The Effects of Evolution are Local: Evidence from Experimental Evolution in *Drosophila*[1]

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Synopsis.   One of the enduring temptations of evolutionary theory is the extrapolation from short-term to long-term, from a few species to all species. Unfortunately, the study of experimental evolution reveals that extrapolation from local to general patterns of evolution is not usually successful. The present article supports this conclusion using evidence from the experimental evolution of life-history in *Drosophila*. The following factors demonstrably undermine evolutionary correlations between functional characters: inbreeding, genotype-by-environment interaction, novel foci of selection, long-term selection, and alternative genetic backgrounds. The virtual certainty that at least one of these factors will arise during evolution shreds the prospects for global theories of the effects of adaptation. The effects of evolution apparently don't generalize, even though evolution is a global process.

INTRODUCTION

Few goals are more fervently espoused by scientists than the creation of a general scientific theory that is predictive over a wide range of circumstances. Many of us would point to the power and elegance of Darwin's theory of evolution by natural selection and claim that we have just such a theory. Is this a fair claim? It might be, if we could make content-laden predictions concerning the long-term outcome of evolution. But can we?

A number of evolutionary theories have been advanced which seem to claim, implicitly or explicitly, to predict long-term and general features of evolution. For example, several researchers have put forward theories of phenotypic evolution that offer a complete predictive package, providing one knows the genetic and phenotypic variances, covariances, and higher order moments between characters (*e.g.,* Lande, 1979, 1980; Lande and Arnold, 1983; Turelli and Barton, 1990; see also Barton and Turelli, 1987, 1991; Kirkpatrick *et al.*, 2002). One thing evolutionary biology does not lack is an abundance of theories.

But do these theories really hold up? An advantage for evolutionary theorists is that few people ever apply strong inference (*cf.*, Platt, 1964) to evolution as a process (but see Lenski *et al.*, 1991, for an important example), leaving few of the predictions or assumptions of most evolutionary theories at much risk of experimental refutation. It is difficult to study evolution. A large amount of time is required in most cases, and many samples have to be taken, creating problems of physical scale in the housing of experimental organisms. Thus there are only a few cases where critical tests of evolutionary theories have actually been performed, and several of these tests have used experimental evolution. The microbial work of Lenski (*e.g.,* Lenski *et al.*, 1991; Lenski and Travisano, 1994; Cooper *et al.*, 2001) and others (*e.g.,* Rainey and Travisano, 1998; Burch and Chao, 1999; Dahlberg and Chao, 2003) has strong tests of evolutionary theory (see reviews in Bell, 1997; Travisano and Rainey, 2000).

We have an outbreeding experimental evolution system, laboratory-evolved *Drosophila* populations (*vid.* Rose *et al.*, 2004). In the first ten years that they were studied, we developed a simple consensus model for the effects of adaptation in these fruit flies. The interesting point for the present purpose is that this model was to be annihilated by the next decade's worth of work. From this destruction, we learned a great deal about the robustness of evolutionary findings, as we will now adumbrate. We are sure that few of our colleagues will mind if we only set ourselves up to be demolished, thereby sparing them the injury or insult.

DROSOPHILA LIFE-HISTORY: THE STANDARD MODEL

The study of *Drosophila* life-history in the laboratory goes back to the 1920s (*e.g.,* Pearl and Parker, 1922), if not earlier (Loeb and Northrop, 1917). Much of this work used mutant or inbred stocks, creating problems that we will discuss shortly. Reasonable fruit fly work on life-history is not much older than the 1960s (*e.g.,* Wattiaux, 1968). A major feature of the modern era of fruit fly life-history research is the use of large quantitative genetics experiments (*e.g.,* Rose and Charlesworth, 1981; Hutchinson and Rose, 1991; Hutchinson *et al.*, 1991; Hughes and Charlesworth, 1994) and replicated experimental evolution (*e.g.,* Rose, 1984*a*; Luckinbill *et al.*, 1984; Service *et al.*, 1988; Partridge and Fowler, 1992; Mueller *et al.*, 1993). Sometimes, the findings from these two types

