Biodiversity loss through speciation collapse: mechanisms, warning signals, and possible rescue

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Abstract

Speciation is the process that generates biodiversity, but recent empirical findings show that it can also fail, leading to the collapse of two incipient species into one. Here, we elucidate the mechanisms behind speciation collapse using a stochastic individual-based model with explicit genetics. We investigate the impact of two types of environmental disturbance: deteriorated visual conditions, which reduce foraging ability and impede mate choice, and environmental homogenization, which restructures ecological niches. We find that: (1) Species pairs can collapse into a variety of forms including new species pairs, monomorphic or polymorphic generalists, or single specialists. Notably, polymorphic generalist forms may

be a transient stage to a monomorphic population; (2) Environmental restoration enables species pairs to re-emerge from single generalist forms, but not from single specialist forms; (3) Speciation collapse is up to four orders of magnitude faster than speciation, while the re-emergence of species pairs can be as slow as *de novo* speciation; (4) While speciation collapse can be predicted from either demographic, phenotypic, or genetic signals, observations of phenotypic changes allow the most general and robust warning signal of speciation collapse. We conclude that factors altering ecological niches can reduce biodiversity by reshaping the ecosystem's evolutionary attractors.

Keywords: Speciation, hybridization, assortative mating, species diversity, warning signals.

Introduction

Species diversity is important for ecosystem functions and services (Naeem et al., 1994; Tilman et al., 1997; Ives and Carpenter, 2007; Mace et al., 2012). As a consequence of human activities, species are being lost at a fast and accelerating pace with consequences rippling through ecosystems (Chapin et al., 2000). To protect the earth's biological diversity, it is necessary to identify and safeguard ecological and evolutionary mechanisms that generate and maintain species diversity. There are two processes that alter the global species pool: extinction and speciation. So far most efforts have been aimed to understand the reasons for species extinction and the conditions that promote speciation.

Recent research shows that the processes that enable speciation may also stop and enable a rapid collapse of two species into one (Seehausen, 2006), implying that loss of biodiversity through this processes could potentially induce significant biodiversity loss. Since population divergence is sustained by a dynamic balance between disruptive selection and gene flow,

changes that weaken disruptive selection or increase gene flow between populations can impede divergence. This is particularly apparent for evolutionarily young species because of their incomplete reproductive isolation (Seehausen et al., 2008). Therefore, any two species that are not firmly separated can be at a risk of reverting back to a primitive ancestral form. The risk is underscored by theoretical work showing how disturbances affecting pre-mating isolating mechanisms can result in bouts of hybridization (Gilman and Behm, 2011). This phenomenon of reverting back from two species to one is usually termed as speciation reversal (Seehausen, 2006), but as seen later, it is just one of several outcomes that can result from environmental change. We here use the broader term speciation collapse to include all possible outcomes that can results from loss of species cohesion.

Potential factors that can weaken disruptive selection or impede pre-mating isolating mechanisms include changes in ecological niches (De Leon et al., 2011; Kleindorfer et al., 2014; Grant and Grant 2014), mating grounds (Vonlanthen et al., 2012; Bhat et al., 2014; Hasselman et al., 2014), and mating choice (Seehausen et al., 1997; Taylor et al., 2006; Gow et al., 2006; Keagy et al., 2016). A well-studied example of incipient speciation collapse is that of the threespine stickleback fish in Enos Lake (Boughman, 2001). The number of hybrids between the benthic and limnetic threespine sticklebacks increased by 16% over a decade (Kraak et al., 2001). The formation of hybrids is thought to have resulted from elevated water turbidity and habitat destruction following the introduction of crayfish (Taylor et al., 2006; Gow et al., 2006). Indeed, experimental studies show that impaired water clarity can alter the pre-mating mechanism of sexual selection to reduce reproductive isolation (Sundin et al., 2010; Glotzebecker et al., 2015).

The risks of biodiversity loss in the wake of evolutionary change may be fairly high. Four-fifths of all coregonid species, a common freshwater fish, are believed to be at risk of

reverting back to a primitive ancestral form. Similar or even higher figures are mentioned for other groups of species (Seehausen, 2006). Unlike demographic extinction, speciation collapse can be difficult to detect because it does not require changes in distribution or abundance, but can manifest through subtle changes in phenotypic variation within multiple species assemblages. Loss of biodiversity through speciation collapse may potentially be more widespread than currently recognized (Vonlanthen et al., 2012). It is thus important to understand to what extent findings from empirical case studies apply to other systems and, more generally, to infer common causes of species collapse.

Here, we aim to broaden understanding of human-induced speciation collapse by developing and analyzing process-based eco-evolutionary models. In addition to identifying causes and possible outcomes of speciation collapse, we develop and investigate warning signals for speciation collapse that may aid ecosystem conservation. We concentrate our efforts on the consequences of two environmental disturbances: deteriorated visual conditions and increased environmental homogenization, both recognized as possible causes of speciation collapse (Seehausen et al., 1997, 2008). Deteriorated visual condition, particularly important in aquatic systems, is a major impediment to mate choice (Sundin et al., 2010, Alexander et al., 2017). Reported examples include the cichlid fish in Lake Victoria (Seehausen et al., 1997), broadnosed pipefish (Sundin et al., 2010) and sand goby (Jarvenpaa and Lindstrom 2004) in the Baltic Sea. Increased environmental homogenization can lead a system to a new evolutionary state with reduced biodiversity. Well-known examples include the increased hybridization of Darwin's finches due to increased abundance of intermediate-sized seeds (Grant and Grant, 1996) and the homogenization of the mating grounds of the threespine stickleback fish in Enos Lake (Taylor et al., 2006) and the Central European whitefish (Alexander et al., 2017).

To study the evolutionary consequences of deteriorated visual conditions and elevated environmental homogenization, we extend an individual-based stochastic predator-prey model by Svanbäck et al. (2009) with genetic detail and mate choice based on the diverging ecological phenotype. We then perform extensive numerical analyses to answer four questions: (1) What conditions promote speciation collapse? (2) How fast is the process of speciation collapse in comparison to the process of speciation? (3) Can the original species diversity be recovered following speciation collapse by restoring the environment? (4) Are there useful warning signals of speciation collapse?

Methods

We first present an individual-based predator-prey model with explicit genetics and assortative mating for the predator population. We then describe the methodology used for our numerical investigation, including the two types of perturbations that we consider, namely 1. altering reproductive isolation by perturbing partner recognition and 2. altering divergent selection by perturbing environmental differences. Parameter values are summarized in Table S1 and motivated in Appendix S1. The implementation of the model is described in Appendix S2.

Model description

Our model is built on the individual-based predator-prey model of Svanbäck et al. (2009). The predator population P lives in a subdivided environment with two habitats and forages on two prey populations N_1 and N_2, that each inhabits one habitat. The structure of the model is inspired by natural lake fish populations in which individuals can specialize on either the near shore littoral habitat or the off shore pelagic habitat. Such habitat specialization has in numerous cases led to the evolution of resource polymorphisms (Smith and Skulasson 1996)

as well as the formation of two species (Barluenga et al., 2006). The speciation process in lake fish populations has furthermore recently been shown to be able to collapse because of changed environmental conditions (Taylor et al., 2006). While the predator individuals in our model are differentiated by their genotypes and sex, prey individuals are assumed to be identical and are two orders of magnitude smaller in body size than the predators. We consider stochastic, individual-based population dynamics for the predator through death and birth at specified rates and deterministic population dynamics for their prey. The assumption of deterministic prey dynamics speeds up numerical simulation without any qualitative changes in the eco-evolutionary dynamics.

