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Experimental tests of minimum viable population size

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Abstract

Fitness and rates of extinction were compared among populations of the housefly, *Musca domestica* L., kept either at constant effective sizes of 50, 500 or 1500 or passed through extreme founder events reducing effective size to 5. Populations were maintained for 24 generations, which for small to medium-sized mammals would be less than the 200 years suggested by Soulé *et al*. (1986) as necessary for maintaining viable populations of endangered species. The results demonstrate that effective population sizes have to be greater than the 50 individuals suggested by Franklin (1980) to retain fitness and escape extinction, even in the short term. In contrast to populations of constant size that exhibited monotonic decreases in fitness through time, populations established with few founders rebounded from initial inbreeding depression. However, they were less adaptable to environmental stress than constant size populations, suggesting that populations founded with few numbers may do well within a single environment but may do far less well if they are reintroduced to natural environments or exposed to rapid environmental changes.

INTRODUCTION

The concept of a minimum size for viable populations, originally articulated by Schaffer (1981), has received considerable attention in conservation biology. Genetic and evolutionary considerations lead to two predictions concerning minimum population sizes for survival. To avoid inbreeding depression in the short term, Franklin (1980) proposed that effective population size should be not less than 50, resulting in a theoretical minimum rate of inbreeding of 1% per generation. This rate of inbreeding has been deemed tolerable for many domestic animal species kept under relatively benign environmental conditions (Franklin, 1980) and, indeed, slow rates of inbreeding in some cases have resulted in less inbreeding depression than more rapid inbreeding (Tantawy, 1957; Latter & Robertson, 1962; Ehiobu, Goddard & Taylor, 1989; Barrett & Charlesworth, 1991)

The maintenance of adequate genetic variation for longer-term evolutionary response depends upon a balance between input of variation by new mutations and loss of variation through selection and drift. Based upon typical levels of mutational variance (Lynch, 1988*a*) and selection against new mutations (Simmons & Crow, 1977), an effective population size of 500 would be sufficient to maintain permanent genetic variance for a typical quantitative trait (Franklin, 1980; Lande & Barrowclough, 1987). Taken together, minimum population sizes for both short- and long-term survival have resulted in the so-called 50/500 rule, which has been widely implemented as a management goal for a large number of endangered and threatened species (Lande & Barrowclough, 1987; Wiese *et al*., 1993; Foose *et al*., 1995).

These population sizes are likely to underestimate the minimum viable population size for survival for several reasons. First, while inbreeding depression is known to affect most endangered species (Charlesworth & Charlesworth, 1987; Ralls, Ballou & Templeton, 1988), it is usually measured within a single and often benign environment. It is well known that inbreeding depression can be significantly exacerbated in stressful environments (Parsons, 1971; Schemske, 1983; Dudash, 1990; Holtsford & Ellstrand, 1991; Wolfe, 1993; Jimenez *et al*., 1994; Pray *et al*., 1994; Latter, Mulley *et al*., 1995), so that the potentially damaging effects of inbreeding may not be manifested completely within any given environment, particularly a favourable one. Second, the mutation rate to potentially adaptive alleles may be an order of magnitude less than the total mutation rate, so the minimum viable population size necessary to strike a balance between mutation, selection and drift may be considerably larger than 500 and possibly as high as 5000 (Lande, 1995; Lynch, 1996; Lynch & Lande, 1998). Third, deleterious mutations may

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accumulate within small populations to cause loss of fitness and eventual extinction (Lynch & Gabriel, 1990; Charlesworth, Morgan & Charlesworth, 1993; Lande, 1994; Lynch, Conery & Burger, 1995*a*,*b*). While short-term population decline is expected to be minimal for populations with effective sizes greater than 100 or so (Lande, 1994), many endangered species are kept under optimal conditions that minimize selection. As a result, accumulated effects of mutations can be rapidly accelerated, leading to losses in competitive ability of 1–2% per generation (Shabalina, Yampolsky & Kondrashov, 1997; Bryant & Reed, 1999). Any detrimental effects might not be evident within a benign captive environment, but these captive populations may not prosper in more natural regimes (Kondrashov & Houle, 1994; Shabalina *et al*., 1997; Bryant & Reed 1999).

