indicated by the regression coefficient, remained similar between years (one-way ANCOVA: year × density, $F = 0.251$, d.f. $= 1$, $P = 0.63$) as was the rate of overwinter survival when the effects of density were removed (one-way ANCOVA: $F = 0.032$, d.f. $= 1$, $P = 0.86$) (Fig. 3b). Therefore, overwinter survival maintained a constant relationship with density regardless of the changes in winter conditions that operated on weaning rate in a density-independent manner.

Though populations rarely, if ever, maintain or achieve an equilibrium density because of fluctuations in population growth induced by stochastic events[3,10], this does not imply that density-dependence is unimportant or does not exist. Processes that operate in a density-dependent manner return a population towards an equilibrium density and are regulating[10]. Experimental density manipulations are the strongest and most robust approach to teasing apart the relative roles of density-dependent and density-independent determination of population density[9,11,12]. Our study demonstrates the power of this approach by showing how different density-dependent processes can operate to sequentially regulate population density in the presence of density-independent processes. Despite environmental differences between years that altered reproduction in a density-independent manner, density-dependent declines in reproduction remained constant between years and thus served as a dampening force against stochastic changes in reproduction. However, density-dependent weaning rate had a more limited role in regulating arctic ground squirrel populations than did a second and sequential density-dependent process, overwinter survival. During our study, density-dependent overwinter survival dominated density-independent changes in weaning rate resulting in strong density-dependent rates of population change—a necessary condition of population regulation[2]. □

Methods

Spring and summer mark–recapture population estimates of arctic ground squirrels were conducted on areas of 8–10 ha from spring 1996 to spring 1998 within each of four control areas and within the following four former experimental areas of the Kluane project (1987–1996) described in detail elsewhere[18,20]: two food-addition areas (36 ha), a predator-exclusion area (1 km²), and a food-addition area (36 ha) enclosed within a predator-exclusion area (1 km²). All fences were dismantled and food addition was discontinued in spring 1996 with the exception of two 2.5-ha areas (Food A, B) within the former predator-exclusion area with food-addition site where we continued weekly food supplementation by hand to all burrow sites during 1996; 20 kg of rabbit food per ha. Food B was not supplemented after 1996 but was monitored as ground squirrel densities on that site converged with those on the other sites. Population estimates and their standard errors were calculated using a closed population mark–recapture heterogeneity model (jack-knife) from program CAPTURE[28]. Weaning success was calculated as the proportion of all females that had litters appearing above ground. Litter size was determined by intensive live-trapping at the natal burrow at the time when young appeared above ground. Dispersal and summer survival was based on radiotelemetry of adult females. Overwinter survival was calculated from trapping records as the proportion of individuals captured in spring that were present in the population before hibernation in the previous year. The details of our procedures for weaning success, litter size, dispersal and survival are described elsewhere[20,22].

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Variation in the reversibility of evolution

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How reversible is adaptive evolution[1–6]? Studies of microbes give mixed answers to this question[6–11]. Reverse evolution has been little studied in sexual populations[12–14], even though the population genetics of sexual populations may be quite different. In the present study, 25 diverged replicated populations of *Drosophila melanogaster* are returned to a common ancestral environment for 50 generations. Here we show that reverse evolution back to the ancestral state occurs, but is not universal, instead depending on previous evolutionary history and the character studied. Hybrid populations showed no greater tendency to undergo successful reverse evolution, suggesting that insufficient genetic variation was not the factor limiting reverse evolution. Adaptive reverse evolution is a contingent process which occurs with only 50 generations of sexual reproduction.

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Over the past 20 years, we have created an artificial evolutionary radiation by imposing demographic and stress selection on large, outbred, replicated populations of *Drosophila melanogaster*. Part of this radiation is shown in Fig. 1. The five different groups of replicated populations are multifold differentiated from each other for several life-history, physiological and biochemical characters[15–21]. One of these groups has been maintained under the ancestral 'Ives' conditions since 1980 (the 'B' populations). One has been selected for reproduction late in life (the 'O' populations). One has been selected for survival under conditions of complete starvation (the 'SO' populations). One has been selected for reproduction in mid-life (the 'CO' populations). Finally, one group has been selected for reproduction very early in life (the 'ACO' populations) (see Fig. 1 legend and Methods). Most of the differentiation occurred within 50 generations of the start of selection.

At the start of the present study, a new population was derived from each of these 25 populations and then returned to the ancestral-Ives environmental conditions for 50 generations. The evolutionary response of these populations was assayed using eight characters which are related to fitness or were highly differentiated from the ancestral condition. By measuring these characters approximately every eight generations we were able to determine reverse evolutionary patterns and level of convergence.

