RESOURCE ACQUISITION AND THE EVOLUTION OF STRESS RESISTANCE IN *DROSOPHILA MELANOGASTER*

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Abstract.—Resistance to environmental stress is one of the most important forces molding the distribution and abundance of species. We investigated the evolution of desiccation stress resistance using 20 outbred *Drosophila melanogaster* populations directly selected in the laboratory for adult desiccation resistance (D), postponed senescence (O), and their respective controls (C and B). Both aging and desiccation selection increased desiccation resistance relative to their controls, creating a spectrum of desiccation resistance levels across selection treatments. We employed an integrative approach, merging data on the life histories of these populations with a detailed physiology of water balance. The physiological basis of desiccation resistance may be mechanisms enhancing either resource conservation or resource acquisition and allocation. Desiccation-resistant populations had increased water and carbohydrate stores, and showed age-specific patterns of desiccation resistance consistent with the resource accumulation mechanism. A significant proportion of the resources relevant to resistance of the stress were accumulated in the larval stage. Males and females of desiccation-selected lines exhibited distinctly different patterns of desiccation resistance and resource acquisition, in a manner suggesting intersexual antagonism in the evolution of stress resistance. Preadult viability of stress-selected populations was lower than that of controls, and development was slowed. Our results suggest that there is a cost to preadult resource acquisition, pointing out a complex trade-off architecture involving characters distributed across distinct life-cycle stages.

Key words.—Desiccation, developmental time, *Drosophila melanogaster*, growth rate, life-history evolution, physiology, starvation, stress resistance, trade-offs.

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Abiotic environmental stresses are key factors determining the distribution and abundance of species. In recent years, increased interest has been paid to general mechanisms whereby organisms resist stress (e.g., Hoffmann and Parsons 1991). Because physiological mechanisms of resistance to stress are likely to consume energy or other resources and because many types of stress involve the lack of resources (e.g., food or water), resource management is a critical issue for the organism.

Our understanding of stress resistance is complicated by the fact that resource acquisition and utilization may be temporally separate from each other. Hibernating mammals, for example, gather and store food in burrows or lipids in adipose tissue during the summer, then consume these over the winter. The situation is still more complicated in organisms with complex life cycles, such as holometabolous insects and amphibians, where ontogenetic changes often result in a niche shift. Some fraction of resources acquired in juvenile stages will be consumed during metamorphosis to the adult form, while the remainder may be available for use by the adult. But even if they can be used by the adult, one can not be certain of the selective forces controlling acquisition of "extra" resources by larvae. Do these reserves evolve as an "insurance policy" against complications during metamorphosis, or are they directly selected for by conditions experienced by the adult?

Laboratory populations of *Drosophila melanogaster* provide an ideal system for the investigation of stage-specific acquisition of resources within a holometabolous life cycle. A number of recent laboratory selection experiments dealing with *Drosophila* stress resistance have demonstrated the ease with which environmental stress resistance can be genetically manipulated by laboratory selection (e.g., Service et al. 1985; Cohan and Hoffmann 1986; Hoffmann and Parsons 1989a,b, 1993a,b; Huey et al. 1991; Rose et al. 1992; Blows and Hoffmann 1993; Partridge et al. 1994; Gibbs et al. 1997; Shiotsugu et al. 1997). Starvation is perhaps the best understood specific stressor. In fruit flies, survival of starvation stress is closely correlated with energetic reserve levels, especially lipids (e.g., David et al. 1975; Service 1987; Da Lage et al. 1989; Zwaan et al. 1991; Chippindale et al. 1996; Djawdan et al. 1996). Chippindale et al. (1996) demonstrated the importance of larval resource storage in the adult starvation selection response. Starvation-selected populations exhibited higher larval growth and lipid acquisition rates, resulting in resources that carried over and directly contributed to the adult selection response. Increased larval acquisition was not without costs: lower viability was observed in selected populations relative to controls, suggesting that selection on adult caloric requirements may impinge upon the balance of selective forces operating in preadult stages.

Desiccation is a pervasive agent of selection, particularly in small terrestrial organisms with high surface area:volume ratios. The Drosophilidae are such organisms, and they provide striking examples of adaptation to the desert, having undergone multiple adaptive radiations into arid regions. In this paper, we investigate the adaptive responses of *D. melanogaster* populations to desiccating conditions imposed on the mature adult. Earlier studies have shown that adult *Dro-*

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sophila populations selected for resistance to desiccation often contain higher levels of stored water and glycogen than their controls (Graves et al. 1992; Gibbs et al. 1997; Djawdan et al. 1998; but cf. Blows and Hoffmann 1993; Hoffmann and Parsons 1993b) and reduced rates of water loss (Hoffmann and Parsons 1989a, 1993b; Gibbs et al. 1997). Whereas the adult may experience desiccation stress, *Drosophila* larvae generally grow up in moist microhabitats. Given these circumstances, there is strong potential for larval sequestering of resources affecting adult stress tolerance.

Here we dissect the life stage- and sex-specific evolutionary responses of desiccation- and aging-selected populations and their matched controls in relation to adult desiccation tolerance. We demonstrate the importance of larval acquisition in the adult desiccation selection response. Desiccation-selected male and female flies exhibit differing patterns of resource acquisition and age-related desiccation resistance. Comparison of populations subjected to differing selection protocols suggests that even very simple selective regimes can have complex effects on the relations between life stages and can affect males and females in different ways.

MATERIALS AND METHODS

Selection Treatments

The 20 *D. melanogaster* populations employed in the present study belong to four different selection treatments designated B, O, C, and D. Figure 1 provides a schematic view of the phylogenetic relationships among these populations. The base population (IV) was maintained in the laboratory as a large, outbred stock on two-week discrete-generations with abundant banana-molasses food, 24:0 (L:D) schedule, at 25°C, and moderate densities for over 100 generations before selection for postponed senescence was initiated in 1980 (Rose 1984). Five early fertility (B) populations were maintained in the same manner as the ancestral IV population, and five populations (O_{1-5}) were selected for late-life fertility by steadily increasing the age of reproduction. Since 1982, the Os have been maintained on a 10-week (egg to egg) selection regime. Leroi et al. (1994) and Chippindale et al. (1994) provide long-term perspectives on this selection experiment.

