Adaptation to the laboratory environment in *Drosophila subobscura*

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Abstract

Adaptation to a novel environment is expected to have a number of features. Among these is a temporal increase in fitness and some or all of its components. It is also expected that additive genetic variances for these fitness characters will fall. Finally, it is expected that at least some additive genetic correlations will decrease, from positive toward negative values.

In a study of several life-history variables in a *Drosophila subobscura* population sampled from the wild and then cultured in the laboratory, we did not find any such longitudinal trends over the first 29 generations. However, a temporal comparison (over 14 generations) of the later generations of this laboratory-adapted population with a new population, derived from a more recent wild-caught sample, indicated clearly that laboratory adaptation was nonetheless occurring. This study suggests the need for extensive replication and control in studies of the features of adaptation to a novel environment.

Introduction

It is a basic corollary of evolutionary theory that populations exposed to a novel environment are expected to undergo adaptation to that environment, where such adaptation can be defined as increasing mean fitness in the new environment due to genetic change. It is known that this effect is not a mathematical certainty; certain types of population genetic models can produce decreasing fitness in the course of selection (e.g. Pollak, 1978; Lewontin, 1979; Charlesworth, 1980, 1994; Pollak, 1995), and some empirical data support this possibility (e.g. Paquin & Adams, 1983). However, it is a common expectation that selection models will usually have the property of increasing mean fitness, in the absence of severe frequency-dependence, density-dependence, epistasis, etc. (cf. Nagylaki, 1977; Endler, 1986; Williams, 1992).

Associated with adaptation is an expectation that additive genetic variance for fitness will fall, following the lines of Fisher's Fundamental Theorem (e.g. Fisher, 1930; Nagylaki, 1977, 1992; Ewens, 1979; Williams, 1992; Falconer & Mackay, 1996). In the simplest possible scenario, as selection proceeds the additive genetic variance for fitness is supposed to approach zero and adaptation then tends to slow or cease (cf. Maynard Smith, 1989). This inference has been generalized from fitness to its components (e.g. Mousseau & Roff, 1987; Roff & Mousseau, 1987; Falconer & Mackay, 1996), but this generalization is not theoretically certain (discussed further below).

It has also been proposed that the additive genetic correlations between fitness-components should tend to fall as adaptation proceeds, owing to convergence on equilibria of balancing selection involving antagonistic pleiotropy (e.g. Caspari, 1950; Wallace, 1968; Rose, 1982). This expectation is derived from the simple point that alleles having uniformly beneficial effects in the new environment should increase in frequency to a point close to fixation, while alleles that exhibit antagonistic effects in the new environment might meet the conditions for convergence on a genetically polymorphic equilibrium. This type of equilibrium may thereby preserve additive genetic variance for individual fitness-components (Rose, 1982, 1985), contrary to the pattern discussed above. This

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does not mean that all additive genetic correlations between fitness components are expected to be negative at equilibrium, owing to the possible role of genes involved in multiple pleiotropic effects (some positive and some negative), or other genetic associations (Charlesworth, 1990; Holloway *et al*., 1993).

In any case, the expectation of a directional trend of exhaustion of additive genetic variance of fitness as well as its components, and a shift of additive genetic correlations from positive towards negative values remains an open question. Empirical data on these issues are still unclear, whether concerning additive genetic variances of life-history traits (e.g. Holloway *et al*., 1993; Carriere & Roff, 1995; Crnokrak & Roff, 1995; Weber, 1996; Campbell, 1997; Holloway *et al*., 1997) or additive genetic correlations (e.g. Holloway *et al*., 1993; Carriere & Roff, 1995; Roff, 1996; Campbell, 1997). These studies are mostly based on either inferences from the analysis of selective responses or comparative studies between populations. A more adequate test should be a temporal analysis of the genetic architecture at the population level, as we propose to do here.

A possible outcome of the evolutionary process when mechanisms involving positive pleiotropic effects coexist with mechanisms involving negative pleiotropy is the occurrence of a biphasic evolutionary pattern for some traits, i.e. an increase in mean values followed by a depression in later generations since establishment in the novel environment. For example, antagonistic pleiotropy between early fecundity and survival might lead to a later drop in longevity after some generations of culture at early ages only.

It is essential to consider the source of genetic variance allowing the adaptation to a novel environment, i.e. genotype–environment interactions. One scenario is that, in a novel environment, new genetic variability is expressed owing to different effects of alleles that had the same effect in the previous environment (see de Jong, 1990a; Stearns, 1992). In this case the additive genetic variance of fitness is higher in the novel environment compared with the previous one, if the population was close to selective equilibrium in the latter, and some empirical studies fit this expectation (e.g. Holloway *et al*., 1990; Silva & Dykhuizen, 1993; Travisano *et al*., 1995; Guntrip *et al*., 1997). Some of the novel genetic variance is plausibly expressed in loci with positive pleiotropic effects. The most likely outcome is positive, or at least less negative, additive genetic correlations between main fitness components (Rose & Service, 1985; Reznick *et al*., 1986; Clark, 1987). Some empirical studies support this possibility (Service & Rose, 1985; Holloway *et al*., 1990; Guntrip *et al*., 1997). The consequence of this scenario is that, other things being equal, the additive genetic variance of fitness and its components, as well as the additive genetic correlations, will drop throughout the adaptation to the novel environment.

