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Scaling up the effects of inbreeding depression from individuals to metapopulations

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Abstract

1. Inbreeding is common in nature, and many laboratory studies have documented that inbreeding depression can reduce the fitness of individuals. Demonstrating the consequences of inbreeding depression on the growth and persistence of populations is more challenging because populations are often regulated by density- or frequency-dependent selection and influenced by demographic and environmental stochasticity. A few empirical studies have shown that inbreeding depression can increase extinction risk of local populations. The importance of inbreeding depression at the metapopulation level has been conjectured based on population-level studies but has not been evaluated.
2. We quantified the impact of inbreeding depression affecting the fitness of individuals on metapopulation persistence in heterogeneous habitat networks of different sizes and habitat configuration in a context of natural butterfly metapopulations.
3. We developed a spatial individual-based simulation model of metapopulations with explicit genetics. We used Approximate Bayesian Computation to fit the model to extensive demographic, genetic, and life-history data available for the well-studied Glanville fritillary butterfly (*Melitaea cinxia*) metapopulations in the Åland islands in SW Finland. We compared 18 semi-independent habitat networks differing in size and fragmentation.
4. The results show that inbreeding is more frequent in small habitat networks, and consequently, inbreeding depression elevates extinction risks in small metapopulations. Metapopulation persistence and neutral genetic diversity maintained in the metapopulations increase with the total habitat amount in and mean patch size of habitat networks. Dispersal and mating behavior interact with landscape structure to determine how likely it is to encounter kin while looking for mates.

5. Inbreeding depression can decrease the viability of small metapopulations even when they are strongly influenced by stochastic extinction-colonization dynamics and density-dependent selection. The findings from this study support that genetic factors, in addition to demographic factors, can contribute to extinctions of small local populations and also of metapopulations.

Keywords: Glanville fritillary butterfly, *Melitaea cinxia*, metapopulation, inbreeding depression, extinction, metapopulation persistence, heterozygosity, individual-based model

Introduction

Inbreeding, defined as mating between related individuals, can cause inbreeding depression, where inbred individuals suffer from reduced fitness due to loss of genetic diversity (Allendorf, Luikart, & Aitken, 2012; Frankham, Ballou, & Briscoe, 2010; Hedrick & Garcia-Dorado, 2016; Keller & Waller, 2002). Studies have documented negative fitness effects of inbreeding on individuals in many taxa (D. Charlesworth & Charlesworth, 1987; Crnokrak & Roff, 1999; Hedrick & Kalinowski, 2000; Keller & Waller, 2002; Ralls, Ballou, & Templeton, 1988). Inbreeding depression may also drive small populations to extinction through feedbacks between small population size and further loss of genetic diversity via inbreeding and genetic drift, as depicted by the extinction vortex (Fagan & Holmes, 2006; Gilpin & Soule, 1986).

Demonstrating the consequences of inbreeding on population growth rate and persistence is more challenging than those in individuals because most populations are believed to be regulated by density- or frequency-dependent (a.k.a. soft) selection (Saccheri & Hanski, 2006). Under such selection, mortality from inbreeding depression can be replaced by higher survival of less inbred individuals, resulting in no significant change in population

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size (Ridley, 2003). When inbreeding depression imposes hard selection, or when population regulation is locally weak or operates at a larger spatial scale, such as a metapopulation, inbreeding depression may reduce local population growth rate and increase the risk of local extinction (Frankham, 2005; 2010; Keller & Waller, 2002; Keller, Biebach, & Hoeck, 2007; Saccheri & Hanski, 2006; Whitlock, 2002). Although only few field studies have so far demonstrated elevated extinction risks of local populations due to inbreeding depression (Keller & Waller, 2002; Newman & Pilson, 1997; Saccheri et al., 1998), laboratory studies have shown extinctions driven by inbreeding depression in various taxa including *Drosophila* (Bijlsma, Bundgaard, & Boerema, 2000; Frankham, 1995; Pekkala, Emily Knott, Kotiaho, & Puurtinen, 2012; Rumball, Franklin, Frankham, & Sheldon, 1994; Wallace & Madden, 1965; Wright, Tregenza, & Hosken, 2007), mice (Bowman & Falconer, 1960; Shorter et al., 2017), a seed-feeding beetle (Fox, Scheibly, & Reed, 2008), *Mimulus* (Willis, 1999), ryegrass (Firestone & Jasieniuk, 2012), and Japanese quail (Sittmann, Abplanalp, & Fraser, 1966). Simulation modeling studies also have shown the plausibility of inbreeding-driven extinction under realistic biological circumstances (Brook, Tonkyn, O'Grady, & Frankham, 2002; Frankham, 2005; O'Grady et al., 2006).

Increased extinction risks of local populations may or may not translate to a higher extinction risk of metapopulations. If a metapopulation consists of small populations, increased local extinctions from inbreeding depression may drive the metapopulation below the extinction threshold (Thrall, Richards, McCauley, & J, 1998). On the other hand, a metapopulation may harbor a large local population that experiences inbreeding infrequently or contains a large number of asynchronous local populations, such that the entire metapopulation is unlikely to go extinct. Alternatively, stochastic extinction and colonization events may overwhelm or even counteract the selection from inbreeding depression (Saccheri & Hanski, 2006). If colonizers or immigrants are unrelated, enhanced colonization and

immigration via heterosis and genetic rescue may increase gene flow to partially alleviate elevated extinction risks (Ebert et al., 2002; Richards, 2000; Saccheri & Hanski, 2006).

Although offspring of the colonists may benefit from increased heterozygosity from outcrossing in the following generations, such effects will be short-lived if subsequent immigration is rare. To understand the impact of inbreeding depression on the persistence of metapopulations, studies should address the complex interactions among population dynamics, genetics, and spatial population structure (Hanski & Gaggiotti, 2004; Richards, 2000; Silvertown & Antonovics, 2004).

Landscape structure also likely modifies the balance between selection strength and stochasticity at the metapopulation level (Hanski, 1999; S. Harrison & Hastings, 1996). Small patches can support only a small number of individuals, and small, isolated populations are prone to demographic and genetic stochasticity, including genetic drift. Related individuals living in small populations are prone to inbreeding if they neither avoid mating with kin nor disperse before mating, and patch size and isolation can affect dispersal rate (Bowler & Benton, 1999; Hanski, Alho, & Moilanen, 2000; Heino & Hanski, 2001). Spatially correlated environmental fluctuations can increase synchrony among nearby local populations (Heino, Kaitala, Ranta, & Lindström, 1997), but greater environmental heterogeneity included in larger habitat networks may mitigate bottlenecks through asynchrony among different parts of the metapopulation (Ehrlich & Murphy, 1987; Hanski, 1999). Connectivity of patches may affect the spatial pattern of dispersal and the amounts of demographic and genetic rescue effects. Therefore, we can expect size distribution and configuration of patches in a landscape to influence the frequency of inbreeding in a metapopulation and how significant inbreeding depression is for the persistence of the metapopulation.

A series of empirical studies on the Glanville fritillary butterfly (*Melitaea cinxia*) residing in the Åland Islands in SW Finland comprise one of the most comprehensive research programs on inbreeding depression in natural populations. Using field data from this system, Saccheri et al. (1998) showed that small inbred populations were more likely to go extinct than less inbred populations. Their finding challenged the well-received theoretical view at that time that inbreeding in habitually inbreeding populations is not detrimental, because most deleterious recessive alleles have been purged (D. Charlesworth & Willis, 2009; Garcia-Dorado, 2012; Hedrick, 1994). Other studies have shown that inbred *M. cinxia* females have lower reproductive fitness in the laboratory (Haikola, 2003; Haikola et al., 2001) and that inbred families overwinter less successfully than outbred ones in the field (Nieminen, Singer, Fortelius, Schops, & Hanski, 2001). Furthermore, a small population of the butterfly in an isolated island located 400 km east of Åland has presumably fixed deleterious mutations during the 75 years of isolation, and its extinction in the near future is predicted due to dramatically reduced individual fitness (Mattila et al., 2012). Hence, wild populations of this species may harbor enough genetic load despite habitual inbreeding, and inbreeding depression can lead to population extinction. It is not yet known whether inbreeding depression is substantial enough to increase extinction risks of the metapopulations of *M. cinxia*.

In this study, we quantified the impact of inbreeding depression at the individual level on metapopulation persistence in heterogeneous habitat networks. We used a genetically explicit, individual-based metapopulation modeling approach to analyze how the effects of inbreeding depression vary with the size and the level of fragmentation of habitat patch networks. Previous modeling studies considered the effects of inbreeding on metapopulation persistence in landscapes with simple spatial population structure (Higgins & Lynch, 2001; Theodorou, Souan, & Couvet, 2009; Thrall et al., 1998). Studies that consider more realistic

spatial structure motivated by empirical systems are needed to draw conclusions relevant to natural metapopulations. We built our model in the context of the *M. cinxia* metapopulations in the Åland islands, making use of extensive demographic, genetic, and life-history data available, including fitness costs of inbreeding.

Materials and Methods

Study species, study area, and survey data

M. cinxia completes its life cycle in one year in Åland (see Murphy, Wahlberg, Hanski, & Ehrlich, 2004 for details). Adults emerge, mate, and lay eggs in June. Most matings occur in natal patches; females mate soon after eclosion and mate almost exclusively only once (Boggs & Nieminen, 2004; Hanski, Saastamoinen, & Ovaskainen, 2006; Saastamoinen, 2007), and a large proportion of males stay in their natal patches (Hanski et al., 2006; Wahlberg, Klemetti, Selonen, & Hanski, 2002). Females lay multiple clusters of 100-200 eggs over their lifetime on host plants (Murphy et al., 2004), but the majority (71%) of females leave only one clutch that survives beyond early autumn (Fountain et al., 2018). Larvae emerge in July and build a silken communal nest in early September for overwintering. The range of overwintering mortality of nests varies from 50 to 84% (Nieminen, Siljander, & Hanski, 2004), and nests with a smaller number of larvae suffer from higher winter mortality (Kuussaari, van Nouhuys, Hellman, & Singer, 2004). In the following April larvae resume feeding and pupate in May (Murphy et al., 2004; Nieminen et al., 2004). On average 6, ranging typically between 1 to 25, adult butterflies may emerge from each surviving clutch in June (P. J. Harrison, Hanski, & Ovaskainen, 2011). In this system, inbreeding depression imposes hard selection and results in elevated mortality of inbred individuals (see *Survival probability of offspring* in Supporting Information Appendix S1 for details).

The number of nests has been surveyed since 1993 (and more completely from 2001) every autumn when newly spun winter nests are conspicuously visible (for the survey protocol, see Ojanen, Nieminen, Meyke, Pöyry, & Hanski, 2013). The butterfly lives as a classic metapopulation (i.e., a metapopulation persisting by extinction and colonization balance; Hanski, 1999) inhabiting annually 300-500 small meadows over the area of 3500 km² (Ehrlich & Hanski, 2004; Hanski, 1999; Ojanen et al., 2013; Fig. 1). The total number of nests recorded during the autumn survey ranged from about 1200 to 11000 between 2001 and 2016 (with 40-50% detection probability of nests; Ojanen et al., 2013). The majority of the local populations are small, consisting of one or two nests, and persist for only one year. Larger populations may persist for several years, and 17 patches have been occupied continuously for 20 years or more. The population size fluctuates mostly due to stochastic abiotic conditions (precipitation and temperature; Ehrlich & Hanski, 2004; Hanski, 1999; Tack, Mononen, & Hanski, 2015). The total number of nests fluctuates around a relatively stable mean, although local populations fluctuate strongly both spatially and temporally and frequently go extinct.