In 1988, five lines selected for increased resistance to desiccation (D_{1-5}) and five control lines (C_{1-5}) were initiated from the O treatment, as described in Rose et al. (1990). Briefly, two weeks after egg collection, D populations were transfered from culture vials to sealed plexiglas population cages containing a bag (150–175 g) of Drierite™ desiccant, and desiccated until the initial population size of 6000–10,000 flies was reduced to 500–1000. An important facet of this experimental design was the construction of a proper control to the desiccation-selected lines. Because desiccation selection consists of at least two direct stresses, food and water deprivation, a true control for the effects of desiccation alone should be watered but not fed. The C treatment constitutes such a control. Controls were maintained in parallel to the selected populations, only they were given non-nutritional agar plates throughout the D selection period. When desiccation selection was stopped on one of the replicate D populations, starvation stress was also stopped on the same-
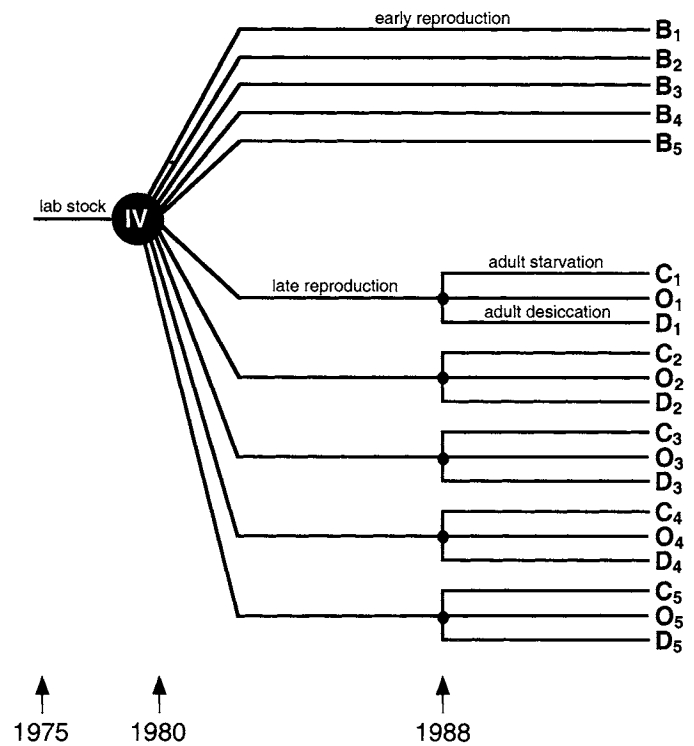

FIG. 1. Phylogeny of the 20 populations employed. B populations were selected for early reproduction whereas O populations were selected for late-life reproduction. The B and O selection treatments were founded en masse from the basal IV population, while the D and C populations of a given replicate number have the same-numbered O population as an ancestor. Each control C population experienced starvation for the same period of time as their matched desiccation (D) selected counterpart.

numbered C population. The Cs are therefore controls for both the demographic and starvation components of desiccation selection. The selection period has steadily increased, so that at the time of these experiments selection took approximately four days. The total generation time of the D and C populations was therefore about three weeks. Survivors of the selection stress were used to found the subsequent generation.

The early part of the life cycle was identical to the founder population (IV) in all populations used in this study. All stocks were kept at regulated densities of 50–150 eggs per vial, with abundant (> 5 ml) food for larval growth for the first two weeks. Thus, despite the substantial differences among the selection treatments in handling under selection, early aspects of their culture have been kept constant.

Preexperiment Standardization of Selection Treatments

Before experimental assays were performed, samples of 1–2000 flies per population were kept for two generations with identical rearing and reproductive schedules. Their usual selection regimes were relaxed to synchronize selection treatments and to remove residual nongenetic effects. The eggs for the experimental generation were derived over a two- or three-hour period from young adults that had been given food plates supplemented with live yeast, refreshed daily, for three

days prior to collection. Females under these circumstances are close to peak reproduction, reducing the incidence of egg retention and preoviposition embryogenesis (Neyfakh and Hartl 1994). For the experimental generation in all of the experiments described below, flies were reared in vials at exact densities of 60 eggs per 5 ml of food.

Desiccation Resistance and Adult Age

A large desiccation assay was performed at generation 102 of D and C selection, generation 61 of O selection, and (approximately) generation 310 of B selection. Flies were reared exactly as outlined above. When adults began emerging from puparium we transferred them every 6 h to temporary holding vials containing food. After the estimated peak eclosion period for a given population, the conditioning period was set up; four males and four females were sorted into each vial, provisioned with standard medium. A total of 80 such vials were set up for each population (1600 vials and 6400 flies in total). Adults were removed from conditioning after 12, 36, 60, and 84 ($\pm$ 3) h for desiccation measurements. We initiated our experiments with morphologically mature (12-hour-old) adults because of the dubious relevance of data for flies lacking a well-formed and tanned cuticle. Desiccation resistance was assayed with four same-sex flies per vial. Flies were sequestered in the closed end of a vial with a thin sponge stopper, desiccant was added, and the vial was sealed with parafilm. Indicator Drierite was used to ensure that the air inside of the test vials was dry. Twenty vials (40 flies of each sex) were used for each population at each adult age. Mortality was scored at 0100, 0400, 0700, 1000, 1300, 1600, 1900, and 2200 h. The D_2 population was lost in the first experiment, so we repeated measurements on the D_2 and C_2 populations two selection-generations later.

Body Weight and Water Content

Wet-weight measurements were made on flies reared in exactly the same fashion as the flies used to measure desiccation resistance, except that the weights were taken at 3 ($\pm$ 3) and 96 ($\pm$ 3) h posteclosion. To minimize water loss, the samples were sorted rapidly into microcentrifuge tubes and flash-frozen using dry ice. Flies were kept on dry ice until weighing less than 18 h later. Preliminary work indicated that water content was not significantly affected by this procedure. For each population, six groups of five flies for each sex were weighed on a Cahn microbalance. Dry weights were obtained after drying the flies overnight at 60°C. Water content was calculated as the difference between wet and dry weights. Thus, 60 flies from each population were weighed at each time interval (three and 96 h old), and in the entire experiment a total of 2400 flies were weighed.

Lipid Content

Lipid was measured for both three- and 96-hour-old samples of all populations using the methods of Chippindale et al. (1996). Whole-body lipid content was measured using ether extraction in a soxhlet apparatus. Flies were sexed, sorted into groups of 10, dried at 60°C for initial dry-weight measurement and then ether extracted for 24 h. Extracted

flies were then dried and reweighed to measure lean weight. The difference between initial and lean dry weights represents the whole-body lipid content. Six samples of each sex were run for each population; 4800 flies were extracted in total.

