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EXPERIMENTAL EVOLUTIONARY DOMESTICATION

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Many millennia before we understood the basic laws governing biological evolution, we bred our commensal species to our liking, whether for economic or leisure purposes. A range of species from plants to animals were thereby domesticated. In a sense, the longest-running experimental evolution projects are those of domestication, and they have produced an astonishing variety of animal breeds and plant varieties. Naturally enough, Darwin used cases of pigeon and dog domestication to illustrate the capacity of selection to produce evolutionary change in *The Origin of Species* (1859), and then he later expanded greatly on this theme in the volumes devoted specifically to domestication (Darwin 1883).

Domestication does not necessarily imply selection directed toward a single goal. In its broader sense, it means evolutionary change when wild populations are maintained in environments controlled, or at least strongly shaped, by human choices. Several features of such environments make the study of domestication interesting from the standpoint of evolutionary biology. Domesticated populations suffer more or less drastic changes in population structure, including size. The environment of domesticated populations is more stable than that of the wild, with a reduction in predation and interspecific competition. Relaxed selection may arise for a wide range of traits. But for other traits, selection may be greatly heightened, sometimes as a result of human intent, but sometimes not. Changes in age structure, a range of abiotic factors from temperature to nutrients, available space, and so on, may lead to significant changes in the components of fitness in domesticated populations. Domestication may thus be a cause of adaptive changes that are well worth analyzing.

Research in experimental evolution often entails the imposition of new selection regimes. These new selection regimes eventually lead to divergent evolution between the populations subject to them and control populations, the latter often being maintained under the antecedent selection regime imposed on the populations directly ancestral to the populations that are subjected to the new selection regime(s). The basic expectation is that sustained directional changes in the phenotypes of the populations subject to the new form of selection, when measured relative to the control populations, can be explained by the imposition of the new selection regime. Replicate populations can test whether such directional changes could be due to genetic drift, which is not expected to produce sustained directional changes on average (Rose et al. 1996). The fundamentals of such experimental evolution are more thoroughly addressed in other chapters of this volume and will not be reviewed further here (Futuyma and Bennett this volume; Huey and Rosenweig this volume; Rhodes and Kawecki this volume; Rose and Garland this volume; Swallow et al. this volume).

Drosophila is, of course, one of the commonly used organisms in experimental evolution. Studies of laboratory natural selection in *Drosophila* have characterized the evolution of populations subject to different densities (e.g., Mueller et al. 1993), demographic regimes (e.g., developmental rate—see Chippindale et al. 1997; age at reproduction—see Luckinbill et al. 1984; Rose 1984; Partridge and Fowler 1992; Roper et al. 1993;

Leroi et al. 1994), several stresses (e.g., starvation resistance—see Rose et al. 1992; Chippindale et al. 1996; Harshman et al. 1999; desiccation resistance—see Hoffmann and Parsons 1993; Gibbs et al. 1997; Folk and Bradley 2005; also see a brief review in Hoffmann and Harshman 1999), different temperatures (Kennington et al. 2003; Santos et al. 2004, 2005), and so on (for reviews, see Prasad and Joshi 2003; Chippindale 2006; Gibbs and Gefen this volume).

One of the goals of such studies is to characterize the potential of populations to respond directly to selection. By now, it is apparent that most *Drosophila* characters will respond significantly to direct selection. Of greater interest, therefore, is the pattern of indirect response to selection. Sometimes the aforementioned studies have revealed declines in functional characters that are not the target of selection, suggesting the presence of trade-offs or, less plausibly for large outbred populations, genetic correlations due to linkage disequilibrium. But such antagonistic indirect responses to selection are not the only possibility. At the start of adaptation to a novel environment, genotype-by-environment interactions are expected to entail significant positive genetic covariances among life-history traits (e.g., Service and Rose 1985; Stearns et al. 1991; de Jong 1993; Matos et al. 2000a; Chippindale et al. 2004).

Many organisms besides *Drosophila* have been studied with the same basic principles and goals: microorganisms (see Elena and Lenski 2003 for a review of studies of adaptation in microorganisms), vertebrates in the wild (e.g., Reznick and Ghalambor 2005; Irschick and Reznick this volume), other insects (e.g., *Tribolium*—see Wool 1987 for an example), among others. The long-term evolutionary studies of adaptation in *Escherichia coli* by Lenski and his collaborators are particularly noteworthy for the large number of generations that they commonly examine (e.g., Lenski 2004). Nevertheless, outbred *Drosophila*, like other sexual diploid organisms that have not been inbred, have the advantage of abundant standing genetic variation. This genetic variation is expected to be most abundant in large natural populations or in laboratory populations at the moment of their foundation from wild samples, since these samples will not have lost genetic variability due to either genetic drift with small population sizes or intense directional selection during initial domestication. This is one reason why studies of the evolutionary domestication of *Drosophila* are of interest, as they allow us to characterize the evolutionary dynamics of local adaptation in populations with considerable genetic variation at the start of selection.

Studies involving convergent evolution in *Drosophila* are much less abundant than studies of divergent evolution. Reverse evolution experiments have been done in lines previously derived from a common ancestor by divergent selection, where phenotypic reversion to the ancestral state is tested when the initial environmental conditions are resumed (e.g., Service et al. 1988; Graves et al. 1992; Teotónio and Rose 2000; Teotónio et al. 2002, 2004; Passananti et al. 2004). Such studies allow us to address the importance of the evolutionary history of populations as a determinant of their capacity to return to ancestral states. For further insight into the relevance of reverse evolution experiments,

see Estes and Teotónio (this volume). In general, convergent evolution among populations subjected to a common selection regime is the intuitive expectation.