The habitat patches are grouped into 125 relatively independent networks (a.k.a. semi-independent networks or SINs) based on connectivity of habitat patches, which is a proxy for the number of immigrants arriving in a focal patch (Hanski et al., 2017). The SINs range in size and spatial structure (Hanski et al., 2017), which we utilized to compare landscapes with different levels of fragmentation. We selected 18 SINs in the north-western quadrant of the mainland Åland as the study area (Fig. 1; covers about 20 km by 20 km). These networks are composed of 849 patches of various area and isolation, and the number of patches in these SINs ranges from 2 to 141 (Table S1). We used metapopulation capacity (Hanski et al., 2017; Hanski & Ovaskainen, 2000) to characterize the degree of fragmentation of the SINs. Metapopulation capacity synthesizes information on habitat area and

connectivity for the dispersal ability of a given species into one quantity to quantify the capacity of habitat networks to support viable metapopulations (Hanski et al., 2017; Hanski & Ovaskainen, 2000). For model fitting, we used data from 549 patches with no missing data in the last 16 years (2001 to 2016). Because more patches were discovered and added to the survey over the years, data are missing from newer patches in the first 9 years. The simulations were run on the 18 SInS and additional surrounding 16 SInS (782 patches) as a buffer to reduce edge effects (Fig. 1). Analyses were conducted on the core 18 SInS.

Model overview

We developed a genetically explicit individual-based simulation model of metapopulations parameterized for *M. cinxia* in Åland. We here briefly summarize the model and the method of model parameterization. A complete description including references is presented in Supporting Information Appendix S1.

The individuals are diploid, reproduce sexually, and carry multiple neutral loci. Their life history is composed of three distinct stages (mating, dispersal, and reproduction) and completes in one year with no overlapping generations (Fig. S1). Individuals emerge in spring, mate in the natal patch (Austin, Ovaskainen, & Hanski, 2011; Hanski et al., 2006) with probability weighted by sex ratio and limited by the maximum number of matings per individual (once for a female, up to three times for a male). They may disperse to one destination patch with distance-weighted probability or stay in the natal patch. Dispersers may die *en route* without reproducing. Emigration rates scale negatively and immigration rates positively with patch size. A female leaves offspring either in her natal patch or destination patch if it disperses. Their baseline fitness is determined by the qualities of natal patches that are stochastically determined at every time step with no temporal but with large-scale spatial autocorrelation. Offspring are subject to elevated mortality due to inbreeding

depression, the severity of which is determined as a function of inbreeding coefficients calculated based on the pedigree recorded during simulations. Inbreeding coefficients are equivalent to the expected probability of any locus containing alleles inherited from a common ancestor (Hartl & Clark, 2007). Offspring mortality is also influenced by regional-scale environmental stochasticity (Nieminen et al., 2004) and local catastrophes (Zheng, Ovaskainen, & Hanski, 2009). Predation and parasitism cause large mortality in the butterfly metapopulations (van Nouhuys & Hanski, 2004) but are not explicitly included and instead subsumed into “patch quality” in this model. Individuals are randomly removed when the number of individuals in a patch exceeds the stochastically fluctuating carrying capacity of the patch (Hanski, 2005; P. J. Harrison et al., 2011).

The number of individuals is recorded after winter mortality but before mating and dispersal; hence the model outputs correspond roughly to the number of adults at eclosion in spring. To match the model results to the nest survey data, the number of adult butterflies from the simulations are probabilistically converted to the number of nests (based on the mean number of adults per nest in spring, 6, estimated in (P. J. Harrison et al., 2011)). We include an observation model to account for incomplete detection by the surveyors (50% detection rate; Ojanen et al., 2013).

Model fitting and validation

We used Approximate Bayesian Computation (ABC; Beaumont, 2010) to estimate the joint posterior distribution of 15 model parameters (Table S2). The simulated populations were characterized by 65 summary statistics calculated at both the SIN and entire metapopulation levels to assess model fit to the summary statistics calculated with the empirical data. In the ABC analysis, we selected the parameter sets that generated simulations closest to the empirical values in terms of summary statistics to approximate the joint posterior distribution

of the parameters. We validated the model fit by comparing various aspects of the outputs with the survey data and other empirical data (Fig. S2). The simulations were 300 generations long, and the first 150 generations were discarded as a burn-in period.

Using outputs from simulations with the parameter sets from the approximate joint posterior distribution, we used logistic regression to examine the dependency of extinctions on population size, connectivity, habitat area, and inbreeding coefficient at the local population scale. We did this to check whether the model outputs were consistent with previous empirical findings by Saccheri et al. (1998) and also newly at the SIN scale for this study. We counted the number of extinction events in the last 150 generations of simulations and fitted logistic regression to each simulation. We sampled extinction events with replacement for each simulation to check the effects of sample size (the number of extinctions) on the variability of the estimated values of the coefficients and to deal with different numbers of extinction events in the simulation runs.

Quantifying the effects of inbreeding depression on metapopulation persistence

To more directly examine the causality between inbreeding depression and metapopulation persistence, we ran the model, which is fitted to data with inbreeding depression in effect, but this time with inbreeding depression turned off. We calculated effect sizes of inbreeding depression by taking the differences between model runs in the presence and absence of inbreeding depression. We quantified the effects of inbreeding depression in terms of metapopulation-wide extinction rate, metapopulation size (total number of individuals), patch incidence (fraction of patches occupied), and mean heterozygosity across the 18 SInS with respect to metapopulation capacity. In addition, we measured the frequencies of females mating with full- or half-siblings.

We then modified landscape structure in a 5 km x 5 km area within the study area (Fig. 1) to examine its effects on how inbreeding depression affected these measures. We focused on 9 selected hypothetical landscape scenarios (10 including the original landscape) to examine the effects of total area, patch size distribution, the number of patches, and patch clustering (Fig. 4, Table 1, S3). In brief, total patch area was equal among scenarios except scenario 10, in which it was doubled. Scenarios 1-3 contrasted different degrees of patch clustering with the original patch size distribution. In Scenarios 4-9, total area was subdivided into 34, 68, or 102 patches of equal sizes clustered at different degrees to reflect increasing fragmentation. Because isolating only one property of a landscape from others is difficult owing to inherent correlations among components of landscape structure, we looked at combinations of these scenarios to deduce the effects of landscape structure (Table 1, S3). The effect size of 0 indicates no effect of inbreeding depression, and a positive effect size indicates an adverse effect. We then compared the scenarios in a pairwise manner and took the difference between the effect sizes of inbreeding depression in each pair. We then examined the posterior predictive distributions of the differences and identified pairs with probability > 0.95 of being above zero. We ran 20 replicates for each of the parameter sets from the approximate joint posterior distribution and took the mean across the replicates to represent the effect size for the parameter set.

Results

The ABC analysis for model fitting

In the ABC analysis, we approximated the posterior distribution of model parameters by selecting 85 simulations that produced summary statistics closest to those of the empirical data (for detail, see Supporting Information). With the parameter sets from the approximate joint posterior distribution, the model predicts realistic ecological dynamics (e.g.,

metapopulation size and patch incidence increase and extinction rate decreases with increasing metapopulation capacity; Fig. S3). We used these simulations to assess the effects of inbreeding depression on population persistence and draw other biologically relevant inferences as following.

Logistic regression for the effects of population size and connectivity on local and metapopulation extinction

In agreement with the empirical findings by Saccheri et al. (1998), our results from the logistic regression show that inbreeding increased extinction risks of local populations after accounting for ecological factors. It increased extinction in 93% of the posterior predictive distribution of the coefficient of F (inbreeding coefficient; Table 2). The logistic regressions at the SIN level revealed the effects of inbreeding on extinction risks also of metapopulations in 84% of the posterior predictive distribution (Table 2). While connectivity (as measured by N_{tend} and N_{neigh} , see the caption in Table 2 for the definitions) reduced extinction risks of local populations generally consistently after accounting for population size, habitat area, and inbreeding coefficient as in Saccheri et al. (1998), SIN-level connectivity did not do so as consistently (Table 2). This result indicates that metapopulations are less dependent on migrants from neighboring SINs for persistence (i.e., rescue effects) than are local populations on migrants from neighboring local populations.

Although metapopulation persistence did not rely on SIN-level connectivity as much, the metapopulations exchanged many migrants (Fig. S4). On average about 17.5% of the migrants dispersed between SINs, mostly to neighboring SINs (Fig. 1, S8). This fraction is close to the value estimated from genetic data (25%, which is likely an overestimate because inter-SIN migrants are more likely to be detected than migrants within a SIN; M. F. DiLeo, *unpublished data*). The largest three SINs (65, 34, 2) were net exporters of migrants, and the

rest were net importers (Fig. S4). The marginal posterior distribution of the parameter adjusting inter-SIN distance relative to intra-SIN suggested modest resistance of the intervening area to migration between SINs, equivalent to about 10% increase in geographical distance on average (Fig. S5, the histogram for SIN_stretch).

The effects of inbreeding depression at the metapopulation scale

The frequency of mating among siblings was higher in smaller SINs, while it was still substantial in large SINs (Fig. 2). As expected, the effect sizes of inbreeding depression on metapopulation persistence reflected the frequency of inbreeding (Fig. 3). The effect sizes in terms of metapopulation extinction rate, metapopulation size, network incidence (fraction of patches occupied), and mean heterozygosity indicated that adverse effects of inbreeding depression on metapopulation persistence were more pronounced in smaller metapopulations. There was a suggestive pattern that the smallest networks may experience less adverse effects than those that are slightly larger. The difference in metapopulation capacity amongst the smallest four networks is small, but habitat area in these networks are 5- to 10-fold different (Table S1). Averaged over time and the whole study region of the 18 SINs, 25.2% of females mated with full- or half-brothers. At the local population level, the mean frequency of mating among siblings was 30.7% (min 5.7%, max 71.0%). The fraction of adult mortality incurred by inbreeding depression varied over time with the mean at 10.1% (min 5.2%, max 20.5%).

Landscape structure scenarios

The effects of inbreeding depression on metapopulation persistence (Fig. 4a-1, b-1, c-1; see Fig. S6 for all 10 scenarios) and mean heterozygosity (Fig. 4d-1) depended on landscape structure with large variation within scenarios. Inbreeding depression consistently incurred larger adverse effects on the landscapes having very small patches (scenarios 1, 2, 4) than on

the landscapes with doubled patch/total habitat area (scenario 10) or those with a half as many but larger patches in clusters (scenario 5; Fig. 4a-2, b-2, c-2). Extinction rate is the most stringent measure among the four, as the others can capture more gradual effects on metapopulation viability. With respect to mean heterozygosity (Fig. 4d-2), inbreeding depression consistently incurred larger effects on the landscapes composed of only small patches (scenario 7). Within the same patch size distribution (lognormal or equally sized), patch arrangement (clustered or random) did not make a difference (scenarios 1-3, analogous comparisons for scenarios 5-7 but results not shown). Neither did patch size distribution (scenarios 1, 4). The effect of inbreeding depression was generally lower when mean patch area was larger (scenarios 5-7; more blue panels for scenario 5 and more red for scenario 7) or when total habitat area was larger (scenarios 1 vs. 10).

Discussion

In this study, we examined how the effects of inbreeding depression in individuals scale up to manifest at the metapopulation level for a well-studied butterfly in realistically complex landscapes. The results show that inbreeding depression reduces the persistence of small metapopulations and degrades the viability (metapopulation size and genetic diversity) of a range of sizes of metapopulations. These effects decline with metapopulation capacity. We conclude that negative fitness consequences of inbreeding depression at the individual level can propagate through the hierarchy of spatial scales and impact the persistence of not only local populations (Saccheri et al., 1998) but also of small metapopulations. Our study provides an example from a field-based system to extend empirically well-supported negative consequences of inbreeding depression in individuals and wild populations (Frankham, 2005; 2010) to metapopulations at a landscape scale.