The patterns of reverse evolution varied among evolutionary histories and among characters. Analysis of the evolutionary trajectories indicated that, except for fecundity, populations with different evolutionary histories had statistically heterogeneous responses to the reimposition of the ancestral environment (analysis of covariance, ANCOVA *F*-tests for evolutionary history and interaction term; $P < 0.05$). Results are shown in Fig. 2. Four kinds of evolutionary trajectories were observed. The first was reversion to ancestral character values with full convergence after little more than 20 generations. This was the case, for example, for IO and ISO female developmental time ('I' indicates a derived population; see Methods). The second kind of trajectory was a pattern of response without convergence within the 50 generations of the study, as in the case of IACO developmental time. The third kind of trajectory was
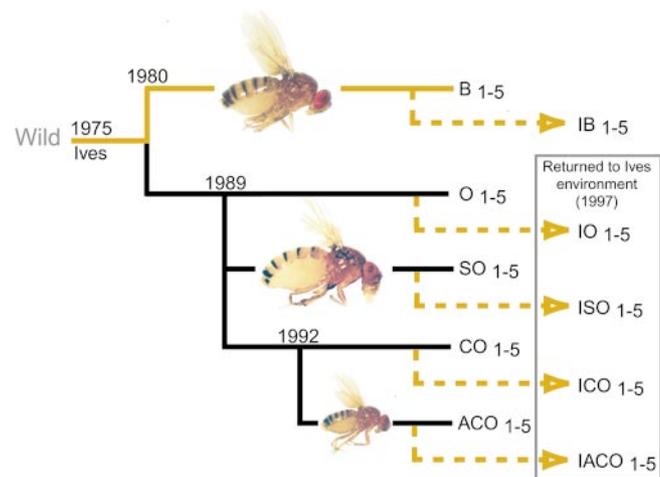
initial rapid reversion, followed by a stalling of evolution, without full convergence to ancestral character values, as for ISO starvation resistance or ICO developmental time. In this case, new stable states may have been attained. Finally, the fourth pattern was no significant change throughout the 50 generations of evolution, the best examples being ICO and IACO fecundity under high-density assay conditions.

The results show that reverse evolution back to an ancestral condition may or may not occur, at least over a 50-generation period of selection. Reverse evolution is neither inevitable nor impossible; it is contingent. This raises the question of the genetic mechanisms responsible for this contingency. Could populations that fail to return to the ancestral state lack genetic variation? Alternatively, could these populations be stuck in the vicinity of
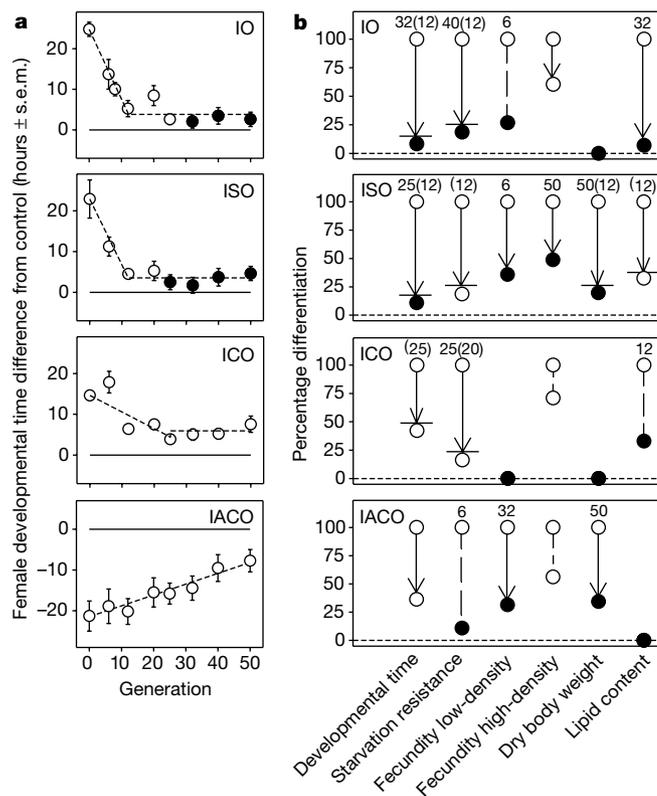
Figure 2 Reverse evolution during 50 generations. **a**, Response to selection in populations of different evolutionary history for female developmental time as a function of time (generations). Open circles show that the average difference from the control levels of the five replicate populations is significant (*t*-tests; $P < 0.05$). Black circles show non-significant differences from the controls, indicating convergence to ancestral levels. Dashed lines represent the best-fit regression models (IO, $P < 0.001$, adjusted $R^2 = 0.92$; ISO, $P < 0.001$, adjusted $R^2 = 0.95$; ICO, $P = 0.008$, adjusted $R^2 = 0.68$; IACO, $P < 0.001$, adjusted $R^2 = 0.93$). **b**, The categorized *x*-axis shows the response of each character separately, and the *y*-axis represents the percentage of differentiation relative to the control populations. Open circles indicate non-convergence to the control levels ($P < 0.05$), and black circles indicate convergence, as before. For all characters in all groups of populations, 100% differentiation was the initial starting condition, with the exception of a few characters which were undifferentiated from the beginning, these being represented by a single black circle at the zero differentiation line. Arrows between circles represent significant one-phase models, and arrow-lines indicate that a significant model with a first linear phase of convergence followed by a second no-response phase was the best fit to the evolutionary trajectory (see Methods). Broken lines show that no significant model was found, that is, that no significant change occurred during evolution. Numbers above the character for each group of populations show the generation of convergence to the control levels, and numbers in parentheses show the generation at which reverse evolution stalled. Reverse evolution patterns differ according to the character considered and according to selection history.

