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# THE SYMMETRY OF CORRELATED SELECTION RESPONSES IN ADAPTIVE EVOLUTION: AN EXPERIMENTAL STUDY USING *DROSOPHILA*

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*Abstract.*—The relationship between the processes of density-dependent and age-specific selection has been investigated by examining a common phenotype, urea resistance, which has apparently evolved in response to each of these selection mechanisms. Twenty populations that have experienced differing levels of age-specific selection show differences in egg-to-adult viability in environments with high levels of urea. Among this group of populations, it appears that resistance to urea is correlated with longevity, but not development time. Ten populations kept at extreme larval densities for many generations also show responses to urea: those kept at high larval densities appear to be most resistant to urea. However, these populations show no differences in adult longevity. An additional five populations were selected directly for urea resistance by adding this compound to the larval food environment. Again, there was a strong response to this artificial selection, with urea resistance increasing dramatically, but these populations showed no response in adult longevity or resistance to crowding when compared to five control populations. There is clearly no simple relationship between longevity and larval urea resistance. It may be that age-specific and density-dependent selection induce similar changes in this phenotype, but do so through different genetic and physiological pathways. We suggest that these data are not consistent with the view of constant and symmetric genetic variance-covariance matrices. These data support a more prominent role for observations of evolutionary trajectories rather than static measurements of genetic components of variance.

*Key words.*—Correlated responses, development, *Drosophila,* evolution, life history, senescence, stress resistance, urea.

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One of the most profound issues in experimental evolutionary biology has been the relationship between the genetic variance-covariance matrix of a set of characters and their long-term evolution. Under one view (Lande 1979), these matrices are stable over time and thus useful evolutionary predictions can be made using variance-covariance matrices. Another view is that selection may cause allele frequency changes that substantially and rapidly alter the magnitude and sign of covariances (Bohren et al. 1966). Under this view, long-term evolutionary predictions will require observations of similar evolutionary trajectories, more elaborate population-genetic theory, or both.

Our particular focus is the evolution of life history. Reasonable theory has been developed for life-history evolution with both age-specific (Charlesworth 1994) and density-dependent natural selection (Roughgarden 1971, 1979; Boyce 1984). Many of the predictions following from these theories have in fact been verified by empirical research (Rose and Charlesworth 1980; Luckinbill et al. 1984; Mueller et al. 1991; Partridge and Fowler 1992). Of particular interest for us has been the interaction between age-specific and density-dependent selection. A prior examination of *Drosophila melanogaster* populations that had been subjected to age-specific or density-dependent natural selection revealed few responses in common (Mueller et al. 1993).

Our previous studies of density-dependent natural selection had focussed on documenting changes in fitness related traits (Mueller and Ayala 1981; Bierbaum et al. 1989; Mueller et al. 1991; Joshi and Mueller 1993) or traits related to larval behavior (Mueller and Sweet 1986; Joshi and Mueller 1988; Guo et al. 1991). Since crowded *Drosophila* cultures will presumably have elevated levels of waste products, a natural direction to continue our studies of density-dependent selection was to examine selected populations for differential ability to tolerate nitrogen waste products. In particular, one of the few published papers on nitrogen waste in *Drosophila* (Botella et al. 1985) suggested that urea was an important nitrogen waste that accumulates in crowded cultures (although recent observations suggest that ammonia, not urea, is the major nitrogen waste product; A. Gibbs unpubl. data). Urea also has effects on *Drosophila* that tend to mimic many of the effects of crowding, such as a reduction in egg-to-adult viability, increased development time (Botella et al. 1985), and decreased adult size (Mueller unpubl. data).

The initial finding in this study was that populations selected for reproduction late in life and populations selected to tolerate high larval densities both show resistance to elevated levels of urea. None of these populations are expected to experience elevated levels of urea in their larval or adult environment. Consequently, the evolution of this resistance is presumably due to changes in underlying correlated characters; a major component of this study involves the examination of correlated traits and their response to different types of selection. Examination of postponed aging characters has revealed a rough symmetry in the response of correlated characters (Rose et al. 1992). For instance, selection for reproduction late in life causes increases in longevity, starvation resistance, duration of flight and a decrease in early fecundity relative to controls (Service et al. 1985; Graves et al. 1992). When these long-lived populations are moved back

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to an early schedule of reproduction, some of these traits also returned to levels typical of the control populations (although not all characters do so, e.g., ethanol tolerance; Service et al. 1988). Likewise, selection on either starvation resistance or desiccation resistance leads to increases in longevity as a correlated response (Rose et al. 1992). An important component of this study is reconciling the degree of asymmetry seen in the correlated responses to selection with different theoretical views of quantitative genetic variation.

## Methods

### *Evolution at High Densities*

Two types of populations were used in experiments investigating the effects of density. The CU populations, derived from the B populations of Rose (1984) in January 1990, were kept at high larval densities (~1000 larvae/6-dram vial) and collected for a period of about one week. During this period, adults were kept at low density (~50/8-dram vial) and were given live yeast three days prior to egg collection in a large population cage. The entire life cycle is complete in about three weeks (Mueller et al. 1993). The UU populations, derived from the Bs in September 1991, were kept at low larval (~50 larvae/8-dram vial) and adult densities. Adults were collected at day 14 of the life cycle, kept in vials until the last three days of the life cycle, and then moved to a large population cage where they also received live yeast. The life cycle of the UUs is complete in three weeks. The life cycle of the UUs is very similar to the life cycle of their parental stock, the Bs. The major difference is that the UUs have a three-week life cycle rather than the two-week B cycle, and the UUs received yeast prior to egg laying. All UU and CU populations were replicated five-fold and handled independently of each other. Adult breeding populations were typically 1000–2000 flies.

### *Evolution of Postponed Senescence*

The B and O populations were derived from a common ancestral population called the IVs in 1980 (Rose 1984). These stocks have been described in detail (Rose 1984; Service et al. 1985; Leroi et al. 1994a). At the time of our assay, the Bs had lived approximately 250 generations since their derivation from the IVs; the Os had lived approximately 60 generations. Relative to the Bs, the Os have evolved increases in mean longevity (Rose 1984; Rose et al. 1992; Leroi et al. 1994a), egg-to-adult developmental time, and egg-to-adult viability (Chippindale et al. 1994).

