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CONVERGENCE TO A NOVEL ENVIRONMENT: COMPARATIVE METHOD VERSUS EXPERIMENTAL EVOLUTION

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Abstract.—Laboratory adaptation allows researchers to contrast temporal studies of experimental evolution with comparative studies. The comparative method is here taken to mean the inference of microevolutionary processes from comparisons among contemporaneous populations of diverse origins, from one or multiple species. The data contrasted here come from *Drosophila subobscura* populations that were introduced to the laboratory at several different times and from two different locations. Two questions were addressed. First, can we correctly infer evolutionary dynamics from comparative data collected simultaneously from disparate populations? In most cases, we could, except for the character of starvation resistance. Second, are the evolutionary dynamics inferred from the comparative approach similar to those revealed by temporal studies of experimental evolution? For fecundity characters, they were. Overall the results show that both comparative and temporal studies are useful, though the former can be uninformative for characters with complex evolutionary trajectories.

Key words.—Comparative approach, convergent evolution, *Drosophila subobscura*, experimental evolution, local adaptation.

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Though central to evolutionary biology, the issue of convergent adaptation has been poorly dealt with in the empirical literature. It is generally assumed that a process of convergence will occur among populations evolving in the same environment, ultimately leading to very similar character states (e.g., Larson and Losos 1996; Futuyma 1998). The empirical evidence supporting this assumption, however, has been very limited.

Most studies of evolutionary convergence are based on a comparative approach that infers microevolutionary processes from comparisons among contemporaneous populations (e.g., Losos et al. 1998; Majerus 1998; Schluter 2000a,b; see also Harvey and Pagel 1991). To infer evolutionary convergence using a comparative approach, it is desirable to have data from independent evolutionary branches and some information concerning the times at which each evolutionary branch was initiated. Data of such quality has been used to make strong inferences about evolutionary processes (see Cohan 1984a,b; Harvey and Pagel 1991). Ideally, these inferences should be tested against actual patterns observed at a microevolutionary level with replicated populations (Matos and Avelar 2001; Matos et al. 2002).

Experimental evolution has mostly been studied using selection under a new culture regime, such as particular culture media (Lenski et al. 1991), high-density conditions (Mueller et al. 1993), altered age at reproduction (Luckinbill et al. 1984; Rose 1984), starvation (Rose et al. 1992), and quicker development (Chippindale et al. 1997). Maintenance of outbred populations under such culture regimes for many generations (> 100) usually leads to considerable differences between experimental populations and their controls. The study of reverse evolution, when differentiated experimental populations return to the ancestral regime for several generations, is another way of testing evolutionary mechanisms of convergence (Service et al. 1988; Teotónio and Rose 2000; Teotónio and Rose 2001; Teotónio et al. 2002).

It has been claimed frequently that there are limits to the extrapolation of findings from laboratory studies to natural populations (Harshman and Hoffmann 2000; Sgrò and Partridge 2000, 2001). Many of these limits indeed present considerable problems for the extrapolation of laboratory findings. But the laboratory can also be seen as just another environment to which populations must adapt (Matos et al. 2000b), regardless of the limits facing the extrapolation of laboratory findings beyond the laboratory. Studies involving the evolutionary processes that occur during the laboratory adaptation of well-known model organisms are particularly important. Temporal studies of laboratory adaptation can be used as a tool to study evolutionary processes arising from novel environments in general and convergent evolution in particular (cf. Harshman and Hoffmann 2000; Matos et al. 2000a,b, 2002).

In *Drosophila*, few studies have characterized the evolutionary trajectories of laboratory adaptation, most being based on few generations or a comparative approach without a temporally detailed trajectory (Dobzhansky et al. 1964; Tantawy and El-Helw 1970; Pascual et al. 1990; Service 2000; Sgrò and Partridge 2000; Hoffmann et al. 2001; but see Matos et al. 2000a,b, 2002; Matos and Avelar 2001).

