

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

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The ecological trade-off between developmental time and starvation resistance, acting in a heterogeneous environment, can promote the coexistence of competing species. Heterogeneity results from variation in the vegetation that influences both abiotic (e.g. temperature, humidity) and biotic (e.g. fruit availability during the year) aspects of the environment. In this study, we investigated whether differences between collection sites have led to local differentiation of the two life-history traits underlying the coexistence model: developmental time and starvation resistance. *Drosophila* were collected from four collection sites, ranging from grassland to secondary forest, along a transect of 15 km. The microclimatic and vegetation differences among these collection sites were considerable. For developmental time, different species showed similar genetic responses to the (habitat) differences between the different collection sites. The shortest developmental times were found in the secondary forest populations and the agricultural area populations, the longest in the grassland populations, and 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 developmental times. Furthermore, the data did not confirm the generality of the positive correlation between developmental time and starvation underlying the coexistence model. © 2006 The Linnean Society of London, Biological Journal of the Linnean Society, 2006, 87, 115–125.

ADDITIONAL KEYWORDS: deforestation – habitat change – intraspecific variation – local selection – spatial heterogeneity.

INTRODUCTION

Sevenster & van Alphen (1993a) developed a coexistence model for fruit-breeding *Drosophila* flies, based on a positive correlation between developmental time and adult life span under starvation. This model also draws on general theoretical studies (Shigesada, Kawasaki & Teramoto, 1979; Shigesada, 1984; Shorrocks *et al.*, 1984; Chesson, 1985; Comins & Noble, 1985; Chesson, 1986; Chesson & Huntly, 1988; Chesson & Huntly, 1989). Fast-developing, short-lived *Drosophila* species are better larval competitors than are slower species (Krijger, Peters & Sevenster, 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, Panama demonstrated a positive correlation between the two traits (Sevenster & van Alphen, 1993b), together with the predicted negative correlation between fruit abundance and prevalent life-history strategy in the community (Sevenster & van Alphen, 1993b; Krijger, 2000).

A change in forest environment often has an impact on the fruit availability during the year (Tabarelli, Mantovani & Peres, 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

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differences in their life-history traits. High starvation resistance facilitates survival during periods of the year when fruit is scarce, but when fruit becomes less scarce during that period, the relative importance of 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; developmental time is now expected to become the sole factor determining the species composition, and a reduction in developmental time due to selection will occur within slower-developing species (Krijger *et al.*, 2001).

Besides changes in the biotic environment, changes in vegetation also lead to changes in the local microclimate. The difference in average air temperature between closed canopy and open vegetation 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 that of the air temperatures as recorded by standard measurement techniques. Vegetation that is more open causes 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 vegetation (Walter, 1984).

Research on large-scale clines has given some insight into the question of whether developmental time responds to climatic variation. James & 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 depended heavily on one population measured at low latitude (A. C. James, pers. comm. in van 't Land, 1997). van 't Land *et al.* (1999) also found a correlation between latitude and developmental time on their *D. melanogaster* cline in South and Central America, but it explained only 0.1% of all the variation. Laboratory-based temperature selection on developmental time has shown that lines adapted to low temperatures have a relatively shorter developmental time compared with those adapted to high temperatures, when measured at the same temperature (Anderson, 1966; Partridge *et al.*, 1994a; Partridge *et al.*, 1994b; James & Partridge, 1995). The latitudinal cline data predict the same pattern as do the temperature selection data, and therefore we would expect opening the canopy (i.e. higher temperatures) to result in longer developmental times.

All studies mentioned by Hoffmann & Harshman (1999) on starvation resistance clines indicate that the

tropical populations of the various *Drosophila* species have better resistance than do the temperate populations (Da Lage, Capy & David, 1990; Shamina, Parkash & Neena, 1993; Parkash & Vandna, 1994; Parkash, Sharma & Sharma, 1994; Karan & Parkash, 1998; Karan *et al.*, 1998). In more recent studies on *D. melanogaster*, Robinson, Zwaan & Partridge (2000) and Hallas, Schiffer & Hoffmann (2002) did not find such a latitudinal cline in either South America or Australia, respectively. Parkash & Munjal (1999) found that for their Indian cline higher starvation tolerance was positively correlated with minimum temperatures, with higher metabolic stress in relation to smaller body size and with higher population density and competition. Thus, we would expect a more open canopy (i.e. higher temperature) to result in higher starvation resistance.