Adaptation to a novel, common environment is another way to test for convergent evolution. One such environment is the laboratory environment, where the evolution in captivity can be characterized as a particular case of evolutionary domestication. Detailed studies of domestication in *Drosophila* have appeared relatively recently in the scientific literature (e.g., Frankham and Loebel 1992; Latter and Mulley 1995; Hercus and Hoffmann 1999a, 1999b; Matos et al. 2000b, 2002, 2004; Sgrò and Partridge 2000; Hoffmann et al. 2001; Krebs et al. 2001; Woodworth et al. 2002; Gilligan and Frankham 2003; Reed et al. 2003; Griffiths et al. 2005; Simões et al. 2007, 2008). Most of these studies indicate that adaptation occurs during domestication, as revealed by improvement in one or several life history traits measured under the conditions of laboratory culture (Frankham and Loebel 1992; Latter and Mulley 1995; Hercus and Hoffmann 1999a, 1999b; Matos et al. 2000b, 2002, 2004; Sgrò and Partridge 2000; Woodworth et al. 2002; Gilligan and Frankham 2003). But there are some disagreements among the authors of such studies (e.g., Frankham and Loebel 1992, cf. Latter and Mulley 1995; Gilligan and Frankham 2003; Hoffmann et al. 2001, cf. Matos et al. 2000b, 2002, 2004; Griffiths et al. 2005; Simões et al. 2007, 2008). In particular, studies that employ a comparative approach (e.g., Frankham and Loebel 1992; Latter and Mulley 1995; Hoffmann et al. 2001; Woodworth et al. 2002; Gilligan and Frankham 2003; Griffiths et al. 2005) have often reached different conclusions from those obtained in studies of evolutionary trajectories (Matos et al. 2000b, 2002, 2004; Krebs et al. 2001). By a comparative approach, we mean the inference of the evolutionary dynamics of a population from comparisons among several contemporaneous populations at different stages of evolution, assuming that each one of them will represent the evolutionary state of a given population at a particular moment. Decoupling between such inferences and direct, real-time studies of experimental evolution may derive from several sources. In particular, evolutionary contingencies associated with founder effects may play an important role, among other sources of nonrepeatability of evolutionary patterns across populations. This will be a major theme of the present review. Another controversy concerns the use of long-established laboratory populations to test several evolutionary theories (see Promislow and Tatar 1998, Harshman and Hoffmann 2000; Sgrò and Partridge 2000; Hoffmann et al. 2001; Linnen et al. 2001). Finally, another common disagreement in the literature concerns the genetic mechanisms that cause the decline of some traits during laboratory domestication, specifically mutation accumulation, inbreeding depression, and genetic trade-offs (e.g., Latter and Mulley 1995; Shabalina et al. 1997; Bryant and Reed 1999; Sgrò and Partridge 2000; Hoffmann et al. 2001; Woodworth et al. 2002; Frankham 2005). We will discuss these issues in light of our own results.

Real-time studies of evolutionary trajectories during domestication test the assumption of convergence, as well as allowing the experimenter to tackle such important issues as the repeatability of the evolutionary dynamics of adaptation, the importance of founder

effects in the process of laboratory adaptation, and the effects of long-term evolution in the laboratory. The study of evolutionary domestication is particularly helpful when it uses as starting populations different collections from the wild, samples that are expected to be highly variable sources of founders each time a study is conducted. By following the dynamic changes that occur within domesticating populations, it is possible to infer evolutionary rates and define evolutionary patterns directly. Though some short-term real-time studies of evolutionary trajectories have appeared in the *Drosophila* literature (e.g., Hercus and Hoffmann 1999b; Krebs et al. 2001), to our knowledge ours are the longest-term real-time studies of domestication in a sexual species that have been published to this point (Matos et al. 2000a, 2000b, 2002, 2004; Simões et al. 2007, 2008).

In this chapter, we start by reviewing our own results and then review other relevant studies, particularly focusing on the points of disagreement between laboratories already mentioned. We end with suggestions for future studies.

EVOLUTIONARY DOMESTICATION: REAL-TIME STUDIES IN *DROSOPHILA SUBOBSCURA*

Since 1990 we have studied the evolutionary changes that occur during laboratory adaptation in the model organism *Drosophila subobscura*. However, we will focus here on experiments that were started in 1998, 2001, and 2005, using the population first domesticated in 1990 as a point of reference. In order to illustrate the type of results that we have obtained, we outline our results for just two adult traits, early fecundity and female starvation resistance, though we have studied a number of other functional characters.

POPULATIONS AND EXPERIMENTAL DESIGNS

All our populations were founded from wild samples collected over one to several days using fermented fruit in traps. The first foundation was done in 1990, in an Adraga pinewood in Sintra, Portugal, from which we established our reference laboratory population for our subsequent studies of domestication (the "NB" populations). Later, in 1998, we collected flies from the same natural location, from which we established a second set of laboratory populations (labeled from here on "NW"). In 2001, we founded two new sets of populations, one from collections again in Adraga, Sintra (called "TW"), and another from a pinewood in Arrábida, some 50 kilometers from Adraga, on the other side of the Tagus River (called "AR") (see figure 5.1). The collections from Arrábida and Sintra were made synchronously, allowing us to follow the evolutionary dynamics of the two sets of populations in parallel simultaneous assays, with the same number of generations in the laboratory, an ideal situation for studying the effects of different wild-source populations on the process of domestication. In 2005, another foundation was made from a Sintra collection, establishing another set of populations (FWA) that will be used in an analysis presented in a later section.

