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Body size, carry-over effects and survival in a seasonal environment: consequences for population dynamics

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Summary

1. In seasonal populations, vital rates are not only determined by the direct effects of density at the beginning of each season, but also by density at the beginning of past seasons. Such delayed density dependence can arise via non-lethal effects on individuals that carry over to influence per capita rates.

2. In this study, we examine (i) whether parental breeding density influences offspring size, (ii) how this could carry over to affect offspring survival during the subsequent non-breeding period and (iii) the population consequences of this relationship.

3. Using *Drosophila melanogaster*, the common fruit fly, submitted to distinct breeding and non-breeding seasons, we first used a controlled laboratory experiment to show that high parental breeding density leads to small offspring size, which then affects offspring survival during the non-breeding period but only at high non-breeding densities.

4. We then show that a model with the interaction between parental breeding density and offspring density at the beginning of the non-breeding season best explained offspring survival over 36 replicated generations.

5. Finally, we developed a biseasonal model to show that the positive relationship between parental density and offspring survival can dampen fluctuations in population size between breeding and non-breeding seasons.

6. These results highlight how variation in parental density can lead to differences in offspring quality which result in important non-lethal effects that carry over to influence per capita rates the following season, and demonstrate how this phenomenon can have important implications for the long-term dynamics of seasonal populations.

Key-words: delayed density dependence, *Drosophila melanogaster*, seasonal density dependence, seasonality

Introduction

Almost all organisms live in seasonal environments, but we still have limited knowledge of how vital rates in different periods of the annual cycle interact to influence fitness and population dynamics (Fretwell 1972; Norris 2005; Holt 2008; Harrison *et al.* 2011; Hastings 2012). In seasonal populations, vital rates are determined not only by the effects of density at the beginning of each season (Fretwell 1972; Boyce, Sinclair & White 1999; Ratikainen *et al.* 2008), but also by densities at the beginning of past seasons (Hansen, Stenseth & Henttonen 1999; Merritt, Lima & Bozinovic 2001). One way in which such delayed density dependence can arise is when variation in population size affects the state of an individual in a non-lethal manner, which can then carry over to influence individual performance in subsequent seasons (Norris 2005; Harrison *et al.* 2011).

An example of a carry-over effect caused by density dependence was recently shown in an experimental seasonal population of *Drosophila melanogaster* (Betini, Griswold & Norris 2013a). Here, high density at the beginning of the non-breeding season negatively influenced breeding output of females that survived the following season, but only when breeding density was below carrying capacity (Betini, Griswold & Norris 2013a). Additional evidence suggested that the mechanism driving this carry-over effect was through variation in physiological condition of surviving individuals and that the interaction between density at the beginning of the previous non-breeding season and breeding density best explained

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breeding output over 23 generations of replicated seasonal *Drosophila* populations (Betini, Griswold & Norris 2013b).

In a similar fashion, variation in density of adults during the breeding season could affect the survival of their offspring during the subsequent non-breeding season. Changes in environmental conditions during early development are known to have short- and long-term effects on offspring survival (Lindström 1999; Lindström & Kokko 2002; Monaghan 2008), and there is evidence that parental breeding density can influence early development (Forchhammer *et al*. 2001). In addition, effects of breeding density on development can either be intensified or weaken depending on the density experienced by offspring in later periods of their life (Clutton-Brock *et al*. 1987; Plaistow & Benton 2009). Despite evidence that parental breeding density could influence offspring performance and interact with offspring density to affect subsequent survival, it is not clear how these processes operate in seasonal environments and there is no information on their potential impact on the long-term dynamics of seasonal populations.

In this study, we experimentally induced seasonality in *Drosophila* to examine how parental breeding density and offspring non-breeding density could affect offspring survival in the non-breeding season. In flies, as in many other organisms (Kingsolver & Huey 2008), high parental density results in offspring with smaller body size compared to offspring from parents that reproduced at lower densities (Miller & Thomas 1958; Mueller & Joshi 2000). Because small body size is typically associated with low survival (Peters 1986), we predicted that high parental breeding density would result in smaller offspring with lower survival in the following non-breeding season compared to offspring born from parents experiencing low breeding densities. In addition, because survival is density-dependent in our laboratory system (Betini, Griswold & Norris 2013a), offspring survival should also be negatively related to the density they experience during the non-breeding period. We also examined the hypothesis that parental density and offspring density interact to influence survival. At low non-breeding density, offspring would not be food limited and therefore survival should be high regardless of parental breeding density, whereas high offspring non-breeding densities would lead to food limitation and a strong effect of parental breeding density on survival. Thus, the interaction between parental breeding density and offspring non-breeding density should best explain offspring survival.

We first tested these predictions experimentally by breeding flies at four different densities and then, for each of these treatments, placing their offspring at three different densities during the subsequent non-breeding period. We then tested these same predictions using replicated seasonal populations of *Drosophila* spanning 36 non-overlapping generations. In these populations, we examined whether variation in offspring survival in each generation could be best explained by parental breeding density, offspring non-breeding density or the interaction between the two. Finally, we developed a biseasonal model to investigate how the parental density–offspring survival relationship influenced population size and stability under different values of maximum growth rate.