Based on the above, we would expect small-scale variation between the collection sites with regard to vegetation and derived aspects such as microclimate and (patterns in) fruit abundance to be considerable and to select for differences between populations. The persistence of the selection effect would depend on the rate of gene flow counteracting it. We would also expect that the differences between the collection sites would 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 would expect a correlated response between degree habitat ranking [as based on the degree of disturbance (van der Linde & Sevenster, 2002)] and realized life histories.

The general existence of a genetic correlation between developmental time and starvation resistance is still under debate. Charnov & Berrigan (1990) showed that the ratio between developmental period and adult life span appears to be constant at the class or family level. In insects, the picture is more complicated. Eijs & van Alphen (1999) found in parasitic wasps that developmental time was not correlated with life span. Zwaan, Bijlsma & Hoekstra (1995a, 1995b) found in *D. melanogaster* that the longevity of adults was not affected by selection for developmental time. In earlier work on the environmental effects of ageing, a similar conclusion was reached (Zwaan, Bijlsma & Hoekstra, 1991, 1992). On the other hand, Sevenster & van Alphen (1993b) found a positive correlation between larval developmental rate and adult survival in a guild of frugivorous *Drosophila* species from Panama (but see Toda & Kimura, 1997). This result was supported by Chippindale, Chu & Rose (1996) and Harshman, Hoffmann & Clark (1999), who found that lines of *D. melanogaster* selected for higher starvation resistance had a longer developmental time. When this

positive genetic correlation does exist, the two traits would be expected to covary and show the same pattern between species and populations. Furthermore, the intraspecific correlation within each collection site would be expected to be positive.

Few studies have investigated the effects of local selection on a small geographical scale, although the small-scale variation in microclimate, vegetation, and related biotic factors can be considerable (Nevo *et al.*, 1998). Our collection sites, in four different habitats, were located on a transect of about 15 km, thus excluding macroclimatic differences, while the different habitats ensured differences in the microclimate, vegetation, and related biotic factors. Our primary goal was to test whether local adaptation in life-history traits occurs, and we discuss whether this variation relates to differences between the habitats in biotic or abiotic factors. We collected flies from different populations and measured the two traits in the F₃ generation in a common laboratory environment. With this set-up, we could show for the two life-history traits whether genetic differences between the populations were present. More specifically, we drew up four expectations. First, we would expect there to be genetic variation within species between populations from different collection sites. Second, we would expect that, if there is variation, the patterns within the single species would be similar within all species. Third, we would expect the pattern between the collection sites to follow 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 would expect the two overall patterns for developmental time and starvation resistance to be similar, and this positive correlation to be found in all four different collection sites.

MATERIAL AND METHODS

COLLECTION AREA

Frugivorous *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 Ocean and to the west by the Cagayan Valley.

The Sierra-Madre has one of the last remaining large areas of tropical rainforest in the Philippines; it is the largest piece of the mere five percent of tropical rainforest that remains there (Danielsen *et al.*, 1993). These days, the central valley area is either grassland or agricultural fields and planta-

tions containing rice and other commercial crops. Towards the mountains, this changes first to kaingins (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 were ranked from most to least disturbed, and from west to east as follows:

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

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

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

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

COLLECTIONS

The collections were made simultaneously at the four different collection sites. The *Drosophila* were collected with oviposition traps. Four traps were placed in each of the four different collection sites with at least 200 m between consecutive traps. The traps were constructed out of 500-mL transparent containers suspended from a thin nylon cord of about 1 m. A hole of diameter 2.5 cm, covered with 1.5-mm mesh, was positioned on one side of the trap. The hole faced slightly downwards to prevent rain from entering. The mesh allowed *Drosophila* access to the bait inside for oviposition, but prevented larger animals from entering. A ‘Manila’ banana was used as bait.

