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ORIGINAL PAPER

Individual heterozygosity and fitness in bottlenecked populations during early colonisation

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Abstract Some populations of alien species, established by a small number of individuals, spread rapidly. This is the 'genetic paradox of invasions' as they must overcome the negative effects of the demographic bottleneck during the establishment phase, which reduces genetic diversity, fitness and evolutionary potential. Using a set of experimentally introduced populations of the Roesel's bush-cricket (*Roeseliana roeselii*), a nuptial gift-giving insect, we investigated this paradox by examining the relationships between individual heterozygosity (SNP markers), body size (an indicator of insect fitness) and population growth. We found that populations with a lower growth rate (annual increase in the number of stridulating males around the introduction patch) also had lower genetic variation and effective size. Females exhibited significantly higher individual heterozygosity than males. Body size (length of hind femur) increased in females with individual heterozygosity, whereas this was not observed in males. However, population growth was related to heterozygosity in males. Since female body size and male heterozygosity in these insects are related to fecundity and nuptial gift quality, respectively, our results suggest that potential selection on fitness-related phenotypic traits may mitigate effects of inbreeding depression and increase population growth during the establishment phase. The present results cannot fully disentangle complex mechanisms underlying the success of colonisation, but we believe that they will stimulate further experimental research in the field of invasion biology.

Keywords ddRAD · Founder effect · Good genes · Invasiveness · *Metrioptera roeselii* · Sexual conflict · Survival

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Introduction

Over the last two centuries, human activities have contributed significantly to the spread of many

species beyond their natural ranges, mainly due to climate change, globalisation of agriculture, trade and travel (Bertelsmeier et al. 2017). During the introduction event and the establishment phase, populations often experience a temporary reduction in population density, the so-called demographic bottleneck, which can lead to a reduction in genetic diversity (Peischl and Excoffier 2015). Unintentionally introduced populations are therefore often small, isolated and may suffer from an Allee effect, where the population growth rate is lower due to lower population density (Kanarek et al. 2013). The segregation of deleterious recessive alleles increases homozygosity and thus reduces genetic diversity, leading to genetic load and inbreeding depression, which ultimately reduces the average fitness of the population (Charlesworth and Willis 2009). The accumulation of deleterious genetic variation and the response to selection are related to effective population size, which is likely to be positively correlated with census size or population density (Söderquist et al. 2020). In small populations, genetic load and inbreeding lower offspring survival which in turn lowers the probability of establishment and the ability to adapt in new environment, reduce dispersal rates and increase the lag in population growth (Hufbauer et al. 2013; Szűcs et al. 2014). Although the likelihood of establishment also depends on non-genetic factors (climate, resources, local competition, etc.), many introductions quickly and successfully overcome negative effects of genetic erosion even if they start from a very small number of individuals. Instead of being threatened with extinction, populations increase in size, gain local dominance and spread invasively. This phenomenon of the 'genetic paradox of invasions' is at the centre of attention of invasion biologists (Schrieber and Lachmuth 2017). Since a depletion of genetic diversity caused by a demographic bottleneck may increase the fitness of the population and boost its growth, this paradox is genuine. It is expected that there are some features of population dynamics that allow the population to overcome the deleterious consequences of low genetic variation, but, experimental studies are needed to test theoretical expectations (Estoup et al. 2016).

The mating system is fundamental to the transmission of genetic variation across generations and has profound effects on adaptations and population dynamics. Offspring of heterozygous individuals are themselves likely to be heterozygous, which may lead to higher fitness (i.e. the theory of 'good-genes-as-heterozygosity'; Andersson 1994). This can mask the effects of deleterious recessive alleles in cases of inbreeding depression (Hoffman et al. 2014). Although determining the origin of individual heterozygosity-fitness correlations (HFCs) is a complex task, its presence in a population is often associated with a recent bottleneck and inbreeding. In a small inbred population, the degree of relatedness varies among random pairs of individuals, leading to identity disequilibrium (correlation in heterozygosity and/or homozygosity across loci) and generating HFCs (Szulkin et al. 2010).

Inbreeding depression is a significant evolutionary force that also drives mate choice (Charlesworth and Charlesworth 1987; Janicke et al. 2014). Females may prefer heterozygous, less related males to maximise the genetic diversity of their offspring (Neff and Pitcher 2005), while heterozygous females may be better able to engage in costly mate search and therefore be more selective in choosing potential mates (Kempenaers 2007). One mechanism for increasing heterozygosity in depleted populations is the selection of fitness-related phenotypic traits. Since adult body size in insects is influenced by egg size, larval nutrition and developmental conditions in addition to the genetic basis, it is an important determinant of their fitness (Beukeboom 2018). Females with a larger body size are associated with greater reproductive success (Whitman 2008). Larger insect females tend to produce more eggs (Honěk 1993), while species that mate multiple times and give nuptial gifts have also direct fitness benefits in addition to genetic ones (Arnqvist and Nilsson 2000). These females favour high-quality males that produce spermatophores with more sperm to increase offspring heterozygosity and that contain more proteins (spermatophylax) to replace the energy lost from copulation (Gwynne 2001; Dorková et al. 2019). However, there is little evidence for a direct correlation between the size of advantageous body traits and heterozygosity of females in insect mate choice (Husseneder and Simms 2008; Browne and Gwynne 2023).