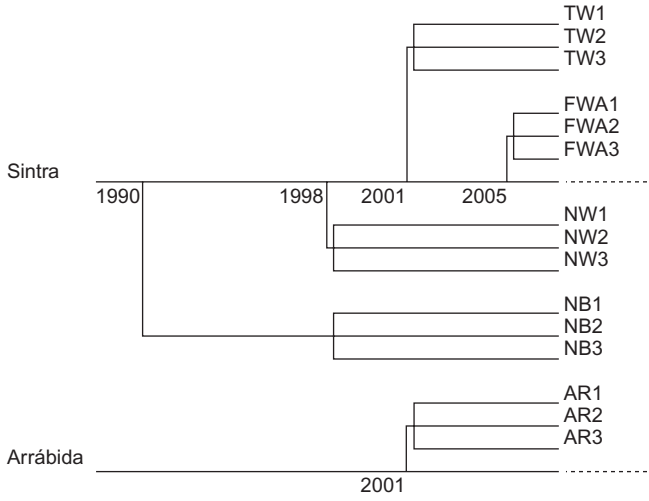

FIGURE 5.1

Phylogeny of the fifteen laboratory populations used in this chapter, indicating the original wild location. Our populations were obtained from the collection of flies of 1990 done in a pinewood of Adraga (Sintra), from which all foundations from Sintra were derived. By the time the last foundation was performed (FWA, 2005) the populations established in 1990, 1998, and 2001 were, respectively, at generations 181, 91, and 45. The foundations of Arrábida and Sintra, in 2001, were carried out synchronously. Three replicate populations were derived from each of the five foundations.

Each population was split into several replicate populations two generations after the collection of individuals from the wild, with the exception of the long-domesticated population founded in 1990, which was split up into replicates in 1998 at the same time as the then newly sampled flies were split. We label each replicate by a number. Thus, the NW1, NW2, and NW3 are the three populations derived from the "NW foundation" started with wild samples collected in Adraga in 1998.

From the moment our laboratory populations were founded, they were maintained in standard conditions, at discrete generations of twenty-eight days, close to the time of peak fecundity in *D. subobscura*, with control of medium, temperature, and population density. Our populations were maintained in numerous vials placed in racks within incubators, with care taken to avoid handling differences among populations during both maintenance and assays. Population sizes were typically about 1,200 (see details in Matos et al. 2002, 2004; Simões et al. 2007, 2008).

Adult assays were done periodically, both on the more recently introduced populations and on the longer-established (NB) populations. We will present here data for mean fecundity during the first week of life and female starvation resistance over generations 4 to 94 of the populations founded in 1998, and the corresponding generations 94 to 184 of the longer-established populations, as well as generations 3 to 48 of the populations founded in Sintra and Arrábida in 2001, when the longer-established populations were in

their generations 139 to 184. Because the unit of evolutionary studies is the population and not the individual, our data analysis focuses on the averages of each replicate population using as source of error the heterogeneity among replicate populations.

LONG-TERM EVOLUTIONARY DOMESTICATION

Early Fecundity There was no significant phenotypic trend among the longer-established NB populations between generations 94 and 184, while the NW populations founded in 1998 showed a significant improvement in performance relative to the NB controls between their generations 4 and 94 (figure 5.2; average slope = 0.411, *t*-test, $p = 0.02$). A log-linear trend is even more significant ($p = 0.007$, data not shown). Altogether, these data indicate a clear, though not very quick, process of adaptation in early fecundity.

Female Starvation Resistance In figure 5.3, we present the changes in female starvation resistance shown by the long-established populations between generations 94 and 184. Contrary to the data on fecundity, these populations show a significant decline in female starvation resistance (*t*-test, $p = 0.03$), with an average slope close to -0.05. This corresponds to a decline of about 0.11 percent per generation, and a decline of about 10 percent during the entire period.

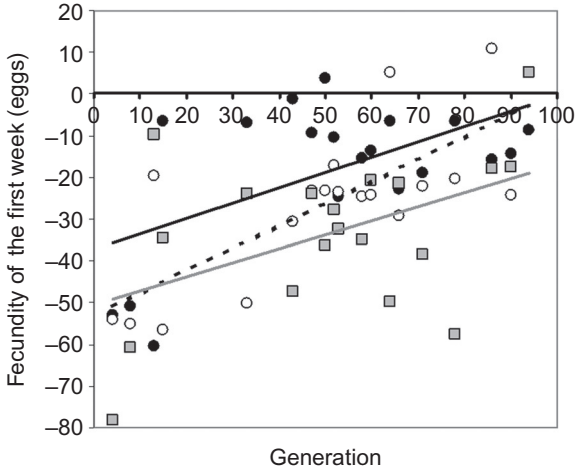

FIGURE 5.2
Fecundity during the first week of life in the populations founded in 1998 (NW) relative to the longer-established (NB) populations. Each data point is the difference between the average absolute values of each population and the same numbered longer established population. Replicate population 1: black circles, full black line; replicate population 2: open circles, broken line; replicate population 3: gray circles, gray line. All analyses of linear regressions used the individual slopes as data points in a *t*-test. NW populations show a steady increase in early fecundity throughout laboratory culture, corresponding to a significant pattern of convergence to longer-established reference populations.

