Effects of assay conditions in life history experiments with *Drosophila melanogaster*

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Abstract

Selection experiments with *Drosophila* have revealed constraints on the simultaneous evolution of life history traits. However, the responses to selection reported by different research groups have not been consistent. Two possible reasons for these inconsistencies are (i) that different groups used different environments for their experiments and (ii) that the selection environments were not identical to the assay environments in which the life history traits were measured. We tested for the effect of the assay environment in life history experiments by measuring a set of *Drosophila* selection lines in laboratories working on life history evolution with *Drosophila* in Basel, Groningen, Irvine and London. The lines measured came from selection experiments from each of these laboratories. In each assay environment, we measured fecundity, longevity, development time and body size. The results show that fecundity measurements were particularly sensitive to the assay environment. Differences between assay and selection environment in the same laboratory or differences between assay environments between laboratories could have contributed to the differences in the published results. The other traits measured were less sensitive to the assay environment. However, for all traits there were cases where the measurements in one laboratory suggested that selection had an effect on the trait, whereas in other laboratories no such conclusion would have been drawn. Moreover, we provide good evidence for local adaptation in early fecundity for lines from two laboratories.

Introduction

To understand the evolution of life histories, we must know the relations between life history traits that constrain the simultaneous evolutionary change of two or more of these traits. These constraining relationships can be estimated via phenotypic correlations, experimental manipulation, genetic correlations from sib analysis or as correlated responses to selection. Selection experiments on life histories in *Drosophila melanogaster* have contributed to the controversy about correlated responses to selection. Inconsistencies concerning correlated responses arose from experiments about the evolution of senescence. One issue has been whether selection for increased lifespan in *Drosophila* inevitably results in a decrease in fecundity early in life, as expected under William's (1957) theory of antagonistic pleiotropy. Several studies (Luckinbill *et al.*, 1984; Rose, 1984; Zwaan *et al.*, 1995b; Partridge *et al.*, 1999) found a decline in early fecundity correlated with the responses to selection

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for late reproduction or longevity and therefore seem to support this theory. In contrast, Partridge & Fowler (1992) found an increase in longevity but no correlated decrease in early fecundity in populations selected for late reproduction.

Another issue concerns the correlated response of development time to an evolutionary change in lifespan (or vice versa). An increase in development time has been found in some populations selected for late reproduction (Partridge & Fowler, 1992; Chippindale *et al*., 1994). However, direct selection on longevity (Zwaan *et al*., 1995b), on development time (Zwaan *et al*., 1995a; Nunney, 1996), and on body size (Hillesheim & Stearns, 1992) did not yield a positive correlation between lifespan and development time.

Further inconsistencies arose in the relationship between development time and body size. Direct selection for development time showed a clear response in body size (Zwaan *et al*., 1995a; Nunney, 1996). This relationship, however, was not supported by selection for small body size, which did not result in a shorter development time (Partridge & Fowler, 1993).

There are several potential reasons for these differences in the results of the groups working on life history evolution using *Drosophila*. First, the *Drosophila* populations used in these studies may have differed in genetic variances and covariances and thus in the direct and correlated responses to selection. Second, in some experiments there might have been inadvertent selection on other life history traits (Partridge & Fowler, 1992; Roper *et al*., 1993; Chippindale *et al*., 1994; Zwaan *et al*., 1995a). Third, inbreeding in some lines might have changed the genetic variances and covariances (Rose, 1984) and therefore, the direct and correlated responses to selection. For instance, Roper *et al*. (1993) reported evidence for inbreeding depression in lines selected for age at reproduction (Partridge & Fowler, 1992). Fourth, the environment where the selection experiment was carried out might have influenced the responses to selection. Both direct and correlated responses to selection in life history experiments can depend on the environment in which the selection is performed (Bell & Koufopanou, 1986; Clark, 1987). An effect on the direct response to selection was observed by Luckinbill & Clare (1985), who selected on longevity in *Drosophila* under two juvenile densities and found a response to selection only at high density. Dependence of correlated responses on the selection environment also has been reported by Falconer (1960), who selected on growth in mice in two environments, and by Hillesheim & Stearns (1991), who selected on body mass in *Drosophila* in two larval food environments. Both found quantitative differences between the two environments in the correlated responses to selection.