To our knowledge, no data exist in the literature contrasting the evolutionary trajectories of different stocks with a comparative approach to the same stocks. Yet laboratory adaptation is an obvious opportunity to test the similarities and differences between the comparative approach and the study of experimental evolution during adaptation, since the relevant data come from populations evolving in the same en-

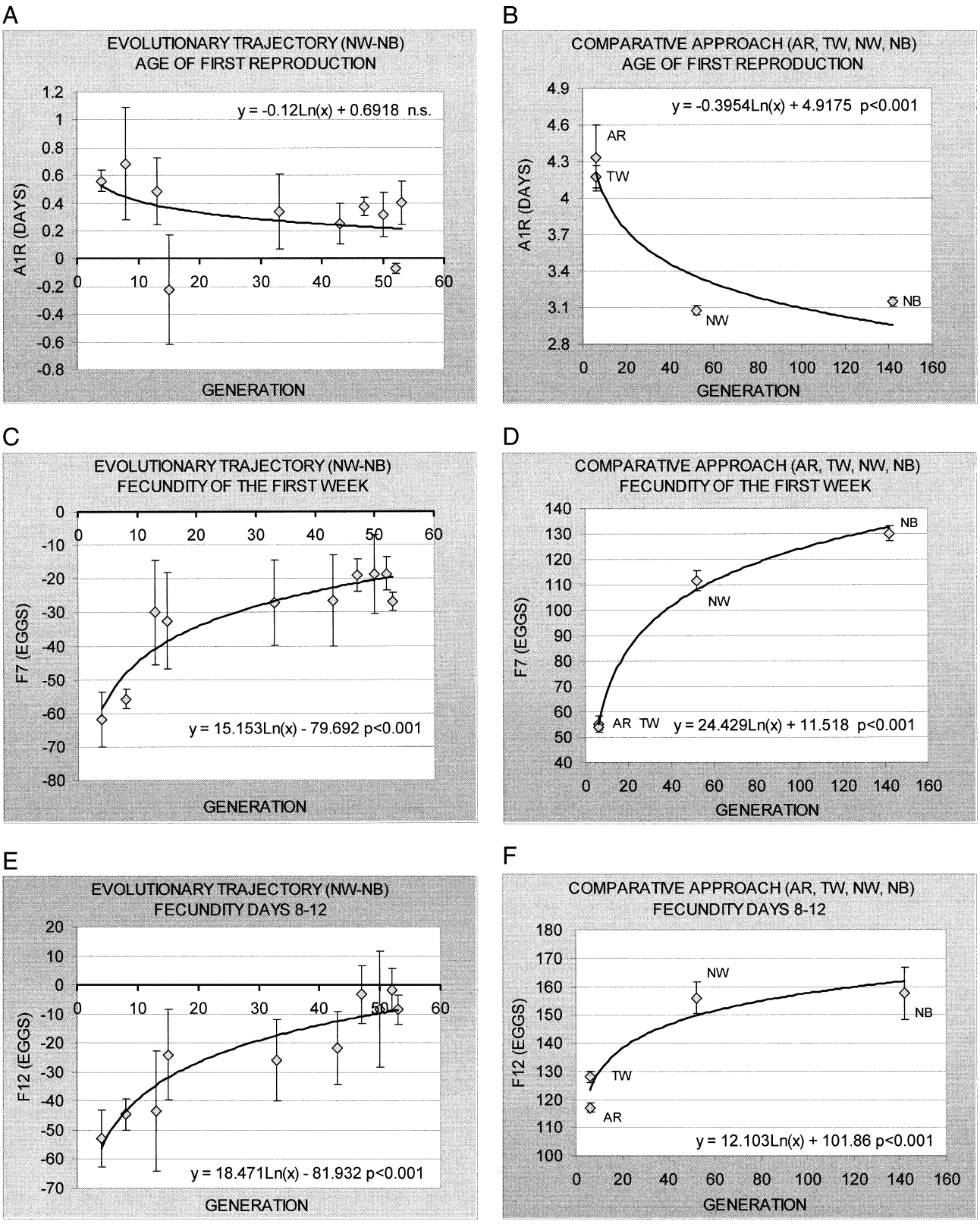

FIG. 1. Evolutionary trajectories for NW-NB and comparative plots of the average values of independently founded populations (AR, TW, NW, and NB), as a function of number of generations in the laboratory. The error bars correspond to the standard errors using the three replicate pairs as datapoints (NW_1-NB_1, etc.) for the evolutionary trajectories and to the standard errors using the three replicate populations of each foundation as datapoints for the comparative approach. Only the results of the assay done when AR and TW were at generation 6 (and NW and NB were at generations 52 and 142, respectively) are shown for the comparative study. See details in Materials and Methods.