Landscape structure, stochasticity, selection, and movement behavior

In the study system, the large metapopulations contain large local populations that do not readily go extinct. However, lower but still substantial inbreeding occurred even in the large SIs. This can be ascribed to the assumptions about the butterfly's reproductive behavior and landscape structure: they mate mostly in natal patches (in the model, they exclusively do so), show no evidence for inbreeding avoidance (Haikola, Singer, & Pen, 2004), lay large clusters of eggs, and the majority of patches, hence local populations, are small. These contribute in concert to the probability of encountering siblings and other closely related individuals while looking for mates. On the other hand, the small metapopulations lack large long-lasting local populations. Although their dynamics are quite stochastic, increased hard selection from inbreeding depression can reduce metapopulation persistence. There is a suggestive pattern that the smallest metapopulations may experience less negative effects of inbreeding depression. Extinctions in the smallest metapopulations are likely governed more by demographic and environmental stochasticity, so that added mortality from inbreeding depression may cause only a minor increase in extinction rate.

The results from testing the different landscape scenarios (Fig. 4) suggest that both total habitat amount and patch size can influence the negative impact of inbreeding depression on metapopulation persistence in this butterfly. The results show that mean patch area is an important characteristic of landscapes determining the magnitude. The scenarios where mean patch size is twice as large as the original (scenarios 5 and 10 vs. other scenarios; Fig. 4) led to lower negative effects of inbreeding depression. In landscapes with numerous small patches (scenario 7), inbreeding depression incurs greater effects on mean heterozygosity largely due to rampant inbreeding and genetic drift in small populations (Fig. 4d-2). It is important to note that landscape structure is multifaceted and the components are

correlated due to geometric constraints (especially in a fixed area), so that varying one aspect of landscape structure is almost always accompanied by other changes especially in a realistic landscape context (e.g., patch size and patch proximity may trade off when the same total habitat area is subdivided into different numbers of patches). We interpreted the results from differently manipulated landscapes with a stringent criterion (95% consistency) to deduce some of the causal effects of landscape structure on the effects of inbreeding depression.

Given the mating and oviposition behavior of this butterfly, mean patch size (through population size) per se may contribute the most to the probability of mating with relatives. Therefore, habitat amount alone is not necessarily the best predictor of species occurrence or abundance or genetic diversity for this butterfly metapopulation. These results illustrate that dispersal ability, mating and oviposition behavior, and landscape structure together determine susceptibility of a metapopulation to elevated extinction due to inbreeding depression.

Because inbreeding depression is not uncommon in nature (Crnokrak & Roff, 1999; Keller & Waller, 2002), and because many butterfly species presumably mate in natal patches (females often mate only once soon after eclosion (Rutowski, 1991; Wickman, 2009) and males often stay in natal patches (Stevens, Turlure, & Baguette, 2010)), we expect our findings to give insights into extinction risks in metapopulations of other species of butterflies experiencing inbreeding depression.

Whether habitat fragmentation independent of habitat amount influences population persistence and species richness in a landscape has been controversial (Fahrig, 2013; Hanski, 2015). The habitat amount hypothesis proposes that the number of species in a sampled area, due to the sample area effect, can be predicted by habitat amount alone in a local landscape scaled appropriately to the focal species' mobility (Fahrig, 2013). Our study does not directly test the habitat amount hypothesis but provides one illustration that habitat amount and configuration both can influence species occurrence through inbreeding depression and

metapopulation persistence. The results show that landscapes with twice as much habitat amount (scenario 10) were never worse than others, but habitat amount per se would not identify landscapes with larger mean patch size (scenario 5) as potentially effective in alleviating negative effects of inbreeding depression (Fig. 4). In this butterfly, doubling mean patch area by merging habitat into a half as many patches could reduce extinction risks from inbreeding depression. Therefore, we argue that habitat amount alone in a local landscape is not necessarily the best predictor of species occurrence or abundance at least in these butterfly metapopulations. So far, support for the habitat amount hypothesis is not very strong (7 out of 15 studies at the time of this writing provided evidence for it. e.g., (Haddad, Holt, Jr Fletcher, Loreau, & Clobert, 2017; Seibold et al., 2017); also see a meta-analysis by Martin, 2018). These equivocal results suggest the need for more theoretical and mechanistic understanding of the effects of landscape structure considering species' life-history characteristics and genetics on species richness and occurrence in fragmented landscapes (Hanski 2015).

Approaches to modeling the genetics of inbreeding depression

Details about the genetic basis of inbreeding depression and fitness consequences are not fully known for *M. cinxia*. One well-studied locus harbors the *Pgi* gene (Saccheri & Hanski, 2006). Heterozygotes at the locus have a fitness advantage, and one of the homozygotes is detrimental in Åland (Saccheri & Hanski, 2006). Deleterious mutations are also suggested to partly underlie inbreeding depression (Mattila et al., 2012). In this study, we adhered to the basic definition of inbreeding (mating among relatives) and used inbreeding coefficients calculated from the pedigree. This approach enabled us to parameterize the fitness cost function using previous empirical results of the butterfly. Previous modeling studies that considered the effects of inbreeding on metapopulation persistence either quantified the

degree of inbreeding at the population level, understating the importance of genetic stochasticity, or assumed deleterious recessive alleles (Higgins & Lynch, 2001; Theodorou et al., 2009). Our study broadens the condition for metapopulation extinction caused by inbreeding depression to individual-level relatedness (i.e., increased autozygosity).

Deleterious recessive alleles and heterozygote advantage are two main genetic mechanisms discussed in literature for inbreeding depression due to increased homozygosity (Keller & Waller 2002; Charlesworth & Willis, 2009). Although deleterious recessive alleles are a plausible explanation for the butterfly (Mattila et al., 2012), it would involve more demanding computation, and essential data such as mutation rate and effect sizes of deleterious mutations are lacking. Another approach could have been to impose inbreeding depression on individuals based on a fitness-heterozygosity relationship. While the advantage would be to incorporate genetic stochasticity associated with recombination, inferring inbreeding from heterozygosity may not be as accurate as inbreeding coefficients calculated from high quality pedigrees (Balloux, Amos, & Coulson, 2004; Pemberton, 2004; Slate et al., 2004). The results would have been similar, as simulated heterozygosity and inbreeding coefficients are negatively linearly correlated in our model.

Conclusion

We conclude that negative fitness consequences of inbreeding depression at the individual level can impact the persistence of small metapopulations in a realistic context. The results exemplify the conjecture that elevated extinction rates of local populations due to inbreeding depression could be strong enough to cause extinction at the landscape scale, despite stochastic dynamics and density-dependent selection (Roslin, 2001; Saccheri & Hanski, 2006). Habitat configuration, apart from habitat amount, can influence the magnitude of the effect of inbreeding depression on metapopulation persistence in this butterfly species. The

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results from this study reinforce the idea that genetic factors, in addition to demographic factors, can contribute to extinctions of small populations (Frankham, 2005; 2015; Saccheri et al., 1998; Spielman, Brook, & Frankham, 2004) and also of metapopulations.

Data accessibility

The MATLAB code for the simulation models and the necessary input data as well as for the ABC analysis and logistic regressions are available through Dryad Data Repository (<https://doi.org/10.5061/dryad.c3618g7>). The approximate joint posterior distribution of the model parameters is also available. Simulation data used in the paper are available upon request from the first author.

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Author contributions

Late Professor and Academician Ilkka Hanski sadly passed away during the preparation of this manuscript. I.H. conceived the original idea, and I.H., O.O. and E.N. initiated the study and developed the model. E.N. implemented and analyzed the model and wrote the paper. J.S. assisted E.N. with Approximate Bayesian Computation and contributed texts regarding the methods. P.S. wrote the code for calculating inbreeding coefficients in C. L.R. and O.O. provided guidance for the model analysis and interpretation. All the authors except I.H. commented on the manuscript and approved the final version.

References

- Allendorf, F. W., Luikart, G. H., & Aitken, S. N. (2012). *Conservation and the Genetics of Populations*. Wiley-Blackwell.
- Austin, A., Ovaskainen, O., & Hanski, I. (2011). Size and genetic composition of the colonizing propagules in a butterfly metapopulation. *Oikos*, 120, 1357–1365. doi:10.1111/j.1600-0706.2010.18992.x
- Balloux, F., Amos, W., & Coulson, T. (2004). Does heterozygosity estimate inbreeding in real populations? *Molecular Ecology*, 13, 3021–3031. doi:10.1111/j.1365-294X.2004.02318.x
- Beaumont, M. A. (2010). Approximate Bayesian Computation in Evolution and Ecology. *Annual Review of Ecology, Evolution, and Systematics*, 4, 379–406. doi:10.1146/annurev-ecolsys-102209-144621
- Bijlsma, R., Bundgaard, J. & Boerema, A. C. (2000). Does inbreeding affect the extinction risk of small populations?: predictions from *Drosophila*. *Journal of Evolutionary Biology* 502–514. doi:10.1046/j.1420-9101.2000.00177.x

Boggs, C. L., & Nieminen, M. (2004). Checkerspot reproductive biology. In *On the wings of checkerspots: a model system for population biology* (pp. 92–111).

Bowler, D. E., & Benton, T. G. (1999). Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. *Biological Reviews*, 80, 205–225. doi:10.1017/S1464793104006645

Bowman, J. C. & Falconer, D. S. (1960). Inbreeding depression and heterosis of litter size in mice. *Genetics Research* 1, 262-274.

Brook, B. W., Tonkyn, D. W., OGrady, J. J., & Frankham, R. (2002). Contribution of inbreeding to extinction risk in threatened species. *Conservation Ecology*.
<http://www.ecologyandsociety.org/vol6/iss1/art16/main.html>

Charlesworth, D., & Charlesworth, B. (1987). Inbreeding Depression and its Evolutionary Consequences. *Annual Review of Ecology and Systematics*, 18, 237–268.
doi:10.2307/2097132?ref=search-gateway:f79bdea72f220638c065a6c7afebaf75

Charlesworth, D., & Willis, J. H. (2009). Fundamental Concepts in Genetics: The genetics of inbreeding depression. *Nature Reviews Genetics*, 10, 783–796. doi:10.1038/nrg2664

Crnokrak, P., & Roff, D. A. (1999). Inbreeding depression in the wild. *Heredity*, 1–11.

Ebert, D., Haag, C., Kirkpatrick, M., Riek, M., Hottinger, J. W., & Pajunen, V. I. (2002). A selective advantage to immigrant genes in a *Daphnia* metapopulation. *Science*, 295, 485–488. doi:10.1126/science.1067485

Ehrlich, P. R., & Hanski, I. (2004). *On the wings of checkerspots: a model system for population biology*. Oxford University Press.

Ehrlich, P. R., & Murphy, D. D. (1987). conservation lessons from long-term studies of checkerspot butterflies. *Conservation Biology*, 1, 122–131. doi:
10.2307/2385828?refreqid=search-gateway:e79c8618334a061e31702f1cc069312b

Fagan, W. F., & Holmes, E. E. (2006). Quantifying the extinction vortex. *Ecology Letters*, 9, 51–60. doi:10.1111/j.1461-0248.2005.00845.x

Fahrig, L. (2013). Rethinking patch size and isolation effects: the habitat amount hypothesis. *Journal of Biogeography*, 40, 1649–1663. doi:10.1111/jbi.12130

Firestone, J. L. & Jasieniuk, M. (2012) Reduced genetic diversity in the parental population decreases offspring quality in an invasive grass 1. *The Journal of the Torrey Botanical Society* 139, 349–355.

Fountain, T., Husby, A., Nonaka, E., DiLeo, M. F., Korhonen, J. H., Rastas, P., et al. (2018). Inferring dispersal across a fragmented landscape using reconstructed families in the Glanville fritillary butterfly, *Evolutionary Applications*, 11, 287-297. doi:0.1111/eva.12552

Fox, C. W., Scheibly, K. L. & Reed, D. H. (2008) Experimental evolution of the genetic load and its implications for the genetic basis of inbreeding depression. *Evolution* 62, 2236–2249.