One reason for differences in the direct and correlated responses to selection in different environments might be that the expression of genetic variance and covariance differs between environments because of genotype–environment interactions (Clark, 1987; Stearns *et al*., 1991). Several studies have shown that genetic correlations between traits can change across environments (Giesel, 1986; Gebhardt & Stearns, 1988; Holloway *et al*., 1990). If genetic correlations between characters constrain their simultaneous evolution, then differences in the expression of genetic correlations in different environments might lead to differences in the simultaneous evolution of the corresponding traits between the different environments (Lande, 1982; Reznick, 1985; Charnov, 1989; Spitze *et al*., 1991).

Fifth, the environment in which the life history is assayed might influence the direct and correlated responses to selection reported. It is this fifth reason that is the focus of this study.

The conclusions drawn from life history studies can depend on the environment where the traits are measured. This point becomes relevant whenever the assay environment is not identical to the environment where the selection was performed and probably applies to some extent to most of the selection experiments on life history evolution with *Drosophila* (Partridge & Barton, 1993). Common differences between assay and selection conditions include adult and larval density, nutrition of the flies and handling. If the effect of the (novel) assay environment differs for lines from different selection regimes, then the differences between selection regimes measured in the assay might not represent the situation during selection. Such an interaction between assay environment and selection regime was reported by Luckinbill & Clare (1986).

An additional complication arises when the conditions that the organisms experience during selection are not the same for the different selection regimes, i.e. if there are differences in the culture conditions between the two treatments other than the differences deliberately imposed. In both Rose's (1984) and Luckinbill *et al*.'s (1984) lines selected for age at reproduction, a negative correlation between fecundity and longevity was measured in one assay environment (that resembled the selection environment of the first regime) but not in another (that resembled that of the second regime: Leroi *et al*., 1994b; Pletcher *et al*., 1997).

We tested whether differences in the assay environment influence the results, and repeated life history measurements with the same lines in different assay environments. Although the motivation for this study arose mainly from experiments about the evolution of senescence, this study addresses the more general question of whether the differences between selection regimes depend on the environment where the life history assay is carried out. This makes our results relevant for all those interested in life history evolution.

We were also able to test whether lines are locally adapted to the conditions of their laboratory of origin. By local adaptation, we mean that lines perform better in the

environment in which their ancestors were selected than do lines that have been selected in other environments.

Methods

Fly stocks and selection procedures are described in web material. We assayed *D. melanogaster* lines from selection experiments from several research groups in different assay environments. The *Drosophila* lines came from four laboratories working on life history evolution – S. Stearns' laboratory at the University of Basel, L. Partridge's laboratory at University College London, R. Bijlsma's laboratory at the University of Groningen and L. Mueller's laboratory at the University of California, Irvine. From each laboratory, we took three lines from each of two selection regimes – a total of 24 lines. All lines were measured in Basel, Groningen and London. Only the Irvine lines were measured in Irvine, where no foreign *Drosophila* populations could be brought to avoid transmission of parasites.

These four selection experiments differ in mode of selection. The lines from Basel have been selected under high or low extrinsic adult mortality (Stearns *et al*., 1996, 2000), the lines from Groningen have been selected for fast or slow development (Zwaan *et al*., 1995a), the lines from Irvine have been selected under different larval densities (Mueller *et al*., 1993; Joshi & Mueller, 1995), and the lines from London have been selected under different temperatures (Huey *et al*., 1991).

Life history traits measured

For mated females we measured lifespan and fecundity and for males and females, egg to eclosion development times and dry weight at eclosion. The lifespan data was right censored, for some flies escaped or got stuck during the experiment. SAS procedure 'LIFETEST' was used to estimate the mean lifespan per line (SAS Institute, 1990). Fecundity of mated females was measured individually for 10 days. The start of the fecundity assay varied among labs from 1 to 4 days after eclosion. If a female died during the assay, her fecundity was treated as a missing value, not as zero. This was made to account for the fact that mortality early in life is likely to have a strong extrinsic component (as opposed to intrinsic mortality, for example as consequence of intensive reproduction). In this study, the total egg production during the first 5 days of the fecundity assay is termed 'fecundity 1–5', the production during the second 5 days 'fecundity 6–10'.