Population dynamics and foraging behavior

We assume, for simplicity, that prey population size is a continuous variable with dynamics within each habitat given by

$$\frac{\mathrm{d}N_i}{\mathrm{d}t} = r_i N_i \left(1 - \frac{N_i}{V_i K_i} \right) - \sum_j F_{i,j}, \tag{1}$$

where the prey populations grow following logistic dynamics with a habitat-specific intrinsic growth rate r_i, carrying capacity K_i, and volume V_i. The predation-induced mortality amounts to $\sum_j F_{i,j}$, where $F_{i,j}$ is the foraging rate in habitat i by predator individual j and the sum is taken over all predator individuals.

Each predator allocates its foraging time between the two habitats depending on their relative profitability (see below). A predator j spending time in habitat i catches prey type i at a rate given by the functional response

$$f_{i,j} = \frac{a_{i,j}N_i / V_i}{1 + a_{i,j}h_{i,j}N_i / V_i} \qquad (2)$$

with predator-specific attack rate $a_{i,j}$ and handling time $h_{i,j}$. We assume that predator j spends a proportion q_j of its time in habitat 1 according to

$$q_j = \frac{V_1}{V_1 + V_2 \exp(-s(Q_{1,j} - Q_{2,j}))}. \qquad (3)$$

Here the parameter s describes the sensitivity of predators to habitat differences and $Q_{i,j} = c_i f_{i,j} / m$ is the profitability of habitat i for predator individual j, and c_i is the prey-specific conversion efficiency. The larger the value of s, the more likely the predator is to spend time in the more profitable habitat. When $s=0$, the predator allocates time purely proportional to volume (de Roos et al., 2002) and if the two habitats are also equal in size, equation (3) reduces to the case considered by Svanbäck et al. (2009). Finally, we can state the foraging rate of predator j on the two prey types as (cf. eq. 1)

$$F_{1,j} = q_j f_{1,j}, \quad F_{2,j} = (1 - q_j) f_{2,j}. \qquad (4)$$

Genotype to phenotype mapping

The prey populations do not evolve while each predator individual has an ecological phenotype u_j that is subject to evolution and determines its attack rate $a_{i,j}$ and handling time $h_{i,j}$ of prey type i (eq. 2). Specifically,

$$a_i(u_j) = a_i \exp\left(-\frac{\left(u_j - u_i\right)^2}{2\sigma_a^2}\right), \qquad (5)$$

and

$$h_i(u_j) = h_i + \delta \left(1 - \exp \left(-\frac{\left(u_j - u_i \right)^2}{2\sigma_h^2} \right) \right), \tag{6}$$

where a_i and h_i are the maximal attack rate and the minimal handling time, respectively. In each habitat the realized attack rate declines and the handling time increases with the phenotypic difference from the optimum in that habitat as scaled by the parameters σ_a^2 and σ_h^2, respectively. The parameter δ in equation (6) scales the maximal increase in handling time. We assume that the optimal phenotypes in the respective habitats are at $u_1 = -1$ and $u_2 = 1$.

To allow for sexual reproduction and mate choice, we first introduce a trait v that determines whether and how strongly a female prefers to mate with regards to the ecological trait u. Second, we assume that each trait (u and v) is determined from L unlinked additive diallelic loci, and assign an allele of value of +1 or -1 to each ecological locus in each predator individual and an allele of value 0 or 1 to each mating locus. Mutations are symmetric and occur with the same independent probability for all loci. Each locus contributes equally, but independently, to the phenotype (Gilman and Behm, 2011). We scale the range of the ecological trait u to values from -2 to 2, which means that predator individuals can overshoot the optimal phenotype in each habitat. While there is only one way to code for the extreme phenotypes, the overshooting allows for multiple genetic paths to code each optimal phenotype. Alleles at the mating loci are either neutral or positive, and we scale the range of the mating trait v from 0 to 1. The use of unlinked loci makes the degradation of assemblages of beneficial loci faster, and may increase the probability of species collapse.

Mating

We consider a global mating scenario, which means that individuals find mates from the whole population regardless of which habitats they are using. Each female and male mate only once or not at all. At a birth event, one female is chosen at random from the whole population, with probability proportional to her birth rate. The chosen female is approached by a certain number of potential males chosen at random from the entire male population. The number of males approaching the female is, up to any discrepancy introduced by rounding off to the nearest integer, assumed proportional to the number of males in the population, with proportionality constant β. The mating probability between the selected female with phenotype u_f and an approaching male with phenotype u_m is determined by the mating kernel

$$A(v_f, u_f, u_m) = \begin{cases} \exp\left(-\dfrac{(2v_f - 1)^2}{2\sigma_A^2}(u_f - u_m + \varepsilon)^2 \right) & \text{if} \quad v_f > 0.5, \\ 1 & \text{if} \quad v_f = 0.5, \\ 2 - \exp\left(-\dfrac{(2v_f - 1)^2}{2\sigma_A^2}(u_f - u_m + \varepsilon)^2 \right) & \text{if} \quad v_f < 0.5. \end{cases} \qquad (7)$$

Here, v_f is the mating phenotype of the female individual. It determines if the mating is assortative ($v_f > 0.5$), random ($v_f = 0.5$), or disassortative ($v_f < 0.5$). Assortative mating means that the female is more likely to mate with a male with phenotype similar to her own, while disassortative mating means that she prefers males with phenotype different from her own. Although we mostly observe assortative mating, we allow for both assortative and disassortative mating in order not to bias evolution. The parameter σ_A characterizes the strength of female preference. Finally, ε is visibility noise, a normally-distributed random variable with a mean 0 and a variance σ_v^2. By drawing an independent value for each encounter, we model impaired partner choice as a result of deteriorated visibility. Apart from

the introduction of visibility noise, equation (7) is identical to the mating-kernel used by Thibert-Plante and Gavrilets (2013).

Reproduction

If a male is accepted, a single offspring is produced and no further males are considered. The genotype of the offspring is produced by drawing one allele at random from each locus in the genome of each parent. Each allele mutates to the opposite allele with a probability μ, and the sex of the offspring is assigned to be male or female with equal probability. Since a female will always find a mate, this is a non-costly global mating scenario and we take it as the default mating strategy in this paper. In the robustness section we study the sensitivity of our results to other mating strategies including costly mating, local mating, and combinations thereof. In the local mating scenario, the mating procedure is similar to the global mating scenario, but a female can only choose among a subset of males living in her habitat. The habitats of the selected female and all males at the time of mating are assigned at random according to their probabilities of foraging in the two habitats.

Genetic signals

To identify possible genetic signals of divergence and collapse, we track 40 neutral marker loci. They are analogous to microsatellite loci with a high mutation probability of 0.001 per birth event (Dallas, 1992; Weber and Wong, 1993; Brinkmann et al., 1998; Drake et al., 1998). Each mutation changes the number of repeats by one, either plus or minus (Kimura and Ohta, 1975; Valdes et al., 1993; Di Rienzo et al., 1994). Each locus has a range of allowed repeats from 0 to 15 and at the boundaries of the range of allowed repeats we have reflective conditions (i.e., mutation from 0 and 15 is 1 and 14, respectively). We assume that

all neutral loci are unlinked among themselves and with any loci under selection, as in Thibert-Plante and Hendry (2010).

Numerical investigation

Using the model described above, we allow an incipient speciation pair to form and investigate possible speciation collapse and re-emergence of the species pair as a consequence of deteriorated and restored environmental conditions. We quantify the time of collapse and recovery in terms of generations. We furthermore investigate the possibility of predicting speciation collapse from genetic and phenotypic signals. We now describe this procedure in detail.

