

RESEARCH ARTICLE

Fast evolutionary genetic differentiation during experimental colonizations

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Abstract

Founder effects during colonization of a novel environment are expected to change the genetic composition of populations, leading to differentiation between the colonizer population and its source population. Another expected outcome is differentiation among populations derived from repeated independent colonizations starting from the same source. We have previously detected significant founder effects affecting rate of laboratory adaptation among *Drosophila subobscura* laboratory populations derived from the wild. We also showed that during the first generations in the laboratory, considerable genetic differentiation occurs between foundations. The present study deepens that analysis, taking into account the natural sampling hierarchy of six foundations, derived from different locations, different years and from two samples in one of the years. We show that striking stochastic effects occur in the first two generations of laboratory culture, effects that produce immediate differentiation between foundations, independent of the source of origin and despite similarity among all founders. This divergence is probably due to powerful genetic sampling effects during the first few generations of culture in the novel laboratory environment, as a result of a significant drop in N_e . Changes in demography as well as high variance in reproductive success in the novel environment may contribute to the low values of N_e . This study shows that estimates of genetic differentiation between natural populations may be accurate when based on the initial samples collected in the wild, though considerable genetic differentiation may occur in the very first generations of evolution in a new, confined environment. Rapid and significant evolutionary changes can thus occur during the early generations of a founding event, both in the wild and under domestication, effects of interest for both scientific and conservation purposes.

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Introduction

Population size plays a key role in determining the relative importance of natural selection and genetic drift, with small isolated populations more exposed to stochastic loss of genetic variability, potentially reducing their subsequent response to selection (Robertson 1960). During a colonization event, a population may experience a considerable reduction in size. The effect of a census population-size bottleneck on effective population size (N_e) is expected to be strong even when a population expands quickly after the initial colonization event (Wade and McCauley 1988;

Charlesworth 2009). Genetic drift arising from a founder-generation bottleneck is thus expected to lead to divergence between the source population and newly founded populations. In a classic paper, Carson and Templeton (1984) proposed that ‘under some circumstances the founder event may set the stage for speciation by altering genetic conditions in the gene pool’, due to genetic changes such as gametic disequilibrium. Moreover, quantitative genetic models show that a strong bottleneck may increase the evolutionary potential of a population, due to conversion of nonadditive into additive component of genetic variance (Goodnight 1988). This may also lead to divergence from the ancestral population. Empirical evidence of such increases in evolutionary potential after a bottleneck comes from several species and traits (see Bryant *et al.* 1986; Regan *et al.* 2003). Such

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stochastic effects are also expected to lead to genetic differentiation when several independent colonizations from the same source take place. When populations colonize a novel environment, high variance in reproductive success may contribute to low N_e (Hedrick 2005), further increasing the importance of drift. Natural selection can thus augment the effects of genetic drift during colonization of a novel environment.

There is abundant evidence for the loss of variability among neutral genetic markers during colonization events (e.g. Pascual *et al.* 2007). Both theory and simulations have shown that when populations grow quickly after a founder event, differences in founding gene frequencies tend to be maintained even in the presence of gene flow (Boileau *et al.* 1992). Only over long time scales will gene flow from the source population partly compensate for initial losses of genetic diversity (Dlugosch and Parker 2008). Moreover, rare alleles may get amplified to high frequencies by a combination of founder effects and rapid population growth (surfing), contributing to genetic differentiation between populations (Excoffier and Ray 2008). The effect of initial historical events may thus play an important role in the evolution of differences between populations derived from independent colonization events, even when a common source population is involved.

While the success of colonization does not depend on genetic variation among neutral markers, the latter can still provide a measure of how strong initial bottleneck drift effects are. Colonization history inferred from neutral genetic markers is thus a useful tool when testing the context for adaptive evolution in particular, when population sizes are expanding and have not reached equilibrium between gene flow and selection (Keller *et al.* 2009).

Reduced population size can have two well-defined genetic sampling effects on a newly founded population: a reduction in allele number (Nei *et al.* 1975) and a bias in allele frequencies (Waples 1998). Even high frequency alleles can be underrepresented in the new population, and relative allele frequencies will be rearranged (Ryman 1997; Palm *et al.* 2003). This can then result in a shift in the mean phenotypes of the colonizers relative to the source population (Keller and Taylor 2008). Differences in the evolutionary dynamics among multiple new colonies derived from the same ancestral population may then result.

Ultimately, laboratory populations of model organisms are founded from collections in the wild, with a limited number of founder individuals that yield lab N_e values which can be orders of magnitude smaller than those of the natural populations of origin. Thus, the initial generations in the laboratory can be seen as a type of colonization process (Matos *et al.* 2000). Since this scenario of both reduced initial population size and new selective pressures will lead to lower effective population size, it is a general expectation that multiple laboratory introductions will lead to genetic differentiation among lab populations, even when they share a common source and undergo parallel selection.

Genus *Drosophila* is widely studied in the laboratory not only for genetics, for which it has long been a leading model organism, but also in experimental evolution (e.g. Prasad and Joshi 2003). Disparities among the experimental results found among different laboratories or with different experimental stocks in the same laboratory may be partly due to stochastic events that took place during the early steps of founding the various *Drosophila* populations that have been employed. This should be taken into account before offering more elaborate interpretations (cf. Ackermann *et al.* 2001). This does not mean that laboratory studies are useless, in terms of their value for generalization. The fact that laboratory evolution may be a 'local' process (vid. Rose *et al.* 2005) illustrates material complexities of evolutionary dynamics that will often be important for the correct interpretation of both genetic and evolutionary research, even in laboratory models.

We have repeatedly performed studies of evolutionary trajectories during laboratory adaptation of *Drosophila subobscura* populations derived from collections in the wild. Two sets of populations derived from geographically close locations revealed significant differences in the laboratory evolution of fitness-related traits after their founding from the wild (Simões *et al.* 2007). Further, these founding populations were differentiated at microsatellites by generation three of laboratory culture (Simões *et al.* 2008a). Nevertheless, the design of that study did not allow us to determine whether this differentiation was due to distinct geographic locations or initial sampling effects, whether at the founding generation or during the next two generations. To disentangle these two possible effects at the level of life-history traits, we sampled two new sets of populations from each of the locations previously used. These new populations again exhibited location-dependent differences in the laboratory evolution of life-history traits. Moreover, we also detected some sampling effects within locations both at the start of adaptation and in initial evolutionary rates, for weakly selected traits (Simões *et al.* 2008b). An important analysis still missing from that study is the systematic characterization of the genetic differentiation of neutral markers between populations, whether among the first founders or at an early generation after laboratory introduction. Data of this kind will allow the estimation of the possible effects of founder events on genetic variability and on differentiation that may reveal the role of early colonization events on laboratory adaptation.

Here we deepen a previous analysis of the genetic variability and differentiation of these six foundations both at the founders and at laboratory generation three (Santos *et al.* 2012), taking into account their natural sampling hierarchy involving two locations, two years and two samples from each location in one of the years, each one three-fold replicated by generation two. We show that striking stochastic effects occur in the first few generations of laboratory culture, effects that produce immediate differentiation between populations derived from different foundations, independently of their source of origin and despite similarity among all founders.

