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From nature to the laboratory: the impact of founder effects on adaptation

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Abstract

Most founding events entail a reduction in population size, which in turn leads to genetic drift effects that can deplete alleles. Besides reducing neutral genetic variability, founder effects can in principle shift additive genetic variance for phenotypes that underlie fitness. This could then lead to different rates of adaptation among populations that have undergone a population size bottleneck as well as an environmental change, even when these populations have a common evolutionary history. Thus, theory suggests that there should be an association between observable genetic variability for both neutral markers and phenotypes related to fitness. Here, we test this scenario by monitoring the early evolutionary dynamics of six laboratory foundations derived from founders taken from the same source natural population of *Drosophila subobscura*. Each foundation was in turn three-fold replicated. During their first few generations, these six foundations showed an abrupt increase in their genetic differentiation, within and between foundations. The eighteen populations that were monitored also differed in their patterns of phenotypic adaptation according to their immediately ancestral founding sample. Differences in early genetic variability and in effective population size were found to predict differences in the rate of adaptation during the first 21 generations of laboratory evolution. We show that evolution in a novel environment is strongly contingent not only on the initial composition of a newly founded population but also on the stochastic changes that occur during the first generations of colonization. Such effects make laboratory populations poor guides to the evolutionary genetic properties of their ancestral wild populations.

Introduction

The founding of a new population in a new habitat often entails a reduction in population size. Such a reduction in population size then leads to genetic sampling effects. The subsequent evolution of the newly founded population will then depend on three factors: interactions between the genetics of fitness-related traits, the pattern of selection in the new environment and any additional genetic drift effects of continuing small population sizes (Keller & Taylor, 2008). In extreme cases, the confluence of these interacting forces can lead to extinction or speciation (Templeton, 1980), the former particularly depending on the availability of genetic variation that can respond to the new pattern of selection.

A population subjected to a bottleneck event can undergo rapid genetic change due to the loss of rare alleles (Maruyama & Fuerst, 1985), with more impact on allelic richness than gene diversity (Nei *et al.*, 1975; Templeton, 1980; Leberg, 1992; Cabe, 1998). This stronger effect on allelic richness has been empirically established in multiple studies (Leberg, 1992; England

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et al., 2003; Dlugosch & Parker, 2008). Nevertheless, common alleles can also be subjected to frequency changes, leading to shifts in gene diversity (England *et al.*, 2003; Simões *et al.*, 2008a). Gene diversity is expected to affect short-term response to selection, but allelic richness may affect long-term responses to selection, and eventually population survival (Allendorf, 1986). Nevertheless, both the absolute and relative importance of gene diversity and allelic richness for the evolutionary potential of a population is still a controversial issue.

Sampling effects take place not only when migrants colonize a new habitat, but also throughout the subsequent evolutionary process, when chance depletion of alleles occurs due to simple genetic drift when the newly founded population is small (Willi *et al.*, 2006). Moreover, sampling effects during the first few generations after colonization of a new environment may be inflated by differences in reproductive success among the colony founders (Hedgecock, 1994). If these effects shift the standing genetic variation of fitness-related phenotypes, heterogeneous responses to the novel selection regime of the new habitat can be expected among populations colonizing the same new habitat, even when those populations share a common prior evolutionary history (Waples, 1998; Keller & Taylor, 2008).

Wright (1931) demonstrated that the smaller the effective population size (N_e) of a finite population, the faster the decay in gene diversity through time, with $1/2N_e$ of gene diversity being lost at each generation (see also Crow & Kimura, 1970). By decreasing the genetic variability, one would expect that bottlenecks would also decrease the ability to respond to selection. Robertson (1960) predicted that the total response of a finite population to selection, in the absence of mutation and nonadditive effects, is a function of (i) the initial frequency of genes segregating in the source population, (ii) the distribution of genetic effects on the trait and (iii) the effective size of the population. The response to selection will then reach a limit when all genetic variability is exhausted. But if nonadditive genetic interactions affect the trait under selection, populations might experience an increase in additive genetic variance after a bottleneck, due to the conversion of epistatic and/or dominance variances into additive genetic variance (Wright, 1977; Bryant *et al.*, 1986; Goodnight, 1988; Cheverud *et al.*, 1999; Merilä & Sheldon, 1999; Barton & Turelli, 2004; Buskirk & Willi, 2006). This can then produce an increased response to selection, contrary to what might be expected intuitively (e.g. England *et al.*, 2003). Nevertheless, decreases in genetic diversity and total genetic variance are still to be expected (see review in Dlugosch & Parker, 2008; Box 2). When environmental change also occurs, genotype-by-environment interactions will affect the additive genetic variance of life-history traits (Falconer & Mackay, 1996). Given this complex array of theoretically conceivable evolutionary effects, it is evident that there is a need for experimental appraisal of the relationships between effective population size, genetic variability and the response to selection, when bottleneck-colonization events occur.

So what evidence is there bearing on such issues? One central question is whether neutral genetic variation is correlated with standing genetic variation for fitness-related traits. Such an empirical association would suggest that we can infer the potential of a population to respond to selection using measures of diversity among molecular markers as surrogates. Unfortunately, a variety of studies have found no clear association between these two types of variation (Reed & Frankham, 2001; Palo *et al.*, 2003; Gilligan *et al.*, 2005; Sæther *et al.*, 2007; also see McKay & Latta, 2002; Edelaar *et al.*, 2011; Kirk & Freeland, 2011). Both the generally complex genetic basis of life-history traits and the fact that they are under selective pressures may lead to this decoupling (Reed & Frankham, 2001). Nevertheless, in a meta-analysis involving 34 studies, Reed & Frankham (2003) found a significant positive correlation between the genetic variability of molecular markers and fitness, as well as between population size and fitness.

A key issue is whether we can predict the capacity of a population to adapt to a novel environment based on genetic variability of neutral markers (McKay & Latta, 2002). In particular, can we predict the impact that founder events have on evolutionary dynamics after colonization, based on their effects on neutral genetic variability? Founding events may lead to different levels of neutral genetic variability and to contrasting evolutionary trajectories among populations, even in traits relevant to fitness (Simões *et al.*, 2008b). This illustrates a key empirical problem affecting research that infers evolutionary trajectories from comparisons between contemporaneous populations, where the state of one population is assumed to represent the ancestral state of another (e.g. Sgrò & Partridge, 2000; Hoffmann *et al.*, 2001; Gilligan & Frankham, 2003; Griffiths *et al.*, 2005; but see Matos & Avelar, 2001; Simões *et al.*, 2009). What has been lacking are experimental studies that both characterize the variability of neutral markers and monitor, over multiple generations, the evolutionary trajectories of populations adapting to a common controlled environment, populations that were derived from different samples of the same deme of origin.

With this goal in mind, six newly founded sets of three laboratory populations each were studied in *Drosophila subobscura*. These populations, derived from collections in nearby locations in the wild, have been characterized previously for differences in their adaptive dynamics after introduction in the laboratory (Simões *et al.*, 2007, 2008b). Temporal changes in microsatellites have also been partly presented before (Simões *et al.*,

2008a). Here, we will analyse for the first time the data of both microsatellites and life-history traits, during the first 14–21 generations since laboratory foundation, in order to address the following empirical questions using these 18 newly founded laboratory populations: Do early sampling effects detected among neutral molecular markers predict differences in the subsequent evolutionary trajectories of fitness-related traits? Is there a predictable relationship between initial effective population size and the subsequent evolutionary trajectories of fitness-related traits? Do populations differentiate to a similar degree in life-history traits and molecular markers, both within and across generations? More generally, do laboratory populations provide a useful guide to the properties of the wild populations from which they derive?