Design and schedule of the study

In Basel, Groningen and London, the 24 lines from all four laboratories were measured. However, in London, the fecundity and the dry weight assay with the London lines failed because most of the larvae collected for those assays died. This unusually high mortality might be attributed to the age of the parents – about 20 days after eclosion. In Irvine, only the lines from Irvine were measured, and the data from the longevity assay were lost after termination of the experiment. Therefore, the data from Irvine is restricted to the fecundity, development time and dry weight measurements of the lines from Irvine.

The first author performed the measurements with support from the local research groups in Basel during May and June 1996, in Irvine during July and August 1996, in London during September and October 1996, and in Groningen from November 1996 to January 1997. Before being used in the assays, all lines were kept for three generations in the laboratory where they were to be measured. This was carried out to synchronize the lines, to bolster population sizes, and to remove any parental and grandparental effect arising from the selection.

Assay methods

The assay methods in every laboratory were local standard methods whenever possible. Sometimes the standard methods had to be changed so that the traits that were measured in different laboratories would be comparable. The assay methods are described in web material.

Statistical analysis

All hypothesis testing was carried out using line means rather than individual values. Within assay environment, the number of replicates contributing to the line means was identical for all the lines. Between assay environments, the number of such replicates was similar except for dry weight and development time. Whereas in Groningen and London, six groups of five flies per sex and line were measured, in Basel all the flies from the development time assay were weighed individually (about 50 flies per sex and line) and in Irvine about 15 groups of 20 flies per sex and line were measured. To balance the data between assay environments, the analysis of dry weight data was restricted to 30 randomly chosen flies per sex and line in Basel and to five groups of 20 flies per sex and line in Irvine. Egg counts were log-transformed to achieve homoscedasticity of the variances (Sokal & Rohlf, 1995: 419).

Statistical analyses were carried out with SAS (SAS Institute, 1990) procedure 'GLM'.

The significance level α' was decreased according to the Dunn–Sidak method (Sokal & Rohlf, 1995: 240) to achieve a questionwise error rate α of 0.05. Because decreasing the significance level by this amount is too conservative, whereas not correcting for multiple testing is too liberal, we present the results with and without correction for multiple testing.

Factor	Symbol	Effect	Levels
Assay environment	α_i	Fixed	Basel, Groningen, Irvine, London
Origin	β_j	Fixed	Basel, Groningen, Irvine, London
Selection (origin)	γ_{jk}	Fixed	HAM, LAM (origin Basel), F, S (origin Groningen), CU, UU (Origin Irvine), 18, 25 (origin London)
Line (selection origin)	D_{jkl}	Random	Three per selection

Table 1 Overview of the factors that potentially contribute variation to the measurements in this study.

Design of the analysis

Table 1 gives an overview over the factors that potentially contribute variation to the measurements in this study. Note that the design is unusual insofar as the factor selection is nested within origin and is fixed; the two selection regimes are different in every origin (therefore they are nested within origin rather than crossed with origin), and they are not a random sample of all possible selection regimes, but are chosen deliberately (so that they are fixed rather than random). Consequently, the factor selection does not contribute to the expected mean squares of origin and does, therefore, not appear in the denominator for origin and for the interaction between assay environment and origin. Because the factor selection nested within origin is fixed rather than random, it confounds the factor origin (see below).

We carried out three different analyses of variance that differed according to the data sets on which they were based. They illuminate different aspects of the question, do conclusions from life history measurements depend on the assay environment? For the first two analyses, the measurements of this study are split into subsets that are analysed separately. The third analyses is based on all measurements for a given life history trait.

Analysis of the lines from one origin measured in one assay environment (analysis A)

For this analysis, the data set was split by the factors assay environment and origin. The analysis tested within every combination of assay environment, origin and life history trait for effects of selection in an analysis of variance with the following design (see Table 1):

$$y = \mu + \gamma_k + \varepsilon_{kl}.$$

The results of this analysis are integrated in Fig. 1 (see also web material). This analysis gives an overview on the data on which this study is based and allows comparisons with results reported in the original studies of these lines.