To unravel the complexity of invasion dynamics, it is necessary to focus on the population level rather than the species level (Sousa et al. 2024). Introduction events are ideal natural experiments for observing parallel evolution and rapid adaptation in wild

populations (e.g. Lee 2002; Ottenburghs 2021), while current genome-wide markers have greatly improved the precision and accuracy of estimates of genetic variability and demographic parameters (Allendorf 2017). Population establishment is a prerequisite for invasion, and species with strong colonisation ability are well-suited for studying population dynamics at this stage. In this study, we used a nuptial gift-giving insect that readily establishes populations (Kaňuch et al. 2013, 2022). Recent data from both unintentionally (passively introduced by human-mediated transport) and experimentally established populations indicate that this species can very successfully colonise new habitats thanks to the rapid recovery of genetic variation after a introduction bottleneck (Kaňuch et al. 2014, 2021). In an experimental system of intentionally introduced populations, we aimed to reveal the mechanism associated with the successful establishment of an introduction by investigating (1) the relationship between individual heterozygosity and individual fitness and (2) the effect of these variables on population growth in populations recently depleted by bottlenecks. We hypothesised that individual HFC will explain the increased population growth and thus the success of the species in the early phase of colonisation.

Material and methods

Experimental introductions

The Roesel's bush-cricket, *Roeseliana roeselii* (Orthoptera: Tettigoniidae), is a small (12–18 mm), typically flightless grassland species distributed in central and eastern Europe, whose range is currently expanding in northern latitudes. Its colonisation history and dispersal behaviour on the Scandinavian Peninsula has been thoroughly studied and well documented (e.g. Berggren et al. 2001, 2002; Kaňuch et al. 2013, 2022). Although *R. roeselii* belongs to the tettigonids with a lower degree of polyandry, it has been found that a female can mate up to five times (mean 1.7–2.7; Vahed 2006; Kaňuch et al. 2013). In a stable population with an equal sex ratio, a similar frequency of copulations is therefore also expected in bush-cricket males (Dorková et al. 2019). The species is typically univoltine, and one year can be considered a generation time, however, a semivoltine life cycle may occur in some embryos laid at the end of the season (Ingrish 1984).

In 2008, individuals of *R. roeselii* were collected from a site in the centre of its core distribution in Sweden, southeast of Västerås at the lake Mälaren (N 59°30', E 16°12'). These individuals were released in 40 habitat patches in the north-eastern part of Stockholm County in a mosaic landscape of agricultural fields, grassland and spruce forests (Fig. 1a). The patches were uncultivated, ungrazed, semi-natural grasslands of varying sizes (~ 0.5–1 ha) surrounded by arable land. There were no established populations of the species in this region, and the smallest distance to its current distribution was 24 km. The introduced propagule size was 16 virgin individuals in the last nymphal stage before the final moult with an equal sex ratio. All individuals of this parental generation were introduced in the centre of the habitat patch and the minimum distance between introduction patches was 1.5 km. According to our previous results, such a spatial arrangement was considered sufficient to prevent significant gene flow by natural dispersal between neighbouring introductions (Berggren et al. 2001; Kaňuch et al. 2021), which could influence genetic and phenotypic variation of bottlenecked populations during the early colonisation phase. To find out whether the introductions had successfully established populations, an area of 0.3 km^2 around the introduction patch (a radius of 309 m) was monitored, in which 95% of the individuals should occur (Berggren 2001). Population size monitoring was carried out annually (during the breeding season in July and August) from the third year after introduction (2011). The population census was based on the number of stridulating males, and an ultrasonic detector was used as an aid to better detect individuals in crop fields. In addition, a 1 km stretch along the roads was monitored from a car entering and leaving the areas to detect potentially dispersed individuals. Surveys only took place on warm, dry and sunny days between 09:00 and 16:00 CEST. The same methodology for measuring the population size of this species has also been successfully applied in other ecological studies (Berggren 2001; Berggren et al. 2001, 2002). Relative population growth rate was determined using simple linear regression estimation (slope).

