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Effects of constant immigration on the dynamics and persistence of stable and unstable *Drosophila* populations

Snigdhadip Dey* & Amitabh Joshi

Evolutionary Biology Laboratory, Evolutionary and Organismal Biology Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur P.O., Bangalore 560 064, India.

Constant immigration can stabilize population size fluctuations but its effects on extinction remain unexplored. We show that constant immigration significantly reduced extinction in fruitfly populations with relatively stable or unstable dynamics. In unstable populations with oscillations of amplitude around 1.5 times the mean population size, persistence and constancy were unrelated. Low immigration enhanced persistence without affecting constancy whereas high immigration increased constancy without enhancing persistence. In relatively stable populations with erratic fluctuations of amplitude close to the mean population size, both low and high immigration enhanced persistence. In these populations, the amplitude of fluctuations relative to mean population size went down due to immigration, and their dynamics were altered to low-period cycles. The effects of immigration on the population size distribution and intrinsic dynamics of stable versus unstable populations differed considerably, suggesting that the mechanisms by which immigration reduced extinction risk depended on underlying dynamics in complex ways.

With an increasing number of species declining in abundance and population sizes, there is considerable interest in understanding the factors affecting the propensity of small populations to go extinct, especially the interplay between dynamics and persistence, in part because of conservation concerns[1,2]. However, greater persistence due to reduced extinction propensity is only one of two major senses in which the term stability is used in population ecology, the other being constancy, which refers to a reduced degree of generation-to-generation change in population size[3,4]. Historically, constancy has been at the centre of most discussions of stability in population ecology, especially in the decades following May's[5,6] demonstration that simple one-dimensional population growth models can show a range of dynamic behaviour from stable points to limit cycles to chaos. As a result, most theoretical work tended to focus on factors and perturbations that alter constancy by affecting the dynamics of population size [reviewed in 7]. In particular, much theoretical effort was devoted to identifying factors and perturbations that might ameliorate chaos in isolated populations[8–11] as well as in metapopulations[12–15]. Yet, for many population ecologists, persistence has been a more pressing issue than constancy per se[1,2,16–18]. Given an intuitive expectation that constancy and persistence are correlated[19–23], ecologists focusing on stabilizing populations have often been more interested in reducing the magnitude of population size fluctuations, rather than stabilizing the dynamics from chaos to limit cycles[16,23–26]. More recently, there has been an increase in studies trying to understand the dynamics of extinction per se [reviewed in[27]], and also a realization that constancy and persistence are not necessarily correlated[28].

It is, therefore, of some interest to examine whether easy-to-implement perturbations that are predicted to enhance constancy in populations with unstable dynamics will also enhance persistence. Moderate levels of constant immigration have been shown to ameliorate chaos over a wide range of parameter values in simple population growth models[8,11,29–31]. Global stabilization of spatially structured populations (metapopulations) by the repeated immigration of individuals into a subset of local subpopulations (pinning) has also been observed theoretically[13,14]. However, the one experimental study of the stabilizing effects of immigration on constancy in discrete generation *Drosophila melanogaster* metapopulations with unstable local dynamics did not support the prediction of global stabilization via pinning[25]. Nevertheless, that study did reveal enhanced constancy in the local subpopulation that received immigrants each generation, relative to the unpinned subpopulations[25]. Similarly, a study on overlapping generation populations of *Tribolium castaneum* showed that the addition of a few individuals in a sensitive zone of the phase space defined by a joint threshold of both larval and adult numbers enhanced the constancy of the populations[32]. Thus, there is some evidence that the immigration of a constant number of

individuals each generation into a small unstable population can stabilize it with regard to constancy and the qualitative dynamics. If constant immigration into a small population also has a stabilizing effect with respect to persistence, it would be particularly helpful from a conservation viewpoint as it would also add genetic variation to the target population.

In the present study, we examined the effects of Low Magnitude Immigration (two females per generation; henceforth, LMI) and High Magnitude Immigration (five females per generation; henceforth, HMI) on persistence, constancy and dynamics in two types of *D. melanogaster* populations whose intrinsic dynamics were manipulated via different food regimes to be either relatively stable or unstable. Provision of Low levels of food for larvae and High quality food for adults (henceforth, LH) is known to induce two-point oscillatory dynamics in both large[33] and small[34] laboratory populations of *D. melanogaster*. On the other hand, a combination of Low levels of larval food and Low quality of adult food (henceforth, LL) yields relatively stable dynamics with erratic fluctuations in population size that are of smaller amplitude relative to the mean population size than the oscillations induced by the LH food regime[35]. The experiment, thus, involved eight replicate populations in each of six combinations of intrinsic dynamics (LH/LL) and immigration (no immigration control/LMI/HMI), whose population size was recorded for 17 generations. Most statistical analyses used a two-factor analysis of variance (ANOVA), treating intrinsic dynamics and immigration as crossed fixed factors, and measures of constancy, persistence and average population size from the eight replicate populations in each combination as within-cell replicates. Extinct populations in LMI and HMI treatments got re-colonized by immigration, whereas extinct control populations were restarted with eight flies (see Methods for details). Overall, we found that immigration significantly enhanced persistence relative to control populations that did not receive immigrants. Unlike in the case of persistence, the effects of immigration on constancy, average population size and dynamics varied according to the intrinsic dynamics, based on the food regime, and there was no clear correlation between constancy and persistence. That the introduction of even two females per generation resulted in a significant reduction in the number of extinctions in both relatively stable (LL) and unstable (LH) populations suggests that constant immigration may be a robust and relatively simple method of enhancing the persistence of small populations.