Analysis of the lines from one origin measured in all assay environments (analysis B)

For this analysis the data set was split by the factor origin. The effect of selection for the lines from one origin was, therefore, tested for all the measurements in all the assay environments pooled together. The corresponding analysis of variance had the following design (see Table 1):

$$y = \mu + \alpha_i + \gamma_k + D_{kl} + (\alpha\gamma)_{ik} + \varepsilon_{ikl}.$$

The results of this analysis are integrated in Fig. 1. The analysis allows us to conclude for each combination of origin and life history trait whether there is an overall effect of selection manifested over all assay environments. Furthermore, it gives for each of these combinations a statistical measure for the interaction between assay environment and selection regime. This interaction indicates for each origin how much the effect of selection depended on the assay environment.

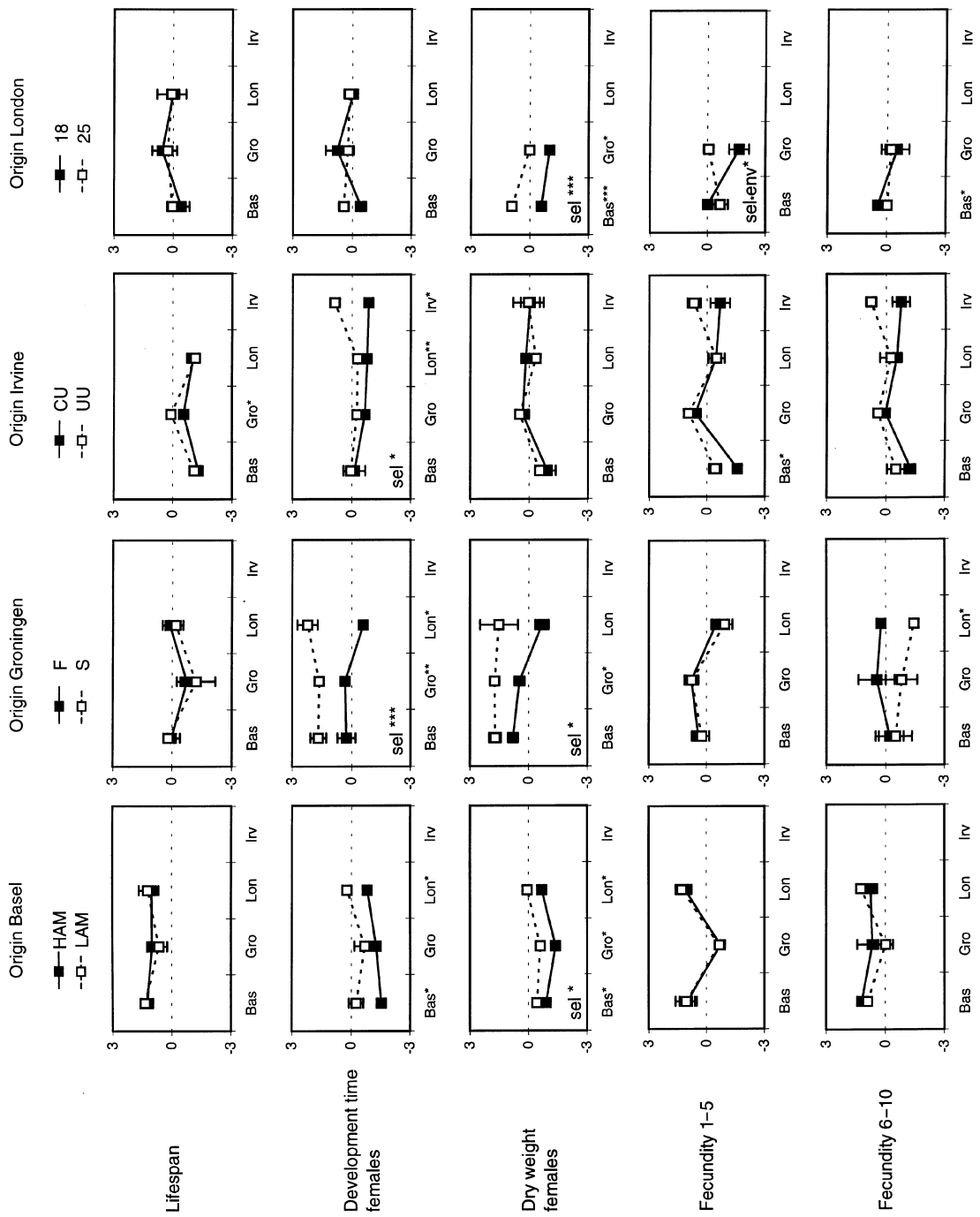
Analysis of the lines from all origins measured in all assay environments (analysis C)