Incipient species pairs

To obtain an incipient species pair, we let the evolution of genetic traits of the predator start from the case in which all predator individuals are homozygous with an ecological phenoytpe 0 and a mating phenotype 0.5, and there is no genetic variation across individuals. This setting means that all individuals are initially generalists, mating randomly and feeding on prey in both habitats. Figure 1 shows that the population initially settles on a convergence stable branching point under the default parameters (Fig. 1). Finally, each predator individual is randomly assigned to be either male or female with equal probability. We initiate each simulation with $P = 1000$, $N_1 = N_2 = 20000$.

After initiation we let the system run until the mean values of the ecological and mating phenotypes do not undergo any qualitative changes. In our study this happens when the generalist predator has diversified into two specialists feeding on their respective resources in different habitats. An example evolutionary trajectory of the ecological and mating phenotypes is illustrated in Fig. S1 in Appendix S3. Due to the large parameter space that we

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are going to explore and the heavy computational load, we simulated five incipient species pairs to calculate an average speciation time, and for each pair, we ran two replicate simulations of the evolution following disturbance, which gives 10 replicates for each considered set of parameters.

Environmental disturbance

The two disturbances of deteriorated visual conditions and increased environmental homogenization are introduced as follows. We independently consider two plausible consequences of deteriorated visual conditions. These are a reduction in individual attack rate which we implement by multiplying a_i (i.e., maximal attack rate) in equation 5 with $(1-r_a)$ and vary r_a from 0 (no reduction) to 1 (reduction to zero), and an increase in the variance of the noise distribution (i.e., σ_v), ranging from 0 (no perturbation at all) to 1, which always suffices to induce speciation collapse (see results below). Environmental homogenization is modeled by moving the two optimal phenotypes (i.e., u_1 and u_2) closer to each other. In practice, we fix u_2 and move u_1 from -1 to 1. Disturbance is introduced to the model system after an incipient species pair has formed and reached eco-evolutionary equilibrium as described above.

Speed of speciation collapse and re-emergence of species pairs

Evolutionary time is measured in generations, which is given as the ratio of the total time divided by the predator generation time, calculated as $1/m$. To quantitatively assess how fast the processes of speciation collapse and re-emergence of species pairs are, we measure the collapse time as the time from disturbance to the formation of a unimodal distribution of the ecological phenotype, and the re-emergence time as the time from removing the disturbance

to the formation of bimodal distribution. The modality of the phenotypic distribution is determined from its skewness and kurtosis (Rohatgi and Szekely 1989). An example is presented in Fig. S2 in Appendix S3.

Warning signals

We consider the potential of phenotypic signals (changes in the phenotype distribution of predator), genetic signals (changes in neutral genetic differentiation, Fst), and demographic signals (changes in predator abundance) as warning signals to infer the occurrence of ongoing speciation collapse. We use data from the first 50 predator generations from the time the disturbance is introduced. In the event that speciation collapse unfolds in less than 50 generations, we use the entire time interval from onset of disturbance to the end of collapse, which is when the Fst drops down to zero. For genetic and demographic signals, we, respectively, consider the temporal rates of change in Fst and in the logarithmic value of population abundance. We derive from those temporal rates a threshold slope for each signal, above which speciation collapse is considered more likely than not (Appendix S7). Here, Fst is tracked, according to Weir (1996), for each neutral locus as a simulation unfolds. We calculate Fst 1000 times based on independent random samples with replacement of individuals from each population, and use the mean value of these 1000 bootstrap replications of Fst as our genetic signal. Furthermore, our simulations show that the standard deviation of these replications is marginally small, and does not affect our conclusion on the genetic signal.

Results

We performed extensive numerical simulations to explore how disturbances of visual conditions and environmental homogenization affect the speciation process and present our

results in six sections. The first section focuses on the role of natural selection in speciation collapse through the analysis of pairwise invadability plots (PIPs), while the second section summarizes the evolutionary outcomes caused by the two types of disturbances. The next two sections, respectively, study the conditions under which species pairs can re-emerge after speciation collapses when the environment is restored, and the evolutionary time required for speciation collapse and species re-emergence. The final two sections, respectively, explore whether warning signals can be inferred from neutral genetic differentiation, phenotypic distribution and demographic abundance, and whether our findings are robust under other parameters and in selected alternative model variants.

Natural selection can be a driver of speciation collapse

Disruptive natural selection and assortative mating are two key factors underlying speciation in our model. To disentangle their respective roles in speciation collapse, we study PIPs of a simplified model that ignores genetic details and assortative mating (Appendix S4). Figures 1A and 1B present bifurcation diagrams of the adaptive-dynamics limit of the simplified model for a range of disturbance intensities. Using baseline parameters (no reduction in attack rate), there are two local evolutionarily stable strategies (ESSs), one being $u = -1.0168$ and the other $u = 1.0168$ (Fig. 1C). In the central region, there are three singular strategies (Fig. 1D). The middle one is an evolutionary branching point and the other two are evolutionary repellers. A generalist with trait value $u = 0$ will experience disruptive selection and the two emergent populations will eventually evolve to a two-species ESS (Fig. S1) with trait value $(-1.0003, 1.0003)$. Disturbance in the form of reduced attack rate does not alter the two local ESSs (Fig. 1A) but restructures the fitness landscape in the central region by shrinking the viable region of the generalist and, consequently, the generalist is ultimately unable to survive (grey region in Fig. 1A, Fig. S3). If disturbance instead increases

environmental homogenization, then the entire fitness landscape is continuously reshaped (Fig. S4) from multiple singular strategies to a single singular strategy, implying a change from two specialists to a single generalist and finally to a single specialist, which is a global ESS (Fig. 1B, Fig. S4).
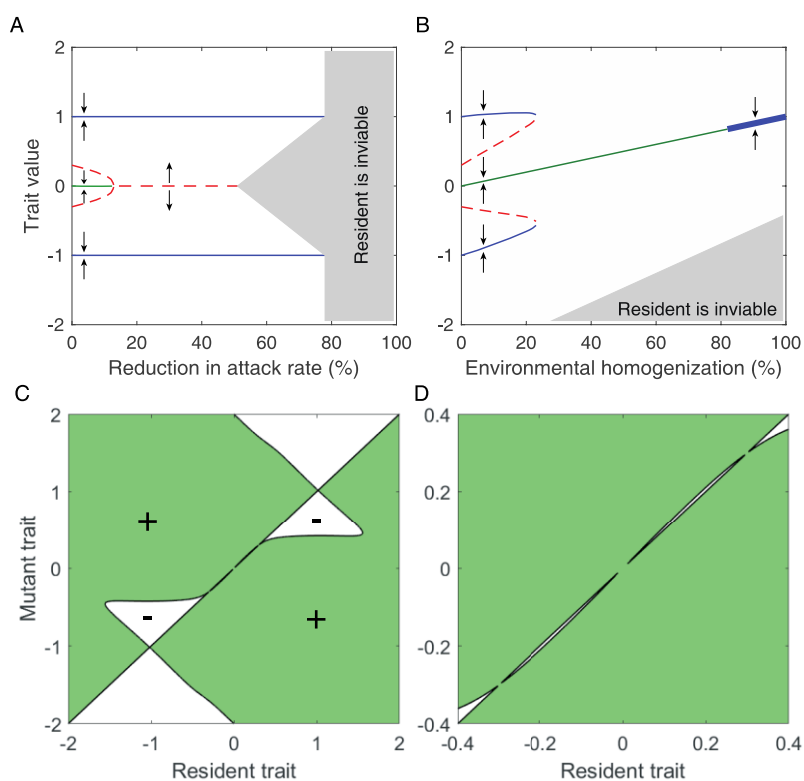