Carbohydrate Content

Carbohydrate was assayed using the anthrone method of Van Handel (1965), as modified by Djawdan et al. (1996). The samples were reared and handled in identical fashion to the lipid-extracted group described above. Groups of five same-sex flies were ground in distilled water and boiled for 3 min. Anthrone reagent was then added and the tubes were incubated at 90°C for 30 min. Carbohydrate content was estimated by comparison with standard curves prepared in each experimental assay.

Developmental Time and Viability

Development time and viability assays were conducted at generations 101 and 126 of D and C selection, generations 59 and 69 of O selection, and approximately generations 300 and 340 of B selection. Egg-to-adult development time and viability were measured at exact densities of 60 eggs per 8 dram shell vial, using the technique described in Chippindale et al. (1994). All 20 populations were assayed simultaneously. As in all of the experiments, selection treatments were interspersed by housing same-numbered replicates together in the same experimental rack. The racks were randomly distributed on the incubator shelves, and repositioned and rotated several times daily to minimize the effects of temperature and lighting gradients that may exist in incubators. Checks for emerging adults were made every six hours after pupal darkening, at 0100, 0700, 1300, and 1900 h. Checks were terminated when no adults had emerged in the assay for three days. Pupal counts were then performed on each vial. By using the proportional survival of eggs to pupae and pupae to adults, it was possible to divide preadult survivorship into embryonic/larval and pupal components.

RESULTS

Desiccation Resistance

Desiccation-selected flies resisted death by desiccation three to four times longer than their controls at 4 days, with the last female dying after 83 h in dry air. Regressions were computed for each population to establish the relationship between desiccation resistance and adult age. The five equations generated for each sex within selection treatment were used to compute mean regression equations (Fig. 2). There was a striking dichotomy between the males and females of the D selection treatment. Desiccation resistance in females was unaffected by age (average slope of -0.016 ($\pm$ 0.046) h desiccation/h of age; not significantly different from zero), whereas D males had a slope of -0.241 ($\pm$ 0.021) h desiccation/h age. Differences between the sexes in the D selection treatment may be the result of different selection pressures imposed on the sexes (see Discussion). Because strong interactions with both age and sex were expected, we chose to handle most analyses of adult traits with a series of one-
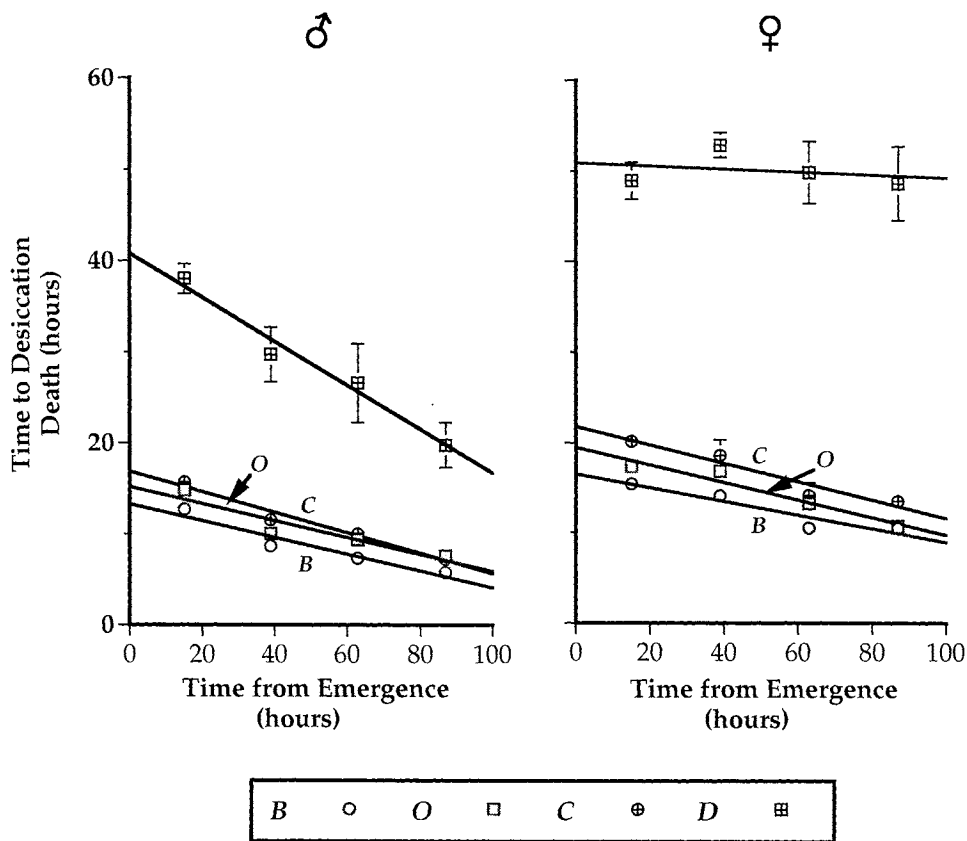
FIG. 2. Differentiation of adult desiccation resistance with age (posteclosion). Each point is the mean of the five replicate populations for a selection treatment (± SE). The lines represent the average least squares linear regression for the five replicates. Part A depicts the male pattern with age, part B the female. The slope, intercept, and bivariate means differed between the D selection treatment and all other selection treatments (B, O, and C) at $P < 0.001$ by the Student-Newmann-Keuls post hoc method, but there were no other significant differences between treatments. These results emphasize the strong differentiation of desiccation selected lines from matched controls and ancestors, as well as the differentiation of males and females within the D selection treatment.

factor analyses of variance to test for differences among selection treatments within each age class and sex. The Student-Newman-Keuls (SNK) post hoc test was used to distinguish differences between selection treatments.

Analysis of variance for male desiccation resistance detected significant variation for the slope ($F_{3,16} = 32.2$; $P < 0.0001$), the intercept ($F_{3,16} = 115.9$; $P < 0.0001$), and for the bivariate mean ($F_{3,16} = 46.1$; $P < 0.0001$). However, the only significant between-treatment differences occured between the D selection treatment and all other treatments. For females, ANOVA revealed no significant differences among selection treatments ($F_{3,16} = 2.6$; $P = 0.09$), but both the intercept ($F_{3,16} = 102.2$; $P < 0.0001$) and the bivariate mean ($F_{3,16} = 251.4$; $P < 0.0001$) displayed significant variation among selection treatments. In the case of the latter two characters, as in males, the only significant pairwise differences were between the D selection treatment and all others.

