

Evolutionary domestication in *Drosophila subobscura*

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Abstract

The domestication of plants and animals is historically one of the most important topics in evolutionary biology. The evolutionary genetic changes arising from human cultivation are complex because of the effects of such varied processes as continuing natural selection, artificial selection, deliberate inbreeding, genetic drift and hybridization of different lineages. Despite the interest of domestication as an evolutionary process, few studies of multi-cellular sexual species have approached this topic using well-replicated experiments. Here we present a comprehensive study in which replicated evolutionary trajectories from several *Drosophila subobscura* populations provide a detailed view of the evolutionary dynamics of domestication in an outbreeding animal species. Our results show a clear evolutionary response in fecundity traits, but no clear pattern for adult starvation resistance and juvenile traits such as development time and viability. These results supply new perspectives on the confounding of adaptation with other evolutionary mechanisms in the process of domestication.

Introduction

The domestication of plants and animals is historically one of the most important topics in evolutionary biology, figuring prominently in Darwin's *Origin of Species*. Traditionally, the term 'domestication' refers to the genetic changes undergone by our commensal species, from dogs to agricultural animals to grains to legumes, sometimes with an additional connotation related to behavioural change, especially reduction in 'wildness' (Soanes, 2003). A more useful definition, however, for scientific purposes is that *domestication is the evolutionary genetic change arising from the transition of a population from nature to deliberate human cultivation*. In some laboratory populations, such as those of *Drosophila* or *Escherichia coli*, the 'state of nature' may be laboratory culture under a sequence of ill-defined, arbitrarily or haphazardly changing conditions (cf. Matos *et al.*, 2002; Lenski, 2004).

Domestication, as defined here, is of both practical and theoretical scientific interest. One of the enduring problems in the breeding of both plants and animals is the interpretation of the evolutionary conditions that they

have been subjected to, a topic that was a particular favourite of Darwin (1859, 1883). The development of modern animal and plant breeding has depended, in part, on the spread of this type of evolutionary understanding from theorists, like Darwin, to practical breeding. Understanding the impact of captivity is also becoming prominent in conservation genetics, as more and more species are being maintained in *ex situ* conservation programmes (Frankham *et al.*, 2002).

For evolutionary biology itself, domestication provides one of the more important contexts for experimental evolution. It is both a background to evolutionary studies of diversification under selection (e.g. Rose *et al.*, 2004) and an important topic in itself. In studying domestication in well-defined laboratory experiments, we can measure in detail the evolutionary process with replication and specific environmental controls. In this context, such key evolutionary processes as adaptation and inbreeding occur transparently and reproducibly, a fruitful setting for testing biological hypotheses (see Mueller & Joshi, 2000; Houle & Rowe, 2003; Prasad & Joshi, 2003).

Domestication of *Drosophila* populations that have been founded from wild samples has been studied using two different approaches. First, comparison of populations that have and have not been subject to particular domestication regimes (e.g. Sgrò & Partridge, 2000;

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Hoffmann *et al.*, 2001; Krebs *et al.*, 2001; Gilligan & Frankham, 2003; Griffiths *et al.*, 2005). Secondly, temporal analysis of the evolutionary trajectories of domesticated populations since their foundation from the wild (e.g. Matos *et al.*, 2000b, 2002), our approach for the last 15 years.

In particular, we have been studying evolutionary convergence between the recent and the long-established populations, suggesting laboratory adaptation, particularly in fecundity traits (Matos *et al.*, 2000b, 2002). In the present study, we extend these previous studies to include more generations, more fitness-related traits, and two new, independent, synchronous foundations. Here we offer the most detailed view yet of domestication in an outbreeding animal species, with new information on the confounding of adaptation, founder effects and inbreeding in the process of domestication.

In this study these specific questions were addressed:

- Are there directional patterns of adaptation across traits?
- Are there plateaus in long-term domestication?
- Do long-maintained populations show progressive inbreeding depression?
- Is there a temporal increase in divergence between replicate populations?
- How important are effects of foundation for evolutionary patterns and processes during local adaptation?

Finally, we address the relevance of these laboratory studies to practical domestication and conservation issues, such as *ex situ* breeding programmes.

Materials and methods

Foundation and maintenance of the laboratory populations

Four sets of wild-caught samples of *Drosophila subobscura* were obtained (Fig. 1). In 1990, the 'B' population was founded from collections in a pinewood near Sintra, Portugal (Matos *et al.*, 2000b). In 1998, another popula-

tion, 'NW', was founded, also from Sintra collections, by which time the B population was in its 90th generation. Two generations later, B and NW were split into five replicate populations, referred to as NB₁₋₅ and NW₁₋₅ respectively (Matos *et al.*, 2002).