Fig. 1: Bifurcation diagram of the adaptive-dynamics limit for reduction in attack rate (A) and environmental homogenization (B). Arrows indicate the direction of natural selection and the grey region indicates that the resident is inviable. The lines indicate singular strategies: branching points (green), local ESSs (thin blue), repellers (red dashed), and global ESSs (thick blue). The lower row shows an example of a PIP under the baseline parameters in Table S1 (C) as well as a magnification of its central region (D).
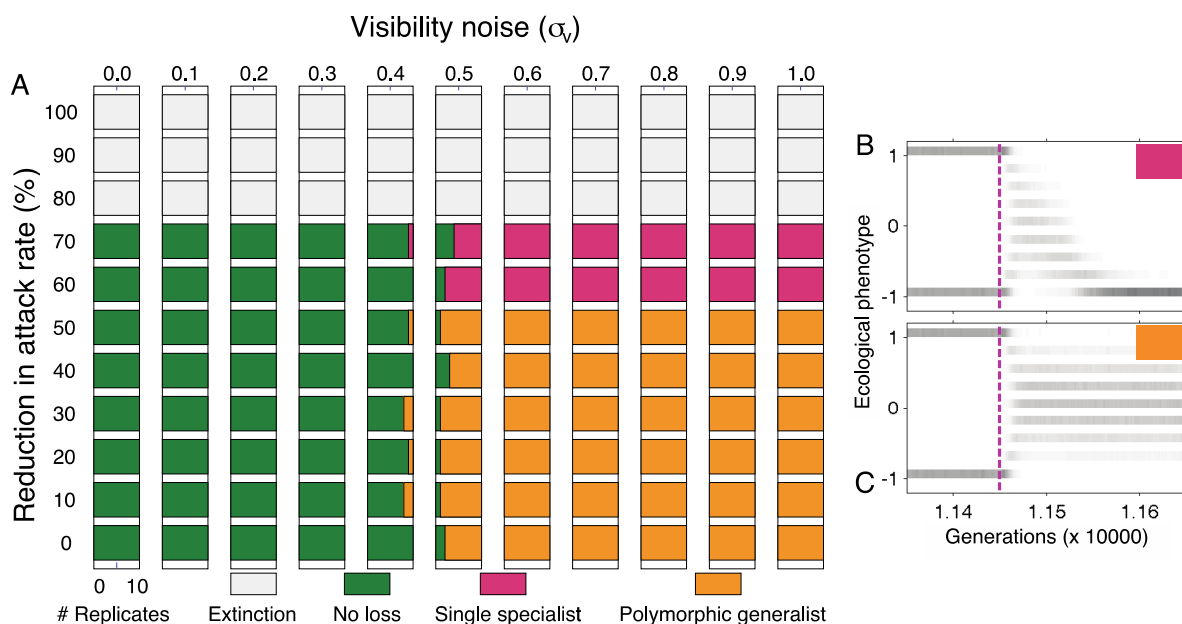
Fig. 2: Evolutionary outcomes following deteriorated visual conditions and reduced attack rate (A) and examples of speciation collapse into a single specialist (B, 70% reduction in attack rate and $\sigma_v = 0.6$) as well as into a polymorphic generalist (C, 40% reduction in attack rate and $\sigma_v = 0.6$). In panel B and C, the dashed lines indicate the time of introducing disturbance and dark areas represent phenotypes at high density while light areas represent phenotypes at low density. The gaps between the evolutionary trajectories are due to the fact that phenotypes are discrete. Other parameters are as in Table S1.

Variety of forms emerge following speciation collapse

Figure 2 summarizes the evolutionary outcomes as a consequence of deteriorated visual conditions. This disturbance promotes speciation collapse given that the visibility noise affecting partner choice exceeds a threshold amplitude (at $\sigma_v = 0.5$, approximately). An incipient species pair can collapse either into a polymorphic generalist, when the reduction in attack rate is small, or into a single specialist through a transient stage of a polymorphic population, when the reduction in attack rate is intermediate. With further reduction of the

attack rate (> 70%), the generalist species becomes inviable (Fig.1A). The speciation collapse into polymorphic generalist results from the increased gene flow between the two subpopulations caused by impaired partner choice and the overall flatness of the positive invasion fitness around the phenotype u=0 (Fig. 1D). With decreasing attack rate, the emerging polymorphic generalists suffer from a narrowing viable region in the phenotypic space or even become inviable (Fig. 1A, Fig. S3), and are therefore driven by natural selection to a single specialist with phenotype u =1 or -1 (Fig. 1A). To which specialist the polymorphic generalist will evolve to depend on the available resource density in the two different habitats.
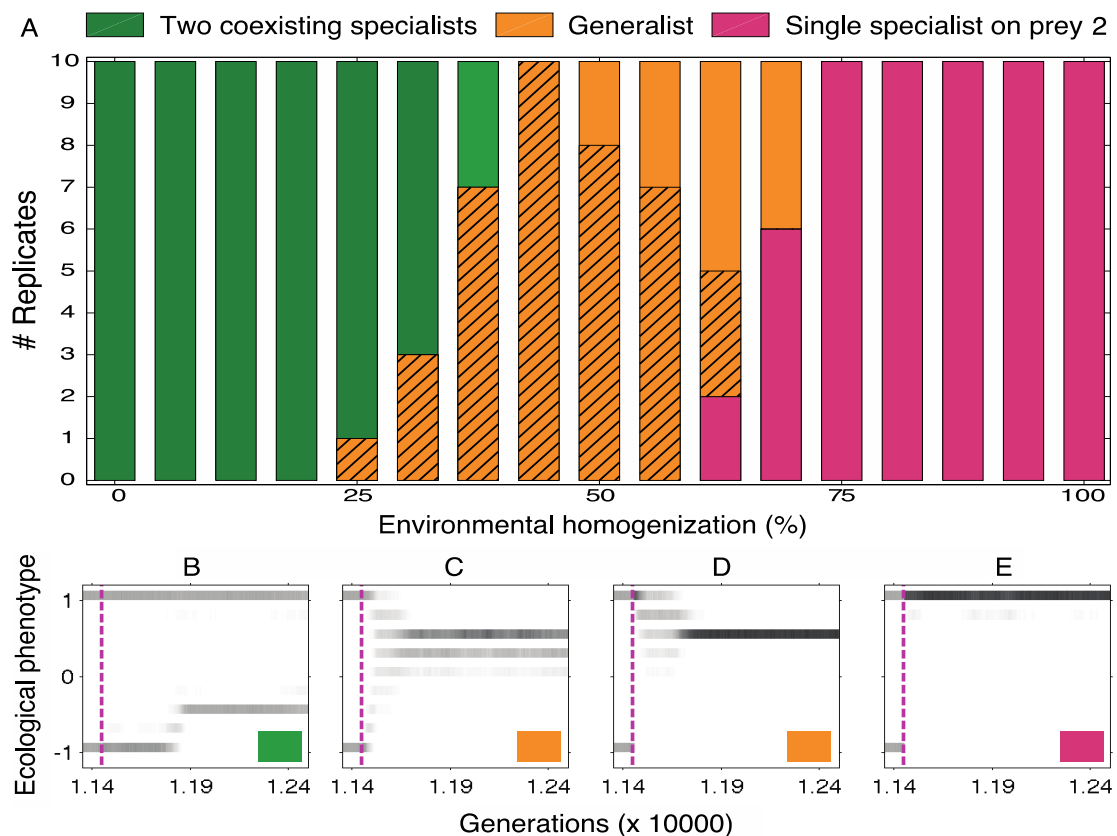

Fig. 3: Evolutionary outcomes following environmental homogenization measured as $1-\left|u_1-u_2\right|/2$ with the optimal phenotype of the first habitat $\hat{u}_1$ moving from -1 to $\hat{u}_2 = 1$. The

top panel shows the frequency of each outcome (A) while the lower panels show typical examples of the different outcomes: speciation collapse into a new species pair (B, 31.25%), a single generalist (C, 37.5% and D, 43.75%), or a single specialists (E, 75%). The hatched areas indicate that the original species pair can re-emerge if disturbance is removed after 100 generations. Other parameters are as in Table S1.