Significant differences between the B and O selection treatments for mean desiccation resistance were predicted from previous studies but were not apparent in the post hoc SNK analysis. When we conducted separate t-tests (unpaired, one-tail) on the bivariate mean data, we found that the O treatment had significantly higher desiccation resistance in both males ($t_8 = 3.9$; $P < 0.01$) and females ($t_8 = 2.7$; $P < 0.01$). The

differentiation of these selection treatments, however, was not as pronounced as in some other reports (e.g., Service et al. 1985; Djawdan et al. 1996, 1998).

Body Weight and Water Content

Selection treatment means for wet weight, dry weight, bulk water content, and proportional water content are presented in Figures 3 (males) and 4 (females). Table 1 presents results from ANOVAs conducted separately for each sex and age class. Highly significant differences among selection treatments were detected for all traits in all analyses. For all weight measures across age groups, the following rank-order progression was preserved: B < O < C < D. The demographically selected (B and O) treatments did not differ significantly in any weight or water comparison of newly eclosed flies. At 96 h of age, males and females of the O treatment were significantly heavier than B (SNK test; $P < 0.05$) in terms of dry mass, water mass, and wet mass, and contained more proportional water. The change with age reflects a faster posteclosion loss of resources by the early-reproduction (B) selection treatment compared to the late-reproduction (O) treatment.

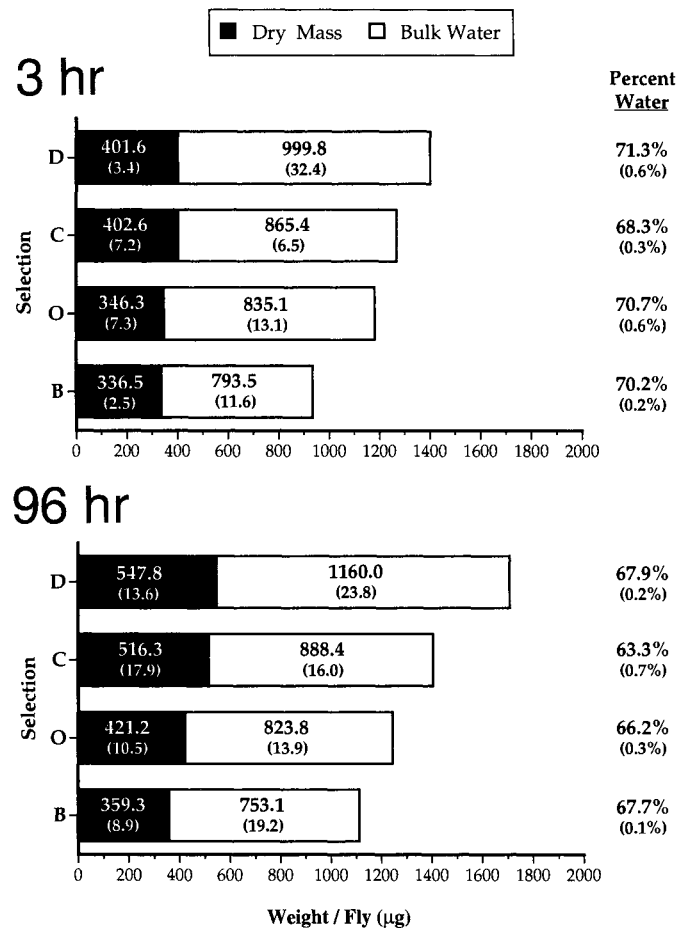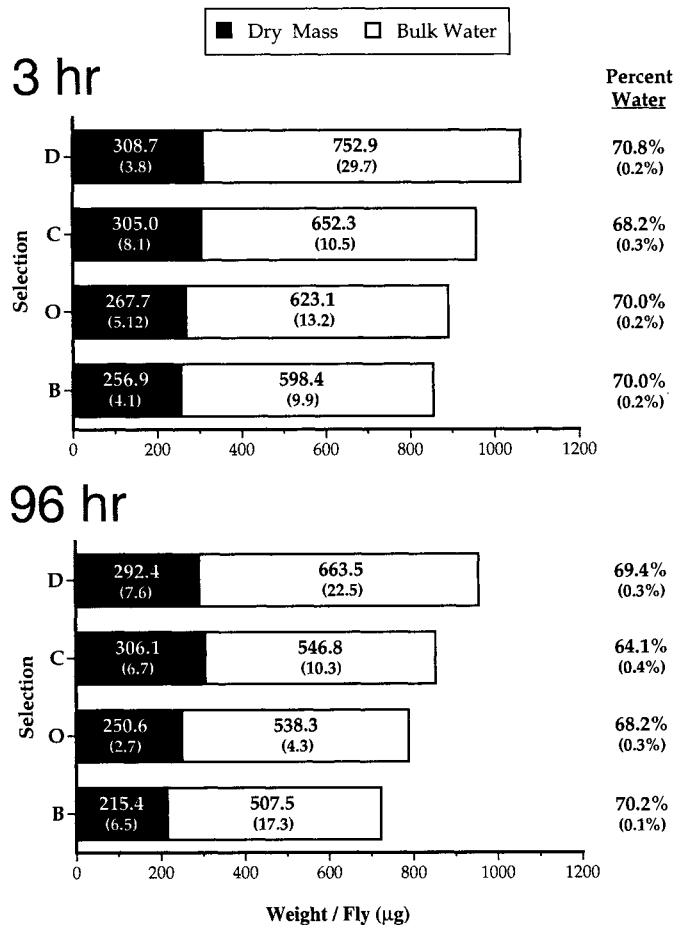Stress-selected (C and D) treatments were significantly

FIG. 3. Dry weight, bulk- and percentage-water content for males of the D, C, O, and B selection treatments at two adult ages, 3 and 96 h posteclosion. Each bar gives the mean of the five replicate populations within a selection treatment.

FIG. 4. Dry weight, bulk- and percentage-water content for females of the D, C, O, and B selection treatments at two adult ages, 3 and 96 h posteclosion. Each bar gives the mean of the five replicate populations within a selection treatment.

heavier than demographic selection treatments (B and O) in both wet and dry weights at both ages measured for all paired comparisons (SNK test; $P < 0.05$). The C and D selection treatments did not differ in dry weight in any comparison, however D had significantly higher water content in both absolute and proportional terms for both sexes at both ages (SNK test; $P < 0.05$).

