ORIGINAL ARTICLE

The evolution of age-specific choosiness and reproductive isolation in a model with overlapping generations

Olivier Cotto,[1,2,3] (iD) Maria R. Servedio,[4] (iD) and Troy Day[1] (iD)

[1]*Departments of Mathematics and Statistics, Department of Biology, Queens University, Kingston, Ontario, Canada*

[2]*Center for Interdisciplinary Research in Biology (CIRB), College de France, CNRS, INSERM, Université PSL, Paris, France*

[3]*E-mail: olivier.cotto2@gmail.com*

[4]*Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599, United States*

The strength of mate choice (choosiness) often varies with age, but theory to understand this variation is scarce. Additionally, theory has investigated the evolution of choosiness in speciation scenarios but has ignored that most organisms have overlapping generations. We investigate whether speciation can result in variation of choosiness with age, and whether such variation can in turn affect speciation. We develop a population-genetic model of the evolution of choosiness in organisms with overlapping generations in the context of secondary contact between two divergent populations. We assume that females choose males that match their phenotype, such that choosiness evolves by sexual selection. We demonstrate that speciation can result in the evolution of age-specific choosiness when the mating trait is under divergent ecological selection and age is not used as a mating cue. The cause of this result is that allele frequencies differ between choosy females and males. However, we find that the evolution of age-specific choosiness does not affect the overall level of reproductive isolation compared to a case without age-structure, supporting previous speciation theory. Overall, our results connect life history and speciation theory, and the mechanisms that we highlight have implications for the understanding of the role of sex-specific selection in the evolution of choosiness.

KEY WORDS: Age, choosiness, life history, reproductive isolation, speciation.

Mate choice, a critical component of both sexual selection and reproductive isolation, is often decomposed into a preference function and choosiness (Jennions and Petrie 1997). The former determines the type of individual that is preferred for mating, and the latter the extent to which this type is preferred relative to other types. Describing and understanding the variation in both preference and choosiness has been the object of a large empirical and theoretical corpus (Rosenthal 2017).

Age has been documented as a factor of variation in mate choice. For example, younger virgin female guppies prefer ornamented males whereas older virgin or experienced females do not present preference for ornaments (Kodric-Brown and Nicoletto 2001). In wandering albatrosses, females prefer to mate with males matching their age (Jouventin et al. 1999). Female choosiness has been shown to decline with age in cockroaches

and crickets (e.g., Gray 1999, Moore and Moore 2001). In vertebrates, sensory perception of a mating cue often increases until the peak reproductive age and declines toward the end of life (reviewed in Ronald et al. 2012). The ultimate factors underlying the variation of mate choice with age have seldom been investigated.

Most theory developed to understand conspecific mate choice does not investigate variation of choosiness with age (Etienne et al. 2014, Priklopil et al. 2015, Henshaw 2018). Mate choice theory has traditionally studied the problem of mate choice by optimizing decisions depending on assumptions regarding their costs and benefits (Janetos 1980). Conclusions from these models have often been extrapolated in empirical studies to predict that choosiness should decline with age (Kodric-Brown and Nicoletto 2001, Moore and Moore 2001, Gilman et al. 2018): If marginal reproductive values decline with age, the cost of

being choosy increases with age. Models of age-specific choosiness, however, yield more complicated predictions. For instance, when both external hazards and internal physiological state determine the benefits and costs of waiting for the best partner, middle-aged adults are predicted to display stronger choosiness than either young and old ones (Sozou and Seymour 2003).

The evolution of age-specific mate choice in the context of speciation, corresponding to the build-up of preference and choosiness for partners of the same population rather than from other populations, has not been investigated. Empirical evidence suggests that as individuals age they have an increasing capacity for discriminating between conspecific and heterospecific partners. For example, in their study of hybridization between collared (*Ficedula albicollis*) and pied flycatchers (*Ficedula hypoleuca*), Alatalo et al. (1990) found evidence that old females are less involved in mixed pairs than young ones. Baugh and Ryan (2010) found that conspecific acoustic recognition increased from metamorphosis to the adult age in tungara frogs (*Physalaemus pustulosus*), a species that is known for its lack of vocal and auditory learning. There is currently no theoretical framework to understand lifetime variation in choosiness in the context of speciation.

