Density-dependent selection and the maintenance of colour polymorphism in barn owls

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Abstract

The capacity of natural selection to generate adaptive changes is according to the Fundamental Theorem of Natural Selection proportional to the additive genetic variance in fitness. In spite of its importance for development of new adaptations to a changing environment, processes affecting the magnitude of the genetic variance in fitness-related traits are poorly understood. Here we show that the red-white colour polymorphism in female barn owls is subject to density-dependent selection at the phenotypic and genotypic level. The diallelic melanocortin-1 receptor (MC1R) gene explained a large amount of the phenotypic variance in reddish colouration in the females (R^2 = 59.8 %). Red individuals (RR genotype) were selected for at low densities, while white individuals (WW genotype) were favoured at high densities and were less sensitive to changes in density. We show that this density-dependent selection favours white individuals and predicts fixation of the white allele in this population at longer time scales without immigration or other selective forces. Still, fluctuating population density will cause selection to fluctuate and periodically favour red individuals. These results suggest how balancing selection caused by fluctuations in population density can be a general mechanism affecting the level of additive genetic variance in natural populations.

Keywords: Population density, individual fitness, natural selection, reproductive value, *Tyto alba*

1 Introduction

One of the most important contributions in evolutionary biology is Fisher's [1] Fundamental Theorem of Natural Selection, stating that the partial rate of increase in fitness is equal to its additive genetic variance in fitness [2,3]. This implies that consistent natural selection favouring genotypes of high fitness may deplete the additive genetic variance in fitness [4], reducing the capacity of a population to change genetically from one generation to the next. Still, comparative analyses of heritability and evolvability suggest that there is substantial potential for evolutionary changes in most traits, but also reveal large variation between traits [5, 6]. Factors affecting this large variation in additive genetic variance are poorly understood and is a major reason for why evolutionary biologists have found it difficult to develop reliable predictions of evolutionary responses to changes in the environment [7].

Fluctuations in environmental conditions and population density affect the vital rates of natural populations, causing populations to fluctuate in size [8]. The close link between the population growth rate and expected absolute fitness means that feedbacks between population density and adaptive evolution may induce density-dependent selection [9–17]. Then mean fitnesses of different phenotypes and genotypes depends on population density and selection will have both density-dependent and density-independent components [14, 16–20]. MacArthur and Wilson [21] introduced the concept of r- and K-selection, where r is the population growth rate in the absence of density regulation (very small values of N) and K is the carrying capacity of the population. This model proposes that those phenotypes favoured at small population densities are selected against at population sizes close to the carrying capacity [18, 21–26]. Theoretical models have shown that this can lead to fluctuating selection around an intermediate value of the phenotype [14, 18, 19, 23–25] (but see Asmussen [27]), which in a fluctuating environment facilitates maintenance of genetic variance. Accordingly, a combination of density-dependent and stabilizing selection in great tits *Parus major* causes the mean clutch size per season to fluctuate around an intermediate optimum maximizing the expected population size [16]. Thus, fluctuations in population size may provide a general agent of selection which also

may maintain additive genetic variance in fitness-related characters.

Analyses of colour polymorphisms have been popular study systems in evolutionary biology for a long period of time [28, 29]. Such polymorphisms are often determined by one or a few genes of large effect [30–32] and frequently related to variation in individual fitness components [29, 33–37]. Importantly, in several natural populations of animals there exists colour polymorphisms which seems to be relatively stable over time [30,38,39]. This suggests the action of balancing selection, which is any form of natural selection that promote the maintenance of polymorphisms at a higher level than would be expected from genetic drift and mutation rates alone [23, 39–42].

Barn owl *Tyto alba* populations harbours a distinct colour polymorphism, where the ventral body parts vary in colouration between individuals from white to dark red and also have a variable number and size of black spots [31,32,43]. Colouration and spottiness are largely heritable [43] and the diallelic melanocortin-1 receptor (*MC1R*) gene has been identified as a gene of large effect [31,32]. The variation in these two traits are to different degrees phenotypically correlated to individual differences in behaviour and to variation in several life-history traits [44–46]. In addition, plumage colouration affects the perception of individuals by predators, conspecifics and prey species such that performance of the individuals may depend on their colouration [29, 46, 47].

In this study, we examine density-dependent selection on colouration and spottiness in female barn owls in a Swiss study population. Individuals have their phenotype scored for the degree of eumelanic spottiness and reddish pheomelanic colouration, and are genotyped for the *MC1R*-gene. We explore whether balancing selection caused by fluctuations in population size is able to maintain a colour polymorphism at the genetic and phenotypic level. To do this we apply a new evolutionary maximization principle showing that in populations subject to density regulation evolution tends to maximize the expected population size [14, 19].

2 Material and methods

Data collection

The data were collected from 1990 to 2016 in a population of barn owls *Tyto alba* on the plains south-west of lake Neuchâtel in western Switzerland (46° 49'N, 06° 56' E). The birds are sexually mature at age one year and mainly breed in nest boxes on farms in the area. Females can lay one or two broods of two to 11 eggs (median clutch size: 6 eggs) from late February to mid-August and incubate the eggs for approximately 32 days. Nestlings are fed in the nest until they fledge at approximately 55 days of age. The total annual population size (N) of the barn owls ranged from 52 to 187 individuals in the study period with a mean of 113.1 (SD = 40, Fig. A1).

Females were captured at the nest during incubation. Nestlings and any unmarked adults were marked with a numbered metal ring, then blood and feather samples were drawn for DNA. Nestling sex was identified using sex-specific molecular markers (the SPINDLIN-gene [48]), while adult breeding females were recognized based on the presence of a brood patch. Year of birth was known for females marked as nestlings, for other individuals age (in years) was deduced based on moult patterns in coverts and flight feathers. Since 1994, in the plumage for each of m body parts on adult females, the number (ν_m) and mean diameter ($d_m \pm 0.1$ mm) of eumelanic black spots and the degree of reddish pheomelanic colouration (c_m) were recorded. The black spots were assessed within a 60×40 mm (2400 mm^2) frame and the reddish coloration was scored using eight-colour chips ranging from -8 (white) to -1 (dark reddish). The measured body parts were the breast, belly, each flank and each of the undersides of the wings, to account for the differential expression of the traits. For each individual we standardized the traits to age 1 year old using a mixed-effects model with three age classes (1, 2 and 3+ years old) and random slope and intercept for each individual. Then we calculated the mean spottiness (area of the plumage covered by black spots $= 100 \times (\Sigma_1^m \pi r_m^2 \nu_m/2400)/m$, where $r_m = d_m/2$) and mean colour ($\Sigma_1^m c_m/m$) across the m measured body parts.