A different scenario, which leads to 'unexpected' evolutionary patterns, is a genetic trade-off between the novel environment and the previous one, specifically if the genotypes with higher fitness in one environment have the lowest in the other (see Via, 1987; de Jong, 1990b; Falconer, 1990; Stearns *et al*., 1991; Leroi *et al*., 1994a,b; Roff, 1997; but see Hawthorne, 1997). Under this scenario, the temporal change of both additive genetic variances and correlations throughout the adaptation to the novel environment may be highly unpredictable (see Charlesworth, 1990; Falconer & Mackay, 1996). The same will obviously apply to the evolutionary patterns of mean values of life history traits.

Nevertheless, the expectation of trends leading to an overall decrease in additive genetic variance and an increase in the expression of genetic trade-offs between life-history traits remains the simplest working hypothesis concerning evolution in a novel environment.

Adaptation to the laboratory has been mostly studied at the phenotypic level, in relation to the changes in mean values of life-history traits. Furthermore, few generations have been analysed, which does not allow a characterization of the trajectory (e.g. Souza *et al*., 1988; Harris & Okamoto, 1991; Economopoulos, 1992; Thomas, 1993; Cacoyianni *et al*., 1995).

Experimental evolution in *Drosophila* has been carried out by selection in a new culture regime. Such experiments show adaptation to novel environments involving high-density conditions (e.g. Mueller & Ayala, 1981; Mueller *et al*., 1993), altered age at reproduction (e.g. Luckinbill *et al*., 1984; Rose, 1984), starvation (e.g. Rose *et al*., 1992), temperature (e.g. Huey *et al*., 1991), etc. Most of these studies are also only at the phenotypic level (but see Hutchinson *et al*., 1991; Engstrom *et al*., 1992). Temporal changes of the genetic architecture of an evolving *Drosophila* population have been analysed more in terms of changes in gene frequencies than of quantitative genetic parameters (e.g. Buzzati-Traverso, 1955; McKenzie *et al*., 1992, 1994; but see Tantawy & El-Helw, 1966; Tantawy & Tayel, 1970).

Adaptation to the laboratory environment has practically not been investigated in *Drosophila*. The few cases that have been published do not characterize the evolutionary trajectories of genetic parameters, being based on few generations or a comparative approach (Dobzhansky *et al*., 1964; Tantawy & El-Helw, 1970; Pascual *et al*., 1990) or involving parallel selective regimes that hamper the interpretation (Tantawy & El-Helw, 1966). More empirical studies about the tempo and mode of the evolution of the mean values and, most of all, genetic parameters of life-history traits as a population adapts to a novel environment are thus needed.

We endeavoured to test whether the predictions referred to above applied to the evolution of *Drosophila subobscura* Collin newly sampled from the wild. Two of these samples were studied, the first in more detail than

the second. Our findings support the notion of adaptation during laboratory culture, but we are unable to test other expectations concerning the adaptive process.

Materials and methods

Source of laboratory samples

Both laboratory cultures of *Drosophila subobscura* were sampled from a pine wood near the village of Sintra, Portugal. Each sample was obtained from several collections, over 2 days, from early morning till late afternoon. About 140 females and 50 males were collected in the sample giving rise to the first laboratory culture, in January 1990, and about 230 females and 100 males were collected for the second, in March 1992.

Culture methods

The flies were maintained at about 18 °C, with a 12-h L : 12-h D photoperiod. Culture medium was similar to that described by David (1959), being composed of agar, corn meal, dead brewer's yeast, charcoal colouring and nipagine. Flies were kept in 10×2-cm vials, with an adult density of around 50 individuals per vial. Larval densities were 60–80 per vial. The reproductive regime involved discrete generations, with egg collection at around 2 weeks 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. CO_2 anaesthesia was used. The culture vials were maintained in the same incubators during the entire period of this study, except during manipulation, when they were handled at room temperature (normally around 22 °C). Adult population sizes were in general higher than 1000 individuals, never dropping below 400.

Assay methods

Fecundity characters

Flies were transferred to laying vials containing freshly prepared medium every day. Total egg-laying for periods of 1 week was used in the data analysis (female's age being estimated using the day of emergence of the imago as reference). Female fecundity records were kept during their entire lives in the half-sib study (see below), which gave additional data on total and mean fecundity, as well as other traits (age of last egg laying, number of eggs laid during the last week of life, number of eggs of the last day of egg laying, number of days without egg laying before death). Females that did not lay a single egg during their entire lifespan were not used in the half-sib study (at most four females having been discarded from each sample).

Age of first reproduction

This trait was estimated as the number of days before first egg laying, counted since the emergence of the imago. In the comparison of new and established populations (from here on called W and B, respectively), we assigned the value 14 for age of first reproduction of the few females (maximum four, in generation 5) that did not lay eggs during the 2 weeks of life covered by the analysis. This way these females (which were mainly of population W) were not discarded from the analysis of this trait.