Results

Persistence. Overall, the LH populations underwent more extinctions (i.e. had lower persistence) compared to the LL populations (Fig. 1A). In both LL and LH populations, the mean number of extinctions over the 17 generation experiment tended to be lower in the immigration treatments than controls (Fig. 1A). The ANOVA on the LH populations revealed a significant main effect of immigration ($F_{2,21} = 10.974$; $p < 0.001$). Pair-wise multiple comparisons (Tukey's HSD tests) revealed that LMI had significantly lower mean number of extinctions than HMI ($p = 0.012$) or controls ($p < 0.001$), and that controls and HMI did not differ significantly ($p = 0.364$). In the LL populations, there were no extinctions at all in the two immigration treatments, precluding the inclusion of LL data in the ANOVA. The mean number of extinctions in the LL controls was 2.25 with a 95% c.i. of $\pm$ 0.89, which does not include zero. Thus, in the LL populations, the two immigration treatments had significantly less extinctions than controls and did not differ among themselves. The mean time to extinction, given that extinction occurred, in the LL controls was 4.79 generations, whereas in LL-LMI and LL-HMI populations there were no extinctions at all over the 17 generations of the experiment, also indicating a substantial decrease in the intrinsic risk of extinction due to immigration in the LL populations.
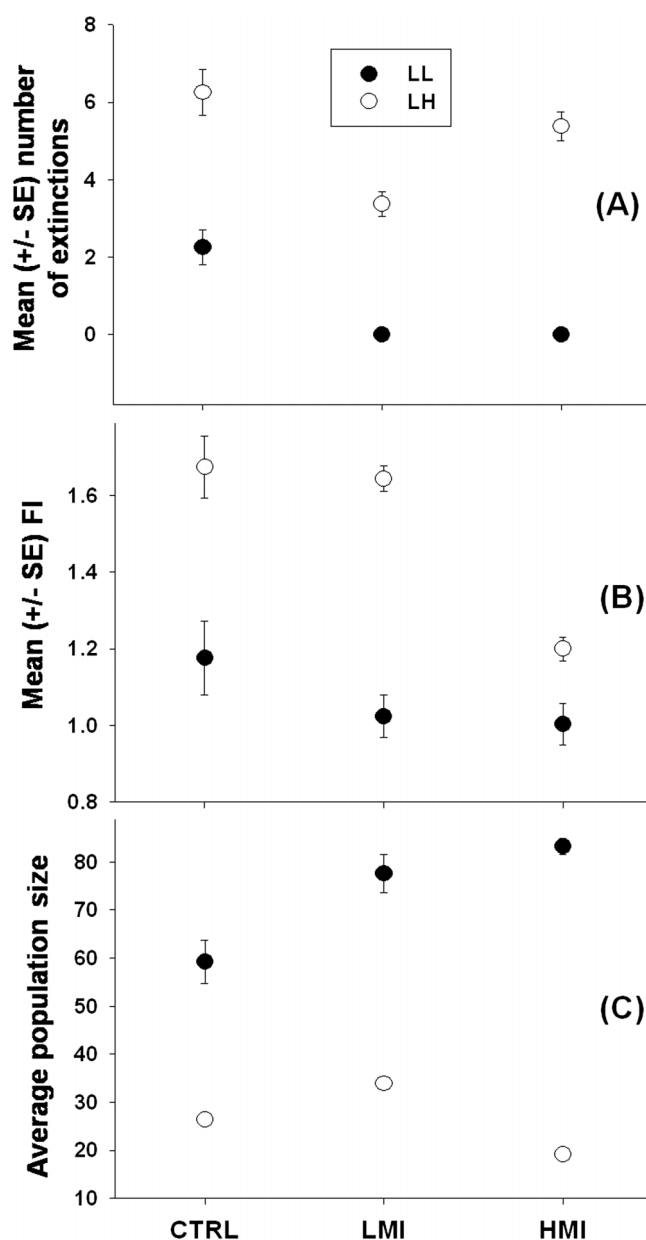

Figure 1 | (A) Persistence: mean number of extinctions suffered by the LL and LH populations during the course of the 17 generation experiment. Pattern of significant ($p < 0.05$) differences is LL-CTRL > LL-LMI = LL-HMI; LH-CTRL = LH-HMI > LH-LMI. (B) Constancy: mean fluctuation index (FI) of the LL and LH populations. Pattern of significant ($p < 0.05$) differences is LL-CTRL = LL-LMI = LL-HMI; LH-CTRL = LH-LMI > LH-HMI. (C) Average population size of the LL and LH populations over 17 generations. Pattern of significant ($p < 0.05$) differences is LL-CTRL < LL-LMI = LL-HMI; LH-CTRL = LH-LMI = LH-HMI. Bars are the standard errors around the mean of 8 replicate populations.

Constancy. Overall, the LH populations showed larger values of FI (i.e. lower constancy in the sense of how large the generation-to-generation fluctuations were, relative to mean population size) compared to the LL populations (Fig. 1B). There was a general tendency for FI to decrease with increasing migration, but the pattern differed between the LL and LH populations (Fig. 1B). The ANOVA results revealed significant effects of local dynamics ($F_{1,42} = 72.397$; $p < 0.001$), immigration ($F_{2,42} = 13.987$; $p < 0.001$) and the local dynamics $\times$ immigration interaction ($F_{2,42} = 5.966$; $p <$

0.01). In the case of immigration main effect on FI, only HMI was significantly lower than both LMI ($p < 0.01$) and controls ($p < 0.001$). Pair-wise multiple comparisons (Tukey's HSD) showed that none of the three immigration treatments in the LL populations differed significantly in FI (all $p > 0.05$). In the LH populations, control and LMI did not differ significantly whereas the FI in HMI was significantly lower than in either control or LMI ($p < 0.001$ in both cases). In fact, the mean FI of LL and LH populations subjected to HMI did not significantly differ ($p = 0.258$).