In the period 1996-2016, genomic DNA was extracted from blood samples of dried

feathers using the DNeasy Tissue and Blood kit or the Biosprint robot (Qiagen, Hombrechtikon, Switzerland). Then the melanocortin-1 receptor (*MC1R*) genotype was determined using allelic discrimination. Each individual was genotyped twice using PCR products from independent duplicated runs. Detailed protocols on DNA extraction and genotyping can be found in San-Jose et al. [31]. A total of 540 females were genotyped, resulting in 374 WW, 155 WR and 11 RR individuals in our sample. For various reasons, a few females each year were not available for genotyping. In 1996, 20 individuals were not genotyped, but for the years 1997-2016 there were 0 (5 years), 1 (4 years), 3 (2 years), 5 (1 year), 6 (1 year) or 7 (1 year) females that were not genotyped.

We structured the data using pre-breeding census. Hence, age 1 year old was the first age class, survival was recorded as 1 if an individual was alive in the beginning of the next years breeding season (otherwise 0) and reproduction was determined as the number of nestlings which were alive to recruit into the next years breeding population (i.e. the number of recruits). Juvenile (age < 1) emigration is common in this population; here emigrants are treated as locally dead individuals (which reduced the estimated fecundity rates). The recapture rate has previously been estimated as 0.84 for adults in this population [49].

Density-dependent selection

Model

The population vector of an age-structured population is denoted as $\mathbf{n} = (n_1, n_2, ..., n_k)^T$, where T denotes matrix transposition, and the total population size $N = \Sigma n_x$ for age classes $x = (1, 2, ..., k)$. As we only work with the female subset of the population, we assume that there are always adequate numbers of males present for all females to be mated. In fluctuating environments, the population growth of density regulated age-structured populations is governed by the stochastic projection matrix $\mathbf{L}$ such that $\mathbf{\Delta n} = \mathbf{Ln} - \mathbf{n}$ [50], where $\mathbf{L}$ in general is a function of $\mathbf{n}$. We now assume that density regulation only act through the total population size such that $\mathbf{L} = \mathbf{L}(N)$. For a large population we then have $\mathbf{L}(N) = \bar{\mathbf{L}}(N) + \boldsymbol{\varepsilon}$, where $\bar{\mathbf{L}}(N)$ is the expected projection ma-

trix at population size N and $\boldsymbol{\varepsilon}$ is an environmental noise term. The non-zero elements of $\bar{\mathbf{L}}(N)$ are the fecundities $f_x(N) = f_x^* F_x(N)$ for all ages in the first row and the survivals $s_x(N) = s_x^* S_x(N)$ for ages 1 to k-1 on the subdiagonal. Additionally, one may have $s_k(N)$ in the lower right element $\bar{L}(N)_{k,k}$ if age class k collects all individuals aged $\geq k$. Here f_x^* and s_x^* are the density-independent vital rates and $F_x(N)$ and $S_x(N)$ are density-dependent functions for fecundity and survival. For logistic density regulation, $F_x(N) = e^{-a_x N}$ and $S_x(N) = e^{-b_x N}$, where a_x and b_x measures the sensitivity of the vital rates in age class x to population density.

At the carrying capacity $N = K$ we have the equilibrium projection matrix $\hat{\mathbf{L}} = \bar{\mathbf{L}}(K)$ with growth rate $\lambda = 1$ given by its real dominant eigenvalue. The stable age distribution $\mathbf{u}$ and reproductive values $\mathbf{v}$ at equilibrium are given as the left and right eigenvectors of $\hat{\mathbf{L}}$ scaled to $\Sigma u_x = 1$ and $\Sigma v_x u_x = 1$ [8, 51, 52]. At equilibrium, when the population has reached its stable age distribution the total reproductive value V of the population equals the carrying capacity, $V = \mathbf{v}\hat{\mathbf{n}} = K$, where $\hat{\mathbf{n}} = K\mathbf{u}$ (Appendix 1, 'Dynamics of reproductive values under density dependence'). The reproductive value v_x is then the expected contribution of an individual of age x to the growth of the equilibrium population [1].

We assume weak density dependence, such that the population mostly experience small deviations from equilibrium, to allow the reproductive values at N to be approximated by the reproductive values at equilibrium [51]. The annual contribution of individual i in age class x to the population next year can now be defined as [52, 53]

$$\Lambda_i = W_i / v_x = \frac{J_i v_{x+1} + B_i v_1 / 2}{v_x}, \tag{1}$$

where Λ_i is the individual fitness, W_i is the individual reproductive value [52], J_i is a dichotomous indicator of survival (1/0), B_i is the number of recruits produced and the v's are age specific reproductive values at equilibrium. B_i is multiplied by 1/2 to account for sexual reproduction, assuming sex ratio 1:1. The scaling by the reproductive value ensure that $\tilde{\mathrm{E}}(\Lambda_i) = \Sigma v_i \Lambda_i / \Sigma v_i = \Sigma W_i / \Sigma v_i = (\Delta V + V)/V = \lambda = e^r$ independent of age at equilibrium [52, 53], where λ is the deterministic growth rate and r is the Malthusian

parameter. Henceforth, notations with ˜ indicates reproductive value (rv) weighting, as originally proposed by Fisher [1].

Let there be variation among individuals in a fitness related phenotype z. Furthermore, assume weak selection such that changes in z only cause minor changes to the elements of the equilibrium projection matrix $\hat{\mathbf{L}}$ [54, 55]. Then the expected fitness of an individual of phenotype z at population size N in environment ε can be written as $\tilde{\mathrm{E}}(\Lambda|z,N,\varepsilon) = e^{\tilde{M}(z,N,\varepsilon)}$, where $\tilde{M}(z,N,\varepsilon)$ is the conditional Malthusian parameter [14, 16]. Taking the expectation over the fluctuating environment we have [14, 16]

$$\tilde{\mathrm{E}}_{\varepsilon}\ln(\Lambda|z,N,\varepsilon) = \tilde{m}(z,N) \approx \tilde{r}(z,N) - \frac{1}{2}\sigma_e^2, \tag{2}$$

where $\tilde{m}(z,N)$ is the mean Malthusian parameter and $\tilde{r}(z,N)$ is the deterministic growth rate of a population fixed for phenotype z and population size N, and σ_e^2 is the environmental variance [16].