The CBs and COs were respectively derived from the Bs and Os in early 1989 (Rose et al. 1992). Each CB or CO population was derived from a single B or O population (e.g., $CB_1$ from $B_1$); both sets of stocks are, therefore, also five-fold replicated. At the time of assay, the CBs and COs had been under five-week generation times for approximately 63 generations.

### *Evolution at Toxic Urea Levels*

In October, 1991, 10 new populations were derived from five B populations. One of the two new populations derived from each ancestral $B_i$ population was called $MC_i$, and the other was called $MX_i$ where $i$ is the number of the ancestral population (e.g., $MC_1$ and $MX_1$ were derived from $B_1$). These MC and MX populations are described here for the first time. The MCs and MXs were maintained in the following way. Each generation, adults kept in population cages oviposited on a non-nutritive agar surface onto which live yeast had been applied to stimulate laying. The flies were allowed to lay eggs for approximately six hours; the eggs were then placed into vials containing approximately 5 mL banana-molasses medium at densities of 60–80 eggs per vial, well below crowding densities. The following day, plastic sheets were inserted into each vial. Once most larvae had pupated onto the plastic inserts, these were removed and placed into cages with petri dishes containing yeasted banana-molasses medium. This procedure ensures that the adult flies that emerge a few days later will not be exposed to the same dietary environment as the larvae. Food was changed daily for five or six days after peak eclosion until egg collection for the following generation took place.

The MXs were selected for resistance to urea by the addition of toxic levels of urea to their rearing medium. Selection was begun at 12 mg urea/mL banana-molasses medium. This was increased to 14 mg/mL at generation 5, 17 mg/mL at generation 15, and 18 mg/mL at generation 25. Because we were interested in correlated selection responses of imaginal longevity to urea resistance, it was necessary that the pattern of age-specific selection upon the adult phase be the same in the two stocks. This, in turn, necessitated that the time between eclosion and egg-collection for the following generation be the same for the MX and MCs: four to five days. Since high levels of environmental urea slow development (Botella et al. 1985), the absolute time to complete one generation in the MC population is often several days less than the MX population. An attempt was made to standardize effective population size in the two stocks by collecting 40 and 60 vials of eggs each generation, for the MCs and MXs, respectively.

### *Larval Stress Resistance Assay*

We examined the effects of rearing in urea-containing medium on three larval life-history traits: egg-to-puparium viability, egg-to-adult viability, and egg-to-adult development time. In each experiment, three urea treatments (0, 12, and 18 mg urea/mL medium) and two selection treatments were assayed. The selection treatments were run in pairs: B and O, CU and UU, MX and MC, B and CB, and O and CO (Table 1). Nongenetic parental effects were eliminated by raising all experimental populations for two generations under standard conditions. Exactly 50 eggs collected from young parental generation flies were counted out into each experimental vial, each containing 5 mL banana-molasses medium. One week after egg collection, checks at six-hour intervals for newly eclosed flies were begun, with the adults then being sexed and counted. Checks ceased after 72 h without eclosion. Egg-to-puparium viability was estimated from counts of pupae. Ten replicate vials were assayed per population per treatment for a total of 300 vials (2 selection treatments × 5 replicate populations × 3 urea treatments).

TABLE 1. Summary of experiments cmopleted in this study. See text for detailed descriptions of each experiment.

| Experiment | Variable | Populations | Generation of selection |
|---|---|---|---|
| Viability/development time | Urea | B and O | B (321, 327, 334); O (67, 70, 73) |
| | | MX and MC | 10, 20, 35 |
| | | B and CB | B (321, 334); CB (59, 64) |
| | | O and CO | O (67, 73); CO (59, 64) |
| | | UU and CU | UU (36); CU (60) |
| Viability/development time | Density | B and O | B (274); O (49) |
| | | MX and MC | 56 |
| | | UU and CU | UU (2); CU (26) |
| Longevity | Urea | B and O | B (306); O (63) |
| | | MX and MC | 30 |
| Fecundity | Urea | B and O | B (306); O (63) |
| | | MX and MC | 30 |

### *Larval Crowding Tolerance Assay*

We examined the effects of larval density on egg-to-adult survivorship and egg-to-adult development time. Synchronized first-instar larvae were placed into vials at one of two densities: 50 larvae per vial or 500 larvae per vial. For high densities, five replicate vials were used per population; for low densities, 10 replicate vials (except for the B and O experiments, with 20 replicates) were used for a total of 150 vials (2 stocks × 5 replicate populations × 15 replicate vials). Crowding experiments were conducted on the CU and UU, B and O, and MX and MC populations (Table 1).

### *The Effects of Larval Stress on Adult Traits*

We examined the effects of rearing in urea-containing medium on two adult life-history traits: early fecundity and imaginal longevity. As above, experimental populations were first raised under standardized conditions for two generations. Eggs were then collected at densities of 60–80 eggs per vial on either 0 or 14 mg urea/mL medium; 18 mg urea/mL was not used as a treatment because viabilities are low in these conditions, making a longevity assay difficult. Very early eclosing flies were discarded so that the bulk of the flies used in the longevity assay would start on the same day. Subsequently eclosing flies were placed in pairs (one male and one female) into 8-dram vials containing freshly yeasted charcoal-sucrose medium (Rose 1984), and transferred daily onto fresh food until death. Twenty-four-hour fecundities were assayed at days 2, 4, and 6 after eclosion. Forty pairs of flies were assayed per treatment per population for a total of 800 (2 selection treatments × 5 replicate population × 2 urea treatments × 40 pairs). These experiments were also done on the B and O, and MX and MC populations (Table 1).

### *Statistical Analysis*

Analysis of variance (ANOVA) was performed with SAS for Windows version 6.08. Age, sex, and urea concentration were treated as fixed, cross-classified effects. The sampling procedure used during the derivation of the B and O populations permits treating each B and O population as an independent replicate of the selection treatment. For all other populations, those with a common subscript constitute a block that must be analyzed as such in the ANOVA. Likewise, temporal blocks were created in those experiments that were repeated on two or more calendar dates. Viability data were transformed using the variant of the arcsine transformation described by Freeman and Tukey (1950). All means are given with ±95% confidence intervals.