Average population size. Overall, the average post-immigration population size was greater in the LL populations, and they also showed a pattern of increasing average population size with immigration (Fig. 1C). The ANOVA results showed significant effects of local dynamics ($F_{1,42} = 482.842$; $p < 0.001$), immigration ($F_{2,42} = 12.549$; $p < 0.001$) and the local dynamics × immigration interaction ($F_{2,42} = 18.418$; $p < 0.001$). In the case of the immigration main effect on average population size, only controls were significantly lower than both LMI ($p < 0.001$) and HMI ($p < 0.01$). Pair-wise multiple comparisons (Tukey's HSD) indicated that in the LL populations the controls had significantly lower average population size than either LMI or HMI (both $p < 0.001$) whereas the two immigration treatments did not differ significantly. In the LH populations, none of the immigration treatments (LMI and HMI) differed significantly from the controls for average population size (both $p > 0.3$). LL populations had significantly greater average population size than the LH populations subjected to the same immigration treatment (all $p < 0.01$).

Periodic properties of the populations. The effects of immigration on the periodic behaviour of population size dynamics were different in the LL and LH populations (Fig. 2). In control LL populations, five out of eight replicate populations did not show a major contribution of any of the periodicities examined, indicating the lack of strong low-period cycling in these populations. In both LMI and HMI, however, almost all the LL populations showed clear evidence of low-period (2 or 2.286) cycling. Constant immigration, thus, appeared to induce dynamics close to two-point cycles in the LL populations (Fig. 3). The control LH populations, as expected from

previous studies of population dynamics under different larval and adult food regimes[33–35], showed clear evidence of low-period cycling as compared to the LL controls (Fig. 2). LMI appeared to sharpen the two-point cycles in LH populations, with all replicates showing a major contribution of only periods 2 and 2.286 (Fig. 2). On the other hand, HMI resulted in considerable damping (reduction of the peak population sizes only) of the amplitude of the intrinsically oscillatory dynamics in LH populations (Fig. 3), and also resulted in only two replicate populations showing major contributions of period 2 or 2.286 and two replicates not showing a major contribution of any of the periodicities examined (Fig. 2). The overall spectral densities obtained for the LH-HMI populations were also considerably lower than the other local dynamics × immigration combinations (data not shown), which is consistent with the damping of the intrinsically oscillatory dynamics. This pattern of LMI and HMI inducing sharper low-period cycling in LL populations, and of the LH populations exhibiting clear low-period cycling in controls and LMI but undergoing damping in HMI was also clear in the time-series (Fig. 3).

Population size distribution. As in the case of dynamic behaviour (Figs. 2,3), the distribution of pre-immigration population sizes also showed a clear LL-LH difference, as well as a differing pattern of immigration effects on the two types of population (Fig. 4). In the LH populations, regardless of immigration treatment, the distribution of population sizes tended to be L-shaped, with population sizes falling in the 0–10 individuals bin in about half the generations. The remaining half of the time, population sizes were relatively evenly distributed across the next four to five bins (Fig. 4). Thus, the low-period cycling seen in the LH populations was not the canonical two-point type in which the population oscillates between one low and one high size. The LH populations appeared to follow dynamics in which a very low population size was attained practically every alternate generation, but these troughs were separated by peaks in a range of moderate to high population sizes (Fig. 3). This basic L-shaped pattern did not change much with immigration, though the damping of oscillations via reduction of peak population sizes in HMI (Fig. 3F) was reflected in the absence of any entry into the highest (>90) population size class and
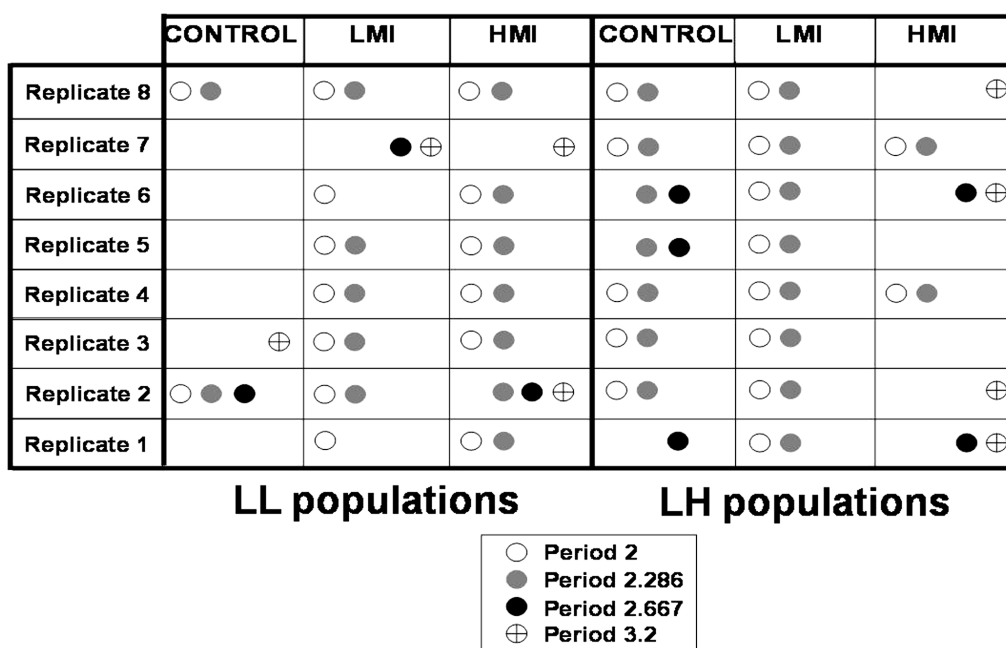
	LL populations			LH populations		
	CONTROL	LMI	HMI	CONTROL	LMI	HMI
Replicate 8	○ ●	○ ●	○ ●	○ ●	○ ●	⊕
Replicate 7		● ⊕	⊕	○ ●	○ ●	○ ●
Replicate 6		○	○ ●	● ●	○ ●	● ⊕
Replicate 5		○ ●	○ ●	● ●	○ ●	
Replicate 4		○ ●	○ ●	○ ●	○ ●	○ ●
Replicate 3	⊕	○ ●	○ ●	○ ●	○ ●	
Replicate 2	○ ● ●	○ ●	● ● ⊕	○ ●	○ ●	⊕
Replicate 1		○	○ ●	●	○ ●	● ⊕

