Variation in the rate of convergent evolution: adaptation to a laboratory environment in *Drosophila subobscura*

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Abstract

Adaptation to novel environments is a central issue in evolutionary biology. One important question is the prevalence of convergence when different populations adapt to the same or similar environments. We investigated this by comparing two studies, 6 years apart, of laboratory adaptation of populations of *Drosophila subobscura* founded from the same natural location. In both studies several life-history traits were periodically assayed for the first 14 generations of laboratory adaptation, as well as later generations, and compared with established, laboratory, control populations. The results indicated: (1) a process of convergence for all traits; (2) differences between the two studies in the pattern and rate of convergence; (3) dependence of the evolutionary rates on initial differentiation. The differences between studies might be the result of the differences in the founder populations and/or changes in the lab environment. In either case, the results suggest that microevolution is highly sensitive to genetic and environmental conditions.

Introduction

An important question concerning adaptation to novel environments is the prevalence of convergence or of parallel evolution when different populations adapt to the same or similar environments (see Cohan, 1984a). It is generally assumed that a process of convergence will occur, ultimately leading to very similar character states (e.g. see Larson & Losos, 1996; Futuyma, 1998). Most studies of evolutionary convergence are based on a comparative approach (e.g. see Losos *et al*., 1998 for a study in natural populations of lizards; Majerus, 1998 for a review of studies of industrial melanism in different populations; Schluter, 2000a,b for studies of replicate radiations in fish). However, this approach has several limitations not always taken into consideration (Lauder *et al*., 1993; Leroi *et al*., 1994). The empirical literature lacks detailed temporal studies testing the expectation of convergence. Also, the rates and patterns of evolutionary convergence in particular are often neglected issues (but see Dobzhansky, 1948 for an early exception in *Drosophila*; see Travisano *et al*., 1995 for examples in bacteria; see also Cohan, 1984a, for other examples), although they are at the core of evolutionary genetics (Matos *et al*., 2000a).

One experimental approach to the study of convergence is the comparison of the evolutionary trajectories of recently derived laboratory populations since their introduction to the novel, laboratory environment. *Drosophila* is a well-known model organism in studies carried out under laboratory conditions. Given that the expression of many traits changes with the environment, leading to differences both at the phenotypic and genetic level, there are limits to extrapolation from laboratory studies to natural populations. However, at the same time the dynamics of adaptation to the laboratory may be used as a tool to study evolutionary processes to novel environments (cf. Harshman & Hoffmann, 2000; Matos *et al*., 2000a, b), without any necessary claims concerning processes of evolution in the wild populations from which laboratory derivatives are obtained.

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The few published cases studying adaptation to the laboratory in *Drosophila* do not characterize in detail the evolutionary trajectories, being based on few generations or a comparative approach (Dobzhansky *et al*., 1964; Tantawy & El-Helw, 1970; Pascual *et al*., 1990; Service, 2000; Sgrò & Partridge, 2000; Hoffmann *et al*., 2001; but see Matos *et al*., 2000a,b; Matos & Avelar, 2001), or they involve parallel selective regimes that make interpretation difficult (Tantawy & El-Helw, 1966). Thus more empirical studies about the tempo and mode of how *Drosophila* populations adapt to a novel environment are needed.

Experimental evolution in *Drosophila* has been mostly carried out by selection under a new culture regime. Such experiments include adaptation to novel regimes involving high density conditions (e.g. Mueller *et al*., 1994), altered age at reproduction (e.g. Luckinbill *et al*., 1984; Rose, 1984), starvation (e.g. Rose *et al*., 1992), and faster development (Chippindale *et al*., 1997). Such regimes usually result in considerable differentiation of populations. When the populations subjected to these different regimes start from the same ancestral population, they can also be used to study reverse evolution (see Service *et al*., 1988; Teotónio & Rose, 2000, 2001).

Convergence is often assumed to occur with adaptation to the same or similar environments. However this assumption needs to be tested, in particular when one infers evolutionary patterns and processes from a comparative approach (see above). Therefore we need direct evidence of how repeatable evolution is. This question can only be answered by comparing evolutionary trajectories as different populations evolve (see Cohan, 1984a, b; Matos & Avelar, 2001).

We compared the evolutionary trajectories of laboratory populations of *Drosophila subobscura* Collin founded from the same wild location, 6 years apart. Our findings support the notion of a process of convergence during laboratory adaptation, but we detected significant differences in patterns and rates of convergence, as well as in the dependence of evolutionary response on initial differentiation.