Materials and methods

Sampling design and maintenance

Two samples of *Drosophila subobscura* were collected in 2001, TW in Adraga (Sintra) and AR in Arrábida. In 2005, two independent samples were also derived from each of the two natural locations, with few days apart: FWA and FWB from Sintra and NARA and NARB from Arrábida (see Simões *et al.*, 2008b for more details). All foundations were three-fold replicated at generation two, and maintenance was similar for all the 18 resulting populations. Populations were maintained with synchronous discrete generations of 28 days, reproduction close to peak fecundity, photoperiod of 12 h of light/12 h of darkness at 18 °C, with census sizes generally between 600 and 1200 individuals (see details in Table S1). Flies were kept in vials with controlled density for both eggs and adults. At each generation, flies emerging from the several vials of a given population were thoroughly randomized 4–5 days after emergence, using CO_2 anaesthesia. Egg collection for the next generation was carried out 1 week later.

Microsatellite extraction and amplification

The 2001 foundations (TW and AR) were genotyped at the founder generation (G0) and at generations 3 (G3) and 14 (the two last generations three-fold replicated), and the 2005 foundations were analysed at the founder generation and at generations 3 and 15 (again the latter three-fold replicated). From now on, the term 'generation 15' (G15) will be used in broad sense to refer to both generation 15 from the 2005 populations and generation 14 from the 2001 populations.

All populations were genotyped for 9 microsatellite loci, *dsub01*, *dsub02*, *dsub05*, *dsub10*, *dsub19*, *dsub20*, *dsub21*, *dsub23* and *dsub27*. Such a number of highly polymorphic microsatellites are satisfactory for the purposes of our study (e.g. Spencer *et al.*, 2000). These loci were previously identified and characterized by Pascual *et al.* (2000) and cytologically localized in the five *Drosophila subobscura* chromosomes (Santos *et al.*, 2010). The distribution of our microsatellites among the *D. subobscura* chromosomes was the following: *dsub05*, *dsub19* and *dsub21* – A (sexual) chromosome; *dsub20* – E chromosome; *dsub23*, *dsub27* – J chromosome; *dsub01*, *dsub02* – O chromosome; *dsub10* – U chromosome. These loci did not present linkage disequilibrium in any of the founders as tested by Genepop version 4.0 (Rousset, 2008). DNA extraction of single flies followed the protocol of Gloor *et al.* (1993), and microsatellite's amplification was performed as described in the study by Simões *et al.*, (2008a).

Thirty females were genotyped *per* population at each generation, amounting to 1260 analysed genotypes. Some of these data have already been presented, for generations 3 and 14 of the 2001 populations (Simões *et al.*, 2008a). Here, we analyse the whole set of data obtained from founders, generation three and generations 14–15 for the 18 populations.

Tests of microsatellite deviation from neutrality

To screen for deficit of heterozygotes per locus and sample, F_{IS} coefficients were obtained and tested by randomization in FSTAT 2.9.3 (Goudet, 1995). False discovery rate (FDR) correction for multiple testing was carried out following Benjamini & Hochberg (1995).

No significant effect of null alleles on the global differentiation was detected using the FreeNA software (Chapuis & Estoup, 2007). Thus no correction for the presence of null alleles was made.

Microsatellite allele frequency changes between generations were used to test for effects of selection. Two outlier approaches were implemented: the LnRH test statistic described in Kauer *et al.* (2003); (see also Simões *et al.*, 2008a) and the FDIST2 approach (Beaumont & Nichols, 1996).

Microsatellite variability

Microsatellite variability was estimated by determining allelic richness (A) and gene diversity (expected heterozygosity, H_E corrected for sample size – Nei, 1978), with the software FSTAT 2.9.3 (Goudet, 1995).

The effect of foundation at each generation on microsatellite variability was tested at generations three (G3) and 15 (G15) with a simple ANOVA model where the differences between replicate populations within foundations are used as error (ε). In the founders (G0), as there were no replicates at that initial generation, a Friedman ANOVA using the data of the nine loci as error (ε) was performed to test the differences due to foundation.

Changes in microsatellite variability through time were analysed over two generation ranges: from

generation zero to generation three (G0–G3) and from generation three to generation 14 or 15 (G3–G15, in broad sense), respectively, for the 2001 and 2005 populations. In each period, percentage decline *per* generation was calculated for both gene diversity (dH_E) and allelic richness (dA). The effect of foundation on the decline of both allelic richness and gene diversity, at each of the two periods (G0–G3 and G3–G15) and also at the whole generation range (G0–G15), was tested again by simple ANOVA as described above. The effects of foundation and generation range on the decline of allelic richness (dA) and of gene diversity (dH_E) were also tested considering the two periods (G0–G3 and G3–G15). The mixed ANOVA model used was

$$Y = \mu + Found + Gen + Found * Gen + Pop\{Found\} + \varepsilon,$$

where Y refers to the decline in variability (whether dA or dH_E), *Found* to the six foundations, *Gen* to the two generation ranges, *Found*Gen* to the interaction between those two factors and *Pop*{*Found*} to the three replicate populations nested inside each of the 6 foundations (random factor).

To test for correspondence of genetic variability (whether A or H_E) between generations, Pearson's correlations were performed. Moreover, we tested whether pairwise differences between foundations presented a similar pattern between generations. For this, we estimated the square matrices of differences in variability between foundations at each of the three analysed generations and compared them using the signed Mantel test. The signed Mantel test takes into account the sign of the vectors of each difference matrices comparing the direction of effects found in the data, because they are not absolute differences (e.g. distances), most commonly used in Mantel tests (see Oberrath & Böhning-Gaese, 2001). Significant correlations between difference matrices indicate a nonindependence of the compared traits, whether they change in the same or in opposite directions (for positive and negative correlations, respectively). Moreover, such associations have a higher statistical power relative to the simple Pearson's correlations. We also use this approach for testing associations involving other traits.

All Mantel tests presented in this study were run with GenAlex 6.4 with 9999 permutations (Peakall & Smouse, 2006). For the sake of clarity, we will use the term *simple* or *direct correlation tests* for analyses of the direct association using raw data while mentioning *signed Mantel tests* for comparisons between square matrices of pairwise differences.

Genetic differentiation

Genetic differentiation between foundations (F_{CT}) – differentiation between groups of populations – was estimated by AMOVA (Weir & Cockerham, 1984) at both generations three and 15 using ARLEQUIN 3.5.1.2 (Excoffier & Lischer, 2010; CMPG lab, University of Bern, Switzerland) and compared considering the 95% confidence intervals estimated by bootstrap. A Mantel test to the correlation between pairwise F_{CT} values at the two generations (involving 9999 permutations) was also performed.

Global F_{ST} values within foundations – differentiation between the three replicate populations – were estimated in FSTAT 2.9.3 (Goudet, 1995), both at generations three and 15.