The traps were exposed in the field for 1 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% relative humidity and 13 : 11 light : dark, roughly corresponding with the natural microclimate. The long-term (1994–1998) macroclimatic temperature average for Tuguegarao was 26.8 °C (PAGASA, Tuguegarao, Cagayan, unpubl. data, see also PAGASA, 2001), and this site is comparable with the Campus collection site, while the higher canopy cover in the

Table 1. Population averages for all species and populations

Species	Campus		Kaingin		Forest edge		Secondary forest	
	DT	SR	DT	SR	DT	SR	DT	SR
<i>Drosophila ananassae</i> Doleschall	9.74	1.98	8.97	1.90				
<i>D. atripex</i> Bock & Wheeler	11.01	1.27	8.67	2.45				
<i>D. barbarea</i> Bock & Wheeler					10.01	2.27		
<i>D. bicornuta</i> Bock & Wheeler	10.83	2.50						
<i>D. bipectinata</i> Duda	8.44	2.20	8.23	2.05	9.50	1.50	8.21	2.08
<i>D. eugracilis</i> Bock & Wheeler			8.50	2.25	8.69	2.68		
<i>D. malerkotliana pallens</i> Bock & Wheeler	9.61	1.42	8.72	1.90			8.51	1.84
<i>D. species 1*</i>							8.74	3.34
<i>D. parabiptectinata</i> Bock	8.53	2.28						
<i>D. pseudoananassae pseudoananassae</i> Bock			8.83	2.56	9.59	1.92	8.29	2.04
<i>D. sulfurigaster albostrigata</i> Wheeler	9.75	2.91			9.94	3.18	10.08	2.79
<i>D. takahashii</i> Sturtevant					8.53	2.29	8.5	1.83

For a species overview of the Philippines, see Baltazar (1991) and <http://www.kimvdlinde.com/professional/biology/drosophila/philippines/> for an updated checklist.

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

DT, developmental time in days; SR, starvation resistance in days. All rearing was at 25 °C.

other collection sites should 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 collection site 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 than ten lines. In total, 25 stocks belonging to 12 species were established (Table 1).

The available fruits differed 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 generally accepted breeding substrate for many fruit-breeding *Drosophila* species, contrary to standard breeding media (J. G. Sevenster, C. L. Krijger, K. van der Linde, E. Baldal, unpubl. data). The use of one standard substrate made comparison between populations possible, avoided 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 40 F_2 flies were put on a fresh slice of banana dipped in yeast suspension, which was on a layer of moist vermiculite. Some species lay eggs on, and most species pupate in, vermiculite. In some

insect species, stored mature eggs start developing before laying, thus decreasing the measured developmental time; therefore, to prevent stowage of eggs, the flies were put on a slice of fresh banana dipped in a yeast suspension for 2 days. For the actual experiment, the flies were allowed to lay eggs for 1 h (14 : 00–15 : 00 h) in order to synchronize the egg laying. Furthermore, this time window eliminated the potential impact of time-of-day-specific egg laying preferences between populations (Dahlgaard, Hasson & Loeschcke, 2001). The newly emerged offspring (F_3) were collected once a day at 14 : 00 h. The time of day was chosen based on the observation that emerging flies show clear diurnal rhythms (Pavan, Dobzhansky & Burla, 1950; Bakker & Nelissen, 1963; Belcher & Brett, 1973); most individuals emerge during early morning, in the first hours after sunrise. The collection of flies at several times in the day did not improve the accuracy of the developmental time measurements in a previous experiment (K. van der Linde, unpubl. data), probably due to these diurnal rhythms.

Developmental time was measured as the time from oviposition until adult eclosion. Starvation time was measured as the length of time that freshly emerged adults lived after eclosion from the pupae under the availability of water but no food (see Sevenster & van Alphen, 1993b). The newly emerged adults were transferred, in batches of no more than ten 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-h period, either between two subsequent collections of the emerged flies or two subsequent counts of the deceased flies, introduced a bias as the flies emerged and died during the whole 24-h period. Taking the midpoint between two observations would not give a more accurate estimate and the bias was the same for all species, so the data were not corrected in any way.

STATISTICAL ANALYSIS

We calculated average developmental and starvation times for each species and replicate. Stock averages were calculated from these three replicate averages to eliminate sample size effects. Therefore, standard deviations could not be estimated. The stock averages were used to test our last three predictions, while the individual data were used to test our first prediction. The possible influence of density on the life-history traits was tested with linear regression analyses.

