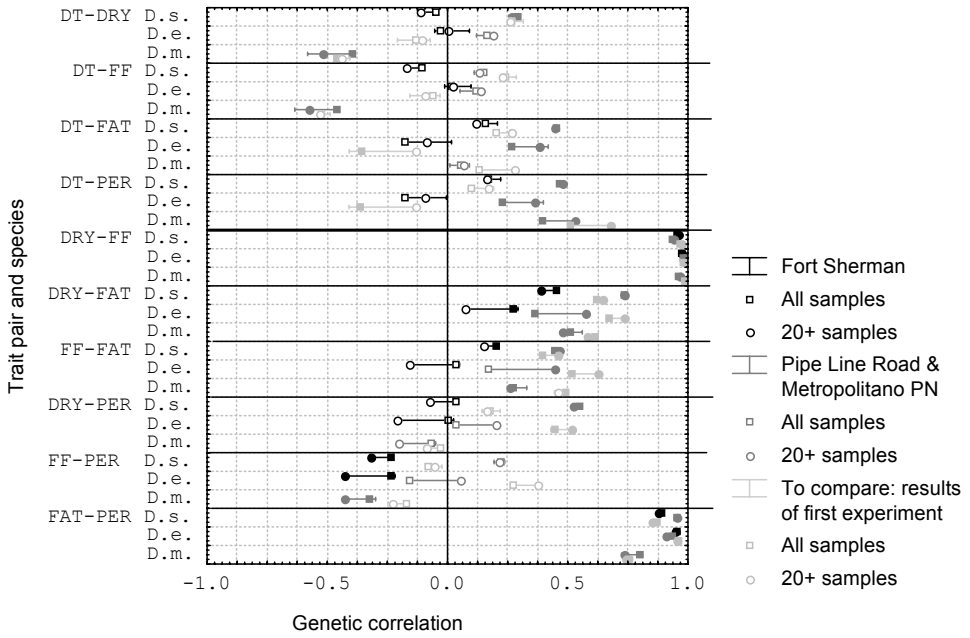


Figure 6: Estimated genetic correlations for different trait combinations based on family means, for females of two different populations of three different species. On the x-axis are indicated the trait combination with the species. D. s. = *D. saltans*; D. e. = *D. equinoxialis*; D. m. = *D. malerkotliana*. 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*.

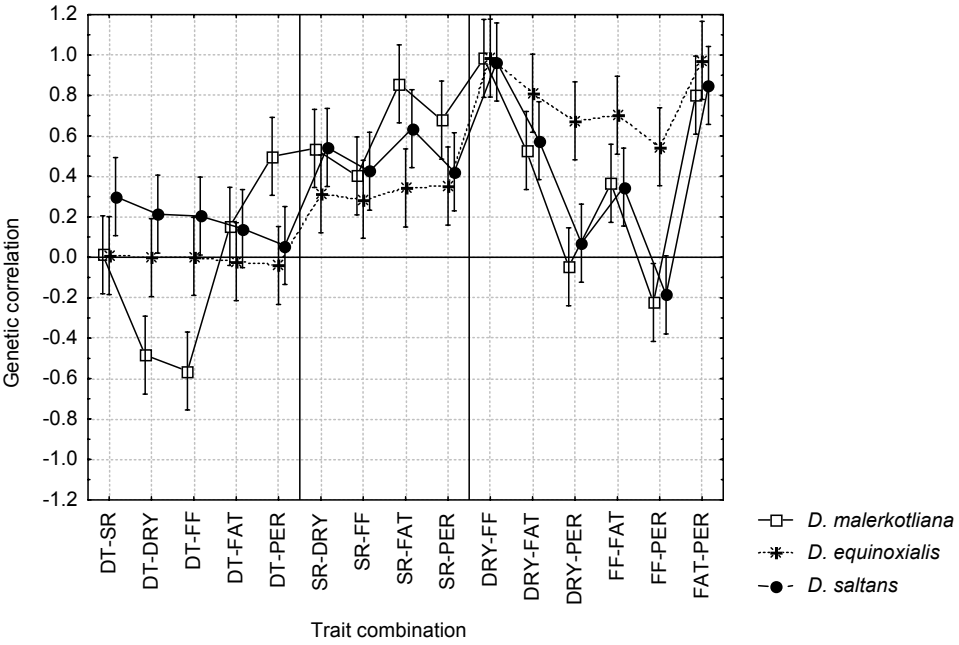


Figure 7: Species specific variation in estimated genetic correlations. Trait combinations are at the x-axis; genetic correlations are at the y-axis. Bars indicate standard errors. DT = development time; DRY = total dry weight; FF = fat-free dry weight; FAT = aft weight; PER = percentage fat relative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