Figure 1 Laboratory radiation due to five selection treatments. Each group of populations was fivefold replicated (shown as subscript numbers). All populations descend from the Ives population, sampled from nature in 1975 (see Methods). We show female flies of the B, SO, and ACO treatments. These were cultured in identical conditions and are shown at the same relative scale. The direct descendants employed in our reverse evolution study are shown by dashed branches. Populations represented by yellow branches were maintained in the ancestral-Ives environment. Hybrid populations were derived either from crosses within or between different selection histories. A total of 44 populations were returned *de novo* to the ancestral-Ives environment and followed for fifty generations. Eight highly differentiated characters were measured during reverse evolution.

selective equilibria at which epistasis and linkage disequilibrium prevent their return to an earlier evolutionary state at which higher fitness would be attained? Both of these hypotheses can be tested by creating hybrids of the differentiated populations before reverse evolution. Hybrids should have more genetic variation (if the uncrossed experimental populations have become genetically impoverished and they should have undergone perturbation away from a strong evolutionary attractor (if that is their initial condition) through drastic gene frequency changes, subsequently coupled with recombination. If these genetic mechanisms are responsible for constraints on reverse evolution then we expect that hybrids should show more reverse evolution in the same 50 generations.

Nineteen hybrid populations were derived (see Methods). These underwent reverse evolution for 50 generations in parallel with the uncrossed derivatives already discussed. The appropriate test for improved convergence of the hybrids compared to the uncrossed must take into account the quantitative differentiation from the ancestral state in the two types of population. Many of the hybrids are less differentiated from the ancestral condition at the outset of reverse evolution, because of their hybridization. Therefore, we examined whether the hybrids and the uncrossed populations fall on the same regression of evolutionary rate on initial differentiation. Figure 3 shows this relationship among the 44 populations. There is no detectable difference between hybrid and uncrossed populations in their reverse evolution, once initial differentiation is allowed for. This indicates that the factors limiting reverse evolution are probably not related to a lack of genetic variation, epistasis, or linkage disequilibrium.

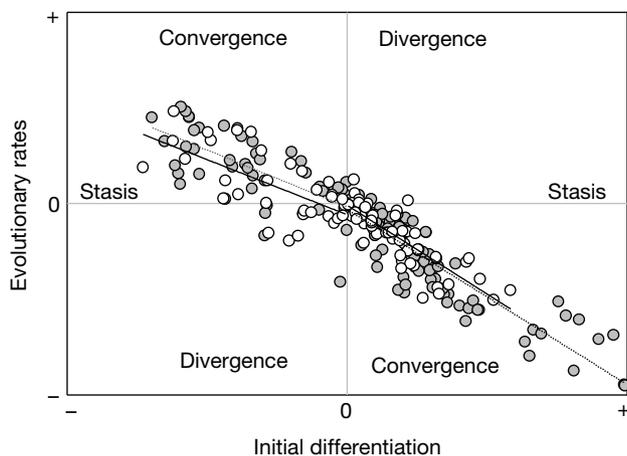To account for these results we propose instead that the return to