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TABLE 1. *The Irvine* Drosophila *experimental evolution system.*

- started from a SINGLE endemic fruit fly population, IV, in 1975; large sample
- Reasonable Ne's-about 1,000 plus
- Adapted to lab for about five years first
- All selection with five populations
- All selection regimes paired with controls
- Long sustained selection regimes

Some of the Laboratory Evolution Regimes:
- B-selected for day 14 fertility in vials
- O-selected for day 70 fertility in cages
- D-selected to survive extreme desiccation
- C-selected to survive moderate starvation
- SO-selected to survive extreme starvation
- CO-selected for day 28 fertility in cages
- SB-selected to survive extreme starvation
- CB-selected for day 28 fertility in cages
- ACO-selected for early (day 7–9) fertility
- ACB-selected for early (day 7–9) fertility
- RSO-relaxed selection, like CO's
- NDO-new D stocks
- NDCO-new C stocks

of experiments reinforce each other, as in cases of negative genetic correlations and antagonistic indirect responses to selection. However, most of our knowledge of the functional interrelations between *Drosophila* life-history characters has come from studies of experimental evolution (*cf.* Rose *et al.*, 2004); see Table 1 for a brief summary.

From the mid-seventies to 1990, the overall pattern in the results from *Drosophila* work on life-history was fairly clear. In outbred fruit flies, early fecundity generally traded-off with longevity. Longer-lived flies had reduced early fecundity, and vice versa. Longer-lived flies had increased later fecundity. Longer-lived flies were more robust under several stressors: starvation, desiccation, and ambient ethanol. Starvation resistance appeared to trade-off with fitness, while desiccation resistance did not. A number of additional subtleties could be added to this model, but it contains the highlights. (See Table 2 for a rough summary of the initially inferred patterns.) Several labs contributed to these basic findings, and quite a few more individual investigators, using flies of different origins. (Rose *et al.* [2004] supply an introduction to this research.) It would have been reasonable to conclude that some fundamental truths had been discovered about *Drosophila* life-history, and perhaps life-history in general.

Below we will destroy this standard model, mostly

using the data that we collected in the period after 1990. Since we are undermining our own pet hypotheses, rather than anyone else's, we can afford to be brutally matter-of-fact.

Inbreeding

The ideal finding in science is one that applies regardless of initial conditions. Many of the theories of physics apply with absolute generality, such as the velocity-dependent transformations of special relativity. Some theories in biology have this property. Darwinian evolution implies that extinct species will never reappear unaltered millions of years later. It would be nice if, for example, such an important idea as a negative genetic correlation between early reproduction and later survival was always true (*cf.* Williams, 1957). Such a conclusion is especially attractive when a result of this kind has been obtained repeatedly (Rose and Charlesworth, 1980; Rose, 1984*a*; Luckinbill *et al.*, 1984) and there are explicit mathematical studies that predict the occurrence of such negative correlations at evolutionary equilibrium (*e.g.,* Rose, 1982).

But it was not to be. One of the anomalies facing this result is that a common observation in studies of fruit fly life-history has been generally positive correlations among life-history characters, especially positive genetic correlations (*e.g.,* Giesel *et al.*, 1982). Perhaps there is no general trade-off pattern?

In a sense, this conclusion was correct. When Rose (1984*b*) derived inbred flies from the stock that had shown a trade-off (*e.g.,* Rose, 1984*a*), the genetic correlations became generally positive. Rose interpreted this result as a reflection of inbreeding depression, with some inbred lines more inbred than others and so generally having reduced life-history characters. As inbred lines vary in their degree of inbreeding, and thus in the depression of their life-history characters, life-history characters will positively co-vary. This effect apparently swamps the negative genetic correlation between early reproduction and longevity. Such trade-offs are not robust under inbreeding. Rather, they are "local" to outbred populations.