Frankham, R. (1995). Conservation genetics. *Annual Review of Genetics*, 29, 305–327. doi:10.1146/annurev.genet.29.1.305

Frankham, R. (2005). Genetics and extinction. *Biological Conservation*, 126, 131–140. doi:10.1016/j.biocon.2005.05.002

Frankham, R. (2010). Inbreeding in the wild really does matter. *Heredity*, 104, 124. doi:10.1038/hdy.2009.155

Frankham, R. (2015). Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. *Molecular Ecology*, 24, 2610–2618. doi:10.1111/mec.13139

Frankham R., Ballou, J. D. & Briscoe, D. A. (2010). *Introduction to Conservation Genetics*. Cambridge University Press.

- Garcia-Dorado, A. (2012). Understanding and Predicting the Fitness Decline of Shrunk Populations: Inbreeding, Purging, Mutation, and Standard Selection. *Genetics*, 190, 1461–1476. doi:10.1534/genetics.111.135541
- Gilpin, M., & Soule, M. E. (1986). Minimum viable populations: processes of species extinction. In *Conservation Biology: The Science of Scarcity and Diversity* (pp. 19–34).
- Haddad, N. M., Holt, R. D., Fletcher, R. J. Jr., Loreau, M., & Clobert, J. (2017). Connecting models, data, and concepts to understand fragmentation's ecosystem-wide effects. *Ecography*, 40, 1–8. doi:10.1111/ecog.02974
- Haikola, S. (2003). Effects of inbreeding in the Glanville fritillary butterfly (*Melitaea cinxia*). *Annales Zoologici Fennici*, 40, 483–493.
- Haikola, S., Fortelius, W., O'Hara, R. B., Kuussaari, M., Wahlberg, N., Saccheri, I. J., ... Hanski, I. (2001). Inbreeding depression and the maintenance of genetic load in *Melitaea cinxia* metapopulations. *Conservation Genetics*, 325–335.
- Haikola, S., Singer, M. C., & Pen, I. (2004). Has inbreeding depression led to avoidance of sib mating in the Glanville fritillary butterfly (*Melitaea cinxia*)? *Evolutionary Ecology*, 18, 113–120.
- Hanski, I. (1999). *Metapopulation Ecology*. Oxford University Press.
- Hanski, I. (2005). *The shrinking world: ecological consequences of habitat loss*. Vol. 14 of Excellence in Ecology, International Ecology Institute.
- Hanski, I. (2015). Habitat fragmentation and species richness. *Journal of Biogeography*, 42, 989–993.
- Hanski, I., & Gaggiotti, O. E. (2004). *Ecology, genetic, and evolution of metapopulations*. Elsevier.
- Hanski, I., & Ovaskainen, O. (2000). The metapopulation capacity of a fragmented landscape. *Nature*, 404, 755–758. doi:10.1038/35008063

Hanski, I., Alho, J., & Moilanen, A. (2000). Estimating the parameters of survival and migration of individuals in metapopulations. *Ecology*, 81, 239–251.

Hanski, I., Saastamoinen, M., & Ovaskainen, O. (2006). Dispersal-related life-history trade-offs in a butterfly metapopulation. *Journal of Animal Ecology*, 75, 91–100. doi:10.1111/j.1365-2656.2005.01024.x

Hanski, I., Schulz, T., Wong, S. C., Ahola, V., Ruokolainen, A., & Ojanen, S. P. (2017). Ecological and genetic basis of metapopulation persistence of the Glanville fritillary butterfly in fragmented landscapes. *Nature Communications*, 8, 1–11. doi:10.1038/ncomms14504

Harrison, P. J., Hanski, I., & Ovaskainen, O. (2011). Bayesian state-space modeling of metapopulation dynamics in the Glanville fritillary butterfly, *Ecological Monographs*, 81, 581–598. doi:10.1890/11-0192.1

Harrison, S., & Hastings, A. (1996). Genetic and evolutionary consequences of metapopulation structure. *Trends in Ecology & Evolution*, 1, 180–183.

Hartl, D. L., & Clark, A. G. (2007). *Principles of Population Genetics*. 4th ed. Sinauer Associates Incorporated.

Hedrick, P. W. (1994). Purging inbreeding depression and the probability of extinction: full-sib mating. *Heredity*, 73, 363–372.

Hedrick, P. W., & Garcia-Dorado, A. (2016). Understanding Inbreeding Depression, Purging, and Genetic Rescue. *Trends in Ecology & Evolution*, 3, 940–952. doi:10.1016/j.tree.2016.09.005

Hedrick, P. W., & Kalinowski, S. T. (2000). Inbreeding depression in conservation biology. *Annual Review of Ecology and Systematics*, 31, 139–162. doi:10.2307/221728

Heino, M., & Hanski, I. (2001). Evolution of migration rate in a spatially realistic metapopulation model. *The American Naturalist*, 157, 495–511. doi:10.1086/319927

Heino, M., Kaitala, V., Ranta, E., & Lindström, J. (1997). Synchronous dynamics and rates of extinction in spatially structured populations. *Proceedings of the Royal Society B: Biological Sciences*, 264, 481–486.

Higgins, K., & Lynch, M. (2001). Metapopulation extinction caused by mutation accumulation. *Proceedings of the National Academy of the United States of America*, 98, 2928–2933.

Keller, L. F., & Waller, D. M. (2002). Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, 17, 230–241. doi: 10.1016/s0169-5347(02)02489-8

Keller, L. F., Biebach, I., & Hoeck, P. E. A. (2007). The need for a better understanding of inbreeding effects on population growth. *Animal Conservation*, 10, 286–287. doi:10.1111/j.1469-1795.2007.00133.x

Kuussaari, M., van Nouhuys, S., Hellman, J. J., & Singer, M. C. (2004). Larval biology of checkerspots. In *On the wings of checkerspots: a model system for population biology* (pp. 138–160).

Martin, C. A. (2018) An early synthesis of the habitat amount hypothesis. *Landscape Ecology*, 33, 1831–1835.

Mattila, A. L. K., Duploux, A., Kirjokangas, M., Lehtonen, R., Rastas, P., & Hanski, I. (2012). High genetic load in an old isolated butterfly population. *Proceedings of the National Academy of Sciences of the United States of America*, 109, E2496–505. doi: 10.1073/pnas.1205789109

Murphy, D. D., Wahlberg, N., Hanski, I., Hanski, I. K., & Ehrlich, P. R. (2004). Introducing checkerspots: taxonomy and ecology. In *On the wings of checkerspots: a model system for population biology* (pp. 17–33).

Newman, D., & Pilson, D. (1997). Increased probability of extinction due to decreased genetic effective population size: Experimental populations of *Clarkia pulchella*. *Evolution*, 51, 354–362.

Nieminen, M., Siljander, M., & Hanski, I. (2004). Structure and dynamics of *Melitaea cinxia* metapopulations. In *On the wings of checkerspots: a model system for population biology* (pp. 63–91).

Nieminen, M., Singer, M. C., Fortelius, W., Schops, K., & Hanski, I. (2001). Experimental confirmation that inbreeding depression increases extinction risk in butterfly populations. *The American Naturalist*, 157, 237–244. doi:10.1086/318630

Nonaka E., Sirén, J., Somervuo, P., Ruokolainen, L., Ovaskainen, O., Hanski, I. (2019). Data from: Scaling up the effects of inbreeding depression from individuals to metapopulations. Dryad Digital Repository. <https://doi.org/10.5061/dryad.c3618g7>

Ojanen, S. P., Nieminen, M., Meyke, E., Pöyry, J., & Hanski, I. (2013). Long-term metapopulation study of the Glanville fritillary butterfly (*Melitaea cinxia*): survey methods, data management, and long-term population trends. *Ecology and Evolution*, 3, 3713–3737. doi: 10.1002/ece3.733

O’Grady, J. J., Brook, B. W., Reed, D. H., Ballou, J. D., Tonkyn, D. W., & Frankham, R. (2006). Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. *Biological Conservation*, 133, 42–51. doi:10.1016/j.biocon.2006.05.016

Pekkala, N., Emily Knott, K., Kotiaho, J. S. & Puurtinen, M. (2012) Inbreeding rate modifies the dynamics of genetic load in small populations. *Ecology and Evolution* 2, 1791–1804.

Pemberton, J. (2004). Measuring inbreeding depression in the wild: the old ways are the best. *Trends in Ecology & Evolution*, 19, 613–615. doi:10.1016/j.tree.2004.09.010

Ralls, K., Ballou, J. D., & Templeton, A. (1988). Estimates of Lethal Equivalents and the Cost of Inbreeding in Mammals. *Conservation Biology*, 2, 185–193.

doi:10.2307/2386104?refreqid=search-gateway:bd0f4a1207db1a10b0f5b671b1e8fd6d

Richards, C. M. (2000). Inbreeding Depression and Genetic Rescue in a Plant Metapopulation. *The American Naturalist*, 155, 383–394. doi:10.1086/303324

Ridley, M. (2003). *Evolution*. 3rd ed. Blackwell.

Roslin, T. (2001). Inbreeding in nature: brothers and sisters, do not unite! *Trends in Ecology & Evolution*, 16, 225–225.

Rumball, W., Franklin, I. R., Frankham, R. & Sheldon, B. L. (1994) Decline in heterozygosity under full-sib and double first-cousin inbreeding in *Drosophila melanogaster*. *Genetics* **136**, 1039–1049.

Rutowski, R. L. (1991). The Evolution of Male Mate-Locating Behavior in Butterflies. *The American Naturalist*, 138, 1121–1139. doi:10.1086/285273

Saastamoinen, M. (2007). Life-history, genotypic, and environmental correlates of clutch size in the Glanville fritillary butterfly. *Ecological Entomology*, 32, 235–242.

doi10.1111/j.1365-2311.2007.00865.x

Saccheri, I., & Hanski, I. (2006). Natural selection and population dynamics. *Trends in Ecology & Evolution*, 21, 341–347. doi:10.1016/j.tree.2006.03.018

Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W., & Hanski, I. (1998). Inbreeding and extinction in a butterfly metapopulation. *Nature*, 392, 491–494.

doi:10.1038/33136

Seibold, S., Bässler, C., Brandl, R., Fahrig, L., Förster, B., Heurich, M., et al. (2017). An experimental test of the habitat-amount hypothesis for saproxylic beetles in a forested region. *Ecology*, 98, 1613–1622. doi:10.1002/ecy.1819

Shorter, J. R., Odet, F., Aylor, D. L., Pan, W., Kao, C.-Y., Fu, D.-P., ..., de Villena, F. P.-M.

(2017) Male Infertility Is Responsible for Nearly Half of the Extinction Observed in the Mouse Collaborative Cross. *Genetics* **206**, 557–572.

Silvertown, J., & Antonovics, J. (2004). *Integrating ecology and evolution in a spatial context*. Blackwell.

Sittmann, K., Abplanalp, H. & Fraser, R. A. (1966) Inbreeding depression in Japanese quail. *Genetics* **54**, 371–379.

Slate, J., David, P., Dodds, K. G., Veenvliet, B. A., Glass, B. C., Broad, T. E., & McEwan, J. C. (2004). Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. *Heredity*, *93*, 255–265. doi:10.1038/sj.hdy.6800485

Spielman, D., Brook, B. W., & Frankham, R. (2004). Most species are not driven to extinction before genetic factors impact them. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 15261–15264.