Figure 3 Reverse evolutionary rates as a function of differentiation at the start of the study. For each character and population, we estimated both the slope and the intercept of a one-phase linear regression, which were then used as the data points plotted, standardized to a common scale. Standardization involved dividing the estimated slopes and intercepts for each character by the largest slope or intercept for all data from that character, respectively. Data from fecundity under low adult density conditions were not used in this analysis. Uncrossed populations are shown by grey circles and hybrids by open circles. Out of 238 independent data points 209 fall in the two convergence quadrants, while only 29 fall in the two divergence quadrants. Assuming that stasis occurs when the evolutionary rate is less than 1% per generation, only 6 points fall in this class. The probability of chance occurrence of this pattern is much lower than 0.001 (sign test for convergence versus divergence). Hybrid and uncrossed evolutionary rate comparisons by ANCOVA (see Methods) did not reveal significant differences, when all characters were analysed either together or separately. Hybrids are not more likely to converge than uncrossed populations. For a significance level $\alpha = 0.05$, the statistical power to detect a difference between these groups, as estimated from the ANCOVA model, was larger than 95%, when global analysis was undertaken. Similar dependences of reverse evolutionary rates on initial differentiation are observed in the hybrid (solid lines) and uncrossed populations (dotted lines), in both global and separate linear regressions.

the ancestral-Ives environment did not produce uniform selection pressures among populations of different evolutionary histories. This can be attributed to variation in the relationship of each character with fitness in the ancestral environment according to the specific genetic background of each experimental population. Selection in the environments imposed before reverse evolution could have created different genetic architectures among groups of populations, in turn producing different genetic relationships between these characters and fitness in the ancestral environment. For example, populations which take longer to develop than the controls respond faster to natural selection on developmental time than populations which began by taking less time to develop. Thus, in the former populations, developmental time is intimately connected with fitness in the ancestral environment, whereas in the latter, the relationship with fitness is weaker in the ancestral environment (see Fig. 2).

Despite the cases in which reverse evolution fails to occur, or is stalled, our study shows an overwhelming tendency to evolutionary reversal during 50 generations of selection for adaptation to the ancestral environment. This demonstrates the power of selection in sexual populations to achieve adaptation[14,22–25]. History still plays a significant role in the observed evolutionary dynamics and outcomes[3,4,26,27], although this history is only a few hundred generations in duration. □

Methods

Experimental populations

We studied four different evolutionary histories, fivefold replicated, all derived from a common ancestor (Ives). These independent evolutionary groups included populations with 95 generations of selection history for increased late-life fertility (the O_{1-5})[15], with 100 generations of selection for increased starvation resistance (the SO_{1-5})[17], with 4–5-week intermediate generation times (the CO_{1-5})[17], and 190 generations of selection for decreased developmental time (the ACO_{1-5})[21]. Derived populations were designated by 'I' followed by the acronym for their evolutionary history. The five populations which had always been maintained in the ancestral-Ives conditions served as the control populations: IB_{1-5} (Fig. 1). These have been maintained for approximately 440 generations[15]. At the start of the experiment, hybrids within each selection treatment were created by five-way crosses of all five replicate populations. One of these five-way crosses used the B populations, which served as our control hybrid populations after replicating the cross five times. Hybrids were also created by crosses among all possible combinations of different selection treatments. Hybrid and uncrossed populations were followed in parallel during the 50 generations of reverse evolution. The ancestral-Ives environmental conditions include discrete two-week generations in vials, with egg and adult density controlled, and egg laying taking place during about two hours after mixing all adults of the same population. The amount of nutrients, temperature, humidity and light schedule were controlled. Census population sizes were always above 1,000 individuals per replicate population.

Assay designs

Eight characters were analysed in this study, most measured under conditions close to those of the ancestral-Ives environment. Developmental time in both genders was measured as the time from egg to adult emergence[21]. A total of 600 eggs per population were measured in each assay. Starvation resistance in both males and females was mesured as the time until death under starvation in high humidity conditions[17]. A total of 40 flies per gender and per population were measured in each assay. Early fecundity under high adult density conditions was measured as the number of eggs laid by 20 pairs of flies per vial during one hour. Sample sizes for thsi character were eight vials per population per assay. Early fecundity under low adult density conditions was measured as the number of eggs laid by a pair of flies per vial during 24 hours, the sample size being 40 such vials per population. Female dry body weight was calculated from the weight of 8 samples of 10 flies each per population, to the nearest 0.001 mg. Female whole-body lipid content was measured as the difference in dry weight before and after lipid extraction with a Soxhlet petroleum ether extractor.