It is also difficult to predict population extinction from current population fitness. It is widely accepted that inbreeding depression in small populations, for example, decreases fitness and increases the risk of extinction (Soulé, 1980; Ralls *et al*., 1988; Miller & Hedrick, 1993; Thornhill, 1993; Frankham, 1995; Falconer & Mackay, 1996). Indeed, the rate of extinction within small populations appears to be greater than large populations, for both experimental and natural populations (Pimm, Jones & Diamond, 1988; Stacy & Taper, 1992; Latter, Mulley *et al*., 1995; Frankham, 1996; Newman & Pilson, 1997). Nevertheless, if extinction bears a non-linear relationship to level of inbreeding, as suggested by Frankham (1995), it may be particularly difficult to predict, based on current levels of inbreeding and/or genetic variation, which populations are under risk of extinction. Moreover, it is often difficult to separate genetic from environmental causes of extinction (Pimm *et al*., 1988; Schoener & Spiller, 1992), so there are few documented cases where inbreeding depression has directly led to extinction in wild populations (e.g. Saccheri *et al*., 1998). This has led some authors to question any important link between genetic variation, inbreeding depression and population extinction (Harcourt, 1991; Young, 1991; Shields, 1993; Caughley, 1994).

Most of the work on establishing minimum viable population size has been theoretical, and there is a need for additional empirical evidence to parallel these theoretical predictions and establish clearer relationships between population size, fitness and extinction. While empirical tests of long-term effects are beyond the scope of most experiments, the relationship of population size to short-term population health is directly amenable to experimental verification. The purpose of this study was to develop an empirical assessment of fitness and extinction in relation to population size in experimental populations of the housefly, *Musca domestica* L. Specifically, we compared life histories among populations derived from a large outbred population of this species and kept at constant effective population sizes of 50, 500 or 1500, respectively, for a total of 24 generations. In addition, some managed populations of endangered species have been initiated with few founders (Foose, 1990; Hedrick & Miller, 1992). To investigate the effect of small founder size separately from maintenance size, we also included experimental populations that were founded with few individuals and allowed to recover to large size. Finally, we compared the fitness of experimental populations in benign as well as stressful environments, to extrapolate our results to more natural conditions. For mammals weighing $\leq$ 330 kg, 24 generations translates into $\leq$ 200 years (Millar & Zammuto, 1986), and thus our experiment directly addresses the minimum of 200 years suggested by Soulé *et al*. (1986) necessary for the management of endangered species.

METHODS

A sample of 141 female houseflies (and an excess of males to ensure mating by all females) were captured by sweep net from a landfill site near Houston, TX. Eggs were collected from these wild-caught flies to establish a control population, that was expanded to over 4000 flies and maintained at this level for the duration of the experiment. After five generations to allow for initial adaptation to the laboratory environment, treatment lines were initiated from this control population (generation 0 of the experiment). (1) Three lines, designated LARGE, were initiated and maintained at each generation using a random sample of eggs to produce approximately 1300 adults. Population size was monitored each generation by obtaining a mean weight per fly (based on a random sample of 100 adult flies) and dividing this into the total biomass of flies produced each generation, which yielded a harmonic mean population size of 1267 individuals over the experiment. (2) Six lines, designated SMALL, were initiated and maintained using a random sample of eggs sufficient to produce approximately 130 adults. Each generation individuals were counted to determine the exact number of adults, yielding a harmonic mean population size of 126 individuals. (3) Six lines, designated FOUNDER-FLUSH, were founded by mating two males each to two separate females (yielding an effective bottleneck size of five), and then allowing the population to grow to a large size of approximately 2500 individuals (in approximately seven generations). During the growth phase after the bottleneck each female was limited to four offspring per generation, to better mimic the slower population growth rates of higher organisms (Bryant, Backus *et al*., 1999). As with the other treatment lines, after the initial flush the FOUNDER-FLUSH lines were monitored by computing total biomass per line per generation, yielding a harmonic mean population size over the duration of the experiment of 76 individuals. The larger number of lines tested for the SMALL and FOUNDER-FLUSH treatments than for the LARGE treatment reflected the greater variance in fitness expected among these smaller lines (Lynch, 1988*b*). All lines were maintained at standard densities and 27 °C.

An effective population size to census population size ratio (N_e/N) of 0.38 was assumed for all populations, based on previous electrophoretic variation among

experimental lines in our laboratory (McCommas & Bryant, 1990; Bryant, Backus *et al*., 1999), so that approximate effective population sizes for the treatments were 50 (SMALL), 500 (LARGE) and 1500 (CONTROL). Effective population size for the FOUNDER-FLUSH lines was determined from the harmonic mean of the number of flies during the 24 generations of the experiment, to yield an approximate effective population size of 30 individuals.