Materials and methods

Foundation of the control population

The control population of *D. subobscura* – from herein called B – was obtained in a pinewood near the village of Sintra, Portugal, from several collections, over 2 days, from early morning till late afternoon in January 1990. About 140 females and 50 males were collected. From the moment the population was brought to the laboratory, it was maintained in the general conditions described below. In all foundations the identification and separation of individuals was performed using ether anaesthesia, which helps to remove mites. All other anaesthesia used CO_2.

Foundation of the experimental populations

First study (started in 1992)
In March 1992 about 230 females and 100 males were collected for the second population – from herein called W – in the same natural location. From then on both populations were kept under the same maintenance regime (see below). By the time of the foundation of population W, the control, B, population was in its 24th generation, i.e. about 2 years in the laboratory (see details in Matos *et al*., 2000a).

Second study (started in 1998)
In March 1998 about 300 females and 280 males were collected from the same pinewood. These were the founders of a new laboratory population, here called NW. By the time of its foundation the control, B, population was in its 90th generation.

Formation of replicate populations in the second study

Our second study was five-fold replicated. For this, in the second generation of NW, the original B population was split into five populations, the same being performed to the NW population. From thereon all these 10 populations were maintained without any interpopulation crosses during the whole study. Each replicate population was labelled with a number from 1 to 5. To distinguish them from the first study (not replicated), the replicate populations derived directly from the B population will be called NB. Thus, the recent populations will be referred to as $NW_{1–5}$ and the control populations as $NB_{1–5}$.

Culture methods

As described in Matos *et al*. (2000a), the flies were maintained in an incubator at 18 ± 1 °C, with a 12 h L : 12 h D photoperiod (except during handling, which occurred at room temperature, in general around 22 °C). The culture medium was similar to that described by David (1959), being composed of agar, corn meal, dead brewer's yeast, charcoal colouring and nipagin. Flies were kept in 10 × 2 cm vials, with an adult density of about 50 individuals per vial. Larval densities were 60–80 per vial. The reproductive regime involved discrete generations, with egg collection at a young age of the imagoes' life, close to peak fecundity. Maximum developmental time allowed was 21 days, some slight truncation selection for rate of development being involved. Adult population sizes were in general higher than 1000 individuals, never dropping below 300. Populations were maintained in separate racks in both studies, being periodically randomized within the incubator.

Some changes occurred between the first and the second study: the incubators were not the same, and generation time changed from 33 to 28 days

(corresponding to a change in the age of the females by the time of egg collection, from around 12 to 7 days) in 1996, around 2 years before the start of the second study.

Life-history traits assayed

Fecundity characters
Flies – in mated pairs – were transferred daily to laying vials containing freshly prepared medium. The total number of eggs laid per female was counted everyday for the first 2 weeks of the female's life (since emergence) in the first study and for the first 12 days in the second study. For the sake of uniformity of traits used, two fecundity measures were used here: the total number of eggs laid during the first week of the female's life as imago, and the number of eggs laid between days 8 and 12.

Age of first reproduction
This trait was estimated as the number of days between emergence and the first egg laying. For the few females that did not lay eggs during the period covered by the analysis, we assigned the total number of days involved in the fecundity study (14 in the first study and 12 in the second) for age of first reproduction. This way these females (which were mainly of the recent populations, W and NW_{1-5}, during the first generations) were not discarded from the analysis of this trait.

Starvation resistance
After the period of fecundity assay, mated pairs were placed in vials with a non-nutritive medium (plain agar) and deaths were recorded every 6 h.

Assay methods

First study – comparison between B and W populations
In each assay, the mating pairs (formed under CO_2 anaesthesia) of each sample of the W and B populations were randomized between racks in the incubator. After the 2 weeks of egg counting, the mating pairs, remaining in the same place of the rack, were placed in plain agar, to assay starvation resistance.

Assays were performed periodically during the first 14 generations after foundation of the W population (generations 4, 5, 7, 9, 10, 12 and 14). Finally, in the 47th generation of the W population, corresponding to the 71st generation of the B population, another comparison was made, using the same traits.

Sample sizes ranged between 20 and 23 individuals per population and generation (starting sample size = 24), except at generation 9 and 47, with around double sample size (see details in Matos *et al.*, 2000a).