Materials and methods

Sampling design

To screen the occurrence of sampling effects in the founders and during the early stages of laboratory evolution, we derived two independent foundations of *D. subobscura* a few days apart in 2005, in each of two natural sites, Adraga, in Sintra (from here on referred as FWA and FWB) and Arrábida (here named as NARA and NARB). These are the same Portuguese locations sampled in 2001, samples of *D. subobscura* which gave rise to the TW (from Sintra) and AR (from Arrábida) foundations (Simões *et al.* 2007, 2008a, b) (figure 1). The sampling locations are 50 Km apart, being separated by the Tagus river. Fermented fruit baits were used for all collections. To assure that female founders were fertilized (less than 30% being virgin upon collection), groups

of around five females and two males, derived from the same collections, were formed and maintained in vials. The TW foundation was collected on October 12th and 13th of 2001, being composed of 110 females and 44 males, while the AR foundation derived from 59 females and 24 males collected on 10th, 14th and 15th of October of the same year. All 2005 collections presented more males than females, the excess being randomly discarded. The FWA foundation was composed of 60 females and 28 males, collected from 5th to 7th of April, while FWB derived from 75 females and 30 males collected on 9th and 10th of April. In Arrábida, 55 females and 24 males collected on April 4th originated the NARA foundation, and 68 females and 30 males collected on April 8th gave rise to the NARB foundation (see figure 1). All foundations were three-fold replicated during the collection of the eggs that gave rise to the second

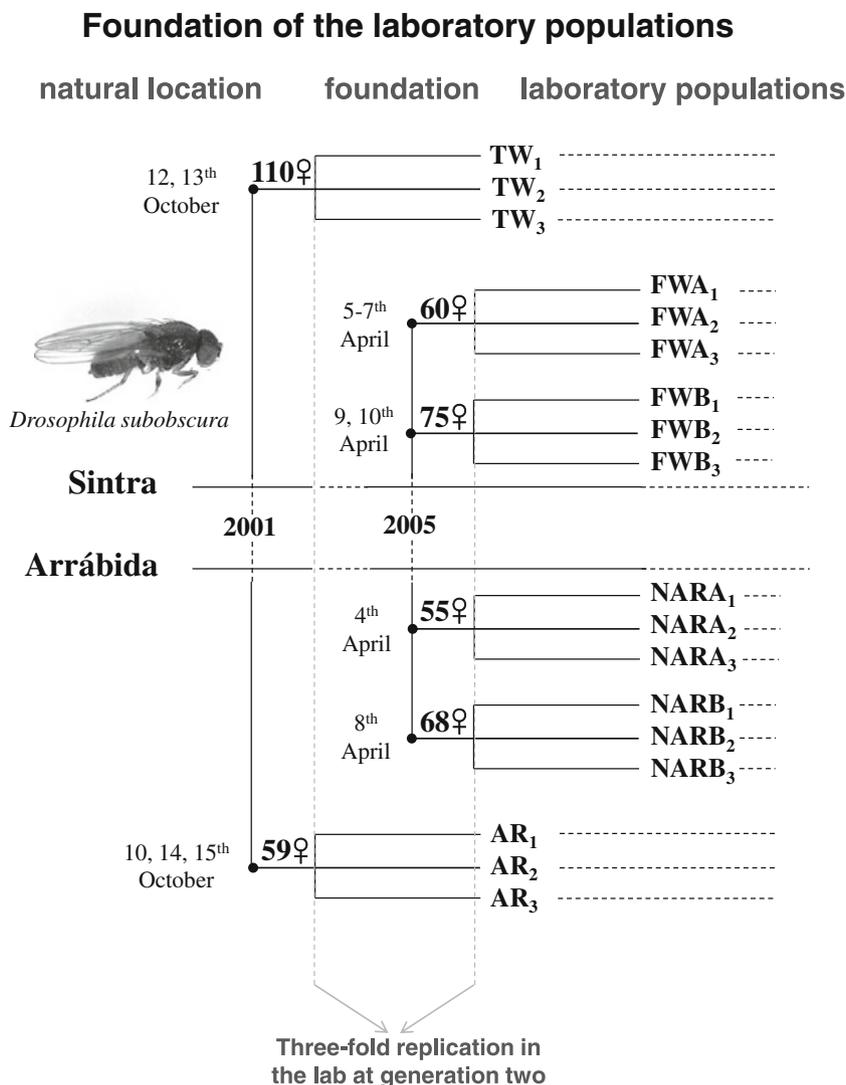


Figure 1. Founding scheme from the natural locations of Sintra and Arrábida used to derive *D. subobscura* laboratory populations. Three-fold replication took place in the collection of eggs to generation two. The number of female founder flies (♀) is given as well as the days of collection from the wild.

generation. Maintenance conditions were same for all populations as described in Simões *et al.* (2008b), involving discrete generations of 28 days, reproduction timed to be close to the age of peak fecundity, controlled temperature of 18°C and a 12 : 12 h L : D cycle. Flies were kept in vials with David axenic medium stained with animal charcoal. Density was controlled for both eggs and adults at about 80 and 50 individuals per vial, respectively. This corresponded to 24 vials with 80 eggs each, at the start of each generation, and to a variable number of adults as a function of developmental success. Census population sizes were generally between 600 and 1200 adult individuals distributed among 12 to 24 vials. In each generation, adults that emerged from several vials corresponding to each population were mixed together with CO₂ anesthesia before the collection of eggs for the next generation.

DNA extraction and amplification

All 18 laboratory populations were genotyped for nine microsatellites at the third generation after the founding event (TW₁₋₃, AR₁₋₃, FWA₁₋₃, FWB₁₋₃, NARA₁₋₃ and NARB₁₋₃). For each of the six foundations, founders collected from the wild (generation zero) were also genotyped for the same markers. This is an adequate number of markers, as Spencer *et al.* (2000) showed that eight highly polymorphic microsatellites are enough to detect bottleneck effects. Our markers' sequences have the accession numbers GU732209–GU732280 at GenBank. The markers: dsub01, dsub02, dsub05, dsub10, dsub19, dsub20, dsub21, dsub23 and dsub27, used were previously identified and characterized by Pascual *et al.* (2000), and cytologically localized in the five *D. subobscura* chromosomes (Santos *et al.* 2010). For each population, 30 randomly picked female flies were analysed. Altogether, the DNA of 720 females was thus extracted and amplified following the general protocol described in Simões *et al.* (2008a). Fragment analysis was carried out always in the same ABI PRISM 310 sequencer (Applied Biosystems, Foster City, USA). Allele sizes were estimated by comparing it with the standard GeneScan-500 ROX, using the software GeneMapper ver. 3.7 (Applied Biosystems). In a separate publication (Santos *et al.* 2012) we used these genotypic data together with other sets of data to analyse how initial variability and its decline may affect subsequent evolution of populations. Data presented here is deposited in the Dryad repository with doi:10.5061/dryad.0fm71.