Life-history traits were assayed at experimental generation 0 for the CONTROL and at generations 6, 12 and 24 for all populations. Adults from all lines were collected from a minimum of three larval cultures set up with a standard density of 80 eggs/18 g CSMA® larval medium (Purina, St. Louis, MO), representing an optimal density for larval survival (Bryant, 1969). Upon emergence, virgin male–female pairs were isolated in single-pair cages consisting of an 8 oz plastic cup (with air holes) inverted over a petri dish. The number of pairs/line were 30 (LARGE), 20 (SMALL, FOUNDER-FLUSH) and 60 (CONTROL), totaling 90, 120 and 120 pairs for the LARGE, SMALL and FOUNDER-FLUSH treatments, respectively. All pairs were supplied daily with diluted evaporated milk and CSMA® larval medium. Individual egg clutches were counted and placed into culture bottles with 0.225 g CSMA® larval medium/egg, reflecting the optimal larval density of 80 eggs/18 g medium. Emerging adults were counted to assess larval viability/male–female pair. Thus, we obtained a daily record of deaths, fecundity (total eggs laid) and larval survivorship; the latter was used to obtain a measure of total production of adult progeny/pair. Progeny production/female is a combination of total eggs laid/female and their subsequent viability, but it is not simply a product of fecundity and viability, due to possible correlation between the two measures across individual pairs and across lines within treatments.

Eggs from population cages were collected 4–5 days after adults reached sexual maturity, and then populations were discarded, resulting in a 21 day discrete generation length. Thus, selection on late-life fitness (>21 days) was relaxed or eliminated in these populations, allowing any late-acting age-specific deleterious mutations to accumulate freely and much faster than when under selection (Crow & Kimura, 1979; Kimura, 1983). As a result, when the entire life-history profile is analyzed late-life fitness traits erode much more rapidly than early-life traits (Bryant & Reed, 1999). To avoid accelerated erosion of late-acting fitness due to the experimental protocol *per se*, we compared life history fitness measures among treatments for early life only (≤ 21 days). In any event, overall fitness depends to a large extent on early contributions and to a far lesser extent on late-life contributions (Charlesworth, 1994), so our use of fitness components for early-life only should not seriously bias results.

At generation 28, larval viability (percentage of emerging adults) was tested simultaneously for all 16 lines in three environments: a standard larval environment consisting of CSMA® larval medium supplemented with yeast and a larval rearing temperature of 27 °C (**ST**), a nutrient stress environment in which the standard yeast supplement was omitted from the CSMA® medium (**D**), and a standard CSMA® media environment in which temperatures fluctuated between 8 °C and 32 °C on a diurnal 12:12 cycle (**T**). Five replicate cultures for each line and environment were set up for the SMALL and FOUNDER-FLUSH treatments, 10 for the LARGE treatment and 25 for the CONTROL, totaling 345 cultures across the three environments.

To determine times to extinction, all lines were continued under the same protocol beyond the 28 generations of the experiment, for a total of 68 generations, at which time five out of the six SMALL lines had gone extinct. None of the lines from the other treatments went extinct during this extended time.

Results were analyzed using analysis of variance procedures on untransformed data, since the data was approximately normal, using the Kolmogorov–Smirnov test (Sokal & Rohlf, 1995). The same lines were tested over generations or across environments, and thus we employed a repeated measures hierarchical (split-plot) analysis of variance (Winer, 1962; Lindeman, 1974).

RESULTS

There was significant variation among individual lines within treatments for all three measures (Table 1, Figs 1–3). Nevertheless, the among-line variances were small relative to other effects, constituting only 5% of the total variance across the three measures, and there were no significant line-by-generation interactions for any measure. As a result, line variation within treatments did not obscure the highly significant effects among treatments and generations (Table 1). In addition to significant main effects for treatment and generation, the relative position among the treatments differed between generations, resulting in a significant treatment-by-generation interaction. The results are clarified below by applying the Tukey–Kramer procedure (Sokal & Rohlf, 1995) to individual generations.