Longevity

These data were collected in the half-sib study, from the same flies as those used in fecundity assays. Longevity was estimated as the number of days between the emergence of the female and the day in which the female was recorded as dead (this last day included). Females that escaped during the study or died from handling were excluded from all the analysis (never more than 4% of each sample). Females dying during the first 2 weeks of life were also excluded (always less than 5% of each sample, their longevities being deviant from the overall distribution of values).

Time of development

As the females of the half-sib samples did not emerge all in the same day, we estimated this trait as the day of formation of each pair, relative to the time of emergence of the first imagoes. For instance, if a female emerged on the second day after the start of emergence in the whole sample, that female was assigned the value 2 for this trait.

Thorax length

The thorax of the females of the half-sib study was measured as the distance between the most anterior point of the mesonotum and the most posterior point of the scutelum. An arbitrary scale was used, allowing the discrimination of around 15 size-classes.

Starvation resistance

In the second study, after the first 2 weeks of adult life (during which fecundity data were collected), mated pairs were placed in vials with a non-nutritive medium (plain agar) and deaths were recorded every 6 h.

Half-sib studies

The first laboratory population was subjected to a half-sib analysis every 4–6 generations, from the second generation to the 29th. To obtain each sample, we did an extra egg collection, applying the same procedures as for the maintenance of the population. From the first emergence of the imagoes, males and females were kept apart until the end of the fourth day of emergence. At that time, harems of one male and six females each were formed. These were kept for 2 weeks, after which the dams were

placed in individual vials and an egg collection over 48 h took place. Finally, we removed, when required, some of the eggs from the vials to minimize differences in larval densities (around 15–25 larvae per vial, in general).

Development of the half-sib families took place in the same incubator. Flies from the first day of emergence were in general discarded, formation of pairs for assay starting on the following day and continuing for three consecutive days. CO_2 anaesthesia was only used in the formation of mating pairs. Matings were made at random over the whole collection of families. Each sample was formed of 67 or 68 families (with family sizes ranging between two and six half-sibs each). As families were not randomized between racks, a higher block effect was defined to extract common environmental effects. Flies were transferred to fresh vials daily. Females remained with males until 30 days of life, by which time all males were removed (until that age dead males were replaced by other males of the same age). This procedure was necessary because males had a shorter life than females in these samples. A preliminary study of this population did not indicate a significant effect of removal of males on female egg laying.

Comparison of new and established samples

After 24 generations had elapsed from the formation of the first population, a new sample was obtained, as described above, in order to compare this new laboratory population with the established one. For generations 1–14 of the second population, a variety of characters were compared in mated pairs collected from the two laboratory cultures, especially fecundities during the first 2 weeks of life, as well as starvation resistance of males and females. In each assay, the samples of the two populations were randomized between racks in the incubator. Daily transfers and egg countings took place during the first 2 weeks of adult life, after which the mating pairs were placed in plain agar to assay starvation resistance. Finally, when the most recent culture was in its 47th generation, and the oldest was in its 71st generation, another comparison was made, using the same traits.

Data analysis

All data analysis was performed using IBM-compatible computers running SYSTAT, STATISTICA and EXCEL. No data interpolation was used in unbalanced ANOVA. Half-sib analysis involved a two-level nested ANOVA, with a higher block level (corresponding to the racks), a sire level (corresponding to the families within racks) and a dam (or error) level (corresponding to the half-sibs within each family). The formulas used to estimate additive genetic variances and correlations were those given by Kempthorne (1957). Sampling errors of additive genetic estimations were calculated using the Hammond & Nicholas (1972) approximate formulas. Estimations of genetic correlations involved only the females for which data were available for both traits. All regressions were Type I least-squares linear regressions.

Results

Half-sib analysis

For sake of brevity, we discuss in detail the analysis of only nine of the 18 measured traits, and only 18 trait correlations, particularly those that were considered *a priori* more interesting in terms of laboratory adaptation.

Table 1 gives the sample sizes as well as the degrees of freedom of the several levels of the nested ANOVA model used in the estimation of components of additive genetic variance and covariance, as well as a list of the traits on which we will focus our analysis.

Sample mean values

Table 1 gives the sample mean values of the traits on which we focus our analysis. The fecundity of the third week of life was close to peak fecundity, while that of the sixth week of life can be considered a 'late' fecundity. To minimize sampling effects due to mortality, fecundities after that age were not used. From least-squares linear regression, none of the traits in Table 1 showed a statistically significant change of means during the 29 generations of the sib analysis study. Further tests involving nine other traits gave significant linear trends in three cases (number of days without egg laying before death, two-tailed, $P = 0.03$; fecundity in the last seven days of life, one-tailed, $P = 0.03$; and fecundity on the last day of egg laying, one-tailed, $P = 0.05$). Considering the overall number of statistical tests involved (18), any of the sequential Bonferroni techniques (Sokal & Rohlf, 1995) lead to the conclusion that none of the linear correlations is actually significant. Moreover, the numbers of positive and negative regression coefficients do not depart significantly from a binomial distribution. Total regression analysis including all mean values of Table 1 as data points (each divided by the average mean of the trait across generations, to reduce scale effects), as well as within-cell regression in an analysis of covariance (in which the traits were defined as categories and generations as the covariate), gave no indication of significance. This conclusion was reinforced by canonical analysis of subsets of traits, which also did not give consistent significance. Finally, we examined the amount of variance explained by regression, and saw no clear tendency for traits supposed to be more connected with fitness (e.g. early or peak fecundity) to have greater temporal variation. In spite of all these results, the average statistical power of the regressions (around 24%, Zar, 1984) indicates that it would have been possible to detect a higher number of significant regressions than the number actually found.