Similarly as Engen et al. [14] and Sæther et al. [16], we define the model $\tilde{r}(z,N) = \tilde{r}(z) - \tilde{\gamma}(z)g(N)$, where $\tilde{r}(z)$ governs the growth rate as the population size approach zero, $\tilde{\gamma}(z)$ defines the strength of density regulation and $g(N)$ is a function for the form of density regulation. Henceforth, we define $g(N) = N$ for logistic density regulation. In this model, the mean Malthusian fitness is $\bar{m}(\tilde{z},N) = \bar{s}(\tilde{z}) - \bar{\gamma}(\tilde{z})N$, where $\bar{s}(\tilde{z}) = \bar{r}(\tilde{z}) - 1/2\sigma_e^2$ (the long-run growth rate in the absence of density regulation), $\bar{r}(\tilde{z}) = \Sigma_{i=1}^{N}\tilde{r}(z)/N$ and $\bar{\gamma}(\tilde{z}) = \Sigma_{i=1}^{N}\tilde{\gamma}(z)/N$ [14]. With density dependent selection, the rv-weighted mean phenotype $\tilde{z}$ is expected to evolve towards the value $\tilde{z}_{opt}$ that maximize the function $Q(\tilde{z}) = \bar{s}(\tilde{z})/\bar{\gamma}(\tilde{z})$, which is the expected value of N [14]. The selection gradient on the phenotype in this model is given by $\nabla\bar{m}(\tilde{z},N) = \nabla\bar{s}(\tilde{z}) - \nabla\bar{\gamma}(\tilde{z})N$ [14, 16, 19], where ∇ indicates the derivative with respect to $\tilde{z}$.

Let the variation in the phenotype partly be caused by a diallelic locus, with alleles 1 and 2. The total reproductive value of allele 1 in the population is then $V_1 = \mathbf{v}\mathbf{X}_1$ [54], where $\mathbf{X}_1$ is the column vector for the numbers of allele 1 for each individual and $\mathbf{v}$ is the row vector with the age specific reproductive values at equilibrium for each individual. Thus, the rv-weighted mean allele frequency $\tilde{p} = V_1/(V_1 + V_2)$. The expected fitness of

an individual with genotype kl at population size N in environment ε can be written as $\tilde{\mathrm{E}}(\Lambda|kl,N,\varepsilon) = e^{\tilde{M}(kl,N,\varepsilon)}$. Taking the expectation over the environment we have

$$\tilde{\mathrm{E}}_\varepsilon \ln(\Lambda|kl,N,\varepsilon) = \tilde{m}(kl,N) \approx \tilde{r}(kl,N) - \frac{1}{2}\sigma_e^2, \tag{3}$$

where $\tilde{r}(kl,N) = \tilde{r}(kl) - \tilde{\gamma}(kl)N$, and we assume that the environmental variance σ_e^2 is independent of the genotype. Similarly to the phenotypic model, $\tilde{r}(kl)$ governs the growth rate in the absence of density regulation and $\tilde{\gamma}(kl)$ defines the strength of density regulation. We assume that the locus is pleiotropic and affects both the density-independent and the density-dependent component of the Malthusian fitness. Then, following Falconer [56] we can define the genotypic values for each genotype as $(\tilde{a}_r, \tilde{a}_\gamma)$, $(\tilde{d}_r, \tilde{d}_\gamma)$ and $(-\tilde{a}_r, -\tilde{a}_\gamma)$, where $\tilde{a}_r = \tilde{r}(11) - \tilde{r}_0$, $\tilde{a}_\gamma = \tilde{\gamma}(11) - \tilde{\gamma}_0$, $\tilde{d}_r = \tilde{r}(12) - \tilde{r}_0$ and $\tilde{d}_\gamma = \tilde{\gamma}(12) - \tilde{\gamma}_0$. Here, $\tilde{r}_0 = (\tilde{r}(11) + \tilde{r}(22))/2$ and $\tilde{\gamma}_0 = (\tilde{\gamma}(11) + \tilde{\gamma}(22))/2$, i.e. the midpoints between the expectation for components of the growth rate of the homozygotes. The expected mean Malthusian fitness can then be given as

$$\bar{m}(\tilde{p},N) = \bar{s}(\tilde{p}) - \bar{\gamma}(\tilde{p})N, \tag{4}$$

where $\bar{s}(\tilde{p}) = \bar{r}(\tilde{p}) - 1/2\sigma_e^2$, $\bar{r}(\tilde{p}) = \tilde{a}_r(\tilde{p}-\tilde{q}) + 2\tilde{p}\tilde{q}\tilde{d}_r + \tilde{r}_0$, $\bar{\gamma}(\tilde{p}) = \tilde{a}_\gamma(\tilde{p}-\tilde{q}) + 2\tilde{p}\tilde{q}\tilde{d}_\gamma + \tilde{\gamma}_0$ and $\tilde{q} = 1 - \tilde{p}$. Hence, we can define a function $Q(\tilde{p}) = \bar{s}(\tilde{p})/\bar{\gamma}(\tilde{p})$, which is a maximization principle for the evolution of the allele frequency $\tilde{p}$ under density-dependent selection (Appendix 1, 'Maximization principle for allele frequency under density-dependent selection'). Adaptive evolution is expected to maximize the Q-function as the population mean allele frequency evolve towards $\tilde{p}_{opt}$. The selection gradient on allele frequency in this model is given by $\nabla\bar{m}(\tilde{p},N) = \nabla\bar{s}(\tilde{p}) - \nabla\bar{\gamma}(\tilde{p})N = 2[\tilde{a}_r - \tilde{a}_\gamma N + (\tilde{q}-\tilde{p})(\tilde{d}_r - \tilde{d}_\gamma N)]$, where ∇ indicates the derivative with respect to $\tilde{p}$.