Legend:
○ Period 2
● (gray) Period 2.286
● (black) Period 2.667
⊕ Period 3.2

Figure 2 | Summary of the results of the spectral (Fourier) analyses on all 48 populations. Symbols represent major periodicities that contributed 20% or more to the total spectral density.
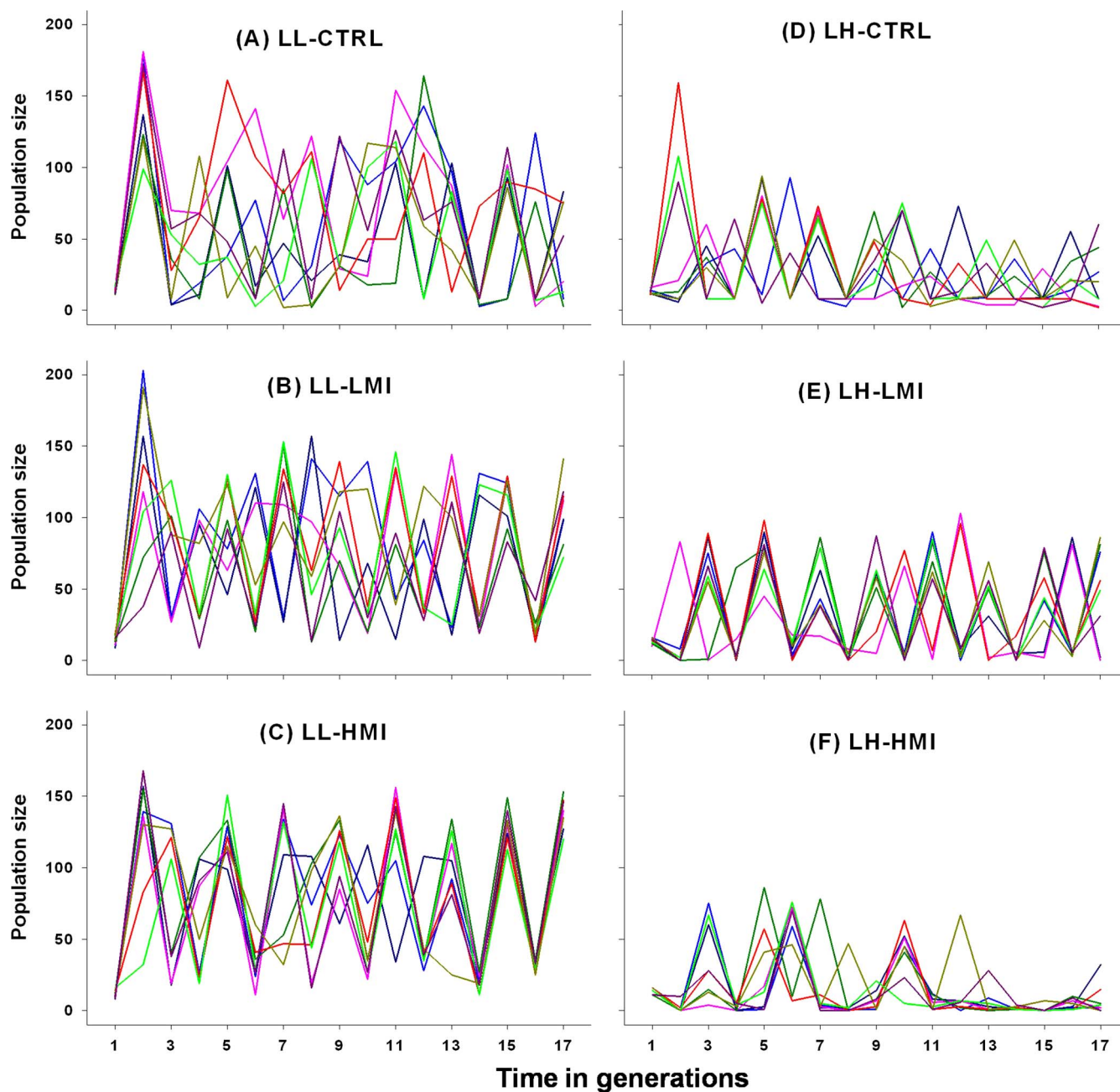
Figure 3 | Time-series of population size in the six combinations of intrinsic dynamics and immigration treatment. The population size values used were the census data prior to imposing constant immigration. Thus, a very low size corresponds to increased extinction risk.

generally lower occurrence of population sizes > 50, compared to LMI or controls (Fig. 4). Moreover, in LMI, the distribution was closer to an asymmetric U-shaped pattern, with the largest population size bins showing a relatively greater proportion of hits (Fig. 4), presumably reflecting the sharper low-period cycling in these populations compared to the HMI or controls (Figs. 2,3). The overall L-shaped distribution of population sizes due to asymmetric two point cycles in the LH populations was likely due to the yeasting of females in this food regime. Yeasted females tend to be very fecund for their size and therefore can probably precipitate population crashes even from moderate population sizes in the previous generation due to intense levels of larval competition. The LH populations subjected to HMI also showed the highest proportion of hits (67%) in the lowest (0–10) population size bin, presumably because the introduction of five well-fed (due to rearing

at low larval density) yeasted back-up females each generation, even to a population with a small number of adults, raised the egg density sufficiently to cause a severe crash in population size in the next generation.