This was the first demonstration of the lack of universality of the "standard model" for *Drosophila* life-history evolution. However, at the time it was felt that barring cases of inbred flies was a reasonable qualification to the standard model. This lack of robustness was not treated as a source of concern. Worse was to follow.

TABLE 2. *The matrix of evolutionary genetic correlations that make up part of the Standard Model.*

	Longevity	Fecundity	Starv. resist.	Desic. resist.	Development	Viability
Longevity	-	neg	pos	pos	neg	pos
Fecundity	neg	-	neg	x	pos	neg
Starvation resistance	pos	neg	-	pos	pos	x
Desiccation resistance	pos	x	pos	-	x	x
Development	neg	pos	pos	x	-	pos
Viability	pos	neg	x	x	pos	-

(x - no correlation inferred; - same character).

Genotype-by-environment interaction

Flies that have been recently sampled from nature are not near evolutionary equilibrium with respect to the laboratory (*vid.* Matos *et al.*, 2000). They undergo a process of rapid adaptation to the laboratory during which several life-history characters improve. Since fly populations inevitably vary in the degree to which they initially are adapted to the laboratory, they will vary up and down for many of their life-history characters in laboratory assays, again producing positive genetic correlations between life-history characters. This was shown in laboratory-adapted fruit flies by giving them a novel environment, and comparing genetic correlations in their normal lab environment *versus* the novel environment (Service and Rose, 1985). As expected, under novel environmental conditions the genetic correlation between fecundity and starvation resistance shifted toward positive values.

This illustrated the dependence of genetic correlations on the environment to which organisms are exposed, in addition to the dependence of these correlations on the degree of inbreeding. Change the environment and the genetic correlation changes. If the environment is novel, there is a tendency to express positive genetic correlations. This result is probably not as robust as the inbreeding result—some novel environments might preserve negative genetic correlations by chance. Still, there is a circumscription of the standard model.

Further evolution of stocks selected for postponed aging led to a reduction in the trade-off between longevity and early fecundity (Hutchinson and Rose, 1991; Chippindale *et al.*, 1993; Leroi *et al.*, 1994*a*). Eventually, the longer-lived stocks even exhibited *increased* early fecundity, compared to the ancestral type of stock. This posed an obvious problem for our understanding of trade-offs in life-history. No inbreeding or novel environment appeared to be involved. But extensive testing for genotype-by-environment interaction revealed that the early fecundity of long-lived stocks was nonetheless reduced specifically under the environmental conditions used to culture the ancestral fruit fly stock: crowding, bad food, and a short opportunity for egg laying (Leroi *et al.*, 1994*a*). Under appropriate environmental conditions, the original trade-offs would reappear (Leroi *et al.*, 1994*a, b*).

Novel and long-term selection

Up to this point, it was still possible to regard these difficulties for the standard *Drosophila* life-history model as experimental artifacts (*cf.* Rose, 1991, Ch. 3–4). But greater difficulties were to come.

One of the areas that the standard model was extended to was the evolution of development. We found an apparent trade-off between rate of development and viability (Chippindale *et al.*, 1994). This was a natural elaboration of the standard model in that it suggests a trade-off between rapidly developing an adult and the survival of the larva. Chippindale *et al.* (1997) successfully selected for accelerated development in the *Drosophila* stocks that had been used to develop the standard model. The rapidly developing flies had reduced viability, too. Borash *et al.* (2000) also found that these faster developing flies were more vulnerable to noxious environments. In these respects, the larval evolutionary patterns seemed to fit the kind of trade-off pattern built into our standard model of *Drosophila* life-history evolution.