Carbohydrate, Lipid, and Energy Content

The absolute and proportional quantities of carbohydrate and lipid, and the sum of these metabolic reserves (in caloric equivalents) are given in Table 2, with analysis presented in Table 3. Carbohydrate content increased with age in all selection treatments. On average, this gain was 11.7 μg. Car-

TABLE 1. Single-factor analysis of variance for weight traits: wet weight, dry weight, bulk water, and relative water content. (a) Newly eclosed males, (b) newly eclosed females, (c) mature males, and (d) mature females.

			Trait						
	Source of variation	df	Wet weight		Dry weight		Bulk water		% Water	
			MS	F	MS	F	MS	F	MS	F
(a) male; 3 h	Selection	3	41,133.13	17.57***	3415.08	22.18***	22,986.03	14.56***	6.3E-04	8.197***
	Residual	16	2340.54		153.99		1578.00		8.0E-05	
(b) female; 3 h	Selection	3	70,499.55	33.60***	6229.71	40.47***	39,833.83	23.05***	8.6E-04	8.37**
	Residual	16	2098.11		153.93		1728.00		1.0E-04	
(c) male; 96 h	Selection	3	49,150.66	22.92***	8493.19	44.60***	23,421.59	20.15***	3.7E-03	91.86***
	Residual	16	2144.63		190.42		1162.23		4.0E-05	
(d) female; 96 h	Selection	3	32,8851.39	73.09***	37,548.65	43.34***	158,266.05	91.42***	2.3E-03	30.85***
	Residual	16	4499.38		866.37		1731.11		7.0E-05	

** $P < 0.01$; *** $P < 0.001$.

TABLE 2. Metabolites stored in the fly at two ages: (a) 3 h, (b) 96 h. Carbohydrate and lipid weights are given in μg. Percent values are the ratio of metabolite weight to whole-body dry weight. Total calories are the sum of metabolites, in J. All values are per single fly.

| | | Trait | | | | | | | | | |
| | | Carbohydrate | | Percent carbohydrate | | Lipid | | Percent lipid | | Total metabolic energy | |
Selection	n	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
(a) 3 h											
B	5	10.00	8.48	3.86%	3.87%	55.45	68.38	21.52%	20.44%	2.36	2.84
SE		1.73	1.17	0.60%	0.60%	3.71	1.66	0.60%	0.60%	0.16	0.07
O	5	10.68	12.45	3.90%	5.07%	62.15	80.68	23.43%	23.12%	2.63	3.39
SE		1.17	2.85	0.50%	1.20%	2.48	4.16	0.50%	1.20%	0.10	0.13
C	5	11.56	14.44	3.89%	4.94%	79.15	99.84	26.54%	25.24%	3.32	4.18
SE		1.13	3.54	0.30%	1.10%	3.82	3.14	0.30%	1.10%	0.15	0.12
D	5	14.60	35.05	4.83%	11.46%	77.65	87.93	25.25%	22.47%	3.31	4.07
SE		2.09	15.11	0.60%	4.70%	2.31	2.88	0.60%	4.70%	0.10	0.37
(b) 96 h											
B	5	17.24	17.84	5.14%	5.29%	36.79	94.72	17.10%	26.39%	1.75	4.04
SE		2.28	3.11	0.70%	0.90%	1.66	10.84	0.70%	0.90%	0.09	0.40
O	5	14.48	24.24	4.16%	6.43%	53.67	110.70	21.82%	27.25%	2.37	4.78
SE		2.08	5.29	0.60%	1.30%	2.46	0.60	0.60%	1.30%	0.11	0.45
C	5	17.12	31.96	4.27%	6.76%	100.21	182.23	32.07%	35.41%	4.24	7.73
SE		2.26	7.83	0.60%	1.40%	3.85	0.06	0.60%	1.40%	0.17	0.44
D	5	22.12	57.88	5.51%	12.51%	62.23	121.90	20.34%	23.01%	2.84	5.81
SE		3.07	17.38	0.60%	3.10%	4.24	31.50	0.60%	3.10%	0.19	0.58

bohydrate scaled isometrically with body weight, as the proportional change was a modest 1.2% and an ANOVA (not shown) did not detect a significant age effect for percentage carbohydrate. Most conspicuous in Table 2 is that desiccation-selected females had very high levels of stored carbohydrate, emerging from puparium with two to four times the absolute amount of the other selection treatments and maintaining this advantage through the early adult period. Although D females increased by 22.83 μg in carbohydrate content (approximately twice the gain of the ancestral O treatment), D males gained only 7.5 μg of carbohydrate, an increase similar to the other selection treatments. For both ages, the proportional carbohydrate content of the D females was approximately double that of their controls or ancestors.

Analysis of variance (Table 3) indicated significant variation among selection treatments for both absolute and relative carbohydrate content in females but not males. In post hoc paired comparisons, females of the D selection treatment differed significantly from all other selection treatments ($P < 0.05$) at both ages, but none of the other selection treatments differed significantly from one another. Females generally carried more carbohydrate than males ($P < 0.001$; analysis not shown) and had higher relative levels (female − male = 2.5%; $P < 0.05$).

Lipid levels were pronouncedly differentiated by selection treatment, age, and sex (Table 2). Most notable are the high levels of lipid measured in the desiccation controls (C), the low levels found in early-reproduction (B) populations, and the unremarkable levels stored by the desiccation-selected (D) populations. Females increased in lipid storage with age in all populations, whereas males tended to decline. The B-selected males displayed the greatest drop in lipid, losing 18.7 μg (about one-third of their initial reserves) of lipid over the first four days of adulthood. The only selection treatment in which both females and males increased in lipid content with age was the starvation-selected C treatment. C-

TABLE 3. Multiple single-factor analyses of variance for physiological traits: carbohydrate, lipid, their relative values, and their sum in terms of energy content (J).