The LL populations, regardless of immigration treatment, showed a shallow U-shaped distribution, with both LMI and HMI making the distribution steeper and moving it to the right of the controls along the population size axis (Fig. 4). In both LMI and HMI, the LL populations rarely fell into the lowest (0–10) population size bin (Fig. 4). Although LMI and HMI rendered the dynamics of the LL populations more periodic (Fig. 2), the oscillations were shifted up along the axis of population size compared to controls (Fig. 3A,B,C). The somewhat shallow U-shaped distribution of the LL populations is a signature of the strong density-dependence that this food regime also entails due to the low level of food available for larvae, but the
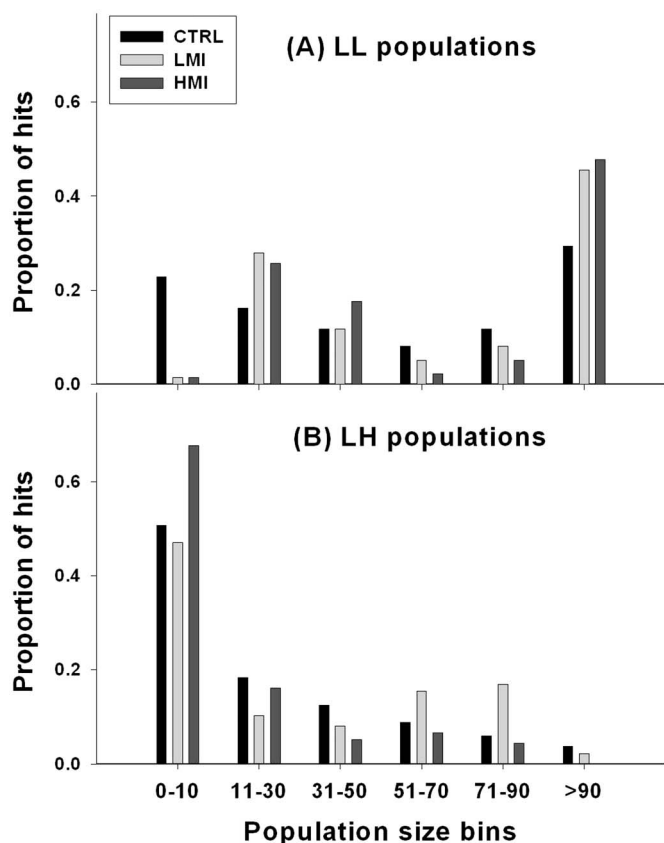
Figure 4 | **The population size distribution of the (A) LL, and (B) LH populations in the three immigration regimes over the 17 generations.** The population size values used were the census data prior to imposing constant immigration. Thus, a very low size corresponds to increased extinction risk.

absence of yeast for the adult females makes the tendency to crash even from moderate population sizes less severe than it is in the LH food regime.

Discussion

We chose to test whether constant immigration would enhance the persistence of small populations at risk of extinction because, in addition to theoretical work on stabilization via immigration[8,11,29-31], there was some evidence that even low levels of constant immigration enhanced constancy in unstable laboratory populations of *Drosophila*[25] and *Tribolium*[32]. However, given that constancy and persistence are not necessarily correlated[28], we thought it worthwhile to examine experimentally whether different levels of constant immigration could enhance persistence in *Drosophila* populations with different dynamics with regard to constancy, and whether any effects of persistence might be subject to interactions between immigration level and local dynamics.

It is clear from our results that constant immigration of either two or five females per generation enhanced persistence via reducing the intrinsic extinction propensity of our small populations, and that this effect was seen in both LH and LL populations despite their differing underlying dynamics (Figs. 1A,4). At the gross level of comparing between food regimes, constancy and persistence were correlated: on the whole, LL populations had greater constancy (Fig. 1B) and persistence (Fig. 1A), compared to LH populations. However, in the LH populations, the enhancement of persistence was dependent on the level of immigration, with only LMI but not HMI showing a significant reduction in the number of extinctions, relative to controls (Fig. 1A). It is also clear from the results on constancy (Fig. 1B),

distribution of population sizes (Figs. 1C,4) and dynamics (Figs. 2,3), that the enhancement of persistence in LL and LH populations probably occurred through different mechanisms. In the LL populations, immigration primarily caused an increase in average population size (Fig. 1C) by shifting the population size distribution to the right (Fig. 4). While low-period cycling in the LL time series was enhanced by immigration (Figs. 2,3), there was a small (non-significant) decline in FI (Fig. 1B) due to the concomitant increase in average population size by which the average absolute one-step fluctuations are scaled. Thus, although LL populations did show large oscillations under immigration, the oscillations were shifted up on the population size axis (Fig. 3) and their population sizes reached very low levels only rarely (Fig. 4), thereby resulting in increased persistence. Thus, the mechanism of immigration-induced persistence was, in case of the LL populations, fairly intuitive. Moreover, in the case of LL populations, persistence and constancy seemed to be affected in tandem, at least qualitatively (Figs. 1A,B).

In the LH populations, on the other hand, the cause(s) of the enhanced persistence under low immigration were not so clear. In particular, persistence and constancy were not correlated across immigration treatments in the LH food regime. In the LMI populations, constancy was not different but persistence was significantly enhanced, compared to controls (Figs 1A,B). Conversely, in HMI, constancy was significantly enhanced but persistence was not different, compared to controls (Figs 1A,B). In LH, unlike in LL, average population size of the LMI and HMI populations also did not differ significantly from the controls (Fig. 1C). The one possible difference between LMI and the controls/HMI in the LH food regime that appeared coincident with the enhanced persistence of LMI populations was seen in the distribution of population sizes (Fig. 4). LMI populations hit the lowest population size (0–10) bin somewhat less frequently than control or HMI populations. Moreover, within this bin, LMI populations hit the 6–10 individuals zone more frequently (27% of the times) compared to controls (10%) or HMI (19%) populations (data not shown). This might be the reason for the reduction in extinctions in LMI compared to controls (Fig. 1A). In the case of HMI populations, the increased proportion of times population size fell to very low levels between 0–5 (54% in HMI, 36% in LMI, 45% in controls, data not shown) was probably responsible for the high number of extinctions (Fig. 1A). Here, we also believe that yeasting of females played a possible role. In LH populations under HMI, five well-fed yeasted females were being introduced to the population each generation. Such females would be extremely fecund and the number of eggs they laid would be enough to cause severe larval overcrowding and hence a crash in adult numbers in the next generation, largely irrespective of the adult population size in the generation when they were added to the population.