## RESULTS

### *The Fitness Effects of Crowding and Urea Toxicity*

Larval crowding has long been known to affect the development time and viability of *Drosophila* (Chiang and Hodson 1950; Bierbaum et al. 1989). This is true for flies from all selection treatments examined here, although, as discussed below, some selection treatments are less susceptible to the effects of crowding than others. In all experiments, as density increases from 50 to 500 larvae/vial, development time increases (results not shown). While the degree of increase may vary among experiments (5–50%), in all cases it is significant. None of the selection treatment by density interactions are significant for development time. Figure 1 shows the comparable effect of crowding on larval viability. For any given selection treatment, viability declines by 37–57% from low to high density, this effect being significant for all selection treatments.

High concentrations of environmental urea have much the same phenotypic effects as crowding: increasing development time and decreasing viability (Botella et al. 1985). Again, this is true for flies from all selection treatments. In general, an increase in added urea from 0 to 12 mg/mL slows development time by 10%; increasing the added urea concentration to 18 mg/mL slows development time by a further 20% (Table 2). These effects are significant for all selection treatments.

The effects of added urea on egg-to-adult viability are shown in Figures 2, 3, and 4 for all selection treatments. In general, viability declines massively between 0 and 18 mg/mL; no stocks have more than 50% survivorship at the highest concentration of added environmental urea; most are much lower. To determine at which stage this mortality occurs, we also measured egg-to-puparium viability (Figs. 2, 3, and 4). For all selection treatments, this measure of viability is relatively unaffected by added urea, declining on average by only 0.3% at 12 mg/mL relative to controls, and a further 9% by 18 mg/mL. This decline in egg-to-puparium viability, always small, was significant in some experiments,

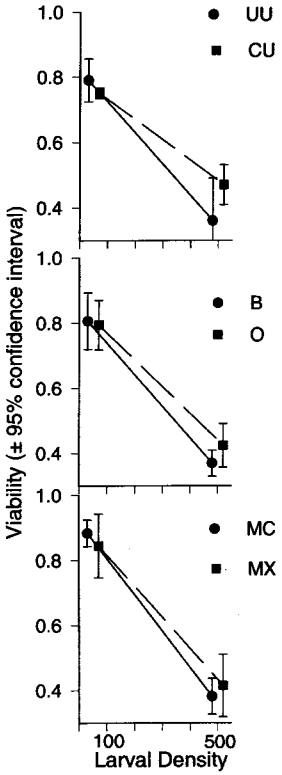


FIG. 1. The fraction of first instar larve that become adults at two different densities in six different selection treatments. Confidence intervals are calculated using the means for the five replicate populations within each selection treatment. Above: UU vs. CU: CUs are selected for crowding tolerance, UUs are not. ANOVA shows a significant effect of density ($P < 0.001$), a nonsignificant effect of selection treatment ($P = 0.273$), and a significant density × selection interaction ($P = 0.026$). Center: B vs. O: Os are selected for late-life (10 wk) reproductive success, Bs for early-life (2 wk) reproductive success. ANOVA shows a significant effect of density ($P < 0.005$), but no effect of selection treatment ($P = 0.96$), or density × selection interaction ($P > 0.9$). Below: MXs are selected for fitness in a toxic urea environment, MCs are selected for fitness in a low urea environment. ANOVA shows a significant effect of density ($P = 0.0001$), but no effect of selection treatment ($P = 0.14$), or density × selection interaction ($P = 0.16$).

but not others. These results, taken with the egg-to-adult viability measure, demonstrate that the pernicious effects of urea toxicity are most acute *in puparium.* At high urea levels, larvae seem to be able to grow and even pupate successfully, but then die in the course of metamorphosis.

### *Evolution at High Densities*

The five replicate populations of the CU selection treatment have been maintained at high larval densities relative to their UU controls. We expect, therefore, that they will have high fitness under crowded conditions. Indeed, the CU selection treatment shows significantly elevated larval viability at high densities (Fig. 1). This result is consistent with earlier results found in other density-selected populations of *D. melanogaster* (Bierbaum et al. 1989).

Given that the CUs survive so much better under crowded conditions, do they survive better when exposed to toxic levels of urea? The answer is that they do. Figure 2 shows

TABLE 2. Effect of environmental urea on development time. ANOVAs for development time under different selection and urea regimes: UU vs. CU: selection treatment $P > 0.05$, urea treatment $P < 0.001$, selection × urea $P > 0.25$; B vs. O: selection treatment $P < 0.001$, urea treatment $P < 0.0001$, selection × urea $P > 0.27$; B vs. CB: selection treatment $P < 0.0001$, urea treatment $P < 0.0001$, selection × urea $P > 0.05$; O vs. CO: selection treatment $P < 81$, urea treatment $P < 0.0001$, selection × urea $P > 0.99$; MC vs. MX: selection treatment $P < 0.0001$, urea treatment $P < 0.0001$, selection × urea $P < 0.05$.

| Selection treatment | 0 mg/ml | | 12 mg/mL | | 18 mg/mL | |
|---|---|---|---|---|---|---|
| | Mean | ± 95% CI | Mean | ± 95% CI | Mean | ± 95% CI |
| UU | 270 | 11.0 | 299 | 5.5 | | |
| CU | 269 | 18.0 | 299 | 15.0 | | |
| B | 224 | 9.3 | | | 289 | 18.6 |
| O | 239 | 14.8 | | | 302 | 23.7 |
| B | 230 | 4.8 | 252 | 4.4 | 299 | 13.9 |
| CB | 236 | 9.9 | 260 | 10.1 | 314 | 17.0 |
| O | 242 | 5.9 | 262 | 7.1 | 321 | 9.4 |
| CO | 241 | 8.8 | 263 | 9.6 | 322 | 10.3 |
| Generation 10 | | | | | | |
| MC | 210 | 22.0 | 227 | 20.8 | 261 | 38.7 |
| MX | 223 | 15.7 | 244 | 13.5 | 286 | 14.6 |
| Generation 20 | | | | | | |
| MC | 219 | 10.4 | 233 | 12.2 | 259 | 19.1 |
| MX | 221 | 13.8 | 225 | 13.5 | 254 | 13.5 |
| Generation 35 | | | | | | |
| MC | 239 | 2.4 | 285 | 4.9 | 354 | 17.8 |
| MX | 241 | 4.6 | 287 | 6.9 | 342 | 9.9 |

that, at 12 mg/mL, the CUs have a 23% superiority in egg-to-adult survivorship relative to their controls. This superiority is a function of increased survival over metamorphosis, as there is no significant interaction between selection treatment and urea level for egg-to-puparium survivorship, the difference remaining very small.