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 developmental 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.

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

The second question, that patterns within different species would be similar, implied no a-priori order in the collection sites. Therefore, we used an index to test for overall concordance of the within-species patterns for the different species. Our concordance index first counted the number of times a value was highest in each of the two collection sites and then took the absolute value of the subtraction of these two values. The higher this concordance value, the more similar the species reacted. An uneven number of species within a two-collection site comparison resulted in a minimum value of one. With four collection sites, this resulted in six two-collection site comparisons, which were com-

binated to one single value for overall concordance. The second step was to randomize the available populations within each species separately. The concordance index for the randomized combination was calculated and repeated 10 000 times. A theoretical distribution of concordance indices was created from the calculated values. Due to three (out of the six) two-collection site comparisons with odd numbers of species, the minimum value for our datasets was 3 and the values ranged between 3 and 19 (with steps of two), with 317, 1512, 2589, 2665, 1846, 790, 231, 47 and 3 hits, respectively (Fig. 1). The fraction of the 10 000 runs that had the same value as the original value or larger, indicated the probability of finding that value. The one-sided critical (5%) value of the overall concordance index was 15 ($P = 0.0281$).

For the third question, the index should accurately indicate the overall matching between an overall pattern and 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 randomized. 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 steps of two), 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 and 0 hits, respectively, out of 10 000 runs. A result was significant with a score equal to or larger/smaller than ± 16 (two-sided, $P = 0.0497$) or ± 14 (one-sided, $P = 0.04695$).

The two traits would be expected to covary in response to local selection if the positive correlation between the two traits were present as predicted. In

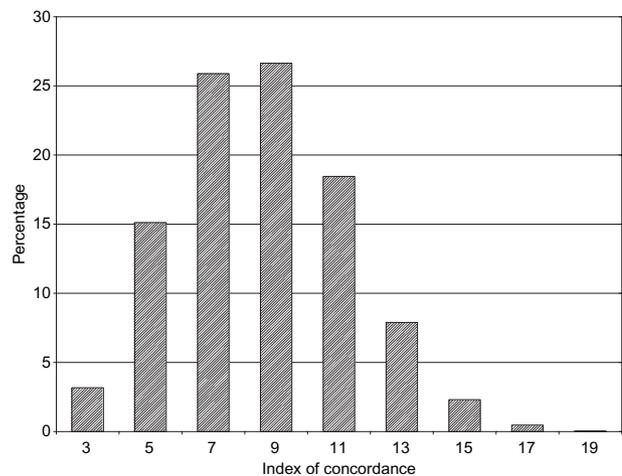


Figure 1. Expected distribution of the overall concordance indices as generated by the randomization test. Total number of runs was 10 000.

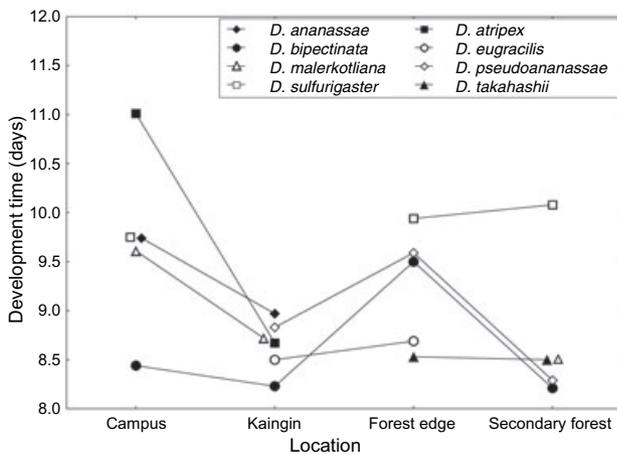


Figure 2. Developmental time averages (in days) per stock vs. habitat. No error bars are given (see explanation in Material and Methods: Statistical analysis).

that case, the two patterns of the developmental time (Fig. 2) and starvation resistance should be similar or completely opposite. We again used an index with randomization to test this hypothesis. For the index, each time we compared two populations within a species, and scored whether or not both traits showed both a simultaneous increase or a simultaneous 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 four species with one comparison (two populations), three species with three comparisons (three populations) and one species with six comparisons (four populations). The theoretical distribution was generated running the model 10 000 times, randomizing 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 and 0 hits, respectively. The patterns of the two traits were expected to be similar and a one-sided significant result was obtained with a test value equal to or larger than 14 ($P = 0.0471$). When the predicted positive interspecific correlation was present, correlations between the two traits across species within collection sites were expected to be significantly positive.