In 2001, two additional foundations were carried out, one from Sintra, called 'TW' here, and another from a new location, Arrábida (about 50 km from Sintra), called 'AR' here. The TW population was founded from 110 female and 44 male insects and the AR population from 59 female and 24 male insects. After two generations in the laboratory they were both split into three replicate populations, TW₁₋₃ and AR₁₋₃.

From the moment the populations were brought into the laboratory, they were all maintained under the same conditions: discrete generations of 28 days, reproduction close to the time of peak fecundity, a controlled temperature of 18 °C, and controlled densities. Population sizes were usually between 600 and 1200 individuals (Matos *et al.*, 2000b, 2002).

Life-history trait assays

Assays of adult traits

Assayed flies were transferred daily as mated pairs to laying vials containing freshly prepared medium. The total number of eggs laid per female insect was counted every day for the first 12 days, after which starvation resistance was assayed. Five characters were analysed: age of first reproduction (number of days between emergence and the first egg laying – 'A1R'), early fecundity (total number of eggs laid during the first week – 'F1-7'), peak fecundity (total number of eggs laid between days 8 and 12 – 'F8-12'), female and male starvation resistance (number of hours until death, registered every 6 h after transfer to a vial with plain agar – 'RF' and 'RM' respectively).

For the NW populations, assays were carried out during their generations 4, 8, 13, 15, 33, 43, 47, 50, 52, 53, 58, 60, 64, 66, 71, 78 and 86, with corresponding assays of NB populations that had already been

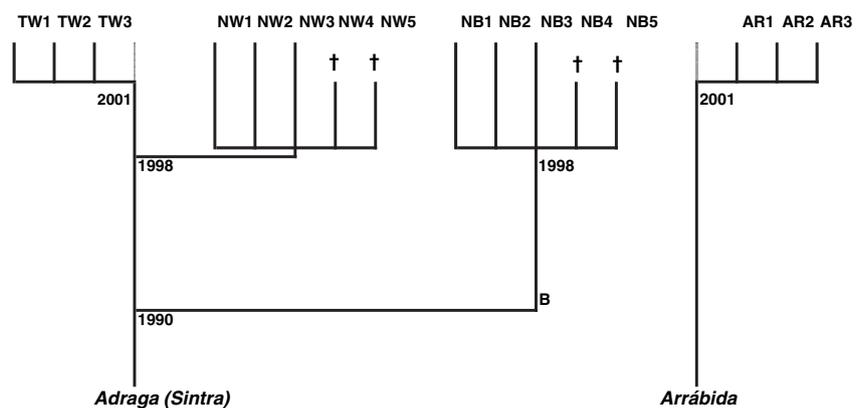


Fig. 1 Phylogeny of the laboratory populations; each main line starting at the natural location (Sintra and Arrábida) represents the natural population where collections were made; three collections were done in Sintra and one in Arrábida, the years being marked in the derived branches representing the foundation of the laboratory populations.

maintained for that number of generations plus 90. Sample sizes per replicate population were between 14 and 21 pairs (Matos *et al.*, 2002). Because of the accidental loss of two replicates of both NW and NB at generation 50 of the former, only the data of replicates NW₁ to NW₃ and NB₁ to NB₃ will be used in all analyses.

For the AR and TW populations, assays were carried out during their generations 3, 4, 6, 7, 12, 14, 18, 20, 25, 32 and 40, with sample sizes between 14 and 18 pairs per replicate population. All assays of AR and TW flies involved simultaneous assays of NB and NW populations (with an additional 136 and 46 generations after introduction in the laboratory respectively), except for the assay at generation 3, when the NW populations were not assayed.

Assays of juvenile traits

For each assay a collection of about 70 eggs per vial was made using eggs laid over a period of 4–6 h. Sample sizes were usually eight vials per replicate population. Development time for female (FDT) and male (MDT) insects was estimated as the number of hours from egg to emergence of imago.

Juvenile viabilities (VIAB) were estimated for each vial as the total number of adults collected per vial divided by 70. Assays for juvenile traits of NW populations were done at generations 3, 4, 6, 11, 20, 48, 51, 54, 59, 65, 73 and 81. NB populations were assayed in parallel at the corresponding generations (i.e. at generation 93, 94, 96 etc.). AR and TW populations were assayed at their generations 5, 8, 13, 19, 27 and 35.

Statistical methods

All data analysis was performed using STATISTICA and EXCEL. All regressions were Type I least-squares linear regressions (Sokal & Rohlf, 1995). The regression analysis was carried out using the mean values of traits for each replicate population as the dependent variable and generation number as the independent variable. The analyses used *both* actual values for each population and the paired differences from NB populations between same arbitrarily numbered replicates for the evolutionary trajectories of NW, AR and TW populations. The analysis of paired differences relative to the longer established NB populations was used to minimize environmental noise that might reduce statistical power to detect evolutionary trends (Matos *et al.*, 2002). In all cases, the significance of the linear trajectory was determined by *t*-test using the average slope of the replicate populations, with the variation of these slopes among replicate populations as the sample variation.