The crowding and urea stress experiments (Table 2) give apparently inconsistent results for development time: in the former, the CUs develop significantly slower than the UUs, but this difference is not apparent later in the urea experiment. The initial differentiation is, however, almost certainly an artifact that stems from the UUs being more recently derived from the Bs than the CUs. The Bs are a very fast developing stock, and the UUs apparently retained that property for some while until adapting to the three-week generation cycle of the CUs; the difference is, therefore, not necessarily attributable to selection for crowding tolerance, and in any case disappears by the time of the urea-resistance assay.

### *Evolution of Postponed Senescence*

The evolution of longevity in the B and O populations has been examined many times before (Rose et al. 1982; Chippindale et al. 1993; Leroi et al. 1994a). The O populations, selected for postponed senescence, invariably live much longer than their controls, the B populations, and this is true here too (Fig. 5). The Os also currently have a higher early-life fecundity than the Bs when raised with yeasted medium (Leroi et al. 1994a,b), and this remains so (Table 3).

Bs and Os differ in their susceptibility to environmental

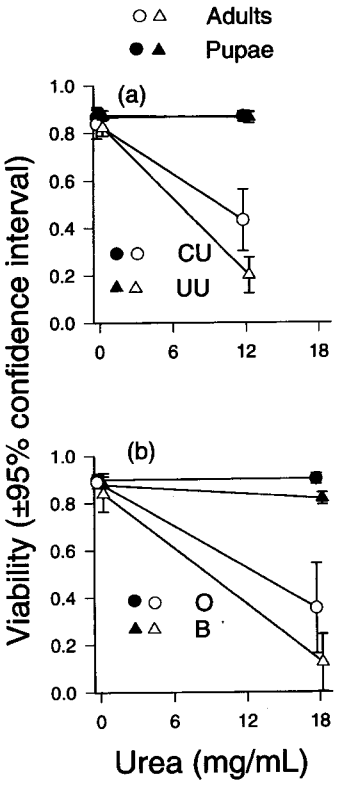


FIG. 2. Effects of urea on egg-to-puparium and egg-to-adult viability in four different selection treatments. Confidence intervals are calculated using the means for the five replicate populations within each selection treatment. Above: UU vs. CU: CUs are selected for crowding tolerance, UUs are not. ANOVA for egg-to-puparium viability shows no significant effect of urea level ($P = 0.75$), selection treatment ($P = 0.90$), or urea × selection interaction ($P = 0.17$). ANOVA for egg-to-adult viability shows a significant effect of urea level ($P = 0.0001$), a significant effect of selection treatment ($P = 0.0001$), and a significant urea × selection interaction ($P = 0.0001$). Below: B vs. O: Os are selected for late-life (10 wk) reproductive success, Bs for early-life (2 wk) reproductive success. ANOVA for egg-to-puparium viability shows no significant effect of urea level ($P = 0.21$), selection treatment ($P > 0.05$), or urea × selection interaction ($P > 0.5$). ANOVA for egg-to-adult viability shows a significant effect of urea level ($P = 0.0001$), selection treatment ($P < 0.005$), and urea × selection interaction ($P < 0.005$).

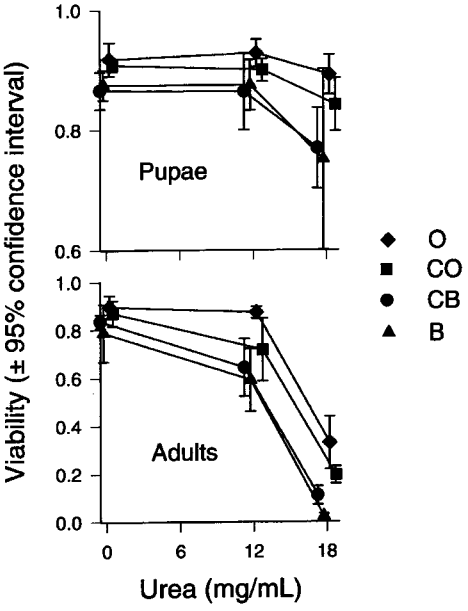


FIG. 3. Effects of urea on egg-to-puparium and egg-to-adult viability in four age-specific selection treatments. Confidence intervals are calculated using the means for the five replicate populations within each selection treatment. CBs and COs are selected for mid-life (5 wk) reproductive success, and are derived respectively from the Bs, selected for early-life (2 wk) reproductive success, and the Os, selected for late-life reproductive success. Egg-to-puparium viability: B vs. CB: ANOVA shows a significant effect of urea level ($P < 0.0001$), none for selection treatment ($P = 0.24$), and a marginally significant urea × selection interaction ($P = 0.046$). O vs. CO: ANOVA shows a significant effect of urea level ($P < 0.001$), selection treatment ($P < 0.0001$), and a marginally nonsignificant urea × selection interaction ($P = 0.056$). Egg-to-adult viability: B vs. CB: ANOVA shows a significant effect of of urea level ($P < 0.001$), selection treatment ($P < 0.0001$), and urea × selection interaction ($P < 0.05$). O vs. CO: ANOVA shows a significant effect of urea level ($P < 0.0001$), selection treatment ($P < 0.0001$), and urea × selection interaction ($P < 0.0001$).

urea as larvae. Figure 2 shows that the Os are superior to the Bs in surviving high concentrations of environmental urea. As with the CUs relative to their controls, this superiority is not manifest in egg-to-puparium survival, rather it must be due to a mitigation of the deleterious effects of urea during metamorphosis (Fig. 2). We have also asked whether the greater resistance of the Os to urea in the larval medium is carried over to the adult phase. It appears not, for regardless of whether the B and O populations are raised on standard medium or urea, the difference in longevity and early fecundity between them remains about the same (Table 3; Fig. 5), with none of the selection by urea treatment terms for these traits being significant.