Essentially, one implication of these results is that constant immigration at low or even somewhat high rates is likely to be a very robust technique for enhancing persistence by reducing intrinsic extinction propensity in populations that represent a combination of high juvenile competition/mortality and relatively low female fecundity (as in our LL populations). Such a combination of juvenile and adult life-history traits is likely to be common in large vertebrates in the wild. In populations with a combination of high juvenile competition/mortality and relatively high female fecundity (as in our LH populations or, more generally, in many invertebrates or fish/amphibians), care may have to be taken to implement constant migration at relatively low levels in order to enhance persistence.

Our results also support the notion that constancy and persistence are not necessarily correlated[28]. Constancy as a measure of stability is essentially concerned with population size fluctuations[3,4]. However, population size fluctuations can be deterministically driven by strong density-dependence and/or be due to demographic stochasticity[7]. In general, larger populations tend to be less susceptible to demographic stochasticity[36], and demographic stochasticity has been shown

experimentally to be an important factor affecting extinction rate[37]. Overall, however, the evidence for smaller or more variable populations being more extinction prone is equivocal[38–44]. Essentially, for a given level of demographic stochasticity, the population size zone in which fluctuations occur is the most critical in terms of extinction risk[45]. When populations in their established phase, wherein population size fluctuations follow a quasi-stationary distribution[46], remain above a critical extinction threshold even when they fall to low values, there is little additional risk of extinction due to increased magnitude of fluctuation in population size[45]. This pattern is clearly seen in the LL populations that have enhanced persistence (Fig. 1A) and hit very low sizes less often than controls at both immigration levels (Figs. 3,4).The opposite effect is seen in the LH populations where population size fluctuations are of reduced amplitude under HMI (Fig. 3F), but the population sizes hit very low values frequently (Fig. 4), leading to greater risk of extinction than under LMI in the same food regime (Fig. 1A). This is also why the relationship between persistence and any measure of constancy, whether CV (coefficient of variation of population size) or FI, is likely to be context-specific and may not hold in general. In fact, even the relationship of measures of constancy (like FI) with the simplicity/complexity of dynamics is not straightforward across the full range of dynamic behaviours shown by populations[47].

Overall, our results clearly suggest that constant immigration, especially at low levels relative to average population size, could be a useful technique for reducing extinction risk in small populations. A major advantage of constant immigration as a perturbation for enhancing persistence would be ease of implementation. Unlike many other classes of stabilizing perturbations[48,49], constant immigration needs no knowledge of either system parameters or the specific time series of the state variable (population sizes), and captive breeding programmes can, in principle, provide a convenient source of individuals for immigration. For example, the persistence-enhancing effect of intermittently introducing immigrants into small populations of African wild dogs (*Lycaon pictus*) is being actively considered in developing strategies for the conservation of this endangered species[50]. However, the dynamics of wild populations are also subject to complex effects due to spatial structuring[51], social behaviour[50] and the presence of multiple interacting species[51,52]. Consequently, these factors also need to be considered while setting up conservation strategies for stabilizing small populations[50].

Methods

Experimental populations and design. Forty eight small populations of *D. melanogaster* subjected to one of two food regimes (LH and LL) that induce different dynamics were used for this study. Each small population consisted of a single-vial culture, initiated with exactly 20 eggs and thereafter maintained on a 21-day discrete generation cycle, with census of adults on day 21, followed by egg laying in a fresh food vial to initiate the next generation [following 34]. The small populations used in this experiment were derived from a large long-term laboratory population of *D. melanogaster* (JB1), whose ancestry and maintenance have been described in detail previously[53] and that has been used for many studies on small population and metapopulation dynamics[24,25,35].

In the LH regime, larval food levels were low (~1 mL per vial) whereas the adults were given high quality food, in the form of yeast paste, for three days prior to egg laying. This combination of Low larval and High adult food (hence, LH) is known to induce two-point oscillations in adult numbers[28,33–35,54]. The other regime, Low larval and Low adult food (hence, LL), provided about 1.2 mL food per vial for the larvae whereas adults did not receive any yeast paste supplement prior to egg laying. The LL food regime results in greater constancy of population dynamics relative to the LH regime, with erratic fluctuations in population size that are of smaller amplitude relative to the mean population size than the oscillations induced by the LH regime, and is hence considered relatively stabilizing[35]. Eight replicates each of the LL and LH populations were subjected to one of three immigration treatments in a fully factorial design: Control (no immigration), Low Magnitude Immigration (LMI) and High Magnitude Immigration (HMI).

In the two immigration treatments, mated females from back-up single-vial cultures run in parallel with the experimental populations were manually added to the LL or LH populations just after census and prior to egg laying on day 21. The females were taken from cultures providing high larval food (6 mL per vial) in combination with either high (yeast paste) or low (no yeast paste) quality adult food i.e. from an HL regime for immigration into LL and from an HH regime for immigration into LH,

respectively. The high larval food levels in the back-up vials were used to ensure an adequate supply of females for the two immigration treatments. In the LMI treatment, two females were introduced into each experimental population every generation, whereas five females per generation per population were introduced in the HMI treatment. The control experimental populations received no immigrants. The experiment ran for 17 generations.