Data analysis

Tests of Hardy-Weinberg equilibrium: Deficit of heterozygotes per locus and sample was estimated by F_{IS} coefficients and tested based on 4320 randomizations in FSTAT 2.9.3 (Goudet 1995). False discovery rate (FDR) corrections for multiple testing were carried out following theorem 1.3 of

Benjamini and Yekutieli (2001). The adjusted α was determined by $\alpha_{adj} = \frac{\alpha}{\sum_{i=1}^m \frac{1}{i}}$, m being the number of tests.

Microsatellite variability: Microsatellite variability was characterized by estimating allelic richness (A) and gene diversity (or expected heterozygosity, H_E) with the software FSTAT 2.9.3 in the founders and at generation three. The variability of the six foundations in each generation was analysed by a Friedman's ANOVA (analysis of variance) across nine loci. Wilcoxon tests were also performed to test for differences between pairs of foundations at the same generation (averaging data of the three replicates of each foundation at generation three).

At generation three, we performed simple ANOVAs to test differences in each of the variability estimates across foundations using data of the three replicates within each foundation as individual data. Variability estimates were also tested across years and locations by a bi-factorial ANOVA, with year corresponding to the years of founding, 2001 and 2005, and location for both sites of origin of foundations, Sintra and Arrábida.

The experimental design of the 2005 foundations involves two independent founder events from each of the two locations (FWA and FWB, in Sintra and NARA and NARB, in Arrábida). To test for the effect of such founder events on the genetic differences of variability at generation three, a nested ANOVA was used with the two foundations (each three-fold replicated) nested inside each location (Foundation{Location}). The significance of the term Foundation {Location} indicates the occurrence of founder effects between the two events of founding within each location. When differences between the two foundations derived from different sampling events within location were not significant, simple ANOVAs were performed pooling together the six populations inside each location as if it is derived from the same founder event.

For each foundation, allelic richness and gene diversity were compared between founders and populations at generation three using a Wilcoxon matched pair-test performed across the nine loci. Decline in both variability measures in this period (dA for allelic richness and dH_E for gene diversity) was calculated by subtracting from unity, the ratio between variability of each population at generation three and variability in the respective founders. To test the differences in decline across foundations, year and location, as well as due to sampling, the same four ANOVA models described above for generation three were used.

Genetic differentiation: Genetic differentiation was estimated by determining the fixation indexes both between foundations (F_{CT}) at generation three and between populations (F_{ST}) at generations zero and three. The differentiation between foundations (F_{CT}) was estimated by AMOVA (analysis of molecular variance) with the software Arlequin 3.5.1.2 (Excoffier *et al.* 1992), which follows the Weir and

Cockerham (1984) framework. The analysis allows the definition of groups of populations, generating a hierarchical structure with the total variance partitioned into covariance components due to different levels of variation: individuals, populations (each replicate is a population) and groups of populations (foundations i.e., groups of three replicates). F_{CT} significance was tested after 1000 permutations of populations among foundations and confidence intervals were obtained by 20,000 bootstraps over loci.

Genetic differentiation at the third generation involving data of the 2005 foundations allowed testing for founder effects due to differences between locations, taking into account the possible role of sampling effects of foundation within locations. To perform this test, these foundations were analysed with a design involving nesting of foundations (samples) inside each location (FWA and FWB, from Sintra and NARA and NARB, from Arrábida). Differentiation between locations was estimated by defining a four-level hierarchical AMOVA with individuals, populations (replicates), foundations (A and B) and locations (Sintra and Arrábida), using the software GDA 1.1 (Lewis and Zaykin 2001). Differentiation relative to the variance at the more inclusive level (location) was given by a Theta-P (θ -P) value, equivalent to F_{CT} , which confidence intervals were obtained after 5000 bootstraps over loci. To understand how founder effects may affect differentiation we also calculated genetic differentiation between locations with a three-level hierarchical analysis pooling together the six populations of the two samples inside each location as if it is derived from a unique sampling effort.

Pairwise F_{ST} values between all populations were also obtained for all populations, including the founders, using FSTAT 2.9.3 software (Goudet 1995). Exact G -tests were performed using GenePop ver. 4.0 (Rousset 2008). FDR correction was applied for multiple testing, considering the number of comparisons inherent to each question, such as: Are the 2005 populations derived from the four foundations differentiated between foundations at generation three (54 comparisons)? Are the 2005 populations differentiated within foundations at generation three (12 comparisons)? Are the founders (including those from 2001 and 2005) differentiated among them (15 comparisons)? Are populations at generation three differentiated from their founder-generation (18 comparisons), and from other founders (90 comparisons)?

A principal coordinate analysis using pairwise F_{ST} values and including generation three as well as the founders of the 2005 populations was performed in GenAlex 6.4 (Peakall and Smouse 2006).

Given the recent controversy as to what is the best way to estimate genetic differentiation between populations using highly variable molecular markers such as microsatellites (Jost 2008; Ryman and Leimar 2009), we also estimated pairwise Jost's D_{est} values using SMOGD (Crawford 2010). After FDR adjustment, the average D_{est} across the nine loci was estimated in each comparison, with significance tested

by t -test (with differences between microsatellites as source of error). Significance of correlations between F_{ST} and D_{est} matrices were also estimated (by Mantel test) using GenAlex 6.4 software (Peakall and Smouse 2006).

Effective population size: Effective population sizes (N_e) were estimated both for founder populations and populations at generation three by the linkage disequilibrium (LD) method, using the software NeEstimator 1.3 (Ovenden *et al.* 2007). Differences between the effective population size estimates in the founders and mean values between replicates at generation three were tested using the Wilcoxon matched pair-test.

The decline in N_e between generations zero and three was estimated from $dN_e = (1 - N_{e(3)}/N_{e(0)}) * 100$. The ratio N_e/N (with N being the census size) was also calculated at generation three.

ANOVA tests for detecting effects of foundation, year and location using the models described above were performed for the estimates of effective population size at generation three, its decline since founding (dN_e), and its ratio with census size (N_e/N).

Results

Hardy-Weinberg equilibrium

In general, most loci were in Hardy-Weinberg equilibrium (HWE) in the founders, with some exceptions. Locus dsub02 gave an indication of heterozygote-deficit in TW and in NARA. At generation three, most populations significantly deviated from HWE for one or more loci, with the exception of AR₁, AR₂, FWA₃, NARA₁ and NARB₁. Nevertheless, only dsub02 in TW and dsub10 in FWB showed consistent heterozygote deficits for all replicates at generation three (see table 1 in electronic supplementary material at <http://www.ias.ac.in/jgenet/>).

Microsatellite variability in the founders and at generation three

Variability levels were globally high both in the founders and at generation three (table 1; details per microsatellite are presented in Santos *et al.* 2012). Overall the six foundations did not differ significantly either in allelic richness (A) or in gene diversity (H_E), both at the founders and at generation three, tested by Friedman ANOVA. Only the comparison between AR and FWA founders gave significantly higher allelic richness for the former (Wilcoxon $Z = 2.19$, $P = 0.028$). However at generation three significant differences for allelic richness were found across foundations by parametric ANOVA ($F_{(5,12)} = 4.71$, $P = 0.013$).