Having shown that crowding resistant populations (CU) are relatively urea resistant, it might be expected that the Os, being urea resistant as larvae, are also tolerant of crowding. As Figure 1 shows, however, this appears not to be the case. At 500 larvae/vial, the Os show a superiority in first instar larva-to-adult viability of 5.5% relative to Bs, a small, but nonsignificant, improvement over the negligible stock differences at low densities (see also Chippindale et al. 1994). Thus, it appears that O resistance to urea does not necessarily confer significant resistance to crowding.

The Os develop slower than the Bs (Table 2). Here, too, there is no evidence that the slowing of development associated with stressful environments affects the Bs and Os differently.

Comparison of the Bs and Os, involving populations kept on two-week generations (B) versus those kept on 10-week generations (O), is a relatively extreme one. The CB and CO populations, derived from the Bs and Os, were kept on an intermediate generation cycle of five weeks, and might, therefore, be expected to evolve levels of urea resistance intermediate to that of their ancestors. Figure 3 shows that this is indeed the case: the CBs and COs, being maintained on a common generation time intermediate to those of their respective ancestors, have converged to nearly the same level of urea resistance: the CBs increase relative to the Bs, and

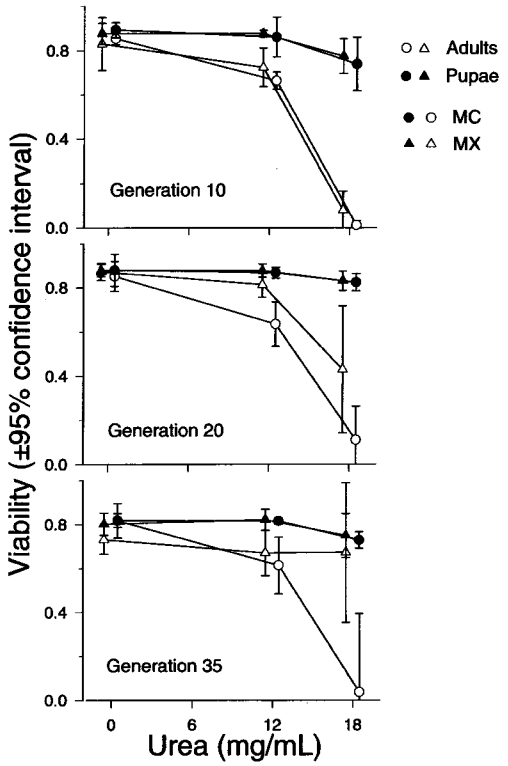


FIG. 4. Effects of urea on egg-to-puparium and egg-to-adult viability in two urea-resistance selection treatments. MXs are selected for fitness in a toxic urea environment, MCs are selected for fitness in a low urea environment.

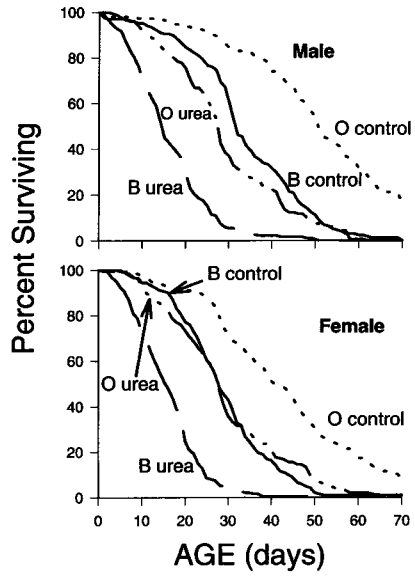


FIG. 5. Adult survivorship of B and O flies that have been raised at two different larval urea concentrations. Os are selected for late-life (10 wk) reproductive success, Bs for early-life (2 wk) reproductive success. B vs. O: considering control (0 mg/mL urea) treatment alone, Os live longer than Bs (males $P < 0.0005$, females $P < 0.005$; two tailed $t$-distribution with $n - 2 = 8$ df). ANOVA shows a significant effect of selection treatment ($P < 0.0001$), sex ($P < 0.01$), and urea ($P < 0.001$), but neither the selection × urea ($P = 0.14$) or selection × urea × sex ($P = 0.41$) terms were significant.

the COs decline relative to the Os. In contrast, for egg-to-adult viability there is no evidence that the development times of the CBs and COs converge to an intermediate value (Table 2). While the CBs have a generally extended development time relative to the Bs, the COs have not reduced their development time relative to the Os.

### *Evolution at Toxic Urea Levels*

The MXs were maintained for approximately 35 generations in a larval medium containing ever-increasing levels of urea. Over the first 10 generations, little adaptation to this toxic environment was seen (Fig. 4, generation 10). However, by generation 35 the MXs adapted specifically to the urea environment, and survived high levels of urea almost 20-fold higher than the unselected controls (Fig. 4: 18 mg/mL at generation 35).

One interpretation of the slow change in resistance in the MX populations may be that resistance is due to only one or a few genes. However, another possibility is that the response to selection was initially very weak because selection was weak. Recall that urea levels did not reach the final level of 18 mg/mL until generation 25. Recent work on the genetics of tolerance in the MX populations (Joshi et al. 1996) suggest that the resistance genes are largely dominant, which would suggest a more rapid increase when these alleles were initially rare, and at least some, but not all, of the genes involved in urea resistance are located on the X-chromosome.

Adaptation to urea causes a great improvement in egg-to-adult viability in urea-laden environments and at least some of this is attributable to an improvement in egg-to-puparium survivorship (Fig. 4). We also investigated whether the MXs and MCs have evolved increased longevity as adults. When raised on standard medium (containing no added urea), the MCs and MXs do not differ in mean longevity (Fig. 6). As might be expected, however, exposure to urea as larvae shortens adult life, and after 20 generations of selection, the MXs were less affected than the MCs. This result is also true for

TABLE 3. Effect of environmental urea on early-life fecundity. B versus O: ANOVA shows a significant effect of selection treatment ($P < 0.0001$), urea ($P < 0.001$), but no selection × urea interaction ($P > 0.1$). MC versus MX: ANOVA shows no significant effect of selection treatment ($P > 0.1$), a significant effect of urea ($P < 0.001$), and a significant selection × urea interaction ($P < 0.05$).