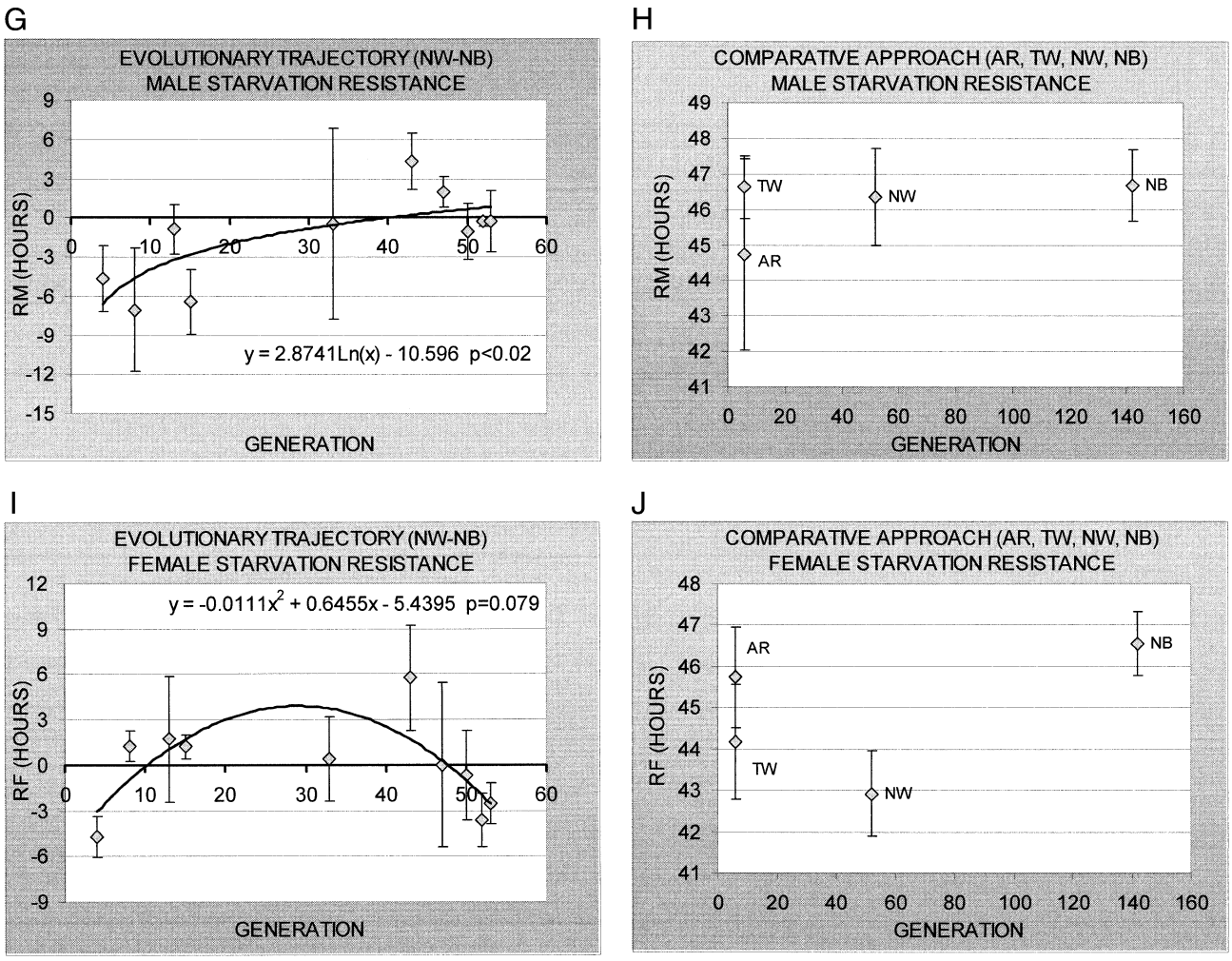

FIG. 1. Continued.

vironment. Such a comparison of comparative and temporal methods is the goal of the present study.

We have been studying adaptation to the laboratory in *Drosophila subobscura* Collin since 1990. At present, we have populations that have been introduced to the laboratory at three different times (1990, 1998, and 2001), with the most recent samples (both in 2001) being from two different natural locations. Thus, four independent founding events led to the laboratory populations studied here.

The general aim of this study is to discover to what extent evolutionary change can be inferred correctly from the comparative method, that is, from simultaneous comparisons of disparate populations. More specifically, two subsidiary questions will be addressed: (1) Given that a clear temporal pattern of convergence occurred in our prior studies of laboratory adaptation (Matos et al. 2000a, 2002), can we infer convergence from the comparative data? (2) Are the evolutionary dynamics inferred from the comparative approach similar to those inferred in the temporal study?

To answer these questions, we carried out a temporal study involving periodic assays of the first 53 generations of laboratory adaptation in stocks founded in 1998, plotting the temporal data against the generation since introduction to the laboratory. We also carried out a comparative study of four disparate sets of laboratory populations, plotted as a function of the number of generations since their founding. The data from the two studies were then used to construct two sets of best-fit models, one set for each method. These models were then compared.

MATERIALS AND METHODS

Founding of the Control Population

The control population of *D. subobscura* was obtained in a pine wood near the village of Sintra, Portugal, from several collections over two days, from early morning until late afternoon in January 1990. About 140 females and 50 males were collected. From the time the population was brought to the laboratory, it was maintained in the general conditions described below (see also details in Matos et al. 2002).