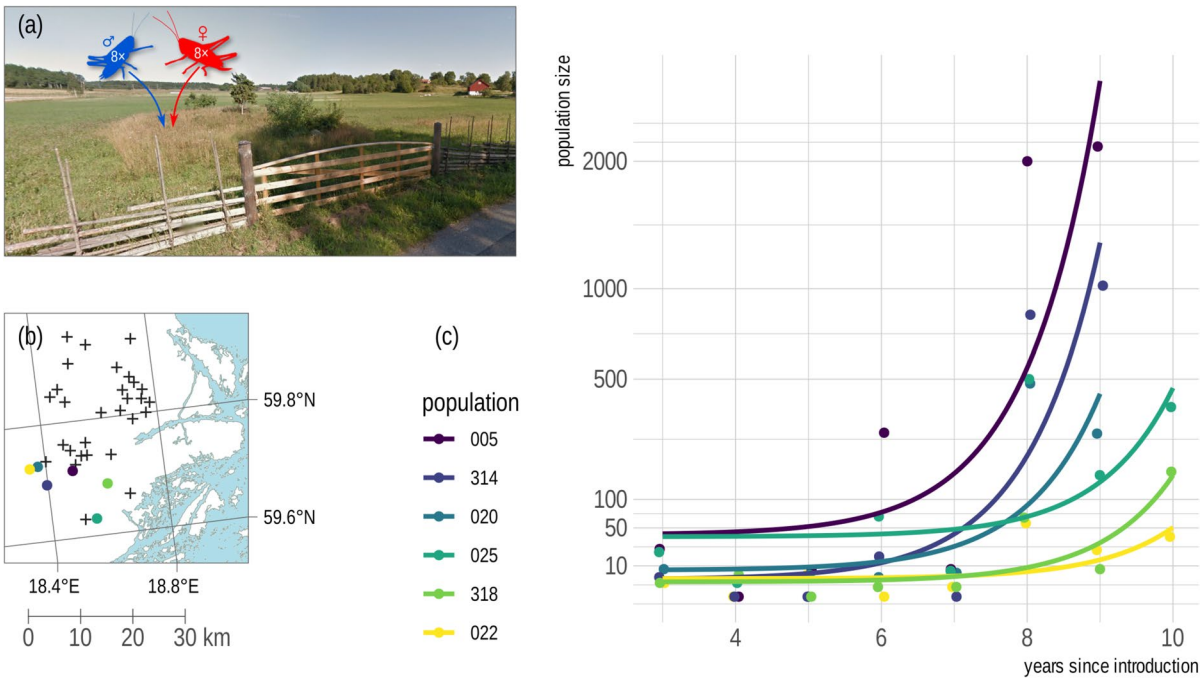

Fig. 1 **a** Each population of *Roeseliana roeselii* was established by the introduction of 16 individuals with equal sex ratio in a small patch of uncultivated habitat surrounded by a matrix of open landscape (photo by © Google Street View). **b** Six study populations (full circles) introduced in 2008 in the north-eastern part of Stockholm County (Sweden) and other populations introduced at the same time in this region and successfully established (crosses). **c** Population growth was monitored from the third year after introduction over seven or eight generations (for better visualisation, the y-axis is square root transformed)

Sampled populations

From the available options (40 original introductions), we selected a set of six successfully established introductions (in the southern periphery of the experimental area) where we observed different demographic trends (Fig. 1b, Table S1). While some populations increased in size relatively slowly, others showed rapid expansion (Fig. 1c). The average annual increase in population size ranged from six to 370 adult males (Table 1). To confirm that the populations developed under similar environmental conditions, we surveyed the habitat surrounding their introduction patches within a radius of 309 m (0.3 km^2; Berggren et al. 2001). Habitat was categorised on the map using the 10 m raster layer of the National Land Cover Database (Nationella marktäckedata 2018, https://www.naturvardsverket.se/; Fig. S1), and various metrics of landscape diversity (Shannon's Diversity Index, Shannon's Evenness Index, Simpson's Diversity Index), the proportion of open habitat

Table 1 Results of the simple linear regressions of population growth

Population	Term	Estimate	SE	*t*	*p*
022	(intercept)	−12.3	12.9	−0.96	0.375
	year	**6.2**	2.5	2.45	0.050
318	(intercept)	−41.5	36.4	−1.14	0.297
	year	**16.1**	7.2	2.24	0.067
025	(intercept)	−109.8	114.6	−0.96	0.375
	year	**56.0**	22.7	2.47	0.049
020	(intercept)	−142.4	124.8	−1.14	0.306
	year	**63.5**	27.9	2.28	0.072
314	(intercept)	−406.4	251.1	−1.62	0.167
	year	**168.7**	56.2	3.00	0.030
005	(intercept)	−840.3	533.0	−1.58	0.176
	year	**369.4**	119.2	3.10	0.027

The populations are listed in ascending order of estimated growth, which indicates the expected annual increase in population size (in bold)

(arable land, vegetated other open land, temporarily non-forest not on wetland) and the number of other introductions in the adjacent area were calculated using QGIS 3.22.4 software (https://qgis.org/). None of these variables correlated with population growth (Fig. S2). In August 2019, we sampled individuals of *R. roeselii* of the eleventh filial generation for genetic and morphological data. We collected 16 adults with equal sex ratio exactly at the place of their introduction (patch of uncultivated habitat) in each site.