Second study – comparison between NB_{1-5} and NW_{1-5} populations
In each assay, the mating pairs (formed under CO_2 anaesthesia) of the samples of the NW and NB replicate populations were randomized between racks in the incubator. Samples were distributed in the racks as a function of their arbitrarily assigned numbers – that is, NB_1 sample in the same rack(s) as NW_1, etc. In each rack the two samples were distributed in alternate rows, the order changing from rack to rack. After the 12 days of egg counting, the mating pairs were assayed for starvation resistance. Assays were performed during the first 47 generations after foundation of the NW population (generations 4, 8, 13,15, 33, 43 and 47). Sample sizes per replicate population were on average between 14 and 21 individuals per generation (average number of individuals per regime between 68 and 110), starting with 18 or 24 individuals, except in the 33rd generation, with sample sizes around eight individuals per replicate population.

Statistical methods

All data analysis was performed using STATISTICA and EXCEL.

All regressions were type I least-squares linear regressions (Sokal & Rohlf, 1995). Loglinear regressions, with ln (generation number + 1) as the independent variable, were also estimated for the data covering the 47 generations of the second study. Both intercepts and slopes were tested for each study and trait. In the two studies the analyses were carried out on the differences between the more recently introduced populations and their controls. This method was used to minimize environmental effects that reduce the power to estimate evolutionary trajectories, and is a standard procedure in experimental evolution in *Drosophila (*e.g. see Teotónio & Rose, 2000). In the first study the analysis was performed on the differences in the average of W and B individuals for each generation (see Matos *et al.*, 2000a; for details). In the second study two approaches were used: (1) a regression analysis using as data points the average of the differences between the five pairs of replicates $NW_i - NB_i$ per generation; (2) an analysis using as data points the values of each difference between pairs. In (2) the test for significance of intercepts involved a simple linear regression model using the entire data set of pairs, whereas the test for significance of slopes was carried out using the ANCOVA model (see Sokal & Rohlf, 1995):

$$Y_{ij} = \mu + \alpha_i + \beta(G_{ij} - \bar{G}_i) + \varepsilon_{ij}$$

with the several replicate differences as categories of the factor α and generation (G) as the covariate (β being the slope of the within-cells regression). For the loglinear regressions the same approach was used, using ln(generation number + 1) as the independent variable.

All comparisons between studies used the first 14 generations of W and the first 15 generations of NW. Two-tailed t-tests (4 d.f.) used the residual error of regression for W–B and the standard error of the

differences of estimates (either intercepts or slopes) between replicates for NW–NB. ANCOVA tests for differences between studies followed the above model involving one factor (α) with two categories: NW–NB and W–B, with generation (G) as the covariate (the slope of the within-cells regression symbolized by β). Two analyses were carried out: one involved the average of the five replicate pairs NW_i–NB_i for each generation in the values of category NW–NB. Another involved all replicate values per generation, i.e. all values NW_1–NB_1, NW_2–NB_2, …., NW_5–NB_5 were discriminated, without grouping same pairs between generations. In both analyses, the P-values for the differences in intercepts were based on the ANOVA differences between categories after extracting the effect of generation (8 d.f. and 24 d.f. for the error, respectively). The P-values for the differences in slope by ANCOVA were determined through a test for parallelism of regressions between categories (7 d.f. and 23 d.f. for error in the first and second ANCOVA tests).

The analysis of the dependence of evolutionary rates on initial differentiation was carried out by type I least-squares linear regressions of the several linear slopes on the respective intercepts of the five life-history traits analysed (see above).

Linear regressions were performed with: (1) the data of both W–B and the averages of the replicates NW–NB together (8 d.f.); (2) the data of the W–B plus all the data set of the NW–NB replicates not discriminated (28 d.f.); (3) the W–B values alone (3 d.f.); (4) the NW–NB average values alone (3 d.f.); and finally (5) the entire data set of the NW–NB replicates not discriminated (23 d.f.).

A comparison between W–B and NW–NB with respect to the linear regressions of slopes vs. intercepts was carried out by means of two types of tests:

1 Two-tailed t-tests on the differences between the average estimate of the five NW replicates and the estimate of W, with 4 d.f., using as source of error the residual error of the linear regression for W and the standard error of the estimates of the regression of slopes vs. intercepts of each of the five replicate NW populations as source of error for NW.

