Do Species Converge during Adaptation? A Case Study in *Drosophila*

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ABSTRACT

Adaptation to novel environments is a crucial theme in evolutionary biology, particularly because *ex situ* conservation forces populations to adapt to captivity. Here we analyze the evolution of life-history traits in two closely related species, *Drosophila subobscura* Collin and *Drosophila madeirensis* Monclús, during adaptation to the laboratory. *Drosophila madeirensis*, an endemic species from Madeira, is here shown to have less ability to adapt to the laboratory. Early fecundity was the only trait where this species showed a significant improvement with time. By comparison, *D. subobscura* improved in most traits, and its early fecundity increased faster than that of *D. madeirensis*. Our findings suggest that different species, even closely related ones, may adapt at different rates to the same environment.

Introduction

Many studies of experimental evolution in *Drosophila* have focused on adaptive divergence in response to a diversity of selection regimes starting from common ancestral populations (for reviews, see Rose et al. 1996; Prasad and Joshi 2003; Chippindale 2006). Adaptive convergence has also been studied using experimental evolution; the typical experimental strategy is to follow the evolutionary trajectories of initially divergent populations undergoing selection in a common environment (e.g., Matos et al. 2000, 2002, 2004; Teotónio and Rose 2000; Teotónio et al. 2002).

In general, studies of convergence in single species suggest that the functional characters of initially evolutionarily differentiated populations usually converge when they are maintained in a common environment for many generations. However, whether or not convergence occurs depends on the trait analyzed and on the previous history of selection (Teotónio et al. 2002). The rate of convergence varies as a function of the initial differentiation between the populations (e.g., Teotónio and Rose 2000; Matos et al. 2002; Simões et al. 2007). It is possible that different genetic backgrounds might eventually lead to divergence between populations adapting to similar environments (Cohan 1984*a*, 1984*b*), particularly in different species (Cohan and Hoffmann 1989). What happens when populations of different species come together in a similar environment? Will they converge, adapting in the same manner to similar conditions, or will they evolve toward different adaptive peaks, as a consequence of different genetic backgrounds?

Understanding the evolutionary changes involved in adaptation to controlled environments is particularly important for conservation efforts, especially when captive breeding is involved. Captive breeding is essential for the conservation of many species (Frankham et al. 1986; Ralls and Ballou 1986; Soulé et al. 1986; Tudge 1995; Frankham 2002). Captivity over multiple generations involves evolutionary changes, and some of these changes can be detrimental to reintroduction (Woodworth et al. 2002). For example, the genetic changes that maximize fitness in captivity could be deleterious in the native environments (Frankham et al. 1986; Frankham 2002). Inbreeding, accumulation of deleterious mutations, and loss of genetic variation are other types of genetic problems that can arise among captive breeding populations (Frankham 2002; reviewed in Frankham 2005*a*). A common approach to studying the problems associated with adaptation to captivity has been to study model organisms like *Drosophila* species rather than endangered species themselves (e.g., Frankham 1995; Woodworth et al. 2002; Gilligan and Frankham 2003).

Here we study the laboratory evolution of populations of two *Drosophila* species derived from collections in the wild. *Drosophila madeirensis* Monclús is an endemic species from Madeira Island. *Drosophila subobscura* Collin, its close relative, is a species with a much wider distribution. Both species coexist in sympatry on Madeira Island, despite being morphologically very similar (Monclús 1984). The estimated time of divergence for this species pair is 0.6–1 million years (Ramos-Onsins et al. 1998). Their reproductive isolation is incomplete, as it is possible to obtain viable and fertile hybrids, especially when *D. madeirensis* is the mother species (Khadem and Krimbas 1991, 1993; Papaceit et al. 1991; Rego et al. 2006).

Drosophila madeirensis is associated with a particular type of habitat, the Laurisilva forest, considered a relic of the subtropical

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Table 1: Differences between species tested by nested ANOVA (with replicates nested within species)

Generation	a1r	F1–7	F8–12	RM	RF
7	.039[a]	4.15[a]	1.16[a]	8.401*	3.392[a]
14	1.685[a]	13.24*	32.971**		
23	15.888*	68.47**	49.248**		
43	15.069*	254.793***	2246.344***	17.902*	17.668*

Note. The $F_{1,4}$ values and significance level are presented. a1r = age of first reproduction; F1–7 = early fecundity; F8–12 = peak fecundity; RM = male starvation resistance; RF = female starvation resistance.

[a] Not significant.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

forests that in the Tertiary era covered the Circum-Mediterranean area. Madeiran Laurisilva has recently been inscribed in the World Heritage List (IUCN 1999) due to its outstanding natural value as the largest surviving area of this type of ecosystem. However, its distribution is decreasing drastically as a result of human activity, a fact that is endangering many animal and plant species, particularly species that are endemic, like *D. madeirensis*.

By bringing populations of both of these *Drosophila* species into a new common environment with controlled laboratory conditions, we seek to answer the following questions: How differentiated are these species when reared in a common environment, and is this differentiation maintained during laboratory evolution, or do *D. subobscura* and *D. madeirensis* converge or diverge during adaptation to captivity?

Material and Methods

Populations

In April 2001, laboratory populations of *Drosophila madeirensis* and *Drosophila subobscura* were established using as founders wild individuals collected in a patch of Laurisilva forest near Ribeiro Frio, Madeira. Since their foundation, these populations were maintained in controlled conditions of 18°C with a photoperiod of 12L : 12D. The maintenance regime involved discrete generations of 30 d, with controlled adult and larval densities (50 adults/vial, 70–90 eggs/vial). The average number of individuals per generation was 1,000, never dropping below 400 for each population (for more maintenance details see Matos et al. 2000, 2002). When the populations were in their third generation of laboratory culture, they were split into three replicate populations for each species (from here on called m1, m2, and m3 for *D. madeirensis* and s1, s2, and s3 for *D. subobscura*).