When tested for year and location effects at the third generation, a significant effect of year and of the interaction term year \times location was found for allelic richness ($F_{(1,14)} = 11.40$, $P = 0.0045$; and $F_{(1,14)} = 6.78$, $P = 0.021$, respectively) but not for gene diversity. Tukey tests showed a significantly lower allelic richness of AR when compared to FWA, NARA and NARB ($df = 12$, $P = 0.018$, $P = 0.019$

Table 1. Variability estimates for foundations at generation three and the respective founders.

Year	Location	Foundation	Allelic richness (<i>A</i>)		<i>dA</i> (%)	Expected heterozygosity (<i>H_E</i>)		<i>dH_E</i> (%)	
			Founders	Generation 3		Founders	Generation 3		
2001	Sintra	TW	16.81	14.39** ±0.09	14.44	0.913	0.896 ^{n.s.} ±0.0008	1.95	
	Arrábida	AR	17.07	13.63** ±0.33	20.12	0.907	0.885 ^{n.s.} ±0.0037	2.41	
2005	Sintra	FWA	15.85	14.80* ±0.15	6.66	0.91	0.892* ±0.0016	1.99	
		FWB	15.52	14.26 ^{n.s.} ±0.10	8.12	0.894	0.891 ^{n.s.} ±0.0042	0.29	
	Arrábida	NARA	16.64	14.78** ±0.32	11.15	0.914	0.895* ±0.0065	2.09	
		NARB	16.5	14.73* ±0.07	10.74	0.909	0.893* ±0.0019	1.8	
	Mean			16.4	14.43 ±0.18	12	0.908	0.892 ±0.002	1.76
	± SEM			±0.24		±1.98	±0.003		±0.30

Wilcoxon tests for differentiation between generations: n.s., nonsignificant $P > 0.05$; * $0.01 < P < 0.05$; ** $P < 0.01$. Decline in allelic richness (*dA*) and heterozygosity (*dH_E*) from the founders to the third generation; *A* values are averages between the nine loci; mean values (±SEM) between the three replicates are given at generation three and over all foundations. N.S., nonsignificant.

and $P = 0.026$, respectively). ANOVA tests on the 2005 foundations taking into account the hierarchical structure (the two foundations in each location) gave no significant differences either between or within locations, at generation three for both allelic richness and gene diversity.

Most foundations showed a significant reduction in allelic richness across loci between generations (table 1). For some foundations, gene diversity also declined significantly. Both the decline of allelic richness and of gene diversity were significantly different across foundations ($F_{(5,12)} = 15.24$, $P = 0.0001$; and $F_{(5,12)} = 3.38$, $P = 0.039$, respectively). The decline in allelic richness was also significantly different across years as well as across locations ($F_{(1,14)} = 62.49$, $P = 0.0001$; and $F_{(1,14)} = 20.25$, $P = 0.0005$, respectively). The 2005 foundations suffered a mean decline of allelic richness of 3.06% per generation compared to the 5.76% observed for the 2001 foundations. Further, the Sintra foundations showed a mean decline of 3.25% per generation in allelic richness while Arrábida foundations had a decline of 4.67%. There was also a significant difference in decline of allelic richness between locations with the 2005 data alone ($F_{(1,10)} = 11.07$, $P = 0.008$). Differences between locations remained significant taking into account the hierarchical structure involving the two foundations in each location ($F_{(1,2)} = 22.40$, $P = 0.042$), while foundations within location did not differ significantly ($F_{(2,8)} = 0.439$, $P = 0.659$). No significant effect of year or location on the decline of gene diversity was detected, both with the whole set of foundations and with the 2005 data alone.

Patterns of genetic differentiation

There was a high concordance between estimates of genetic differentiation using F_{ST} versus Jost's D_{est} , as shown by the

significant Mantel test correlations between F_{ST} and D_{est} matrices among all populations ($r = 0.977$, $P < 0.001$), between founders ($r = 0.751$, $P = 0.003$) and between populations at generation three ($r = 0.971$, $P < 0.001$) (see figure 2). Thus, in spite of the bigger values obtained with D_{est} , the rankings of the estimates of genetic differentiation across population pairs were very similar using either index. Nevertheless, few cases of significant differentiation were detected using D_{est} probably due to a lower statistical power of the *t*-tests used in this case (cf. table 2; tables 3 and 4 in electronic supplementary material with tables 5–7 in electronic supplementary material). Below we will focus the results obtained

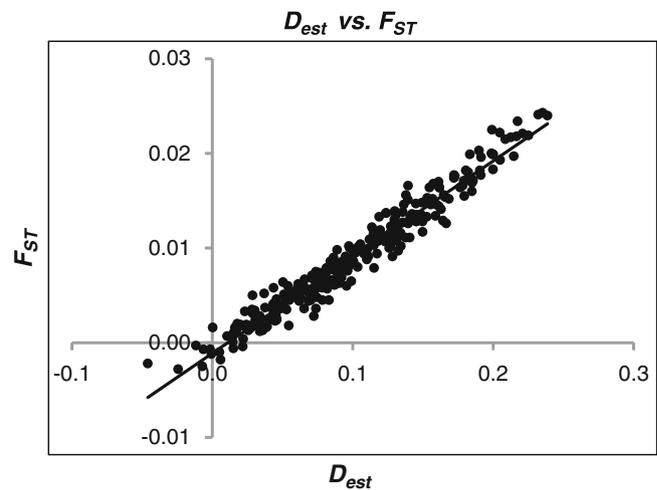


Figure 2. Plot of estimates of genetic differentiation by D_{est} versus F_{ST} across all pairs of populations (276 comparisons). The line of the best linear model is also presented. The correlation (r) between the two dissimilarity matrices was: $r = 0.997$ and the significance (by Mantel test) $P < 0.0001$.

Table 2. Pairwise differentiation (F_{ST}) between the founders and populations at generation three.

	TW _f	AR _f	FWA _f	FWB _f	NARA _f	NARB _f
TW1	0.0018	0.0044*	0.0041	0.0047	0.0013	0.0045
TW2	0.0065	0.0095**	0.0050*	0.0034*	0.0067*	0.0055
TW3	0.0029	0.0079**	0.0019*	0.0048**	0.0034	0.0039
AR1	0.0135**	0.0148†	0.0182**	0.0199**	0.0166**	0.0146**
AR2	0.0106**	0.0100†	0.0125**	0.0104**	0.0141**	0.0063**
AR3	0.0077**	0.0123†	0.0123**	0.0112**	0.0058**	0.0088**
FWA1	0.0045*	0.0066*	0.0023	0.0091**	0.0066**	0.0025
FWA2	0.0094*	0.0080*	0.0018	0.0107**	0.0076**	0.0088*
FWA3	0.0076*	0.0107**	0.0015	0.0137**	0.0087**	0.0036
FWB1	0.0052	0.0115**	0.0083**	0.0053	0.0057*	0.0077*
FWB2	0.0017*	0.0075*	0.0016**	0.0016	−0.0004	0.0024
FWB3	0.0045	0.0091*	0.0025	0.0033	0.0061**	0.0001
NARA1	0.0045	0.0047	0.0063**	0.0051*	−0.0010	0.0081*
NARA2	0.0066	0.0053	0.0048	0.0072	−0.0025	0.0036
NARA3	0.0064**	0.0094**	0.0044**	0.0119**	0.0018	0.006**
NARB1	0.0017	0.0020	0.0024**	0.0046**	0.0005	−0.0003
NARB2	0.0061**	0.0104**	0.0024*	0.0064**	0.0075**	0.0017
NARB3	0.0101**	0.0122*	0.0064**	0.0058*	0.0068*	0.0058

Significance levels after FDR correction: * $0.002 < P < 0.0098$ (for $0.01 < \alpha < 0.05$), ** $P < 0.002$ (for $\alpha < 0.01$) between founders and generation three of populations from other foundations (90 pairs); † $P < 0.0029$ (for $\alpha < 0.01$) between founders and generation three of derived populations (18 pairs); *f*, founder populations.

by F_{ST} , comparing them with D_{est} when appropriate. Statistical significance was in all cases adjusted for multiple testing by FDR.