2 ANCOVA tests, using as model:

$$b_{ij} = \mu + \alpha_i + \beta(a_{ij} - \bar{a}_i) + \varepsilon_{ij}$$

with α as factor with W–B and NW–NB as categories, b as slope of the regression of slopes of single traits on intercepts, β as the slope of the within-cells regression, and a (intercept of single traits) as the covariate. Two analyses were carried out: one involved the average of the five replicate pairs NW_i–NB_i for each generation in the values of category NW–NB (7 d.f. involved in the error of intercepts and 6 d.f. in the error of the test of parallelism of studies). Another involved all replicate values per generation (6 and 26 d.f. involved in the respective tests).

Results

Evolutionary trajectories of each trait

First study (W–B)

There was in general a significant initial difference between the recently founded population (W) and the control (B), followed by linear convergence of age of first reproduction and fecundity. Male and female starvation resistance did not follow this pattern (Fig. 1, Table 1; see also Matos *et al*., 2000a for details and other fecundity traits). A later assay at generation 47, however, suggested eventual convergence for starvation resistance (given by a nonsignificant negative difference between W and B; see details in Matos *et al*., 2000a).

Second study (NW–NB)

The initial differentiation between NW and their control populations was clear for all traits, whether tested on the average of replicates or using the data of all the replicate pairs discriminated (Table 1). Consistent convergent patterns appeared for all traits except for female starvation resistance. The best fit model for all traits (except male starvation resistance), corresponding to a higher coefficient of determination, was a loglinear one (see Table 1 and Fig. 1). Male starvation resistance showed a linear pattern of convergence, although a loglinear model was also significant (but with a smaller coefficient of determination). The convergence process of fecundity was slower, suggesting that a final state of convergence will only occur when more generations elapse (Fig. 1). Female starvation resistance showed a more complex pattern because the best fit model crossed the zero line around generation 23, suggesting a phase of divergence after the initial phase of convergence, as in the first study.

Comparisons of evolutionary trajectories between studies

The significance of two-tailed t-tests on the differences between the average intercepts of the five NW replicates and the intercept of W, as well as between slopes, is presented in Table 1, for all traits analysed. Age of first reproduction was significantly different both in intercept and slope, whereas the fecundity of the first week was only significantly different in intercept, and male starvation resistance showed a marginally significant difference in slope. ANCOVA tests were also carried out (see details in Statistical methods), indicating in general the significant differences in intercepts but not in slopes, and whether the average of all replicates or each replicate value per generation in the second study were used (Table 1). More significant estimates were obtained by ANCOVA than by t-tests. This is expected because our t-tests were more conservative, not taking into account the degrees of