DNA extraction and genotyping

Genomic DNA was extracted from hind femur muscle tissue using a modified high-salt extraction protocol (Paxton et al. 1996; Appendix S1). ddRAD libraries were assembled following a protocol developed by IGA Technology Services (Italy) with minor modifications based on Peterson's Double Digest restriction site-associated DNA preparation (Peterson et al. 2012; Appendix S2). Restriction endonucleases (SphI, EcoRI) were selected based on in silico analyses of reference genomes of related species of the family Tettigoniidae. The ddRAD sequencing was performed in pair-end mode using a Novaseq6000 sequencer (Illumina, California), together with an initial bioinformatic analysis by IGA Technology Services. The Illumina raw reads were demultiplexed using process_radtags, and then the short-reads were assembled, catalogued and matched de novo using the ustacks, cstacks, sstacks and tsv2bam utilities implemented in Stacks 2.61 (Catchen et al. 2013). Subsequently, the gstacks add-on software from Stacks was used to detect and genotype all aligned single nucleotide polymorphisms (SNPs).

To ensure high quality of the genotypes, filtering of SNPs was performed according to the recommendations of Marees et al. (2017) using PLINK 1.90 (Chang et al. 2015). First, we excluded SNPs and individuals with missing data of more than 20%. This removed 22% of 124,440 SNPs and 15% of 96 individuals. Secondly, we opted for a stricter threshold and excluded SNPs and individuals with missing data of more than 2%. This excluded 77% of 96,568 SNPs and 5% of 82 individuals. Subsequently, 14% of SNPs with minor allele counts below the threshold of three were excluded. In the next step, the next 10% of SNPs that were not in Hardy–Weinberg equilibrium (HWE) were removed. To filter out SNPs that deviated extremely from HWE, filtering was performed with a –hwe threshold of 1e-6. Subsequently, the test for heterozygosity was performed with a set of SNPs that were not highly correlated. Therefore, to create a list of non-(highly) correlated SNPs, regions of high linkage disequilibrium (LD) were excluded using the –indep-pairwise threshold of 50 5 0.7, and only selected variants were retained after LD pruning. However, some SNPs in strong LD are likely included in the analyses, as the loci are not arranged along a genome, but are assembled de novo. Finally, we found no heterozygosity outliers (i.e. individuals deviating more than three standard deviations from the mean heterozygosity rate). Using these thresholds, we obtained two datasets for the analyses (Table S2). The first dataset had a higher quality (HQ) of genotypes at the expense of a lower number of individuals and loci (78 individuals and 9,787 SNPs) compared to the other, lower quality (LQ) dataset (91 individuals and 37,495 SNPs), which has a higher percentage of missing data.

Population genetic analyses

The signatures of the bottleneck induced in all populations eleven generations ago were examined using two tests for the excess in heterozygosity under a stepwise mutation model (SMM). In the software INEST 2.3 (Chybicki 2017) we conducted the Z-test based on combined Z scores for particular loci and the Wilcoxon signed-rank test (permutations for the p-value were deactivated). To determine whether our introductions originating from the same source population are still genetically homogeneous or whether a founder effect (genetic drift) is visible, we used an individual-based clustering method implemented in the software Structure 2.3.4 (Pritchard et al. 2000; Hubisz et al. 2009). We ran the admixture model with correlated allele frequencies without the prior information about the population and the degree of admixture $\alpha = 1$. For each value of K (range 1–6), we performed five independent runs with uniform priors using a burn-in of 25,000 iterations followed by 75,000 Markov chain Monte Carlo iterations. The number of genetic clusters K was inferred in two steps. First, we used the ΔK method (Evanno et al. 2005), which finds the breakpoint in the slope of the likelihood distribution for different K values, using Structure Harvester Web 0.6.94 (Earl and von Holdt

2012). We then also identified the stable *K* solutions by Q-matrix correlations (average maximum correlation coefficient and the rows-and-columns method), which are implemented in the package 'CorrSieve' 1.6–9 (Campana et al. 2011) of the R 4.1.2 software environment for statistical computing (R Core Team 2021), which help to identify anomalous runs. The outputs of the Structure analysis were visualised using the Clumpak programme (Kopelman et al. 2015). For the bottleneck and structure analysis, we used minimum required settings and only analysed the HQ dataset due to the very large number of loci and our hardware limitations.

At the population level, the observed heterozygosity (H_O), the expected heterozygosity (H_E) and the inbreeding coefficient (F_{IS}) were calculated using the R package 'diveRsity' 1.9.90 (Keenan et al. 2013). Confidence intervals (95%) for the F_{IS} were determined by 9,999 permutations. Nucleotide diversity (π) and scaled Tajima's *D* in the populations were calculated using the R package 'SambaR' 1.10 (de Jong et al. 2021). To calculate the effective population size (Ne), we used the linkage disequilibrium method with nonparametric jack-knifed confidence intervals implemented in the software NeEstimator 2.1 (Do et al. 2014). Along with these population characteristics, we also estimated the heterozygosity of each individual averaged across a large panel of SNPs markers. Using the R function GENHET (Coulon 2010), we calculated five estimates of individual heterozygosity: proportion of heterozygous loci in an individual (PHt), standardised heterozygosity derived from the mean observed heterozygosity (Hs_obs), standardised heterozygosity derived from the mean expected heterozygosity (Hs_exp), internal relatedness (IR) and homozygosity by locus (HL). As all estimates were fully ($\geq$99%) correlated (Fig. S4), we used Hs_obs as the only representative of individual heterozygosity in the statistical modelling.