Assays

Fecundity assays were carried out in the following manner: in each of the generations, analyzed mating pairs were formed using virgin individuals from each replicate population, and the sample sizes varied from 12 to 24 pairs. Pairing was done with CO_2 anesthesia during the first 6 h after adult eclosion to guarantee that the individuals were virgin at the time of sample formation. The daily fecundity of each mating pair was recorded over 12 d. In some generations, starvation resistance was also analyzed. To do this, after the fecundity assay, the mating pairs were transferred to vials containing plain agar, and the time of death was estimated using observations every 6 h. Fecundity assays were carried out on generations 7, 14, 23, and 43. Generations 7 and 43 also included starvation resistance assays. At generation 43, the Madeiran populations of *D. madeirensis* and *D. subobscura* were assayed in synchrony with three sets of threefold replicated continental populations of *D. subobscura*. One set of populations, derived from a foundation in Sintra in 2001, is called TW. Another set was founded simultaneously from a collection in Arrábida and is called AR. The third set of populations also derived from Sintra from an earlier foundation in 1990 and is called NB. By the time this assay was done, the TW and AR continental populations had undergone 40 generations in the laboratory while the NB populations were in their 176th generation of laboratory culture (see details in Matos et al. 2004).

The traits analyzed were age of first reproduction (a1r; number of days before the first egg laying), early fecundity (F1–7; number of eggs laid in the first 7 d), peak fecundity (F8–12; number of eggs laid in the last 5 d of the study), and starvation resistance (RF and RM, for females and males, respectively; number of hours an individual resisted without food). Daily fecundity, the number of eggs laid by the females in each day, was also analyzed.

Statistical Analysis

For each assayed generation, a two-way nested ANOVA was performed to test whether the differences between species were significant, with replicate populations (random effect) nested within species. This analysis was done separately for each trait assayed.

Daily Fecundities. For each assay, daily fecundities over multiple days were analyzed by plotting the mean daily fecundity values against the age of the females and estimating the best regression model for each replicate population of both species. Two types of regression models were estimated: linear and second-degree polynomial. The best-fit model was chosen according to the Akaike Information Criterion (AIC; i.e., Bieri and Kawecki 2003). When a linear model was the best-fit model for all replicate populations of each species, *t*-tests were applied to test whether the trend was significant. These *t*-tests were done on the average slopes for each species using the variation of slopes between replicates as the sample variation. The same criterion was applied when comparing the two species.

The mean daily fecundities of both species were also compared with *t*-tests performed for each day. Significance levels were adjusted with a sequential Bonferroni technique using available software (Rice 1989).

Starvation Resistance. At generations 7 and 43, *t*-tests were used to compare starvation resistance between species, using the heterogeneity between replicates as the source of error. We also did a two-way ANOVA with species (fixed) and assayed generation (random) as factors, testing for overall differences between species, differences between assayed generations, and changes between species across the generations assayed.

Comparison with Continental Populations. At generation 43, Madeiran populations were compared with continental populations of *D. subobscura* during a synchronous assay. *Drosophila madeirensis* was compared with each replicated set of *D. subobscura* (Madeira, Sintra [TW and NB], and Arrábida [AR]) populations using *t*-tests on the mean values for all traits assayed. The same procedure was applied when comparing *D. subobscura* (Madeira) with continental populations. *P* values were adjusted by a sequential Bonferroni method (Rice 1989). Specifically, for comparisons between *D. madeirensis* and *D. subobscura* from each foundation, *P* values were adjusted considering the use of four tests, and for comparisons between *D. subobscura* from Madeira and the continent, *P* values were adjusted considering the use of three tests. In all cases, the estimated error was based on the heterogeneity between replicates within each set of populations. *Drosophila madeirensis* was also compared with all *D. subobscura* populations founded at a similar time (*D. subobscura* [Madeira], TW, and AR), using the average of the three mean values estimated for each trait and set of populations. In these tests, the variance for *D. subobscura* was estimated as the heterogeneity between different foundations (differences between the means of the three sets of populations independently founded).

Evolutionary Trajectories. Type I least squares linear regressions were carried out to analyze the evolutionary trajectories of each species, with the mean values of each trait as the dependent variable and generation as the independent variable (Sokal and Rohlf 1995). Regression models were obtained independently for each of the three replicate populations of each species. To evaluate whether there was a consistent, directional, linear change over evolutionary time, a *t*-test was performed on the