Estimation

Age classes 8-15 were collapsed to age class 8+ to ensure that there were sufficient numbers of individuals in each age class in the analyses ($n_x \geq 10$). First we estimated the observed

mean projection matrix $\bar{\mathbf{L}}(N)$ by taking the average of annual age specific survival (J) and recruit production ($B/2$) over years (Table A2a). Then to estimate the elements $(\mathbf{s}(N), \mathbf{f}(N))$ of the expected equilibrium projection matrix $\bar{\mathbf{L}}(K)$ we applied separate generalized linear models (GLMs) for survival and recruit production with age categories and $\Delta N/N$ as explanatory variables (Table A1). For survival we fitted a GLM with binomial error distribution and a logit link function, and for fecundity we fitted a GLM with Poisson error distribution, a log link function, and an offset of ln2 and weights 1/2 to account for sexual reproduction (on average, half of the recruits are females). $\mathrm{E}(\Delta N/N|N) = \lambda(N, \phi) - 1$, where ϕ collects all parameters affecting the population growth rate [8]. Hence, we predicted survival and fecundity rates at K from the GLMs by setting $\Delta N/N = 0$ (Table A2b). Due to emigration of juveniles, which result in reduced estimates of fecundities in the study population, the growth rate of $\bar{\mathbf{L}}(K)$ was lower than one ($\lambda_K = 0.58$). Accordingly, to obtain a stationary model at K we scaled the recruit production $\mathbf{f}(K)$ by a constant c to obtain $\bar{\mathbf{L}}^*(K)$ (Table A2c). The c was estimated by solving the Euler-Lotka equation, $c\sum \lambda(K)^{-x} l_x(K) f_x(K) = 1$, using the Newtons method. Here, $l_x(K) = \prod_{x=1}^{k-1} s_x(K)$ and $\lambda(K) = 1$. Given the equilibrium projection matrix $\bar{\mathbf{L}}^*(K)$, reproductive values ($\mathbf{v}$) and the stable age distribution ($\mathbf{u}$) were estimated as the scaled left and right eigenvector [50,52]. We also estimated reproductive values for all observed $\Delta N/N$ to investigate the difference from the reproductive values at equilibrium (Fig. A2). There were no evidence for a difference on average between the reproductive values at the observed population densities and the reproductive values at equilibrium (ANOVA: mean difference = -0.0001±0.0043, $F_{7,200} = 1.67$, $P = 0.118$, Fig. A2). Hence, the reproductive values at equilibrium were good estimates of the expected contributions of the age classes to the future population growth when averaged over N in our population.

We fitted generalized mixed effects models (GLMMs), using the R package *lme*4 (version 1.1-21), with a random intercept for year to estimate the density dependent and density independent parameters in our population (equations 2 and 3). For each phenotype (z), mean spottiness or mean colour, we define $\tilde{r}(z) = \beta_1 + \beta_2 z + \beta_3 z^2$ to allow

a test for an intermediate phenotypic optimum in the growth rate as a function of z and $\tilde{\gamma}(z) = -\beta_4 - \beta_5 z$ to test for a decrease in $\tilde{\gamma}(z)$. The selection gradient on the phenotype with this parametrisation is found to be $\nabla\bar{m}(\tilde{z}, N) = \beta_2 + 2\beta_3\tilde{z} + \beta_5 N$ [14]. To ease model convergence, the traits were standardised to a mean of 0 and unit variance prior to analyses (colour: mean = -4.586±0.045, sd = 1.412, spottiness: mean = 3.898±0.100, sd = 2.560). Parameter estimates were backtransformed and are reported for mean centered traits. Thus, the selection gradients are unscaled. Similarly for the MC1R-genotype, we define $\tilde{r}(kl) = \beta_1 + \beta_2 x_{WR} + \beta_3 x_{RR}$ to test for an effect of genotype (kl) on the growth rate and $\tilde{\gamma}(kl) = -\beta_4 - \beta_5 x_{WR} - \beta_6 x_{RR}$ to test for a difference in the density-dependence for each genotype. Here, the x_{kl}'s are dummy variables which take to value 1 for individuals of genotype kl (otherwise 0). The selection gradient on the allele frequency $\nabla\bar{m}(\tilde{p}, N) = 2[\tilde{a}_r - \tilde{a}_\gamma N + (\tilde{q} - \tilde{p})(\tilde{d}_r - \tilde{d}_\gamma N)]$ with this parametrisation is given by $\tilde{a}_r = (\beta_1 - (2\beta_1 + \beta_3)/2)$, $\tilde{a}_\gamma = -\beta_4 - (-2\beta_4 - \beta_6)/2$, $\tilde{d}_r = (\beta_1 + \beta_2) - (2\beta_1 + \beta_3)/2$ and $\tilde{d}_\gamma = (-\beta_4 - \beta_5) - (-2\beta_4 - \beta_6)/2$.

The model, specified for a given year t, was $\ln\tilde{\mathrm{E}}(\mathbf{\Lambda}_t) = \mathbf{X}_t\boldsymbol{\beta} + \mathbf{1}_t\mathbf{u}_t$, where $\mathbf{1}$ is a column vector of ones, $\mathbf{u}$ is a normal environmental noise with zero expectation and temporal variance σ_e^2, $\mathbf{X}$ is a design matrix and $\boldsymbol{\beta}$ is a column matrix with the parameters. In the model for a phenotype, $\mathbf{X}$ had column vectors $(\mathbf{1}, \mathbf{z}, \mathbf{z^2}, \mathbf{N}, \mathbf{Nz})$ and $\boldsymbol{\beta} = (\beta_1, \beta_2, \beta_3, \beta_4, \beta_5)^T$, while in the model for genotypes, $\mathbf{X}$ had column vectors $(\mathbf{1}, \mathbf{x_{WR}}, \mathbf{x_{RR}}, \mathbf{N}, \mathbf{Nx_{WR}}, \mathbf{Nx_{RR}})$ and $\boldsymbol{\beta} = (\beta_1, \beta_2, \beta_3, \beta_4, \beta_5, \beta_6)^T$. Individual fitness, $\mathbf{\Lambda}$, does not follow any well characterized distribution. Hence, for a record of individual i we define $2W_i^* = 2J_i + B_i$, which takes integer values and may be modeled using Poisson regression with a log-link function. We then define a scaling variable C_i such that $2W_i^* C_i^{-1} = \Lambda_i$ and find that

$$\begin{aligned} \ln\tilde{\mathrm{E}}(\mathbf{\Lambda}_t) &\equiv \ln\tilde{\mathrm{E}}(\mathbf{2W^*}_t\mathbf{C}_t^{-1}) = \mathbf{X}_t\boldsymbol{\beta} + \mathbf{1}\mathbf{u}_t, \\ &\equiv \ln\tilde{\mathrm{E}}(\mathbf{2W^*}_t) = \mathbf{X}_t\boldsymbol{\beta} + \ln\mathbf{C}_t + \mathbf{1}\mathbf{u}_t \end{aligned} \tag{5}$$

where $\ln\mathbf{C}_t$ is an offset with parameter value fixed at 1 and the model is fitted with weights $\boldsymbol{\omega}_t = \mathbf{v}_t\mathbf{C}_t^{-1}$, where $\mathbf{v}_t$ are the age specific reproductive values for each individual

in year t.