Measurement of body size

We used the body size to estimate individual fitness of bush-crickets (Beukeboom 2018) and measured the lengths of four morphological traits for each individual: head, pronotum, hind femur and total body (excluding genital appendages). Only successfully genotyped specimens (HQ dataset) were measured. The sex ratio was almost equal in the populations, but in the low-density population 318 we could only find males. Dorsal and lateral views of the insect were digitally photographed using a Canon EOS 1000D camera with Canon Macro EFS 35 mm lens (Canon, Japan) mounted on a copy stand with standard focal length. Several photos were taken of each individual. The distance between the landmarks (Fig. S5) was measured using ImageJ 1.53e software (Rasband et al. 1997–2018). With one exception (a male from population 318), all individuals measured were short-winged. Exclusion of this long-winged individual had no effect on the results; so it was left it in the dataset. Repeated measurements on multiple traits of the same specimen were made due to the complex allometry among different body structures (Whitman 2008). From the repeated measurements, we calculated the mean and the coefficient of variance (CV) to see the accuracy of the measurements of a particular trait. The trait with the highest measurement accuracy was femur (mean CV = 0.014), followed by pronotum (0.040), total body (0.066) and head (0.077). Furthermore, the measurement of the total body was difficult because it comprises a flexible abdomen, the size of which varies due to the development of the gonads. As the Pearson coefficients between all measured body traits showed significantly high correlations ($r = 0.65–0.82$, $p < 0.001$; Fig. S6), we used femur length as the only estimate of body size (fitness) in the statistical modelling.

Statistical modelling

We used a linear mixed model (LMM) to test the association between individual heterozygosity (explanatory variable) and body size (response variable), and two univariate linear models (LMs) to test the association between individual heterozygosity or body size (explanatory variables) and population growth (response variable). When including both explanatory variables in a single model, there was high collinearity ($\geq$10) between the model terms, which could increase parameter uncertainty, so we analysed them in separate models. Population identity was used as a random effect (i.e. random intercept) in the LMM. Population identity was not considered as a random factor when analysing the effects of explanatory variables on population growth, as individuals from the same population have the same value for population growth and the models were therefore overfitted. Sex

was used as a categorical explanatory variable in all models. The LMM was fitted using the lmer function of the R package 'lme4' 1.1–35.1 (Bates et al. 2015); the summ function of the R package 'jtools' 2.2.2 (Long 2022) was used to produce and format model summaries. The Anova function of the package 'car' 3.1–0 (Fox and Weisberg 2019) was used to calculate Type III ANOVA tables for the objects of the models. The diagnostics (uniformity, dispersion tests and outliers) of the models were performed with the check_model function of the R package 'performance' 0.9.2 (Lüdecke et al. 2021). For the LMM, the adjusted marginal R^2, which represents the variance explained by fixed factors, was calculated using the function r.squaredGLMM from the R package 'MuMIn' 1.15.1 (Bartoń 2023).

Results

Genetic variation, population bottleneck and founder effect

The average population values of genetic variation were 0.171–0.214 for observed heterozygosity, 0.204–0.240 for expected heterozygosity, 0.19–0.23 for nucleotide diversity and 0.088–0.178 for the inbreeding coefficient (Table 2; values for LQ dataset are in Table S3). Eleven generations after introduction, we found statistically significant signatures of a demographic bottleneck in all but one population (314; Table S4). This population had rapid population growth (Fig. 1c), the highest effective population size (Ne = 220.8) and the lowest value of the Tajima's statistic (D = -0.220), indicating the strongest expansion (Table 2). Although there was no significant Spearman's rank correlation ($p > 0.05$) between population growth and the mean estimates of genetic variation and effective size using either the HQ or the LQ dataset, the three populations (318, 025, 022) with lower growth had lower values of these estimates and therefore appear to be more genetically eroded compared to the other populations. However, the level of inbreeding did not exceed the threshold of 0.2 in any populations, suggesting a low to moderate level of mating between close relatives (but see F_{IS} values calculated from the LQ dataset; Table S3). The ΔK method inferred the optimal number of genetic clusters $K = 2$, but the average maximum Q-matrix correlations (r = 0.99, $p < 0.05$) showed a stable solution even with three and four clusters (Fig. 2). As the populations originated from the same source, the separate clustering of the smallest populations (318, 022) suggests a founder effect.