Table 1 Sample sizes and related data, sample means and confidence intervals (± $t_{(0.05,\ d.f.B)}$ × error) of several traits analysed in each half-sib sample (columns correspond to generations); *n* – sample size; B,S,D – degrees of freedom of the block, sire and dam level; d_0 – coefficient of the variance and covariance component at the sire level; NF42 – subsample size surviving more than 42 days. All the following symbols refer to sample means and respective confidence intervals: F7, F21 and F42 – fecundity of the first, third and sixth week of life; TL – thorax length; A1R – age of first reproduction; FT and FM – total and mean fecundity; L – longevity; TD – time of development.

	Generation						
	2	6	10	15	19	25	29
n	235	342	360	335	376	375	374
B	12	16	16	16	16	16	16
S	55	50	51	51	51	51	51
D	167	275	292	267	308	307	306
d_0	3.411	5.014	5.238	4.819	5.478	5.288	5.409
NF42	235	322	352	301	370	344	302
F7	12.97 ± 2.46	43.30 ± 3.37	65.39 ± 4.93	48.92 ± 4.14	43.74 ± 3.07	46.20 ± 5.82	42.89 ± 5.33
F21	61.04 ± 4.92	114.17 ± 4.01	103.4 ± 3.4	79.62 ± 4.01	110.5 ± 3.3	130.95 ± 4.53	76.43 ± 3.35
F42	64.65 ± 5.17	78.10 ± 4.35	68.48 ± 5.65	36.89 ± 1.78	66.01 ± 4.03	65.38 ± 4.17	54.10 ± 3.99
TL	33.33 ± 0.13	33.28 ± 0.16	33.35 ± 0.10	33.45 ± 0.11	33.59 ± 0.14	33.43 ± 0.15	33.31 ± 0.17
A1R	6.48 ± 0.49	4.26 ± 0.16	3.78 ± 0.22	4.28 ± 0.51	4.37 ± 0.31	4.28 ± 0.28	4.01 ± 0.24
FT	567.0 ± 40.8	726.2 ± 26.1	801.7 ± 51.1	489.3 ± 25.4	686.5 ± 35.30	705.4 ± 35.50	494.3 ± 36.9
FM	6.496 ± 0.236	9.38 ± 0.39	8.52 ± 0.26	6.69 ± 0.21	8.28 ± 0.22	9.29 ± 0.46	8.21 ± 0.40
L	87.92 ± 4.27	82.16 ± 3.44	95.48 ± 4.09	81.19 ± 4.02	84.66 ± 3.98	81.50 ± 5.90	61.83 ± 3.87
TD	2.00 ± 0.14	2.61 ± 0.16	2.46 ± 0.19	2.46 ± 0.16	2.73 ± 0.15	2.36 ± 0.15	2.26 ± 0.16

Additive genetic variances

Figure 1 shows the estimated additive genetic variances, as well as their standard errors, estimated using Hammond & Nicholas's (1972) formulas. The errors associated with these estimates were in general very high. Only one trait presented a significant, negative linear trend for additive genetic variance – total fecundity ($P = 0.024$, one-tailed test). Regressions involving nine other traits were not significant either, except for the fecundity of the fifth week of life (negative slope, $P = 0.003$, one-tailed), which was the only trait retaining significance with a sequential Bonferroni test. On balance, as for the character means, the additive genetic variances do not give any indication of directional trends. The number of positive and negative regression coefficients do not depart significantly from a binomial distribution. Total regression analysis (standardized values) and within-cell regression in an analysis of covariance were not significant. Canonical analysis only presented a significant value for a set including total fecundity, fecundity of the third week and longevity ($P < 0.04$) which, given the results mentioned above, does not constitute additional information. Finally, the order of magnitude of the amount of variance explained by regression has no clear connection with the supposed relevance of the traits in terms of fitness. Here too the average power of the linear regressions (25%, Zar, 1984) would have allowed us to detect a higher number of significant regressions.

Additive genetic correlations

Some of the additive genetic correlations among the traits defined in Table 1 are presented in Fig. 2, together with their associated standard errors (Hammond & Nicholas, 1972). The errors were in general high. In the overall regression analysis, there were no clear directional temporal patterns in the correlations. Only two gave indications of being significant – the correlation between longevity and total fecundity (negative slope, $P = 0.04$, one-tailed), and the correlation between fecundity of the third week of life and time of development (positive slope, $P = 0.02$, one-tailed test). Additional regressions with 26 other correlations gave only one other significant regression, the correlation between fecundity of the first week of life and thorax length ($P = 0.02$, two-tailed). Considering the whole set of estimates, there is no indication of a tendency for more positive or negative regression coefficients, relative to expectations from a binomial distribution. Additionally, we did a total regression analysis using the 18 sets of correlations of Fig. 2 as data points, and within-cell regression in an analysis of covariance, none of which gave significant results. Canonical analysis only presented a significant value for the following set of correlations: fecundity of the first week of life vs. longevity as well as fecundity of the sixth week of life, longevity vs. time of development and longevity vs. total fecundity ($P < 0.001$), which is not additional information, considering the univariate results. Finally, the order of magnitude of the amount of variance explained by regression presented no clear connection with the expected change considering expectations of relevance of the correlations in terms of fitness (e.g. early fecundity vs. longevity changed less than 10 other, supposedly less relevant, correlations). The average statistical power of the full set and of the subset