| Selection treatment | | 0 mg/ml | | 14 mg/ml | |
|---|---|---|---|---|---|
| | | Mean | ± 95% CI | Mean | ± 95% CI |
| B | d2 | 53.6 | 9.8 | 8.1 | 4.9 |
| | d4 | 68.2 | 3.7 | 11.1 | 1.6 |
| | d6 | 65.8 | 5.9 | 10.0 | 2.5 |
| O | d2 | 61.8 | 13.8 | 14.1 | 1.0 |
| | d4 | 78.9 | 8.2 | 20.3 | 7.2 |
| | d6 | 83.6 | 1.0 | 19.5 | 4.3 |
| MC | d2 | 42.4 | 9.1 | 8.1 | 8.3 |
| | d4 | 46.0 | 28.9 | 10.1 | 18.3 |
| | d6 | 43.3 | 24.5 | 17.4 | 6.7 |
| MX | d2 | 31.1 | 16.7 | 16.7 | 11.2 |
| | d4 | 52.6 | 5.6 | 23.4 | 7.3 |
| | d6 | 28.3 | 10.6 | 29.4 | 23.3 |

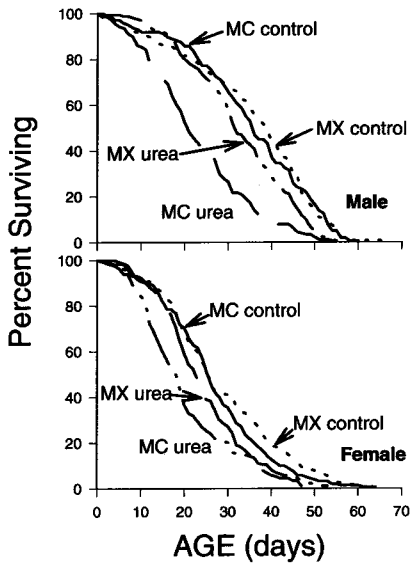


FIG. 6. Adult survivorship of MC and MX flies that have been raised at two different larval urea concentrations. MXs are selected for fitness in a toxic urea environment, MCs are selected for fitness in a low urea environment. MC vs. MX: considering control (0 mg/mL urea) treatment alone, MXs do not live longer than MCs (males $P > 0.1$, females $P > 0.1$; one tailed $t$-distribution with $n - 2 = 8$ df). ANOVA shows a significant effect of selection treatment ($P < 0.01$), sex ($P < 0.001$), and urea ($P < 0.001$) and selection × urea ($P < 0.05$) terms.

early fecundity in which, under control conditions, the MCs and MXs do not differ. They do differ when exposed to urea as larvae, with MXs being less susceptible (Table 3).

Since the MXs are the most resistant populations to urea, it might be expected that they, at least, might find this superiority to their advantage under crowded conditions. At low larval densities, the MC populations show a slightly higher viability (Fig. 1), which is reversed under crowded larval conditions. However, the interaction between population and density in this experiment is not significant.

There is also some evidence (Table 2) that the MXs have a generally increased egg-to-adult development time relative to the MCs. But at later generations, the MXs develop faster than the MCs under high urea conditions (Table 2). Considering all generations and the control urea treatment (0 mg urea/mL medium) alone, the MXs are slower than the MCs by about four hours, though this difference in not statistically significant. In the larval crowding experiment, there is no overall significant stock difference in development time, although in the control treatments the MXs appear to be slightly, but nonsignificantly, slower ($t = -1.7$, $P > 0.1$).

## DISCUSSION

### *Repeated, Independent Evolution of Urea Resistance*

Several sets of the populations studied here, Os, CUs, and MXs, have independently evolved resistance to toxic levels of urea in the larval environment, and have done so in response to a variety of selection regimes. Surprisingly, in one case, Bs and Os, this larval-pupal character has evolved in response to selection on adults.

The MXs have evolved a considerable resistance to urea after having been exposed to toxic levels of urea for thirty five generations. The CU populations have evolved urea resistance relative to their controls over 60 generations of crowded larval environments. This may be an indirect selection response to an environment that contains high levels of nitrogen waste products, like ammonia. Yet a third pattern of differentiation is presented by the B, O, CB, and CO populations, which also differ from each other in urea resistance, even though all have experienced larval environments with identically low larval densities and no added urea. Consequently, it is very unlikely that these populations have experienced different levels of environmental urea during the course of their history; differences among them would appear to be due to selection for some correlated trait. These results suggest that the traits that confer fitness at high larval densities, toxic urea levels, and old age are genetically correlated in some fashion. But how, exactly? We examine the relationships among these various traits below.

### *Genetic Relationship between Crowding Tolerance and Urea Resistance*

The superior viability of the MXs, CUs, and Os relative to their controls when exposed to toxic levels of urea varies from 23% (CU vs. UU) to 20-fold (MX vs. MC at generation 35). At this time, it is unclear to what extent crowded *Drosophila* cultures may create conditions that directly select for urea tolerance. Previous reports (Botella et al. 1985) have suggested that urea levels increase in crowded cultures. Recent measurements on our CU populations suggest that ammonia may be the major nitrogenous waste product (A. Gibbs pers. comm.). Ammonia is a common nitrogen waste product, while urea is not common among insects (Cochran 1985). Thus, whether exposure to high levels of ammonia may cause resistance to urea to also evolve, or whether there is some other aspect of adaptation that causes urea resistance to evolve is unknown at this time. Nevertheless, it might be expected that other stocks with high urea resistance would also be able to tolerate crowded conditions, but in our study, this was not the case. Neither the MXs nor the Os show much ability to weather crowding at densities of 500 larvae/8 dram vial (any trends in that direction are not significant). We are forced therefore to conclude that larval urea resistance per se confers little if any tolerance to crowding. Our data raise the possibility that CU urea resistance is not an adaptation to larval crowding at all, but rather an unselected trait that has evolved by virtue of being correlated with some other aspect of larval physiology undergoing selection.