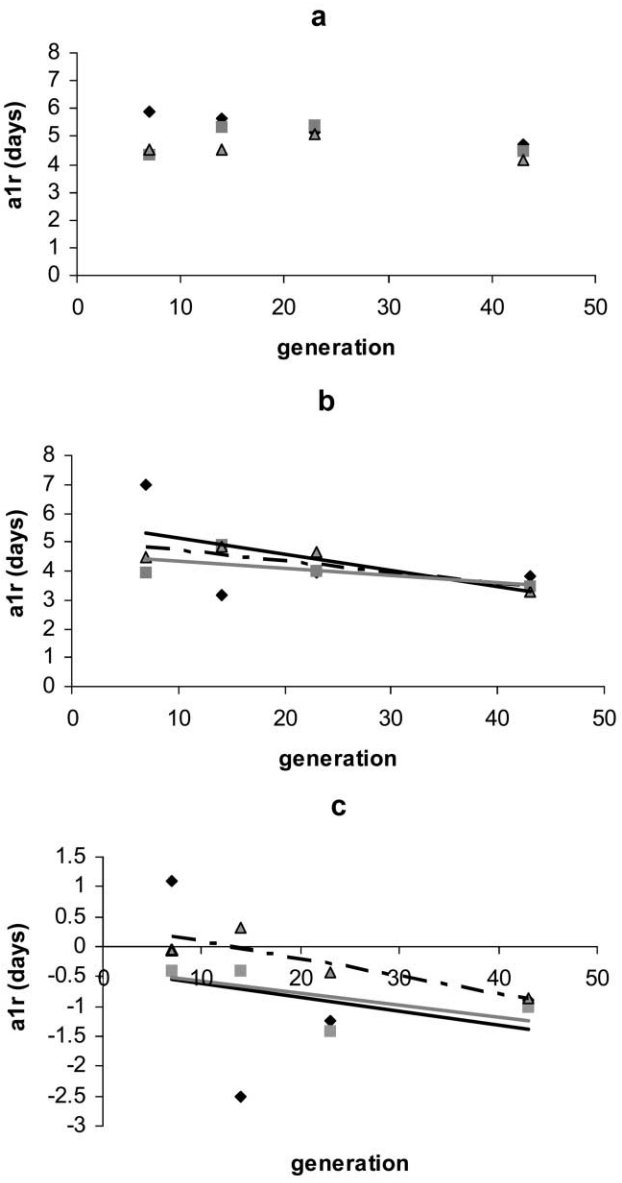

Figure 1. Evolutionary trajectories for age of first reproduction. Plots of means of age of first reproduction (a1r) as a function of generation number for *Drosophila madeirensis* (*a*), for *Drosophila subobscura* (*b*), and for differences between them (*D. subobscura* − *D. madeirensis*; *c*). Data points show the mean values of replicate populations of each species. Significant linear trends (presented) were obtained for *D. subobscura* ($P < 0.05$; *b*) and for the differences between the two species ($P < 0.01$; *c*). *Black line*, *diamonds*, replicate 1; *gray line*, *squares*, replicate 2; *broken black line*, *triangles*, replicate 3.

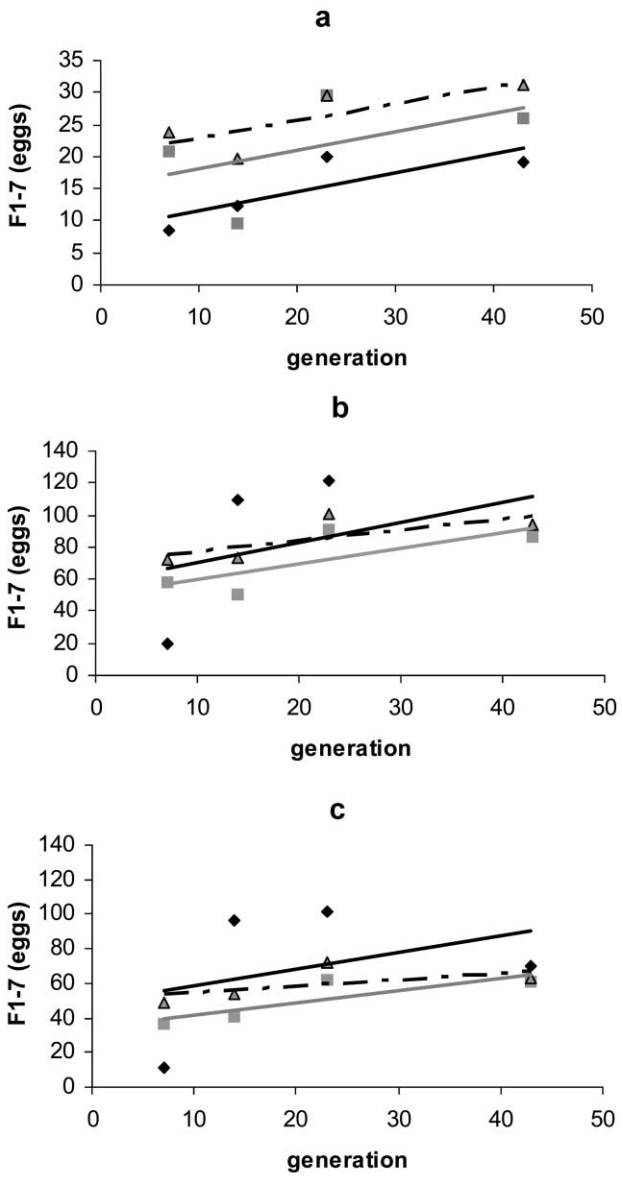

Figure 2. Evolutionary trajectories for early fecundity. Plots of means of early fecundity (F1–7) as a function of generation number for *Drosophila madeirensis* (*a*), for *Drosophila subobscura* (*b*), and for differences between them (*D. subobscura* − *D. madeirensis*; *c*). Data points show the mean values of replicate populations of each species. Significant linear trends (presented) were obtained for *D. madeirensis* ($P < 0.001$; *a*), for *D. subobscura* ($P < 0.05$; *b*), and for the differences between the two species ($P < 0.05$; *c*). *Black line*, *diamonds*, replicate 1; *gray line*, *squares*, replicate 2; *broken black line*, *triangles*, replicate 3.

average slope among the populations of each species, using the variation of slopes as sample variation.