Migration is an important component of the dynamics in this barn owl population [49]. Hence, we had to estimate the migration rate μ needed to obtain a stable population at the carrying capacity and replace $\bar{s}(\tilde{z})$ and $\bar{s}(\tilde{p})$ by $\bar{s}(\tilde{z})+\mu$ and $\bar{s}(\tilde{p})+\mu$ in the expressions for the Q-function and the mean Malthusian fitness $\bar{m}$. The log growth rate at the carrying capacity can be estimated as $\ln\lambda_K - 1/2\sigma_e^2 + \mu = 0$, where λ_K is the deterministic growth rate obtained from the unscaled equilibrium projection matrix $\bar{\mathbf{L}}(K)$. Thus, the migration rate was estimated as $\mu = -\ln\lambda_K + 1/2\sigma_e^2$.

The significance of parameter estimates were assessed using likelihood ratio tests, in which twice the difference in log likelihood between two nested models is χ^2-distributed with degrees of freedom (df) equal to $df_1 - df_2$. Parameter estimates are provided with 95% confidence intervals (CI). All analyses were performed in the statistical software R (version 4.0.5).

3 Results

The MC1R genotype significantly affected the expression of the degree of spottiness (number and size of spots, ANOVA: $F_{2,342} = 13.09$, $P < 0.0001$, Fig. A3a and b) and the degree of reddish coloration (ANOVA: $F_{2,526} = 391.10$, $P < 0.0001$, Fig. A3c and d). Homozygote RR individuals were less spotted and more red than individuals with the WW genotype, while heterozygote WR individuals were as spotted as RR individuals and intermediate in the degree of reddish colouration (Fig. A3). Overall the MC1R genotype explained 7.1 % of the variation in spottiness and 59.8 % of the variation in reddish colouration.

There was clear evidence of density-dependence in the vital rates, with a negative relationship between $\Delta N/N$ (the multiplicative growth rate - 1) and N in the time series (regression: $b = -0.007 \pm 0.002$, $F_{1,24} = 18.48$, $P = 0.0002$, Fig. 1a). The multiplicative growth rate was positively associated with both recruit production ($b = 1.631 \pm 0.171$, $\chi^2 = 89.49$, $df = 1$, $P < 0.0001$, Fig. 1b, Table A1a) and survival ($b = 1.377 \pm 0.158$, $\chi^2 = 84.69$, $df = 1$, $P < 0.0001$, Fig. 1c, Table A1b). Thus, both fitness components contributed similarly to changes in growth rates.

Phenotypic selection

There was significant density-dependent selection on the degree of reddish colouration (β_{Nz} = -0.0018, $CI_{Nz} = [-0.0035, -0.0001]$, $\chi^2 = 4.2$, $df = 1$, $P = 0.040$, Table A3a, Figs 2 and A4), but no significant density-dependent selection on spottiness ($\beta_{Nz} =$ 0.0011, $CI_{Nz} = [-0.0003, 0.0026]$, $\chi^2 = 2.21$, $df = 1$, $P = 0.137$, Table A3b). Red individuals were favoured at low densities, while white individuals were favoured at high densities (Fig. 2a and c). The population growth rate generally decreased with increased population density due to density regulation, but white individuals were less sensitive than red individuals to changes in density (Fig. 2a). There was no significant stabilizing selection on the degree of reddish colouration (β_{z^2} = -0.0032, $CI_{z^2} = [-0.0475, 0.0394]$, $\chi^2 = 0.02$, $df = 1$, $P = 0.883$, Table A3a). Accordingly, the mean phenotype in the

population is expected to move towards white individuals as evolution maximize $Q(\tilde{z})$, the maximum expected population size as function of the mean phenotype (Fig. 2b).

Genotypic selection

At the genetic level, there was an overall trend for density-dependent selection on the MC1R genotype ($\chi^2 = 4.91$, $df = 2$, $P = 0.086$, Table A4, Figs 3 and A5). The strength of density regulation was significantly stronger in red individuals (RR genotype) than in white individuals (WW genotype) ($\beta_{Nx_{RR}}$ = -0.0275, $CI_{Nx_{RR}} = [-0.0723, -0.0006]$, Table A4), such that red individuals again were the most sensitive to changes in population density (Fig. 3a). Similarly to the results on the phenotype, red individuals had higher Malthusian fitness than white individuals at low densities, while white individuals were superior at high densities and had the highest estimated equilibrium population size (Fig. 3a and c). Over a narrow range of variation in population size, just below the mean population size ($\bar{N} = 113.1 \pm 7.7$ SE), the estimated model show a slight overdominance for mean Malthusian fitness (Fig. 3a). However, the difference between WR and WW individuals was not significant ($\beta_{Nx_{WR}}$ = -0.0030, $CI_{Nx_{WR}} = [-0.0084, 0.0024]$, Table A4). Accordingly, maximizing the value of $Q(\tilde{p})$ the population was expected to evolve towards fixation of the W-allele (Fig. 3b). The strength of selection depended on the population size and became weaker as the allele frequency ($\tilde{p}$) approached 1 (Fig. 3c).

4 Discussion

The dual role of the population growth rate in evolution and ecology means that density-dependent selection can be a particularly direct and important part of the eco-evolutionary dynamics of natural populations [12, 14–17, 19, 20, 26, 51]. Empirically, the study of this process is much more challenging than studying density-dependence and adaptation separately. It requires long-term collection of high quality data on both population parameters, such as population size and composition, and evolutionary parameters, such as phenotypes and individual fitness. The present study was facilitated by the availability of long-term individual-based level data from an intensively monitored population of barn owls. These owls have a high degree of fidelity to their breeding sites and home range, maintaining similar home ranges from year to year [49]. Thus, population size and composition could be estimated with high accuracy and precision, and individuals that were established in the population could be monitored throughout their life.