The third analysis was carried out on the pooled data from all four origins measured in all four assay environments. The analysis had the following design:

$$y = \mu + \alpha_i + \beta_j + \gamma_{jk} + D_{jkl} + (\alpha\beta)_{ij} + (\alpha\gamma)_{ijk} + \varepsilon_{ijkl}$$

(see Table 1). This analysis yielded Table 2. It determined for every life history trait whether the effect of selection

Fig. 1 Overview of the measurements made in this study. Each column represents measurements with the lines from a particular origin. Each row represents one life history trait. Each chart represents the expression of a particular life history trait measured with the lines from one origin. The horizontal axis on each chart represents the assay environments where the lines from the particular origin where measured. The vertical axes represent standardized life history measurements: all measurements (all lines from all origins together) within one assay environment were standardized by adjusting the mean to 0 and the SD to 1. Each chart shows the mean and SE for the two selection regimes from every origin (each selection regime consists of three replicate populations). Asterisks associated with assay environments (on the vertical axis) indicate that there was a significant effect of selection for this particular combination of assay environment, origin and life history trait (analysis A). Asterisks indicate probability values from the analysis. $*P < 0.05$; $**P < 0.005$; $***P < 0.00084$ (this reduced significance level leads to a questionwise error rate α of 0.05). Significant overall effects of selection (sel; analysis B) or significant interactions between selection regime and assay environment (sel *env; analysis B) are indicated in the lower left corner of the charts. Asterisks indicate probability values from the analysis. $*P < 0.05$; $**P < 0.005$; $***P < 0.0026$ (this reduced significance level leads to a questionwise error rate α of 0.05).


Origin Basel
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Lifespan
Development time females
Dry weight females
Fecundity 1–5
Fecundity 6–10
Bas
Gro
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Irv
sel *
sel ***
sel-env*
Bas***
Gro*
Lon**
Irv*

Table 2 Analysis of variance (analysis C). After correction for multiple testing the significance level α' becomes 0.0127. The effects of origin and selection(origin) were tested over line MS; all other effects were tested over error MS. The effects involving origin are confounded and are therefore not tested.

Trait	Source of variation	d.f.	Mean square	*F*-value	Pr > *F*
Lifespan	Assay environment	2	2160.41	134.71	0.001
	Origin	3	578.98	18.80	
	Selection (origin)	4	5.34	0.17	0.949
	Line (selection origin) (*D*)	16	30.79	1.92	0.057
	Assay environment × origin	6	83.99	5.24	
	Assay environment × selection (origin)	8	8.43	0.53	0.828
	Error	32	16.04		
Development time females	Assay environment	3	10 626.23	355.29	0.001
	Origin	3	1090.82	30.23	
	Selection (origin)	4	549.33	15.21	0.001
	Line (selection origin)	16	36.12	1.21	0.309
	Assay environment × origin	6	111.44	3.73	
	Assay environment × selection (origin)	9	37.08	1.24	0.303
	Error	36	29.91		
Dry weight females	Assay environment	3	426 077.56	115.77	0.001
	Origin	3	180 860.02	32.81	
	Selection (origin)	4	78 410.03	14.14	0.001
	Line (selection origin)	16	5546.81	1.51	0.158
	Assay environment × origin	5	41 634.36	11.31	
	Assay environment × selection (origin)	8	3833.43	1.04	0.427
	Error	32	3680.46		
Fecundity 1–5	Assay environment	3	24.26	3739.00	0.001
	Origin	3	1.87	143.61	
	Selection (origin)	4	0.04	2.88	0.057
	Line (selection origin)	16	0.01	2.02	0.044
	Assay environment × origin	5	0.16	25.36	
	Assay environment × selection (origin)	8	0.03	4.55	0.001
	Error	32	0.01		
Fecundity 6–10	Assay environment	3	51.79	2112.00	0.001
	Origin	3	2.29	144.26	
	Selection (origin)	4	0.05	3.50	0.031
	Line (selection origin)	16	0.02	0.64	0.827
	Assay environment × origin	5	0.05	1.88	
	Assay environment × selection (origin)	8	0.01	0.48	0.858
	Error	32	0.02		