RESULTS

Before we could test whether there was genetic variation between the populations of different collection sites, we needed to verify whether density effects played a role in the data. Both the correlation between developmental time (residuals within species to correct for species effects) and sample size ($r = 0.09$,

$P = 0.49$), as well as the correlation between starvation resistance residuals and sample size ($r = 0.177$, $P = 0.19$), were not significant.

VARIATION WITHIN SPECIES

The average developmental 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 and 3.18 days (Table 1). For developmental 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 developmental time was higher than the expected type 1 errors using a binomial test ($P = 1.54 \times 10^{-5}$), but lower than expected for the starvation resistance ($P = 0.33$). Replicate was nested within collection site, and showed a significant effect in five and six out of eight species for developmental time and starvation resistance, respectively. Based on this, we concluded that genetic differentiation is present between populations for developmental time, but not for starvation resistance. Consequently, starvation resistance was not tested further.

SIMILARITY WITHIN TRAITS

The combined measure of concordance for the developmental times was 15, thus falling within the 5% probability level of the random model. This result supports our hypothesis that differences between collection sites would 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 kaingins in particular supported fast-developing populations, while the slowest populations were found in the grasslands (Campus site). The forest edge showed intermediate values. This figure also clearly shows that there was no correlation between the ranking of the developmental times within all species separately and the ranking of the habitats based on disturbance and canopy cover.

Most species belonged to the subgenus *Sophophora*, with only one species in the subgenus *Drosophila*. *D. 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 randomization test was applied again for only the *Sophophora* subgenus, the observed overall pattern became much stronger. The minimum value in this distribution was four (four comparisons with odd numbers) and the maximum was 16 (with steps of two), with 873, 2660, 3255, 2157, 861, 182 and 12, respectively. The overall concordance index for this dataset was 16 and was significant ($P = 0.0012$). This

Table 2. *F*-values and *P*-values for the interpopulation variation for intercept, habitat, and replicate nested in habitat