At the first test (generation 6), there were no significant differences among the constant population size treatments for any fitness measure. However, in subsequent tests the SMALL lines were significantly lower in all three fitness measures than either the CONTROL or

Table 1. Analyses of variance for fitness components

		Mean squares		
Source of variation†	d.f.	Fecundity	Viability	Progeny
Treatments	3	163 882***	2.42***	140 379***
Generations	2	10 297ns	0.42*	16 981*
Treat. × Gen.	6	21 470***	0.37**	12 015**
Lines (Treat.)	12	7254*	0.36***	9577***
Lines × Gen. (Treat.)	24	3102ns	0.10ns	3053ns
Error	342	3625	0.11	2424

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$.
†In the mixed model split-plot analysis of variance, Treatments were tested over lines (Treat.); Generations and Treat.× Gen. were tested over Lines × Gen. (Treat.); Lines (Treat.) and Lines × Gen. (Treat.) were tested over Error.

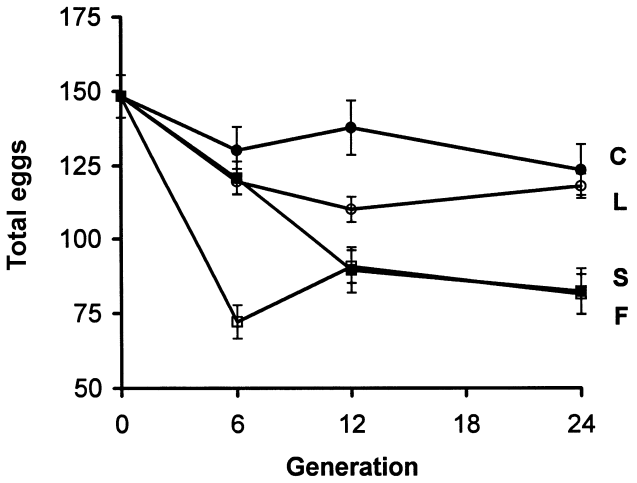

Fig. 1. Total eggs laid (fecundity)/female for the four treatments over the 24 generations of the experiment for the CONTROL (C), LARGE (L), SMALL (S) and FOUNDER-FLUSH (F) lines. Standard errors of means are indicated by vertical bars.

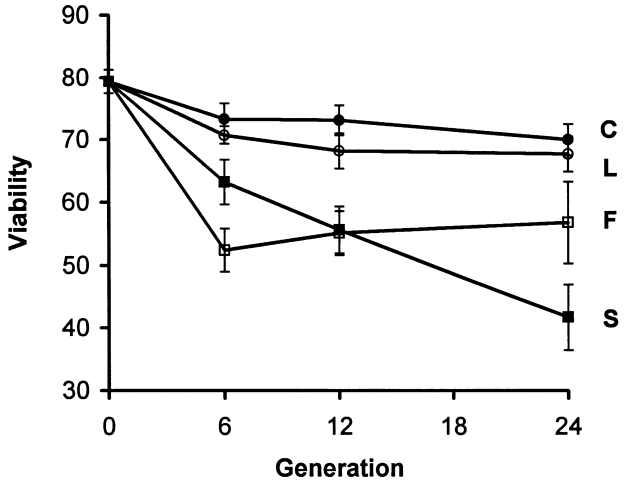

Fig. 2. Percentage larval viability for the four treatments over the 24 generations of the experiment for the CONTROL (C), LARGE (L), SMALL (S) and FOUNDER-FLUSH (F) lines. Standard errors of means are indicated by vertical bars.

the LARGE lines; the CONTROL and LARGE lines were not significantly different from each other for the duration of the experiment (Figs 1–3).

The responses of the FOUNDER-FLUSH lines were somewhat different. At the first test, the FOUNDER-FLUSH lines were significantly lower in all three fitness measures from the constant population size treatments, including the SMALL lines, reflecting the effects of the initial bottleneck (Figs 1–3). By the second test (experimental generation 12), the FOUNDER-FLUSH lines had rebounded, so they were no longer significantly lower in any fitness measure than the SMALL lines (Table 1, Figs 1–3). The rebound in fitness was most pronounced for viability, where by generation 24 the FOUNDER-FLUSH lines were intermediate between the LARGE and the SMALL lines, and were not significantly different from either (Fig. 2). None of the six FOUNDER-FLUSH lines went extinct, so the rebound in fitness for these lines was not due to differential extinction of less fit lines.