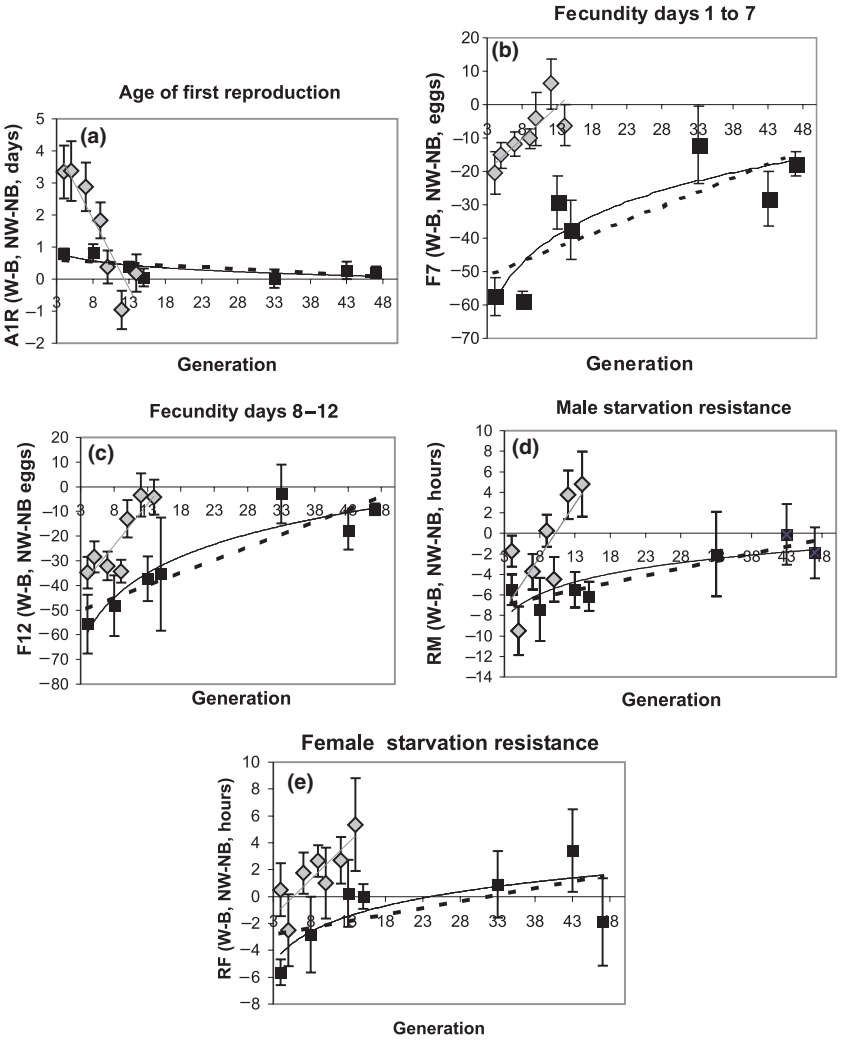

Fig. 1 Panel figure with the evolutionary trajectories for W–B and NW–NB. W: Experimental population and B: control population, for the first study; NW: replicate experimental populations and NB: replicate control populations, for the second study.(a) A1R: age of first reproduction; (b) F7: fecundity between days 1 and 7; (c) F12: fecundity between days 8 and 12; (d) RM: male starvation resistance; (e) RF: female starvation resistance; Diamonds, grey: W–B values and linear regression line for generations 4–14. Squares, black: NW–NB (average of the differences between the five pairs of NW and NB replicate populations) values; both the linear regression (dotted) and loglinear regression (full) lines are plotted. The error bars were the standard errors of the five replicate pairs in the case of NW–NB and the standard errors based on the sample variances of W and B in the case of W–B. Almost all regressions were significant or marginally significant (see Table 1); for age of first reproduction and female starvation resistance the loglinear regression for NW–NB was significant whereas the linear regression was not.

freedom added by the number of generations involved in the regressions.

It is worth noting that there was a higher initial differentiation for fecundity in the second study, but there were parallel patterns of convergence between studies. This is explained by the fact that full convergence took in general more generations to occur in the second study (see also Fig. 1). Age of first reproduction presented different slopes in all tests, as well as intercepts tested by *t*-test and one of the ANCOVA models (although it is important to point out the limitations of ANCOVA testing of adjusted means when the regressions on the covariate are not parallel).

Evolutionary rates as a function of initial differentiation

Table 2 presents the results of a general linear regression, using NW and W data, of the slopes vs. intercepts obtained from the linear evolutionary trajectories of the

Table 1 Regression of the evolutionary trajectories of W–B and NW–NB and comparisons between studies.

	Linear regressions of evolutionary trajectories				Differences between studies		
	W–B	NW–NB (15 gen.)	NW–NB (47 gen.)	Ln(gen. + 1) NW–NB (47 gen.)	*P* of *t*-test	*P* of ancova 1	*P* of ancova 2
Intercepts							
A1R	5.346***	1.18**,***	0.639**,***	1.219**,***	0.008***	0.288 n.s.	0.02**
F7	−25.452***	−70.934**,***	−53.761***,***	−88.832***,***	0.007***	0.000***	0.000***
F12	−50.489***	−63.264***,***	−53.656***,***	−94.104***,***	0.333 n.s.	0.000***	0.008***
RM	−10.496**	−6.217**,**	−7.398***,***	−11.913***,***	0.344 n.s.	0.052*	0.022**
RF	−3.095 n.s.	−7.502**,***	−3.14 n.s.*	−8.226**,**	0.150 n.s.	0.001***	0.014**
Slopes							
A1R	−0.433***	−0.067*,**	−0.012 n.s.*	−0.293**,***	0.016**	0.011**	0.000***
F7	1.909**	2.529 n.s.**	0.834**,***	18.697***,***	0.52 n.s.	0.587 n.s.	0.728 n.s.
F12	3.331**	1.912 n.s.***	1.039***,***	22.183***,***	0.346 n.s.	0.234 n.s.	0.577 n.s.
RM	1.029*	0.008 n.s.	0.142***,**	2.686**,**	0.068*	0.079*	0.063*
RF	0.543**	0.544**,***	0.099 n.s.*	2.540*,**	0.996 n.s.	0.995 n.s.	0.998 n.s.