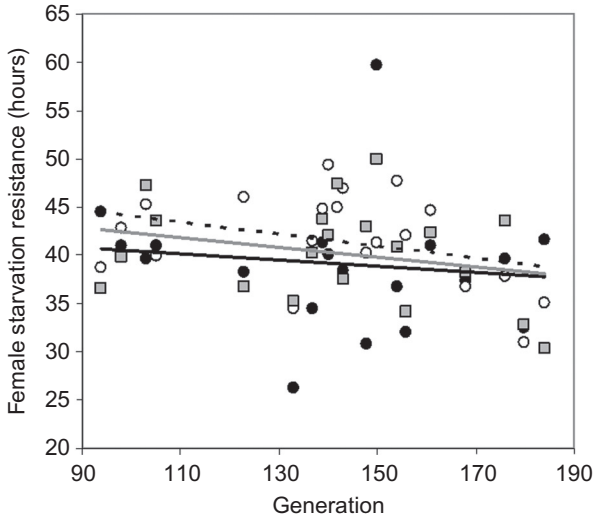

FIGURE 5.3
Female starvation resistance in the longer-established (NB) populations between generations 94 and 184. Replicate population 1: black circles, full black line; replicate population 2: open circles, broken line; replicate population 3: gray circles, gray line. The analysis used the individual slopes as data points in a *t*-test. NB populations show a significant decline in female starvation resistance with an average slope of –0.05, which corresponds to a decrease of about 0.11 percent per generation.

Ehiobu et al. (1989) found that viability in *D. melanogaster* decreased by about 0.96 percent for every 1 percent increase in the inbreeding statistic, *F*. In our case, assuming an effective population size of about five hundred individuals, we expect between generations 94 and 184 an increase in *F* value of about 8 percent, which corresponds to a decrease of 1.2 percent in female starvation resistance for every 1 percent increase in the inbreeding statistic *F*. These values can thus be explained by inbreeding depression alone.

There is no significant temporal change of female starvation resistance in the NW populations founded in 1998 relative to the long-established NB populations (average slope = −0.006, n.s.). Nevertheless, the NW populations also show a significant decline in starvation resistance when absolute values are analyzed (average slope = −0.05; *t*-test, $p < 0.01$, data not shown).

SHORTER-TERM EFFECTS OF FOUNDATION AND REPEATABILITY OF EVOLUTION

Early Fecundity Figure 5.4 presents the changes in fecundity over the first week of life for the populations founded in Sintra (TW) and Arrábida (AR) in 2001, relative to the

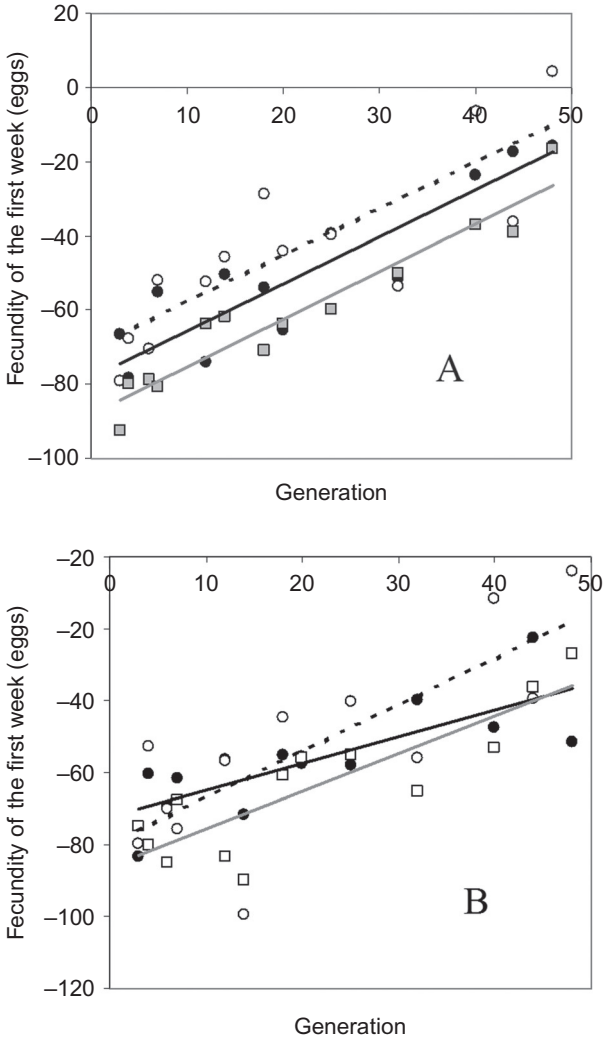

FIGURE 5.4
Fecundity of the first week of life in the populations founded in Sintra (A) and Arrábida (B) in 2001, between 3 and 48, relative to the longer-established populations. The analysis of each set of replicate populations used the individual slopes as data points in a *t*-test. Early fecundity significantly increases during domestication of both sets of populations. There are no significant differences in evolutionary rate between the two sets of populations.

long-established NB populations. Both regimes show significant linear improvement during the generations under study (average slope = 1.27, $p < 0.001$ for TW; average slope = 1.03, $p = 0.024$ for AR).

It is interesting to compare the slopes of these 2001 populations with the pattern of adaptation shown by the NW populations founded in 1998, over the equivalent generations 4 and 47. The NW populations had an average slope of 0.76 in that period relative to the reference NB populations, a result not significantly different from that obtained with the populations founded in 2001.