Recent speciation theory points to cases where choosiness evolves toward intermediate values, resulting in evolutionary stable gene flow between divergent lineages (reviewed in Servedio and Hermisson 2020). The fact that incomplete reproductive isolation can occur as an evolutionary equilibrium raises questions about (i) whether incomplete reproductive isolation can result in age-specific variation in choosiness and (ii) whether variation in choosiness with age can affect the level of reproductive isolation between divergent populations. Complete reproductive isolation at the population level would mean that all females should be very choosy, regardless of their age. However, the evolution of incomplete reproductive isolation between divergent populations can be achieved in several different ways. For example, it could result from weak choosiness at all ages or by strong choosiness at some ages and no choosiness at others.

Previous theory showed that the evolutionary equilibrium level of choosiness by females depends on the frequency of males carrying the local trait in a population (Servedio 2011). In an age-structured population, selection on survival can result in an increase in frequency of a locally adapted phenotype with age. This process corresponds to selective disappearance and is commonly observed in wild populations (e.g., Bouwhuis et al. 2009, and references therein). If a mating trait is also involved in local adaptation, selective disappearance can induce a change in frequency of this mating trait in the different age cohorts, which can in turn select for age-specific choosiness. Such a process is likely to occur in populations undergoing ecological speciation (see figure 2 in Nosil et al. 2003, for an empirical example).

In the present study, we follow classical speciation theory to focus on the case where females choose their male partners based on phenotype matching (see a review in Servedio and Boughman 2017). This mating scheme results from a number of processes (reviewed in Kopp et al. 2018), such as self reference (e.g., for color pattern, Summers et al. 1999, Jiggins et al. 2001) or pleiotropy in the genes controlling for preference and mating traits (e.g., Jiggins et al. 2001, Kronforst et al. 2006). Phenotype matching further generates assortative mating, a widespread pattern in natural populations (Jiang et al. 2013, Janicke et al. 2019). We expand previous theory to model an organism with multiple reproductive age classes. Our primary goal is to investigate whether speciation can result in the variation of choosiness with age. We find that it can cause quite significant variation, and that the mechanism for this evolution hinges on the way that variation in choosiness affects population divergence. This study bridges a gap between the evolution of reproductive isolation and life history theory for mating strategy.

Model

We consider two habitats connected by migration. The habitats are symmetrical regarding migration and selection (Moran 1959, and see below). The organism is haploid with overlapping generations. A single bi-allelic locus with alleles *A* and *a* controls a trait that is subject to mating preference and ecological selection. This trait locus thus codes for a magic trait (sensu Gavrilets 2004, Servedio et al. 2011), but recent research shows that strong associations of loci coding for mating preference and coding for traits under ecological selection work similarly for enhancing divergence (Servedio and Bürger 2020). The trait locus is expressed throughout the whole lifespan in both males and females. A second bi-allelic locus, with alleles *M* and *m*, controls female choosiness for the preferred trait and is not expressed in males (see below). The choosiness locus is not subject to direct selection (it is selectively neutral), but can evolve by indirect selection through linkage disequilibrium with the trait locus. The trait locus and the choosiness locus independently segregate during meiosis. We track the frequencies separately in females and males, and we will use an "*" to refer to the frequency in males throughout. The frequencies of the genotypes AM, Am, aM, and am are denoted v_1 to v_4, respectively. Further, we use p_i to denote the frequency of allele i. The life cycle consists of breeding, migration and selection. The order of the life cycle events does not affect our general conclusions.