Pairwise F_{ST} values considering all populations at the three generations (G0, G3 and G15) were also obtained in FSTAT 2.9.3. A principal coordinate analysis was performed for each foundation, using F_{ST} values as distance between all populations and generations, for example for TW the distance matrix F_{ST} between TW_t, the three replicate populations $TW_{1–3}$ at generation three and the three replicate populations $TW_{1–3}$ at generation 15 (7 by 7 square matrix). Another principal coordinate analysis was accomplished across foundations and generations (18 by 18 square matrix), considering mean F_{ST} values within or across foundations at generations 3 and 15, for example the F_{ST} between TW at generation three and AR at generation 15 was calculated as the average of the nine F_{ST} estimates between the three TW replicate populations at generation three and the three AR replicate populations at generation 15.

Effective population size

Single-point effective population size was estimated by the linkage disequilibrium method (Hill, 1981), using the information on the nonrandom association between alleles at different microsatellite loci, both in the founders and at generation three ($N_{e\ (G3)}$). As the effective population size reflects the number of individuals that gave rise to a given generation, the N_e calculated with the founders is an estimate of N_e of the source population, being thus bigger than the census size at foundation. The rate of coalescence of alleles between generations was modelled to estimate effective population sizes for the period between generations three and 15 ($N_{e\ (G3–G15)}$) using the Bayesian method (Berthier *et al.*, 2002). This method is more conservative than the likelihood-based estimators and thus more likely to include the true N_e (Tallmon *et al.*, 2004). Both methods were run using the software NEESTIMATOR 1.3 (Ovenden *et al.*, 2007; Department of Agriculture, Fisheries and Forestry, Queensland, Australia). Tests for associations between N_e and the decline in genetic variability (whether dA or dH_E), as well as the slopes of life-history traits (and early differentiation – see below), were carried out with the simple Pearson's correlation test. Correlations between the square difference matrices calculated with the same data were analysed by signed Mantel tests.

Life-history trait evolutionary trajectories

The analysis of life-history traits reported here was mostly already presented elsewhere (Simões *et al.*, 2008b). Periodic assays covered the first 20 to 21 generations after introduction to the laboratory (for the 2001 and 2005 foundations, respectively). The traits were age of first reproduction (number of days of life until laying the first egg – A1R), early fecundity (number of eggs laid from day one of life to day seven – F1–7), peak fecundity (number of eggs laid from day eight to twelve – F8–12) and female starvation resistance (number of hours of life without food after day twelve, registered every 6 hours – RF). Data points per generation and population were calculated as differences from the long laboratory-established NB population, with the same arbitrary number (e.g. average TW_1 minus average NB_1), which was synchronously assayed. Using re-sampling methods, we tested for differences both in slopes and initial differentiation between the several foundations (see details in Simões *et al.*, 2008b). A composite phenotype (CPhen) was also analysed, being generated, as in the study by Simões *et al.* (2008b), by discriminant analysis between two groups, 'START' (first assay data for all populations) and 'END' (last assay data for all populations), considering the different contribution of the four traits. For the six foundations of the present study, the coefficients of the linear discriminant function were A1R = −0.76; F1–7 = 0.47; F8–12 = −0.15 and RF = −0.60.

Both the estimates of slopes and of early differentiation (ed) for each population were used to test an association with the neutral molecular data. Early differentiation was obtained as an average between phenotypic assays at generations three and six.

Relating life-history traits to microsatellite data

We tested the association between slopes and early differentiation (for each of the five traits) and microsatellite genetic variability (A or H_E), both among the founders (G0) and generation three (G3), estimating the significance of the simple Pearson's correlation. Signed Mantel tests were also applied to inspect the relation of pairwise differences between the same parameters. Similarly, we tested for associations with the decline of genetic variability (both dA and dH_E) at the generation ranges G0–G3, G3–G15 and G0–G15, as well as with the number of founders (n) and the effective population size (whether $N_{e\ (G3)}$ or $N_{e\ (G3–G15)}$), both by simple correlation and by signed Mantel test. False discovery rate due to multiple testing was controlled following the FDR procedure of Benjamini & Hochberg (1995), considering five to fifteen tests, for example 10 tests were considered in the analysis of the relationship between each genetic parameter and the slopes of five life-history traits, both in the founders and at generation three. Because age of first reproduction (A1R) is expected to be negatively correlated with early fecundity (F1–7), as it is defined by the number of days until laying the first egg, for the sake of comparisons of correlations with microsatellite data, we will invert the sign of the correlations with A1R, so that magnitude of effects can be compared readily across traits.

Mantel tests were also performed to test for an association between variance components, obtained for pairs of foundations and each phenotype, and both the F_{CT} matrix between pairs of foundations at generation three (G3) and the F_{ST} matrix between founder populations (G0).

Moreover, the heterogeneity between replicates within foundations for both microsatellite and phenotypic data, after several generations of adaptation to the laboratory, was compared by a simple regression relating global F_{ST} values between replicates of each foundation at generation 15 to the variance of slopes among the evolutionary phenotypic trajectories (along the first 21 generations), also within foundations.

Results

Testing for H-W equilibrium and selective hitchhiking on microsatellites

No consistent departures from Hardy–Weinberg equilibrium or effects of selection were detected at any loci over the three replicates of each foundation over any interval of time; thus, those data are not presented here.

Microsatellite data at generations zero, three and 15

All microsatellites presented high variability for both allelic richness (A) and gene diversity (H_E) in all generations (Fig. 1; Table S2). No significant differences were encountered between founder populations (G0), whether in allelic richness or in gene diversity (Friedman ANOVA χ^2 (N = 9, d.f. = 5) <8, P >0.25). On the other hand, at generation three (G3), significant differences between foundations were found in allelic richness ($F_{5,12}$ = 4.71, P = 0.013), but not in gene diversity. At generation 15 (G15), again no significant differences in variability levels (either A or H_E) were observed between foundations (Table S2).

Mean allelic richness over all foundations declined from 16.40 (SEM 0.24) in the founders to 14.43 (SEM 0.18) at generation three and to 11.17 (SEM 0.18) at generation 15 (Fig. 1a). Mean gene diversity decreased from 0.908 (SEM 0.003) to 0.892 (SEM 0.002) and to 0.843 (SEM 0.006) over the same periods (Fig. 1b). There was a highly significant difference between foundations in the decline of allelic richness in the periods between generations zero and three (G0–G3) and

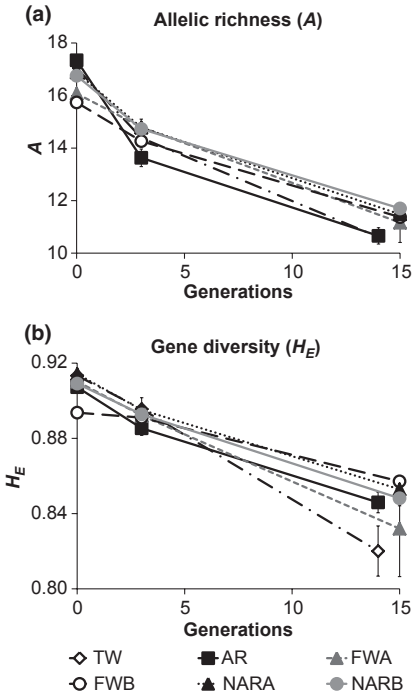

Fig. 1 Mean genetic variability estimates for the six foundations after introduction in the laboratory. Mean allelic richness (a) and mean gene diversity (b) are shown both for the founders and for generations three and 15. Standard error bars are given for each foundation (based on variation across replicates).

between generations zero and 15 (G0–G15) ($F_{5,12} > 5.8$, $P < 0.006$ for both), but not in the period between generations three and 15 (G3–G15). Gene diversity, on the other hand, only showed a significant difference in its decline between foundations during the first period (G0–G3: $F_{5,12} = 3.38$, $P = 0.039$; Fig. 1).