Founding of the Experimental Populations

In March 1998, about 300 females and 280 males were collected from the aforementioned pine wood. These were

TABLE 1. Models that fit the data best in the comparative study (three assays, corresponding to generations 4, 6, and 7 of TW and AR) and the evolutionary trajectories of NW-NB (data of 10 assays covering generations 4–53). A1R, age of first reproduction; F7, fecundity of the first week of life; F12, fecundity between days 8 and 12; RM and RF, male and female starvation resistance. g4, g6, and g7 are the three assays used for the comparative study. The symbol given in front of each model indicates the corresponding degree of significance (*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ‡$0.05 < P < 0.1$).

Generation (TW, AR)	Replicates	A1R	F7
Comparative study			
4	1	no model	$y = 21.9021\ln(x) + 15.498$‡
4	2	$y = -0.3917\ln(x) + 4.9581$‡	$y = 16.349\ln(x) + 14.021$*
4	3	$y = -0.3688\ln(x) + 4.3645$*	$y = 1.1612x^2 - 0.004(x) + 42.485$**
All data assay g4		$y = -0.4362\ln(x) + 4.8041$***	$y = 19.79\ln(x) + 15.355$***
6	1	no model	$y = 24.873\ln(x) + 13.028$*
6	2	$y = -0.4431\ln(x) + 5.0813$‡	$y = 22.752\ln(x) + 14.3$**
6	3	no model	$y = 25.663\ln(x) + 7.2259$**
All data assay g6		$y = -0.3954\ln(x) + 4.9175$***	$y = 24.429\ln(x) + 11.518$***
7	1	$y = -0.4686\ln(x) + 5.0458$**	$y = 18.781\ln(x) + 17.657$**
7	2	no model	$y = 20.833\ln(x) + 11.322$*
7	3	$y = -0.4239\ln(x) + 5.0575$**	$y = 23.737\ln(x) + 5.6757$*
All data assay g7		$y = -0.4277\ln(x) + 5.0324$***	$y = 21.117\ln(x) + 11.552$***
All data of three assays		$y = -0.414\ln(x) + 4.9013$***	$y = 21.692\ln(x) + 13.201$***
Evolutionary trajectories			
	1	$y = -0.2545\ln(x) + 1.2311$‡	$y = 20.207\ln(x) - 86.101$**
	2	no model	$y = 0.5966x - 54.374$*
	3	no model	$y = 13.037\ln(x) - 78.876$‡
All replicates		no model	$y = 15.153\ln(x) - 79.692$***