Stevens, V. M., Turlure, C., & Baguette, M. (2010). A meta-analysis of dispersal in butterflies. *Biological Reviews*, *16*, doi:10.1111/j.1469-185X.2009.00119.x

Tack, A. J. M., Mononen, T., & Hanski, I. (2015). Increasing frequency of low summer precipitation synchronizes dynamics and compromises metapopulation stability in the Glanville fritillary butterfly. *Proceedings of the Royal Society B: Biological Sciences*, *282*, 20150173–20150173. doi:10.1098/rspb.2015.0173

Theodorou, K., Souan, H., & Couvet, D. (2009). Metapopulation persistence in fragmented landscapes: significant interactions between genetic and demographic processes. *Journal of Evolutionary Biology*, *22*, 152–162. doi:10.1111/j.1420-9101.2008.01634.x

- Thrall, P. H., Richards, C. M., & McCauley, D. E. (1998). Metapopulation collapse: the consequences of limited gene-flow in spatially structured populations. In J. Bascompte & S. R V (Eds.), *Modeling spatiotemporal dynamics in ecology* (pp. 83–104).
- van Nouhuys, S., & Hanski, I. (2004). Natural enemies of checkerspots. In *On the wings of checkerspots: a model system for population biology* (pp. 161–180).
- Wahlberg, N., Klemetti, T., Selonen, V., & Hanski, I. (2002). Metapopulation structure and movements in five species of checkerspot butterflies. *Oecologia*, 130, 33–43.
doi:10.1007/s004420100775
- Wallace, B. & Madden, C. (1965). Studies on inbred strains of *Drosophila melanogaster*. *The American Naturalist* **99**, 495–510.
- Whitlock, M. C. (2002). Selection, load and inbreeding depression in a large metapopulation. *Genetics*, 160, 1191–1202.
- Wickman, P.-O. (2009). Mating behaviour in butterflies. In *Ecology of Butterflies in Europe* (pp. 9–16). Cambridge University Press.
- Willis, J. H. (1999) The role of genes of large effect on inbreeding depression in *Mimulus guttatus*. *Evolution* **53**, 1678–1691.
- Wright, L. I., Tregenza, T. & Hosken, D. J. (2008). Inbreeding, inbreeding depression and extinction. *Conservation Genetics* **9**, 833–843.
- Zheng, C., Ovaskainen, O., & Hanski, I. (2009). Modelling single nucleotide effects in phosphoglucose isomerase on dispersal in the Glanville fritillary butterfly: coupling of ecological and evolutionary dynamics. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 364, 1519–1532. doi:10.1098/rstb.2009.0005

Tables and figures

Table 1. Landscape structure for 7 of the 10 hypothetical landscape scenarios (the complete table is in Supporting Information Appendix S1). The patch size distribution for the scenarios are shown in Fig. S9. Scenario 1 is the original landscape, and the patch size distribution is nearly lognormal (Fig. S9). When “Original” is indicated in the table, the feature is the same as it is in the original landscape (shaded with gray). Example landscapes are shown in Figure 4.

Scenarios	Landscape structure				
	Total area (TA)	No. of patches (NP)	Patch size distribution (PD)	Mean patch area (MA)	Patch clustering (PC)
1	Original	Original (68)	Original (close to lognormal; Fig. S9)	Original	Original patch locations
2					Clusters of 8 patches, *
4					Original patch locations
5		34 (half as many)	Equal size	Doubled	Clusters of 8 patches, *
6		Original		Original	
7		102 (50% more)		2/3 of original	
10	Original x 2	Original	Each patch is twice as large as the original	Doubled	Original patch locations

*random locations of cluster centers

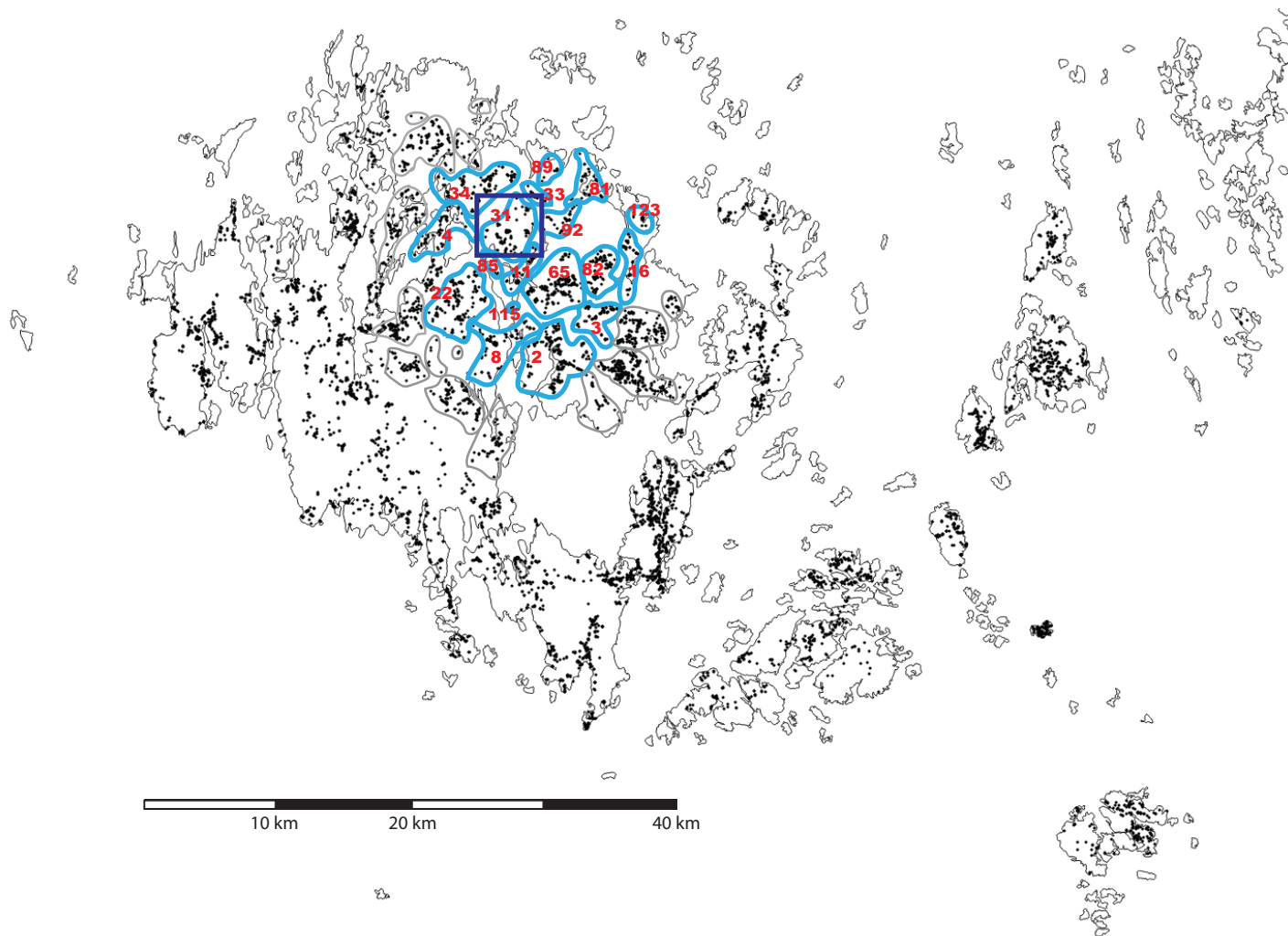


Figure 1. A map of 4400+ habitat patches (black dots) in the Åland Islands. Light blue polygons delineate the 18 study semi-independent networks (SINs; their ID numbers are indicated in red), and gray the buffer networks. The dark blue square encloses the 5 km x 5 km area where landscape structure was manipulated.

Table 2. The mean (95% confidence limits) of the coefficients of the multiple logistic regression models predicting the probability of extinction at the local and the SIN levels. The posterior support for increasing extinction probability is calculated as the proportion of the approximate joint posterior distribution that yields positive coefficients. The explanatory variables: $\log N$ = log-transformed population size, N_{neigh} = the sum of distance-weighted sizes of neighboring populations or metapopulations, N_{trend} = the temporal trends in the size of neighboring populations or metapopulations ($N_{\text{trend},t-1} = N_{\text{neigh},t-1} - N_{\text{neigh},t}$), Area = habitat area (patch area or the sum of patch area in SINs), F = inbreeding coefficient. The results from simple logistic regression models predicting the probability of extinction are also presented in the row named “F alone.” The logistic regression model was fitted separately to extinction events for each of the 85 simulation runs with parameter sets sampled from the approximate joint posterior distribution. Extinction events are sampled 10000 times with replacement. The mean values are very similar across different sample sizes (100 to 20000), and the spread stabilized after sample size of 5000.

Explanatory variables	Local	Posterior support for increasing extinction probability of local populations	SIN	Posterior support for increasing extinction probability of metapopulations in SINs	759 760 761 762 763 764 765
LogN	-0.64 (-0.83, -0.45)	0%	-1.47 (-1.99, -0.96)	0%	766 767
N_{neigh}	-0.31 (-0.81, 0.43)	16.5%	-0.0016 (-0.0070, 0.0019)	24.7%	768 769
N_{trend}	-0.18 (-0.54, 0.19)	15.3%	0.0016 (-0.032, 0.046)	48.2%	770 771
Area	-0.40 (-0.62, -0.16)	0%	-0.41 (-1.1, 0.21)	10.6%	772 773
F	0.77 (-0.31, 1.94)	92.9%	1.31 (-1.48, 4.24)	83.5%	774 775 776
F alone	2.44 (1.16, 3.75)	100%	8.4 (5.0, 11.5)	100%	

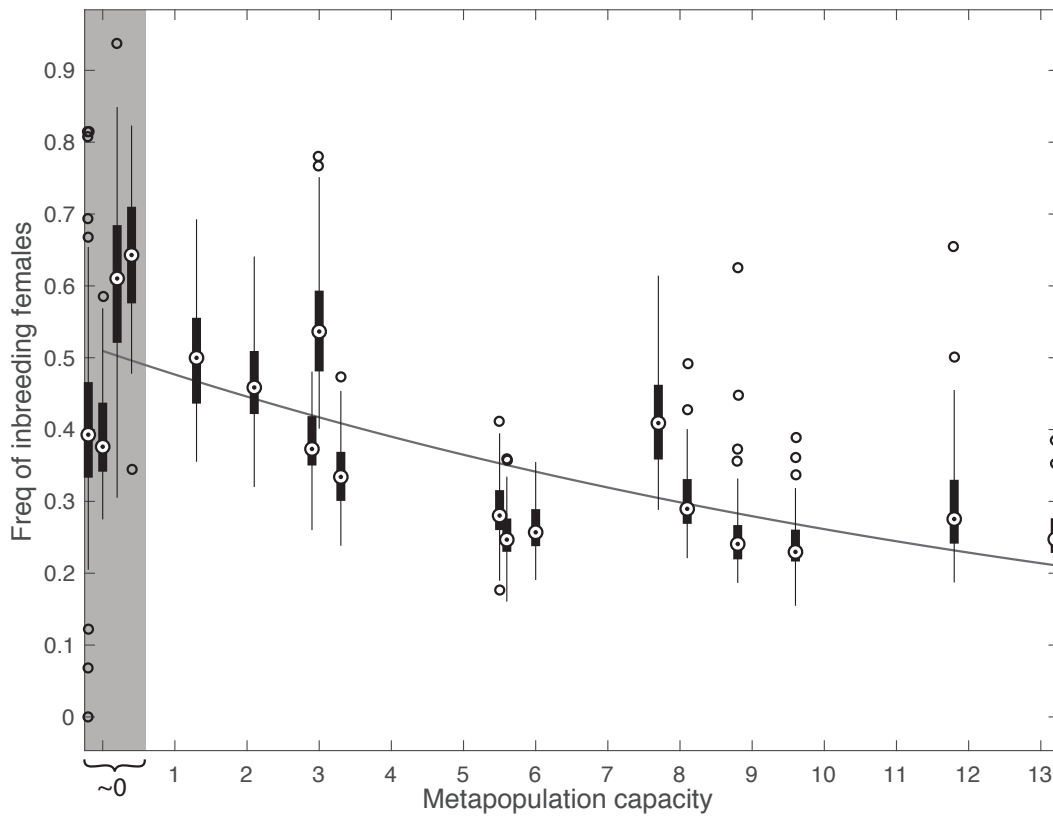


Figure 2. The frequencies of inbreeding females plotted by SINS in the order of ascending metapopulation capacity. Metapopulation capacity is a measure that integrates the effects of patch area and configuration on the capacity of the habitat network to support a viable metapopulation (in the unit of $\text{ha}^{0.89}$; Hanski et al. 2017). The boxplots represent the posterior distributions of the frequency of inbreeding females. The circles with a dot inside are the medians and the boxes demarcate the 75th and 25th percentiles. Outliers are those data points located beyond 1.5 times the corresponding interquartile range (IQR). The line is the best fit Gaussian curve $\left(y = ae^{-\left(\frac{x-b}{c}\right)^2} \right)$, where x = metapopulation capacity, to guide the eye (a , b , and c are fitting parameters). For clarity, the smallest 4 SINS are plotted slightly further apart from each other than the values of their metapopulation capacity, but the Gaussian curve is drawn on the original scale.