### *Genetic Relationship between Fitness at Old Age and Urea Resistance*

Selection for postponed senescence generally causes increased longevity and increased late fecundity; here, this is shown by the Os and Bs (Rose 1984; Leroi et al. 1994a). The finding that urea resistance has improved so dramatically in the Os relative to the Bs suggests, then, that late-life reproductive success, possibly longevity itself, is genetically correlated with urea resistance. We expected, therefore, that the MXs and CUs would also be long lived relative to their

TABLE 4. Summary of evolved difference among selection treatments. All evolved gains are significant unless stated (ns). When the first population does differ from the second, the symbols are defined as follows: ≪, much less than; <, less than; and >, greater than. MX/MC urea resistance and development time data come from generation 35. The high urea level data are taken from 18 mg/mL except for the UU/CU comparison, which is 12 mg/mL. Longevities are females only. CU/UU longevity data from Mueller et al. (1993); B/CB/O/CO data from Nusbaum et al. (1996).

| | Larval urea resistance | Larval crowding tolerance | Longevity | Development time |
|---|---|---|---|---|
| UU vs. CU | ≪ | ≪ | ns | </ns |
| MX vs. MC | ≪ | ns | ns | </ns |
| B vs. O | ≪ | ns | ≪ | < |
| B vs. CB | < | | < | < |
| O vs. CO | > | | > | ns |

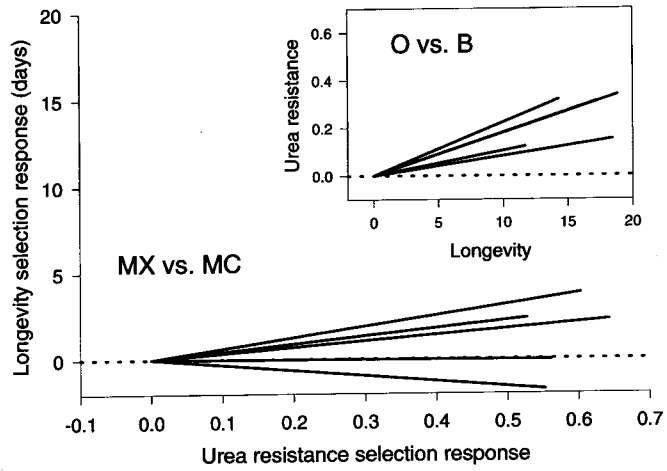


FIG. 7. The bivariate response to selection for each of the five replicate MX and O populations. Urea resistance is measured as viability at 18 mg/mL of urea. The O populations, relative to the Bs, show an indirect response with a slope that is significantly greater than zero. The slope of the indirect response of the MXs, relative to the MCs, is not different from zero.

controls, but this appears not to be the case. Adult MX longevity is the same as their controls when the larvae are raised under control (low density and no added urea) conditions. Mueller et al. (1993) have shown, similarly, that there is no difference in UU and CU longevity. Our failure to detect a correlated response in longevity in these stocks is not likely due to their being insufficiently differentiated, for the difference in urea resistance between the MXs and MCs is far greater than between the Os and Bs. We discuss below possible reasons for these incongruent selection responses.

### *Genetic Relationship between Development Time and Urea Resistance*

Previous work has shown that the increased development time of Os is *not* due to a genetic correlation with longevity, but simply because the Os are under less selective pressure to develop rapidly (being only required to mate late in life) than the Bs. This caused us to think that the Os urea resistance may have evolved because of a genetic correlation with developmental time. But again this does not seem to be true. As discussed above, the COs and CBs (derived from the Os and Bs, respectively) are on an intermediate generation time (five weeks), and they have largely converged in egg-to-adult urea resistance (Fig. 3). They have not, however, done so for development time, for which the COs remain indistinguishable from the Os. The COs and CBs *are,* however, converging in adult longevity. When assayed at generation 71 (Chippindale et al. unpubl. data), the COs had declined in longevity considerably relative to the Os (means and standard errors: males: O 62.2 [± 2.4] vs. CO 46.9 [± 0.03], $P < 0.005$; females: O 54.3 [± 2.4] vs. CO 46.5 [± 2.5], $P = 0.06$; one tailed $t$-distributions with $n - 1 = 4$ df). Assayed at the same time, the CB females, but not the males, appear to be increasing in longevity relative to the Bs (males: B 36.7 [± 0.7] vs. CB 36.6 [± 0.9], $P > 0.95$ NS; females: B 32.8 [± 1.3] vs. CB 38.8 [± 0.9], $P < 0.025$; one tailed $t$-distributions with $n - 1 = 4$ df). Consequently, these results do not reveal a consistent relationship between development time and urea resistance even among demographically selected populations. Finally, we note that neither the MXs nor CUs show a consistent difference in development time relative to their controls, suggesting that if such a difference exists, it is very small and does not contribute to urea resistance. However, the relationship between longevity and urea resistance is not symmetric and we discuss the implications of this finding next.

### *Asymmetrical Correlated Selection Responses*

When we initiated this study, we fully expected to find patterns of correlated selection responses to be roughly symmetrical. By this we mean that if trait $T_1$ evolves as a consequence of selection on $T_2$, then the reverse would be true as well. Under this scenario, our initial findings that (1) lines selected for longevity (Os) are urea resistant; and (2) lines selected for crowding tolerance (CUs) are urea resistant, would lead naturally to the prediction that lines selected for increased urea resistance (MXs) would be both long lived and crowding tolerant. In fact, they are neither [Table 4]. What is more, long-lived lines are not crowding tolerant, and crowding tolerant lines are not long lived.

We seem to have encountered two examples of asymmetrical correlated selection responses. We compare one such asymmetrical correlated response in greater detail in Figure 7, which shows the relationship between the direct and correlated selection responses in populations selected for late-life reproductive success (O vs. B) and urea resistance (MX vs. MC). We can calculate slopes of the bivariate selection responses, $\Delta\bar{z}_u/\Delta\bar{z}_l$ for instance, as follows: $(S_{i,u} - C_{i,u})/(S_{i,l} - C_{i,l})$, where $S$ and $C$ are the means of some trait for a selected ($S$) and control ($C$) population, $i$ is population subscript (e.g., $B_1$ or $O_1$), and $u$ and $l$ denote the traits urea resistance and longevity, respectively. The trait in the numerator is the correlated character and the trait in the denominator is the directly selected trait. Thus, the mean (and standard error) of the slopes of the selection response in populations selected directly for urea resistance, $\Delta\bar{z}_l/\Delta\bar{z}_u$, is 2.2 (± 1.7); that of the populations selected directly for late-life reproductive success, $\Delta\bar{z}_u/\Delta\bar{z}_l$, is 0.015 (± 0.0026). While $\Delta\bar{z}_u/\Delta\bar{z}_l$ is small and significantly greater than zero, $\Delta\bar{z}_l/\Delta\bar{z}_u$

is large but not significantly greater than zero due to the small and inconsistent changes in longevity. A consequence of this asymmetry and the similar one shown by the crowding selected lines (CU vs. UU), is that no solid connection between fitness at high densities and fitness at old age can be established.