In addition to straightforward analysis of the effect of domestication on individual characters as a function of the number of generations of domestication, we analysed the dependence of *evolutionary rate* on *early differentiation*. The *evolutionary rate* (slope of evolutionary trajectories

during the first 14–15 generations) was estimated using, in each generation, the difference in character values between experimental populations and control populations divided by the latter. To characterize *early differentiation*, we averaged the character values for several assays centred around generation 6. This standardization ensured that scale effects did not bias the dependence of evolutionary rate on early differentiation. We then estimated the best linear model relating evolutionary rate to early differentiation and tested it using *t*-tests and ANCOVA *F*-tests.

Results

Long-term domestication: NW and NB populations

Adult traits

Early fecundity (days 1–7). We tested for a directional change in NB early fecundity data from generations 94 to 176 using a *t*-test. At a confidence value of $P < 0.05$, there was no significant deviation from zero, suggesting an absence of directional, evolutionary change among the NB populations during this period (see Table 1). The NW data over generations 4 to 86 showed a significant increase in early fecundity (see Table 1). The differences between NW and the reference NB populations are shown in Fig. 2, for NW generations 4 to 86. The analysis showed no significant directional trend for the difference between NW and NB with respect to early fecundity (see Table 1 and Fig. 2).

Peak fecundity (days 8–12). At a confidence value of $P < 0.05$, no directional change was obtained for NB populations (see Table 1). The analysis of NW populations showed no directional trend in NW peak fecundity using actual values, but a highly significant increase in the difference between NW and NB populations (see Table 1 and Fig. 3).

Age of first reproduction. For all NW and NB populations, there was no suggestion of a significant evolutionary trend for the trait, analysed as absolute values or as differences between NW and NB replicates (see Table 1).

Starvation resistance. The NB data showed no significant linear trend for female starvation resistance (see Table 1). NW female starvation resistance showed a significant decline, both in absolute terms and in comparison with NB. NW and NB male starvation resistance showed no significant evolutionary trend (see Table 1).

Tests for temporal stabilization and population divergence of adult traits. NB populations showed no significant directional trend over generations 94 to 176, in contrast to the consistent changes for fecundity traits in the NW populations assayed synchronously for their generations 4 to 86 (see Table 1). This suggests that a domestication plateau had already been reached in the NB populations, whereas an adaptive response to domestication occurred in the NW populations assayed at the same time, but over earlier generations of domestication.

Table 1 Slopes of least squares linear regressions of the several traits for each NB and NW replicate population. The analysis of each set of populations used the individual slopes as data points in a *t*-test; at the bottom line for each set of populations the average slope of the linear model is presented.

	Adult traits					Juvenile traits			
	NB populations, generations 94–176					NB populations, generations 93–171			
	A1R	F1–7	F8–12	RF	RM	FDT	MDT	VIAB	
NB1	-0.0083	0.1855	-0.1685	-0.0277	-0.0424	0.4038	0.2820	0.0016	
NB2	0.0046	-0.1664	-0.3392	-0.0090	-0.0803	0.0226	-0.0346	0.0048	
NB3	-0.0042	0.0721	-0.0951	0.0090	-0.0299	-0.1833	-0.0084	0.0038	
Average slope	-0.0026 n.s.	0.0304 n.s.	-0.2010 n.s.	0.0090 n.s.	-0.0509 m.s.	0.0810 n.s.	0.0797 n.s.	0.0034 m.s.	
	NW populations, generations 4–86					NW populations, generations 3–81			
	A1R	F1–7	F8–12	RF	RM	FDT	MDT	VIAB	
NW1	-0.0157	0.6254	0.3791	-0.0554	0.1235	0.7072	0.6596	0.0030	
NW2	-0.0021	0.4169	0.1655	-0.0433	-0.0055	0.2286	0.1510	0.0038	
NW3	0.0024	0.2774	0.4039	-0.0355	-0.0252	-0.6593	-0.6903	0.0052	
Average slope	-0.0051 n.s.	0.4399*	0.3162 m.s.	-0.0447*	0.0309 n.s.	0.0922 n.s.	0.0401 n.s.	0.0040*	
	NW-NB, generations 4–86 (of NW)					NW-NB, generations 3–81 (of NW)			
	A1R	F1–7	F8–12	RF	RM	FDT	MDT	VIAB	
NW1-NB1	-0.0074	0.4399	0.5476	-0.0277	0.1659	0.3034	0.3776	0.0014	
NW2-NB2	-0.0067	0.5833	0.5047	-0.0344	0.0748	0.2060	0.1856	-0.0010	
NW3-NB3	0.0066	0.2053	0.4991	-0.0446	0.0047	-0.4761	-0.6819	0.0013	
Average slope	-0.0025 n.s.	0.4095 m.s.	0.5171***	-0.0356*	0.0818 n.s.	0.0111 n.s.	-0.0396 n.s.	0.0006 n.s.	