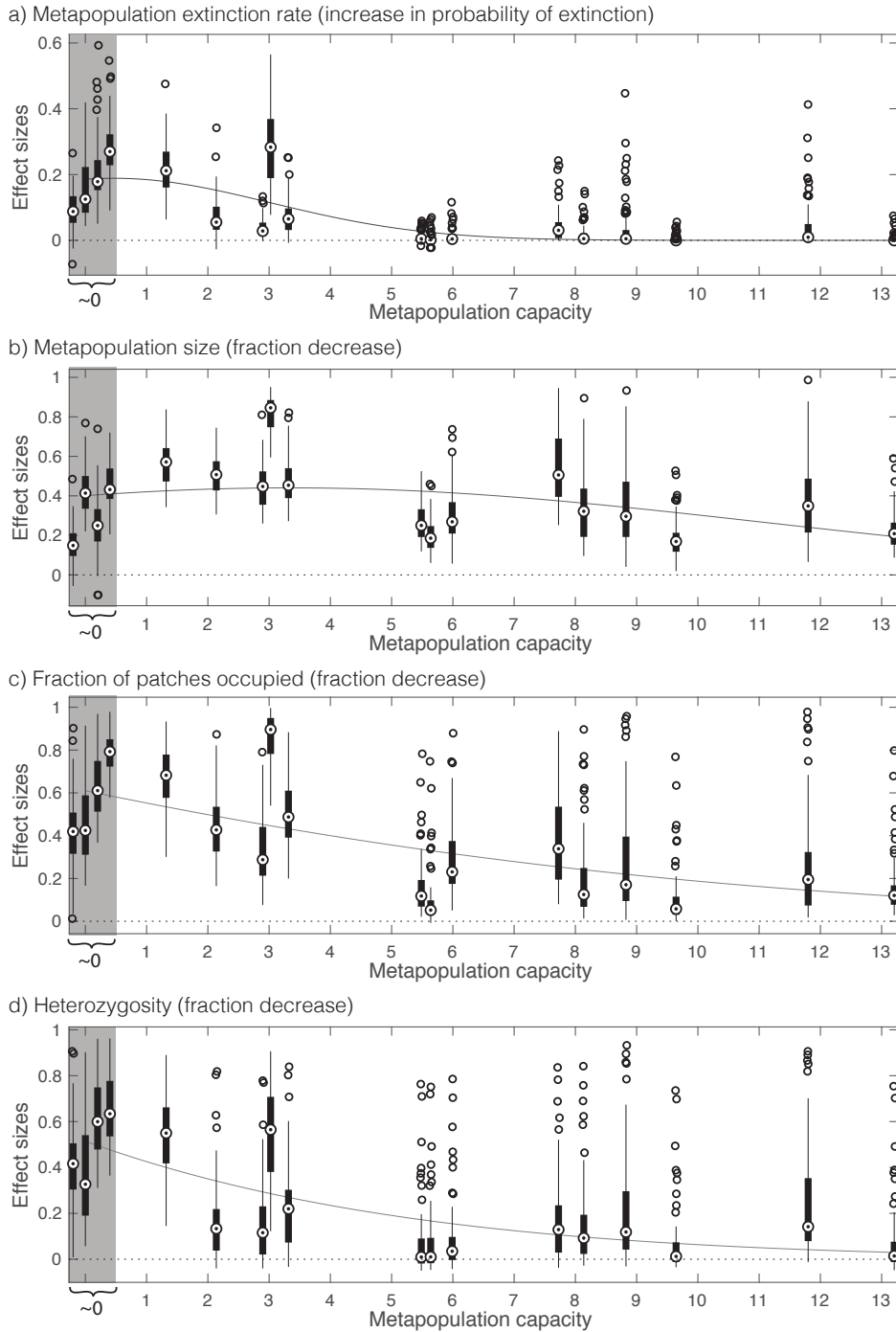


Figure 3. Effect sizes of inbreeding depression in terms of a) metapopulation extinction rate, b) metapopulation size, c) patch incidence (fraction of patches occupied), and d) mean heterozygosity. The effect sizes are calculated as pair-wise differences in these measures between simulations where inbreeding depression is turned on and off. “Fraction decrease” refers to the reduction in these measures due to inbreeding depression relative to its absence and is calculated as $G = 1 - Z^I/Z^O$, where G is the fraction decrease for the properties, Z^I and Z^O are the values of the property in the presence and absence of inbreeding depression, respectively. The circles with a dot inside are the medians and the boxes demarcate the 75th and 25th percentiles. Outliers are those data points located beyond 1.5 times the corresponding interquartile range (IQR). The dotted line at effect size of zero indicates no effect of inbreeding depression. The solid lines are the best fit Gaussian curves $\left(y = ae^{-\left(\frac{x-b}{c}\right)^2}\right)$, where x = metapopulation capacity, to guide the eye (a , b , and c are fitting parameters). The

smallest four SInS are plotted slightly further apart from each other along metapopulation capacity for clarity, but the Gaussian curves are drawn in the original scale.

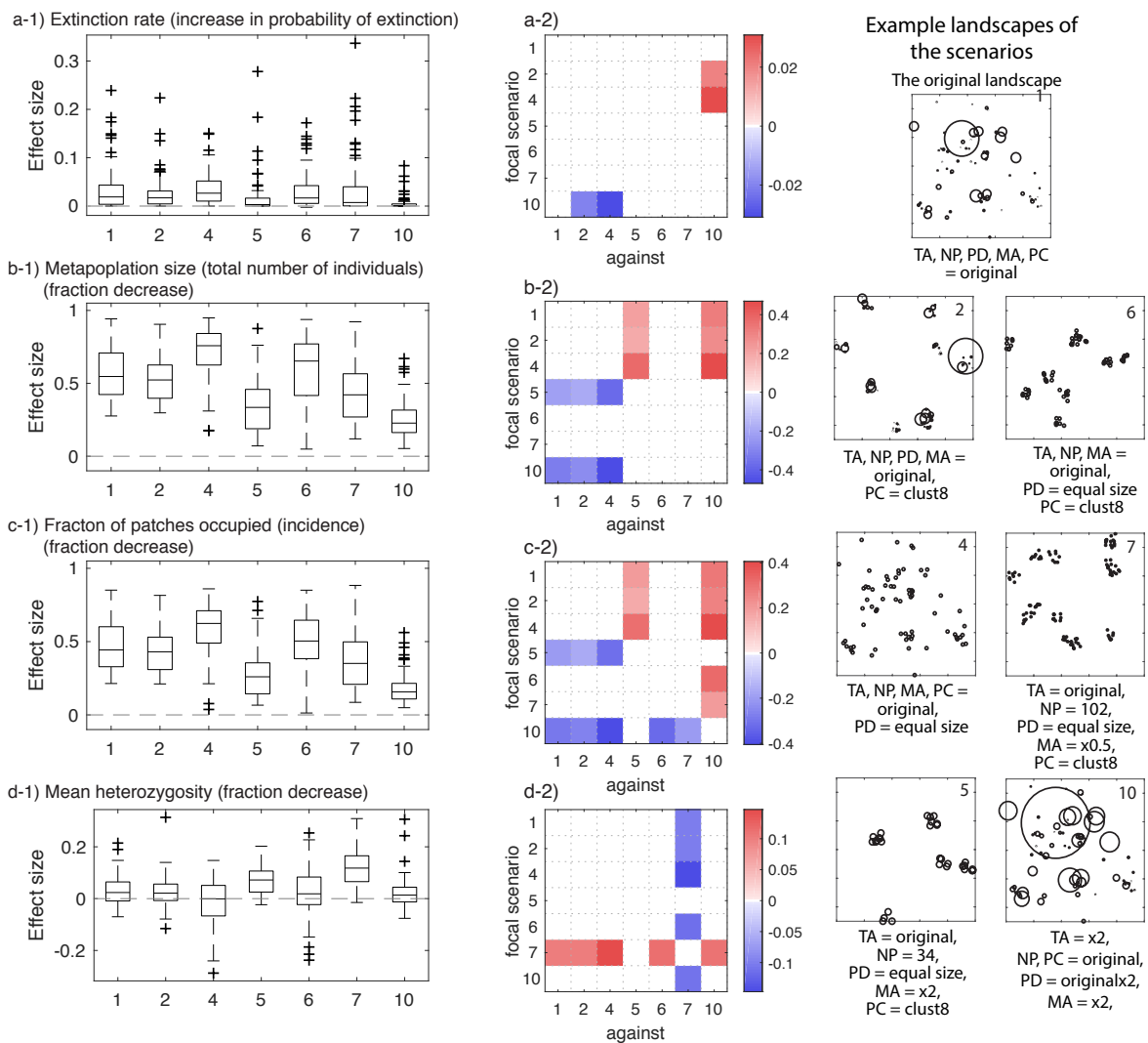
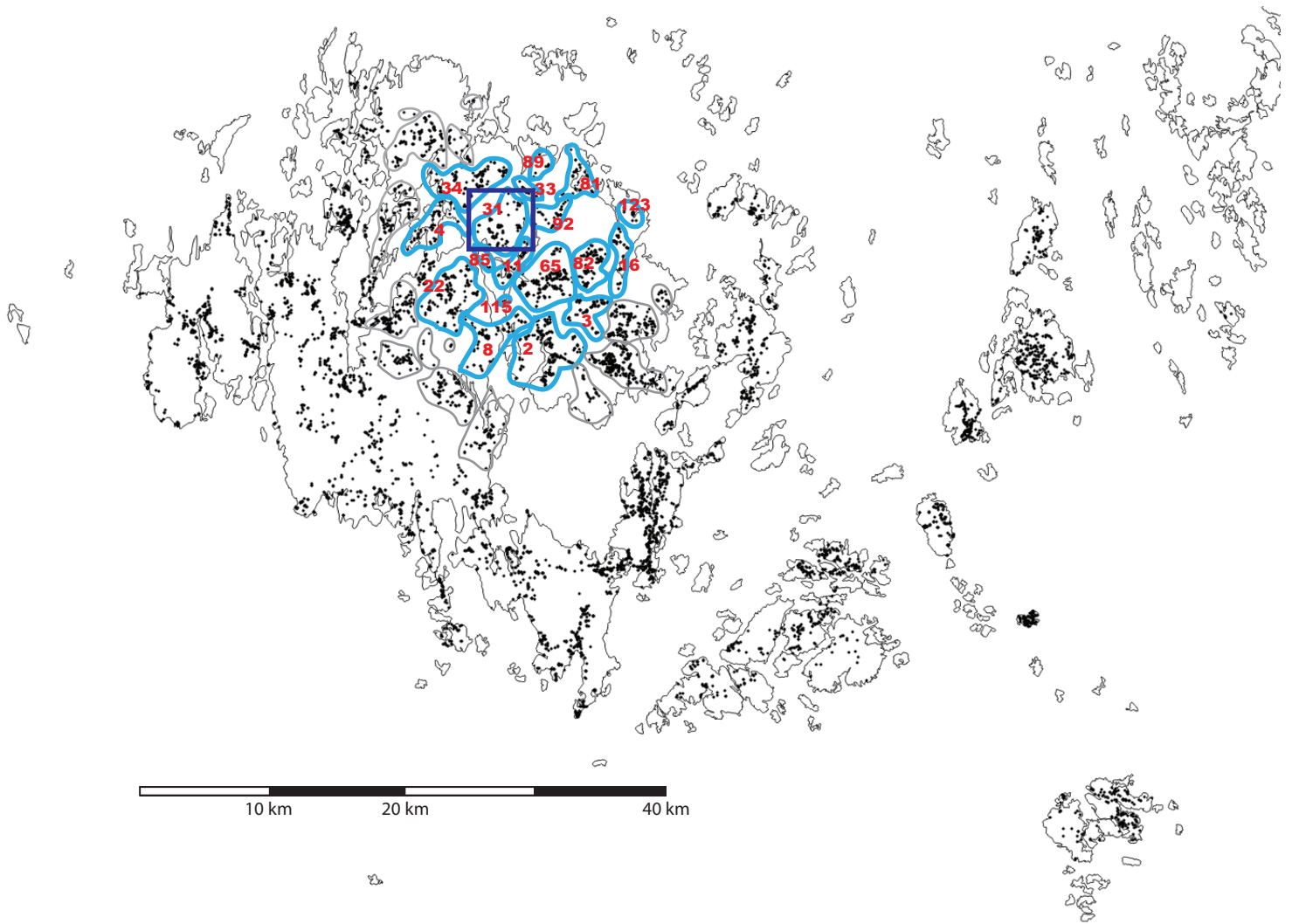
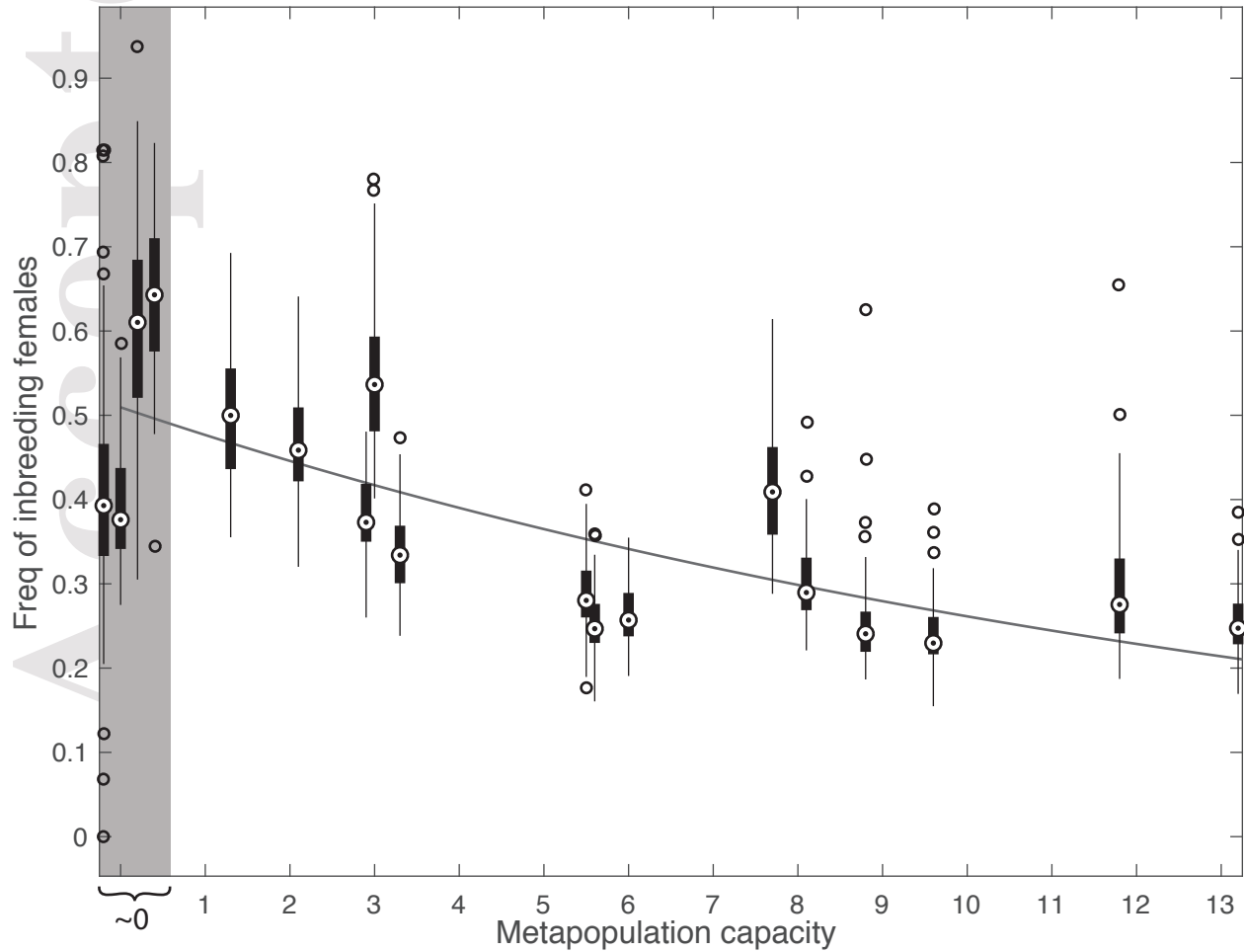