| | | | Trait | | | | | | | | | |
| | Source of variation | df | Carbohydrate | | Proportion carbohydrate | | Lipid | | Proportion lipid | | Total energy | |
			MS	F	MS	F	MS	F	MS	F	MS	F
(a) male; 3 h	Selection	3	20.60	1.65	1.00E-04	0.71	679.64	13.64***	2.46E-03	4.86*	1.18	13.67***
	Residual	16	12.47		1.40E-04		49.82		5.10E-04		0.09	
(b) female; 3 h	Selection	3	596.88	2.67†	4.90E-03	2.17	859.96	21.56***	1.92E-03	12.03***	1.89	11.77***
	Residual	14	222.51		2.26E-03		39.89		1.60E-04		0.16	
(c) male; 96 h	Selection	3	50.74	1.69	2.00E-04	1.04	3598.33	69.31***	2.08E-02	45.15***	5.62	53.44***
	Residual	16	30.07		1.90E-04		51.92		4.60E-04		0.11	
(d) female; 96 h	Selection	3	2152.38	5.50**	5.69E-03	4.11*	5846.69	10.39***	1.09E-02	6.93**	10.93	11.11***
	Residual	14	391.90		1.36E-03		562.88		1.58E-03		0.98	

† $0.05 < P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 4. Analysis of variance for developmental traits. Developmental time and viability were both measured from egg to adult. The entire 20-population experiment was repeated twice, a factor that we call "Block" in the present analysis; 2400 individuals were measured in total.

| | | Trait | | | | | |
| | | Male developmental time | | Female developmental time | | Egg-adult viability | |
Source of variation	df	MS	F	MS	F	MS	F
Selection	3	777.09	28.86***	915.16	35.06***	2.61E-03	4.37**
Block	1	22.07	0.82	60.89	2.33	1.00E-03	1.68
Selection × block	3	21.39	0.79	36.07	1.38	2.55E-03	4.28*
Residual	32	26.92		26.10		6.00E-04	

$* P < 0.05;$ $** P < 0.01;$ $*** P < 0.001.$

selected males increased by 21.1 μg in lipid content, adding approximately 25% to their initial stores.

Total available metabolic energy was estimated by converting lipid and carbohydrate weights into caloric equivalents using the formulae provided by Schmidt-Nielsen (1990,
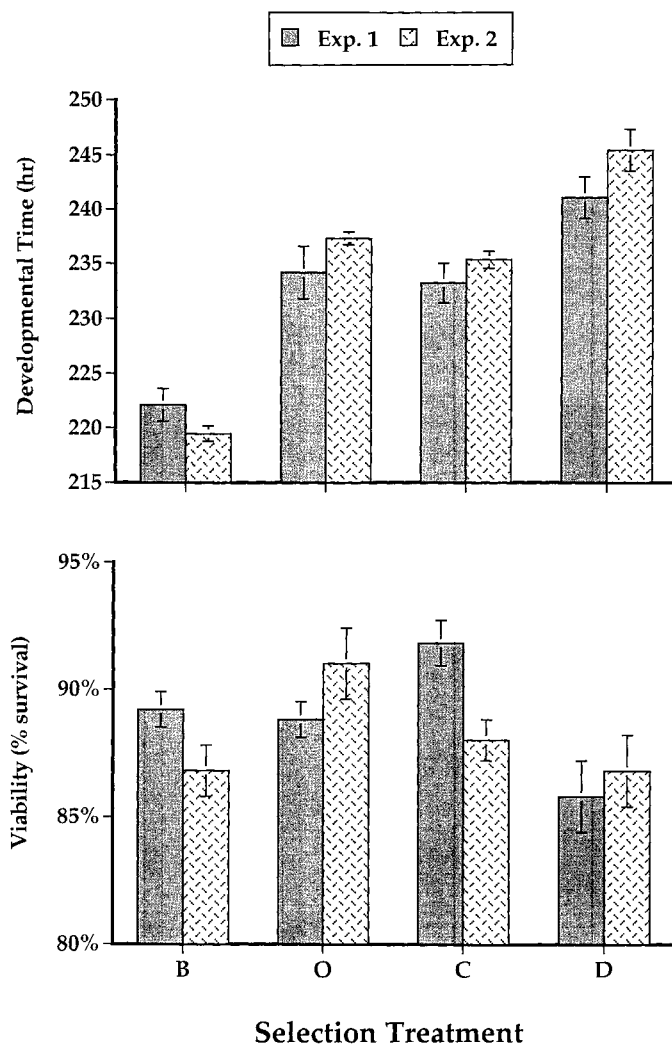

FIG. 5. Developmental time and egg-to-adult viability in the four selection treatments. As indicated by the legend, this experiment was repeated. Each bar gives the mean of the five replicate populations within a selection treatment (± SE). Development time is the male/female mean.

p. 175). Energy content was highly variable by selection treatment, age, and sex (Tables 2 and 3). Because lipid yields approximately twice the energy per unit weight that glycogen (carbohydrate) does, populations storing more fat tended to have correspondingly higher energy content. This point is illustrated by the comparison of the C and D selection treatments, which did not differ in dry weight, but did differ in body composition: Mature C females stored lipid:glycogen at a ratio of 5.7:1 by weight, whereas D-selected females had a ratio of 2.1:1. For females, most of the age-specific increase in energy content from three to 96 h was accounted for by the accumulation of lipid. Unlike females, males generally declined in stored energy content over the first four days of life. The exception among males was the C selection treatment, where males, on average, increased in total caloric content with age.

Developmental Time and Viability

An analysis of variance on the collected development data (development time and viability) is given in Table 4. Highly significant differences were detected among selection treatments in developmental time, which displayed the following order: B < C < O < D (Fig. 5). Post hoc pairwise comparison using the SNK test indicated that the B selection treatment was significantly faster developing than all other treatments ($P < 0.01$), and the D treatment developed more slowly than all other treatments ($P < 0.01$). Males were, on average, 2.4 h slower developing than females across all selection treatments.

Analysis of variance on the egg-to-adult viability data (Table 4) revealed a significant effect of selection. The selection treatments followed the following rank ordering: D < B < C < O (Fig. 5). The SNK test on these data showed that the D populations were, on average, significantly less viable than either the O or C populations ($P < 0.01$). Desiccation-selected populations had particularly high pupal mortality: When averaged across both experiments, the difference in pupal viability of 3.1% between the C and D equalled the overall egg-to-adult viability difference of 3.0%. This pupal survivorship difference was statistically significant by ANOVA (experiment and selection as factors; $F_{1,16} = 6.60$; $P < 0.05$), and there was no difference between experiments nor interaction between factors.