Heterozygosity-fitness and population growth associations

Using the HQ dataset, the mean Hs_obs was 0.934 (range 0.728–1.057) for males and 1.100 (0.692–1.210) for females. The paired t-test revealed that all estimates except Hs_obs indicate significantly higher heterozygosity in the HQ dataset compared to the LQ dataset (Fig. S3). This difference is due to incomplete data (up to 20% of missing alleles in genotypes) in the LQ dataset, which leads to incorrect estimates of individual heterozygosity. The mean femur length was 15.95 mm (range 15.00–17.14 mm) for males and 17.82 (15.09–19.45 mm) for females. The effect of individual heterozygosity on body size was sex-dependent. The body size of females was positively

Table 2 Observed (H_O) and expected (H_E) heterozygosity, nucleotide diversity (π), inbreeding coefficient (F_{IS}) and effective population size (Ne) calculated from 9,784 SNPs and statistical testing (scaled Tajima's D) whether populations evolve neutrally at constant size

Population	n_m / n_f	H_O (SD)	H_E (SD)	π	F_{IS} (95% CI)	Ne (95% CI)	Tajima's D
005	6 / 6	0.209 (0.190)	0.230 (0.175)	0.22	0.088 (−0.280–0.366)	169.2 (99.5–526.5)	−0.199
020	8 / 6	0.208 (0.172)	0.238 (0.167)	0.23	0.107 (−0.247–0.383)	170.2 (103.4–454.0)	−0.217
022	8 / 6	0.199 (0.194)	0.217 (0.183)	0.20	0.087 (−0.273–0.374)	105.2 (68.4–217.9)	−0.179
025	7 / 5	0.193 (0.172)	0.231 (0.172)	0.22	0.135 (−0.248–0.417)	33.9 (21.4–70.0)	−0.211
314	5 / 8	0.214 (0.177)	0.240 (0.169)	0.23	0.098 (−0.263–0.373)	220.8 (139.3–516.3)	−0.220
318	13 / 0	0.171 (0.195)	0.204 (0.186)	0.19	0.178 (−0.158–0.445)	32.5 (14.8–473.6)	−0.165

n_m, number of genotyped males; n_f, number of genotyped females

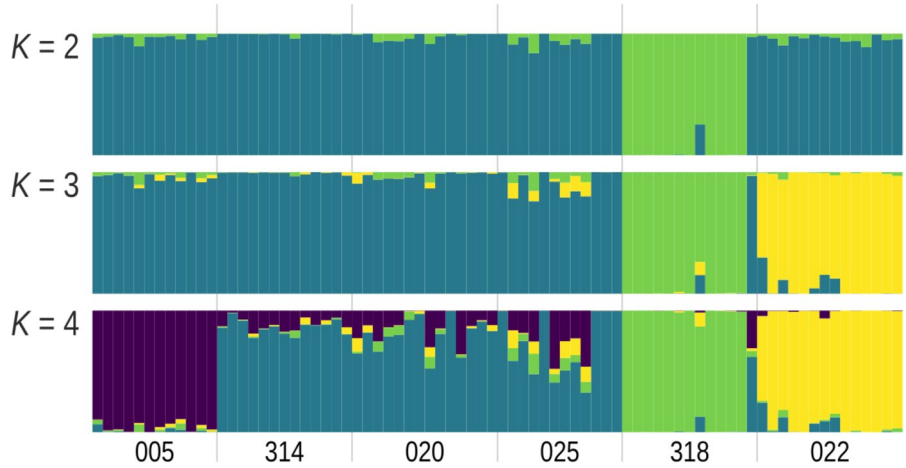

Fig. 2 Genetic structure of the populations 11 years after their introduction, inferred by the Structure admixture analysis. Individuals in the stacked bar charts of the different *K* are represented by vertical bars divided into parts proportional to their proposed ancestry in determined genetic clusters

related to their heterozygosity, whereas this pattern was not observed in males. Females showed significantly higher individual heterozygosity and larger body size than males (Table 3, Fig. 3a). There was a significant interaction effect between heterozygosity and sex on population growth. Specifically, population growth was positively influenced by individual heterozygosity of males, whereas this was not the case for females (Table 3, Fig. 3b). On the other hand, the effect of body size on population growth was not observed in either males or females (Table 3). The investigation of these patterns using lower quality genotypes resulted in non-significant models (Table S5).