Values for the intercepts and slopes are presented, with the *P*-values symbolized by asterisks (*$0.05 < P < 0.1$; **$0.01 < P < 0.05$; ***$P < 0.01$; n.s.: $P > 0.1$); for NW–NB the first symbol refers to regression involving the average of the five pairs of replicates; the second symbol for the intercept refers to regression involving the values of each replicate and for the slope corresponds to the *P*-value of an ANCOVA with the replicates as categories and generation as covariate; both linear and loglinear [ln(gen. + 1)] regression results are presented for the entire (47 gen.) NW–NB study. ANCOVA 1 involves the average of the five replicate pairs; ANCOVA 2 involves all replicate values per generation; *P* for intercepts is the ANOVA difference between categories after extraction of the effect of generation; for slopes *P* relates to the test for parallelism of regressions between categories (see details in the Statistical methods). A1R: Age of first reproduction; F7: fecundity between days 1 and 7; F12: fecundity between days 8 and 12; RM and RF: male and female starvation resistance.

five traits studied here. Both analyses used all data obtained in the two studies (discriminating or not the replicate populations in the second), and analysis only with W or NW data gave highly significant negative slopes and no significant intercepts (Fig. 2). That is, there is a significant effect of the initial differentiation on evolutionary rate, in the direction of convergence, and the expectation of no evolution when this difference is not present.

As previously mentioned, convergence took more generations to occur in the second study, given that, although the initial differentiation was higher, the evolutionary trajectories were not significantly different when the slopes are considered. It is thus important to compare the linear regressions of slopes vs. intercepts in the two studies. Table 2 presents the *P*-values of several *t*-tests and ANCOVA tests on the slopes of the regressions obtained in the two studies. All indicate a significantly lower value of slope for the second study, that is, the effect of initial differentiation on the evolutionary rate is smaller in this case (Fig. 2), as expected from the previous analysis of the evolutionary trajectories (see Fig. 1).

To forestall the possibility that the differences observed might be at least partly because of scale effects (e.g. fecundity data generally involve considerably higher initial differences between experimental populations and controls, particularly in the second study), another analysis was carried out, this time on the slopes and intercepts obtained in evolutionary trajectories when the data points used were the ratios between experimental populations and controls. The conclusions were the same as those presented above for the differences.

Discussion

Convergence

Two general conclusions can be drawn from both studies analysed here. First, there is a clear indication of adaptation to a novel environment. All the adult traits analysed presented clear patterns of improvement throughout the first 14–15 generations assayed (with the exception of male starvation resistance in the second study, which took more generations to indicate clear convergence). This general improvement agrees with the

Table 2 Linear regressions of the evolutionary rates (slopes of linear evolutionary trajectories) as a function of initial differentiation (intercepts of linear evolutionary trajectories) and tests for differences between studies.

	W: Single estimates NW: Average of estimates	Replicate data points used
Intercept of linear regression		
NW and W	0.241 n.s.	0.055 n.s.
W	0.197 n.s.	–
NW	0.012 n.s.	−0.064 n.s.
Slope of linear regression		
NW and W	−0.039***	−0.033***
W	−0.065***	–
NW	−0.033***	−0.032***
P *t*-test – NW vs. W		
Slopes	0.017**	–
Intercepts	0.250 n.s.	–
P_{ANCOVA} analysis – NW vs. W		
P parallelism	0.003***	0.035**
P adj. means	0.037**	0.033**

The regressions of slopes vs. intercepts involved the data from the linear evolutionary trajectories of A1R, F7, F12, RM and RF for the first 14 (W–B) and 15 (NW–NB) generations. ***$P < 0.01$; **$0.01 < P < 0.05$; *$0.05 < P < 0.1$; n.s. $P > 0.1$ (see details in the Statistical methods).