We were initially rather surprised by these findings, for in the past we have generally found correlated selection between life-history and stress resistance traits to be at least *qualitatively* symmetrical. For example, Service et al. (1985, 1988) showed that selection for late-life reproductive success is associated with increases in starvation and desiccation resistance. Conversely, Rose et al. (1992) showed that direct selection for either increased desiccation or starvation resistance causes longevity to increase. But if we consider the matter more carefully, we can see that this kind of a symmetry is in fact likely to be the rule, rather than the exception, for complex traits such as those studied here. Suppose, for example, that numerous loci contribute to each of urea resistance, crowding tolerance, and longevity (e.g., Hutchinson and Rose 1991 for longevity and other *Drosophila* characters). Suppose further that some of the loci that affect urea resistance also affect longevity, while many others do not. It easy, then, to see how asymmetrical correlated responses might arise. Where selection for longevity might always entail selection for alleles that also improve urea resistance, it is quite possible that direct selection for urea resistance mostly affects alleles that have no influence on longevity. It is a kind of genetic complexity that simply arises from the existence of many possible physiological mechanisms by which high urea resistance might be achieved.

There are two ways in which physiological complexity enters into quantitative and population genetic models capable of yielding asymmetrical selection responses. First, one may assume constant genetic variances and covariances; from Lande (1979):

$$\frac{\Delta\bar{z}_\ell}{\Delta\bar{z}_u} = (r_{u,\ell})^{-1}\frac{h_\ell\sigma_\ell}{h_u\sigma_u} \tag{1}$$

and

$$\frac{\Delta\bar{z}_u}{\Delta\bar{z}_\ell} = (r_{u,\ell})^{-1}\frac{h_u\sigma_u}{h_\ell\sigma_\ell}, \tag{2}$$

where $r_{u,l}$ is the genetic correlation between urea and longevity, and $h$ and $\sigma$ are the square root of the heritability and phenotypic variance of each trait, respectively. It is evident that if $h_u\sigma_u \neq h_l\sigma_l$ then $\Delta\bar{z}_l/\Delta\bar{z}_u \neq \Delta\bar{z}_u/\Delta\bar{z}_l$, that is, asymmetrical correlated responses arise. However, we contend that the level of asymmetry seen in the current study is too extreme to be accounted for by this model and thus our experimental results are not consistent with a theory that assumes constant variance-covariance matrices.

We demonstrate this first by noting that from the above theory the product of $\Delta\bar{z}_l/\Delta\bar{z}_u$ and $\Delta\bar{z}_u/\Delta\bar{z}_l$ is $(r_{u,l})^{-2}$. This product then should be no smaller than one and often should be much greater than one. While this result depends on a theory that assumes a constant variance-covariance matrix, it does not require that we be able to estimate this matrix. Nevertheless, it yields a prediction about the upper limit of the magnitude of the selection responses (the $\Delta\bar{z}_l$) that we can estimate from these experiments. The product of the slopes from the MX, MC, B, and O data is 0.034. Even if we use the upper 95% confidence interval of each slope, the product is still too small (0.12). Consequently, we conclude that the results of these experiments are inconsistent with the simple theory of evolution of quantitative traits we have outlined above. Below we consider some alternative explanations of the asymmetries seen in this study.

Another way of producing asymmetrical correlated responses from physiological complexity is demonstrated by population genetic models that do not assume constant genetic variances and covariances (Bohren et al. 1966; Gromko 1995; Shaw et al. 1995). Bohren et al. (1966) review instances of asymmetrical correlated responses and also develop a simple model in which four loci affect two different traits. One locus affects only trait $T_1$, the second locus affects only trait $T_2$, the third locus affects both traits in the same manner (positive correlation), and the last locus affects both traits in an opposite fashion (negative correlation). A common observation from this research was that as allele frequencies change due to selection the magnitude and sign of the covariance between traits often changed rapidly, much more so than the variance of each trait. Consequently, the covariance matrix for a given set of traits was not particularly useful for predicting the outcome of evolution except for very short (one generation) periods of time. With this model, traits $T_1$ and $T_2$ may initially show a positive genetic correlation, and selection on $T_1$ can lead to an increase in $T_2$. Yet selection on $T_2$ can have no effect on $T_1$. This is an outcome entirely analogous with the results of this study.

Another possible explanation for the asymmetrical responses seen in our study is that during the course of selection, linkage disequilibrium may develop if genes that affect urea resistance are closely linked to genes that affect longevity (Ewens 1979). Such linkage disequilibrium may cause correlations to develop that are specific to the particular type of selection involved. Thus, different sets of correlated characters may change in the course of selection for late-life reproductive success as opposed to selection for resistance to urea in the larval environment.

Finally, it remains possible that there is some other unmeasured trait whose selection responses, if visualized, would make sense of the pattern of correlated responses. Such a trait would respond positively to selection for urea resistance, late-life reproductive success, and crowding tolerance. An obvious candidate for such a trait was development time, which we know to have increased independently of longevity in the long-lived Os. As discussed above, however, at least two lines of evidence suggest that development time does not affect urea resistance or crowding tolerance.

### *General Implications*

Of these explanations for the observed asymmetrical responses, we consider the theory suggested by Bohren et al. (1966) most likely. We hypothesize that the physiological basis of urea resistance in the MXs is, at least in part, different from urea resistance in the Os, and possibly even from that of the CUs; and that the evolution of this response gives rise

to asymmetrical correlated responses (cf. King 1955). Specifically, the force of selection changes the covariance between traits in a substantial fashion that makes it impossible to generalize about the genetic relationship between the suite of traits examined here except through the observation of the evolutionary trajectories. The most direct way to test this hypothesis would be to identify these mechanisms and how they affect life history.

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