Figure 4. (left column) Effect sizes of inbreeding depression from seven landscape scenarios (see Table 1 for the descriptions of the scenarios). Positive values indicate the adverse effects of inbreeding depression. Scenarios 3, 8 and 9 are omitted (see Supporting Information). The patch configuration and area of a landscape enclosed in a 5 km x 5 km area in the middle of the study region (Fig. 1) are manipulated to examine the effects of landscape structure on the effects of inbreeding depression. The boxplots show the posterior distributions of the effect sizes of inbreeding depression on a) metapopulation extinction rate (proportion increase due to inbreeding depression), b) metapopulation size, c) patch incidence (fraction of patches occupied), and d) mean heterozygosity. “Fraction decrease” refers to the reduction in these measures due to inbreeding depression relative to its absence and is calculated as $E = 1 - V^I/V^O$, where E is the effect size of the given property, V^I and V^O are the values of the property in the presence and absence of inbreeding depression, respectively. The line in the box signifies the median and the upper and lower sides of the box demarcate the 75th and 25th percentiles. Outliers are those data points located beyond 1.5 times the interquartile range (IQR). (middle column) The plots show pairwise comparisons of the scenarios. Colored panels indicate where the effect sizes of the focal scenarios (along the vertical axis) are consistently greater (red) or smaller (blue) than those of the scenarios compared against (along the horizontal axis) for 95% or more of the approximate joint posterior distributions. Hence, a red panel indicates that the focal scenario consistently experiences greater negative effects of inbreeding depression than the scenario that is compared against. White panels indicate that the scenario pairs are not consistently different for 95% of the approximate

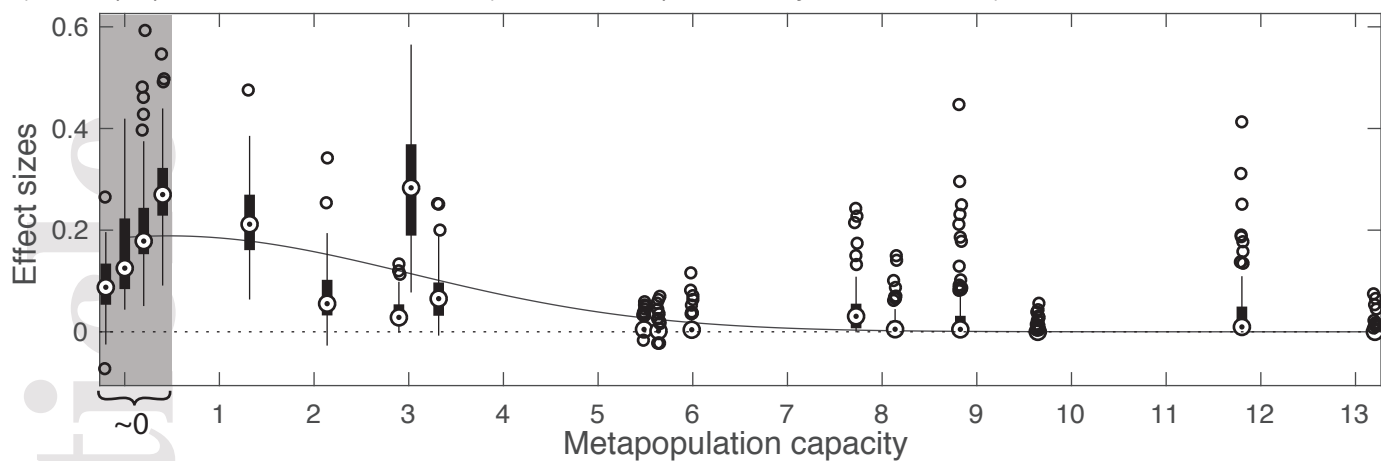
posterior distributions of the model parameters. (right columns) Example landscapes of the 7 scenarios are shown with key manipulated features (For the two letter abbreviations, see Table 1). The size of circles reflects the relative size of the patches, and overlapping circles do not imply that the patches are geographically overlapping.