When considering the mixed ANOVA model with a factor for foundation and a factor (*Gen*) for the two generation (*Found*) ranges (G0–G3 and G3–G15), there were significant differences in decline across foundations for both allelic richness and gene diversity ($F_{5,12} > 5.16$, $P < 0.01$). The decline of allelic richness was significantly different between generation ranges ($F_{1,12} > 127.12$, $P < 0.0001$), and this difference varied significantly between foundations (*Found*Gen*, $F_{5,12} > 8.45$, $P < 0.002$). There were no significant effects of foundations, generation ranges or their interaction on the decline of gene diversity (see Table 1 and Fig. 1).

There was no significant simple Pearson's correlation between generations in either allelic richness or gene diversity. Signed Mantel tests gave a significant positive correlation for gene diversity between generations zero and three ($r = 0.452$, $P = 0.015$), a negative significant correlation between generations zero and 15 ($H_{E(G0)}$ vs. $H_{E(G15)}$, $r = -0.617$; $P = 0.003$) and no correlation between generations three and 15 ($H_{E(G3)}$ vs. $H_{E(G15)}$). In contrast, for allelic richness the only significant correlation was between generations three and 15 ($r = 0.550$, $P = 0.046$).

No direct Pearson's correlation was observed between variability decline (dA or dH_E) and effective population size at generation three ($N_{e\ (G3)}$), or with the number of founders (n) (Tables 1 and 2; Table S3). Nevertheless, signed Mantel tests gave highly significant negative associations between both dA and dH_E during G0–G3 and differences in effective population size. Differences in dA in the period G0–G15 also showed a strong negative correlation with differences in $N_{e\ (G3)}$ (Table S3). On the other hand, no association of this type was observed in the period G3–G15 for both estimates.

Genetic differentiation across foundations and generations

Foundations were significantly differentiated at generation three (G3), although not at generation zero – G0 (see Fig. S1). The differentiation between all foundations (F_{CT}, estimating the differentiation between groups of three replicated populations) increased at generation 15 (G15), being significantly higher relative

Table 1 Decline per generation in allelic richness (dA) and in gene diversity (dH_E) after a founding event. Data from six foundations at three generation ranges (G0–G3, G3–G15 and G0–G15) are presented.

	dA (%)			dH_E (%)		
Foundation	G0–G3	G3–G15	G0–G15	G0–G3	G3–G15	G0–G15
TW	5.21	2.38	2.70	0.65	0.77	0.73
AR	7.13	1.98	2.75	0.80	0.40	0.48
FWA	2.66	2.04	2.04	0.66	0.56	0.57
FWB	3.12	1.69	1.85	0.10	0.32	0.27
NARA	4.15	1.85	2.13	0.70	0.39	0.45
NARB	4.03	1.71	2.01	0.60	0.42	0.45
Mean	4.38 ± 0.66	1.94 ± 0.11	2.25 ± 0.16	0.59 ± 0.10	0.48 ± 0.07	0.49 ± 0.06

Mean values ± SEM across foundations are given.

Table 2 Effective population size (N_e) of six foundations at different generations (Gi) after a founding event with n female founders. At generations zero and three, N_e was estimated using the information on linkage disequilibrium at a single generation, whereas from generations three to 15 N_e was obtained by modelling the temporal changes in allele frequency at each population using a backward coalescent approach (see material and methods).

		N_e *per* generation		
Foundation	*n*	G0*,†	G3*,‡	G3–15‡,§
TW	110	231.2 [141.2; 598.1]	90.2 ± 8.7	132.00 ± 13.28
AR	59	149.6 [105.8; 248.9]	55.2 ± 8.6	144.73 ± 27.39
FWA	60	271.8 [151.7; 1130.3]	115.9 ± 29.1	143.53 ± 46.94
FWB	75	236.3 [135.6; 820.3]	86.3 ± 18.0	164.70 ± 46.19
NARA	55	334.3 [171.5; 3802.3]	104.4 ± 10.8	155.77 ± 15.17
NARB	68	665.2 [236.5; infinite]	129.2 ± 7.6	151.00 ± 7.5
Mean	71.17 ± 8.30	314.7 ± 74.3	96.9 ± 10.6	148.6 ± 24.59

*N_e estimated by the single-point linkage disequilibrium method (Hill, 1981).
†Limits for 95% confidence interval of N_e in the founders are in brackets.
‡Mean values ± SEM (across replicate populations) for each foundation.
§N_e estimated by the temporal Bayesian method (Berthier *et al.*, 2002).

to the one observed at generation three [$F_{CT\ (G3)}$ = 0.009; 95% CI (0.007; 0.012); $F_{CT\ (G15)}$ = 0.019; 95% CI (0.013; 0.026); Fig. 2]. Nevertheless, no significant correlation was found between pairwise differentiations at both generations. Furthermore, principal coordinate analysis was carried out considering pairwise F_{ST} values between foundations and/or generations, that is, mean values of F_{ST} between pairs of populations from different foundations and/or generations, for example $TW_{1–3}$ at generation three vs. $FWA_{1–3}$ at generation 15. That analysis shows how the time in the laboratory (axis 1, explaining 47.66% of the total variation) led to increasing divergence between foundations (Fig. 3a) as well as between replicates within each foundation (Fig. 3b), although they were not differentiated at the moment of laboratory introduction. FWA, TW and AR foundations at generation 15 seem to have diverged more from the other populations and also with respect to the founders (Fig. 3).

As expected, the mean global F_{ST} value estimating differentiation within foundations (between replicates) was significantly higher at generation 15 than at generation three ($F_{ST\ (G15)}$ = 0.041 (SEM 0.0058); $F_{ST\ (G3)}$ = 0.003 (SEM 0.0003); Z = 2.20, P = 0.028).

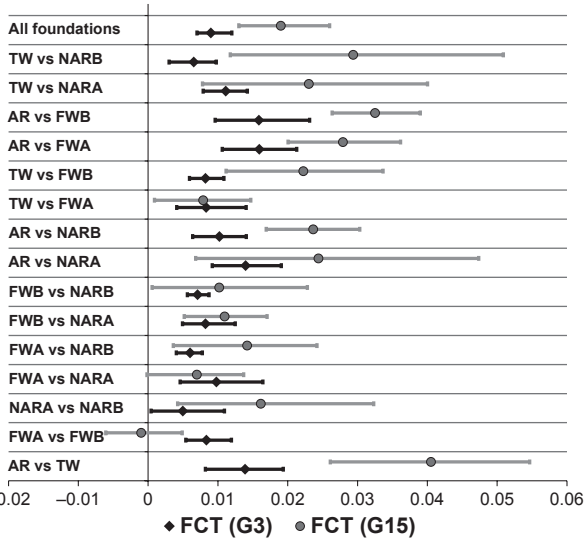

Fig. 2 Genetic differentiation (F_{CT}) between pairs of foundations, each consisting of three replicated populations, at generations three and 15, with 95% confidence interval.