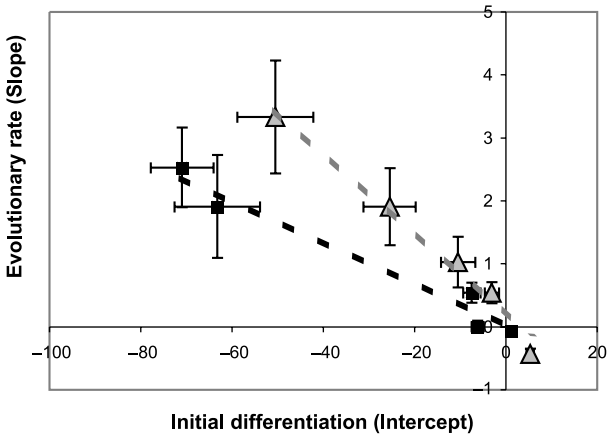

Fig. 2 Plot of slopes of the linear evolutionary trajectories of five traits as a function of intercepts. The first 14–15 generations were used. NW–NB (average of the five replicates): black squares; W–B: grey triangles; the error bars were defined by the differences between replicates for NW and by the residuals of the regressions for W. Dotted lines: linear regressions of the data sets; W data: $y = -0.0644x + 0.1915$; $P < 0.0005$; NW data: $y = -0.0332x + 0.0121$; $P < 0.003$.

expectations of positive covariances among life-history traits when populations start evolving in a novel environment (see Service & Rose, 1985; Matos *et al*., 2000a). But it is not in accordance with what might be expected considering Partridge and collaborators' study of laboratory adaptation in *D. melanogaster*, which indicated that adaptation to the novel, laboratory environment involved an improvement of early adult performance at the cost of late performance and starvation resistance (Sgrò & Partridge, 2000; Hoffmann *et al*., 2001). Although we did not measure either late fecundity or mortality rates, we did measure starvation resistance, which is expected to be strongly correlated with longevity, at least in *D. melanogaster* (e.g. see Rose *et al*., 1992) and plausibly in our species as well. This trait showed an increase throughout generations in both studies, and although female starvation resistance did give indications of a biphasic evolutionary trajectory in both studies (corresponding to higher values of the more recent populations from a certain phase onwards, which suggests the occurrence of a later drop if a convergent state is reached in relation to established populations), this is not the same as observing a drop of values since foundation. It seems to us that the most likely explanation for the disagreement between our results and those of Hoffmann *et al*. (2001) is a different methodological approach, i.e. inferences from a comparative approach in their case and a direct analysis of evolutionary trajectories in our case (see also below).

Secondly, in both our studies the evolutionary trajectories indicate a process of convergence towards the values presented by long-established populations. The fact that the best fit models for most traits in the second study were loglinear ones is in accordance with the expectation of an approach to an evolutionary equilibrium (plausibly a stable convergent state). Thus, although fecundity is still differentiated between experimental and established populations, it is likely that a state of convergence will be achieved.

Although convergent evolution is an important corollary of Darwinian evolution, adequate testing of this expectation is surprisingly lacking in the literature, particularly the empirical study of evolutionary trajectories such as those presented here (see introduction). A clear exception is the study by Teotónio & Rose (2000) of reverse evolution in *D. melanogaster*. In that study, 25 replicated populations (from five different selective regimes, starting from a common ancestral population) were followed for 50 generations, after being placed again in the ancestral environment. Their conclusion was that reverse evolution (which is a particular type of convergent evolution) occurred, but was not universal, being dependent both on previous evolutionary history and the traits involved. It would have been interesting to have more generations of reverse evolution data from this study in order to check whether the difference between our study and the reverse evolution study was because of the peculiarities of the selective regimes imposed, the traits analysed, or general differences of reverse evolution vs. convergent evolution to an overall novel environment.

There are different convergent patterns between the first and second study

Although there was a clear convergence process in both our studies, there were also differences in the initial differentiation and the rate of convergence, resulting in a higher number of generations (observed or expected) to attain full convergence in the second study.

These differences may have been the result of different genetic backgrounds at foundation (e.g. genetic changes in the natural population and genetic sampling effects, although foundation involved a relatively high number of individuals) and/or environmental factors, that, although apparently slight, might have affected the adaptive process to the laboratory environment between studies (see also Cohan, 1984b).