Female Starvation Resistance There is no significant temporal linear increase in starvation resistance among the populations founded in Sintra and Arrábida in 2001, relative to the

longer-established populations (TW, average slope = 0.114, n.s.; AR, average slope = 0.124, n.s.; data not shown). Interestingly, the analysis of the temporal changes during the first forty generations reveals a significant improvement in TW populations, though with a low rate (average slope = 0.06, $p = 0.04$; see Simões et al. 2007), which suggests that heterogeneity between replicates with further analysis may have affected the statistical power. As for absolute values, both sets of populations showed a suggestion of a decline during the study, though it was not as clear as we had previously obtained for the NW populations founded in 1998 (TW, average slope = −0.09, n.s.; AR, average slope = −0.08, n.s.). Also, in contrast to the populations founded in 1998, there is no significant improvement of female starvation resistance when considering the first fourteen generations, relative to longer-established populations. This suggests that any improvement that may occur in the initial period of domestication differs among populations. In view of this result, it is apparent that generalizing from short-term studies of domestication can be misleading. This may explain some of the disparities in the conclusions of different studies of domestication (see the last section).

BALANCE OF OUR STUDIES

In the three studies summarized here, there is clear adaptation to the laboratory, in the steady increase in early fecundity. The long-term study of the populations founded in 1998 also suggests that an adaptive plateau is being reached, indicated by a slowing down of the evolutionary rate. Our data indicate that inbreeding depression may play some role in the changes observed under domestication.

One of the odd features of our data is that starvation resistance initially increases and then decreases. This is the expected outcome if the additive genetic covariance between traits undergoing domestication changes through time, from positive (or less negative) to negative (or more negative) values. Initial positive covariances in a novel environment can derive from genotype-by-environment interactions, given the new selective scenario involved (see Service and Rose 1985; Chippindale 2006). As a consequence of the approach to an evolutionary equilibrium, most variation involving positive pleiotropy among traits will be likely exhausted, while antagonistic pleiotropy may allow the maintenance of additive variance, expressed as a negative genetic correlation in those traits. This change in genetic correlation may lead to a nonlinear, biphasic evolutionary pattern. In fact, the laboratory populations founded from Sintra in 1998 and 2001 show an initial phase with a significant increase in starvation resistance, while more generations show a shift to a negative slope. But it is possible that both inbreeding depression and selection act during the evolutionary changes of starvation resistance. The relative importance of these mechanisms of selection and inbreeding may in general change as a function of the initial composition of the population, selective pressures, and how long studies are conducted.

COMPARATIVE STUDIES OF DOMESTICATION

Several studies have used the comparative method to study evolutionary patterns in laboratory adaptation as opposed to the analysis of evolutionary trajectories that we illustrated in the previous section. Here we discuss briefly such comparative studies.

STATIC COMPARISONS OF LONG-ESTABLISHED VERSUS RECENTLY INTRODUCED POPULATIONS

Several studies of laboratory adaptation compare populations maintained in the laboratory for several generations with other populations, recently introduced from the wild. These studies do not present data on evolutionary dynamics. In some of these studies, the effects of population size, degree of inbreeding, and so forth, are also analyzed.

Hercus and Hoffmann (1999a) conducted a study involving interspecific hybrids between *Drosophila serrata* and *Drosophila birchii*. This study was short in duration and lacked adequate reference populations, but the results are suggestive. Populations that had been kept in the lab for seventeen to twenty generations were compared with populations derived from the same location that had spent just seven generations in the laboratory. Both fecundity and desiccation resistance were higher in the populations that had been in the lab longer, suggesting that desiccation resistance had increased without a trade-off with fecundity. It is a pity that these authors did not analyze the changes of these traits within each population over multiple generations, as they did for juvenile viability between generations 17 and 30, which showed a temporal increase in performance (Hercus and Hoffmann 1999b).

Woodworth et al. (2002) also analyzed the effects of both adaptation and inbreeding during evolutionary domestication. They founded laboratory populations of *D. melanogaster* at population sizes ranging from twenty-five to five hundred individuals and compared their performance after fifty generations in the laboratory, both in "benign" captive conditions and in "wild" competitive conditions. Several control populations were used in this study, some derived from the same location in later years. In benign conditions, populations of bigger size showed a higher performance, while those with the smallest population size performed poorly. In "wild" conditions, all laboratory populations had a lower performance than the recently derived populations. The authors concluded that both genetic adaptation and inbreeding depression were responsible for the poor performance of laboratory populations in the "wild" environment.

Another interesting study was conducted by Latter and Mulley (1995) using *D. melanogaster*. These authors analyzed the effects of both adaptation and inbreeding on reproductive ability in competitive and noncompetitive environments. They compared

the performance of populations derived from the same wild-source population, but differing in the degree of inbreeding. Comparisons with recently introduced populations were also performed. Long-established populations were superior in competitive ability (assessed in competition experiments with a mutant, marked stock) in the laboratory relative to both recently introduced and inbred populations. Over about two hundred generations, there was a doubling of competitive fitness (estimated as the ratio between the competitive index of the longer-established populations and the more recent ones), even in populations with a population size of fifty during most generations. Comparing differences in performance as a function of the amount of inbreeding, the authors were able to disentangle effects of inbreeding from effects of selection. They concluded that both processes had acted in the inbred populations. Interestingly, fitness differences were minor in a noncompetitive environment, indicating the presence of genotype-by-environment interactions for these characters.