Temporal changes of life-history traits

As we indicated before, the analysis of the evolutionary trajectories of life-history traits has been already presented (see Simões *et al.*, 2008b), and so we will just offer a brief summary of our earlier conclusions here.

Laboratory adaptation during the first 20–21 generations was consistently reported across our foundations, with a general improvement of more fitness-relevant traits such as early fecundity (F1–7) and the composite phenotype (CPhen), which increased through time. Age of first reproduction (A1R) significantly declined with time in the laboratory, again corresponding to an improvement in fitness. Nevertheless, we also detected significant differences between foundations, both at the start of adaptation and in evolutionary rates for all traits, more pronounced for less-relevant fitness traits such as female starvation resistance – RF – (see Tables 3 and 4 in Simões *et al.*, 2008b). Those differences illustrate the importance of evolutionary contingencies for adaptation to a novel environment.

The evolutionary trajectories for the six foundations and five traits that we analyse here are presented in Fig. 4.

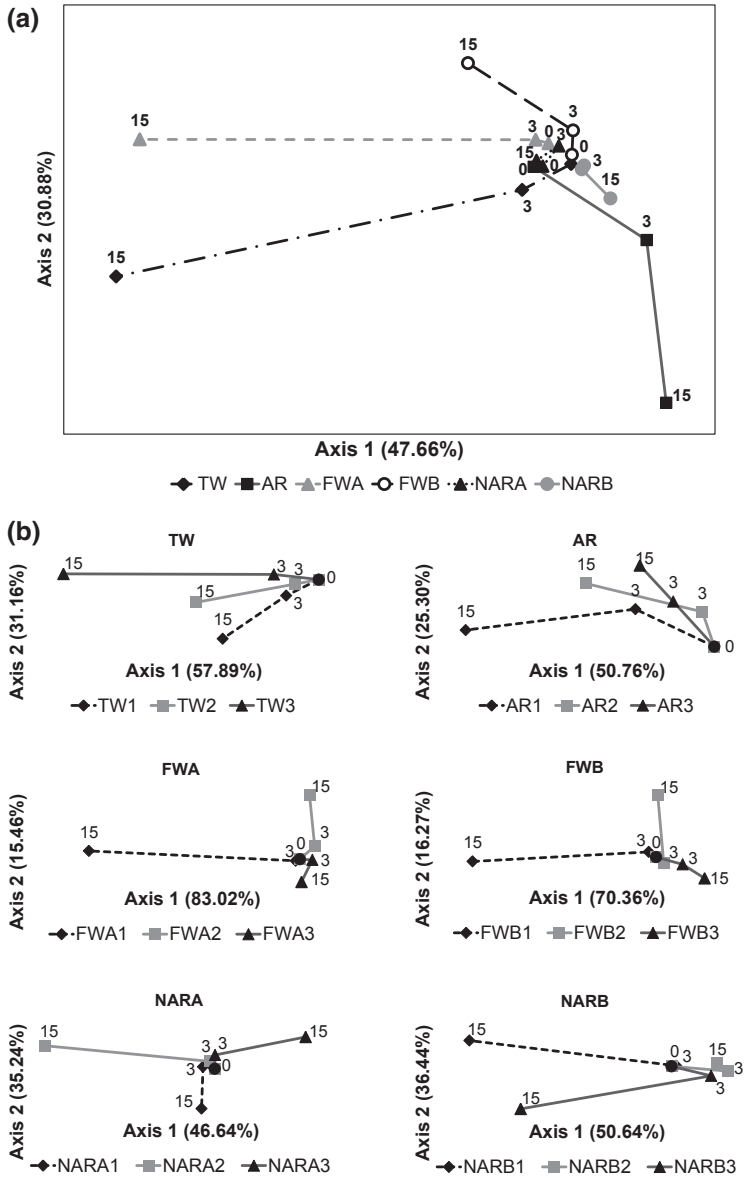

Fig. 3 Principal coordinate analysis (PCoA) using F_{ST} values as distance across populations and generations, including founders (0) and generations three (3) and 15. The coordinates that explain most of the variation (first two axes) are presented. Lines connect the dots of each foundation (population) through successive generations; 3(a) Principal coordinate analysis across foundations and generations (18 by 18 square matrix); mean F_{ST} values between replicate populations across foundations and/or generations were considered for data involving generations 3 and 15, for example average of the 9 F_{ST} estimates between the three TW replicate populations at generation three and the three AR replicate populations at generation 15; 3(b) Principal coordinate analysis within foundations, across replicate populations and generations (7 by 7 square matrix), for example for TW PCoA involved the square matrix of F_{ST} between TW_i, the three replicate populations $TW_{1–3}$ at generation three and the three replicate populations $TW_{1–3}$ at generation 15.

Relating life-history traits to initial microsatellite data

Most simple correlations between early performance (ed) and average slopes and genetic variability across foundations were not significant (Table S4). By contrast, we found many significant associations between the difference matrices of these parameters, when tested by signed Mantel tests (Table 3). It is worth noting that there was in general a correspondence in sign, and frequently a similarity in value, of the Pearson's coefficient for the two types of tests,

Table 3 Correlations between pairwise differences (for the six foundations) in life-history parameters and pairwise differences in microsatellite parameters. Microsatellite data involve the founders (G0) and generation three (G3). Other correlations presented: between pairwise differences in life-history parameters and differences in number of founders (n), as well as in effective population size (N_e); between pairwise variance components of slopes and differentiation between founder populations (F_{ST}) and between foundations at generation three (F_{CT}). For ease of comparisons, the signs of correlations with edA1R and A1R were inverted. See details in Materials and Methods.

	Life-history traits									
	Early differentiation					Slopes				
Microsatellite data	edA1R	edF1–7	edF8–12	edRF	edCPhen	A1R	F1–7	F8–12	RF	Cphen
Founders (G0)										
H_E	0.57*†	0.78*†	0.88**†	−0.12	0.36	−0.39	−0.54*†	−0.81**†	−0.07	−0.09
A	−0.05	0.37	0.34	−0.66	0.75**†	−0.63**†	−0.82**†'	−0.59**†	0.41	−0.63*
F_{ST}	–	–	–	–	–	−0.32	0.64	0.43	0.17	0.38
Generation three (G3)										
H_E	0.61**†	0.09	0.54**†	0.29	−0.43	0.53	0.47*	−0.09	−0.41	0.64**†
A	0.88**†	0.43*	0.64**†	0.62*	−0.46	0.29	0.42	−0.25	−0.52*	0.70*
F_{CT}	–	–	–	–	–	−0.60*	0.76*†	0.37	0.34	0.73*
n	0.02	−0.30	0.16	−0.39	0.01	0.58	0.45**†	0.55**†	0.34	0.05
$N_{e(G3)}$	0.48	−0.11	0.08	0.61	−0.66*	0.62	0.85***†	0.43	−0.36	0.72
$N_{e(G3–G15)}$	0.42	−0.19	−0.03	0.68	−0.75*	0.67**†	0.91**†	0.49	−0.41	0.76

A, allelic richness; H_E, gene diversity.
*0.01 < P-value < 0.05; **0.001 < P-value < 0.01; ***P-value < 0.001 (significance by Mantel test).
†Significant after false discovery rate correction (for $\alpha = 0.05$) considering 25 tests for H_E and A at each generation and the 30 tests for n and N_e.