The degree of reddish pheomelanic colouration and the MC1R genotype in females were found to be under density-dependent selection in our barn owl population (see Figs 2 and 3). Directional selection was estimated to be zero at a population size around 100 and shifted between favouring red individuals at low population densities to favouring white individuals at high population density (see Figs 2 and 3). Thus, fluctuations in population size cause temporal fluctuations in directional selection, where 12 years had very high population size ($N > 120$) and 6 years had very low population size ($N < 80$, see Fig. A1). Differences in colouration generally affect the perception of individuals by conspecifics, predators and prey [29]. Accordingly, the main prey of barn owls, the common vole *Microtus arvalis*, have been shown to respond with longer freezing times when approached by a white owl compared to a dark red owl [47]. In addition, the population sizes of predators is likely to have large consequences for their prey species, modifying their abundances, anti-predator behaviours or both [47,57]. This suggests that density-dependent selection can be directly related to variation in colouration through density-dependence in predator-prey interactions. However, melanin-based colouration is generally part of a complex network of correlated traits [29, 44, 46], which include

behaviour, physiology, morphology and life-history. In barn owls, the degree of reddish colouration is related to habitat choice, but only weakly correlated to other phenotypes [44]. The size of black spots is on the contrary negatively associated with aggressiveness and the susceptibility to stress [46]. In addition, the correlation between the degree of reddish colouration and spottiness was low among females in this study ($r = 0.027$, $n = 439$). This may explain why we do not find the same pattern of selection on these two traits in this study.

Theoretical and empirical studies have shown that density-dependent selection may facilitate the existence of a stable polymorphism through balancing selection [10, 11, 14–16, 18]. In the present study, density-dependent selection in females was not associated with stabilizing selection on colouration or any clear overdominance at the equilibrium population size (see Figs 2c and 3c). Accordingly, in the long-term the density-dependent selection was not balancing and in the absence of immigrating red individuals, adaptive evolution was expected to fixate the white (W) allele in the population (see Figs 2b and 3b, Tables A3 and A4). Still, short-term fluctuations in population size could temporally maintain the red-white polymorphism in the barn owls by alternately favouring red and white individuals and make the process of fixating the white allele slow. The analysis in this study is based on the assumption that there is no environmental autocorrelation in the population dynamics [14]. Understanding the effect of any autocorrelated population dynamics on the results of this study and the evolution of colouration would require future analyses. However, Altwegg *et al.* [58] have shown that there are no significant temporal autocorrelation in the different components of survival and reproduction in this barn owl population. In terms of mean Malthusian fitness, the heterozygote WR individuals and the homozygote WW individuals did not significantly differ (see β_2 and β_5 in Table A4), suggesting that the W-allele was dominant with respect to fitness (Fig. 3a). Such dominance would additionally slow down the rate of evolution towards white individuals as directional selection would become weaker when the allele frequency approached fixation [1].

Mating in the barn owls has been found to be random with respect to the degree

of reddish colouration [59]. However, the response to selection on colouration in female barn owls also depends on the pattern of selection in males. Plumage colouration is genetically correlated between the sexes [60] and while males are less red on average, the colour polymorphism in male barn owls is otherwise similar to that in females [31]. Earlier studies have found that reddish coloured males had a higher brood size at fledging than white coloured males [60], while white coloured males had higher recruitment rate than red coloured [61]. Density-dependent selection on the degree of reddish colouration is likely to be similar in both sexes when related to the response in rodent prey to the colour of the owls [47]. Still, at a given population density and allele frequency, with identical selection surfaces, selection will not be equally strong in both sexes due to the difference in the mean colouration between males and females.

Colour polymorphisms in many species display spatial variation [30, 37, 62], which in several cases are thought to have adaptive value [37, 62]. In barn owls, the reddish colouration display a marked latitudinal gradient in North America and Europe, with a preponderance of red individuals in northern and north-eastern populations and white individuals in the southern populations [62, 63]. The maintenance of this gradient have been suggested to be due to local adaptation to prey [63] and our results suggest a role for density-dependent selection as a mechanism that affect the variation in colouration also at a spatial scale. For instance, larger environmental stochasticity in population dynamics in northern populations could lower the mean Malthusian growth rate and shift the equilibrium population density in favour of red individuals. Such latitudinal increases in environmental stochasticity has been found in two species of passerine birds [64] and in several species of ducks there were geographic differences in the magnitude of environmental stochasticity [65, 66]. Gene flow probably also contributes to the maintenance of the latitudinal gradient in the barn owls, as there is a low genetic differentiation at neutral markers between populations across Europe [67]. In addition, Ducret et al. [68] showed that the immigration rate is relatively high for both sexes in our population, but that slightly more of the females are immigrants than the males. With respect to the MC1R genotype, female immigrants were more often heterozygotes than female residents, while

male immigrants and residents had similar frequencies of the genotypes [68]. Immigration is positively correlated to emigration and the population size in the study population [49]. In terms of dispersal distance, darker reddish individuals of both sexes have been found to move a longer distance during natal dispersal than more white individuals [45, 69]. Breeding dispersal is extremely rare for barn owls in our population [49]. Overall the gene flow, especially due to female immigrants, would increase the effective population size relative to an isolated population and contribute to the maintenance of the R-allele in the local population. This gene flow would probably be quite important as the relatively rare R-allele could easily be lost by chance due to genetic drift. Generally, the impact of genetic drift on evolutionary trajectories increase with decreased population size [70]. Thus, the chance of random loss of the rare allele is increased by population crashes and periods with low population size, such as seen in the years 2009 and 2013 in our barn owl population (see Fig. A1).

Our results emphasize the importance of considering population density as an agent of selection. Specifically, the maintenance of polymorphisms within populations can be made possible by differences in density-dependent selection and reciprocal gene flow between spatially distributed populations.

Data accessibility

The data and R code for the analyses are available on Dryad (https://doi.org/10.5061/dryad.prr4xgxpd).

Author Contributions

T.K. did the analyses and wrote the paper together with B.-E.S and S.E. A.R. initiated the project, and managed and performed the fieldwork and genotyping. All authors contributed to the intellectual content through comments and edits when writing up the manuscript.

Competing interests

The authors of the present study have no competing interests to declare.

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Figure legends

Fig. 1: Density-dependence in a time series of (**a**) population size (N), and in individual records of (**b**) recruit production and (**c**) survival in in female barn owls in western Switzerland. ΔN equals N - N_{t+1} and $\Delta N/N = \lambda - 1$, where λ is the multiplicative growth rate. Small displacements have been added to the data points in **b** and **c** to avoid overlapping and improve visualisation. The solid lines are predictions of the relations between the variables from a linear regression (**a**), a Poisson regression (**b**) and a binomial regression (**c**), with dashed lines showing 95 % confidence intervals (CI). Predictions of recruit production and survival are given for barn owls in age class three.