Materials and methods

PARENTAL DENSITY AND OFFSPRING SURVIVAL IN A CONTROLLED EXPERIMENT

To simulate seasonality in populations with non-overlapping generations, we changed food composition to generate two distinct 'seasons'. During the '*breeding season*', we placed adults in vials with a dead yeast-sugar medium to lay for 24 h (day 0), after which adults were discarded and larvae were allowed to mature to adults. The '*non-breeding season*' consisted of an empty vial of the same size as the breeding vials and a pipette tip filled with 0·200 ml of 5% water–sugar solution per day, which provided food for the flies but prevented females from producing eggs. To experimentally estimate the effects of parental density on offspring survival, we placed adults (50 : 50 sex ratio) to lay eggs for 24 h in four different densities: 2, 10, 80 and 180 individuals (Fig. S1, Table S1, Supporting information). These offspring were moved from old to fresh vials every 2 days from day 10 to day 16 to avoid high offspring mortality (Dey & Joshi 2006). During this period, densities of offspring were not manipulated, that is, their densities were a function of the number of flies that emerged in each of the four parental breeding density treatments. On day 16, they were lightly anesthetized with CO_2 and counted. We then combined all offspring from our replicates (i.e. vials) from each parental breeding density into three different densities in the non-breeding season (20, 180 and 300 individuals per vial) so that offspring from low (2 and 10 individuals), medium (80) and high (180) parental breeding density were exposed to low (20 individuals), medium (180) and high (300) non-breeding density (Fig. S1, Supporting information). The '*non-breeding season*' lasted 4 days when all the survivors from each of the non-breeding density were counted. More details about the system can be found elsewhere (Betini, Griswold & Norris 2013a,b).

To examine whether offspring survival at the end of the non-breeding season was affected by parental breeding density, we compared three models using Akaike Information Criterion (Burnham, Anderson & Huyvaert 2011). The first model had only offspring density at the beginning of the non-breeding season as a predictor (termed the '*offspring model*'), representing the hypothesis that survival was only explained by direct density dependence. The second model had both parental density and offspring non-breeding density as predictors (the '*parental model*'), which represented the hypothesis that parental density (i.e. delayed density dependence) could also affect offspring survival. The third model had the interaction between offspring non-breeding density and parental density as a predictor (the '*interaction model*'), because we expected survival to be high at low non-breeding density regardless of parental breeding density.

To examine whether parental density influenced offspring weight, we used an ANOVA with female dry weight as a response variable and parental breeding density as the predictor. To obtain an estimate of dry weight, we collected 2 females from 5 arbitrarily selected vials (replicates) from each parental breeding

density (2, 10, 80, and 180) on day 16, just before offspring were moved to the non-breeding season. Individuals were placed in vials in a freezer at −20 °C until they were dried in an oven at 60 °C for 72 h and weighed in a microbalance. We measured females instead of males because population dynamics of fruit flies is largely influenced by female size (Mueller 1987; Mueller & Joshi 2000). An analysis of males with same sample size as the female analysis yielded similar results, although the effect of parental breeding density on male weight was less pronounced (Fig. S2, Supporting information). For both sexes, we used a Tukey honest significant difference (HSD) test to investigate the differences between breeding density treatment pairs.

PARENTAL DENSITY AND OFFSPRING SURVIVAL IN LONG-TERM SEASONAL POPULATIONS

To assess whether parental breeding density had an effect on offspring survival in unmanipulated populations, we used 45 replicated populations of *Drosophila* submitted to two distinct seasons, during which population size was allowed to vary naturally (Betini, Griswold & Norris 2013b). During the '*breeding season*', adults were allowed to lay eggs for 24 h (day 0) in dead yeast–sugar medium, after which they were discarded and larvae were allowed to mature to adults. On day 16, flies were marked with a fluorescent dust (as part of another experiment) and left overnight in large bottles with fresh food so that they could remove the excess of dust. On the morning of day 17, flies were lightly anesthetized with CO_2, counted and placed into '*non-breeding season*' vials. After 4 days, flies were counted and the cycle was repeated for 36 generations. One generation or cycle was completed in 21 days. We randomly removed 5% of the population each time they were moved between seasons to mimic events such as migratory mortality and dispersal (Betini, Griswold & Norris 2013b).

We examined the variation in offspring survival in the long-term seasonal populations using the same models considered in the controlled experiments: the '*offspring model*', the '*parental model*' and the '*interaction model*'. Because the time series had 45 replicates, all models had population (or vial) as a random effect. As in the controlled experiment, we then compared the three models using Akaike Information Criterion (Burnham, Anderson & Huyvaert 2011).

As in the experiment, we also investigated whether parental density influenced offspring size in the replicated long-term seasonal populations. To do this, we obtained dry weight from 5% of offspring before they went to the non-breeding season from an arbitrary number of populations (16 to 25 different vials) in generations 15, 16, 21, 22, 25, 26, 28–33. We then identified and weighed females (n = 1374) and examined the correlation between parental breeding density with female dry weight with a Pearson's product-moment correlation test.