No other paired comparisons between selection treatments were statistically significant. The absence of a significant difference between the B and O selection treatments for vi-

ability was surprising, given the weight of evidence for their differentiation presented in earlier work (Chippindale et al. 1994; Shiotsugu et al. 1997). In the first experiment, the mean viability the O treatment was aberrently low, and that of the B treatment slightly higher than usual. The results of the second experiment were much more typical of these treatments, with the O selection treatment displaying significantly higher egg-to-adult viability than the B treatment upon separate analysis by t-test. As Figure 5 shows, the two experiments yielded uniform results for development time; the second experiment produced a grand mean for development time that was just 1.9 h slower than the grand mean of the first experiment.

DISCUSSION

Desiccation is a ubiquitous stress for terrestrial organisms. Small insects like *Drosophila* are particularly subject to dehydration stress because of their high surface area:volume ratio. Adaptation to dry conditions is therefore likely to be a key feature in determining the evolution and distribution of insects (e.g., Fairbanks and Burch 1970; Arlian and Eckstrand 1975; Hadley 1994). The rapid initial increase in desiccation resistance in the D populations (Rose et al. 1990) indicates the existence of substantial additive genetic variation for this trait in the founder (O) populations, consistent with high heritability estimates for desiccation resistance in *D. melanogaster* (Hoffman and Parsons 1989a) and *D. serrata* (Blows and Hoffmann 1993). After 102 generations of selection, our desiccation-selected populations were able to survive completely arid conditions several times longer than control populations (Fig. 2).

Previous work demonstrated substantial differences in desiccation tolerance among populations selected for adult age at reproduction (B and O flies). In addition, desiccation-selected flies exhibited greater longevity than their controls (Rose et al. 1992), despite the lack of demographic selection. These results indicated a genetic correlation of components of stress resistance with adult life-history traits. Several important questions arise from these findings. First, what specific physiological mechanisms are involved in generating the spectrum of desiccation resistance times we have observed? Second, what are the age- and sex-specific dynamics of stress resistance evolution? Third, what are the life-history consequences of increasing resistance to desiccation?

Physiology of Desiccation Resistance

Resistance to desiccation can be increased in three ways: flies can carry more water (in the form of bulk or metabolic water), they can restrict water loss, or they can be more tolerant of the loss of a given amount of water. We have previously demonstrated that only the first two mechanisms contribute significantly to higher resistance in young (four-day-old) flies from the desiccation-selected populations (Gibbs et al. 1997). The current study specifically addresses the issue of water acquisition: In what form (bulk or metabolic) and during what stage is water accumulated?

Water comprises 65–70% of the total mass of *Drosophila*, and the D flies contain significantly more bulk water than any other population. Figure 6 shows the positive relationship between bulk water content and desiccation resistance across populations, sexes, and ages, which suggests that increased water storage is an important mechanism for increased desiccation resistance. The physiological significance of water storage is supported by the fact that only desiccation selected females gain water as adults and maintain the same ability to resist desiccation as they mature. All other treatment groups studied exhibit a posteclosion decrease in water content and desiccation resistance.

Metabolism of either glycogen or lipid results in the formation of metabolic water, with glycogen providing ~18% more water for a given metabolic rate (Schmidt-Nielsen 1990, p. 333). Under desiccating conditions, selected flies preferentially metabolize carbohydrates, rather than lipids (Djawdan et al. 1996). Although these differences suggest that glycogen accumulation in the D populations is associated with metabolic water production (Graves et al. 1992), three factors indicate otherwise. First, flies from all populations in this study gained carbohydrate in the first four days posteclosion, yet all groups except the D females declined in desiccation resistance (Fig. 2). Second, the amount of metabolic water available was not sufficient to significantly increase desiccation resistance. Four-day-old desiccation-selected females contained 25.9 mg of carbohydrate more than control females. Using previously measured rates of water loss (Gibbs et al. 1997) and standard conversion factors, the extra metabolic water available could support the flies for only 1.25 h of desiccation stress. Third, flies selected for resistance to starvation store approximately twice the energy than either the C or D populations (Djawdan et al. 1998). However, despite this large store of potential metabolic water, starvation-selected populations are not notably desiccation-resistant (Djawdan et al. 1998). We conclude that metabolic water production from glycogen is not an important factor in resistance to desiccation. Flies must have enough energy stored to survive the simultaneous, mild starvation selection, but the value of glycogen as an energetic source during desiccation stress is more likely to result from its ability to bind three to five times its weight in bulk water (Schmidt-Nielsen 1990, p. 173). This water would be released when glycogen is metabolized, and would account for seven to eight times more available water than metabolic water alone (Gibbs et al. 1997). The importance of glycogen in the evolution of desiccation resistance appears to result from its role as a "sponge," binding some of the additional bulk water stored by desiccation selected flies, with metabolic water production as a secondary, minor factor.

Differences in glycogen storage may also contribute to increased desiccation resistance in the later-reproduced O populations relative to their B controls. For reasons not well understood, selection for postponed senescence in *Drosophila* results in increased storage of both lipid and glycogen (Service 1987; Graves et al. 1992; Djawdan et al. 1996). If one assumes that these populations lose water at the same rate as the C flies (44 mg/h; Gibbs et al. 1997), and that glycogen binds 4 g H_2O/g, then we calculate that glycogen-bound water may account for half of the difference in desiccation resistance between four-day-old B and O flies. Water conservation also contributes to increased desiccation resistance, because
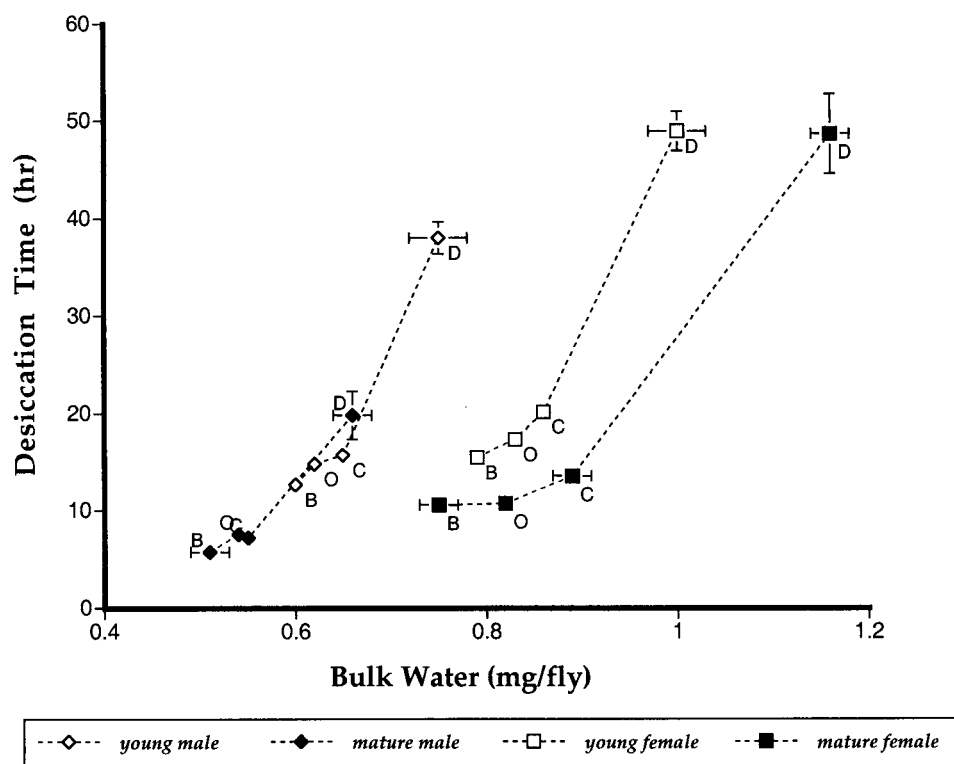
FIG. 6. Desiccation survival as a function of bulk water, with age classes and sexes shown separately. We found that bulk water was the dominant character influencing stress resistance.