The same procedure was applied to the differences between pairs of replicates from each species. Least squares linear regressions were done using as data points the differences between same numbered replicate populations from each species (e.g., s1-m1) to test for temporal variation in the differences between them. Finally, an ANCOVA was used to test for homogeneity of slopes between the two species, with species as a factor with two categories (*D. madeirensis* and *D. subobscura*) and generation as the covariate.

Temporal change in the patterns of daily fecundity was also analyzed. The slopes of the linear regressions of daily fecundity obtained for each replicate population were plotted against generation number. A linear regression was estimated to see whether there was a trend in the temporal change of this trait, the significance of this trend being estimated by a *t*-test. A similar procedure was used to test for temporal changes in the differences between slopes of the two species. All statistical analysis was done using STATISTICA and EXCEL.

Results

Fecundity Traits

The results of the nested ANOVA indicate that in general the species differ significantly in all fecundity-related traits in all generations. In the cases where the differences were not significant, this was mostly due to a higher heterogeneity between replicates, which was common in the earlier generations, particularly generation 7 (Table 1). The same may explain the lack of significant differences for age of first reproduction at generation 14. In all instances, *Drosophila subobscura* had higher fecundity and quicker maturation time relative to *Drosophila madeirensis* (Figs. 1–3).

Daily Fecundities

Figure 4 presents a plot of daily fecundities against age for each species and generation. The change of daily fecundity with age differs between the species. *Drosophila madeirensis* presents the same pattern in all assayed generations, with an initial stage without laying eggs followed by a steady increase in fecundity. In all generations the best-fit model for daily fecundity is a linear regression for this species (Fig. 4). *Drosophila subobscura* has a similar pattern in generation 7, but in subsequent generations, it presents a pattern that is best fit by a second-degree polynomial regression (after application of AIC). This pattern includes two phases: an initial maturation period including the beginning of egg laying followed by an increase in fecundity, reaching an apparent peak around days 8–10 of the assay, after which there is a drop in fecundity (Fig. 4).

All the models obtained for each replicate population were highly significant. The *t*-tests performed on the average slope for *D. madeirensis* in all assayed generations also gave significance (generation 7: average slope = 2.184, $t = 20.798$, $P < 0.01$; generation 14: average slope = 0.609, $t = 10.263$, $P < 0.01$; generation 23: average slope = 1.418, $t = 11.766$, $P <$

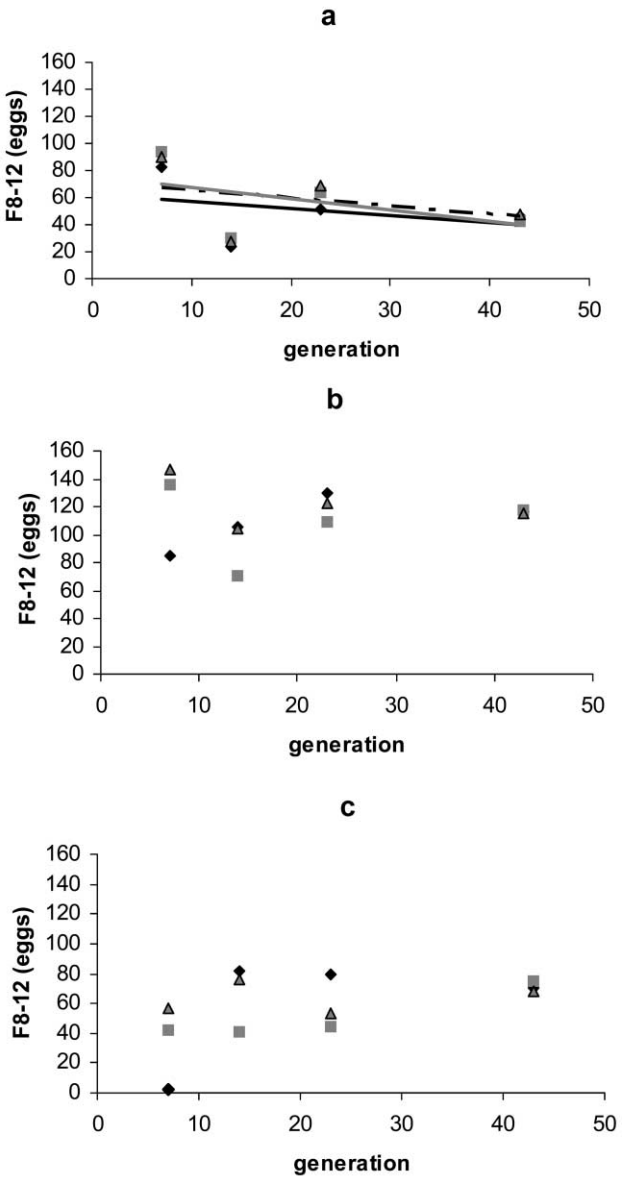

Figure 3. Evolutionary trajectories for peak fecundity. Plots of means of peak fecundity (F8–12) as a function of generation number for *Drosophila madeirensis* (*a*), for *Drosophila subobscura* (*b*), and for differences between them (*D. subobscura* − *D. madeirensis*; *c*). Data points show the mean values of replicate populations of each species. A significant linear trend (presented) was obtained for *D. madeirensis* ($P < 0.05$; *a*). *Black line*, *diamonds*, replicate 1; *gray line*, *squares*, replicate 2; *broken black line*, *triangles*, replicate 3.

0.01; generation 43: average slope = 0.952, $t = 22.504$, $P < 0.01$). The same test performed with the data from *D. subobscura* for generation 7 presents similar results (average slope = 2.727, $t = 4.927$, $P < 0.05$). There were no significant differences between the slopes obtained for both species in generation 7 ($t = 0.965$, $P > 0.5$).