Adult traits: age of first reproduction (A1R); early fecundity (F1–7); peak fecundity (F8–12); female starvation resistance (RF); male starvation resistance (RM). Juvenile Traits: female and male development time (FDT and MDT); viability (VIAB).

n.s., $P > 0.1$; m.s., $0.05 < P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

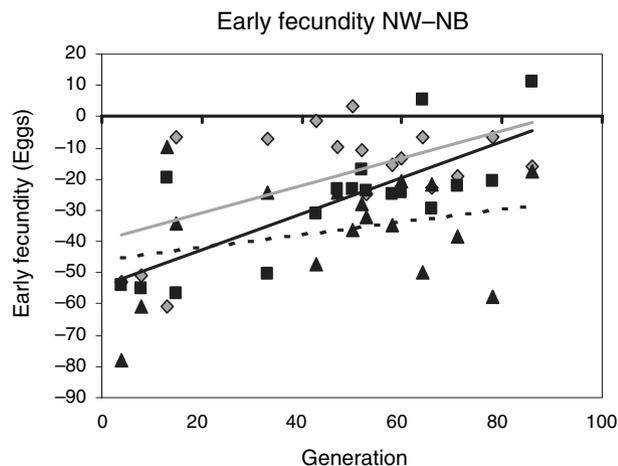


Fig. 2 Early Fecundity NW minus NB, NW Generations 4 to 86. Gray, diamonds, full line – first replicate population; Black, squares, full line – second replicate population; Black, triangles, dashed line – third replicate population.

It is worth noting that log-linear models applied to the NW data usually did not show statistically improved fit over a linear model, which would be expected if evolutionary rates did decrease clearly with time. The exception was early fecundity, for which statistical analysis of log-linear models indicated a significant increase for NW populations relative to NB populations ($t = 6.465$, d.f. = 2, $P < 0.05$). This contrasts with the

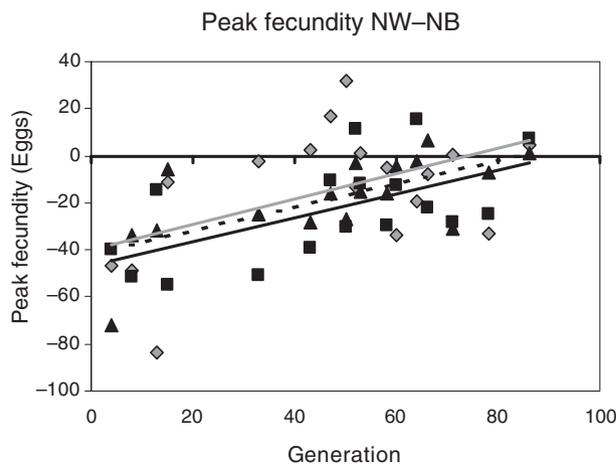


Fig. 3 Peak Fecundity NW minus NB, NW Generations 4 to 86. Gray, diamonds, full line – first replicate population; Black, squares, full line – second replicate population; Black, triangles, dashed line – third replicate population.

nonsignificant result obtained when the linear model was applied ($P = 0.066$). This result suggests a decrease in the rate of evolutionary change of early fecundity in the NW populations.

We failed to detect a consistent increase in the amount of divergence (measured by the coefficient of variability) among replicates over the course of domestication (data not shown). The only statistically significant result was a

decrease in divergence for peak fecundity among the NW replicates ($t = -2.21$; d.f. = 15; $P < 0.05$).

Juvenile traits

Development time. We found no significant longitudinal trends for this character in the NB and NW populations, for both males and females (see Table 1).

Viability. The NB viability data did not show any significant directional pattern (see Table 1). NW viability showed a significant upward trend, but there was no significant change in differences between NW and NB populations (see Table 1).

Test for replicate population diversification of juvenile traits. Data analysis of NW and NB populations showed no clear temporal changes in variability among replicate populations for all juvenile traits (data not shown).

Effects of wild source on domestication: TW vs. AR populations

Adult traits

Early fecundity (days 1–7). Figure 4 shows the temporal changes in TW and AR early fecundity, relative to NB values, during their first 40 generations of domestication. Both absolute values and differences relative to NB populations indicated a significant increase in fecundity for both AR and TW (see Table 2). A comparison of TW and AR populations did not indicate a significant difference in evolutionary rates between them (data not shown).