Table 4 Correlations between pairwise differences in slopes of life-history traits and variability decline in three generation ranges over six foundations. Significances of Mantel tests are given. For ease of comparison, the signs of correlations with A1R were inverted.

	Slopes of life-history traits				
Generation range	A1R	F1–7	F8–12	RF	Cphen
dA					
G0–G3	−0.60**†	−0.80**†	−0.29	0.56	−0.81*
G3–G15	0.15	−0.26	−0.28	0.10	−0.12
G0–G15	−0.47**†	−0.83**†	−0.38	0.54	−0.78*
dH_E					
G0–G3	−0.73**†	−0.87**†	−0.86**†	0.15	−0.46
G3–G15	0.22	0.02	−0.09	0.15	0.02
G0–G15	0.10	−0.32	−0.40*	0.18	−0.17

dA, decline in allelic richness; dH_E, decline in gene diversity.
*0.01 < P-value < 0.05; **0.001 < P-value < 0.01.
†Significant after false discovery rate correction (for $\alpha = 0.05$) considering 30 tests (dA and dH_E across traits and periods).

particularly when the Mantel test gave significant results. This suggests that it was lack of statistical power that prevented the detection of a dependence of effects when using the first approach. Except when indicated otherwise, we will from here on focus on our analyses that used the Mantel approach.

In general, pairs of foundations with smaller variability differences in microsatellites had smaller differences in early performance in the laboratory (ed), both in the founders (G0) and at generation three (G3) – one to three (of four tested) positive correlations for most traits even after FDR correction (Table 3). Differences in genetic variability between founders were in general negatively correlated with differences in evolutionary rate, with many negative correlations remaining significant after FDR correction (Table 3). In contrast, genetic variability at generation three was generally positively correlated with the slopes of most traits, although with few statistically significant results: only the correlation between gene diversity and slope of the composite phenotype remained significant after FDR correction.

Genetic differentiation between founder populations (pairwise F_{ST} at generation zero) showed no significant correlation with the variances among slopes of any of the five fitness-related traits, whereas genetic differentiation at generation three (F_{CT}) had a positive significant correlation with the variance of several traits, although only the correlation with early fecundity remained significant after FDR correction (Table 3).

Overall starvation resistance contrasted with other traits in not showing significant correlations with genetic variability at either generation.

Relating phenotypic traits with effective population size and number of founders

Most simple correlations between effective population size (or number of founders) and life-history parameters

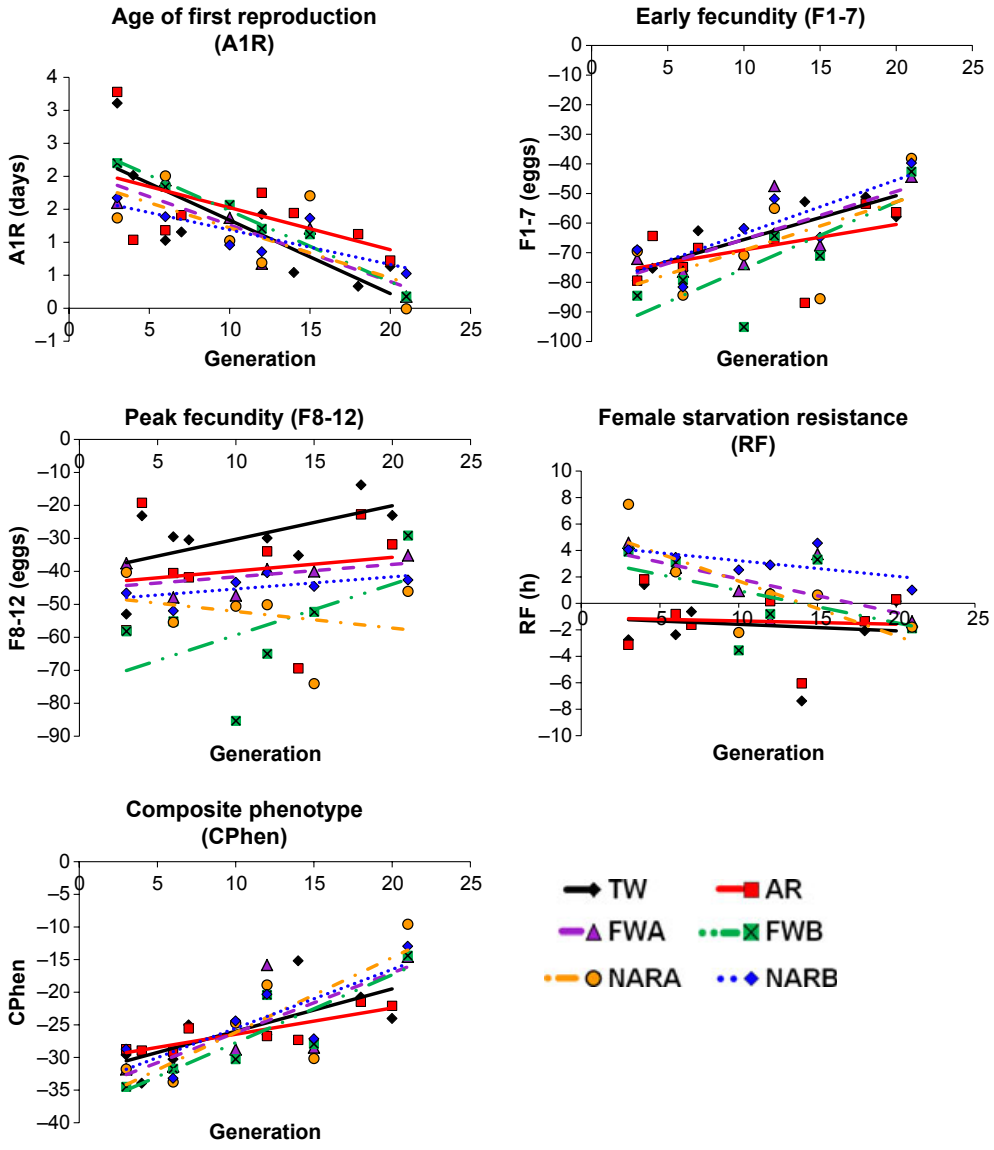

Fig. 4 Evolutionary trajectories of several life-history traits during the first 20/21 generations in the laboratory. At each generation, the values plotted were calculated as difference from the reference (control) NB populations. Markers indicate mean values between the three replicates of each foundation at each assay, and trendlines are the mean tendency through time for each foundation.

were not significant (Table S4). Both number of founders and the two estimations of N_e presented significant positive correlations, as estimated by signed Mantel tests, with the slopes (although not early differentiation) of all traits, except starvation resistance. After FDR adjustment, five correlations remained significant, three involving F1–7 (Table 3).