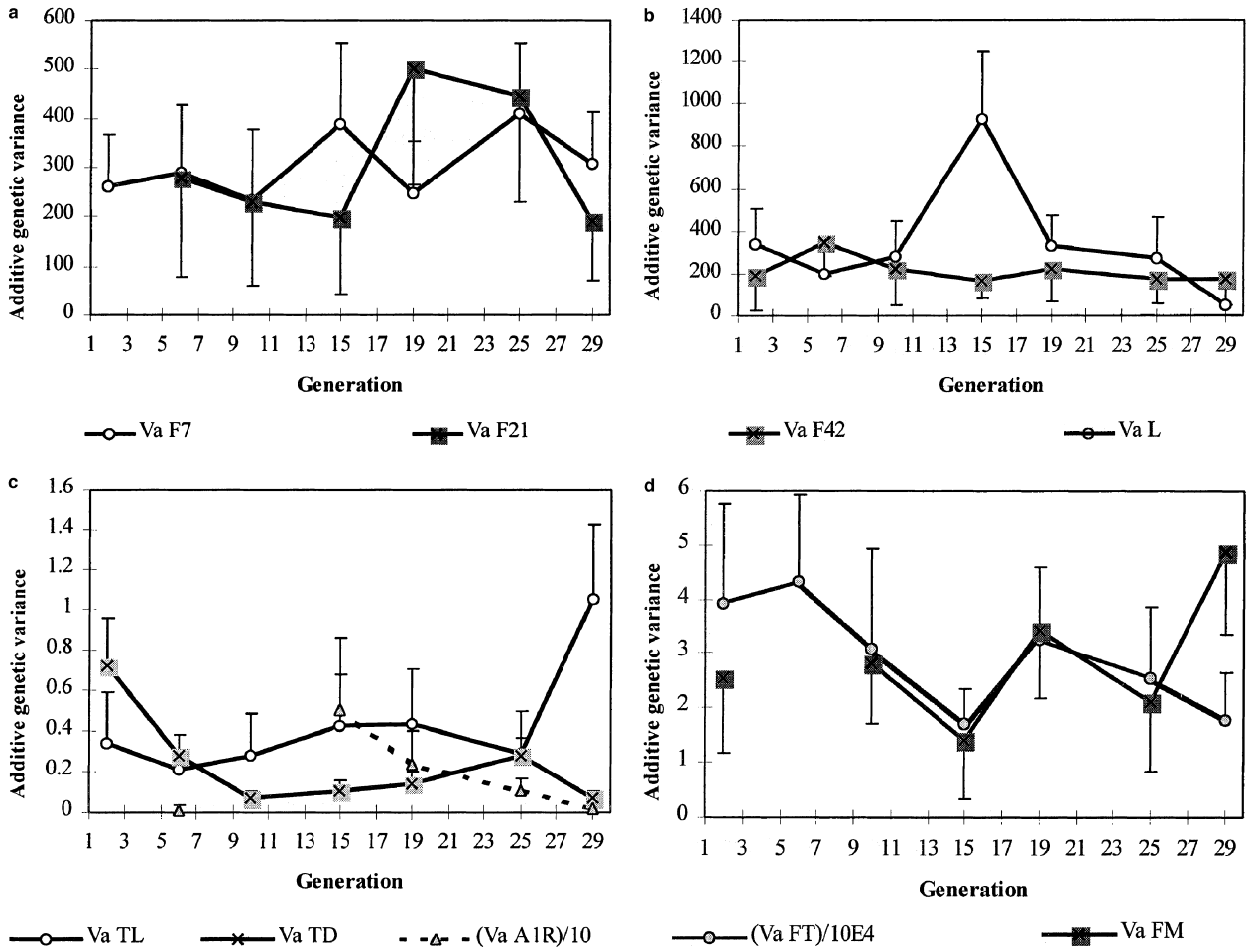

Fig. 1 Temporal change of additive genetic variances. (a) Fecundity of the first week of life (F7) and fecundity of the third week of life (F21). (b) Fecundity of the sixth week of life (F42) and longevity (L). (c) Thorax length (TL), time of development (TD) and age of first reproduction (A1R, variance values and error bars divided by 10). (d) Total fecundity (FT, variance values and error bars divided by 10 000) and mean fecundity (FM). For clarity of presentation only the upper or lower limit of the error bars is presented for each estimate.

represented in Fig. 2 was 15–18%, which would allow a higher number of significant regressions to be detected.