SEASONAL DELAYED DENSITY DEPENDENCE AND THE LONG-TERM DYNAMICS OF POPULATIONS

To investigate whether carry-over effects on non-breeding survival caused by changes in parental density could increase fluctuations in population size between seasons, we used a biseasonal Ricker model with season-specific parameters based on Betini, Griswold and Norris (2013a). A Ricker model for an aseasonal environment or a single census period is expressed as $(N_{(t+1)} = N_{(t)}e^{r_{\max}(1-N_{(t)}/K)})$. In the biseasonal model, population size at the end of the breeding season in generation $t + 1$ can be written as:

$$N_{\mathrm{b}(t+1)} = N_{\mathrm{nb}(t)}e^{r_{\mathrm{b(max)}}(1-N_{\mathrm{nb}(t)}/K_{\mathrm{b}})} \quad \text{eqn 1}$$

and the population size at the end of the non-breeding season in generation $t + 1$ can be expressed as:

$$N_{\mathrm{nb}(t+1)} = N_{\mathrm{b}(t+1)}e^{r_{\mathrm{nb(max)}}(1-N_{\mathrm{b}(t+1)}/K_{\mathrm{nb}})} \quad \text{eqn 2}$$

where r_{b} and r_{nb} are the maximum rate of increase, and survival and K_{b} and K_{nb} are the carrying capacity in the breeding (b) and non-breeding (nb) seasons. $N_{\mathrm{b}(t)}$ is population size at the end of the breeding season at generation t, and $N_{\mathrm{nb}(t)}$ is the population size at the end of the non-breeding season at generation t. Parameter values for eqns 1 and 2 were obtained from an independent experiment (Betini, Griswold & Norris 2013a).

Using this model, we then integrated the effect of parental breeding density on survival of the offspring in the following non-breeding season based on results from the experiment described above. To do this, we first had to identify which non-breeding season parameter to vary in the seasonal Ricker model. Based on the experimental results, offspring survival in the non-breeding period was high when non-breeding season density was low, and this occurred regardless of parental density the previous breeding season (see Results). In the context of the Ricker model, this meant that $r_{\mathrm{nb\ (max)}}$ (the intercept of the density-dependent function) in the non-breeding season was the same across different parental densities. Since the strength of density dependence in the Ricker model is determined by the relationship between r and K, we changed the strength of density dependence as a function of parental density by varying K_{nb}.

To estimate how K_{nb} changed with variation in parental density, we first fit a regression line to the relationship between survival and non-breeding density for each parental breeding density based on the experimental results. We then fit a nonlinear relationship between the slope of the regressions obtained in the previous analysis and parental breeding density, so that we could predict changes in the slope as a function of parental breeding density. Because there was little variation in survival for low non-breeding density (20 individuals), we fixed the intercept for all regressions based on the average intercept for all breeding densities when non-breeding density was 20. We used this intercept and the slope function described above to calculate changes in the K_{nb} as a function of parental breeding density. Finally, we replaced K_{nb} for this function in eqn 2 above (shown below in Results).

Because the experimental results turned out to be the opposite from what we initially predicted, and our prediction was based on past work on wild systems, we felt it would be informative to develop another model that had the opposite effect of parental breeding density on offspring survival (i.e. higher parental density = lower survival). To do this, we simply inverted the relationship between parental breeding density and offspring survival. We then compared these two models with a biseasonal model that had only direct density dependence in each season (outlined eqns 1 and 2). For all three of these models, we simulated breeding and non-breeding size over 100 generations and over a range of $r_{\mathrm{b\ (max)}}$ values using bifurcation plots, which investigate the emergence of cycles, and in the case of seasonal populations, also provide information on how population fluctu-

ates between seasons. All analyses were conducted in R (R Core Team 2013).

Results

PARENTAL DENSITY AFFECTS OFFSPRING SURVIVAL VIA CHANGES IN BODY SIZE

In our controlled experiment, the best model to explain variation in offspring survival included the interaction between parental density and density of offspring at the beginning of the non-breeding season (Fig. 1a, Tables 1, S2, Supporting information). As expected, offspring survival during the non-breeding period tended to be negatively related to the density of offspring at the beginning of the non-breeding season. Higher parental breeding densities tended to result in higher offspring survival, but it had no effect on survival when offspring density was low (Fig. 1a). Thus, with the exception of lowest non-breeding density treatment, parental density actually had the opposite effect of what we predicted; high parental breeding density resulted in higher offspring survival (Fig. 1a).

High parental breeding density resulted in female offspring with lower dry weight than females from parents that reproduced at low density ($F_{3,36} = 27{\cdot}79$, $P < 0{\cdot}001$, Fig. 1b), although the post hoc Tukey HSD test revealed that there was no significant difference in female weight when adults reproduced at the two low-density treatments (parental breeding densities 2 and 10, $P = 0{\cdot}999$, Fig. 1b).