Also performed were *t*-tests comparing the average fecundity of both species day by day, the *P* values being adjusted using a sequential Bonferroni correction (Rice 1989). The results indicate that on generation 7, both species had similar fecundity values during the assayed period, the first 12 d of adulthood. However, in the following generations, *D. subobscura* in general presented higher fecundities throughout the assay. At generation 14, *D. subobscura* presented higher fecundities between days 5 and 9, while in the remaining days of the assay, no significant differences were found. Finally, at generations 23 and 43, *D. subobscura* presented significantly bigger fecundities from day 5 on ($P < 0.05$). Overall, these results suggest a tendency for divergence between the two species during adaptation to the laboratory, with *D. subobscura* exhibiting a more rapid increase in the new environment.

Starvation Resistance

Figure 5 presents the average values of male and female starvation resistance for both species during the two assayed generations. *Drosophila madeirensis* had higher starvation resistance in both assays and for both sexes. The nested ANOVA results indicate that *D. madeirensis* flies were significantly more resistant in both generations, the exception being female starvation resistance in generation 7. A bifactorial ANOVA comparing the two species and the two assayed generations indicates that both female and male starvation resistance differed significantly between species (RF: $F_{1,8} = 17.413$, $P < 0.001$; RM: $F_{1,8} = 34.985$, $P < 0.0001$) as well as between assays (RF: $F_{1,8} = 51.069$, $P < 0.0001$; RM: $F_{1,8} = 9.878$, $P < 0.05$). Over both species, starvation resistance achieved higher values in generation 7. The interaction term was not significant, which seems to indicate that both species did not diverge significantly in terms of starvation resistance during laboratory adaptation.

Comparisons with Continental Populations

The assay carried out on generation 43 was done in synchrony with an assay performed on other *D. subobscura* populations derived from the continent: the TW, AR, and NB populations. The results of unpaired *t*-tests on the differences of averages between the different sets of populations (using the heterogeneity among replicates as source of error) are given in Table 2. In general, *D. madeirensis* differs from continental populations of *D. subobscura* for all fecundity-related traits, having a poorer performance in that they started to lay eggs later and laid fewer eggs. *Drosophila madeirensis* males are in general more starvation resistant. On the other hand, no significant differences were found for female starvation resistance between *D. madeirensis* and continental *D. subobscura* populations. The only trait where continental and Madeiran populations of *D. subobscura* differed was peak fecundity, continental populations laying more eggs independently of geographic origin and num-

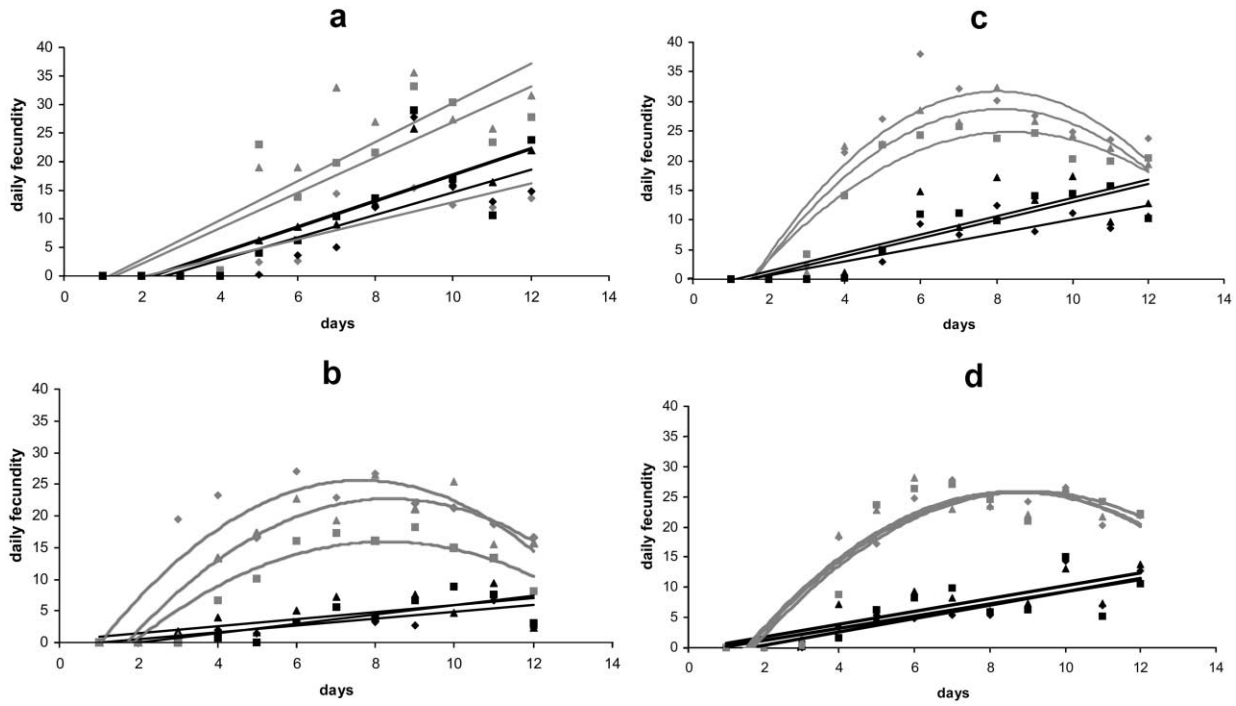

Figure 4. Daily fecundities for each of the generations assayed. Mean daily fecundities of *Drosophila madeirensis* and *Drosophila subobscura* replicate populations in generations 7 (*a*), 14 (*b*), 23 (*c*), and 43 (*d*). The best regression models for each replicate population are also shown. *Diamonds*, s1, m1; *squares*, s2, m2; *triangles*, s3, m3; *gray*, *D. subobscura*; *black*, *D. madeirensis*.