Fig. 2: Density-dependent selection on the degree of reddish pheomelanic coloration in female barn owls. (**a**) Estimated mean Malthusian fitness for different phenotypic means $\tilde{z}$ at five different population sizes, ranging from low ($N = 50$) to high ($N = 150$). Red individuals are favoured at low densities, while white individuals are favoured at high densities. (**b**) The estimated $Q(\tilde{z})$-function for the phenotypic mean $\tilde{z}$. Evolution is expected to maximize the Q-function under density dependent selection. Higher values of $Q(\tilde{z})$ can be interpreted as higher carry capacity. Thus, the population is expected to evolve towards white coloured birds (as indicated by the star and dotted line). (**c**) The relationship between the selection gradient (unscaled) on phenotype and the population size. The effect of migration was accounted for in all panels (see *Estimation* in Materials and methods). Rug plots display individual observations of the degree of reddish colouration (**a**, **b**) and annual population size (**c**).

Fig. 3: Density-dependent selection on genotype in female barn owls. (**a**) The relationship between the mean Malthusian fitness for each genotype of the melanocortin-1 receptor (*MC1R*) gene and the population size. The red genotype (RR) is shown to be most sensitive to changes in population density, such that WW is favoured at high densities and RR is favoured at low densities. (**b**) The estimated $Q(\tilde{p})$-function for the mean allele frequency $\tilde{p}$. Evolution is expected to maximize the Q-function under density dependent selection. Higher values of Q can be interpreted as higher carry capacity. Thus,

667 the population is expected to evolve towards fixation of the white allele (as indicated by
668 the star and dotted line). (**c**) The relationship between the selection gradient and the
669 population size for five different allele frequencies. The effect of migration was accounted
670 for in all panels (see *Estimation* in Materials and methods). Rug plots display observa-
671 tions of annual population size (**a**, **c**) and annual reproductive value weighted average
672 allele frequency (**b**).

Figures

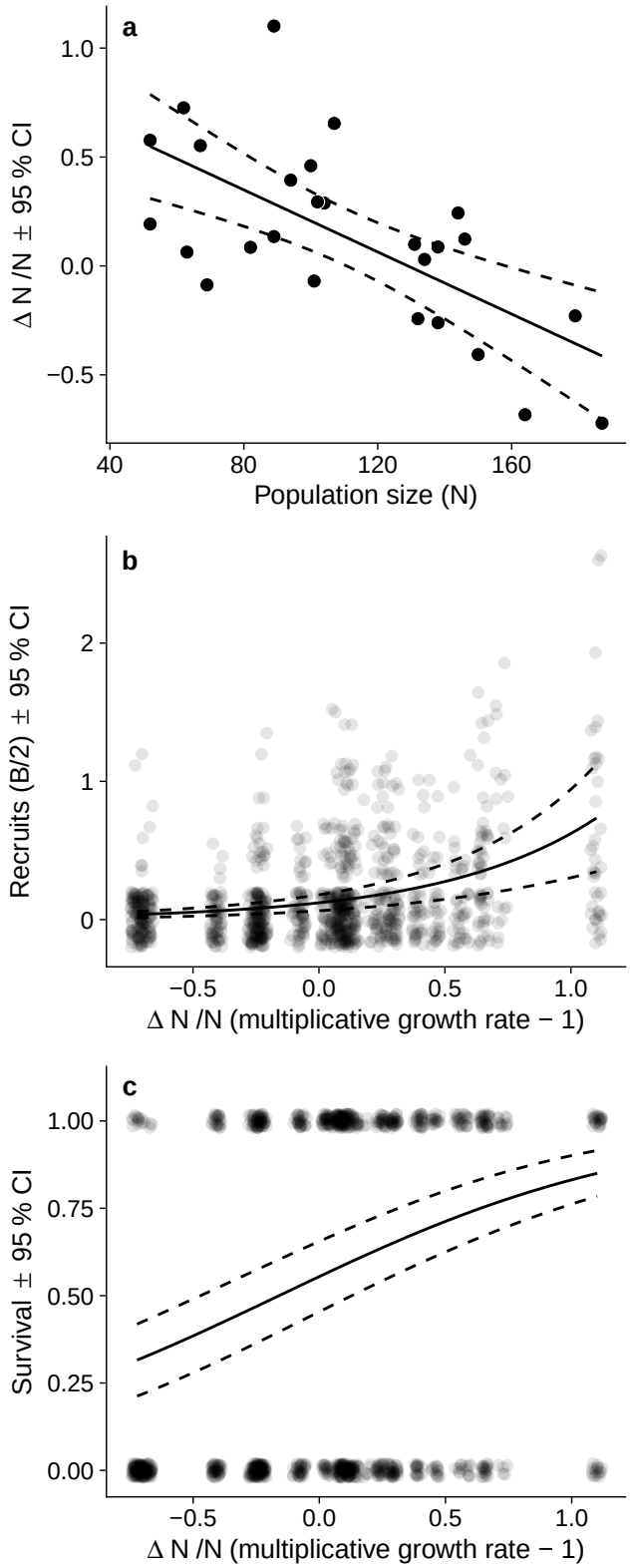
a
1.0
0.5
0.0
−0.5
Δ N /N ± 95 % CI
40
80
120
160
Population size (N)
b
2
1
0
Recruits (B/2) ± 95 % CI
−0.5
0.0
0.5
1.0
Δ N /N (multiplicative growth rate − 1)
c
1.00
0.75
0.50
0.25
0.00
Survival ± 95 % CI
−0.5
0.0
0.5
1.0
Δ N /N (multiplicative growth rate − 1)