Relating temporal changes in microsatellites with dynamics of fitness-related traits

The decline in genetic variability between generations zero and three – and to a lesser extent generation 15 – tended to be negatively associated with the slopes of several life-history traits, both by simple correlation and by Mantel test (Table 4, Table S5). No significant correlation was found with the decline of variability between generations three and 15. After FDR correction, seven correlations estimated by Mantel test (of 30 tested across parameters and periods) remained significant, none involving starvation resistance (Table 4).

Highly significant simple Pearson's correlations were found between the global F_{ST} values (differentiation across replicates within foundations) at generation 15 and the corresponding variance of slopes between repli-

cates, both for female starvation resistance ($r = 0.941$; $F_{1,4} = 31.03$, $P = 0.005$) and for the composite phenotype ($r = 0.863$; $F_{1,4} = 13.99$, $P = 0.027$).

Discussion

Dynamics of molecular markers after a founding event

We have found that founder events entail an immediate loss of genetic variability in six separate foundations of replicated laboratory populations of *Drosophila subobscura* derived from collections in the wild. Dlugosch & Parker (2008) also saw this general tendency when compiling information on several founding events. Genetic drift due to reduced effective population size was the major force causing this loss of variability, and its effects changed through time, being stronger in the early phases of evolution after the founding event, reinforcing the conclusions of our previous studies (Simões *et al.*, 2008a, 2010). As expected, this effect was particularly strong for allelic richness (Fig. 1 and Table 1; also see Table S6) due to the fact that it is more sensitive to the loss of low-frequency alleles during a bottleneck (Nei *et al.*, 1975; Leberg, 1992; Cabe, 1998; Dlugosch & Parker, 2008). This is particularly expected for microsatellites, which have many rare alleles (Spencer *et al.*, 2000). On the other hand, gene diversity is mostly affected by intermediate-frequency alleles, which are more likely to remain in the gene pool of the newly founded population (Nei *et al.*, 1975).

Moreover, there were significant differences in the loss of variability among foundations (see Fig. 1 and Table S6), again with the strongest impact during the first generations (G0–G3). These effects were strong enough for populations from different foundations to achieve significant molecular genetic differentiation after only three generations spent evolving in the new laboratory environment and to increase in genetic differentiation over subsequent generations (Figs 2 and 3; see also Fig. S1). Our results suggest that stochastic sampling effects mediated by changes in effective population size play a major role during the first steps of adaptation to a non-native environment (see review in Keller & Taylor, 2008).

As also observed by Dobzhansky & Spassky (1962), we detect significant evolutionary differences across foundations just a few generations after a founding event. Effective population size is implicated in this divergence, while the number of founders seems to play a less important role (Table S3). The power of sampling effects is particularly shown by the absence of a significant positive correlation between allelic richness at generation zero and after 15, or even after only three, generations of evolution in the same new environment. Furthermore, the significant positive correlation between differences in allelic richness across foundations at generations three and 15 suggests that early random effects that arise during the first three generations have consequences at later generations, particularly due to the irreversible loss of rare alleles. Although such strong reshuffling effects of sampling were not seen for gene diversity between the founders and generation three, it clearly played a role at more advanced generations, leading to the negative association of gene diversity between the founders (G0) and generation 15 (Table S7). In general, the decoupling between the genetic variability of the founders and the genetic variation in the early generations after the establishment of laboratory culture prevents the prediction of future molecular evolutionary dynamics of the population from the variability of the founders.

Adaptation of founder populations to a new common environment: Do sampling effects play along?

There was a general and clear response to the new laboratory selective pressures, particularly in traits more closely related to fitness, like age of first reproduction, early fecundity and the composite phenotype (see Fig. 4). Nevertheless, as we also showed previously (Simões *et al.*, 2008b), adaptation to the laboratory differed across foundations, both in initial state and in adaptive rate (see Tables 3 and 4 in Simões *et al.*, 2008b). Given our analysis of the evolutionary dynamics of molecular markers, it is most likely that the evolutionary contingencies observed for adaptation to a novel environment were due to sampling effects immediately upon foundation. This is in accordance with a study conducted by Regan *et al.* (2003) with housefly populations, which found more diverse evolutionary responses by bottlenecked founder-flushed populations compared to nonbottlenecked ones (but see Kolbe *et al.*, 2012, for a study in lizards with contrasting results).

Do molecular markers predict the evolutionary dynamics of fitness-related traits?

In general, we did not find significant direct associations across foundations between initial genetic variability of molecular markers (both A and H_E at generations zero and three) and subsequent evolutionary rates for life-history traits (see Table S4). Nevertheless, the Pearson's correlation coefficients were similar in sign and often in absolute value to those of the correlations between difference matrices, which gives robustness to our following interpretations based on signed Mantel tests (see Table 3).

Several significant correlations between pairwise differences in genetic variability (both allelic richness and gene diversity) and differences in fitness-related

traits were found, both for early phenotypic performance (ed) and for subsequent adaptive dynamics. The generally positive association between higher variability in microsatellites (A and H_E for both G0 and G3) and higher early phenotypic differentiation (ed) in the laboratory is in agreement with the expectation that bottlenecks can reduce both genetic variability and fitness (Reed & Frankham, 2003). Nevertheless, the correlations found between differences in the genetic variability of the founders (G0) and differences in evolutionary rates were negative (see Table 3). That negative association means that, within pairs of foundations, the one with lower genetic variation tended to exhibit a higher rate of evolutionary response relative to the other. This might seem unexpected, considering that molecular markers are often used as surrogates for quantitative genetic variation of fitness and thus as an indication of potential to respond to selection. However, Reed & Frankham (2001) had already challenged this idea, as in a meta-analysis of 71 data sets, they did not find a correlation between molecular genetic variation and genetic variation for fitness. Curiously here, we did find some association, but in the opposite direction, as just mentioned. As Robertson (1960) suggested, it is possible that a bottleneck may lead to an increase in additive genetic variance due to the conversion of nonadditive genetic variance to additive (Goodnight, 1988; Whitlock *et al.*, 1993; Cheverud *et al.*, 1999). When this is the case, stronger bottlenecks can lead to both reduced genetic variability in neutral markers and increased adaptive response.

But if we use the genetic variability at generation three as a point of reference for predicting subsequent evolutionary change, we find that the association tends to be positive, although a smaller number of significant correlations are detected (Table 3). Among these, it is worth noting the inversion of sign of the significant association between differences in allelic richness and differences in the evolutionary rate of the composite phenotype, as well as an inversion of sign between differences in gene diversity and differences in the evolutionary rate of early fecundity, although this was not significant after FDR adjustment. Nevertheless, excluding the AR foundation, which suffered the highest decline in both genetic variability estimates along the first three generations (Fig. 1 and Table S2), leads to consistent negative correlations whether using the founders or generation three data (Table S8). It is likely that the strong sampling effects suffered by this AR foundation during the first three generations led to a greater overall loss of additive genetic variance among the AR populations, strongly affecting the correlation with evolutionary rate across foundations (Buskirk & Willi, 2006). Nevertheless, a word of caution is required here, because few correlations remain significant after FDR adjustment, probably due to the lower statistical power of such analysis with five foundations.