It was only when more detailed analyses of growth rate were performed that problems appeared. When Chippindale and collaborators analyzed growth rate using measurements of dry body mass instead of thorax length, this trade-off disappeared (Chippindale *et al.*, 2004). The correlation between growth rate and viability went from negative to positive, as a function of the specific trait that was measured, say mass *versus* thorax length. In other words, the evidence for a trade-off was dependent on how the traits were characterized.

The populations that were originally used to develop the standard model underwent continued selection. The total number of generations of selection came to exceed 100 for most of these stocks, as opposed to 20 or 30 generations, the number of generations of selection that characterized the stocks when they were first studied. At that earlier point in the evolution of our populations, we had a positive genetic correlation between stress resistance and longevity. (See Table 2.) After more than 100 generations of experimental evolution, we re-analyzed the relationship between stress resistance and longevity (Phelan *et al.*, 2003), finding that the positive correlation between stress resistance and longevity disappeared at high levels of stress resistance. There was even evidence for a negative relationship between high levels of starvation resistance and longevity. Because this correlation breakdown arose in a miscellany of stocks, we proceeded to select specifically for very high levels of stress resistance to determine its effects on longevity (Archer *et al.*, 2003), without confounding selection. Again, the positive correlation built into the standard model broke down.

The pattern of the selection results was fairly simple. So long as selection didn't push functional characters too far, our standard ideas about viability, development, fecundity, longevity, and stress resistance held up fairly well. But if we pushed selection hard, producing substantial enhancements in these functional characters, the standard model collapsed. In other words, our standard model was only a local finding.

Genetic background

There are some findings that do seem to be highly robust. For example, the effect of delayed reproduction on the laboratory evolution of *Drosophila* appears quite reliable: longevity increases (Wattiaux, 1968; Rose and Charlesworth, 1980; Rose, 1984*a*; Luckinbill *et al.*, 1984; Partridge and Fowler, 1992). Passananti (2000) performed such a late-reproduction study using hybrids of four inbred *rosy D. melanogaster* stocks:

TABLE 3. *The evolutionary effects of postponed reproduction in rosy stocks, generation 22.*

	B_{ry}	O_{ry}
Male longevity* (days)	46.52 ± 1.22	54.76 ± 1.14
Female longevity* (days)	36.98 ± 0.59	47.63 ± 1.49
Early fecundity	31.80 ± 3.51	17.64 ± 2.17
Male starvation resistance (hours)	24.87 ± 2.28	22.08 ± 1.29
Female starvation resistance (hours)	33.68 ± 3.78	28.11 ± 1.22

(* Indicates $P < 0.05$ in paired *t*-tests for treatment differences with 5 replicates; results are given as means ± standard errors).

Canton-S, Oregon-R, Swedish-C, and Lausanne. These stocks and their controls were created using independent crosses. Once the starting stocks were created, five populations were subjected to selection for early reproduction, the B *ry,* while the other five were subject to selection for late reproduction, the O *ry.* Selection proceeded for 22 O generations, and many more B generations, before samples were taken for assay. Two generations of standardized rearing were used before data were collected. The results are shown in Table 3. (Note that all statistical comparisons are between treatments, so that the number of replicate lines [not individuals] limits the degrees of freedom, which in turn makes the inference of statistical significance quite conservative.)

As in earlier studies, average longevity significantly increased in the O *ry* stocks. This is in keeping with the findings of Rose (1984*a*), a study that employed fewer generations. The chief interest of the results of Table 3, however, is that the indirect responses of starvation resistance and early fecundity are not in keeping with the standard model. There is no statistically significant decrease in fecundity or increase in starvation resistance at generation 22. While the linear regression of average fecundity in O *ry* stocks does significantly trend downward when multiple generations of data are used ($P < 0.05$; data not shown here; Passananti, 2000), the starvation resistance results are not even in the right direction. Here, as in the findings of Phelan *et al.* (2003) and Archer *et al.* (2003), the qualitative correlation between starvation resistance and longevity is undermined. Using a different genetic background breaks the positive correlation between starvation resistance and longevity.