Figure 1

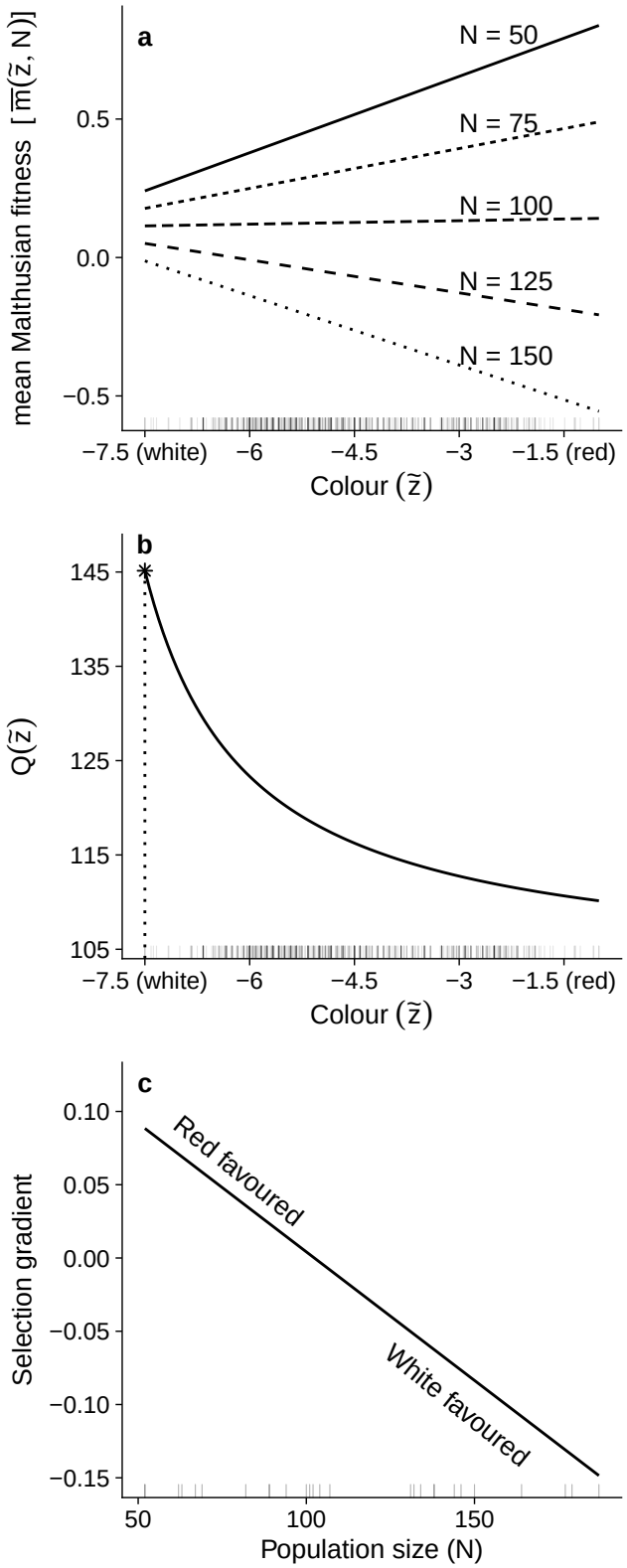
a
N = 50
N = 75
N = 100
N = 125
N = 150
mean Malthusian fitness [m̄(z̃, N)]
0.5
0.0
−0.5
−7.5 (white)
−6
−4.5
−3
−1.5 (red)
Colour (z̃)
b
145
135
125
115
105
Q(z̃)
−7.5 (white)
−6
−4.5
−3
−1.5 (red)
Colour (z̃)
c
Red favoured
White favoured
Selection gradient
0.10
0.05
0.00
−0.05
−0.10
−0.15
50
100
150
Population size (N)

Figure 2

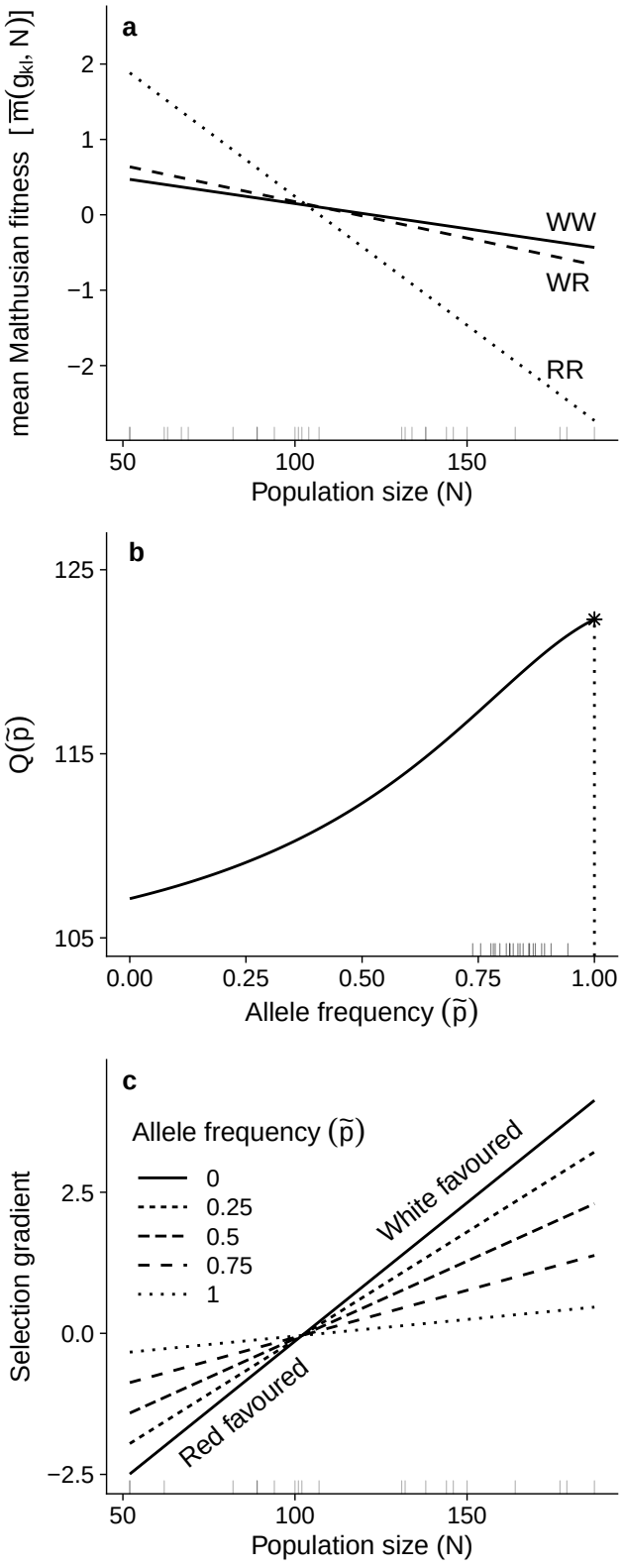
a
mean Malthusian fitness [$\overline{m}(g_{kl}, N)$]
2
1
0
−1
−2
WW
WR
RR
50
100
150
Population size (N)
b
125
115
105
Q($\tilde{p}$)
0.00
0.25
0.50
0.75
1.00
Allele frequency ($\tilde{p}$)
c
Allele frequency ($\tilde{p}$)
0
0.25
0.5
0.75
1
White favoured
Red favoured
Selection gradient
2.5
0.0
−2.5
50
100
150
Population size (N)

Figure 3