Table 3 ANOVA results for a linear mixed model to examine the effects of individual heterozygosity, sex and their interaction on body size (LMM) and for linear models to examine the effects of individual heterozygosity (LM 1) and body size (LM 2) and their interaction with sex on population growth. For model estimates see Appendix S3

LMM (body size)	χ^2	*p*
(intercept)	65.53	< 0.001
heterozygosity	12.97	< 0.001
sex	5.03	0.025
heterozygosity × sex	8.72	0.003
$R^2_{\text{fixed effects}} = 0.58$, $R^2_{\text{total}} = 0.64$		
LM 1 (population growth)	*F*	*p*
(intercept)	0.10	0.750
heterozygosity	0.06	0.802
sex	3.79	0.055
heterozygosity × sex	4.03	0.048
$R^2 = 0.15$		
LM 2 (population growth)	*F*	*p*
(intercept)	0.62	0.432
body size	0.20	0.659
sex	0.01	0.942
body size × sex	0.00	0.985
$R^2 = 0.03$		

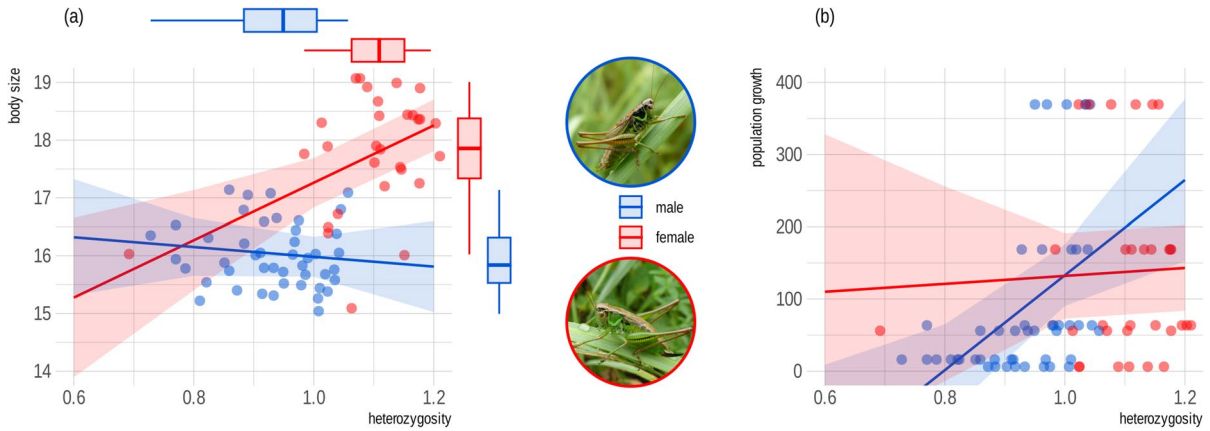

Fig. 3 **a** Predicted body size (femur length in mm) in response to individual heterozygosity (standardised observed heterozygosity) with 95% confidence limits derived from a linear mixed model and **b** predicted population growth (estimate of annual increase in population size) in response to individual heterozygosity with 95% confidence limits derived from a linear model for males and females of *Roeseliana roeselii* in introduced populations (photo by P. Kaňuch)

Discussion

In this original experimental field study, we utilised precise knowledge of the founders' origin, maximum propagule size and complete temporal data on demographic trends since introduction – all information that is constantly lacking when studying the invasion biology of alien species. We observed a positive relationship between heterozygosity and female size, while male heterozygosity was related to population growth, which is a key mechanism that prevents species extinction. Our empirical results also suggest that genetic diversity in an insect species with uniform female-biased sexual size dimorphism (Hochkirch and Gröning 2008) improves female fitness as larger individuals should have higher fecundity (Honěk 1993). Consistent with our hypothesis, this adds to the growing evidence that genome-wide heterozygosity is associated with fitness-related traits (Forcada and Hoffman 2014; Velando et al. 2015), which likely shapes population dynamics.

The populations of *R. roeselii* with higher genetic diversity and effective size showed faster recovery and population growth rates. All populations were however established with ten times fewer individuals, than believed required for successful reintroductions of similar species (Baur et al. 2017). Due to technical limitations, it was not possible to register the genetic make up and phenotypic variation of the released individuals. However, we still do not know which and how many individuals mated and reproduced in the introduction patch after release. Previous research has shown that even a single breeding pair can establish a viable population (Kaňuch et al. 2021). This could most likely also be the case in populations where we have observed a founder effect as this may have occurred immediately after introduction due to predation or unexplained mortality. In a possible extreme case, the population in our experiment could only be founded by a female that had the chance to copulate with one or more males. To understand population growth and long-term viability of introduced species, it is therefore important to determine the causes and effects of evolutionary mechanisms on the fitness of small populations.

In this study, genetically more diverse females showed greater fitness (as indirectly inferred from femur length). Mate choice of female bush-crickets is largely influenced by the signals they receive from stridulating males, which indicate the age, body size, health status or potential quality of their nuptial gifts (Gwynne 2001; Verburgt et al. 2011; Stoffer and Walker 2012). During courtship, the females then select heterozygous males (i.e. males with 'good genes'), which can explain our findings of a significant relationship between male heterozygosity and positive population growth. In the early establishment phase, when selection pressure on introduced populations is strongest, large females may be more successful at locating singing males, as auditory sensitivity