Evaluation of persistence. We evaluated the persistence of each experimental population by recording the number of times it went extinct during the 17 generations. A population was considered extinct when it lacked even a single male-female pair at the time of census, prior to receiving immigrants in the LMI and HMI treatments. Extinctions in this study, thus, are pre-immigration extinctions, reflecting the intrinsic propensity of the populations to go extinct, independent of their "recolonization" by immigration, and the number of extinctions suffered by a population is inversely related to persistence. In the controls, any population that went extinct was restarted with four males and four females from the appropriate back-up vials. In LMI and HMI treatments, immigration sufficed to recolonize an extinct population.

Evaluation of constancy. We evaluated the constancy of experimental populations by using a statistic called fluctuation index (FI)[24], which is the mean absolute value of one-step change in the population size ($N_{t+1} - N_t$), scaled by mean population size over the duration of the experiment. FI is thus inversely related to constancy to the extent that it reflects the average magnitude of generation-to-generation fluctuations relative to the mean population size. This relationship is most relevant when the behaviour is periodic, but FI is not necessarily correlated with qualitative simplification of dynamics, for example stabilization from chaos to periodic behaviour[47]. In case of the LMI and HMI treatments, all population size values used for the FI calculation were the post-immigration N_t values.

Spectral (Fourier) analysis. The periodic properties of the time series of all 48 experimental populations were evaluated by single spectral (Fourier) analysis using STATISTICA for Windows[55]. Only periods that contributed 20% or more to the total spectral density were considered as being representative of the major periodicities in a time series. All time series were of post-immigration population sizes.

Analyses of variance. We tested for significant fixed effects of intrinsic dynamics (LL/LH), immigration (control/LMI/HMI), and their interaction, on FI and average population size by two-way ANOVA in which the eight populations within each intrinsic dynamics × immigration combination served as replicates. In the case of number of extinctions, due to there being zero within-cell variance as a result of no extinctions in any replicate populations in the LL-LMI and LL-HMI treatments, we performed a one-way ANOVA on the LH populations, and tested the mean number of extinctions in the LL-Control treatment for significant differences from zero, to ascertain whether immigration had an effect on persistence. All ANOVAs were implemented on STATISTICA for Windows[55].

1. Soulé, M. E. *Viable populations for conservation.* Cambridge, UK: Cambridge University Press (1987).
2. Morris, W. F. & Doak, D. F. *Quantitative conservation biology: theory and practice of population viability analysis.* Sunderland, MA, USA: Sinauer Associates (2002).
3. Connell, J. H. & Sousa, W. P. On the evidence needed to judge ecological stability or persistence. *Am. Nat.* **121**, 789–824 (1983).
4. Grimm, V. & Wissel, C. Babel, or the ecological stability discussions: an inventory and analysis of terminology and a guide for avoiding confusion. *Oecologia* **109**, 323–34 (1997).
5. May, R. M. Biological populations with non-overlapping generations: stable points, stable cycles and chaos. *Science* **186**, 645–647 (1974).
6. May, R. M. Simple mathematical models with very complicated dynamics. *Nature* **261**, 459–467 (1976).
7. Mueller, L. D. & Joshi, A. *Stability in model populations.* Princeton, NJ, USA: Princeton University Press (2000).
8. McCallum, H. I. Effects of immigration on chaotic population dynamics. *J. Theor. Biol.* **154**, 277–284 (1992).
9. Sinha, S. & Parthasarathy, S. Controlling chaos in unidimensional maps using constant feedbacks. *Phys. Rev. E* **51**, 6239–6242 (1995).
10. Gueron, S. Controlling one-dimensional unimodal population maps by harvesting at a constant rate. *Phys. Rev. E* **57**, 3645–3648 (1998).
11. Stone, L. & Hart, D. Effects of immigration on the dynamics of simple population models. *Theor. Pop. Biol.* **55**, 227–234 (1999).
12. Güémez, J. & Matías, M. A. Control of chaos in unidimensional maps. *Phys. Lett. A* **181**, 29–32 (1993).
13. Doebeli, M. & Ruxton, G. D. Controlling spatiotemporal chaos in metapopulations with long-range dispersal. *Bull. Math. Biol.* **59**, 497–515 (1997).
14. Parekh, N., Parthasarathy, S. & Sinha, S. Global and local control of spatiotemporal chaos in coupled map lattices. *Phys. Rev. Lett.* **81**, 1401–1404 (1998).
15. Parekh, N. & Sinha, S. Controlling dynamics in spatially extended systems. *Phys. Rev. E* **65**, 036227–1–9 (2002).
16. Pimm, S. L. *The balance of nature? Ecological issues in the conservation of species and communities.* Chicago, IL, USA: University of Chicago Press (1991).

17. Sala, O. E. *et al.* Global biodiversity scenarios for the year 2100. *Science* **287**, 1770–1774 (2000).

18. Abbott, K. C. A dispersal-induced paradox: synchrony and stability in stochastic metapopulations. *Ecol. Lett.* **14**, 1158–1169 (2011).

19. Thomas, W. R., Pomerantz, M. J. & Gilpin, M. E. Chaos, asymmetric growth and group selection for dynamic stability. *Ecology* **61**, 1312–1320 (1980).

20. Shaffer, M. L. Minimum population sizes for species conservation. *BioScience* **31**, 131–134 (1981).

21. Berryman, A. A. & Millstein, J. A. Are ecological systems chaotic – and if not, why not? *Trends Ecol. Evol.* **4**, 26–28 (1989).

22. Shulenberger, L., Ying-Cheng, L., Yalçinkaya, T. & Holt, R. D. Controlling transient chaos to prevent species extinction. *Phys. Lett. A* **260**, 156–161 (1999).

23. Pimm, S. L., Jones, H. L. & Diamond, J. On the risk of extinction. *Am. Nat.* **132**, 757–785 (1988).

24. Dey, S. & Joshi, A. Stability via asynchrony in *Drosophila* metapopulations with low migration rates. *Science* **312**, 434–436 (2006).