The six foundations were not significantly differentiated from each other among the founders (table 2a in electronic supplementary material). Moreover, populations at generation three were generally not significantly differentiated from the founders of origin with the exception of the three AR replicates (table 2). Nevertheless, there were significant differences between some of the founders and populations of other foundations at generation three (table 2). The same general conclusions are suggested by the D_{est} estimates (cf. tables 2b and 5 in electronic supplementary material).

At generation three, the two foundations of 2001 were significantly differentiated, whether estimating pairwise F_{ST} between populations of distinct foundations ($F_{ST} = 0.022$; $P < 0.0001$) or F_{CT} between the two groups ($F_{CT} = 0.0139$; $P < 0.0001$). By contrast, of the six pairwise comparisons between replicates within foundations, only AR₁ ver-

sus AR₂ showed significant differentiation ($F_{ST} = 0.0086$; $P < 0.002$). Similar results were obtained when estimating differentiation by D_{est} (data not shown).

When comparing the 2005 populations at the third generation, a four-level hierarchical model was used to test for founder effects between locations taking into account sampling effects within each location. For this purpose, we defined an AMOVA hierarchy with the following levels: individuals, replicate populations within foundations (e.g. FWA₁₋₃), the two foundations within each location (i.e. FWA₁₋₃ versus FWB₁₋₃ and NARA₁₋₃ versus NARB₁₋₃; see figure 1), and locations (FWA and FWB versus NARA and NARB). When using this hierarchical analysis, no significant differentiation was observed between locations (θ - $P = 0.0011$; 95% CI (−0.00092, 0.00305)). Nevertheless, low but significant differentiation between locations was detected when pooling together the six populations in each location (i.e. six Sintra populations versus six Arrábida populations; θ - $P = 0.0038$; 95% CI (0.00196, 0.00568)). By contrast,

Table 3. Genetic differentiation (F_{CT}) between the 2005 foundations at generation three.

Location	Foundation	F_{CT}
Sintra sample A vs B	FWA vs FWB	0.00836** (0.00540, 0.01193)
Arrábida sample A vs B	NARA vs NARB	0.00498* (0.00041, 0.01094)
Sintra vs Arrábida	FWA vs NARA	0.00979** (0.00459, 0.01645)
	FWA vs NARB	0.00601** (0.00404, 0.00777)
	FWB vs NARA	0.00822** (0.00492, 0.01249)
	FWB vs NARB	0.00709** (0.00561, 0.00873)

* $0.00001 < P < 0.001$; ** $P < 0.00001$; in brackets, 95% confidence intervals obtained over 1000 bootstraps.

significant F_{CT} values were obtained at generation three among both sets of populations (foundations A and B) derived from each location (table 3). Differentiation between sets of populations from different locations (e.g. FWA versus NARB) was also detected in all comparisons.

Pairwise F_{ST} values at generation three were estimated for all pairs of populations derived in 2005 (see table 3 in electronic supplementary material). Of the 12 pairwise comparisons between replicates of the same foundation, only three showed significant pairwise differentiation (FWA₁ versus FWA₂, FWB₁ versus FWB₃ and NARB₁ versus NARB₂). By contrast, all the pairwise generation-three comparisons between populations from different foundations were significantly differentiated, both when comparing populations from Sintra and Arrábida and when comparing populations derived from different foundations of the same location (e.g. NARA₁ versus NARB₃). The few significant results obtained using D_{est} were also between populations of different foundations (table 6 in electronic supplementary material). To visualize the results obtained from all populations derived in 2005, a principal coordinate analysis using pairwise F_{ST} values involving all founders and populations at the third generation was carried out (figure 3). The first two axes explain 82.72% of the variation, with the four founder populations located closely together in a central position, and the derived replicated populations dispersed around them. FWA and FWB are farther apart than NARA and NARB foundations mostly due to FWA, which is farther away from the other foundations.

When comparing the third generation of populations derived from different years (72 pairs), highly significant genetic differentiation was always obtained (F_{ST} ranging

from 0.0058 to 0.0241, $P < 0.001$; see table 4 in electronic supplementary material). Significant D_{est} values were obtained in 38 of the 72 tests (see table 7 in electronic supplementary material).

Effective population size in the founders and at generation three

No significant differences in the initial effective population sizes were found across foundations (see table 8 in electronic supplementary material). At the third generation, N_e did not differ significantly either between foundations or locations, but differed significantly between years of founding (mean N_e 2001 = 72.7; mean N_e 2005 = 108.95; $F_{(1,14)} = 6.57$, $P = 0.023$). This difference was due to the lower N_e of Arrábida 2001 foundation (AR, $N_e = 55.2$) compared to Arrábida 2005 foundations (NARA and NARB pooled together, mean $N_e = 116.8$; Tukey test; $df = 14$, $P = 0.037$). The N_e/N ratio, like N_e , also showed a significant effect of year (mean N_e/N 2001 = 0.105, mean N_e/N 2005 = 0.180; $F_{(1,14)} = 5.20$, $P = 0.039$). When considering the hierarchical design of the 2005 foundations alone, no significant founder effects were detected either between or within locations.

A significant decline in effective population size (dN_e) was found between generations zero and three across foundations ($Z = 2.20$; $P = 0.028$), with N_e changing from a mean number of 314.7 (standard error of the mean (SEM) 74.3) breeders in the founder populations to 96.9 (SEM 10.6) breeders at the third generation (see table 8 in electronic supplementary material). No significant differences were detected in the N_e decline either between foundations, years, or locations. Moreover, no significant differences were detected between the 2005 foundations either between or within locations.