ber of generations in the laboratory (Table 2). Significant differences in all traits are shown in *t*-tests on the overall differences between *D. madeirensis* and *D. subobscura* (using as source of error of the latter different foundations), both continental (specifically TW and AR) and Madeiran. In these comparisons, *D. subobscura* presented quicker maturation, higher fecundity, and lower starvation resistance, paralleling the differences between *D. subobscura* from Madeira and *D. madeirensis* (Table 2). This conclusion is still valid applying a sequential Bonferroni adjustment for five tests.

Evolutionary Trajectories

Figures 1–3 show the temporal changes of age of first reproduction, early fecundity, and peak fecundity for both species as well as differences between species. To check for significance of linear evolutionary trajectories, *t*-tests were performed on the average slope for each species and trait (Table 3). Age of first reproduction (a1r) significantly evolved in *D. subobscura*, with females laying eggs progressively earlier. The same tendency occurred in *D. madeirensis*, though it was not statistically significant (Fig. 1). Early fecundity (F1–7) increased significantly in both species (Fig. 2). On the other hand, peak fecundity (F8–12) declined significantly in *D. madeirensis* but not in *D. subobscura* (Fig. 3).

The analysis of the effect of laboratory evolution on the differentiation of the two species indicates that there is a significant divergence for age of first reproduction and early fecundity, with *D. subobscura* increasing its performance at a higher rate relative to *D. madeirensis* (Table 3; Figs. 1, 2). The same tendency occurs for peak fecundity, though it is not significant (Table 3; Fig. 3). Nevertheless, the average slopes of these two characters did not differ significantly when tested for parallelism by ANCOVA (Table 4). This lack of significance is probably due to a lack of statistical power because the absolute values of replicates were used in this statistical comparison rather than analysis of differences between pairs of populations assayed synchronously.

Evolutionary change was also analyzed using daily fecundity data. The slopes of daily fecundity obtained for *D. madeirensis* replicate populations in each generation were plotted against generation number, and a linear regression was applied. A *t*-test performed on the average slope indicates that the slopes of daily fecundity decreased with time ($t = 6.05$, $\mathrm{df} = 2$, $P = 0.026$). This suggests that the increase in daily fecundity with age decreased during the adaptation to the new environment. This may be due to a drop in fecundity at later ages relative to its level during the first few days of egg laying (see above).

To analyze the temporal change in daily fecundity for *D. subobscura*, a similar approach was used. The slopes of linear

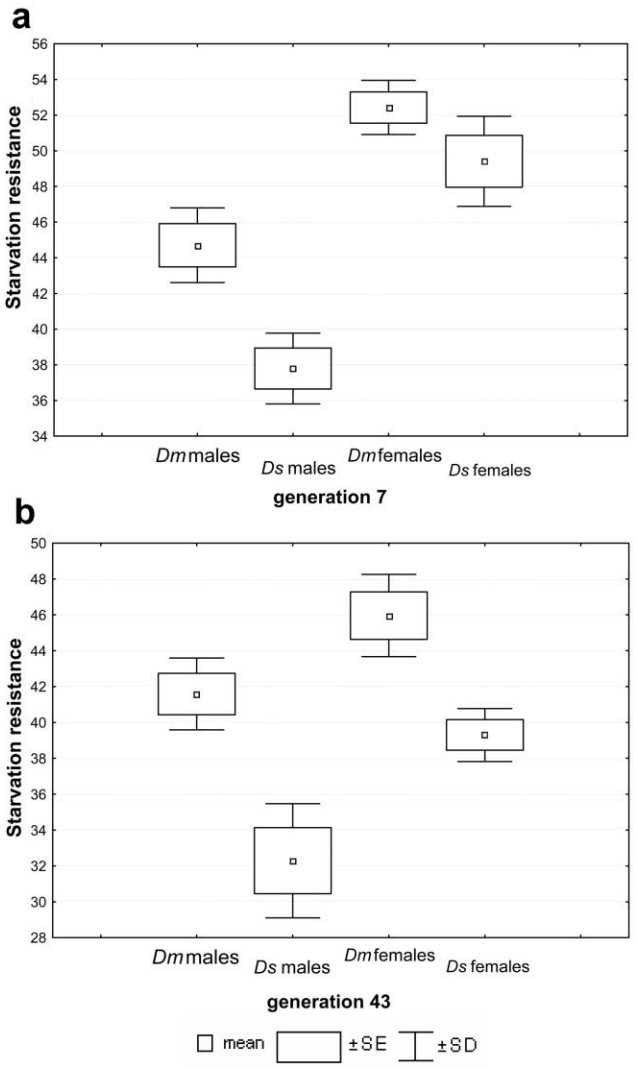

Figure 5. Starvation resistance over different generations. Box plot of female and male starvation resistance values of *Drosophila madeirensis* and *Drosophila subobscura* in generations 7 (*a*) and 43 (*b*). The mean values of each replicate population were used as individual data points.

regressions obtained for daily fecundity for each replicate population were plotted against generation number, and another linear regression was applied. The *t*-test results on the average slope indicate that the pattern of increase in daily fecundity did not change during laboratory adaptation ($t = 1.01$, df = 2, $P = 0.42$). The fact that this species does not show a detectable increase in this character during laboratory evolution may be an artifact arising from a lack of applicability of linear regression models for daily fecundity in this species.