The role of the initial sampling effect on the evolutionary dynamics is also illustrated by the significant negative correlations of pairwise differences in variability decline (in particular allelic richness – dA) from the founders to later generations (G0–G3 or G0–G15) and differences in evolutionary dynamics (slopes) for several functional traits (see Table 4). This effect is not present when considering the decline between generations 3 and 15 (G3–G15). Therefore, most sampling effects with an effect on adaptive rate occurred during the first three generations. The AR foundation seems once more to have played a major role in this contrast, because removing this foundation from the analysis causes this contrast between periods to disappear, as some significant negative correlations were also observed at the generation range G3–G15 (see Table S9). Because no correlations remain significant after the removal of the AR foundation and FDR adjustment, these particular results have to be viewed with caution.

It is interesting to note that starvation resistance strongly contrasts with other traits, in that in general we did not detect significant associations between the evolutionary dynamics of that trait and genetic variability of molecular markers. For instance, in the analysis of the association between decline of genetic variability and the evolutionary rate of life-history traits, we were able to detect 10 significant correlations of the 24 estimated between the rate of decline of genetic variability and fecundity-related traits (of which 7 remained significant after FDR). This ratio of significant results is fairly high, considering the low statistical power expected in our analysis involving only six foundations. Nevertheless, no significant correlation (of the six tested) arose in statistical tests involving starvation resistance, even without FDR adjustment. This may seem unexpected, because we found previously that starvation resistance is in fact one of the traits that shows highly contingent evolution across foundations (Simões *et al.*, 2007, 2008b). But during laboratory adaptation, starvation resistance is expected to change chiefly as a correlated response with other, more relevant fitness-related traits and not by direct selection. Sampling effects on the genetic association between traits are thus expected to play a more important role for starvation resistance than general loss of genetic variability arising from an initial bottleneck.

The role of effective population size in the evolutionary dynamics of molecular markers and of life-history traits

As we have seen, AR was one of the foundations with a smaller number of founders and moreover the one with the smallest effective population size (N_e). This might have contributed to the higher rate of loss of genetic variability, as well as its lower adaptive rate, observed in all life-history traits (see also Simões *et al.*, 2007, 2008b).

In general, across foundations, we saw that effective population size affected both the decline of genetic variability and the adaptive rate in the expected direction. Specifically, foundations with smaller mean effective population size at generation three (G3) tended to have a bigger decline in genetic variability than foundations with bigger population size (see Table S3). Also as expected, foundations that presented higher number of founders and N_e tended to exhibit greater rates of adaptation (Table 3). These results corroborate the hypothesis that, during adaptation to a novel environment, the relative roles of natural selection and genetic drift are mediated by population size (Allendorf, 1986; Weber, 1990; Weber & Diggins, 1990; Frankham, 1999; Buskirk & Willi, 2006).

It is noteworthy that the range of mean effective population sizes in our foundations was relatively small, between 55.2 ± 8.6 and 129.2 ± 7.6 from estimates at generation three, with AR having the smaller N_e and the strongest sampling effects in this study. This suggests that greater differences in the evolutionary dynamics, with respect to both molecular markers and life-history traits, would have been detected among foundations if cases with more variation in the number of founders had been analysed. Studies including more foundations with widely contrasting numbers of founders would allow a test of this expectation.

From Nature to the Laboratory, there is no valid retrodiction

Our findings reinforce the importance of sampling effects for the rate of adaptation to a novel environment, effects that start with the founding event and are inflated during the very first generations after colonization. During this early phase of adaptation, a population finds itself in an 'evolutionary no man's land' (Matos *et al.*, 2000). Some have proposed that the best way to reduce any potential decoupling between evolution in the laboratory and the adaptive process in nature is to study recently introduced laboratory populations as few generations from their foundation as possible (Harshman & Hoffmann, 2000). But sampling effects during the very first generations in the laboratory may lead to significant genetic differentiation between foundations derived from a small number of founders. Here, we further show that these early-generation effects can also lead to differences in the subsequent dynamics of laboratory adaptation, despite founding from the same source population in the wild. These effects in turn explain the patterns that we previously found for the dynamics of laboratory populations of different foundations (Simões *et al.*, 2008b).

One might argue that the sampling effects observed in our populations would not have been detected if larger founding populations were used. Indeed, we do not wish to generalize our conclusions to such evolutionary scenarios. However, most colonization events involve a small number of founders. This also applies to the foundation of captive populations for *ex situ* conservation purposes. Moreover, laboratory studies of populations adapting to a novel, laboratory environment frequently involve a small number of founders from the wild, with values that do not differ much from those used in this study.

Thus, it is systematically difficult to retrodict the properties of a population in its previous circumstances from its evolutionary trajectories and its evolutionary genetics after it colonizes a novel environment. This is particularly relevant when one wants to extrapolate from studies in laboratory populations to evolution in nature. Evolution is local (cf. Rose *et al.*, 2005), and care must be taken with generalizations, as well as with the inference of evolutionary dynamics from comparisons across populations (see a critical review in Simões *et al.*, 2009).

We find that evolution in a novel environment is a very complex process. Although all of our experimental populations adapted to the novel laboratory environment, they did so with contrasting initial states and disparate rates of adaptation. Thus, although highly repeatable in its general outcome of adaptation, the evolution of a newly founded population is strongly contingent not only on its initial composition but also on the immediate stochastic changes that occur during the first generations of colonization. Between ancestral and novel environment, 'all hell may break loose', imperilling the accuracy of scientific inference across environments.

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Data accessibility

Microsatellite sequences are available with the accession numbers GU732209-80 at GenBank. Data is deposited in the Dryad repository with doi:10.5061/dryad.0fm71.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Census size across generations. (A) Sintra & Arrábida, 2001. (B) Sintra, 2005. (C) Arrábida, 2005.

Table S2 Allelic richness and gene diversity at generations zero, three and 15. (A) Mean allelic richness and gene diversity at generations zero, three and 15. (B) Allelic richness and gene diversity for each microsatellite at generations zero, three and 15.

Table S3 Simple Pearson correlation test and signed Mantel test between the variability decline per generation and the number of founders (n) and effective population size at generation three.

Table S4 Simple Pearson correlation coefficient between early differentiation (ed) or slopes of life history traits and microsatellite parameters, number of founders and effective population size.

Table S5 Simple Pearson correlation coefficient between slopes of life history traits and variability decline per generation in allelic richness and gene diversity.

Table S6 ANOVA results. (A) Friedman ANOVA testing differences in variability between foundations at

generation zero. (B) ANOVA testing differences in variability between foundations at generations three and 15. (C) ANOVA testing differences in the decline of variability at each generation range. (D) ANOVA testing differences in the decline of variability across generation ranges and foundations.

Table S7 Correlation coefficients between variability estimates at different generations, tested by simple Pearson correlation coefficient and by signed Mantel test.

Table S8 Correlations excluding AR, between pairwise differences in life-history parameters and pairwise differences in microsatellite parameters, in number of founders, and in effective population size.

Table S9 Correlations excluding AR, between pairwise differences in slopes of life-history traits and variability decline per generation tested by Mantel test.

Figure S1 F_{ST} (Weir & Cockerham, 1984) between pairs of foundations, at generations zero, three and 15.

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