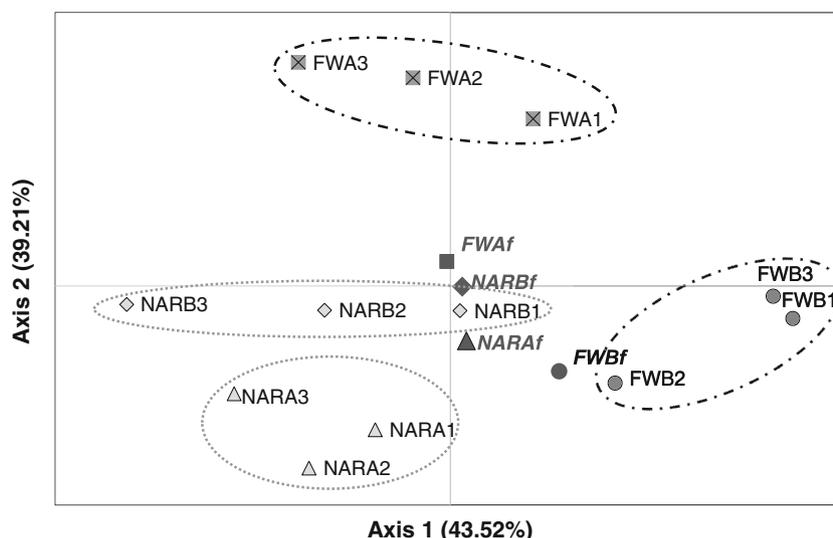


Figure 3. Principal coordinates analysis of the 2005 populations at generations zero and three. Pairwise F_{ST} values (Weir and Cockerham 1984) were used as genetic distance estimate. *f* refers to founder populations and replicate populations at generation three are labelled from one to three.

Discussion

High and consistent genetic variability among initial samples collected from the wild

Genetic variability was very high for all founder populations and in total not significantly different between them, indicating negligible initial sampling effects across multiple collections from the wild. Moreover, these data suggest high stability in the genetic variability of the natural source populations, across both space and time. The high mean genetic diversity levels estimated (from 0.894 to 0.914) place these *D. subobscura* natural populations among the most diverse for this species, when compared to the analysis of similar sets of loci from other European populations (Pascual *et al.* 2001).

Moreover, the lack of differentiation between founders (the initial samples of wild individuals) from Sintra and Arrábida suggests that there is extensive gene flow between the two locations, suggesting in turn that they are a single deme. This natural population has maintained remarkably stable genetic composition for neutral markers across years and seasons involved in the two sampling points of this study (autumn 2001 and spring 2005).

Changes in genetic variability during the first generations of a founding event

During the first three generations after introduction, there was a significant decline in genetic variability across all our laboratory populations, an average decline of 4.00% per generation in allelic richness and 0.59% per generation in gene diversity. The stronger decline in allelic richness is in accordance with expectations, as it is a more sensitive measure of the loss of low frequency alleles when population size is reduced (Nei *et al.* 1975).

In fact, from generation zero to three, the number of alleles with frequency lower than 5% decreased further than the number of alleles in higher frequencies, though more frequent alleles were also affected. This observation is in accordance with studies of the loss of alleles during the colonization of America by *D. subobscura* (Pascual *et al.* 2007). However, there is a 4.6% to 36% probability that some alleles were not lost, but instead were not detected due to their low frequency, given the use of a generation-three sample size of 30 individuals genotyped per population (Gregorius 1980). Nevertheless, since the founders and third generation were characterized using the same sample size this effect is not sufficient to explain all of the difference in the distribution of frequencies of alleles between generations. Thus, we conclude that loss of lower frequency alleles has occurred during the early steps of the founding event, although the present quantitative estimates of the magnitude of that loss are biased upward.

Due to this stochastic loss of alleles after as few as three generations, these newly-founded laboratory populations diverged in allelic richness, though not in gene diversity. In

particular, there was a clear effect of year, and year \times location interaction, for allelic richness, mainly due to the low values for the three replicate populations derived from the Arrábida foundation in 2001. This was also the foundation with the highest decline in allelic richness (6.71% per generation) from initial collection to generation three, and the foundation with the slowest subsequent rate of adaptation to laboratory conditions (Simões *et al.* 2008b; Santos *et al.* 2012). Yet, this was initially one of the most genetically variable founder populations, despite having the smallest effective population size. Drift effects may explain all these observations, in that a stronger bottleneck effect during the first laboratory generations of these populations may have led to a higher loss of low frequency alleles with a corresponding but smaller impact on gene diversity (Nei *et al.* 1975). Differences in the decline of allelic richness were also found between the two locations in 2005, though not between the two foundations from the same location. This particular case suggests that initial founder effects involving different locations or years can inflate later divergence during laboratory evolution.

Thus, our results illustrate the point that multiple populations which derive from the same source population may develop genetic differentiation quickly as a result of an initial bottleneck (cf. Wade and McCauley 1988; Charlesworth 2009), particularly if gene flow from the source populations or among the derived populations does not occur after introduction (Boileau *et al.* 1992; Dlugosch and Parker 2008). Our data thus demonstrate the relevance of stochastic effects for the genetic variability of neutral markers during the establishment of new populations, which in turn provides an important ‘null model’ for the interpretation of observed genetic changes in terms of adaptive evolution (Keller *et al.* 2009) when changes in population structure occur.

Do F_{ST} and D_{est} tell the same story?

F_{ST} and related measures may underestimate genetic differentiation when highly polymorphic molecular markers, such as microsatellites are used leading to the development of alternative estimators such as D_{est} (Jost 2008). Other problems may however arise when estimating genetic differentiation from D_{est} , particularly when the goal is to describe the average amount of differentiation observed over multiple loci (e.g. see Ryman and Leimar 2009, also Leng and Zhang 2011). The fact that F_{ST} may increase as polymorphism drops is relevant to our study, since genetic drift is expected to cause a drop in gene diversity, which may also inflate the expected increase in genetic differentiation between populations. Here we found a high concordance between differentiation measured by F_{ST} and D_{est} across all population pairs (see figure 2), despite the lower statistical significance of the latter estimates. Thus, the two measures of genetic differentiation tell a similar story with respect to early differentiation among our populations. We will chiefly base our discussion on F_{ST} estimates.

Disentangling sources of differentiation between wild and laboratory populations

After three generations of culture in the laboratory environment, populations derived in 2001 from Sintra foundations were highly differentiated genetically from populations derived from Arrábida (Simões *et al.* 2008a). We also detected genetic differentiation at the third generation between the two sets of populations collected in the same location in 2005. Our present results suggest that such differences between foundations could have been caused by sampling effects during the collection from the wild, a problem which has been emphasized by the authors of other studies (Waples 1998; Keller and Taylor 2008). If this is generally the case, laboratory populations derived from the wild may not be representative of their source population, leading to overestimation of differentiation between natural populations. Nevertheless, as we have found no significant differentiation between different sets of founders collected a few days away from the same location, we conclude that overestimation of genetic differentiation due to initial-collection sampling effects is not relevant when analysing as few as 30 individuals from natural populations. Our findings suggest instead that genetic differentiation is more likely to occur during the first generations of evolution in the laboratory due to the effects of low effective population size. This fits the model of Wade and McCauley (1988) for evolution after colonization of a new patch. According to their model, both the number of founders and subsequent effective population size (N_e) across generations are major factors causing genetic differentiation between independent recently founded colonies, with the first factor having a stronger weight. Applying the authors' equations (see equations 1–3, pp. 998 in Wade and McCauley 1988) to our data for the number of founders and effective population sizes, we obtained similar values to those observed in our microsatellite analysis (table 9 in electronic supplementary material). We conclude that the pairwise differentiation we encountered at generation three is easily explained by neutral drift effects during the first three generations of laboratory culture. However, the fact that these populations are adapting to a novel lab environment has probably augmented drift effects, as high variance in reproductive success can result in very low effective population size even in quickly expanding populations (Hedrick 2005).

