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

Abstract:

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

Introduction

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

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

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

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

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

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

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

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

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

Material and Methods

COLLECTION AREA

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

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

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

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

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

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

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

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

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

COLLECTIONS

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

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

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

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

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

LIFE-HISTORY PARAMETERS

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

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

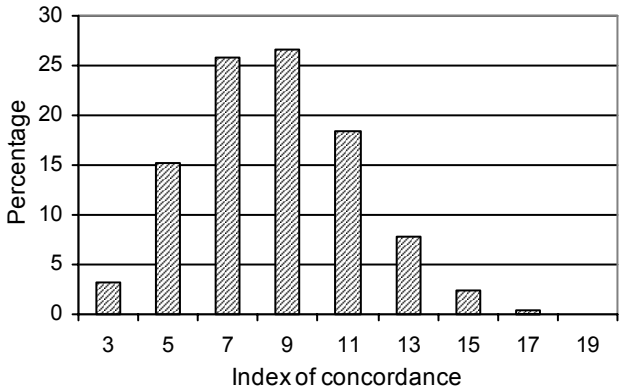

Figure 1: Expected distribution of the overall concordance indices as generated by the randomisation test. Total number of runs was 10.000.

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

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

STATISTICAL ANALYSIS

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

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

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

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

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

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

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

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

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

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

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

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

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

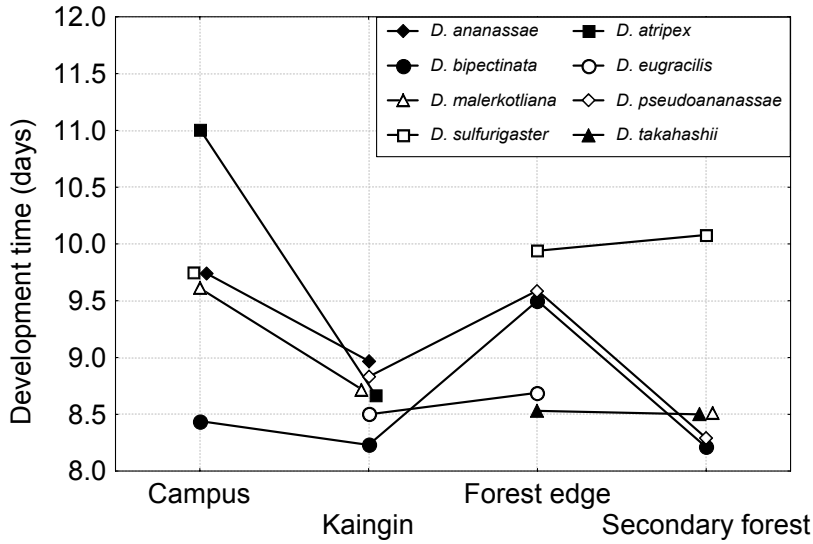

Figure 2: Development time averages (in days) per stock versus habitat. Overlapping points of different species are positioned next to each other to avoid confusion. No error bars are given, see Material and methods.

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

Results

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

VARIATION WITHIN SPECIES

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

SIMILARITY WITHIN TRAITS

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

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

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

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

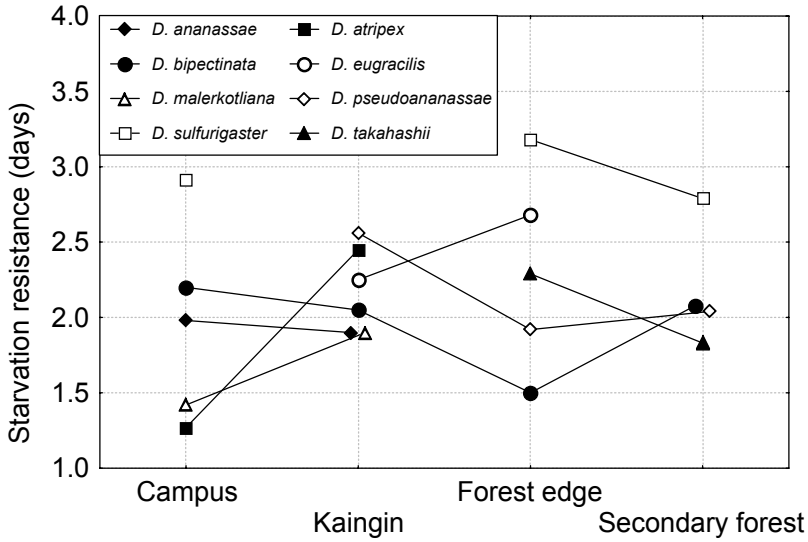

Figure 3: Starvation resistance averages (in days) per stock versus habitat. Overlapping points of different species are positioned next to each other to avoid confusion. No error bars are given, see Material and methods.

HABITAT RANKING - TRAIT COMPARISON

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

COMPARISON BETWEEN TRAITS

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

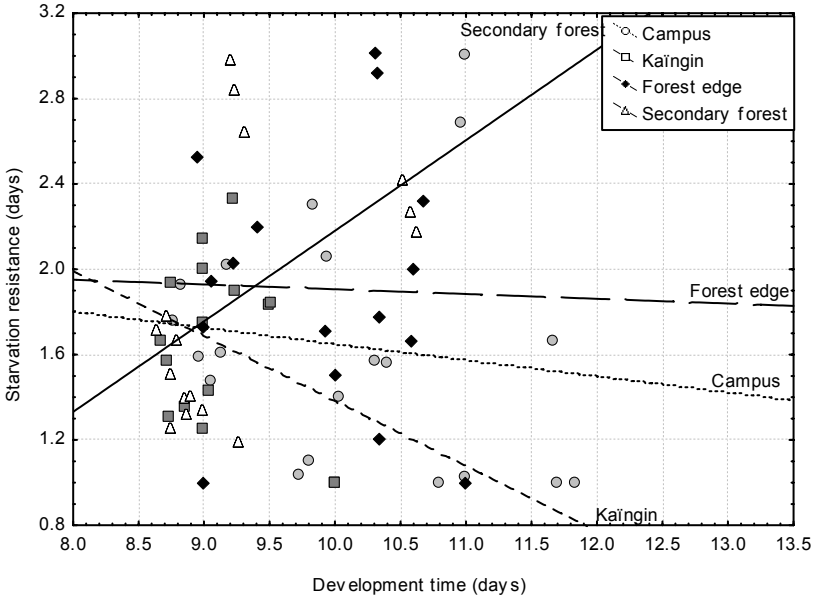

Figure 4: Habitat specific development time - starvation resistance plots, based on vial averages. The different lines represent the correlations between the two traits for the four different habitats, respectively. See text for more details.

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

Conclusion and discussion

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

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

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

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

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

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

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

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

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