CONCLUSION: WHERE DO WE GO FROM HERE?

The standard *Drosophila* model for life-history evolution arose first in the 1970s. It is now more than 30 years old. Much of its history is outlined in Rose *et al.* (2004). But our recent research is inimical to the standard model. As a set of precepts about life-history evolution in a particular system, the standard model should be abandoned.

What is the general import of this conclusion? There is the question of whether or not other evolutionary systems will have the same features. In general, we do not know the answer to this question. There aren't many studies of experimental evolution. Of these studies, very few compare with our *Drosophila* work in terms of the number of generations, replicates, or distinct selection regimes utilized. The Luckinbill laboratory has performed somewhat similar research (*e.g.,* Luckinbill *et al.*, 1984). Interestingly, one of their studies demonstrated the existence of a genotype-by-environment interaction involving rearing density (Clare and Luckinbill, 1985), a finding that was later corroborated in our system (Service *et al.*, 1988).

An experimental evolution system that has been even more replicated is the *Escherichia coli* model system established by Lenski and his colleagues, beginning with Lenski *et al.* (1991). This system has been studied for thousands of generations, and some additional lines have been created that focus on particular characters, such as adaptation to temperature (*e.g.,* Bennett *et al.*, 1992). Like the original standard model for *Drosophila* life-history evolution, it would be fair to say that Lenski and colleagues have created a standard model for *E. coli.* But how global is it? Will it too breakdown as they learn more?

Consider the possibility that the destruction of the *Drosophila* model will prove generally true, if not for all organisms perhaps, then at least for metazoa. That is, what if none of the patterns that we adduce for the effects of evolution on animals hold up when we learn more? Natural selection may be a process that rapaciously exploits new advantageous alleles and allele combinations to increase fitness, often in ways that undermine antecedent limits on adaptation. If so, then it is only appropriate to expect that simple evolutionary just-so stories will not be sustained when enough is learned about the range of pertinent evolutionary dynamics. What can we do about this prospect?

We could track the accomplishments of evolution the way market analysts track the stock market, always searching for the latest pattern. Ever more complex models could explain observed patterns with increasing precision, without gaining predictive power. There might be an alternative, however: focusing only on the dynamical machinery of evolution independently of the outcome of evolution. With this approach, the *workings* of the process would be studied, eschewing any prospect of generally characterizing the *effects* of the evolutionary process. This leads to a focus on testable predictions concerning the evolutionary mechanisms involved: 1) We might test whether standing genetic variation plays a predominant role in the response to selection (*cf.* Teotónio and Rose, 2000); 2) Similarly, we could determine if new mutations were involved in the response to selection (*e.g.,* Mackay, 1985), and if their effect is dependent on population structure (*e.g.,* Estes and Lynch, 2003); 3) The relative role of additive and non-additive gene interactions can be tested for their role in inbreeding depression (*e.g.,* Vassilieva *et al.*, 2000); 4) Hypotheses about how genetic drift and selection change the patterns of genetic variance and covariance can be addressed (*e.g.,* Whitlock *et al.*, 2002); 5) Specific forms of natural selection, such as density and frequency-dependent selec-

tion, can be examined for their prevalence (*e.g.,* Mueller *et al.*, 1993; Reznick *et al.*, 1996); 6) The relationship between evolutionary rate and initial differentiation is sometimes strong, linear, and negative in slope (*e.g.,* Teotónio and Rose, 2000), perhaps because of the greater magnitude of selection differentials when there is more differentiation—a testable finding that may not depend on local features of evolution. But we should always be prepared to discover, and document, that our expectations are not met, even for hypotheses about basic mechanisms of evolution.

Some might conclude that we have shown that experimental evolution is of little value for evolutionary research. On the contrary, we propose that experimental evolution is one of the most powerful techniques in evolutionary biology, powerful enough to reveal the unreliability of most conclusions that have been adduced concerning evolution.

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