25. Dey, S. & Joshi, A. Local perturbations do not affect stability of laboratory fruitfly metapopulations. *PLoS One* **2**, e233 (2007).

26. Caughley, G. Directions in conservation biology. *J. Anim. Ecol.* **63**, 215–244 (1994).

27. Griffen, B. D. & Drake, J. M. A review of extinction in experimental populations. *J. Anim. Ecol.* **77**, 1274–1287 (2008).

28. Dey, S., Prasad, N. G., Shakarad, M. & Joshi, A. Laboratory evolution of population stability in *Drosophila*: constancy and persistence do not necessarily coevolve. *J. Anim. Ecol.* **77**, 670–677 (2008).

29. Sinha, S. & Parthasarathy, S. Behaviour of simple population models under ecological processes. *J. Biosci.* **19**, 245–254 (1994).

30. Stone, L. Period-doubling reversals and chaos in simple ecological models. *Nature* **365**, 617–620 (1993).

31. Ruxton, G. D. The effect of emigration and immigration on the dynamics of a discrete-generation population. *J. Biosci.* **20**, 397–407 (1995).

32. Desharnais, R. A., Costantino, R. F., Cushing, J. M., Henson, S. M. & Dennis, B. Chaos and population control of insect outbreaks. *Ecol. Lett.* **4**, 229–235 (2001).

33. Mueller, L. D. & Huynh, P. T. Ecological determinants of stability in model populations. *Ecology* **75**, 430–437 (1994).

34. Sheeba, V. & Joshi, A. A test of simple models of population growth using data from very small populations of *Drosophila melanogaster*. *Curr. Sci.* **75**, 1406–1410 (1998).

35. Dey, S. *Experimental and theoretical investigation of the dynamics and stability of single populations and metapopulations of Drosophila melanogaster in the laboratory.* Bangalore, India: PhD Thesis, Jawaharlal Nehru Centre for Advanced Scientific Research (2007).

36. Desharnais, R. A., Costantino, R. F., Cushing, J. M., Henson, S. M., Dennis, B. & King, A. A. Experimental support of the scaling rule for demographic stochasticity. *Ecol. Lett.* **9**, 537–547 (2006).

37. Drake, J. M. Density-dependent demographic variation determines extinction rate of experimental populations. *PLoS Biol.* **3**(7), e222 (2005).

38. Drayton, B. & Primack, R. B. Experimental extinction of garlic mustard (*Alliaria petiolata*) populations: implications for weed science and conservation biology. *Biol. Invas.* **1**, 159–167 (1999).

39. Vucetich, J. A., Waite, T. A., Qvarnemark, L. & Ibargüen, S. Population variability and extinction risk. *Conserv. Biol.* **14**, 1704–1714 (2000).

40. Bengtsson, J. & Milbrink, G. Predicting extinction: interspecific competition, predation and population variability in experimental *Daphnia* populations. *Oecologia* **101**, 397–406 (1995).

41. Burkey, T. V. Metapopulation extinction in fragmented landscapes: using bacteria and protozoa communities as model ecosystems. *Am. Nat.* **150**, 568–591 (1997).

42. Belovsky, G. E., Mellison, C., Larson, C. & Van Zandt, P. A. Experimental studies of extinction dynamics. *Science* **286**, 1175–1177 (1999).

43. Berggren, A. Colonization success in Roesel's bush-cricket. *Metrioptera roeseli: the effects of propagule size. Ecology* **82**, 274–280 (2001).

44. Grevstad, F. S. Experimental invasions using biological control introductions: the influence of release size on the chance of population establishment. *Biol. Invas.* **1**, 313–323 (1999).

45. Hildenbrandt, H., Müller, M. S. & Grimm, V. How to detect and visualize extinction thresholds for structured PVA models. *Ecol. Model.* **191**, 545–550 (2006).

46. Grimm, V. & Wissel, C. The intrinsic mean time to extinction: a unifying approach to analyzing persistence and viability of populations. *Oikos* **105**, 501–511 (2004).

47. Sah, P., Salve, J. P. & Dey, S. Stabilizing biological populations and metapopulations through Adaptive Limiter Control. *J. Theor. Biol.* **320**, 113–123 (2013).

48. Kapitaniak, T. *Controlling chaos: theoretical and practical methods in non-linear dynamics.* London, UK: Academic Press (1996).

49. Hilker, F. M. & Westerhoff, F. H. Preventing extinction and outbreaks in chaotic populations. *Am. Nat.* **170**, 232–241 (2007).

50. Gusset, M., Jakoby, O., Müller, M. S., Somers, M. J., Slotoe, R. & Grimm, V. Dogs on the catwalk: modelling re-introduction and translocation of endangered wild dogs in South Africa. *Biol. Conserv.* **142**, 2774–2781 (2009).

51. Hanski, I. *Metapopulation ecology.* New York, USA: Oxford University Press (1999).

52. Bjørnstad, O. N., Ims, R. A. & Lambin, X. Spatial population dynamics: analyzing patterns and processes of population synchrony. *Trends Ecol. Evol.* **14**, 427–432 (1999).

53. Sheeba, V., Madhyastha, N. A. A. & Joshi, A. Oviposition preference for novel versus normal food resources in laboratory populations of *Drosophila melanogaster*. *J. Biosci.* **23**, 93–100 (1998).

54. Mueller, L. D., Joshi, A. & Borash, D. J. Does population stability evolve? *Ecology* **81**, 1273–1285 (2000).

55. *StatSoft. Statistica Vol. I: general conventions and statistics 1.* Tulsa, OK, USA: StatSoft Inc (1995).

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S.D. and A.J. conceived of the experiment, S.D. carried out the experiments, S.D. and A.J. did the data analysis and manuscript writing.

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