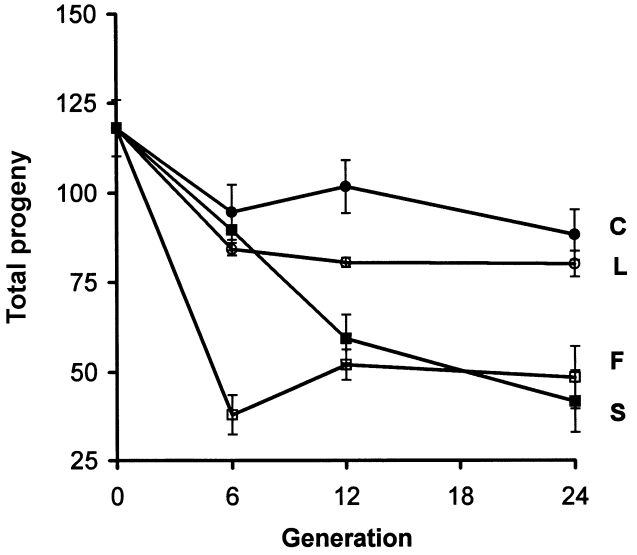

Fig. 3. Progeny production/female for the four treatments over the 24 generations of the experiment for the CONTROL (C) LARGE (L), SMALL (S) and FOUNDER-FLUSH (F) lines. Standard errors of means are indicated by vertical bars.

In order to link individual population fitness to extinction, linear regressions of fitness onto generation were calculated separately for each line within the three constant population size treatments. Even though five generations were allowed for laboratory adaptation before initiating the experiment, there appeared to be a curvilinear loss in fitness across the generations (Figs 1–3), owing to a more rapid loss in fitness from generations 0–6 than for generations 6–24, which, for the CONTROL and LARGE lines would more likely be due to continued laboratory adaptation and not to inbreeding effects. As a result, regressions were calculated for each line and fitness component over generations 6–24 only. Each line then represented an independent estimate of fitness loss over these generations, yielding 2 and 5 degrees of freedom for the LARGE and SMALL treatments, respectively. From these regressions, the times when each fitness component would reach zero (extinction) were extrapolated (Table 2). Standard errors of mean regression slopes/treatment were derived from observed variances among regression coefficients for individual lines, and these were used to obtain bounds (standard errors) around the expected time to extinction.

The loss in fitness was approximately 1.7% per generation for the SMALL lines, resulting in expected extinction within 39–65 generations (Table 2). In contrast, the fitness loss for the CONTROL and LARGE lines of 0.3% per generation was significantly lower than that for the SMALL lines for all three measures ($P < 0.05$), resulting in expected minimum survival of these populations for at least 200 generations. No extinction of lines occurred over the 24 generations for any

Table 2. Linear regressions of fitness onto generation and estimation of extinction time (in generations)

Treatment†	Fitness component	Regression	Extinction (in generations)
N_e = 1500	Fecundity	137.3–0.48g	286
	Viability	74.8–0.19g	394
	Progeny	101.4–0.47g	216
N_e = 500	Fecundity	116.0–0.18g	644
	Viability	70.9–0.15g	473
	Progeny	84.4–0.18g	469
N_e = 50	Fecundity	124.3–1.92g	65
	Viability	70.3–1.20g	58
	Progeny	80.1–2.07g	39

† CONTROL (N_e = 1500); LARGE (N_e = 500); SMALL (N_e = 50).

treatment; however, extinctions of the SMALL lines occurred at generations 37, 39, 44, 51 and 64, yielding a median extinction time of 47.5 generations, which compared well with the extinction time of 54 generations predicted from the regressions.

At generation 28, viability for all lines was tested simultaneously in the standard rearing environment and two stressful environments (Fig. 4). Both population size and environment had highly significant effects on viability (Table 3), with all treatments doing less well in stressful environments. As for generation 24, viability in the standard environment at generation 28 was nearly identical for the CONTROL and the LARGE lines and both were significantly greater than the SMALL lines; the FOUNDER-FLUSH lines were intermediate in viability and not significantly from any of the other treatments using the Tukey–Kramer procedure. However, in the stressful environments the FOUNDER-FLUSH lines were not significantly different from the SMALL lines and both were significantly lower in viability than the CONTROL or LARGE lines, resulting in a significant environment-by-treatment interaction (Table 3). Hence, while the FOUNDER-FLUSH lines showed some rebound in fitness from an initial low fitness after the bottleneck, this was specific to the standard larval rearing environment; in the stressful environments they were indistinguishable from the SMALL lines maintained at a constant effective size of 50.