We have nevertheless tested whether there was a temporal change in the differences between species relative to a linear pattern of increase in daily fecundity. In each generation assayed, we estimated the differences in the slopes of the linear model adjusted for daily fecundity data for both species (pairing same-numbered replicate populations, e.g., s1, m1). A linear regression was applied to these data as a function of generation. The results indicate that overall there were no evolutionary changes in the differentiation of these species with respect to the dependence of daily fecundity on age (data analysis not shown).

Discussion

Comparing Initial Laboratory Adaptation in Two Species

Drosophila subobscura and *Drosophila madeirensis* differ greatly in terms of fecundity; *D. subobscura* has quicker maturation and lays more eggs during the assayed periods. The differences between the two species for daily fecundity could be due to their maturation time. As *D. madeirensis* females start laying eggs later, it is possible that including more days in the assay would give *D. madeirensis* the opportunity to express a similar fecundity pattern to the one observed in *D. subobscura*, albeit with a time delay. For some of the generations analyzed in this study, daily fecundity data for two additional days were available, but these later daily fecundities also showed superiority of *D. subobscura*.

Why are these species so persistently different with respect to fecundity in the laboratory environment? The differences that we have observed could mean that these two species explore different resources in their natural environments. Maintaining both species in the same conditions might favor one species relative to the other. In the case of our study, *D. subobscura* could have been unintentionally favored because the maintenance conditions that we adopted were based on our prior experience with this species (Matos et al. 2000, 2002). Furthermore, the fact that *D. madeirensis* is an endemic species, apparently specialized to the Laurisilva forest, while *D. subobscura* is a widespread species found in a large variety of habitats, suggests that we are dealing with two species that differ in their ability to exploit novel resources (Parsons and Stanley 1981; Parsons 1982). Nevertheless, our field experience indicates that both species can be collected simultaneously in the same baited trap (C. Rego, unpublished data). This suggests that both species share feeding and/or breeding preferences.

Drosophila madeirensis flies in general have higher starvation resistance. This may be partly due to their bigger size (Rego et al. 2006). In fact, such an association was found in a comparison of several *Drosophila* species by Sharmila Bharathi et al. (2003). Nevertheless, more studies are needed to test for this pattern.

An important issue to bear in mind is that the populations studied here are undergoing adaptation to a novel environment. It is possible that fecundity may vary between our two species as a function of how much they are preadapted to recognize our culture medium as adequate for egg laying, while starvation resistance basically differs due to different sizes (Sharmila Bharathi et al. 2003). This does not preclude the possibility that

Table 2: *t*-tests on the differences between mean values of the several life-history traits assayed and their respective significance level

	a1r	F1–7	F8–12	RM	RF
Drosophila madeirensis vs. *Drosophila subobscura*:					
D. madeirensis vs. *D. subobscura* (Madeira)	3.885*	16.164***	47.810***	4.276*	4.223[a]
D. madeirensis vs. NB	4.246*	−13.818***	−35.652***	7.371**	2.647[b]
D. madeirensis vs. TW	7.321**	−9.316**	−23.978***	2.628[a]	2.637[b]
D. madeirensis vs. AR	3.797*	−6.287**	−11.923 ***	6.589**	2.122[b]
D. madeirensis vs. *D. subobscura* (Madeira)+ TW + AR	4.776**	−11.809***	−6.05**	3.952*	4.296*
D. subobscura (Madeira) vs. *D. subobscura* (Continent):					
D. subobscura (Madeira) vs. NB	1.807[b]	−1.236[b]	−10.997***	.0486[b]	−.542[b]
D. subobscura (Madeira) vs. TW	2.677[b]	−1.236[b]	−15.888***	−2.148[b]	−.547[b]
D. subobscura (Madeira) vs. AR	.294[b]	.656[b]	−4.530*	−.559[b]	−.508[b]

Note. *t*-tests compare *Drosophila madeirensis* with *Drosophila subobscura* from different geographical origins, *D. madeirensis* with the average for *D. subobscura* across synchronous foundations, and *D. subobscura* from Madeira with Continental populations. The *P* values were corrected using a sequential Bonferroni method. See note to table 1. NB, TW = *D. subobscura* from Sintra (two independent foundations; see details in the text); AR = *D. subobscura* from Arrábida.

[a] $0.05 < P < 0.06$.

[b] Not significant.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

genetic trade-offs may also play a role. On the other hand, the two species may also differ in their capacity to assimilate the nutrients that we have provided. Such a difference might affect both fecundity and resistance in a similar way, giving rise to a positive covariance between traits (Service and Rose 1985; Matos et al. 2000). Nevertheless, all these inferences deserve a note of caution, given that comparisons between two species do not allow direct inference of the genetic architecture within each population, given that they have different genetic backgrounds (Leroi et al. 1994).

Evolutionary Trajectories during Laboratory Adaptation

Overall, our results indicate a general tendency for improvement in characters related to fecundity, particularly early reproduction. Both species showed a tendency to improve in both early fecundity and age of first reproduction during adaptation to the new environment, the rate being higher in *D. subobscura*. This is in accordance with other studies of laboratory adaptation that indicate an increase in early fecundity; most of these studies focus on a single species (*D. melanogaster*, Sgrò and Partridge 2000; *D. subobscura*, Matos et al. 2000, 2002; but see Hercus and Hoffmann 1999 for a study of *Drosophila birchii* and *Drosophila serrata* and their hybrids). On the other hand, peak fecundity did not show any consistent evolutionary change in *D. subobscura*, while it actually declined in *D. madeirensis*. This is an unexpected result, given our other studies in *D. subobscura* (e.g., Matos et al. 2002). It might be explicable in terms of either differences in genetic background or founder effects, two causes that a subsequent study might unravel. Of course, these results need to be interpreted with caution given that transient assay effects may have also contributed to the patterns observed in these analyses of absolute values.