PARENTAL DENSITY AND OFFSPRING SURVIVAL IN LONG-TERM SEASONAL POPULATIONS

As in the controlled experiments, the best model to explain variation in offspring survival in the long-term seasonal populations ($n = 45$ replicates) included the interaction between parental density and offspring non-breeding density (Fig. 1c, Tables 1, S3, Supporting information). Also similar to the experimental results, high breeding density tended to result in offspring with lower than average weight (*Pearson's product-moment correlation* $= -0{\cdot}30$, d.f. = 1372, $P < 0{\cdot}001$, Fig. 1d), suggesting that smaller offspring had higher survival in the non-breeding season after controlling for density at the beginning of the non-breeding season (Figs 1d, S3, Table S4, Supporting information).

SEASONAL DELAYED DENSITY DEPENDENCE AND THE LONG-TERM DYNAMICS OF POPULATIONS

The strength of the relationship between offspring survival and non-breeding density (i.e. the slope) decreased with increasing parental density, but survival at low densities (i.e. the intercept) was similar to all parental densities (Fig. 1a), suggesting that high parental densities weakened the strength of density dependence survival. The relationship between slope and parental breeding density could be described as

$$a * N_{\mathrm{nb}(t)}^2 + b * N_{\mathrm{nb}(t)} + c \qquad \text{eqn 3}$$

where a, b and c are constants estimated from the data with a nonlinear (weighted) least-squares regression ($a = -1{\cdot}04 \times 10^{-7}$, $b = 3{\cdot}85 \times 10^{-5}$, $c = -4{\cdot}37 \times 10^{-3}$, Fig. 2), and $N_{\mathrm{nb(t)}}$ is the population size at the end of the non-breeding season at generation t, (i.e. parental breeding density). We assumed that our lowest breeding density (2) produced the largest flies possible in our system therefore causing the lowest offspring survival in the non-breeding season. Thus, to estimate a and b in (3), we fixed the intercept c at $-4{\cdot}37 \times 10^{-3}$ (the value for the slope of the relationship between survival and offspring non-breeding density when parental breeding density was 2; Fig. 1a).

Equation 3 could predict a stronger density dependence survival for densities >180, that is, smaller flies would have lower survival, which is opposite to what we observed in our laboratory system. To avoid this, we found the vertex of the quadratic function (3) by solving

Table 1. Akaike Information Criterion model selection parameters and regression coefficients from competing models used to explain offspring survival in both controlled experiments ('Experiment') and long-term seasonal populations ('Time series'). The '*offspring model*' contained just offspring non-breeding density ($N_{\mathrm{nb}(t)}$) as the predictor, the '*parental model*' included offspring non-breeding density and parental breeding density ($N_{\mathrm{b}(t-1)}$), and the '*interaction model*' contained the interaction between those two variables ($N_{\mathrm{nb}(t)} * N_{\mathrm{b}(t-1)}$). LogLik, log-likelihood values for each mode; AICc, Akaike Information Criterion corrected for small samples; **Δ**AICc, difference for model relative to the smallest AICc in the model set; W_j, Akaike weight is the approximate probability in favour of the given model from the set of models considered

Model	Intercept	$N_{\mathrm{nb}(t)}$	$N_{\mathrm{b}(t-1)}$	$N_{\mathrm{nb}(t)} * N_{\mathrm{b}(t-1)}$	d.f.	LogLik	AICc	ΔAICc	W_j
Experiment									
Offspring model	0·99	$-1{\cdot}77 \times 10^{-3}$	–	–	3	50·54	−94·9	150·5	0
Parental model	0·91	$-1{\cdot}89 \times 10^{-3}$	$1{\cdot}52 \times 10^{-3}$	–	4	85·9	−163·5	81·9	0
Interaction model	1·00	$-2{\cdot}54 \times 10^{-3}$	$-1{\cdot}05 \times 10^{-4}$	$1{\cdot}05 \times 10^{-5}$	5	127·9	−254·4	0·0	1
Time series									
Offspring model	1·04	$-1{\cdot}52 \times 10^{-3}$	–	–	4	439·3	−870·6	157·2	0
Parental model	0·91	$-1{\cdot}87 \times 10^{-3}$	$-1{\cdot}45 \times 10^{-3}$	–	5	512·6	−1015·1	12·65	0
Interaction model	1·04	$-2{\cdot}47 \times 10^{-3}$	$-4{\cdot}31 \times 10^{-4}$	$4{\cdot}71 \times 10^{-6}$	6	519·9	−1027·7	0	1