Species	Intercept	Habitat	Replicate (habitat)
Developmental time (days)			
<i>Drosophila ananassae</i>	$F_{1,429} = 5510.56$ $P < 0.0001$	$F_{1,429} = 13.03$ $P = 0.0056$	$F_{4,429} = 3.4$ $P = 0.0094$
<i>D. atripex</i>	$F_{1,85} = 2210.36$ $P < 0.0001$	$F_{1,85} = 28.38$ $P = 0.0092$	$F_{4,85} = 2.33$ $P = 0.0623$
<i>D. bipectinata</i>	$F_{1,175} = 5608.22$ $P = 0$	$F_{3,175} = 3.5$ $P = 0.0433$	$F_{7,175} = 0.84$ $P = 0.5547$
<i>D. eugracilis</i>	$F_{1,96} = 5938.14$ $P = 0.0002$	$F_{1,96} = 0.53$ $P = 0.5426$	$F_{2,96} = 4.28$ $P = 0.0166$
<i>D. malerkotliana</i>	$F_{1,133} = 3536.25$ $P < 0.0001$	$F_{2,133} = 7.46$ $P = 0.016$	$F_{6,133} = 2.33$ $P = 0.0358$
<i>D. pseudoananassae</i>	$F_{1,247} = 24502.42$ $P = 0$	$F_{2,247} = 30.15$ $P = 0.0004$	$F_{6,247} = 1.52$ $P = 0.1735$
<i>D. sulfurigaster</i>	$F_{1,762} = 7945.85$ $P < 0.0001$	$F_{2,762} = 0.58$ $P = 0.5879$	$F_{6,762} = 16.75$ $P = 0$
<i>D. takahashii</i>	$F_{1,103} = 93992.02$ $P < 0.0001$	$F_{1,103} = 0.2$ $P = 0.6872$	$F_{1,103} = 3.99$ $P = 0.0484$
Starvation resistance (days)			
<i>D. ananassae</i>	$F_{1,429} = 416.58$ $P < 0.0001$	$F_{1,429} = 3.36$ $P = 0.0868$	$F_{4,429} = 1.82$ $P = 0.1232$
<i>D. atripex</i>	$F_{1,85} = 1532.3$ $P < 0.0001$	$F_{1,85} = 164.06$ $P = 0.0005$	$F_{4,85} = 2.59$ $P = 0.0423$
<i>D. bipectinata</i>	$F_{1,175} = 149.19$ $P < 0.0001$	$F_{3,175} = 0.47$ $P = 0.7134$	$F_{7,175} = 4.27$ $P = 0.0002$
<i>D. eugracilis</i>	$F_{1,96} = 185.15$ $P = 0.0058$	$F_{1,96} = 2.3$ $P = 0.2698$	$F_{2,96} = 6.37$ $P = 0.0025$
<i>D. malerkotliana</i>	$F_{1,133} = 452.89$ $P < 0.0001$	$F_{2,133} = 4.14$ $P = 0.0535$	$F_{6,133} = 1.35$ $P = 0.2393$
<i>D. pseudoananassae</i>	$F_{1,247} = 256.37$ $P < 0.0001$	$F_{2,247} = 2.97$ $P = 0.1243$	$F_{6,247} = 7.02$ $P < 0.0001$
<i>D. sulfurigaster</i>	$F_{1,762} = 524.81$ $P < 0.0001$	$F_{2,762} = 2.17$ $P = 0.1948$	$F_{6,762} = 16.39$ $P = 0$
<i>D. takahashii</i>	$F_{1,103} = 224.39$ $P = 0.0041$	$F_{1,103} = 4.61$ $P = 0.1447$	$F_{1,103} = 4.88$ $P = 0.0293$

Bold values indicate significant results.

leads to the conclusion that, for developmental time, all but one of the species respond in a way similar to the differences between the collection sites.

HABITAT RANKING–TRAIT COMPARISON

The score for the habitat rank–developmental time comparison was minus eight and non-significant ($P = 0.20$). Excluding *D. sulfurigaster* increased the value for the habitat rank–developmental time comparison to -12 but remained not significant ($P = 0.0673$). This leads to the conclusion that the factor that shapes developmental times is not correlated with any aspect related to habitat ranking such as temperature or humidity (Fig. 1).

INTERSPECIFIC CORRELATIONS BETWEEN TRAITS

The interspecific correlations across species between developmental time and starvation resistance, based on vial averages, varied with collection site (Fig. 3). None of the correlations was significant, and only one was positive (Secondary forest: starvation resistance (SR) = $-2.064 + 0.424 \times$ developmental time (DT), $R^2 = 0.24$, $P = 0.054$), while the others were negative (Forest edge: SR = $2.13 - 0.022 \times$ DT, $R^2 < 0.01$, $P = 0.92$; Kaingin: SR = $4.428 - 0.305 \times$ DT, $R^2 = 0.11$, $P = 0.22$; Campus: SR = $2.406 - 0.076 \times$ DT, $R^2 = 0.018$, $P = 0.58$). The results do not confirm the generality of the positive interspecific correlation underlying the coexistence model of Sevenster & van Alphen (1993a, b).

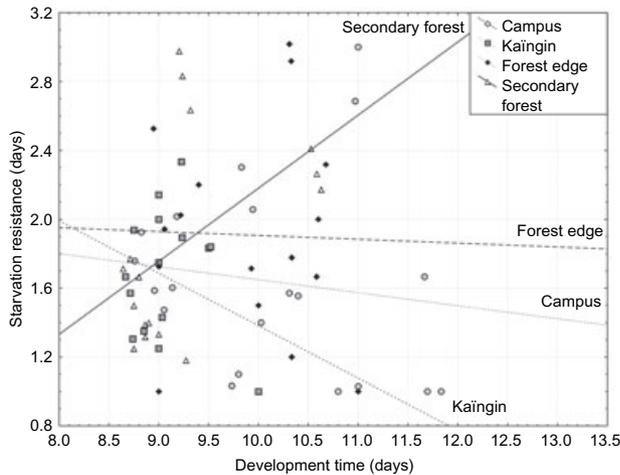


Figure 3. Habitat-specific developmental time (days) vs. starvation resistance (days) plots, based on vial averages. See text for details.