the founders of a new laboratory population. By 1998, the control population was in its 90th generation.

The study was replicated five times, both for the controls (hereafter called NB) and the recently introduced populations (NW; see details in Matos et al. 2002). The data presented here concern only replicates 1–3 of both sets of populations, because the others were lost before our last assays were done.

In October 2001, two new populations were founded: one (TW) from the same place where the founders of the NB and NW populations were collected (Sintra), and another (AR) from a pine wood in Arrábida, a natural reserve about 50 km away from Sintra and on the other side of the Tagus River. The TW population was founded from 110 females and 44 males and the AR population from 59 females and 24 males. Similar methods were applied to obtain three replicate populations from each of these foundings. By this time, the NB and NW populations were in their 136th generation and 46th generations, respectively. The comparative study was carried out when the TW and AR populations were in their fourth, sixth, and seventh generations.

Culture Methods

As described in Matos et al. (2000a), the flies were maintained in an incubator at 18 ± 1°C, with a 12:12 L:D photoperiod (except during handling, which occurred at room temperature, in general around 22°C). The culture medium was composed of agar, corn meal, dead brewer's yeast, charcoal coloring, and nipagin. Flies were kept in 10 × 2-cm vials, with an adult density of about 50 individuals per vial. Larval densities were controlled by placing 60–80 eggs in each vial. The reproductive regime involved discrete generations, with a generation time of 28 days (see details in Matos et al. 2000a, 2002). Since development (egg to imago) lasted 18–22 days, flies reproduced at a relatively early age, around the age of peak fecundity.

Life-History Traits Assayed

Fecundity characters.—Flies were transferred daily in mated pairs to laying vials containing freshly prepared medium. The total number of eggs laid per female was counted every day for the first 12 days of the imago's life.

Age of first reproduction.—This trait was estimated as the number of days between emergence and the first day of egg laying.

Starvation resistance.—After the fecundity assay, mated pairs were placed in vials with a nonnutritive medium (plain agar) and deaths were recorded every 6 h.

Assay Methods

In each assay, the mating pairs (formed under CO_2 anesthesia) of the samples of the several replicate populations were randomized between racks in the incubator. Samples were distributed in the racks as a function of their arbitrarily assigned numbers (e.g., NB_1 sample in the same racks as NW_1, AR_1, and TW_1, etc.). Each rack therefore contained a sample (or subsample) of one of the replicate populations of each regime. In each rack the samples were distributed in alternate rows, the order changing from rack to rack. We nevertheless thoroughly tested for spatial autocorrelations with no indication of any bias due to spatial assignment.

After the 12 days of egg counting, the mating pairs were assayed for starvation resistance. Assays involved in the temporal study covered the first 53 generations after foundation of the NW population (generations 4, 8, 13, 15, 33, 43, 47, 50, 52, 53, and corresponding contemporaneous generations of the NB, control populations). Thus, for each generation tested we had paired data (e.g., NW_1 in its generation 4 with NB_1 in its generation 94) that were used for evolutionary trajectories (see below). The last three generations assayed also involved populations TW and AR, in their fourth, sixth,

TABLE 1. Extended.