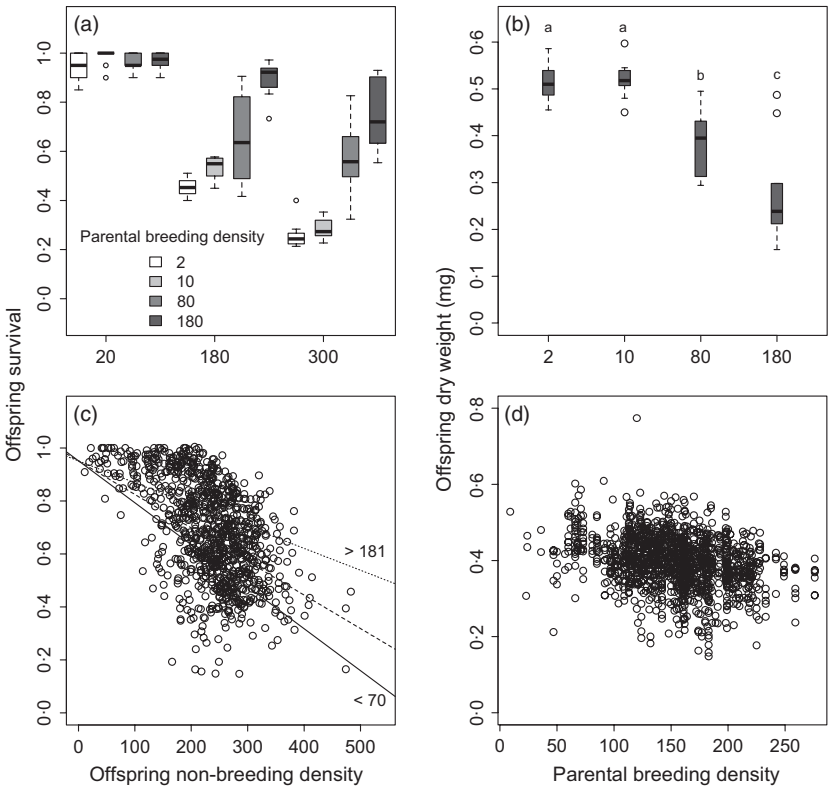

Fig. 1. The effect of parental breeding density on offspring survival and female dry weight. Top panels (a and b) represent results from a controlled experiment, and bottom panels (c and d) represent results from a 45 replicates populations of *D. melanogaster* kept for 36 generations in a seasonal environment. Left panels show the relationship between offspring survival and offspring non-breeding density for (a) different parental breeding densities treatments and for (c) the long-term seasonal population over a natural range of densities. Right panels represent the effect of parental breeding density on offspring dry weight in (b) the controlled laboratory experiment and in (d) the long-term seasonal population. The horizontal line within each box plot in panels (a) and (b) represents the median value, the edges are 25th and 75th percentiles, the whiskers extend to the most extreme data points, and dots are potential outliers. In (c), the lines represent the regression line between offspring survival and offspring non-breeding density when parental breeding density was <70 (solid line), between 71 and 180 (dashed line), and >181 (dotted line). Offspring that came from high parental density tended to have high survival in both (a) controlled and (c) long-term seasonal populations, and high parental density resulted in smaller flies (b, d). In (b), different letters indicate a significant difference between breeding density treatments according to Tukey HSD test ($P < 0.01$).

$2 * a * N_{\mathrm{nb}(t)} + b = 0$ for $N_{\mathrm{nb}(t)}$ ($N_{\mathrm{nb}} = 185$) and found the slope for this value ($-7{\cdot}99 \times 10^{-4}$). Thus, in our simulations, parental densities >185 always resulted in $K_{\mathrm{nb}} = 3{\cdot}92\ 10^{-2}/-7{\cdot}99 \times 10^{-4} = -41$, meaning that the strength of density dependence survival did not get weaker for parental breeding densities >185.

At low levels of $r_{\mathrm{b\ (max)}}$ (i.e. more stable dynamics), the positive relationship between parental density and offspring survival had almost no effect on population size compared to a model without the effects of parental density (Fig. 3a,b). However, as $r_{\mathrm{b\ (max)}}$ increased, the effects of parental density on offspring survival stabilized dynamics by decreasing the differences in population size between seasons (Fig. 3a,b). This happened because the positive relationship between parental breeding density and offspring survival for densities >185 was constant and stronger than the strength of density dependence survival in the model without the effects of parental density ($K_{\mathrm{nb}} = -41$ and -84, respectively). Strong density dependence survival results in high mortality during the non-breeding season, fewer individuals moving to the next breeding season and less fluctuation in population size between seasons.

We then inverted the relationship between parental breeding density and survival, such that low parental breeding density resulted in slopes values closer to 0 (Fig. 2). This relationship could be described as

$$a' * N_{\mathrm{nb}(t)}^2 + b' * N_{\mathrm{nb}(t)} + c' \qquad \text{eqn 4}$$

where a', b' and c' are constants estimated from the data with a nonlinear (weighted) least-squares regression ($a' = -1{\cdot}11 \times 10^{-7}$, $b' = 1{\cdot}75 \times 10^{-6}$, $c' = -7{\cdot}99 \times 10^{-3}$, Fig. 2), and $N_{\mathrm{nb}(t)}$ is as described above. Similar to above, we constrained the minimum and maximum values for the slope. Thus, in our simulations, the slope assumed the maximum value of $4{\cdot}29 \times 10^{-3}$ ($K_{\mathrm{nb}} = -7{\cdot}67$) for parental breeding values ($N_{\mathrm{nb}(t)}$) > 185 and the minimum value of $-7{\cdot}99 \times 10^{-4}$ ($K_{\mathrm{nb}} = -41$) for densities <2, which is the opposite to what we simulated with eqn 3 (Fig. 2b).