Is there a signature of history in the initial laboratory evolution of differentiation?

Though no consistent differentiation was observed between the six initial founding samples by the third generation they had significantly differentiated from other founders but not from their own founding sample except for the case of the AR_{1–3} populations previously discussed. These results suggest the presence of a signature of the founders on the gene pool of some of the derived populations at generation three. Thus, although sampling effects did not lead to statistically inferable differentiation at the time of first collection, genetic

differentiation aligning each new laboratory population with its founder nonetheless arose. This was probably due to the fact that founder populations differ enough in alleles of lower frequencies which are lost preferentially during the first generations of laboratory evolution to have later effects.

The impact of initial genetic drift on subsequent responses to selection

We have concluded this far that genetic sampling effects during a founding event can have repercussions on the genetic variability of newly-founded populations. These evolutionary changes can be misleading if taken as a signature of selection. Moreover, these chance effects can impact the subsequent evolutionary potential of populations, if loss of genetic variability due to bottlenecks affects additive genetic variation in traits relevant for fitness. As an illustration of possible associations between drift events and subsequent response to selection, it is interesting to note that the 2001 Arrábida populations, which had (i) the lowest N_e values, (ii) the fastest decline of genetic variability, as well as (iii) the greatest genetic differentiation after three generations of laboratory culture were also the populations that had the slowest subsequent rate of change in the most relevant fitness traits during laboratory evolution (Simões *et al.* 2007, 2008b). This leads us to suggest that the differences in the experimental evolution of life-history characters inferred in our earlier publications might have been due to stochastic sampling effects that arose in the first few generations of laboratory culture, and not, as we concluded before, due to differences between the wild source populations (cf. Simões *et al.* 2008b). Our finding of a significant association between early genetic variability and subsequent laboratory adaptation supports this scenario (see Santos *et al.* 2012).

General significance for conservation and experimental biology

Most biologists and many conservationists do not reflect much on the evolutionary genetic upheaval that taking individuals out of wild populations necessarily causes. Here we have shown that this upheaval begins immediately within the first three generations of sampling from the wild. In particular, even if a reasonably large sample of organisms is first collected, we have shown here that the much smaller N_e values that will almost always prevail in laboratories and zoos are likely to cause an immediate evolutionary shift away from the population-genetic state of the initial sample.

Some might argue that equalization of family contribution, frequently recommended in captive breeding programmes with conservation purposes might have led to contrasting results. Such a management procedure is expected to approximately double the effective population size in a simple neutral model while further benefiting N_e by removing the between family component of selection (Rodríguez-Ramilo *et al.* 2006). In particular, this method has been shown to improve founder representation in the initial generations

(Loebel *et al.* 1992). Nevertheless, a big contrast between the census size of the natural population and the recent colonizer must still occur leading to the expected divergence between populations in the early steps of colonization.

A different procedure that might reduce the decoupling between the natural population and derived colonizers, at least for the purpose of scientific inference, is to create isofemale lines during the first generations of the founding event. While this is a common practice in order to preserve molecular genetic variability over the entire ensemble of isofemale lines (e.g. Kauer *et al.* 2003), it is unclear what impact such intense inbreeding might have on polygenic traits (Hoffmann and Parsons 1988). Thus, creating isofemale lines too could generate potentially serious problems of its own even for the purpose of scientific inference.

This makes laboratory populations inherently suspect as guides to the properties of wild populations, even their proximally ancestral wild populations. Contrary to the assertions or hopes of Harshman and Hoffmann (2000), we do not feel that many specific inferences about the properties of wild populations are likely to be obtainable from the study of their laboratory derivatives (vid. Matos *et al.* 2000), just as many results obtained from specific laboratory evolution experiments may not generalize to other laboratory populations (e.g. Rose *et al.* 2005). Thus laboratory populations, in our opinion, are best viewed as specific instances of a broad range of possible evolving populations. This makes them useful for strong-inference tests of wide-ranging Popperian scientific theories, but not notably useful as material for the inductive study of innumerable properties of the wild populations from which they were derived.

But, more constructively, studies like the present one provide an opportunity to study the evolutionary possibilities that can arise from vicariance events and large-scale dispersal to novel environments. While the study of the colonization of the New World by a European *D. subobscura* population has provided an important window on the long-term patterns of such events (Gilchrist *et al.* 2004; Pascual *et al.* 2007), experimental evolutionary studies allow us to look in much greater detail at colonization events, with respect to both the evolution of components of fitness (vid. Simões *et al.* 2007, 2008b) and, as provided here, the population genetics at a fine scale of temporal resolution. With the application of genomewide sequencing to such experimental evolution, as well as greater replication of source and derived populations, we may soon be able to determine experimentally just how such key evolutionary processes as adaptation and speciation work genetically as population structure ramifies across geographical landscapes.

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References

- Ackermann M., Bijlsma R., James A. C., Partridge L., Zwaan B. J. and Stearns S. C. 2001 Effects of assay conditions in life history experiments with *Drosophila melanogaster*. *J. Evol. Biol.* **14**, 199–209.
- Benjamini Y. and Yekutieli D. 2001 The control of the false discovery rate in multiple testing under dependency. *Ann. Stat.* **29**, 1165–1188.
- Boileau M. G., Hebert P. D. N. and Schwartz S. S. 1992 Non-equilibrium gene frequency divergence: persistent founder effects in natural populations. *J. Evol. Biol.* **39**, 25–39.
- Bryant E. H., McComas S. A. and Combs L. M. 1986 The effect of an experimental bottleneck upon quantitative genetic variation in the housefly. *Genetics* **114**, 1191–1211.
- Carson H. L. and Templeton A. R. 1984 Genetic revolutions in relation to speciation phenomena – the founding of new populations. *Annu. Rev. Ecol. Syst.* **15**, 97–131.
- Charlesworth B. 2009 Fundamental concepts in genetics: effective population size and patterns of molecular evolution and variation. *Nat. Rev. Genet.* **10**, 195–205.
- Crawford N. G. 2010 SMOGD: software for the measurement of genetic diversity. *Mol. Ecol. Res.* **10**, 556–557.
- Drugosch K. M. and Parker I. M. 2008 Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Mol. Ecol.* **17**, 431–449.
- Excoffier L. and Ray N. 2008 Surfing during population expansions promotes genetic revolutions and structuration. *Trends Ecol. Evol.* **23**, 347–351.
- Excoffier L., Smouse P. E. and Quattro J. M. 1992 Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**, 479–491.
- Gilchrist G. W., Huey R. B., Balanyà J., Pascual M. and Serra L. 2004 A time series of evolution in action, a latitudinal cline in wing size in South American *Drosophila subobscura*. *Evolution* **58**, 768–780.
- Goodnight C. J. 1988 Epistasis and the effect of founder events on the additive genetic variance. *Evolution* **42**, 441–454.
- Goudet J. 1995 FSTAT (ver. 2.9.3.): a computer program to calculate F-statistics. *J. Hered.* **86**, 485–486.
- Gregorious H. R. 1980 The probability of losing an allele when diploid genotypes are sampled. *Biometrics* **36**, 643–652.
- Harshman L. G. and Hoffmann A. A. 2000 Laboratory selection experiments using *Drosophila*, what do they really tell us? *Trends Ecol. Evol.* **15**, 32–36.
- Hedrick P. W. 2005 A standardized genetic differentiation measure. *Evolution* **59**, 1633–1638.
- Hoffmann A. A. and Parsons P. A. 1988 The analysis of quantitative variation in natural populations with isofemale strains. *Genet. Sel. Evol.* **20**, 87–98.
- Jost L. 2008 G_{ST} and its relatives do not measure differentiation. *Mol. Ecol.* **17**, 4015–4026.
- Kauer M. O., Dieringer D. and Schlötterer C. 2003 A microsatellite variability screen for positive selection associated with the “Out of Africa” habitat expansion of *Drosophila melanogaster*. *Genetics* **165**, 1137–1148.
- Keller S. R. and Taylor D. R. 2008 History, chance and adaptation during biological invasion: separating stochastic phenotypic evolution from response to selection. *Ecol. Lett.* **11**, 852–866.