EVOLUTIONARY DYNAMICS INFERRED FROM A COMPARATIVE APPROACH

In this experimental strategy, populations introduced into the laboratory environment at different times are compared synchronously at different stages of the adaptation process; with this data, the evolutionary trajectory of a single population adapting to the laboratory environment is inferred. The assumption is that the evolutionary pattern of the different populations used would be the same if they were compared directly over multiple generations, and so the performance of the most recently founded population will accurately reflect the early stages of adaptation of the previously founded populations. For example, Sgrò and Partridge (2000) compared life-history traits in populations of *D. melanogaster* founded three consecutive times from the same natural location, maintained in either bottles or population cages. The analyses revealed marked changes in some of the traits but few changes in most of them. Differences were found between populations maintained in cages and bottles as a function of time in the laboratory. Development time increased during laboratory culture. The authors advanced the hypothesis that this might be due to higher larval competition in laboratory culture, based on the fact that this increase was particularly seen in cages, with higher larval densities. Early fecundity increased with bottle culture, while late fecundity decreased. However, with cage culture, the fecundity patterns were less clear. This was assumed to be due to the truncation of the adult period in bottle culture, enhancing the relative focus of natural selection on the early adult period. The authors propose that this led to a decrease in late fecundity by either mutation accumulation or antagonistic pleiotropy.

Using the same three sets of populations and a new one from a recent foundation, Hoffmann et al. (2001) tested the hypothesis that stress resistance is lost during laboratory

adaptation. The most recently founded populations showed higher starvation and desiccation resistance than the previously founded ones, a result that was interpreted as a marked evolutionary decline in resistance for both stresses during laboratory adaptation. According to the authors, the rapidity of the response ruled out mutation accumulation as a possible explanation for the pattern obtained. They propose that the most likely explanation is that resistance to starvation and desiccation was lost as a correlated response to selection on early fertility, as a result of a negative genetic correlation between stress resistance and fecundity traits.

To investigate the genetic dynamics of adaptation to captivity, Gilligan and Frankham (2003) also used the comparative approach, measuring the fitness of several independently founded populations of *D. melanogaster*, derived from the same natural site in consecutive years, relative to a genetically marked stock. The authors inferred a curvilinear pattern of adaptation, with an increase of captive fitness reaching 3.3 times the initial fitness after eighty-seven generations of laboratory adaptation.

Griffiths et al. (2005) studied the effects of laboratory adaptation in *D. birchii* using isofemale lines established from collections made in the same four natural locations over three consecutive years. They concluded that time in laboratory culture influenced evolutionary responses for some traits but not others. For example, there was an increase in starvation resistance and development time in the laboratory lines, while recovering time following a cold shock decreased. On the other hand, heat knockdown resistance and wing size were not affected. The authors argue that collections made in different locations and the use of isofemale lines can overcome the limitations of using a classic comparative approach (e.g., Sgrò and Partridge 2000; Hoffmann et al. 2001). Nevertheless, the data on development time presented in this study clearly illustrate some of the limitations of this approach, in that the data of one of the sets of lines were quite different from the others. The authors attributed this to changes in the genetic composition of the wild populations.

Although some traits appear to give consistent results across studies (e.g., increased fecundity and development time during laboratory adaptation), others, such as stress resistance, do not. This may not only be due to the different genetic composition of the populations analyzed but also to methodological issues (see later discussion).

TESTING COMPARATIVE METHODS USING TRAJECTORY DATA

We will now test the validity of the comparative approach with our own data, as we now have several sets of populations founded at different times and know their actual evolutionary trajectories. The question is, can evolutionary dynamics be correctly inferred using comparative data only?

In a recent study, Matos et al. (2004) tested the consistency of results using both the comparative and temporal methods applied to the study of domestication. Although the

comparative method proved to be quite accurate for the analysis of robust evolutionary patterns, such as those of fecundity traits, it can lead to problems with less predictable traits. This applies clearly to starvation resistance. Our own studies of real-time evolution suggest that starvation resistance is a trait that has complex evolutionary trajectories during domestication, rendering short-term and comparative studies problematic. It is also a trait that has given disparate results among laboratories in studies that infer evolutionary changes from comparisons among contemporaneous populations. For example, while Hoffmann et al. (2001) found a consistent decline of this trait over generations with laboratory culture, the study by Griffiths et al. (2005) finds an improvement during laboratory adaptation.

We can illustrate this problem using new data that we have collected from a new 2005 foundation from Sintra, the same location where the TW, NW, and NB populations were derived (see figure 5.1).

At generation 3 after foundation, we made our first assay with these more recently founded populations (which we call FWA), as well as TW (at their corresponding 48th generation), NW (in the lab for 94 generations), and NB (the longer-established populations, for 184 generations in the laboratory). The plots for both early fecundity and female starvation resistance are presented in figure 5.5. In that figure we also plot the data obtained in our previous study of TW populations, when these were in their 4th generation (the earliest assay conducted in that study, involving simultaneous assays of NW and NB populations, by that time in their 50th and 140th generations), using the same methodology.