Peak fecundity (days 8–12). Figure 5 presents TW and AR peak fecundity as differences from NB values. The AR results were not significant, but the TW fecundities significantly increased relative to those of the NB populations. Dropping the use of the NB populations as a standardizing control, both TW and AR populations show a significant directional increase for fecundity (see Table 2). TW and AR did not differ significantly in the rate of temporal change for this trait.

Age of first reproduction. Unlike the NW populations, TW populations showed a significant improvement in this trait both for absolute values and relative to NB populations. For AR populations, no significant improvement was observed (see Table 2). TW and AR did not differ significantly in the rate of temporal change for this trait.

Starvation resistance. Starvation resistance shows a general lack of significant directional change, except for a significant increase in female starvation resistance among TW populations relative to NB. No significant difference was observed between the evolutionary rate of AR and TW populations for these traits.

Juvenile traits

Development time and viability. There were no significant directional trends or differences in evolutionary rates (see Table 2).

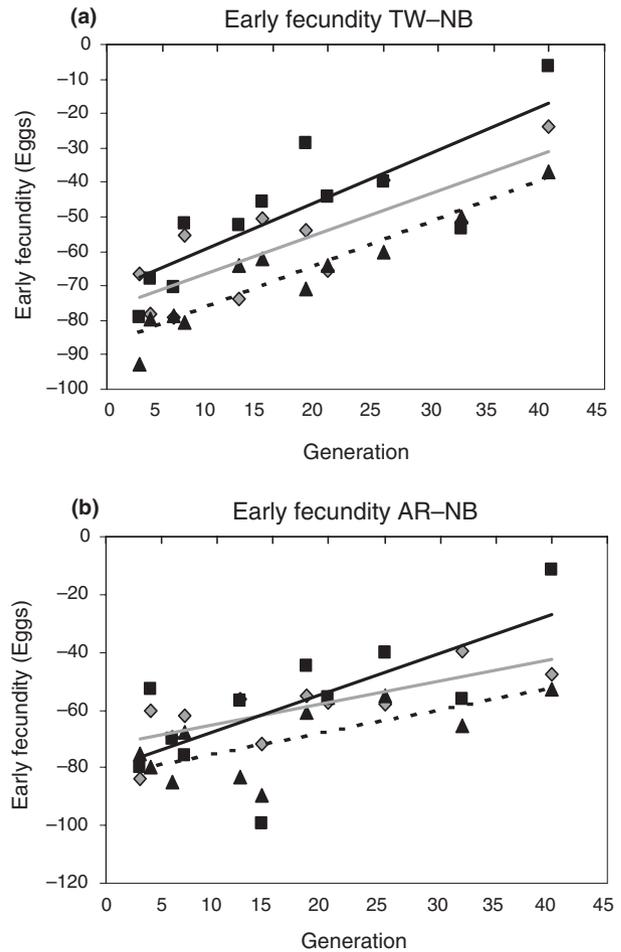


Fig. 4 Early fecundity evolutionary trajectories for TW minus NB and AR minus NB. (a) Early fecundity TW minus NB, Generations 3–40; (b) Early fecundity AR minus NB, Generations 3–40. Gray, diamonds, full line – first replicate population (AR or TW); Black, squares, full line – second replicate population (AR or TW); Black, triangles, dashed line – third replicate population (AR or TW).

Dependence of evolutionary rate on early differentiation

To analyse whether there is a dependence of evolutionary rate on early differentiation we estimated the linear regression of evolutionary rate on initial character value, as described in the *Materials and methods*, using data from simultaneous NB assays to standardize the results over repeated longitudinal assays. Figure 6 presents the results for the NW, TW and AR populations.

The results of ANCOVA and t -tests showed clearly that the replicated foundations differ significantly, with the exception of the NW vs. TW comparison ($P = 0.647$, for ANCOVA F -test with all replicate data points included; $P = 0.562$, for t -test).

In particular, it is worth noting the highly significant difference between AR and TW ($P = 0.000$, for ANCOVA

Table 2 Slopes of least squares linear regressions of the several traits for each TW and AR replicate population. The analysis of each set of populations used the individual slopes as data points in a *t*-test; at the bottom line for each set of populations the average slope of the linear model is presented.