Comparison between new and established population

In Table 2 we present the mean values and confidence intervals (95%) of the traits analysed in several samples of populations B and W, the first (B) established in the laboratory for 24 generations before introduction of the new, W population. The differences that are significant (two-tailed unpaired *t*-tests) are indicated. During the first 14 generations of the W population, both populations were sampled every other generation or in consecutive generations. Finally, they were compared when population W was in its 47th generation and population B in its 71st generation.

Evolutionary trajectories during the first 14 generations of population W

There were significant differences in age of first reproduction and early fecundities during the first nine or 10 generations, and a clear reduction in the significance of the difference throughout the generations. Starvation resistance both of females and males did not present such a pattern, few differences being significant and some appearing at later generations.

In the analysis of the temporal changes in differences between the two populations we only consider the data from generations 4–14. The first two generations were discarded to avoid maternal effects (see Jenkins & Hoffmann, 1994). Like these, the third generation was also discarded from the regression analysis because during this generation environmental effects caused by the recent foundation were still possible, owing to a

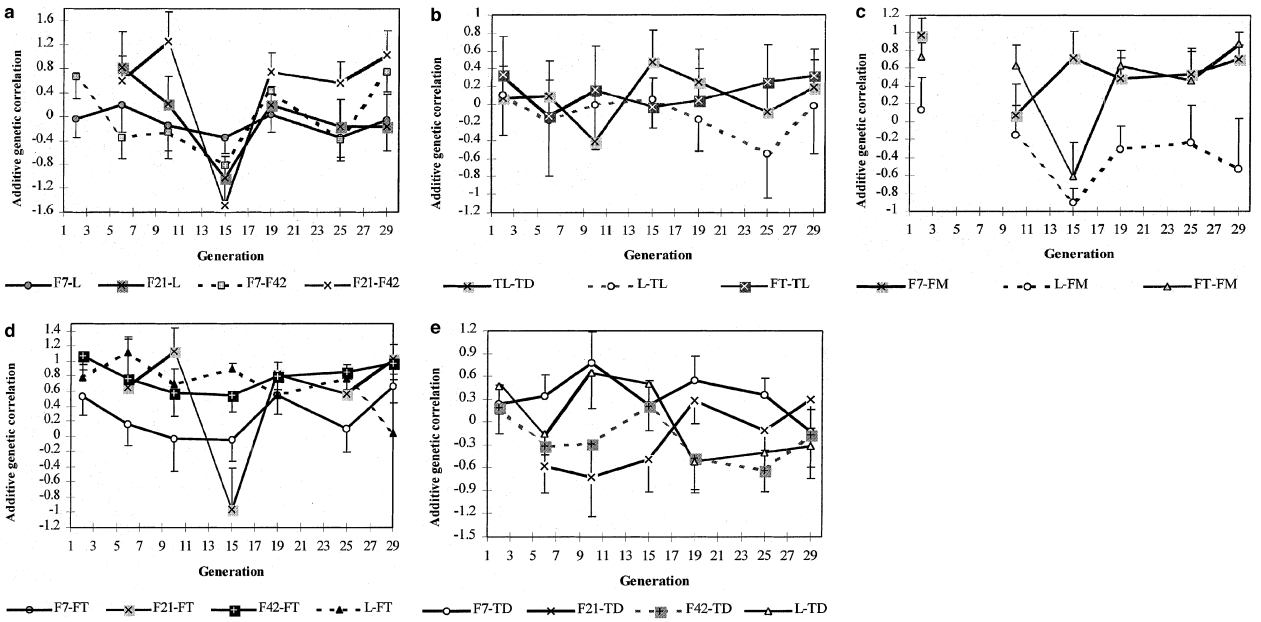

Fig. 2 Temporal change of additive genetic correlations. (a) Between F7 and L, F21 and L, F7 and F42, and F21 and F42. (b) Between TL and TD, L and TL, and FT and TL. (c) Between F7 and FM, L and FM, and FT and FM. (d) Between F7 and FT, F21 and FT, F42 and FT, and L and FT. (e) Between F7 and TD, F21 and TD, F42 and TD, and L and TD. See explanation of symbols in the legend to Fig. 1. Only the upper or lower limit of the error bars is shown for each estimate.

Table 2 Mean values and confidence intervals ($\pm t_{(0.05,n-1)} \times$ error) of the samples of population B and W. In the first column the number of generations corresponds to that of population W (generation B = generation W + 24); *n* – sample size; A1R – age at first reproduction; F7 and F14 – fecundity of the first and second week; RF and RM – female and male starvation resistance (hours). The significant differences (two-tailed unpaired *t*-tests) between W and B are shown in the W lines: ***$P < 0.01$; **$0.01 < P < 0.05$; *$0.05 < P < 0.1$.