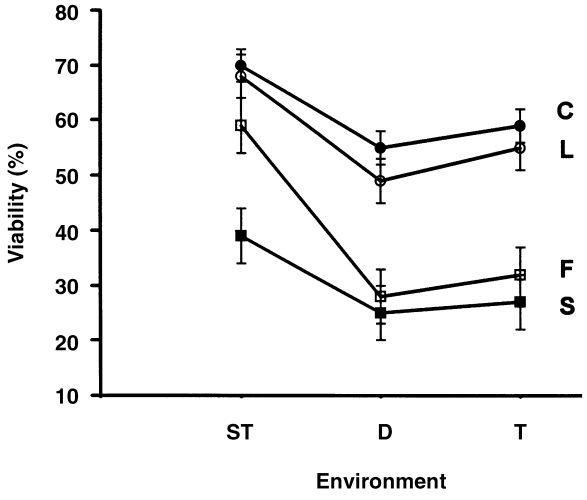

Fig. 4. Larval viability in the standard (**ST**), impoverished diet (**D**) and the thermally variable (**T**) environments for the CONTROL (C), LARGE (L), SMALL (S) and FOUNDER-FLUSH lines. Standard errors of means are indicated by vertical bars.

DISCUSSION

The results of the experiment clearly show that population fitness is closely linked to maintenance population size; within six generations SMALL populations maintained with an effective population size of 50 individuals were significantly lower in all three fitness measures than either populations maintained at effective sizes of 500 (LARGE) or 1500 (CONTROL) and remained so for the 24 generations of the experiment. The total progeny production/female in these SMALL lines was approximately 50% of that for the CONTROL or the LARGE lines. Hence, if the maintenance of fitness is the primary goal of conservation management, an effective population size considerably larger than 50 would be required.

The loss in fitness also decreased the longevity of these SMALL lines, with five out of the six lines going extinct by generation 64, yielding a median extinction time of 47.5 generations. This agreed quite well with the extinction time of 54 generations predicted by linear regressions of fitness up to generation 24, suggesting that extinction times in Table 2 may be reasonable, despite being based on linear extrapolation. In addition to the lines in this experiment, another experiment was being carried out in our laboratory in which populations, derived from a base population taken from the same landfill collection site, were being maintained at constant sizes of 40 (five lines, N_e = 15.1) and 200 (five lines, N_e = 87.1) individuals for 24 generations (Bryant, Backus *et al*., 1999). For these lines, average regression coefficients of viability onto generation were –1.71 and –0.86, resulting in extrapolated extinction times of 32 and 80 generations, respectively. As in the present experiment, standard errors of extrapolated extinction times were derived from standard errors of regression coefficients based on among-line variances within treatments. Taking the two experiments together, the predicted extinction times based on larval viability show

Table 3. Analysis of variance for egg-to-adult viability across the three environments

Source of variation†	d.f.	Mean square
Treatment	3	1.801***
Environment	2	1.160***
Treat. × Env.	6	0.060*
Lines (Treat.)	12	0.213***
Line × Env. (Treat.)	24	0.017*
Error	297	0.010

*, P<0.05; **, P<0.01; ***, P<0.025.
† In the mixed model split-plot analysis of variance Treatment is tested over Lines (Treat.); Environment and Treat. × Env. are tested over Line × Env. (Treat.); Lines (Treat.) and Line × Env. (Treat.) are tested over Error.

that extinction times remained low up to an effective population size of 90 and then increased rapidly thereafter (Fig. 5). Hence, survival of these populations was only insured when effective population sizes were larger than 100 and nearly twice as large as that suggested by Franklin (1980) for short-term survival of endangered species. Because there were no experimental populations between 90 and 500, we cannot be assured of the true inflection point when extinction time became attenuated. However, time to extinction scaled largely with effective population size, with populations of effective sizes of 50 and 500 yielding extinction times of approximately 50 and 500 generations, respectively. Approximately 50% of captive populations of endangered species consist of less than 50 individuals (Magin *et al*., 1994) and wild populations typically number 100–1000 individuals when listed as endangered (Wilcove, McMillan & Winston, 1993). If ratios of N_e/N are as low as 10% (Frankham, 1996; Vecutich, White & Nunney, 1997), identification and management of endangered species occurs at too small a population size to ensure successful propagation, even for the short term. Moreover, to retain evolutionary flexibility, populations may have to be of the order of several thousand individuals and far greater than population sizes of most threatened and endangered species (Lande, 1995; Franklin & Frankham, 1998; Lynch & Lande, 1998).