Adult traits						Juvenile traits		
TW populations, generations 3–40						TW populations, generations 5–35		
	A1R	F1–7	F8–12	RF	RM	FDT	MDT	VIAB
TW1	–0.0486	1.9221	2.0685	–0.0333	0.0514	1.1052	0.7637	–0.0003
TW2	–0.0680	2.0067	1.8781	–0.1584	0.0321	0.6164	0.2850	0.0049
TW3	–0.0577	1.4913	1.8718	–0.0396	–0.0627	0.3798	0.2209	0.0004
Average slope	–0.0581**	1.8067**	1.9395**	–0.0771 n.s.	0.007 n.s.	0.7005 m. s.	0.4232 n.s.	0.0017 n.s.
TW-NB, generations 3–40 (of TW)						TW-NB, generations 5–35 (of TW)		
	A1R	F1–7	F8–12	RF	RM	FDT	MDT	VIAB
TW1-NB1	–0.0387	1.1452	1.1799	0.0787	0.1722	–0.2790	–0.2111	–0.0039
TW2-NB2	–0.0668	1.3540	0.7198	0.0818	0.1693	–1.3616	–1.2568	0.0034
TW3-NB3	–0.0462	1.2177	0.9566	0.0365	0.0256	–1.9679	–1.5102	–0.0071
Average slope	–0.0506*	1.2390**	0.9521*	0.0657*	0.1224 n.s.	–1.2028 n.s.	–0.9927 n.s.	–0.0025 n.s.
AR populations, generations 3–40						AR populations, generations 5–35		
	A1R	F1–7	F8–12	RF	RM	FDT	MDT	VIAB
AR1	–0.0371	1.5328	1.9204	–0.0328	–0.1021	1.3730	1.3045	0.0065
AR2	–0.0723	1.9782	1.9860	–0.0474	0.0243	–0.1931	–0.2634	0.0029
AR3	–0.0298	1.0366	1.2142	–0.1412	–0.1153	1.9177	1.8285	0.0004
Average slope	–0.0464 m.s.	1.5159*	1.7069*	–0.0738 n.s.	–0.0644 n.s.	1.0325 n.s.	0.9565 n.s.	0.0033 n.s.
AR-NB, generations 3–40 (of AR)						AR-NB, generations 5–35 (of AR)		
	A1R	F1–7	F8–12	RF	RM	FDT	MDT	VIAB
AR1-NB1	–0.0272	0.7559	0.9647	0.0792	0.0188	–0.0112	0.3297	0.0030
AR2-NB2	–0.0710	1.3255	0.8159	0.1928	0.1616	–2.1711	–1.8052	0.0014
AR3-NB3	–0.0183	0.7631	0.3367	–0.0651	–0.0271	–0.4300	0.0973	–0.0072
Average slope	–0.0388 n.s.	0.9482*	0.7058 m.s.	0.0690 n.s.	0.0511 n.s.	–0.8708 n.s.	–0.4594 n.s.	–0.0009 n.s.

Adult traits: age of first reproduction (A1R); early fecundity (F1–7); peak fecundity (F8–12); female starvation resistance (RF); male starvation resistance (RM). Juvenile traits: female and male development time (FDT and MDT); viability (VIAB). n.s., $P > 0.1$; m.s., $0.05 < P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

F-test with all replicate data points included; $P < 0.05$, for *t*-test). These two sets of laboratory populations were founded synchronously from different wild source populations, suggesting an effect of their population of origin. Figure 6 shows that AR populations evolved at a slower rate than TW populations, relative to their initial differentiation.

Discussion

Initial adaptation

It is clear from our results that some functional characters evolutionarily respond to domestication in a predictable fashion, improving in an intuitively expected way, particularly in the early generations of the process. Fecundity traits in our laboratory show clear initial improvement during domestication, a pattern that is qualitatively consistent among all domesticated *D. subobscura* populations that we have studied, regardless of time or location of initial sampling (Matos *et al.*, 2000b, 2002 and the present study). During the first 50 generations of domestication in our NW, TW and AR sets of populations we observed 70%, 79% and 60%

increases in early fecundity. These results are in general agreement with those reported by Gilligan & Frankham (2003), who also found rapid adaptation to a novel environment over multiple generations in *Drosophila*, as well as the comparative findings of other laboratories, particularly for early fecundity (Sgrò & Partridge, 2000; Hercus & Hoffmann, 1999).

But other characters that might be expected to improve with domestication do not do so. Developmental rate and juvenile viability showed no clear pattern of improvement in the present study. Starvation resistance does not show consistent directional improvement in the present data. In an earlier study, we observed significant early improvement in the NW populations (Matos *et al.*, 2002), but the present results suggest overall decline. In the present study, we found significant improvement over the first 40 generations of domestication in our TW populations, but no significant improvement in our AR populations. This lack of consistency between populations may be a result of genetic trade-offs only becoming observable at a later phase of adaptation (see Service & Rose, 1985; Matos *et al.*, 2000a, 2002; but see Sgrò & Partridge, 2000; Hoffmann *et al.*, 2001 for a different view). However, it is worth pointing out that the