Generation	Population	*n*	A1R	F7	F14	RF	RM
1	B	24	3.33 ± 0.30	74.21 ± 9.91	138.92 ± 9.67	42.5 ± 3.88	35 ± 3.23
	W	22	5.5 ± 0.58***	18 ± 6.66***	94.77 ± 11.58***	42.27 ± 3.43	37.09 ± 4.32
2	B	23	4.22 ± 0.34	39.56 ± 8.23	119.74 ± 9.56	31.83 ± 1.82	15.13 ± 2.70
	W	22	6.73 ± 0.71***	7.91 ± 6.26***	58.41 ± 11.01***	30.54 ± 2.45	13.636 ± 2.62
3	B	23	3.87 ± 0.33	41.70 ± 7.84	104.52 ± 9.01	38.09 ± 2.16	30 ± 3.41
	W	24	5.12 ± 0.55***	18.79 ± 6.54***	65.83 ± 10.55***	43.5 ± 2.39***	32 ± 2.86
4	B	24	4.75 ± 0.66	32.08 ± 10.13	86.38 ± 10.97	38.5 ± 3.07	26 ± 2.32
	W	22	8.09 ± 1.58***	11.64 ± 8.5***	40.82 ± 13.9***	39 ± 2.69	24.273 ± 2.09
5	B	24	5.21 ± 1.15	20.5 ± 5.79	73.75 ± 14.79	46.75 ± 3.34	35.25 ± 3.02
	W	24	8.58 ± 1.55***	5.42 ± 5.55***	30.75 ± 11.59***	44.25 ± 4.41	25.75 ± 3.84***
7	B	24	4.62 ± 0.43	18.5 ± 5.95	78.21 ± 9.35	35.5 ± 2.47	25.5 ± 2.61
	W	24	7.5 ± 1.51***	6.71 ± 5.03***	40.42 ± 13.16***	37.25 ± 1.97	21.75 ± 2.46**
9	B	43	5.26 ± 0.58	16.02 ± 4.83	110.81 ± 11.59	45.07 ± 1.58	37.12 ± 1.98
	W	44	7.09 ± 0.96***	5.84 ± 3.12***	75.84 ± 12.44***	47.73 ± 1.76**	37.36 ± 2.48
10	B	24	4.08 ± 0.50	46.58 ± 10.86	108.29 ± 13.3	42.5 ± 4.16	30.75 ± 3.28
	W	24	4.46 ± 0.94	42.42 ± 12.21	85.833 ± 15.61**	43.5 ± 3.53	26.25 ± 3.06**
12	B	23	5.35 ± 1.13	29.83 ± 11.38	108.13 ± 21.23	40.96 ± 2.89	28.35 ± 4.20
	W	23	4.39 ± 0.50	36.04 ± 10.84	104.39 ± 13.63	43.65 ± 2.13	32.09 ± 2.55
14	B	18	4.72 ± 1.06	32.78 ± 9.86	92.94 ± 17.0	39.56 ± 3.84	22.67 ± 4.16
	W	22	4.91 ± 0.63	26.5 ± 9.86	85.64 ± 11.88	44.91 ± 6.11	27.46 ± 5.19
47	B	48	4.12 ± 0.66	63.58 ± 9.78	148.96 ± 16.75	64.25 ± 5.54	49 ± 2.7
	W	41	4.76 ± 0.78	52.07 ± 9.24*	137.05 ± 18.47	56.34 ± 6.18*	47.27 ± 4.7

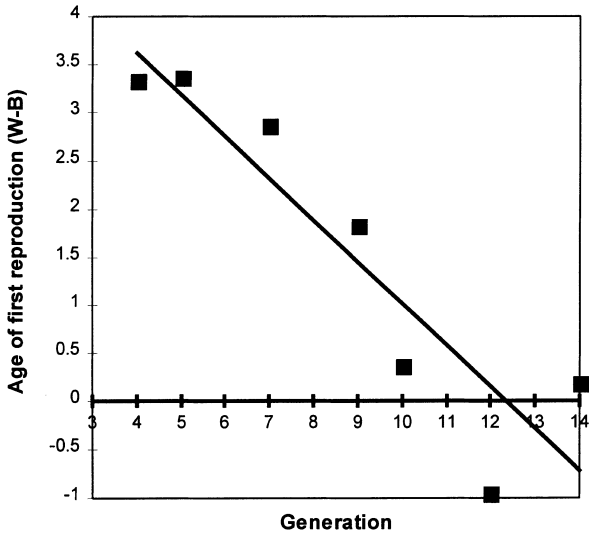

Fig. 3 Temporal change of the differences between populations W and B in mean values of age of first reproduction (days). Generations 4–14 of W are covered. The linear regression is significant: $y = -0.4326x + 5.346$; $P < 0.005$ (two-tailed).

differential contamination of media between the two populations. This problem was not detected from the fourth generation on. Figures 3–5 present the results for age at first reproduction, fecundity during the second week of life and starvation resistance (of females and males), respectively.

It is clear from these figures that there is a linear trend in the differences between the two populations, suggesting a progressive improvement of the W population

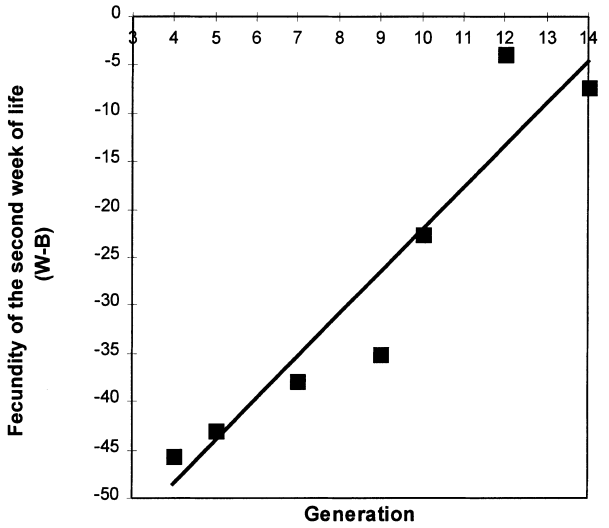

Fig. 4 Temporal change of the differences between populations W and B in mean values of fecundity (number of eggs) of the second week of life. Generations 4–14 of W are covered. The linear regression is significant: $y = 4.4x - 66.176$; $P < 0.002$ (two-tailed).