Our simulations showed that when $r_{\mathrm{b\ (max)}}$ is low (<2), the negative relationship between parental density and

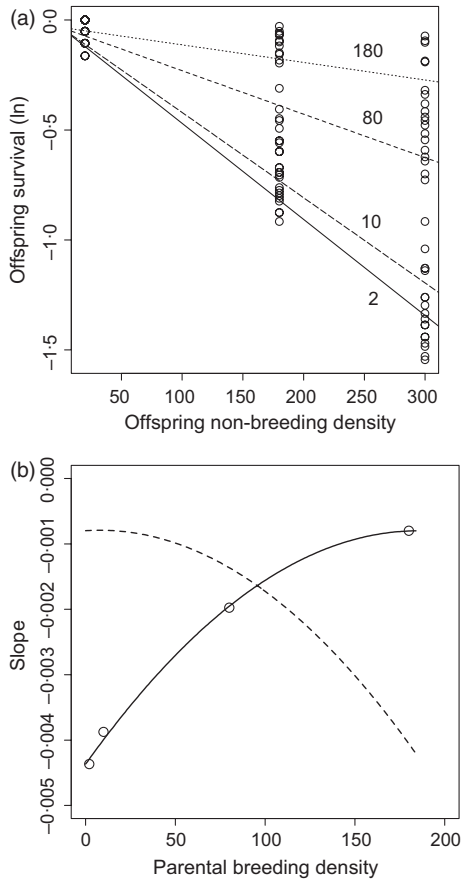

Fig. 2. (a) The relationship between offspring survival (ln) and offspring non-breeding density for different parental breeding densities (2, 10, 80 and 180). The lines represent the regression line between survival and offspring non-breeding density when parental breeding density was 2 ($\beta = -4{\cdot}37 \times 10^{-3}$, SE = $1{\cdot}25 \times 10^{-3}$, $t = -34{\cdot}88$, $P < 0{\cdot}001$, $R^2 = 0{\cdot}98$), 10 ($\beta = -3{\cdot}87 \times 10^{-3}$, SE = $1{\cdot}19 \times 10^{-3}$, $t = -32{\cdot}33$, $P < 0{\cdot}001$, $R^2 = 0{\cdot}96$), 80 ($\beta = -1{\cdot}97 \times 10^{-3}$, SE = $1{\cdot}91 \times 10^{-3}$, $t = -10{\cdot}31$, $P < 0{\cdot}001$, $R^2 = 0{\cdot}78$) and 180 ($\beta = -7{\cdot}98 \times 10^{-4}$, SE = $1{\cdot}14 \times 10^{-3}$, $t = -7{\cdot}02$, $P < 0{\cdot}001$, $R^2 = 0{\cdot}62$). Intercept was held constant in all regressions ($-3{\cdot}29 \times 10^{-2}$) and was calculated from our controlled experiments as the average of survival for all parental breeding density when offspring non-breeding density = 20. (b) Changes in slope of the relationship between offspring survival and offspring non-breeding density as influenced by parental breeding density. The solid line represents the predicted values based on eqn 3, where high parental density resulted in weaker density dependence survival (i.e. high offspring survival), and the dotted line represents the predicted values for eqn 4, where high parental density resulted in stronger density dependence survival (i.e. low offspring survival).

offspring survival caused populations to fluctuate more between breeding and non-breeding seasons, destabilizing dynamics (Fig. 3c), but the second bifurcation and more complex dynamics only happened at higher levels of $r_{b\ (max)}$ and therefore had a stabilizing effect. Finally, to mimic what was done in the long-term time series, we also re-ran all models with an added 5% mortality at the end of each season, and the results were very similar (ESM, Fig S4, Supporting information).

Discussion

Using both a controlled experiment and observations from a replicated seasonal population, our results provide evidence that variation in density during the breeding season influenced offspring state, which then carries over to influence offspring survival and that this has long-term consequences for population dynamics in seasonal environments. Offspring originating from high parental densities were smaller and, contrary to our expectations, had higher survival during the non-breeding season. Although the survival benefits of large body size has been widely recognized (Peters 1986; Kingsolver & Huey 2008), there is evidence that, in some cases, it can be costly to produce and maintain a large size (Stockhoff 1991; Roff 1992; Blanckenhorn 2000; Reznick, Nunney & Tessier 2000; Munch, Mangel & Conover 2003; Gotthard, Berger & Walters 2007; Monaghan 2008). For example, large individuals grow slowly, which is usually negatively related to survival (Kingsolver & Huey 2008). They also require more resources for maintenance, which might be costly if large body does not translate to large fat reserves (Munch, Mangel & Conover 2003). Indeed, in our system, larger flies tended to have lower survival and consume more food than smaller flies (Fig. S5, Supporting information). Thus, in many seasonal environments, smaller individuals might have an advantage over larger individuals, especially for small-bodied species who may not always have sufficient fat reserves to sustain themselves during extended periods of physiologically challenging conditions (i.e. long winters).