F12	RM	RF
no model	no model	$y = -0.046x + 46.32$*
no model	$y = 0.0012x^2 - 0.1681x + 33.300$‡	no model
no model	$y = -1.4352\ln(x) + 37.183$**	no model
$y = 5.7225\ln(x) + 106.91$‡	no model	no model
$y = 12.1604\ln(x) + 102.3317$*	no model	no model
no model	no model	no model
$y = 16.788\ln(x) + 92.37$*	no model	no model
$y = 12.103\ln(x) + 101.86$***	no model	no model
$y = 12.596\ln(x) + 66.336$‡	no model	$y = 0.0013x^2 - 0.2077x + 41.331$*
$y = 13.813\ln(x) + 63.83$‡	no model	$y = 0.0349x + 41.922$**
$y = 10.669\ln(x) + 67.551$*	no model	no model
$y = 12.36\ln(x) + 65.906$***	no model	no model
$y = 9.2638\ln(x) + 94.391$***	no model	no model
$y = 1.3702x - 58.847$**	$y = 4.0123\ln(x) - 12.232$‡	$y = -0.0253x^2 + 1.5165x - 11.3$‡
$y = 0.6536x - 50.007$‡	no model	no model
$y = 15.189\ln(x) - 73.866$*	no model	no model
$y = 18.471\ln(x) - 81.932$***	$y = 2.874\ln(x) - 10.596$*	$y = -0.0112x^2 + 0.6455x - 5.4395$‡

and seventh generation after foundation, used for the comparative study. Sample sizes per replicate population ranged between 14 and 21 individuals per generation.

Statistical Analysis

Two types of plots were done, on which all other analyses were based. For the purpose of clarity, hereafter ''temporal plots'' are those involving evolutionary trajectories (i.e., the changes of NW-NB throughout the generations), and ''comparative plots'' are those involving, in a single assay, the plots of the values presented by all the coexisting populations (AR, TW, NW, and NB), in each of the three assays (e.g., in the assay done at generation 4 of TW and AR, NW was at generation 50 and NB at generation 140).

To test for convergence, regression analysis was carried out on both types of plots. For the temporal study the dependent variables were the differences between experimental populations and their controls as usual in experimental evolution (e.g., Rose et al. 1996), and the independent variable the number of generations since foundation of these experimental populations. In this case, convergence will correspond to an approach to the zero value in the differences between experimental and long-established populations. For the comparative study, the dependent variables were the absolute (average) values of each of the populations assayed simultaneously, and the independent variable the number of generations since foundation of each specific population. In this case, convergence will be inferred when, the more recent the populations are, the more they will differ from the longer established populations.

All regressions were Type I least-squares linear regressions (Sokal and Rohlf 1995). For each dataset we estimated which of three regression models—linear, log-linear, and second degree polynomial—presented the lowest *P*-value (higher significance), and retained it for future analysis (except if $P > 0.1$ in all models, in which case no model was considered). We had an a priori interest in testing these models with our data, given both theoretical expectations and our previous results (see Matos et al. 2002). When the model with lowest *P*-value was a second-degree polynomial, we checked that it was indeed the best model under the Akaike's information criterion (AIC), which takes into account the number of parameters involved in the model (e.g., Bieri and Kawecki 2003).

The analysis was done two ways: (1) using the data from all populations for each type of study (i.e., the three replicate pairs of differences between NW and NB in each generation for the temporal study and the three replicate populations derived from each foundation for the comparative study, in each assay done); and (2) using the data of single replicate sets. In the second case, the analysis employed the paired differences between same-numbered replicate populations for the evolutionary trajectories (e.g., NW_1-NB_1) and the absolute values of sets of same-numbered populations for the comparative study (e.g., AR_1, TW_1, NW_1, and NB_1).

When a similar function was found to fit both study types, collective and by replicate, we tested whether the regression coefficients differed between the two methods by means of *t*-tests. For this we used as error the variation between replicates for the temporal analysis and the variation between each set of replicate populations (e.g., AR_1, TW_1, NW_1, and NB_1 being one replicate) in the comparative analysis.