was overall sensitive to the assay environment. From this one can conclude which traits were most sensitive to the environment in which they were expressed. Note that the factor origin in this analysis is confounded because selection regimes within origins are fixed rather than random. There are aspects that are common to both regimes within one origin (but different from regimes from another origin) so that the origin means are influenced by the particular selection regimes chosen in these origins. This confounding effect also affects the interaction between origin and assay environment.

Local adaptation

To test for local adaptation, we used the data from the lines from Groningen and Basel measured in Groningen and Basel. The analysis was so restricted because these data represent a complete and balanced set of measurements of life history traits of lines from two origins measured in both origins. In this design (two environments and lines from both environments), necessary evidence for local adaptation is that fitness components show significant interaction between the origin and the assay environment (Sork *et al*., 1993), and that lines perform better in the assay environment in which they were selected.

Results

Characterization of the lines

Figure 1 gives an overview of the measurements made in this study (the data for development time and dry weight

of males are similar to those for females and are omitted; web material shows the nontransformed data of all measurements including the data for males). Every combination of origin, assay environment and life history trait was tested for an effect of the selection regime (analysis A). Of the 61 tests conducted (omitting data for males), 16 showed an effect of the selection regime that was significant at $P \leq 0.05$. After correcting for multiple tests, only one of these 16 cases remained significant: dry weight of female flies of the lines from London measured in Basel.

When the measurements of the lines from one origin measured in all the assay environments were analysed together (analysis B), there were five cases (out of 20) where the effect of the selection was significant at $P \leq 0.05$: dry weight of females for the lines from Basel, development time and dry weight of females for the lines from Groningen, development time for the flies from Irvine and dry weight of females for the lines from London (Fig. 1). After correcting for multiple testing, two of these cases remained significant: development time for the lines from Groningen and dry weight for the lines from London.

Interactions between selection regime and assay environment

In several cases, the effect of the selection regime changed in sign when the assay environment changed. In most of these cases the effect of the selection regime was not significant in either of the assay environments, but there were three cases – lifespan and fecundity 1–5 for the lines from Irvine, and fecundity 6–10 for the lines from London – where in one assay environment there was a significant effect (without correction for multiple testing) of the selection regime in one direction, whereas in another assay environment the effect of the selection regime was in the opposite direction, but not significant.

None of these cases corresponded to a significant interaction between selection regime and assay environment in the analysis carried out for every origin separately (analysis B). In only one case did selection regime and assay environment interact significantly (at $P < 0.05$) – fecundity 1–5 for the lines from London (Fig. 1). This case was no longer significant after correcting for multiple testing. In many cases there was a significant effect of the selection regime on a particular life history trait in one assay environment but not in another.

Analysing the measurements from all origins together (analysis C) showed that there was an overall interaction between assay environment and selection regime for fecundity 1–5, but not for other traits (Table 2).

Local adaptation

The interaction between origin and assay environment for Basel and Groningen was significant for fecundity 1–5. This is expressed in a change of the rank of the lines from the two origins – lines from Basel have higher fecundity 1–5 than lines from Groningen when measured in Basel but lower fecundity when measured in Groningen (Fig. 2). No other interactions between origin and assay environment were significant (Table 3).