The five SMALL populations that eventually went extinct at generations 37, 39, 44, 51 and 64 showed variation in progeny production/female by generation 24, with 5.1, 17.3, 25.6, 38.7 and 41.9 progeny produced/female, respectively. The correlation between progeny production at generation 24 for these five populations and the eventual generation when extinction occurred was 0.90 ($P < 0.05$). The remaining SMALL population had the highest progeny production at generation 24 (49.6 progeny/female), but even this was only 60% of the progeny production/female for the CONTROL and LARGE lines at generation 24, so that its decline was also clearly evident. Although Frankham (1995) suggested that extinction might be a threshold effect, whereby inbreeding depression would not be easily detected before extinction was imminent, fitness components, particularly total progeny production, was a clear indicator of a failing population in this experiment long before extinction occurred. It is possible that such populations could be identified by declining fitness components and rescue attempts made before extinction.

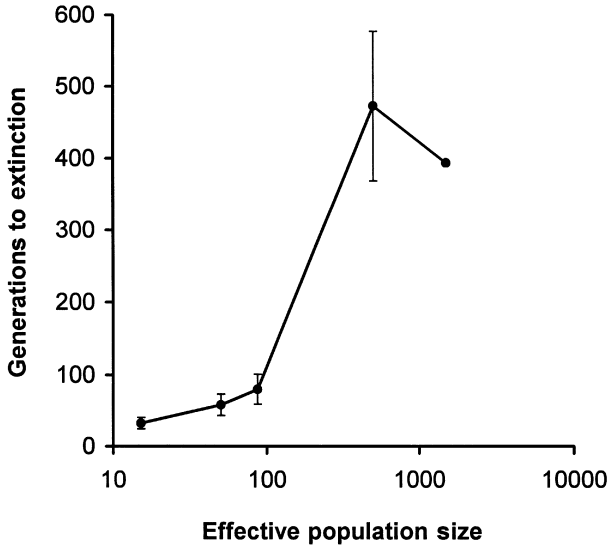

Fig. 5. Estimated generations to extinction for populations of increasing effective sizes, estimated from the regression of viability onto generation for the three constant population size treatments of this experiment (N_e = 50, 500 and 1500), combined with the results of Bryant, Backus *et al*. (1999) for N_e = 15 and 87. Standard errors of extinction times were derived from standard errors of regression coefficients among lines within treatments over generations 6–24.

The FOUNDER-FLUSH lines exhibited some recovery in fitness after the initial bottleneck, particularly for larval viability (Fig. 2); the SMALL lines continued to decline in fitness through the experiment. In addition, the FOUNDER-FLUSH lines also exhibited no extinctions up to 68 generations after recovery, despite a continuing low effective population size near that for the SMALL population. We found a similar recovery in fitness after a population bottleneck in earlier experiments on the housefly (Bryant *et al*., 1990), suggesting that inbreeding effects occurring immediately after a bottleneck can be purged (Hedrick, 1994). However, the FOUNDER-FLUSH lines did worse under both dietary and thermal stress than lines from the other treatments, so their fitness was reduced to the level of that for the SMALL lines in both stressful environments. While a bottlenecked population may adapt well to a particular environment, its *adaptability* may be low. The lack of adaptability in bottlenecked populations may far outweigh any immediate benefit of bottlenecks due to purging.

Fluctuations in population size and even bottlenecks are probably common in many natural populations. A review of the literature by Young (1994) documented 96 severe die-offs in wild populations of large mammals, primarily due to starvation and disease. Species of large body size and long life-spans suffer less severe fluctuations in population size than do smaller short-lived organisms (Williamson, 1972; Pimm, 1991), so it is not surprising that bottlenecks have been reported for many of these smaller organisms, including amphibians (Pounds & Crump, 1994), birds (Stout & Cornwell, 1976; Keller *et al*., 1994), fish (Hedrick & Hedgecock, 1994), insects (Wolda, 1992; Seidl & Opler, 1994; Brookes *et al*., 1997) and a small mammal (Jimenez *et al*., 1994). If such bottlenecks restrict adaptability, as in the housefly, many species, despite currently high population levels, may be compromised in their ability to cope with environmental stress, leading to increased rates of extinction as environments change.

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