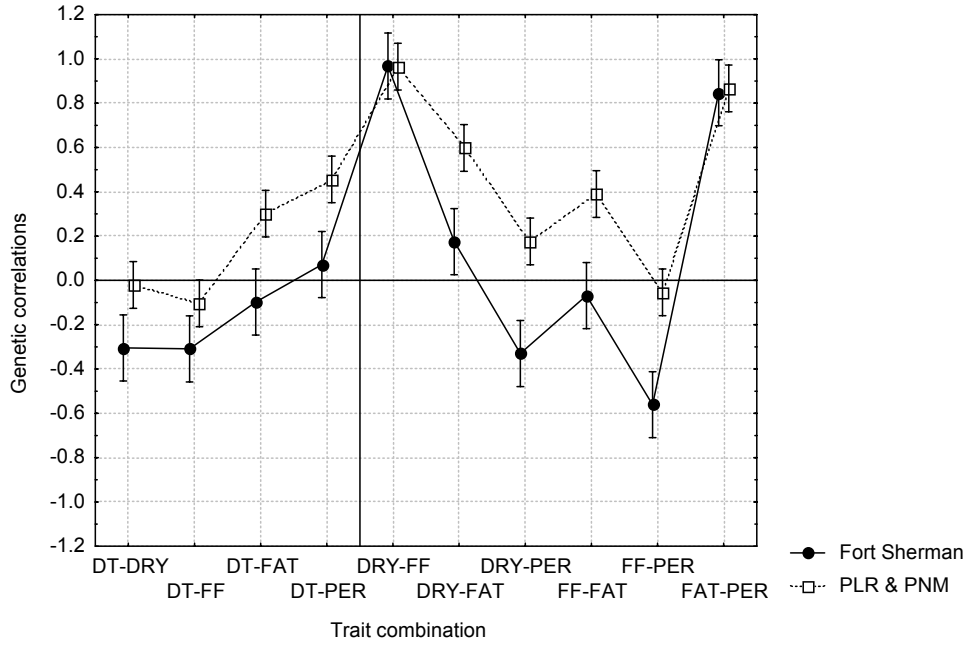


Figure 8: Location specific variation in estimated genetic correlations. Trait combinations are at the x-axis; genetic correlations are at the y-axis. Bars indicate standard errors. DT = development time; DRY = total dry weight; FF = fat-free dry weight; FAT = aft weight; PER = percentage fat relative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

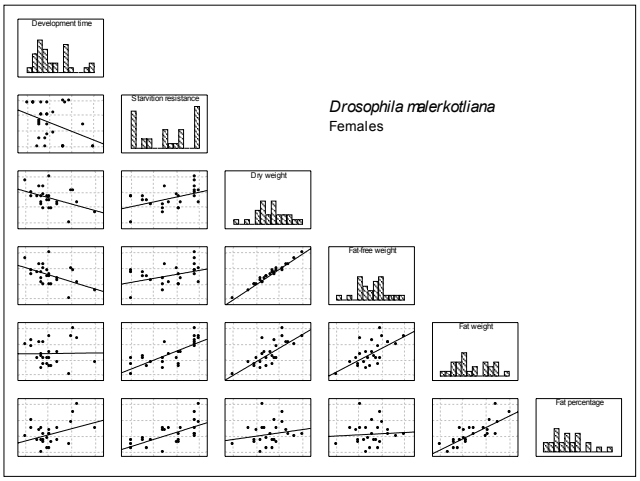
In **chapter 6**, in which I will present a synthesis of the whole thesis, I will briefly present an idea that might shed some light on the underlying genetic mechanism that could explain the results in the variation between and among species and populations. This idea is based on the notion that pleiotropic 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

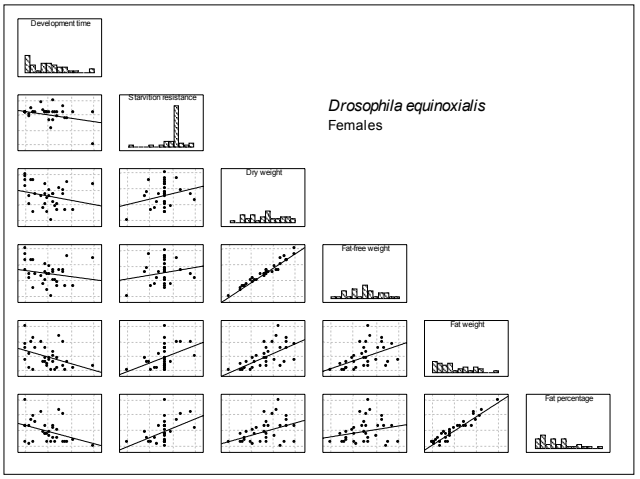
lead to directional selection resulting in changes in the estimated genetic correlations.