Discussion

Characterization of the lines

Most of the differences between selection regimes found in this study relate to dry weight and development time (Fig. 1). Differences in these two traits for the lines from Groningen and Basel were already reported in the original studies from these labs and could be corroborated with this study when measured in the laboratory of origin as well as in the analysis that combined the measurements from all assay environment. Development time of females for the lines from Irvine differed significantly between selection regimes when measured both in Irvine

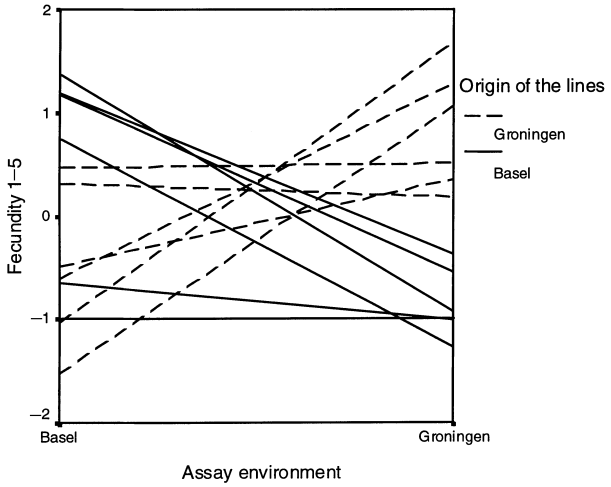

Fig. 2 Local adaptation: Mean fecundity 1–5 for the lines from Basel and Groningen measured in Basel and Groningen. Measurements were normalized within assay environment by adjusting the mean to 0 and the variance to 1.

Table 3 Local adaptation: interactions between the origin of the lines and the assay environment where they were measured, tested over error MS. Only the interaction terms are shown. After correction for multiple testing the significance level α' becomes 0.0102.

Trait	d.f.	Mean square	*F*-value	Pr > *F*
Lifespan	1	27.55	0.30	0.5987
Development time females	1	25.63	2.31	0.1669
Dry weight females	1	617.75	4.03	0.0795
Fecundity 1–5	1	0.01	28.12	0.0007
Fecundity 6–10	1	0.07	0.42	0.5329

and in London, which was not reported previously. This effect remained significant when analysed over all the assay environments. Dry weight for the lines from London differed significantly between the selection regimes when measured in Basel and in Groningen as well as when analysed over both assay environments. This result contrasts with the report that flies selected at 18 °C had longer thoraxes and larger wings than flies selected at 25 °C (Partridge *et al*., 1994). In this study, flies from the 18 °C selection regime had lower dry weight than flies from the 25 °C selection regime. We currently have no explanation for these findings.

The original studies with these lines showed differences in fecundity measurements (reported for the lines from Basel, Groningen and London) and lifespan (reported for the lines from London). These differences could not be corroborated either when analysed only in the laboratory of origin or when analysed over all assay environments. This could be because of either the smaller sample sizes or to slight differences in the methods used in this study. Furthermore, selection on the lines from Groningen had been relaxed for about 5 years and the differences between the lines in development time had been reduced. This might explain why a correlated response in fecundity for the lines from Groningen was no longer found in this study.

Interactions between selection regime and assay environment

The effect of the selection regime on fecundity measurements was sensitive to the assay environment. This suggests that fecundity measurements can be sensitive to assay conditions and that lines from different selection regimes sometimes react differently to different environments, corroborating the findings of Leroi *et al*. (1994a, 1994b) and of Pletcher and Tatar (unpublished data, in Pletcher *et al*., 1997) of genotype–environment interactions for early fecundity in Rose's (1984) and Luckinbill *et al*. 's (1984) lines selected for age at reproduction.

Sensitivity of the fecundity measurements to assay environment implies that fecundity comparisons do not necessarily reflect the situation in the populations under selection when the assay environment and the selection environment are not identical. Thus, differences between selection and assay environment may have contributed to the controversy about the correlated responses of early fecundity to selection on age at reproduction.

For the other traits measured – longevity, development time and body size – no significant interactions between selection regime and assay environment were found. However, for all of these traits there were cases where the measurements in one environment would have led to the conclusion that selection had an effect on this trait, whereas in other environments no such conclusion would have been drawn.

In general, repeating life history measurements in different environments with the same lines led to substantial variation in the estimates of selection effect. This means that differences between the assay and the selection environment might have contributed to the differences in the conclusions drawn by different groups. Do the differences in the expression of life history variation between assay environments reported in this study also indicate that differences between laboratories in the selection and assay environments influenced the evolution of life histories? The fact that the outcome of life history measurements depends on the assay environment means that the expression of genetic variance and covariance of life history traits could depend substantially on the environment. Differences in the expression of genetic correlations in different laboratory environments might lead to differences in the simultaneous evolution of the corresponding traits (Lande, 1982; Reznick, 1985; Charnov, 1989; Spitze *et al*., 1991).