correlates with body size (Strauß et al. 2014). In contrast, smaller females should be at a competitive disadvantage as they are unable to recognise the calls of distant males. Therefore, larger females have the advantage of selecting optimal male phenotypes that provide larger nuptial gifts (Blackenhorn 2000; Fedorka and Mousseau 2002; Dorková et al. 2019). Such females can allocate more resources and energy to reproduction (Pincheira-Donoso and Hunt 2017). Selection for large, highly heterozygous females could positively influence the evolutionary persistence of certain male traits and indirectly improve offspring fitness (e.g. increased hatching success), while this could occur through genetic benefits from mating with heterozygous partners (Mays and Hill 2004). Larger and heterozygous females may therefore have higher mating success (Husseneder and Simms 2008), but interestingly, females in the populations studied also had higher overall heterozygosity than males. In a number of populations of the Roesel's bush-crickets that had become naturally established in the continuous area of the species range and/or were principally older than our experimental introductions, no sex difference in heterozygosity was observed (Fig. S7; Kaňuch et al. 2013). Thus, selection for larger females can mitigate inbreeding depression in small isolated populations without gene flow. We could hypothesise that strong selection on female size, and thus fecundity, leads to higher heterozygosity in females than in males. To mitigate the negative genetic consequences of a temporarily reduced population size in sexually reproducing organisms, resources must be effectively allocated between the sexes (Hedrick 2005; Neff and Pitcher 2005).

The sex-specific effect of individual heterozygosity on population growth is certainly the most interesting result of our study. The fact that males exhibit significantly lower heterozygosity than females could indicate that the detrimental effects of low individual heterozygosity are mostly expressed in males, and on the other hand, could explain the absence of effects on population growth in females. From the perspective of males, heterozygous individuals with better fitness may be more productive in the population because they make larger or more numerous nuptial gifts, emit louder stridulations that attract more females over a greater distance, or are better able to overcome female resistance behaviour during copulation (Gwynne 2001; Wulff et al. 2018; Lehmann et al. 2021). Heterozygous males in these cases may have an influence on the heterozygosity of females in the population, as they should produce heterozygous (also female) offspring (Andersson 1994; Neff and Pitcher 2005). A limitation of our study is the lack of measured phenotypic traits related to male heterozygosity. We found that the body size of males does not have the same explanatory power as that of females. Other morphological structures of males might therefore be more important for their mating success, such as titillators (Wulff et al. 2018), but further phenotypic data, including behavioural and physiological traits, are definitely needed to test possible explanations. As males contribute few or no resources to offspring production, selection against rare, recessive deleterious alleles is generally stronger in males than in females (Mallet and Chippindale 2011; Grieshop et al. 2016). Increased pressure of natural selection in males leads to indirect genetic benefits in the form of higher viability of offspring (Grieshop et al. 2021). Non-random mating in small populations of alien species can thus initiate the purging of deleterious alleles (Glémin 2003), and inbreeding depression promotes their invasion (Facon et al. 2011).

Understanding the genetic paradox of invasions is crucial for the development of effective management strategies to prevent and control the spread of alien species (Wang et al. 2020; He et al. 2024), while every invasion is preceded by successful colonisation and naturalisation in the local ecosystem (di Castri 1989). The genetic variability of introduced individuals influences their response to genetic load from bottlenecks and/or founder events. More diverse populations also have higher growth and viability, so they respond better to genetic load than genetically eroded ones when suffering from founder events (Mathur and DeWoody 2021). Studies on genetic depletion in natural populations have revealed various evolutionary mechanisms that aid the maintenance, and in some cases even the restoration, of genetic diversity despite a severe bottleneck (e.g. Groombridge et al. 2009; Kaňuch et al. 2021). The results presented cannot fully disentangle the complex mechanisms underlying the success of colonisation, but our study of intentionally introduced populations with different demographic trends sheds important light on potential outcomes that may arise from bottleneck-related stochasticity.

We confirmed that individual heterozygosity, rather than population mean-based estimates of genetic diversity, is an important measure for predicting the success of introduction (Scott et al. 2020). However, we emphasise the need for strict quality filtering of SNP loci and thus the quality of genotypes for the purpose of testing HFCs, as estimates of heterozygosity vary in proportion to the amount of missing data (Schmidt et al. 2021). The main reason for the discrepancy in the results of our study lies in the considerable differences in the completeness of the genotypes. For each locus in the HQ dataset, at most one individual had no identified alleles, whereas in the LQ dataset, alleles were missing for up to 19 individuals at some loci. In conclusion, by examining genetic diversity, demographic patterns and mating systems, our study provides a solid framework for future experiments investigating complexities of invasion dynamics. The study of the relationship between heterozygosity and fitness-related traits can contribute to understand the detrimental effects of inbreeding and genetic drift in newly established populations. In addition to the management of alien species, these results can also be used to improve conservation measures and species reintroduction/restoration programmes, ultimately contributing to the conservation of biodiversity and ecosystem health.

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Data availability Data and code are available in GitHub https://github.com/pjeter77/roeseliana.

Declarations

Conflict of interest The authors declare no conflict of interest.

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