O flies lose water less rapidly than their controls (Graves et al. 1992).

The pattern observed in all treatments not directly selected for resistance to desiccation was a decline in resistance posteclosion. Service et al. (1985) documented a similar loss in desiccation resistance with adult age for demographically selected populations between five and 33 days of adult life, and similar changes have been observed in other strains as well (e.g., Clark and Doane 1983). These changes in desiccation resistance were reflected by changes in stored water levels. Newly eclosed D flies contained significantly greater amounts of bulk water than did other selection treatments, and D females were also the only group that gained significant quantities of water in the adult phase. In contrast to bulk water, glycogen levels increased with adult age in all treatment groups, for both sexes. This indicates that age-related decreases in desiccation resistance are not due to the loss of glycogen to support metabolism or provide metabolic water.

Sex Differences in Desiccation Resistance and Resource Management

One might expect a strong genetic correlation between the sexes as a result of the shared autosomal genotype, which seems to be reflected in the similar age-related patterns of desiccation resistance and resource accumulation in flies that were not selected for desiccation resistance. However, male and female flies from the desiccation-selected populations exhibited qualitatively different patterns in several characters. For example, the desiccation resistance of D males declined rapidly with age (significantly more rapidly even than their controls) but D females did not change in desiccation resistance with age.

We believe the origin of this sexual dimorphism is the selection treatment itself, which exerted differing selective pressures on males and females. In unselected populations, female flies are more resistant to desiccation than males, so that 90% culling like that imposed during desiccation selection would eliminate nearly all males long before the cessation of selection. Between-sex differences were apparent in the Ds ancestral populations, the O flies, in studies predating the establishment of the D selection lines in 1988 (Service et al. 1985). Based on these observations and recent studies in extant D males, it appears that essentially all D males died during desiccation selection over their evolutionary history (Chippindale, Ngo, and Brar, unpubl.). Because male D flies do not survive selection, and mating is not observed during selection (AKC., unpubl. obs.), mating must occur before selection. We believe that males are not selected for desiccation resistance at all, but are strongly selected for early reproduction. Depletion of resources associated with courtship and mating could account for the rapid decrease in desiccation resistance in D males.

Evidence from the C flies provides additional insight into sex-specific selective forces in these populations. Only in the control lines did males gain lipid reserves posteclosion. Early in desiccation selection, control flies would have been subjected to relatively mild starvation selection, which both males and females could survive. As the desiccation resistance of the desiccation-selected flies increased, both the control males and females would have been selected for star-

vation resistance. In the extant C populations, large numbers of male flies are consistently observed after selection. We conclude that the C populations have experienced coordinate evolution of both sexes, whereas in the desiccation-selected flies there has been an intersexual antagonism resulting from different requirements for male and female fitness.

Life-History Trade-offs within and between Major Stages

Development of aging- (O) and stress-selected (C and D) populations was slower than that of early-reproduction (B) populations. The difference between the B and O selection treatments has been attributed to selection for B flies to grow faster, and mature and mate earlier, at the cost of reduced viability (Chippindale et al. 1994). As in the case of strong starvation selection described by Chippindale et al. (1996), here stress selection led to higher growth rates: D-selected lines grew 12% faster and C-selected lines 16% faster than the ancestral (O) selection treatment (net egg-to-adult growth, based on dry weight). However, only the strong selection imposed on desiccation-selected populations resulted in reduced preadult viability relative to other selection treatments. Desiccation selection also led to significantly slower egg-to-adult development. Extended development may allow for increased juvenile resource acquisition, as development time often exhibits a positive genetic correlation with body size (Zwaan et al. 1995; Nunney 1996). A combination of accelerated larval growth and extended development led to D and C selected flies emerged from pupa 15% heavier than their ancestors, storing 65% and 41% more metabolic reserve substances, respectively.

Life-history stages may characteristically exhibit physiological and genetic trade-offs that render their evolution intertwined. Like the case of abnormal abdomen in wild D. mercatorum (Templeton and Johnston 1988), development and larval resource acquisition in these populations figure prominently in the performance of the adult under environmental stress. Life-history traits such as developmental time, growth rate, and preadult viability, although governed by their own set of trade-offs, are also intimately bound up in the selection response of the mature imago. Our results indicate that the forces molding the fitness of the desiccation-selected populations and their controls were quite different, with control populations experiencing coordinated evolution of the sexes, whereas desiccation-selected populations experienced disruptive selection. The intersexual antagonism described in the D populations may be considered one in which sexual selection on males opposes natural selection on females. The necessity for matings to occur before the selection bout is likely to have engendered strong selection for early maturation and mating in males, whereas females would be under selection to acquire more resources to resist stress. A trade-off between preadult growth, on the one hand, and adult feeding and early fertility, on the other, is likely. The antagonism may be reinforced if selection for increased resource storage by females is counterbalanced by selection for greater weight economy in males, whose mating success depends upon speed and precise choreography during courtship displays. Directional selection on sexually dimorphic populations may often lead to disruptive selection on the sexes

toward divergent fitness optima, imposing constraints on the overall adaptive response (Lande 1987).

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