However, there are two problems with this view. The first is that in this study the different laboratory environments were novel for the experimental populations and, therefore, might have led to changes in the expression of many genes, which in turn would transiently change the genetic variances and covariances (Service & Rose, 1985; Bell & Koufopanou, 1986; Holloway *et al*., 1990). The second problem is that even if genetic correlations were stable, it is not clear to what extent they constrain the attainment of phenotypes favoured by selection and, therefore, whether they constrain the correlated responses to selection (Zeng, 1988; Houle, 1991). These two problems make it impossible to extrapolate from genetic correlations measured in a novel environment to correlated responses to selection in this environment.

In conclusion, the fact that the expression of variance and covariance of life history traits depended on the assay environment suggests that differences between the environment where the populations are selected might lead to differences in the direct and correlated response to selection. Thus, differences between the selection environments of different groups might have contributed to the differences in the results reported.

Local adaptation

Lines from Groningen and Basel had higher fecundity 1–5 when measured in the environment where they were selected compared with lines selected in the other laboratory. This indicates that these lines were adapted to their local environment. However, there was no indication of local adaptation for fecundity 6–10, longevity or development time.

The fitness of laboratory populations of *Drosophila* is very sensitive to changes in early fecundity (Stearns & Kawecki, 1994) and much less sensitive to changes in

longevity and development time when generations are discrete. This means that the force of natural selection on early fecundity in the environment where the selection is carried out is high. Thus, two potential mechanisms could lead to the observed interaction between the origin of lines and the assay environment. First, selection may favour different alleles in different environments and alleles favoured in the selection environment may have the pleiotropic effect of depressing early fecundity in other environments. Such a trade-off in early fecundity across environments was proposed by Leroi *et al*. (1994a). Second, the interaction between origin and assay environment could be caused by the accumulation of deleterious mutations with environment-dependent effect (Fry, 1990; Kawecki, 1994). Mutations would be quickly eliminated if they depressed early fecundity in the selection environment, but not if they depressed early fecundity in other environments.

Conclusion

Conclusions about direct and correlated responses to selection drawn from life history studies depended on the environment in which the assay is performed. Fecundity measurements were particularly sensitive to the assay environment, so that life history experiments performed in different assay environments lead to qualitatively different conclusions about the effect of selection on fecundity. Therefore, if the environment where the life history assays are carried out is not identical to the environment where the selection is performed, the results obtained from the assay may not correspond to the actual situation in the population under selection.

Furthermore, the sensitivity of the expression of variance and covariance of life history traits to the assay environment suggests that inconsistencies in the response to selection obtained by different groups might, at least in part, be a consequence of differences in the environment where the populations were selected. Moreover, there was compelling evidence for local adaptation in early fecundity in lines from two laboratories, Basel and Groningen.

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Supplementary material

The following material is available from http://www.blackwell-science.com/products/journals/suppmat/JEB/JEB281/JEB281sm.htm

Appendix 1

Description of the assay methods in the different assay environments.

Appendix 2

Description of the selection methods in the different selection environments [(1) Stearns *et al*. (1996); (2) Stearns *et al*. (2000); (3) Zwaan *et al*. (1991); (4) Zwaan *et al*. (1995a); (5) Mueller *et al*. (1993); (6) Joshi & Mueller (1995); (7) Rose (1984); (8) Santos *et al*. (1997); (9) Shiotsugu *et al*. (1997); (10) Huey *et al*. (1991); (11) James & Partridge (1995); (12) Partridge *et al*. (1995); (13) Partridge *et al*. (1994)].

Appendix 3

Expression of life history traits for every selection regime in every assay environment (mean and SE). Every

selection regime consists of three lines. Ori: Origin of the lines, Env: assay environment, Sel: selection regime (abbreviations of the selection regimes: see methods), Bas: Basel, Gro: Groningen, Irv: Irvine, Lon: London. Asterisks indicate probability values from the analysis of variance for the effect of the selection regime (analysis A). $*P < 0.05$; $**P < 0.005$; $***P < 0.00059$ (this reduced significance level leads to a questionwise error rate α of 0.05).