CONCLUSION AND DISCUSSION

We found that for developmental time, five out of eight species had significant differences between the populations, indicating that genetic variation for this trait is present in those species (Table 2). The developmental 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 developmental 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 developmental 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).

Drosophila sulfurigaster belongs to the subgenus *Drosophila*, while the other species belong to the *Sophophora* subgenus (Grimaldi, 1990; Baltazar, 1991). These subgenera diverged from each other long ago (Beverly & Wilson, 1984), while the species of the *Sophophora* subgenus speciated much more recently (Grimaldi, 1990). Therefore, lineage-specific effects due to the early separation of the two subgenera may explain why *D. sulfurigaster* showed a different response from that of 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). Random

sampling of a limited number of individuals can explain such a result; however, most stocks were established using at least ten gravid females. Furthermore, the observed significant differences in developmental time for five out of the eight species as well as the highly consistent pattern within the developmental times suggests that the sample sizes were sufficiently large to detect genetic differences between populations. Another issue is that we scored the dead flies only once a day. We chose this method based on unpublished results of a previous experiment, in which we scored dead flies three times a day. In that experiment, combining the three different scores for each day had little effect on the mean. Although the variances of the combined scores differed, there was not a consistent increase or decrease in variance. This might have influenced the statistical tests in which we tested for differences between the populations, but not the regressions with the developmental times as those were based on averages.

Which environmental factor can explain the consistent differences between the collection sites as observed for developmental times? The habitat ranking–trait comparison was not significant, thus excluding factors 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 developmental times. We were not able to test whether fruit abundance through the year was related to the realized life-history values, as measuring the differences in fruit availability requires year-long sampling to obtain a proper estimate due to habitat-specific differences (Sevenster & van Alphen, 1993b; Krijger, 2000). The use of banana as the breeding substrate could have resulted in the systematic difference between the collection sites if local adaptation was driven by variation in the natural available breeding substrates, and this option cannot be excluded. However, this does not contradict the conclusion that local adaptation within developmental 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 habitats with regard to *Drosophila* diversity. The various biodiversity indices 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 intermediately disturbed habitats (van der Linde & Sevenster, 2002), which was 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 developmental times within species are of a different nature.

Three of the four collection sites were just next to each other, forming a continuous transect of about 2 km. Several studies, both in tropical and temperate *Drosophila* species, indicate that daily travel distances of up to 100 m are possible (Burla *et al.*, 1950; Taylor *et al.*, 1984; van Konijnenburg, 1999). Compared with our transect length, it suggests that either habitat differences or related aspects (e.g. microclimate) between the collection sites form effective barriers for migration, or that there was severe selection against flies migrating to another habitat. Harry *et al.* (1999) demonstrated that populations could differ significantly even over such small distances. In two communities just 100 m from each other on south and north slopes of Evolution Canyon, they found a complex pattern of differences in the taxonomic, genetic, morphological, and behavioural levels of biodiversity. This is consistent with our finding of very large differences in community composition between closely located communities from different habitats (van der Linde & Sevenster, 2002). In contrast, Panhuis, Swanson & Nunney (2003) found no differentiation in accessory gland proteins and sexual behaviour in *D. melanogaster* from the same two communities in Evolution Canyon. However, that study was limited to one single species and two aspects, which makes it difficult to generalize to the whole community or other traits. As our pattern in developmental time was consistent for all but one species, it is more likely that the pattern we found was real.

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 collection sites was positive, but not significant, while the remaining correlations were all negative and not significant. This result casts doubt about the generality of the expected positive correlation. Fischer, Zwaan & Brakefield (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 developmental times in this study was between 8.2 and 11.0 days, which is much narrower than within the Panamanian *Drosophila* community (7.8–15.4 days, Sevenster & van Alphen, 1993b). When the Panamanian dataset is limited to the same range as the dataset 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 developmental 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 has a similar influence on the developmental 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 collection site. 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 developmental 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.

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