Our comparative analysis of fecundity does give similar results to those of our real-time evolution studies, with clear-cut differences between populations as a function of how many generations they have been in the lab, even though they derive from different foundations. There is also robustness of results among the plots using our most recent data and those of the previous study (TW populations at generation 4). But contrary to these fecundity results, starvation resistance shows differences between the two studies: the assay at generation 4 of the TW populations suggests stability of this trait, while the most recent data present evidence for a decline with generations. These data illustrate one of our points about the limitations of a comparative approach: if the values of the TW populations in their generation 4 were close to the ones presented by our most recent populations (assuming the differences to be purely genetic, which is obviously simplifying), the inferred trend might even be positive. In fact, the data of an assay done at generation 6 of the TW populations present such a shift relative to NB values, with TW populations having lower values than these populations, though bigger than NW (see Matos et al. 2004). This does not correspond to any trend in the actual evolutionary trajectories. The problems of a comparative approach are thus clearly revealed by our data. The differences among comparative studies in the evolution of starvation resistance contrast with the more repeatable patterns obtained with evolutionary trajectories. This suggests that the comparative approach to experimental evolution can yield

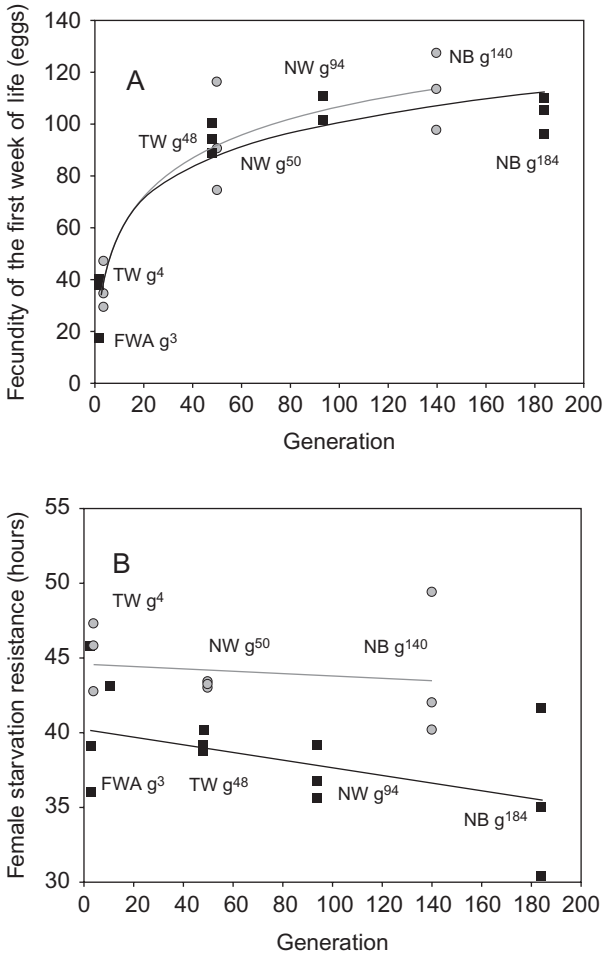

FIGURE 5.5

Comparative plots of the values of fecundity of the first week of life (A) and female starvation resistance (B) of independently founded populations as a function of number of generations in the laboratory. Gray circles and gray lines: data from assays done at generation 4 after foundation of TW (NW at generation 50; NB at generation 140); Black squares and black lines: data from assays done at generation 3 after foundation of FWA (TW at generation 48; NW at generation 94 and NB at generation 184). Fecundity comparative plots are remarkably similar to those obtained using real-time evolutionary trajectories. These results are also robust among comparative plots using data from different studies. Contrary to this, female starvation resistance shows differences between the two comparative studies: the one using data from TW at generation 4 suggests stability for this trait, while the one for FWA at generation 3 shows evidence for a decline with generations.

misleading results. Indeed, the use of contemporaneous populations as "surrogates" for the evaluation of the phenotypic state of a given population through time (e.g., Frankham and Loebel 1992; Hoffmann et al. 2001; Gilligan and Frankham 2003; Griffiths et al. 2005) rests on several untested a priori assumptions that may not always

apply. For example, it is often assumed that founder effects and random genetic drift during adaptation are negligible (as proposed in Sgrò and Partridge 2002; but see Matos and Avelar 2001; Woodworth et al. 2002). Furthermore, comparative studies often lack appropriate reference populations, and this prevents the disentangling of the evolutionary mechanisms involved, particularly in traits exhibiting complex evolutionary trajectories, as our studies of starvation resistance illustrate.

We conclude that the comparative approach is not the appropriate tool with which to study the detailed dynamics of domestication. A proper approach is to follow the temporal, evolutionary changes in captivity of a population, since its foundation from the wild, using the methods of experimental evolution, of which experimental evolutionary domestication is a particular case. This is not to say, obviously, that comparisons of evolutionary dynamics presented by different populations are not a fundamental approach of such studies, as it is by such analyses that the repeatability of the evolutionary dynamics under study can be tested and the degree of predictability of evolution measured.

GENERAL ISSUES

THE PROBLEM OF COMPLEX EVOLUTIONARY TRAJECTORIES

The evolutionary trajectories that we have adduced indicate that starvation resistance is evolving through both selection and drift mechanisms during the domestication of *D. subobscura*. It seems likely to us that these mechanisms might generate nonlinear evolutionary trajectories for any particular functional character during longer-term laboratory evolution. How much each of these mechanisms affects the trajectory of a particular character may be rather unpredictable. Novel environments pose difficult evolutionary challenges for both organism and experimenter, challenges that may give rise to genotype-by-environment interactions that in turn generate novel additive genetic covariances among traits.

How repeatable is evolution? Our data across three different studies of detailed characterization of adaptation to the laboratory suggest general repeatability of evolutionary processes and patterns, though also disparity of results for particular traits. This contingency is apparently related to the relevance of these traits with fitness: early fecundity is clearly a very important fitness component, while this is not necessarily the case for starvation resistance. Also, short- and long-term studies can give different results. Our conclusions add to a body of data indicating that although evolution is a global process, its specific outcomes often cannot be generalized (see Rose et al. 2005).