Our results suggest that the effects of delayed density dependence on population dynamics in seasonal environments might be related to life history. When there was a positive relationship between parental breeding density and offspring survival, population fluctuation between breeding and non-breeding seasons was dampened at high values of intrinsic growth rate ($r_{b\ (max)} > 2{\cdot}5$). High fecundity is expected for small-bodied organisms (Sæther & Engen 2002; Herrando-Pérez *et al.* 2012), and our results suggest that for such life history, smaller individuals might have an advantage during the non-breeding season. In contrast, a model that incorporated the negative relationship between parental breeding density and offspring survival destabilized dynamics at low levels of intrinsic growth rate by increasing fluctuations between seasons. Large-bodied organisms are expected to have low fecundity (Sæther & Engen 2002; Herrando-Pérez *et al.* 2012), and empirical evidence suggest that for such life history, larger individuals have an advantage during the non-breeding season (Clutton-Brock *et al.* 1987; Forchhammer *et al.* 2001). Thus, we expect delayed density dependence to stabilize dynamics for fast-life-history species and destabilize dynamics for slow-life-history species.

Although our results show that size is inversely related to survival during the non-breeding season, it is usually positively related to fecundity (Miller & Thomas 1958; Mueller & Joshi 2000). This has important evolutionary

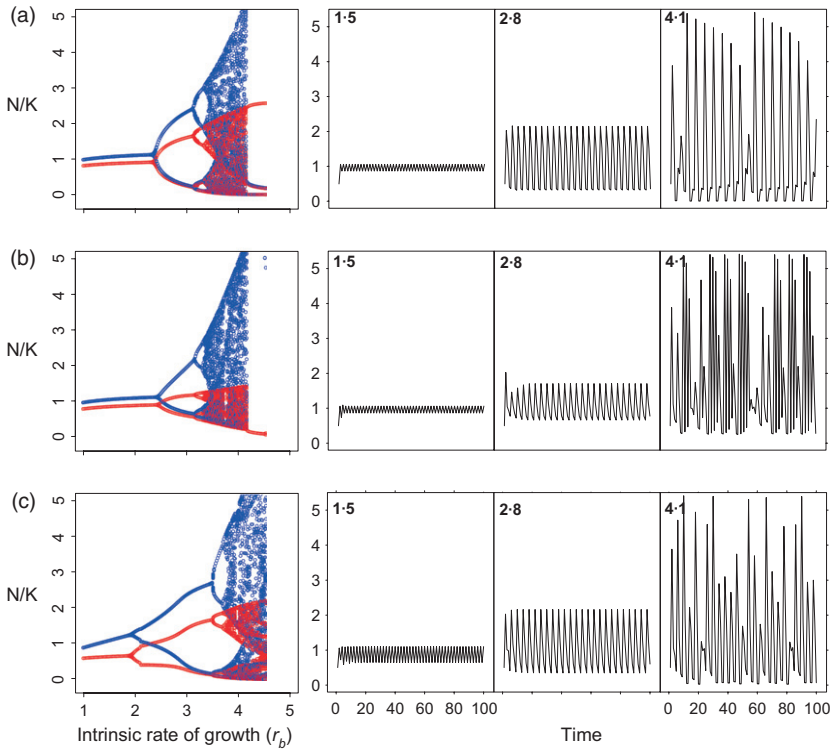

Fig. 3. Dynamics of the (a) biseasonal model, (b) biseasonal model when offspring survival is positively correlated with parental breeding density (as shown in our experimental results) and (c) biseasonal model when offspring survival is negatively correlated with parental breeding density (as shown in other studies). Bifurcation plots are shown on the left, and the corresponding time series on the right, with $r_{b\ (max)}$ values on the top left of each time series. The blue and red trajectories on the bifurcation plots indicate population size at the end of the breeding and non-breeding seasons, respectively. n, population size; K, carrying capacity. Parameters used in the models: $r_b = 2{\cdot}24$, $K_b = 200$ and $r_{nb} = -3{\cdot}29 \times 10^{-2}$ for all models; (a): $K_{nb} = -41$; (b): $a = -1{\cdot}04 \times 10^{-7}$, $b = -3{\cdot}85 \times 10^{-2}$, $c = 4{\cdot}37 \times 10^{-3}$; (c): $a' = -1{\cdot}11 \times 10^{-7}$, $b' = 1{\cdot}75 \times 10^{-6}$, $c' = -7{\cdot}99 \times 10^{-3}$.

consequences because it suggests that two distinct selective pressures could be operating on the same individual in different periods of an annual cycle. In seasonal environments, an individual cannot specialize in only one season, and an intermediate phenotype that does moderately well in both seasons could be favoured (Levins 1968). However, natural fluctuations in density could prevent an intermediate phenotype from becoming frequent in the population and, instead, result in fluctuating selection over time. We have shown that average body size decreases with an increase in population size. Thus, fecundity will also decrease, which would result in lower population size. This could, in turn, release the selective pressure for smaller individuals in the non-breeding season, and larger individuals would be favoured because of the selective pressure for high fecundity. As the frequency of large individuals increases, population size should also increase, causing a new, strong selective pressure for small individuals in the non-breeding season. This type of feedback loop between ecological and evolutionary processes is now believed to be widespread in nature (Yoshida *et al.* 2003; Schoener 2011; Smallegange & Coulson 2013), and our results suggest that they could occur in seasonal environments through the interplay between fluctuations in density and life-history trade-offs between seasons.