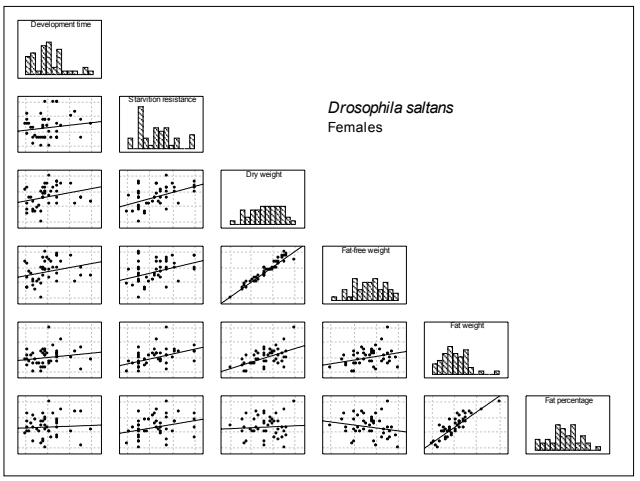
This study showed that genetic correlations between important life-history traits in species of *Drosophila* are unlikely to pose a strong barrier for local adaptation because they are not close to unity. They do however predict the speed at which changes can occur. Overall, these results are in line 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.

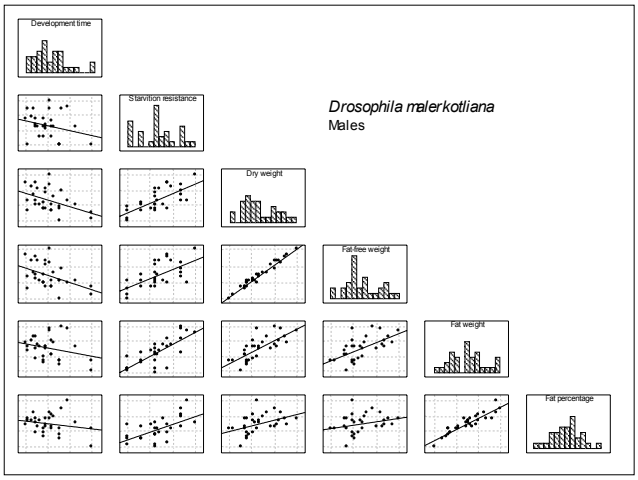
Appendix 1

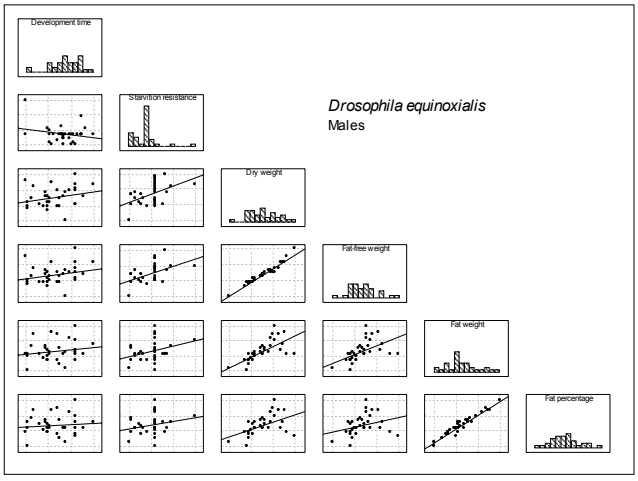
Matrix plots (see next page) for the unweighted data, for each species and sex. Plots on the complete diagonal axis indicate distribution of the values within a trait, while off-diagonal plots are scatterplots between two traits. Data along the x-axis in each scatterplot correspond to the histogram above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

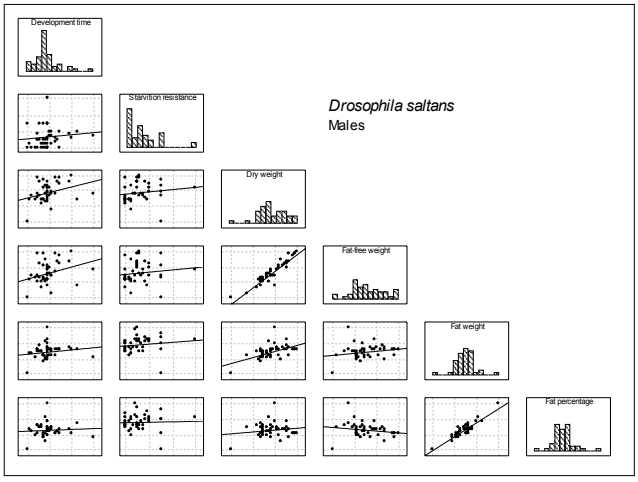

Development time
Starvation resistance
Dry weight
Fat-free weight
Fat weight
Fat percentage
Drosophila melerkotliana
Females


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