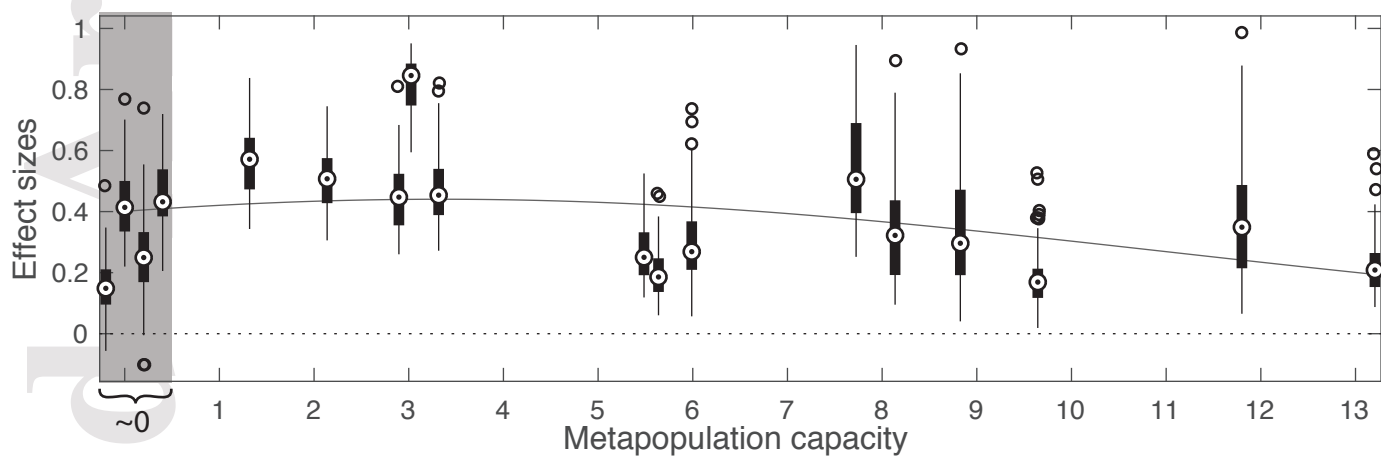
10 km 20 km 40 km



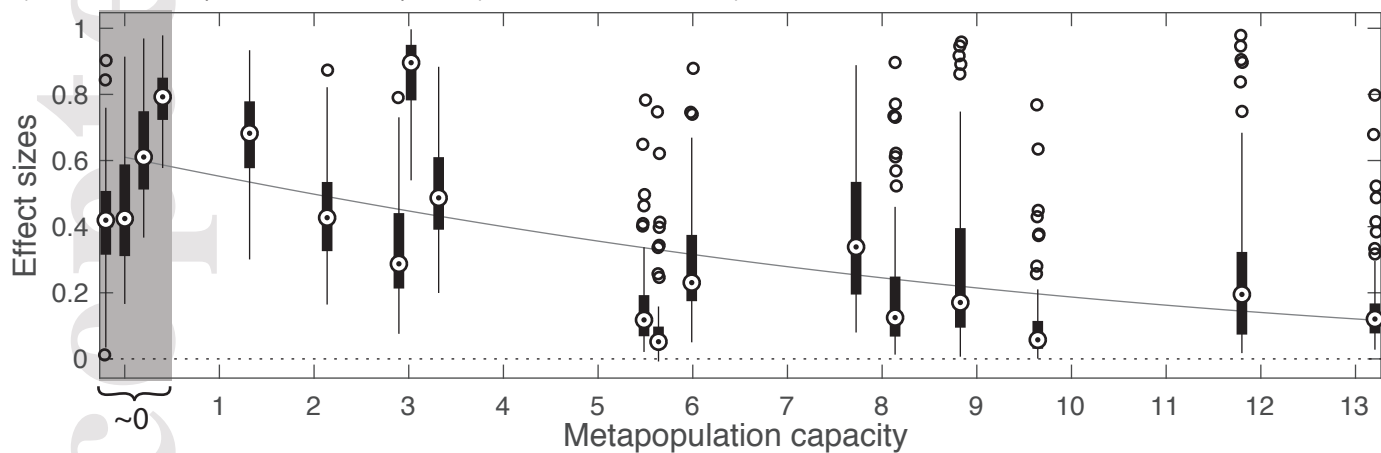
a) Metapopulation extinction rate (increase in probability of extinction)



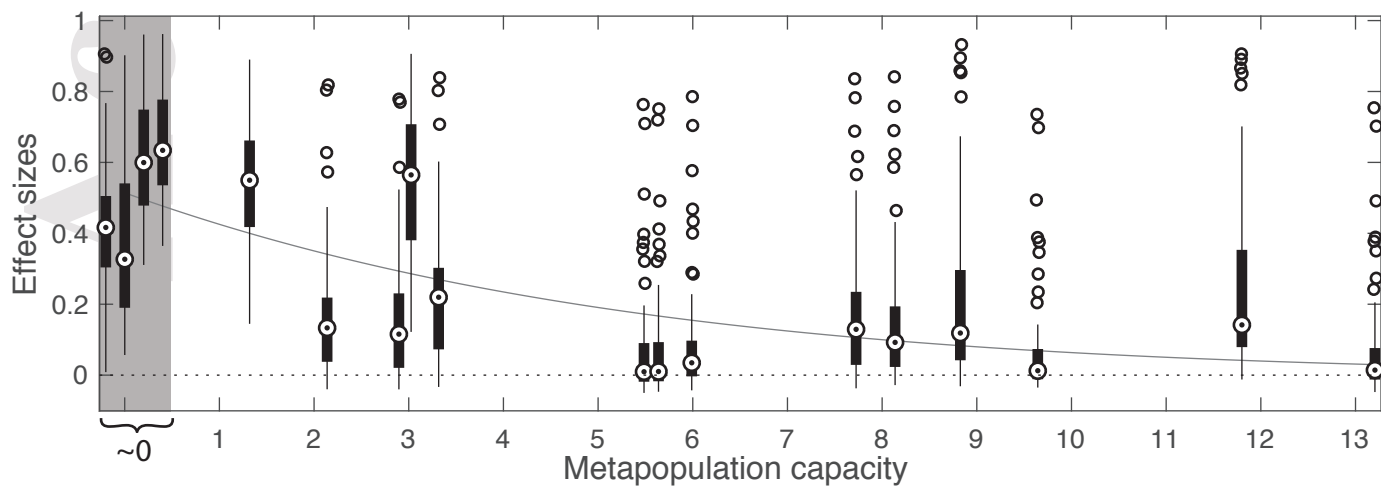
b) Metapopulation size (fraction decrease)



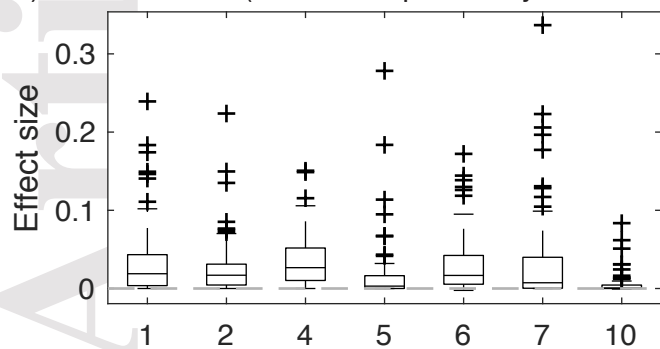
c) Fraction of patches occupied (fraction decrease)



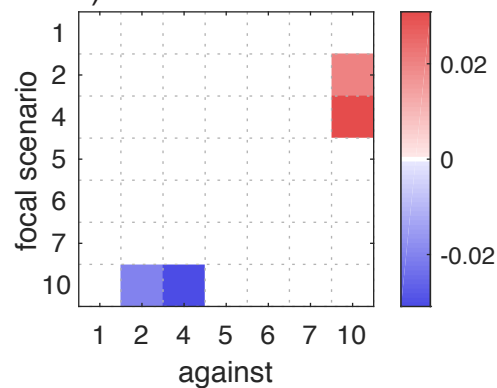
d) Heterozygosity (fraction decrease)



a-1) Extinction rate (increase in probability of extinction)

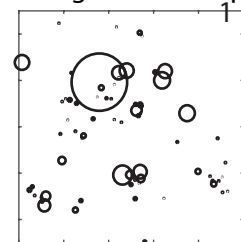


a-2)



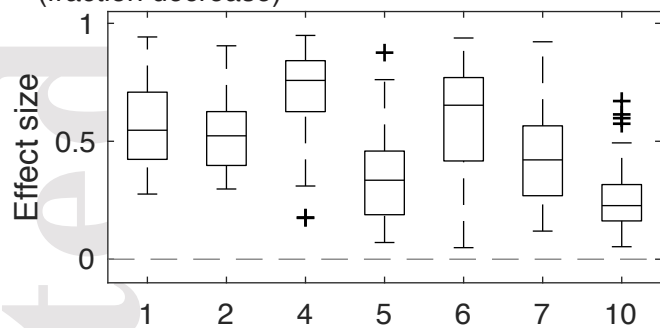
Example landscapes of the scenarios

The original landscape

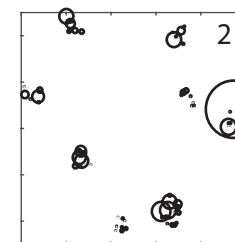
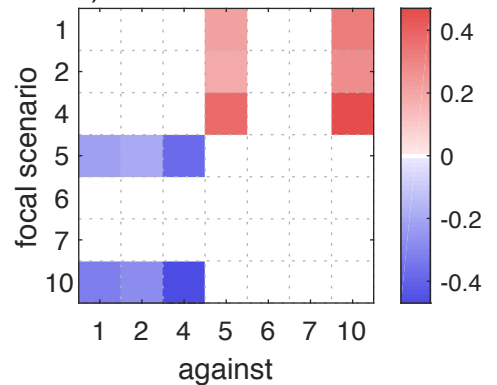


TA, NP, PD, MA, PC = original

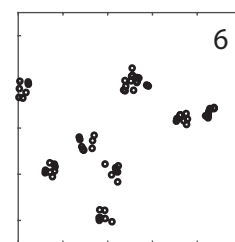
b-1) Metapopulation size (total number of individuals) (fraction decrease)



b-2)

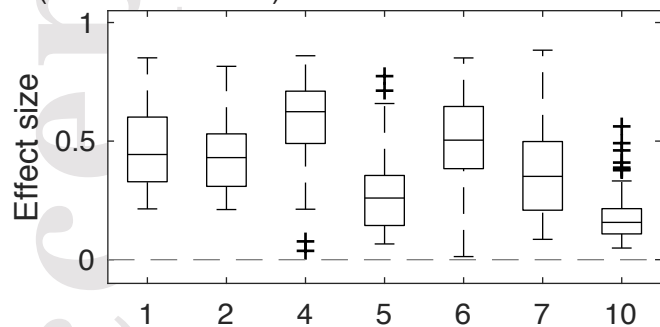


TA, NP, PD, MA = original, PC = clust8

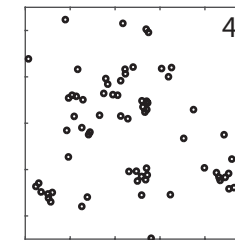
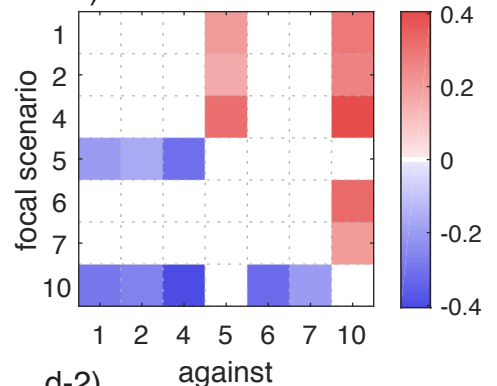


TA, NP, MA = original, PD = equal size, PC = clust8

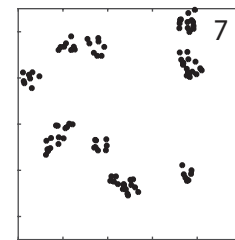
c-1) Fracton of patches occupied (incidence) (fraction decrease)



c-2)

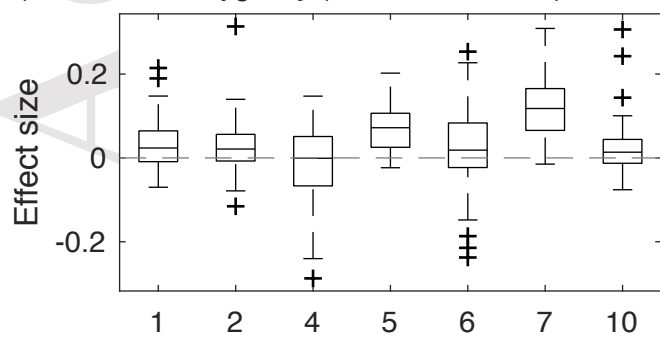


TA, NP, MA, PC = original, PD = equal size

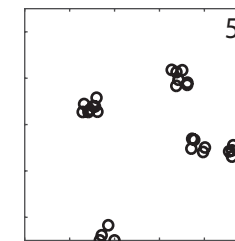
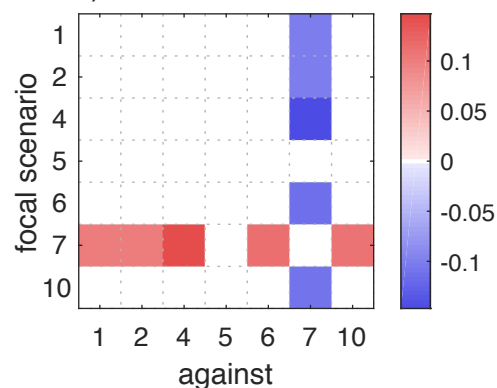


TA = original, NP = 102, PD = equal size, MA = x0.5, PC = clust8

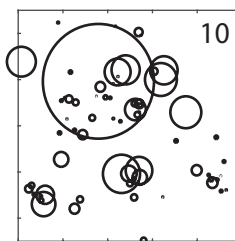
d-1) Mean heterozygosity (fraction decrease)



d-2)



TA = original, NP = 34, PD = equal size, MA = x2, PC = clust8



TA = x2, NP, PC = original, PD = originalx2, MA = x2