Statistical analysis

All the starting populations employed in this study have been maintained independently of one another throughout their history and as such each one is an evolutionary experiment that is treated as a unique data point in our statistical analysis. Among the uncrossed populations, our experimental design allows a separation of genetic sampling effects from the main selection treatment effects. The IB_{1-5} are the control populations used to test the hypothesis of convergence to the ancestral character states. In each assay generation, each group of populations was independently compared for differences from the control populations by one-tailed unpaired Student t-tests. The evolutionary trajectories of the uncrossed populations were analysed with two general least-squares regression models to explain the response to selection: a one-phase linear model, with a constant

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rate of response throughout the experiment, or a two-phase linear model, with a first phase of response to selection followed by a plateau. The generation at which the second phase started was empirically determined[28]. Adjusted R^2, PRESS, Ellner and Turchin V_2, and Mallows C_p were the statistics used to choose the best regression model[29,30]. Heterogeneity among evolutionary trajectories was analysed using ANCOVA. These models used each separate character as the dependent variable, with generation defined as the covariate and evolutionary histories as a design factor with four categories: IO, ISO, ICO and IACO. Comparison of the rates of response under reverse evolution between uncrossed and hybrid populations were done by ANCOVA. In this model, the dependent variable was the slope estimated from one-phase linear regression models (using the response during reverse evolution) and the covariate was the estimated intercept of the regression model. Hybrid condition defined the factor in the ANCOVA, with uncrossed and hybrid categories. Power analyses were performed using the hybrid and uncrossed estimated least-squares means and standard deviations to estimate the power of t-tests for mean differences[30].

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Integration of target and body-part information in the premotor cortex when planning action

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To plan an action, we must first select an object to act on and the body part (or parts) to use to accomplish our intention. To plan the motor task of reaching, we specify both the target to reach for and the arm to use. In the process of planning and preparing a motor task, information about the motor target and the arm to use must be integrated before a motor program can be formulated to generate the appropriate limb movement. One of the structures in the brain that is probably involved in integrating these two sets of information is the premotor area in the cerebral cortex of
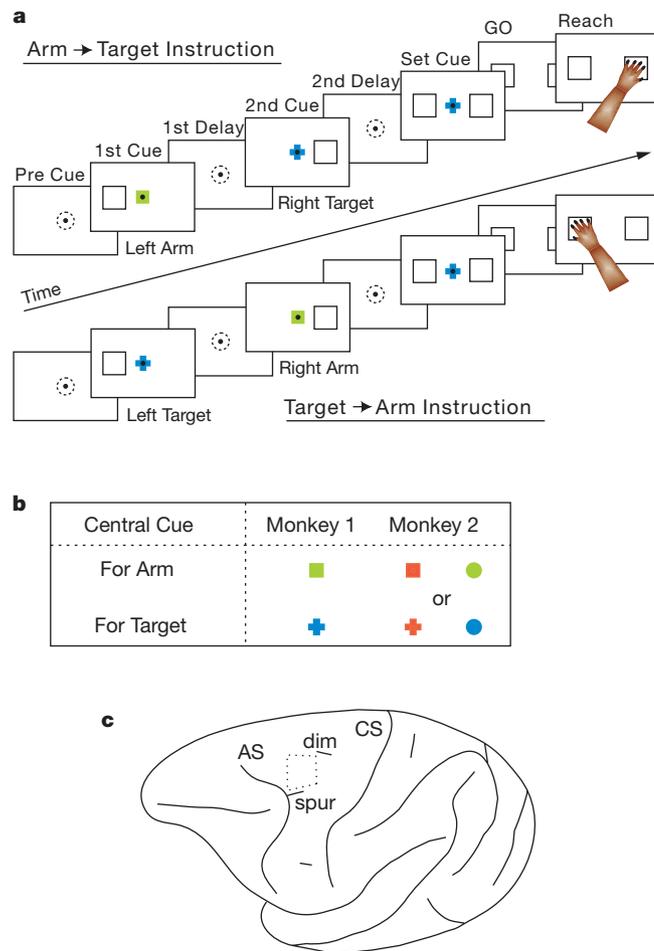

Figure 1 Experimental design. **a**, Temporal sequence of the behavioural events. Top row, a trial in which the two instructions were given in the order 'arm' then 'target'. Bottom row, a trial in which the two instructions were given in the order 'target' then 'arm'. The relative size of the fixation window is shown by the dotted circle. **b**, Central cues for monkeys 1 and 2. **c**, Cortical map of the recording sites. This work refers to the activity of neurons inside the dotted rectangle (PMd). AS, arcuate sulcus; CS, central sulcus; dim, superior precentral dimple; spur, spur of AS.