- Keller S. R., Sowell D. R., Neiman M., Wolfe L. M. and Taylor D. R. 2009 Adaptation and colonization history affect the evolution of clines in two introduced species. *New Phytol.* **183**, 678–690.
- Leng L. and Zhang D.-X. 2011 Measuring population differentiation using G_{ST} or D' ? A simulation study with microsatellite DNA markers under a finite island model and nonequilibrium conditions. *Mol. Ecol.* **20**, 2494–2509.
- Lewis P. O. and Zaykin D. 2001 Genetic data analysis: computer program for the analysis of allelic data, vers. 1.0.d16c. Available at <http://lewis.eeb.uconn.edu/lewishome/software.html>.
- Loebel D. A., Nurthen R. K., Frankham R., Briscoe D. A. and Craven D. 1992 Modeling problems in conservation genetics using captive *Drosophila* populations: consequences of equalizing founder representation. *Zoo Biol.* **11**, 319–332.
- Matos M., Rego C., Levy A., Teotónio H. and Rose M. R. 2000 An evolutionary no man's land. *Trends Ecol. Evol.* **15**, 206.
- Nei M., Maruyama T. and Chakraborty R. 1975 The bottleneck effect and genetic variability in populations. *Evolution* **29**, 1–10.
- Ovenden J., Pee D., Street R., Courtney A., Hoyle S., Peel S. and Podlich H. 2007 The genetic effective and adult census size of an Australian population of tiger prawns (*Penaeus esculentus*). *Mol. Ecol.* **16**, 127–138.
- Palm S., Laikre L., Jorde P. E. and Ryman N. 2003 Effective population size and temporal genetic change in stream resident brown trout (*Salmo trutta*, L.). *Conserv. Genet.* **4**, 249–264.
- Pascual M., Schug M. D. and Aquadro C. F. 2000 High density of long dinucleotide microsatellites in *Drosophila subobscura*. *Mol. Biol.* **17**, 1259–1267.
- Pascual M., Aquadro C. F., Soto V. and Serra L. 2001 Microsatellite variation in colonizing and Palearctic populations of *Drosophila subobscura*. *Mol. Biol. Evol.* **18**, 731–740.
- Pascual M., Chapuis M. P., Mestres F., Balanyà J., Huey R. B., Gilchrist G. W. et al. 2007 Introduction history of *Drosophila subobscura* in the New World: a microsatellite-based survey using ABC methods. *Mol. Ecol.* **16**, 3069–3083.
- Peakall R. and Smouse P. E. 2006 Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* **6**, 288–295.
- Prasad N. G. and Joshi A. 2003 What have two decades of laboratory life-history evolution studies on *Drosophila melanogaster* taught us? *J. Genet.* **82**, 45–76.
- Regan J. L., Meffert L. M. and Bryant E. H. 2003 A direct experimental test of founder-flush effects on the evolutionary potential for assortative mating. *J. Evol. Biol.* **16**, 302–312.
- Robertson A. 1960 A theory of limits in artificial selection. *Proc. R. Soc. London, Ser. B* **153**, 234–249.
- Rodríguez-Ramilo S. T., Morán P. and Caballero A. 2006 Relaxation of selection with equalization of parental contributions in conservation programs: an experimental test with *Drosophila melanogaster*. *Genetics* **172**, 1043–1054.
- Rose M. R., Passananti H. B., Chippindale A. K., Phelan J. P., Matos M., Teotónio H. and Mueller L. D. 2005 The effects of evolution are local: evidence from experimental evolution in *Drosophila*. *Integr. Comp. Biol.* **45**, 486–491.
- Rousset F. 2008 GENEPOP' 007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Mol. Ecol. Resour.* **8**, 103–106.
- Ryman N. 1997 Minimizing adverse effects of fish culture, understanding the genetics of populations with overlapping generations. *ICES J. Mar. Sci.* **54**, 1149–1159.
- Ryman N. and Leimar O. 2009 G_{ST} is still a useful measure of genetic differentiation — a comment on Jost's D . *Mol. Ecol.* **18**, 2084–2087.
- Santos J., Serra L., Solé E. and Pascual M. 2010 FISH mapping of microsatellite loci from *Drosophila subobscura* and its comparison to related species. *Chromosome Res.* **18**, 213–226.
- Santos J., Pascual M., Simões P., Fragata I., Lima M., Kellen B. et al. 2012 From nature to the lab. The impact of founder effects on adaptation. *J. Evol. Biol.* **25**, 2607–2622.
- Simões P., Rose M. R., Duarte A., Gonçalves R. and Matos M. 2007 Evolutionary domestication in *Drosophila subobscura*. *J. Evol. Biol.* **20**, 758–766.
- Simões P., Pascual M., Santos J., Rose M. R. and Matos M. 2008a Evolutionary dynamics of molecular markers during local adaptation: a case study in *Drosophila subobscura*. *BMC Evol. Biol.* **8**, 66.
- Simões P., Santos J., Fragata I., Mueller L. D., Rose M. R. and Matos M. 2008b How repeatable is adaptive evolution? The role of geographical origin and founder effects in laboratory adaptation. *Evolution* **62**, 1817–1829.
- Spencer C. C., Neigel J. E. and Leberg P. L. 2000 Experimental evaluation of the usefulness of microsatellite DNA for detecting demographic bottlenecks. *Mol. Ecol.* **9**, 1517–1528.
- Wade M. J. and McCauley D. E. 1988 Extinction and recolonization: their effects on the genetic differentiation of local populations. *Evolution* **42**, 995–1005.
- Waples R. S. 1998 Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. *J. Hered.* **89**, 438–450.
- Weir B. S. and Cockerham C. C. 1984 Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370.

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