Finally, a test for parallelism of models between the two studies was made using analysis of covariance (ANCOVA), with one factor having two categories, temporal data and comparative data, with generation as the covariate. This was only done with the comparative data of generation 6 of AR and TW (generations 52 and 142 of NW and NB).

We did not adjust *P*-values for multiple testing, specifically sequential Bonferroni adjustments (see Sokal and Rohlf

TABLE 2. *P*-values of comparisons of slopes of log-linear models, estimated as descriptors of evolutionary patterns inferred by the comparative data versus evolutionary trajectories (NW-NB); both *t*-tests (error terms are the differences between the slopes among replicates, df = 4 in all tests) and ANCOVA (using all data/points of the several, not discriminated, replicates within each regime) are used; the symbol given with the *P*-values for the *t*-tests indicates the degree of significance after sequential Bonferroni adjustments for multiple comparisons (* $P < .05$; ‡ $0.05 < P < 0.1$); for ANCOVA only generation 6 was analyzed (df = 1, 38 for tests of parallelism); for details see legend of Table 1.

	NW-NB versus each assay		
t-tests	A1R	F7	F12
NW-NB vs g4	0.023*	0.206	0.08 n.s.
NW-NB vs g6	0.029*	0.026‡	0.409
NW-NB vs g7	0.013*	0.11	0.30

	NW-NB versus assay of generation 6		
ANCOVA	A1R	F7	F12
Within-cell regression	0.00019	0.00	4.00×10^{-6}
Test for parallelism	0.0275	0.0555	0.2624

1995), except when these were at the core of the study—comparisons between temporal and comparative studies (as mentioned in the Results section).

All data analysis was performed using Statistica (StatSoft, Inc., Tulsa, OK).

RESULTS

Evolutionary Trajectories of NW-NB

In Figure 1 we present the temporal changes of age of first reproduction (Fig. 1a), fecundity of the first week of life (Fig. 1c), fecundity between days 8 and 12 (Fig. 1e), and male and female starvation resistance (Fig. 1g,i) for the NW-NB comparison between generations 4 and 53. There is a highly significant fit of the fecundity data to a log-linear model (Table 1). Log-linear models also fit partially to the data of male starvation resistance. Female starvation resistance, however, presents a pattern that is best described by a second degree polynomial, although this polynomial's fit to the data is only marginally significant.

Comparative Approach

Three datasets were analyzed, corresponding to the three assays performed at generations 4, 6, and 7 of the most recently founded (AR and TW) populations. In Figure 1 we present a plot of the values of TW, AR, NW, and NB obtained in the assay done in generation 6, the other plots being quite similar. The data on age of first reproduction and fecundity show a highly significant fit to log-linear models (Table 1; Fig. 1b,d,f). Taken together, these data present a clear pattern of convergence, as do the results of the temporal study.

However, starvation resistance does not show any clear pattern, either for males or females (Fig. 1h,j). Given these results and the purpose of this study, the significance tests of comparisons that follow are with respect to age of first reproduction and fecundity values only.

Evolutionary Patterns Obtained from the Evolutionary Trajectories versus a Comparative Approach

To compare data between the temporal and comparative studies, we focused on the log-linear models for fecundity-related traits. (Table 1 presents some of these models; others are not shown.) The functions defined this way were tested both by *t*-tests and ANCOVA (Table 2).

Of the three traits involved in this analysis, age of first reproduction indicated consistent differences (across assays and tests) of regression coefficients between the temporal and comparative approaches, while both fecundity traits gave nonsignificant differences between approaches in all tests, after sequential Bonferroni correction for multiple testing (Table 2). Fecundity between days 8 and 12 was the trait with the least detectable difference among all tests. Overall, we found similar results for the comparative and temporal approaches for several traits, particularly fecundity between days 8 and 12 (Fig. 1e vs. 1f; Table 2). But there are also dissimilarities, specifically for age of first reproduction (Fig. 1a vs. 1b, Table 2) and for starvation resistance, (Fig. 1g vs. 1h, 1i vs. 1j).