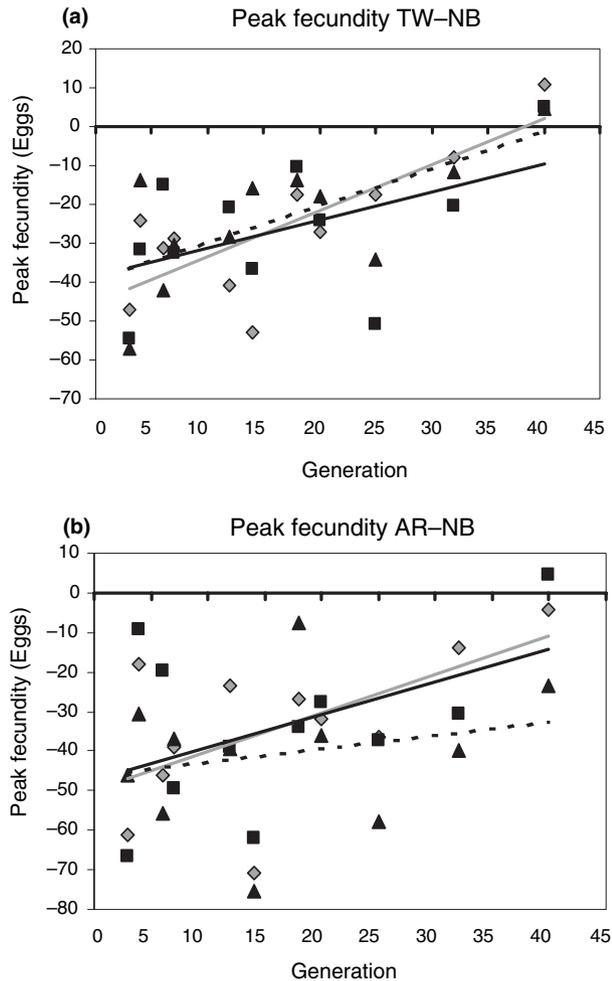


Fig. 5 Peak fecundity evolutionary trajectories for TW minus NB and AR minus NB. (a) Peak fecundity TW-NB, Generations 3–40; (b) Peak fecundity AR minus NB, Generations 3–40. Grey, diamonds, full line – first replicate population (AR or TW); Black, squares, full line – second replicate population (AR or TW); Black, triangles, dashed line – third replicate population (AR or TW).

response of starvation resistance to domestication also shows disparity of results between laboratories, e.g. decreased starvation resistance in a study by Hoffmann *et al.* (2001), whereas Griffiths *et al.* (2005) observed an increased performance for this trait.

The lack of apparent directional response to selection by some functional characters, but not others, was somewhat unexpected, but it continues a tradition in experimental evolution. It is now clear that adaptation involves an unsynchronized mosaic of evolutionary changes. The source of such differences between characters is not yet known, but it is unlikely to be the absence of genetic variation because almost all characters respond to laboratory selection, when it is applied determinedly (see Prasad & Joshi, 2003; Rose *et al.*, 2004).

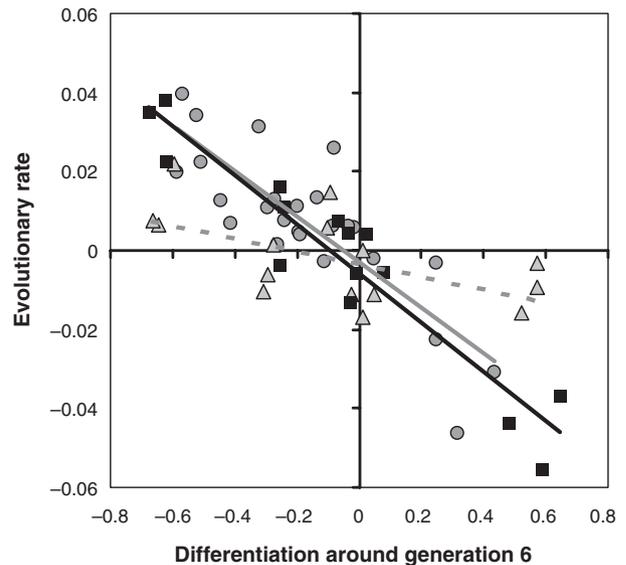


Fig. 6 Linear dependence of evolutionary rate on early differentiation for each set of populations. Each data point of the plot is defined by the early differentiation and the corresponding slope of each evolutionary trajectory (see *Materials and methods*).

Grey, circles, full line – NW populations; regression model: $y = -0.0576x - 0.0028$ ($R^2 = 0.6922$, d.f. = 23; $P < 0.00001$).

Black, squares, full line – TW populations; regression model: $y = -0.0622x - 0.0058$ ($R^2 = 0.8968$, d.f. = 13; $P < 0.00001$).