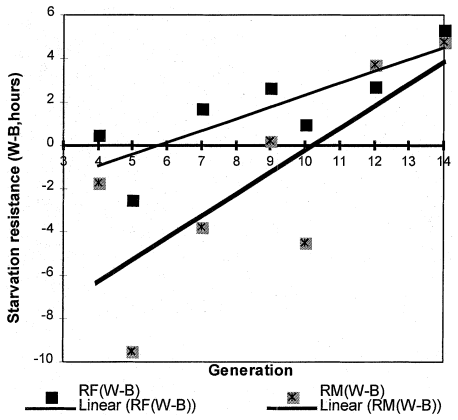

Fig. 5 Temporal change of the differences between populations W and B in mean values of starvation resistance (hours). RF: females; RM: males. Generations 4–14 of W are covered. $RF = 0.543x - 3.0952$; $P < 0.05$, two-tailed; $RM = 1.029x - 10.496$; $0.05 < P < 0.1$, two-tailed.

throughout the 10 generations considered in this analysis. However, there is a considerable difference between the temporal pattern of fecundity and starvation resistance, particularly in females. While the differences between W and B in fecundity (and related traits, like age of first reproduction) are becoming progressively smaller, the differences in female starvation resistance from generation 7 on become progressively bigger, with population W being more resistant than population B, at least until generation 14. There are suggestions that the same pattern occurs in males as well, though later.

Comparison between the two populations at a more advanced generation

In generation 47 of the more recent W population (corresponding to generation 71 of the B population – see Table 2) both fecundity in the first week of life and female starvation resistance present marginally significant differences, with B > W. Other traits analysed did not present significant differences. The suggested tendency for W females (and eventually males) to present higher values of starvation resistance during some of the first generations of adaptation is thus no longer expressed at a more advanced generation, which suggests a later convergence of values between the two populations.

Discussion

Overview of the results

The results of the sequential sib analysis reveal no obvious pattern of means, genetic variances or genetic correlations during 29 generations of domestication to laboratory conditions. It is interesting to note that some striking patterns might have been inferred if our sib

analyses had been stopped at generation 15 (Fig. 2). Specifically, over just 15 generations, observed changes in genetic correlations meet the expectation of a progressive decrease in the frequency of alleles having positive pleiotropic effects (17 out of 18 linear regressions present a negative slope in terms of fitness, which is a highly significant departure from expectations of a binomial distribution).

Nevertheless, an analysis involving more generations reveals that this pattern was not sustained over the entire period of observation. It is an open question whether this loss of the generation 15 patterns is a result of genuine nonlinear effects of laboratory adaptation – see below – or whether it is merely an effect of statistical fluctuation. The latter would presumably have been revealed if there had been replication of evolving lines, as well as controls that had already adapted to the laboratory. It is nevertheless important to note that the average statistical power of the regressions was enough to detect a higher number of linear trends.

In any case, the results over 29 generations clearly do not fit the simplest expectations of the evolutionary theory discussed in the Introduction. That laboratory adaptation does nonetheless occur is revealed by the striking convergence of fecundity traits of B and W lines in the second group of experiments.

Quantitative genetics of adaptation

A laboratory is just another environment in the evolutionary history of a population, where a general pattern of adaptation might be expected.

In the particular example afforded by our experiments, it was possible to infer adaptation when a population already further along in the evolutionary process was compared with one just beginning the process. It was not, however, possible to detect any feature of adaptation, whether phenotypic or quantitative genetic, in a single population undergoing the process on its own. This was the case despite the repeated use of sib analysis, using multiple characters. These results suggest that the detection of adaptation will hinge critically on the availability of controls and replicates.

To our knowledge, almost no studies have tracked the evolutionary trajectories of the genetic architecture of life history traits during extensive adaptation (see Introduction). Had such a detailed analysis been developed in the selective experiments already done, we would suggest that it is open to question whether they would have been able to detect the expected patterns of change in genetic variation that we were unable to detect here.

In any case, population genetics theory already indicates that typical quantitative genetics expectations may not be met. Variances might increase under selection if beneficial alleles start at low frequencies and then take on intermediate frequencies during selection. As mentioned in the introduction, this may be expected if there is a genetic trade-off between the novel and the previous environment (e.g. see Leroi *et al*., 1994b). This, together with the possible coexistence of genetic mechanisms affecting several life-history traits with a multitude of pleiotropic effects, may render any evolutionary pattern possible, and short-term analysis of little predictive power (see Charlesworth, 1990). For now, we offer no firm conclusions on the actual dynamics of genetic variation during adaptation.

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