Figure 3A summarizes the evolutionary outcomes as a consequence of environmental homogenization (focus first only on the color – the hatched areas will be referred to later). At low levels of homogenization there is no species loss, but one of them may adapt to the new optimal phenotype (green bars in Fig. 3A, see also Fig 3B) due to the restructure of evolutionary state (Fig.1B). At intermediate levels of homogenization, the two incipient species collapse to a single generalist (yellow bars, Fig. 3A) that varies from a polymorphic (Fig. 3C) to a monomorphic population (Fig. 3D), which is mainly determined by the restructured fitness landscape where two local ESSs vanish (Fig. 1B). At high homogenization, when the environment in habitat 1 becomes similar to habitat 2, the habitat 1 specialist that is initially maladapted is lost through competitive exclusion (red bars in Fig. 3A, Fig. 3E). During these processes we did not observe any polymorphic transient states.
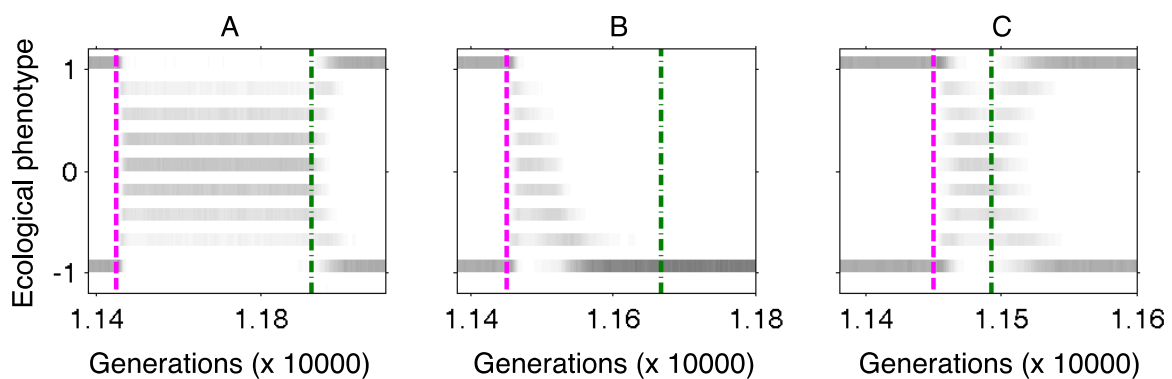

Fig. 4: Examples of evolutionary trajectories of the ecological phenotypes after visual conditions are restored. The strength of visibility noise is $\sigma_v = 0.6$ for all panels and the

reduction in attack rates are 40% (A) and 60% (B, C), respectively. The vertical lines indicate the timing of introducing (dashed) and removing (dash-dotted) disturbance. Other parameters are as in Table S1.

Restoration of the system enables ecological and genetic re-differentiation

If the visual conditions are restored to their original state, two species that have collapsed into a polymorphic generalist can always be recovered in our model (see Fig. 4A), while a collapse into a specialist is irreversible (Fig. 4B) because it is trapped in a local ESS (Fig. 1A). In the latter situation, if the environment is restored during the transient stage of a polymorphic generalist, the original species pair may still re-emerge (e.g., Fig. 4C). Successful recovery of species pairs is possible whenever mating is sufficiently assortative and a considerable amount of genetic variation still remains.
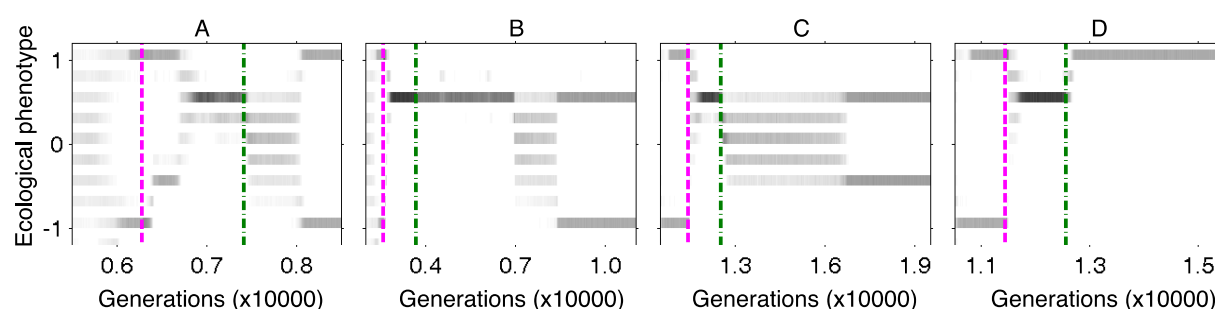

Fig. 5: Examples of evolutionary trajectories of the ecological phenotypes when disturbance of environmental homogenization is removed. The homogenization levels are 43.75% (A, C) and 50% (B, D), respectively. The vertical lines indicate the timing of introducing (dashed) and removing (dash-dotted) the disturbance. Other parameters are as in Table S1.

When environmental homogenization is reverted to the original state, a species pair can re-emerge from a majority of the generalist outcomes (hatched areas in Fig. 3A, Fig. 5A) but not

from the single specialist. The re-emergent species pair can be of the same ecological phenotype as the initial incipient species pairs (e.g., Fig.5A) or dramatically different (Fig.5B and 5C), depending on the genetic variants that can re-emerge once the environment is restored. Actually, these new species pairs are transient states and they can occur with a relatively high probability (Fig. S7 in Appendix S5). Natural selection will ultimately push them to the original ecological phenotypes, but this process can take a very long time because of the strong assortative mating that imposes a strong stabilizing selection on the two ecotypes, preventing further evolutionary change (Fig. S5 and S6 in Appendix S5). In addition to the above, a monomorphic generalist can evolve to become a single specialist after system restoration (Fig. 5D).
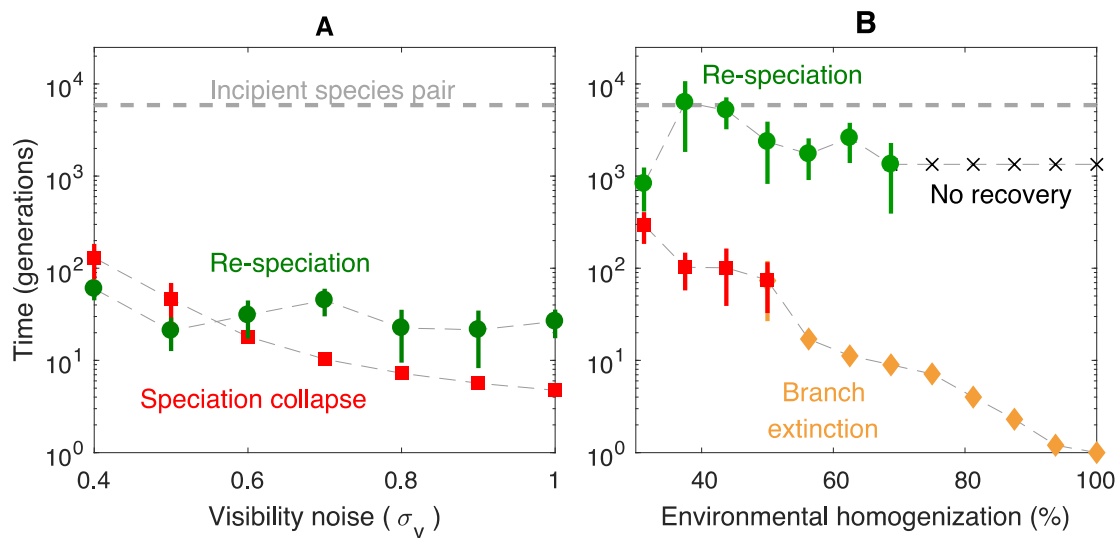