Although we have shown that parental breeding density influences offspring body size, we did not identify the specific mechanism that drives this relationship. One hypothesis is that high parental densities result in high levels of larval competition for food, which then leads to a relatively small adult body size after development (Mueller 1985). Alternatively, high parental breeding densities could result in smaller eggs and therefore smaller offspring. If the latter was the case and females made different decisions about how to invest in their offspring, then carry-over effects from the non-breeding to the breeding season could cause maternal effects, which could intensify or weaken the effects of parental breeding density on offspring survival (Plaistow & Benton 2009). Although high larval densities would most likely to be a result of high parental breeding density resulting in high larval competition, it would be interesting to experimentally separate the effects of offspring survival due to high parental breeding density from the effects of high larval density.

We have previously shown that variation in density during the non-breeding season causes individuals to be in poor physiological condition, which influences their reproductive output the following breeding season (Betini, Griswold & Norris 2013a,b). Here, we have shown that variation in density during the breeding season affects the subsequent sur-

vival of offspring. Thus, there is a potential for these two mechanisms to interact. For example, if density in the non-breeding season was high, then individuals would be in poor physiological condition and have relatively low per capita breeding output in the following breeding season. Fewer eggs (i.e. low larvae competition) would result in larger offspring compared to a scenario where females would not be under physiological stress. Thus, carry-over effects caused by variation in non-breeding density could buffer the effects of parental density on offspring survival. The potential for long lags has been documented in natural population subjected to strong seasonality (Merritt, Lima & Bozinovic 2001; Stenseth *et al*. 2003) and could even be operating in our simple laboratory system.

Negative feedback caused by delayed density dependence has been shown to be an important factor to explain dynamics of natural populations (Turchin 1990; Beckerman *et al*. 2002, 2006; Inchausti & Ginzburg 2009), particularly in seasonal environments (Lima 2001; Stenseth *et al*. 2003). However, little is known about the mechanisms involved, and the hypotheses proposed to explain the presence of such lags in response to density usually evoke an interaction with other species, such as changes in food web structure or response to predators (Hansen, Stenseth & Henttonen 1999; Merritt, Lima & Bozinovic 2001; Stenseth *et al*. 2003). Under this context, delayed density dependence is believed to destabilize dynamics by creating cycles (Beckerman *et al*. 2002, 2006; Stenseth *et al*. 2003). Our model system provides evidence that variation in abundance in both the non-breeding (Betini, Griswold & Norris 2013a,b) and breeding (this study) seasons can affect the state of the individual in a non-lethal manner, which can then carry over to the next season to affect individual performance and population dynamics. Taken together, these results suggest that delayed density dependence can be caused by intrinsic dynamics of the system, such as non-lethal individual carry-over effects, resulting in a strong stabilizing effect by damping cycles that would otherwise happen under direct density dependence.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1. Schematic of the experimental set-up.

Figure S2. Offspring dry weight (male) was negatively related to parental breeding density in both the (a) controlled experiments ($F_{3,36} = 16{\cdot}73$, $P < 0{\cdot}001$) and (b) long-term seasonal populations (Pearson's product-moment correlation = $-0{\cdot}15$, d.f. = 1309, $P < 0{\cdot}001$). In (a) the horizontal line within each box represents the median value, the edges are 25th and 75th percentiles, the whiskers extend to the most extreme data points, and points are potential outliers ($n = 1311$ males). Different letters indicate a significant difference in offspring weight caused by different parental breeding densities according to Tukey HSD test ($P < 0{\cdot}01$).

Figure S3. Offspring dry weight negatively affected offspring survival after controlling for density at the beginning of the non-breeding season.

Figure S4. Dynamics of the (a, b) biseasonal model, (c, d) biseasonal model when offspring survival is positively correlated with parental breeding density (as in experimental results) and (e, f) biseasonal model when offspring survival is negatively correlated with parental breeding density (as shown in other studies). Left panels are as presented in the main text, and right panels represent results from the same models, but accounting for the 5% of individuals that were removed from the populations at the end of each season, as in the long-term seasonal population.

Figure S5. High parental density caused per capita offspring food consumption to increase, after controlling for density at the beginning of the non-breeding season ($F_{3,28} = 21{\cdot}04$, $P < 0{\cdot}001$).

Table S1. Number of replicates used in the controlled experiments to test the effect of breeding density on offspring survival during the following non-breeding season (Fig. S1).

Table S2. Parameter estimates of three competing models to investigate variation in offspring survival during the non-breeding season in controlled laboratory experiments (Table 1 and Fig. 1a in the main text). R^2 for the best model (the '*interaction model*') = 0·88.

Table S3. Parameter estimates for three competing models to investigate variation in offspring survival during the non-breeding season in long-term seasonal populations of *Drosophila melanogaster* submitted to two distinct breeding and non-breeding seasons (Table 1 and Fig. 1c in the main text).

Table S4. Parameter estimates for two mixed-effect models two investigate the effects of offspring dry weight on offspring survival during the non-breeding season.