Grey, triangles, broken line – AR populations; regression model: $y = -0.0164x - 0.0031$ ($R^2 = 0.3414$, d.f. = 13; $P < 0.025$).

Selection response plateaus

In addition to our expectations concerning the characters that should respond under domestication, we also intuitively expected that the response to the selection pressures of domestication would slow progressively. In *Drosophila*, Gilligan & Frankham (2003) found a slowing down of the rate of adaptation to captivity as measured by a competitive index after 87 generations in the lab. Evolutionary plateaus were also observed in the classic Lenski studies of domestication in *E. coli* (e.g. Lenski & Travisano, 1994; Lenski, 2004).

We have evidence for a similar plateau pattern in the fruit flies that we study. For the long-established NB populations, least-squares linear regression of early and peak fecundity over generations 94–176 (see Table 1) shows no significant improvement under continuing domestication.

NW early fecundity presents a progressive drop in the rate of improvement throughout domestication, giving rise to a good fit to a log-linear model, in contrast with previous studies with a smaller number of generations (Matos *et al.*, 2002). For peak fecundity, there is also the suggestion of a slowing down in the evolutionary response of NW populations (see Fig. 3). We interpret this slowing as evidence for the deceleration of functional

improvements after more than 100 generations of domestication, at least for fecundity, if not for all functional characters (see Table 1).

By contrast, Lenski and his colleagues (e.g. Lenski & Travisano, 1994; Cooper & Lenski, 2000) found that *E. coli* took around 5000 generations to reach a plateau. This disparity in the rate of slowing of adaptation between laboratory experiments using *Drosophila* and *E. coli* could be because the *E. coli* experiments relied exclusively on the occurrence of new mutations as each population derived from a single clone (cf. Elena & Lenski, 2003), whereas in outbred populations of *Drosophila* standing genetic variation is almost certain to be the primary factor in the initial response to domestication.

Inbreeding depression and genetic drift

The effect of inbreeding is well-known to be a reduction in the average value of functional characters. In the populations studied here, census population sizes vary from 600 to 1200. Effective population sizes were probably not more than 600, from our unpublished estimates. Over the 176 generations of NB culture, assuming a steady effective population size of only 500, the NB populations can be expected to become about 16% inbred (see Falconer & Mackay, 1996), assuming no countervailing selection. The starvation resistance and developmental time characters were not apparently selected on very strongly during domestication. It might thus be expected that these traits would show a decline in our longer established NB populations as a result of inbreeding depression. Nevertheless, our data do not show that starvation resistance or developmental rate declined over the last 82 generations of domestication in the NB populations. This suggests either a weak phenotypic effect of inbreeding or countervailing selection against deleterious alleles.

Genetic drift can in principle progressively increase the between-line variance among domesticated populations. But between-line variances either decrease or show little directional change in our results. This again suggests that finite population size effects have been small relative to the statistical power of our experiments.

Strict selective breeding processes and maintenance as small-sized populations have predictably led to inbreeding depression during the domestication of most of our commensals. But inbreeding depression may vary considerably from species to species, population to population, trait to trait (Frankham *et al.*, 2002), and is expected to be more severe under stressful environmental conditions (Hedrick & Kalinowski, 2000). We have used moderately large populations in order to highlight the role of selection in domestication. If we had used effective population sizes an order of magnitude smaller, we would probably have detected the action of genetic drift and inbreeding depression (cf. Montgomery *et al.*,

2000; Woodworth *et al.*, 2002; Rodriguez-Ramilo *et al.*, 2006).

Effects of source wild population

There was a significant disparity between the initial rates of response to domestication as a function of initial differentiation among NW, TW and AR populations for the range of characters we have studied (Fig. 6). It is notable that the NW and TW groups are not significantly different from each other, but both are significantly different from the AR populations. The NW and TW populations were sampled from Sintra, Portugal, though during different years, whereas the AR populations were founded from flies collected at Arrábida, Portugal, on the other side of the Tagus river. These results suggest that there may be significant effects of foundation on the evolutionary response to domestication, such that populations founded with samples from different wild populations respond at a different rate, when the magnitude of initial differentiation is eliminated from the analysis.

We are encouraged that three separate samples from the same wild population of *D. suboscuro* over a number of years give remarkably similar results over all. Natural selection in the laboratory can apparently be strong enough to override sampling effects over a short period of time from one wild source population, yet sensitive enough to distinguish differences in domestication among populations founded from different source populations. Nevertheless, this finding of course does not allow ready inferences concerning the adaptive process in the wild populations from which samples are taken (cf. Sgrò & Partridge, 2000 vs. Matos & Avelar, 2001; Matos *et al.*, 2004).

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