Fig. 6: Average time in generations for speciation collapse (red squares), species extinctions (orange diamonds), and re-speciation (green circles) under reduced visual conditions (A) and increased environmental homogenization (B). The time to speciation collapse is measured in generations and taken as the time from adding a disturbance to the formation of a unimodal distribution of the ecological phenotype. The re-emergence time is measured from the removal of a disturbance to the formation of a bimodal distribution of the ecological

phenotype (see the section of **Numerical investigation**). Irrespective of the outcome, simulations are run for 10000 generations at most. Parameters are as in Table S1

Speciation collapse can be several orders of magnitude faster than species pair re-emergence

Figure 6 shows the time required for speciation collapse and the re-emergence of a species pair following the introduction and removal of deteriorated visual conditions (Fig. 6A) and environmental homogenization (Fig. 6B). Compared to a speciation process, a speciation collapse is a fast process, up to four orders of magnitude faster, and the time required decreases with the intensity of the disturbance. The time required for a speciation collapse decreases steadily with the reduction in attack rate (Fig. S8 in Appendix S6). After restoring visual conditions, the original species pair quickly re-emerges if recovery is possible, and the time required for re-emergence is roughly independent of the deterioration of visual conditions (Fig. S8 in Appendix S6). The quick re-emergence of the original species pair can be attributed to the maintenance of considerable genetic variation and strong assortative mating. By contrast, the re-emergence of a species pair following a homogenization disturbance is slow, if at all possible, due to the low level of genetic variation. The reason for the lost genetic variation is strong assortative mating in combination with stabilizing natural selection, which quickly cleanses the population of the alleles that enable individuals to express the old optimal phenotype in habitat 1.

Phenotypic distribution serves as a robust warning signal of ongoing speciation collapse

We see from previous subsections that there in general exists a stage of unimodal, but still polymorphic, distribution of phenotypes, emerging from a collapse of the two incipient species pairs. This stage can be permanent (Fig. 2C) or transient (Fig. 2B, 3D). We also

notice that the original species pair can generally re-emerge if the environment is restored to pre-disturbance condition during this stage (Fig. 4C), which is true for both considered types of disturbance. Thus the formation of a unimodal distribution (Fig. S2) of phenotypes can be taken as a warning signal. We find genetic and demographic signals less reliable, however, as they are critically dependent on the type of disturbance and system parameters (Appendix S7).

Findings are robust to model variants

To verify that our conclusions are robust and generally applicable, we show here that they remain unchanged for both changes in key model parameters and for other salient model variants. First, we vary the number of neutral loci and measure how this affects the effectiveness of our proposed warning signals. We find that a larger number of neutral loci increases the threshold slope of the F_{ST} (Fig. S13 in Appendix S7). Second, we consider alternative mating scenarios. Apart from the default non-costly global mating, mating can be costly and local. Local mating means that individuals mate only with conspecifics in their foraging habitat. Compared to non-costly mating, the costly mating means here that the female assesses the males sequentially. If the female rejects all the potential males, mating fails and the female may die without reproducing before her next mating opportunity, otherwise mating succeeds. We find that our results are robust also under global costly mating. For local random mating, speciation collapse does not occur under reduced visual conditions as cross-habitat mating is impeded, but happens for environmental homogenization when the two optimal habitat traits are sufficiently close. (The Fst value is smaller than 10^{-2}, but still positive, when the level of environmental homogenization is greater than 25%.)

Discussion

Our model predicts that environmental changes can rapidly induce a variety of evolutionary outcomes, including new species pairs, polymorphic or monomorphic generalists, and single specialists. The specific outcome is determined by the type and strength of the environmental disturbance, as well as the balance between natural selection and mate choice. Provided that the environmental disturbance is not too intense, the original species pair can re-emerge if the original environmental conditions are restored, however, the time of re-emergence varies considerably with the type of disturbance and significantly depends on the amount of genetic variation, which in turn depends on the time that has passed since the onset of the disturbance. Since polymorphic generalists are a dominant transient stage of speciation collapse, and usually of short duration, it is critical to act quickly to prevent the establishment of a new evolutionary state with a single species. Early detection of speciation collapse is therefore important and we found that change in phenotype distribution can potentially serve as a general and effective warning signal of speciation collapse.

The risk that environmental changes cause loss of species diversity through hybridization is underscored by a growing number of empirical studies of fish (Seehausen et al., 1997, Bettles et al., 2005, Taylor et al., 2006, Gow et al., 2006, Seehausen et al., 2008, Vonlanthen et al., 2012, Bhat et al., 2014, Hasselman et al., 2014, Rudman and Schluter, 2016) and birds (Grant and Grant, 1996, De Leon et al., 2011, Kleindorfer et al., 2014). A polymorphic generalist is, in fact, only one of the forms to which speciation can collapse in response to environmental changes. If in the new environment a generalist strategy is an evolutionary repeller, natural selection results in directional evolution to one of the specialist ecological niches with the associated collapse to a monomorphic population (Fig. 2B, cf. Fig. 1A). Glotzbecker et al. (2014) showed that elevated turbidity can weaken sexual selection by impairing species

recognition between native and invasive stream fishes, thus resulting in formation of hybridization, but they neglected the impact of water turbidity on fish ecology.

Unlike deteriorated visual conditions, environmental homogenization does not reduce the ability of individuals to identify and discriminate against heterospecific mates, but instead restructures ecological niches, which causes a corresponding adaption of the incipient species pairs, which may give rise to new species pair (Fig. 3B), polymorphic population (Fig. 3C), or monomorphic population (Fig. 3D). Our results suggest that besides the breakdown of reproduction isolation and change in reproductive niches (e.g., Vonlanthen et al., 2012, Bhat et al., 2014), disturbance-induced restructuring of ecological niches (e.g., De Leon et al., 2011) is also an important factor for speciation collapse, which is fairly robust against different approaches to modelling disturbance (Appendix S8).

The empirical studies of the sympatric stickleback fish in Enos Lake show a rapid formation of polymorphic generalists (Taylor et al., 2006, Gow et al., 2006). It has been suggested that the emergence of these generalists is because of crayfish introduction, which causes both increased water turbidity and destruction of aquatic vegetation (Seehausen 2006, Taylor et al., 2006). This observation is in line with our model predictions, which show that polymorphic generalists can emerge due to elevated water turbidity and vegetation destruction. More recent empirical and field studies on the stickleback fish in Enos Lake show that the emergent population is not of intermediate morphology between the extant benthic-limnetic pair that has undergone speciation collapse but the resulting hybrids are closer to the benthic morphology (Rudman and Schluter, 2016). Our model predictions suggest that it might be because of the high level of vegetation destruction (Fig. 3C, 3D). Our model further predicts that if the water turbidity is degraded the polymorphic generalists may evolve by natural selection to a monomorphic population (Fig. 2C and Fig. 3D), permanently

losing the possibility of evolving back even if the environment is restored (Fig. 4B and Fig. 5C). This new observation suggests that polymorphic generalists can be just a transient stage in the process of permanent extinction of populations (Grant and Grant, 2014).