APPLICATION TO CONSERVATION

Recent interest in characterizing the evolutionary changes of populations from the moment they are brought to the laboratory arises from both their general significance for the study of biological evolution and the need to characterize the specific effects of

captivity for the purpose of conservation (Gilligan and Frankham 2003). Not all agree as to what studies of adaptation during captivity can tell us about the impact of such evolution for conservation purposes. Genotype-by-environment interactions will limit considerably extrapolations from the laboratory even to zoo and enclosure environments.

We thus certainly cannot extrapolate the findings of evolutionary change in the laboratory to what will occur when populations are reintroduced in the wild (see Shabalina et al. 1997). The evolutionary genetic complexity of functional traits does not allow reliable inference (cf. Woodworth et al. 2002; Reed et al. 2003). As a safeguard, the best strategy may be to avoid prolonged captivity, minimizing concomitant evolutionary changes (Frankham 1995; Woodworth et al. 2002; Gilligan and Frankham 2003; Rodriguez-Ramilo et al. 2006, cf. with Shabalina et al. 1997).

WHAT HAVE WE LEARNED ABOUT DOMESTICATION FROM EXPERIMENTAL EVOLUTION?

Most studies of evolutionary domestication indicate that adaptation occurs during domestication, as can be inferred from improvement in such traits as juvenile viability (Hercus and Hoffmann 1999b), early fecundity (e.g., Hercus and Hoffmann 1999a; Matos et al. 2000b, 2002, 2004; Sgrò and Partridge 2000), competitive ability (Frankham and Loebel 1992; Latter and Mulley 1995), and noncompetitive fitness (Woodworth et al. 2002). Some studies differ over the rate of adaptation during captivity (e.g., Frankham and Loebel 1992, cf. Latter and Mulley 1995), and short-term studies may be misleading, as we have shown here. Our studies suggest that domestication can involve complex evolutionary trajectories. We have shown that disparate results among studies of domestication may be due to different methodologies, specifically the limitations of a comparative approach (e.g., Latter and Mulley 1995; Hoffmann et al. 2001; Gilligan and Frankham 2003; Griffiths et al. 2005) versus studies of evolutionary trajectories (Matos et al. 2000b, 2002, 2004; Krebs et al. 2001; Simões et al. 2007). In our view, multiple evolutionary mechanisms can be involved in domestication, and their specific relevance will probably vary from case to case.

From an applied standpoint, the study of adaptation to captivity has received progressively more attention in the conservation literature. There is still a substantial need for basic research on the evolutionary and genetic mechanisms relevant to conservation programs, where these mechanisms range from direct and correlated adaptive responses to inbreeding and drift. The experimental study of domestication is a particularly useful vein for such basic research.

ARE LAB FLIES DEGENERATE?

Some have argued that laboratory populations that have been established for many generations are of little use for evolutionary studies (Promislow and Tatar 1998; Harshman and Hoffmann 2000; Linnen et al. 2001). Such a view is based, at least in part, on the idea

that experimental evolution studies necessarily try to extrapolate results from laboratory populations to evolution in the wild. This is not correct. Experimental evolution is about potential genetic changes in response to defined selection regimes. In particular, some have argued that the ability to select for delay of senescence suggests that alleles with different effects at late ages have accumulated in laboratory populations maintained using short generation times, to a much higher extent than would occur in overlapping generations (Promislow and Tatar 1998; Linnen et al. 2001). While this is indeed expected, we find this criticism ironic in that, to our view, this is one more reason why populations maintained with discrete generations may be the best material to test for the mechanism of accumulation of mutations (Rose and Matos 2004; see also Rauser et al. this volume). After all, this is one of the important tools of experimental evolution, allowing selection to generate differences between the average phenotypes of populations that permit us to infer underlying evolutionary mechanisms (see also Futuyma and Bennett this volume).

More generally, there is no reason to assume that the laboratory environment is not a particular kind of environment or that laboratory populations are not simply natural populations evolving in that environment (Matos et al. 2000a; but see Huey and Rosenzweig this volume, for a different view). As a final note, and as Darwin already understood, evolutionary domestication illustrates the power of natural selection as a process that leads both to the adaptation and the diversity of organisms, as a function of the peculiarities of each environment, whether controlled by humans or not.

SUMMARY

This chapter provides a general overview of the field of experimental evolutionary domestication, focusing on studies using *Drosophila* as a model organism. In its general evolutionary sense, domestication means evolutionary change when wild populations are maintained in environments controlled by human choices. It is thus an evolutionary process that is worth analyzing. Here we review the most relevant findings in the field of evolutionary domestication in *Drosophila*, analyzing the evolutionary changes that occur when populations are placed in a human-controlled environment, such as a laboratory. We present our own experiments on evolutionary domestication in the laboratory, going back almost two decades, and discuss our results in the context of relevant literature. We focus on the effects of natural selection, inbreeding, and genetic drift on evolving populations. We compare the results of different research groups, particularly given the common disparities among results involving less relevant fitness traits. We point out the limitations of a comparative approach that relies on inferences based solely on contemporaneous populations, the predominant method used in studies of laboratory adaptation. We argue that the best approach is experimental domestication, in which real-time trajectories are monitored, as is usually the case in experimental evolution studies. After considering applications to conservation, we conclude with a discussion of the debate over the use of long-established laboratory populations for evolutionary research in general.

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