Again, the difference in the rate of adaptation of early fecundity between the two species could be due to the fact that we are dealing with two species with different ecological requirements, *D. subobscura* being a widespread, generalist species and *D. madeirensis* being an endemic species, specialized on Laurisilva forest. Widespread species are expected to be resource generalists and as such to have a higher ability to adapt to new conditions during domestication (Parsons 1982). Adaptive evolutionary potential is in general dependent on quantitative genetic variation (Frankham 1995, 2005*b*). This is in turn expected to be lower in populations with a more restricted geographic distribution, including fragmented habitats (Lienert et al. 2002). *Drosophila madeirensis*, being more specialized and appearing only on Laurisilva forest patches, may indeed give it less potential to adapt to novel environments because of lower genetic variability. The fact that it adapts more slowly than *D. subobscura* in our laboratory corroborates this hypothesis.

The results suggest that starvation resistance decreases during laboratory adaptation in both species. Our previous work and that of others has revealed some inconsistencies in the evolution of starvation resistance during domestication (e.g., Hoffmann et al. 2001; Matos et al. 2002, 2004; Griffiths et al. 2005; Simões et al. 2007). Though in this study we obtained a suggestion of a parallel decline in starvation resistance in the two species, this should be interpreted very cautiously because we only compared starvation resistance at two points in the evolutionary process. Therefore, there is no firm generalization to be made

about the comparative laboratory evolution of starvation resistance. There was no sign of either progressive divergence or progressive convergence between species during the laboratory evolution of starvation resistance in a common environment, unlike previous results with populations of a single species (e.g., Teotónio and Rose 2000).

On balance, the experimental evolution of early fecundity clearly indicates adaptation to the new environment in both species. *Drosophila subobscura* also shows signs of improvement for age of first reproduction. On the other hand, the observed patterns for age of first reproduction, peak fecundity, and daily fecundity for *D. madeirensis* suggest a possible failure to adapt, a failure that might eventually lead to cumulative divergence between the two species (see below).

Table 4: ANCOVA results comparing average values for each species and trait in each generation using generation as a covariate

	a1r	F1–7	F8–12
Generation	6.041*	4.710*	.598[a]
Species	4.478*	59.277***	37.787***
Generation × species	1.072[a]	1.457[a]	1.300[a]

Note. *F* values and significance levels are given. a1r = age of first reproduction; F1–7 = early fecundity, F8–12 = peak fecundity. The *F* values of tests on the homogeneity of slopes (generation × species), comparing both species and their significance levels, are also given.

[a] Not significant.

* $P < 0.05$.

*** $P < 0.001$.

Do Species Converge under Similar Conditions?

Our data indicate that *D. subobscura* and *D. madeirensis* were different with respect to several life-history traits from the moment they were brought into the laboratory. Subsequent laboratory evolution produced no apparent convergence. On the contrary, we found signs of evolutionary divergence between them, though this differentiation varies from trait to trait. It is unlikely that the slower rate of improvement of *D. madeirensis* was due to higher inbreeding levels during laboratory culture compared with *D. subobscura*, given that they were maintained at similar population sizes.

Table 3: Slopes of the linear evolutionary trajectories for each trait and replicate population

	a1r	F1–7	F8–12
m1	−.034	.293	−.510
m2	−.005	.283	−.860
m3	−.010	.263	−.608
Average	−.016[a]	.280***	−.659*
s1	−.057	1.259	.781
s2	−.025	.979	.103
s3	−.038	.654	−.511
Average	−.040*	.964*	.124[a]
s1 − m1	−.028	.966	1.291
s2 − m2	−.019	.696	.962
s3 − m3	−.023	.390	.096
Average	−.024**	.684*	.783[a]

Note. The average values for *Drosophila madeirensis* (m1, m2, m3), for *Drosophila subobscura* (s1, s2, s3), and for the differences between both species as well as the significance levels (*t*-tests) are also given. a1r = age of first reproduction; F1–7 = early fecundity; F8–12 = peak fecundity.

[a] Not significant.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Our data suggest that the evolution in a novel, common environment increases differences between species that are already expressed at foundation. This may be a result of different evolutionary dynamics resulting from different genetic backgrounds, which is particularly expected when dealing with different species (Cohan and Hoffmann 1989). However, variation within species can confound interspecific comparisons, especially in species with wide distributions (Hoffmann and Harshman 1999). We have obtained evidence of effects of foundation in previous studies of the evolutionary dynamics of *D. subobscura* populations (Matos et al. 2002; Simões et al. 2007). It would thus be important to test for repeatability of the results obtained in this study using several independent foundations from both *D. subobscura* and *D. madeirensis*.

Implications for Captive Breeding

There is a lack of previous empirical studies estimating how much species differ in their evolutionary rates during adaptation to captivity, though some studies are tangentially relevant (e.g., Deckert-Cruz et al. 2004). Our data suggest that generalization from one species to another, even to closely related species, can be misleading. Adaptation to captivity may occur generally, but its rate depends on the genetic background of each species. This could be particularly relevant for conservation efforts, because some species may fail to thrive in captivity due to an inability to adapt to such novel conditions.

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