While hybridization has received growing attention for its potential to the loss of species diversity, a practical question is what happens if environment can be restored to the previous conditions (McKinnon and Taylor, 2012). Field studies on the whitefish in Constance Lake and stickleback fish in Paxton Lake show a genetic re-differentiation with the return of the lake to near-natural conditions (Gow et al., 2006, Seehausen et al., 2008). A recent theoretical study investigated how disturbance to premating mechanisms of reproductive isolation influences hybridization and species collapse (Gilman and Behm, 2011). They found that re-emergence of species pairs after collapse into polymorphic generalists is mostly likely when disturbance is of short duration. Their theoretical study focused only on the disturbances that weaken premating barriers of reproductive isolation, but ignored disturbance induced changes in environmental niche space and the changes to population vital rates, which are included in the present work. While in line with their findings, our model gives rise to additionally novel predictions. First, if disturbance alters the ecological conditions of an ecosystem in a way that the incipient species pair collapses into a single specialist that settles down on a stable evolutionary attractor, the re-emergence of a species pair is impossible (Fig. 4B and red bars in Fig. 3). Secondly, when the evolutionary attractors of an ecosystem are altered through restructuring of ecological niches (e.g., our disturbance of environmental homogenization) such that the incipient species pairs collapse into a monomorphic population, the re-emergence of a species pair might be possible but through a rather slow process of re-speciation (Fig. 6). Thirdly, the re-emergent species pair may consist of new ecological phenotypes (Fig. 5). Although these phenotypes are transient, they can persist for sufficiently

long time before completely evolving back to the ecological phenotypes that are the same as the incipient species pairs. Finally, the transient stage from a species pair to an evolutionarily stable single specialist creates a narrow time window during which removing the disturbance can lead from the polymorphic generalists to the re-emergence of species pairs (Fig 4C). However, whether and how fast the ecologically and genetically differentiated species will re-emerge from polymorphic generalists is critically dependent on the extent and duration of hybridization.

Taken together, our findings disclose two general mechanisms behind speciation collapse: hybridization induced by weakened assortative mating and evolutionary regime shifts induced by restructured ecological niches. The former mechanism generally leads incipient species pair to polymorphic generalist, and since it does not alter ecological niches, species pairs can re-emerge quickly after speciation collapse once mate choice is strengthened. The latter mechanism can drive the original incipient species pair to collapse into a new evolutionarily stable state such that species pair can no longer re-emerge even if the original ecological niches are restored. Moreover, the interaction of the two mechanisms is expected to make speciation collapse more likely and more quick, and to make it more unpredictable whether the original species pair can return when the environment is restored.

Demographic decline and introgressive hybridization have been recognized as two different, but potentially interacting, processes in driving species loss. Unlike the demographic process, the hybridization process is probably more widespread than commonly believed, because this process is fast and hard to detect (Vonlanthen et al., 2012). Thus, recognizing when it is too late to recover a species is very important (McKinnon and Taylor, 2012). When we compared the demographic, phenotypic, and genetic signals in terms of their applicability as warning signals for speciation collapse, we found that the genetic signals can be fairly efficient in

predicting speciation collapse for the disturbances considered in our model. The demographic signals can also be efficient for the disturbance of environmental homogenization but not for visual condition. However, both of these two signals are disturbance- and system-dependent and the estimated threshold slope also varies with the window size (Fig. S12 in Appendix S7) and number of neutral loci (Fig. S13 in Appendix S7). In addition, as natural populations vary in density because of environmental variation or natural fluctuations in population abundance (Shelton and Mangel, 2011), neutral genetic differentiation is affected by immigration of individuals (Pringle et al., 2011), and sampling error of the Fst (Weir, 1996), demographic and genetic warning signals might not be applicable in natural systems. Interestingly, the genetic signal discloses a transient stage before the quick drop of neutral genetic differentiation, during which there seems that nothing is happening (no obvious decline in Fst), but actually speciation is on the verge of collapsing (see the increasing part of green lines after the onset of disturbance in Fig. S9B and S9D in Appendix S7). In comparison, we found that changes in phenotype can serve as a robust warning signal. As long as one samples two populations often enough in time to detect a unimodal distribution of phenotypes given that it appears, it is still possible to act to restore species. Thus, we conclude that phenotypic signal can be used as a simple and general warning signal to managers in natural systems where population's phenotypes are clearly known and relatively easy to measure. A caveat is that the potential of this warning signal might be reduced if the phenotypes are plastic. Due to the fact that speciation collapse is a rapid process, action of restoring environment needs to be taken immediately, once warning signal is detected. Delayed response may lead to permanent loss of species diversity (Fig. 4B).

We assumed for this study that the prey species do not evolve, while they may plausibly be assumed to co-evolve with the predator species. The importance of such co-evolutionary

dynamics will likely depend on the specific system considered. The prey may similarly be affected by, and adapt to, altered environmental conditions. With environmental homogenization, it is likely that the prey – having much shorter generation time than the predator – would quickly adapt to the new conditions in a process that would resemble the sudden shift of optimal predator phenotype that we assumed for our investigations. Overall, we thus expect that prey-predator coevolution is at least in part covered by the results presented here, although more directed modelling would be necessary to sort out all possible outcomes.

Finally, notice that there are five singular strategies (one branching point, two repellers and two local ESSs, Fig. S1) under the default parameter values (Table S1). This is not a typical type of PIP under strong specialist-generalist trade-offs where there is usually a repeller at the generalist phenotype and an attractor for each of the two specialist phenotypes (see Rueffler et al. 2006, Fig. 2b). However, this is likely to result in similar outcomes as we found, with the exception that the phenotypically-wide polymorphic generalist we observed is likely to be replaced with a more narrow phenotypic distribution. To have three generalist equilibria as in our model appears less common, but has been observed (e.g., Egas et al. 2004, Fig. 3, third panel from top left). As elegantly demonstrated by Rueffler et al. (2006), other PIPs are also possible and the type of PIP depends on both the trade-off strength and the type of environmental feedback.

In summary, environmental changes that weaken mate choice or restructure ecological niches can both lead to quick speciation collapse into new species pairs, monomorphic or polymorphic generalists, or specialists. Polymorphic generalists can just be a passing stage to the formation of specialist. Restoring the environment can allow the re-emergence of species pair either from the fast process of re-divergence of polymorphic generalists or from a slow

process of re-speciation. The finding of warning signals provides promising guidance for ecosystem managers aiming to save species diversity from loss through hybridization.

Authorship contributions

All authors designed the study, developed the model, and analyzed the data. Lai Zhang performed numerical simulations and wrote, together with all coauthors, the manuscript draft. All authors contributed to discussion of results and the final text.

Data accessibility: There is no data to be archived.

References

Alexander, T. J., P. Vonlanthen, and O. Seehausen. 2017. Does eutrophication-driven evolution change aquatic ecosystem? Philos. Trans. R. Soc. B 372: 20160041.

Barluenga, M., K. N. Stolting, W. Salzburger, M. Muschick, and A. Meyer. 2006. Sympatric speciation in Nicaraguan crater lake cichlid fish. Nature 439: 719-723.

Bettles, C. M., M. F. Docker, B. Dufour, and D. D. Heath. 2005. Hybridization dynamics between sympatric species of trout: loss of reproductive isolation. J. Evol. Biol. 18: 1220 – 1233.

Bhat, S., P. -A. Amundsen, R. Knudsen, K. Ø. Gjelland, S. -E. Efvolden, L. Bernatchez, and K. Præbel. 2014. Speciation reversal in European Whitefish (*Coregonus Iavaretus* (L.)) caused by competitor invasion. Plos One 9, e91208.

Boughman, J. W.. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. Nature 411: 944– 948.

Brinkmann, B., M. Klintschar, F. Neuhuber, H. Huhne, and B. Rolf. 1998. Mutation rate in human microsatellites: influence of the structure and length of the tandem repeat. Am. J. Hum. Genet. 62: 1409 – 1415.

Chapin, F. S., E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavorel, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Diaz. 2000. Consequences of changing biodiversity. Nature 405: 234 – 242.