DISCUSSION

Comparative Studies Can Lead to Valid Evolutionary Inferences

Comparative studies have always been a popular tool for evolutionary studies. This was one of the most powerful approaches used by Darwin (1859), and we do not question the heuristic power of comparisons across taxa to generate hypotheses concerning evolutionary processes (Harvey and Pagel 1991).

In our study, we found that a comparative approach, by which we mean the inference of evolutionary processes from the simultaneous comparison of disparate populations, leads to qualitatively similar conclusions about evolutionary processes as an analysis of actual evolutionary trajectories. In particular, there are clear similarities in the results obtained by the two approaches for fecundity, the phenotype with the strongest pattern of convergent adaptation. (See Table 3 for a summary of our results.)

Decoupling between Temporally Dynamic Studies and Static Comparisons among Populations

Both temporal and comparative methods indicate adaptive convergence for fecundity following a log-linear pattern. These log-linear results suggest that fecundity shows progressively slower evolution as it approaches a stable evolutionary and genetic state. Even when we proceed to more detailed comparisons between models obtained from temporal versus comparative studies, no significant differences were detected between them except for age of first reproduction (Table 2).

Several recent studies have used a comparative approach to infer adaptation to the laboratory (Sgrò and Partridge 2000; Hoffmann et al. 2001; Krebs et al. 2001). These studies rely on the assumption that independent populations will undergo similar processes of Darwinian adaptation in their approach to evolutionary equilibrium. This is a questionable assump-

TABLE 3. Answers to the questions addressed with the temporal and comparative data. Data from each of the three replicate populations and from each of the three assays were used to estimate relative frequencies for the temporal studies and for the comparative studies, respectively. Marginally significant results were considered significant in this balance. The percent similarity between studies refers to statistical comparisons of the slopes estimated with the two approaches, involving all replicate populations and assays, as in Table 2. For details, see the legends of Tables 1 and 2.

Trait	Study	Convergence	Similar evolutionary dynamics
A1R	temporal	33%	
	comparative	100%	0%
F7	temporal	100%	
	comparative	100%	66%
F12	temporal	100%	
	comparative	100%	100%
RM	temporal	33%	
	comparative	0%	no common model to compare
RF	temporal	biphasic pattern (33%)	
	comparative	0%	no common model to compare

tion, one that deserves investigation (Matos and Avelar 2001). Not only may founder effects confound results, but also subsequent changes in laboratory conditions, however slight, might render such comparisons of limited value (see Matos et al. 2002).

How then can we explain the results presented here, particularly the remarkable similarities of the fecundity data across both comparative and temporal studies? In our opinion, these similarities are due to the fact that fecundity traits show similar evolutionary dynamics in all the analyses of laboratory adaptation that we have performed (Matos et al. 2000a, 2002). Fecundity has also shown consistency in inferences of evolutionary change during adaptation across laboratories (e.g., see Sgrò and Partridge 2000). This leads to considerable robustness when making comparisons across populations.

The same consistency is not shown by other traits, such as starvation resistance, which have evolutionary trajectories that are poorly defined (our data), lack coherent comparative patterns (Fig. 1h,j; Tables 1, 3), and present disparate results among laboratories (e.g., Hoffmann et al. 2001). For complex evolutionary trajectories, such as those of starvation resistance in *Drosophila*, detailed temporal studies may be the best tool for evolutionary analysis.

The comparative method and experimental evolution both have been criticized for their limitations. The comparative method has been attacked as lacking the power to infer microevolutionary processes from comparisons among populations (e.g., Leroi et al. 1994). With experimental evolution there is a problem of repeatability, especially disparities in the results obtained by different laboratories working on similar problems and more or less comparable experimental methodologies (e.g., Ackerman et al. 2001). Parallel analysis of data from comparative studies and experimental evolution may alleviate the problems that arise when either method is used in isolation. It allows both direct tests of how repeatable experimental evolution is and of how informative comparative patterns can be. With such a combined approach, we may someday relate adaptive processes to diversity.

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