One cannot dismiss the possibility that changes in the control values may have contributed to the differences observed. There was in fact a generally better performance of NB values, in relation to B values, which can be seen in the averages across generations for most traits. These were, respectively: for age of first reproduction, 3.0 and 4.9; for fecundity of the first week, 119.6 and 28.0; for fecundity between days 8 and 12, 157.4 and 66.3; for male starvation resistance, 39.1 and 29.4; the only exception was female starvation resistance, in which NB had an average of 41.1 and B of 41.3. The differences in the first four traits were significant (two-tailed *t*-tests, 12 d.f., $P < 0.01$). It is unlikely that a different level of inbreeding depression in the B population caused the observed differences, given the population sizes during maintenance (in general above 1000 individuals during the first study, similar to or bigger than the NB replicate population sizes). Differences could also be because of the fact that the B population was still adapting to the standard laboratory environment between the first and second study. The stability of B phenotypic values during the 14 generations of the first study (see Matos *et al*., 2000a) makes this unlikely. Furthermore, an assay performed at generation 47 of W and 71 of B (in which there were no significant differences between populations; see Matos *et al*., 2000a) further suggests the hypothesis that neither inbreeding depression nor evolutionary disequilibrium were involved in the changes of the B population. Finally, a comparison of NW and *W*-values at generation 4 also found a significantly higher performance for the first (two-tailed *t*-tests, 106 d.f., $P < 0.01$ for all traits except female starvation resistance). This suggests environmental changes in laboratory conditions between the first and second studies, affecting both NB and NW performance.

All told, the most likely explanation for the differences between NB and B stocks are environmental factors contributing to a better performance in the second study, and acting simultaneously on the NB and NW populations (e.g. change of incubator, yeast used, occurring at least 2 years before the start of the second study). The interaction between these environmental factors and the genetic background of the founder population, together with possible effects of the slight changes in the culture regime (generation time, slightly shortened more than 2 years before the second study), may have changed the evolutionary scenario, causing a change in the tempo of convergence, both in single traits and in the relationship between the evolutionary rates and initial differentiation.

Our results confirm that microevolutionary processes are highly sensitive, leading to considerable differences in the evolution of life-history traits, whether caused by effects of foundation, changes in environment, or interactions between the two (e.g. see Fontdevila, 1989; Schlichting & Pigliucci, 1998). Although comparative studies may allow synchronous testing (e.g. Sgrò & Partridge, 2000; Hoffmann *et al*., 2001), the fact that different populations are involved obscures evolutionary processes, given the many potential sources of variation in evolutionary patterns (see Leroi *et al*., 1994).

Evolutionary rate is dependent on initial differentiation

In our study evolutionary rates were linearly related to initial differentiation, both in each separate study and when both were plotted together. However, there were differences in the relationship between initial differentiation and evolutionary rate between studies. It is an open question whether these differences were because of the different genetic backgrounds of the founder populations, or because of the effects of genotype vs. environment interactions. Whatever the source of the differences, they show that the linear relation found between these parameters is no mathematical certainty and deserves attention for its potential evolutionary significance. Is such a general linear relationship related to positive genetic covariances among traits, which may arise from the effects of a novel environment (Service & Rose, 1985; Matos *et al*., 2000a)? Only more studies of patterns of convergence among synchronously evolving populations can clarify this point.

The power of studying convergent evolution in action

Unlike divergent selection experiments, evolutionary convergence studies can test for a particular outcome of the evolutionary process. This gives an increased power to define clear predictions in evolutionary biology studies. In particular, they are a fine way of testing the evolutionary limitations possibly caused by genetic constraints, so often mentioned in the literature (see general reviews in Maynard Smith *et al*., 1985; Loeschcke, 1987; Roff, 1992, 1997; Stearns, 1992; Falconer & Mackay, 1996; Stearns & Hoekstra, 2000; Teotónio & Rose, 2001).

Thus, empirical studies involving reverse evolution and convergent evolution in a common environment are powerful tools for evolutionary biology. Few studies have used this approach. The most common approach for

investigating adaptive evolution has been a comparative approach to infer evolutionary patterns and processes. Our data suggest that even in the same laboratory, slight temporal changes in the environment cause significant changes in the features of convergent evolution, suggesting that even more severe problems will likely arise in the comparison of natural populations (cf. Leroi *et al*., 1994; Matos & Avelar, 2001). The results also illustrate the difficulties of evolutionary studies in which the controls are dynamic populations themselves, possibly leading to misleading results (see Rose *et al*., 1996). In particular, tests for repeatability between experiments are important because replication within experiments is not enough to cover the effects of both the foundation and microenvironmental changes, in studies aimed at general evolutionary questions.

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