Dallas, J. F. 1992. Estimation of microsatellite mutation rates in recombinant inbred strains of mouse. Mamm. Genome 3: 452 – 456.

de Roos, A. M., K. Leonardsson, L. Persson, and G. G. Mittelbach. 2002. Ontogenetic niche shifts and flexible behavior in size-structured populations. Ecol. Monogr. 72: 271 – 292.

de Leon, L. F., J. A. M. Raeymaekers, E. Bermingham, J. Podos, A. Herrel, and A. P. Hendry. 2011. Exploring possible human influences on the evolution of Darwin's finches. Evolution 65: 2258 – 2272.

Di Rienzo, A., A. C. Peterson, J. C. Garza, A. M. Valdes, M. Slatkin, and N. B. Freimer. 1994. Mutational processes of simple sequence repeat loci in human populations. Proc. Natl. Acad. Sci., USA 91: 3166 – 3170.

Drake, J. W., B. Charlesworth, D. Charlesworth, and J. F. Crow. 1998. Rates of spontaneous mutation. Genetics 148: 1667 – 1686.

Geritz, S. A. H., J. A. J Metz, E. Kisdi, and G. Meszena. 1997. Dynamics of adaptation and evolutionary branching. Phys. Rev. Lett. 78: 2024-2027.

Geritz, S. A. H., E. Kisdi, G. Meszena, and J. A. J. Metz. 1998. Evolutionarily singular strategies and the adaptive growth and branching of the evolutionary tree. Evol. Ecol. 12: 35-57.

Gillespie, D. T. 1977. Exact stochastic simulation of coupled chemical reactions. J. Phys. Chem. 81: 2340 – 2361.

Gilman, R. T., and J. E. Behm, 2011. Hybridization, species collapse, and species reemergence after disturbance to premating mechanisms of reproduction isolation. Evolution 65: 2592 – 2605.

Glotzbecker, G. J., J. L. Ward, D. M. Walters, and M. J. Blum. 2015. Turbidity alters pre-mating social interactions between native and invasive stream fishes. Freshwater Biol. 60: 1784 – 1937.

Gow, J. L., C. L. Peichel, and E. B.,Taylor. 2006. Contrasting hybridization rates between sympatric three-spined sticklebacks highlight the fragility of reproductive barriers between evolutionarily young species. Mol. Ecol. 16: 2893 – 2909.

Grant, P. R., and B. R. Grant. 1996. Speciation and hybridization in island birds. Philos. Trans. R. Soc. B 351: 765 – 772.

Grant, P. R., and B. R. Grant. 2014. Speciation undone. Nature 507: 178 – 179.

Hasselman, D. J., E. E. Argo, M. C. Mcbride, P. Bentzen, T. F. Schultz, Perez-Umphrey A. A., and Palkovacs E. P. 2014. Human disturbance causes the formation of a hybrid swarm between two naturally sympatric fish species. Mol. Ecol. 23, 1137 – 1152.

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Ives, A. R., and S.R. Carpenter. 2007. Stability and diversity of ecosystems. Science 317: 58 – 62.

Jarvenpaa, M., and K. Lindstrom. 2004. Water turbidity by algal blooms causes mating system breakdown in a shallow-water fish the sand goby *Pomatoschistus minutus.* Proc. R. Soc. Lond. B 271: 2361– 2365.

Keagy, J., L. Lettieri, and J. W. Boughman. 2016. Male competition fitness landscapes predict both forward and reverse speciation. Ecol. Lett. 19: 71 – 80.

Kimura, M., and T. Ohta. 1975. Distribution of allelic frequencies in a finite population under stepwise production of neutral alleles. Proc. Nat.l Acad. Sci. USA 72: 2761 – 2764.

Kleindorfer, S., J. A. O'Connor, R. Y. Dudaniec, S. A. Myers, J. Robertson, and F. J. Sulloway. 2014. Species collapse via hybridization in Darwin's tree finches. Am. Nat. 183: 325 – 341.

Kraak, S. B. M., B. Mundwiler, and P. J. B. Hart. 2001. Increased number of hybrids between benthic and limnetic three-spined sticklebacks in Enos Lake, Canada; the collapse of a species pair? J. Fish Biol. 58: 1458 - 1464.

Mace, G. M., K. Norris, A. H. Fitter. 2012. Biodiversity and ecosystem services: a multi-layered relationship. Trends Ecol. Evol. 27: 19 – 26.

McKinnon, J. S., and E. B. Taylor. 2012. Species choked and blended. Nature 482: 313 – 314.

Naeem, S., L. J. Thompson, S. P. Lawler, J. H. Lawton, and R. M. Woodfin. 1994. Declining biodiversity can alter the performance of ecosystems. Nature 368: 734 – 737.

Pringle, J. M., A. M. Blakeslee, J. E. Byers, and J. Roman. 2011. Asymmetric dispersal allows an upstream region to control population structure throughout a species range. Proc. Natl. Acad. Sci. USA 108: 15288 – 15293.

Rohatgi, V. K., and G. J. Szekely. 1989. Sharp inequalities between skewness and kurtosis. Stat. Probabil Lett. 8: 297 – 299.

Rudman, S. M., and D. Schluter. 2016. Ecological impacts of reversal speciation in Threespine Stickleback. Curr. Biol. 26: 490 – 495.

Seehausen, O., J. J. M. van Alphen, F. Witte. 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. Science 277: 1808 – 1811.

Seehausen, O. 2006. Conservation: losing biodiversity by reverse speciation. Curr. Biol. 16: 334 – 337.

Seehausen, O., G. Takimoto, D. Roy, and J. Jokela. 2008. Speciation reversal and biodiversity dynamics with hybridization in changing environments. Mol. Ecol. 17: 30 – 44.

Shelton, A. O., M. Mangel. 2011. Fluctuations of fish populations and the magnifying effects of fishing. Proc. Nat.l Acad. Sci. USA 108: 7075 – 7080.

Smith, T. B., and S. Skulason. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. Annu. Rev. Ecol. Syst. 27: 111-133.

Sundin, J., A. Berglund, and G. Rosenqvist. 2010. Turbidity hampers mate choice in a Pipefish. Ethology 116: 713 – 721.

Svanbäck, R., M. Pineda-Krch, and M. Doebeli. 2009. Fluctuating population dynamics promotes the evolution of phenotypic plasticity. Am. Nat. 174: 176 – 189.

Taylor, E. B., J. W. Boughman, M. Groeenboom, M. Sniatynski, D. Schluter, and J. L. Gow. 2006. Speciation in reverse: morphological and genetic evidence of the collapse of a Threespine Stickleback species pair. Mol. Ecol. 15: 343 – 355.

Thibert-Plante, X., and A. P. Hendry. 2010. When can ecological speciation be detected with neutral loci? Mol. Ecol. 19: 2301 – 2314.

Thibert-Plante, X., and S. Gavrilets. 2013. Evolution of mating choice and the so-called magic traits in ecological speciation. Ecol. Lett. 16: 1004 – 1013.

Tilman, D., J. Knops, D. Wedin, P. Reich, M. Ritchieand, and E. Siemann. 1997. The influence of functional diversity and composition on ecosystem processes. Science 277: 1300 –1302.

Valdes, A. M., M. Slatkin, and N. B. Freimer. 1993. Allele frequencies at microsatellite loci: the stepwise mutation model revisited. Genetics 133: 737 – 749.

Vonlanthen, P., D. Bittner, A. G. Hudson, K. A. Young, R. Muller, B. Lundsgaard-Hansen, D. Roy, S. Di Piazza, C. R. Largiader, O. Seehausen. 2012. Eutrophication causes speciation reverse in whitefish adaptive radiations. Nature 482: 357 – 362.

Weber, J. L., and C. Wong. 1993. Mutation of human short tandem repeats. Hum. Mol. Genetics 2: 1123 – 1128.

Weir, B. S. 1996. Genetic Data Analysis II: Methods for Discrete Population Genetic Data. Sinauer Associates, Sunderland, MA.