BREEDING

We assume a polygynous mating system where females prefer to mate with males matching their phenotypes (i.e., matching the

allele they carry at the trait locus). Male age is thus not a criterion for female mating preference, and females can choose males from any cohort. We define $\alpha_{x,k}$ to be the choosiness exhibited by an age-x female carrying allele k (either M or m) at the choosiness locus. The probability that an age-x female with genotype i mates with a male with genotype j is given by (omitting for simplicity the index for habitat, as breeding works similarly in both habitats)

$$M_{ij}^x = \frac{(1 + \delta_{m,ij}\alpha_{x,m})(1 + \delta_{M,ij}\alpha_{x,M})\nu_j^*}{\sum_z (1 + \delta_{m,iz})(1 + \delta_{M,iz})\nu_z^*} \tag{1}$$

where $\delta_{m,ij} = 1$ (resp. $\delta_{M,ij} = 1$) if the female genotype i carries allele m (respectively, M) at the choosiness locus and male genotype j carries the same allele as genotype i at the trait locus, and $\delta_{m,ij} = 0$ (respectively, $\delta_{M,ij} = 0$) otherwise. Notice that if $\alpha_{x,k} = 0$ then a female mates randomly, choosing males in proportion to their frequencies in the population, and that larger values of $\alpha_{x,k}$ correspond to a stronger preference for males having a trait that matches her own.

We use $f_{x,i}$ to denote the number of females produced by an age-x female with genotype i (hereafter fecundity), and assume that all females are mated such that they realize their maximum fecundity. Further, assuming that fecundity does not depend on either age or on genotype, the genotypic frequencies in offspring simplify to (the prime indicates frequency after breeding)

$$\nu_{0,k}' = \sum_{x,i,j} u_x \nu_{x,i} \nu_j^* M_{ij}^x \psi_{ij \to k} \tag{2}$$

where the index $\{x, i\}$ denotes age x and genotype i and $\psi_{ij \to k}$ is the probability that a cross between a female with genotype i and a male with genotype j produces an offspring with genotype k, as given by standard population genetics recursions with two loci and free recombination. We use $u_x = \sum_i n_{x,i} / \sum_x n_x$, to denote the frequency of age class x in the population, where $n_{x,i}$ is the number of females with age x and genotype i and n_x is the total number of females with age x.

When choosiness is fixed (or alleles m and M encode for the same choosiness), we need only track the allele frequencies at the trait locus. Equation 2 simplifies to

$$p_{0,A}' = \sum_x u_x p_{0,A}^x \tag{3}$$

with

$$p_{0,A}^x = p_{x,A}(M_{A,A}^x + M_{A,a}^x/2) + p_{x,a}M_{a,A}^x/2 \tag{4}$$

the frequency of A-carrying offspring from age-x females, and similarly for allele a. $p_{x,A} = n_{x,A}/n_x$ denotes the frequency of allele A within age-x females.

Notice that in Equations 2 and 3, the right-hand side depends on age-specific genotypic frequencies for females but on overall frequencies for males. Therefore, any change in allele frequency with age will likely induce a difference in frequency between choosy females and their male partners, as we develop below (unless age is an absolute criterion for mate choice).

MIGRATION

Here, we need to introduce a third index for genotype frequencies corresponding to habitat. We assume that age-specific migration is symmetric between habitats, such that it does not affect the number of individual in the local populations. We further assume that the migration rate is the same for both sexes. The genotypic frequencies are then

$$\nu_{x,i,1}'' = (1 - m_x)\nu_{x,i,1}' + m_x \nu_{x,i,2}' \tag{5}$$

and similarly for habitat 2 (by reversing indices 1 and 2).

VIABILITY SELECTION

Selection occurs similarly on both sexes and affects survival only. We use $s_{x,i}$ to denote the probability of survival from ages x to $x + 1$ for an individual with genotype i. Survival depends only on the trait allele with allele A (respectively, a) favored in habitat 1 (respectively, 2). For simplicity, we assume that selection is symmetric between habitats (we thus omit the index for habitat). The genotypic frequencies after viability selection follow

$$\nu_{x+1,i}''' = \nu_{x,i}'' s_{x,i}/\bar{s}_x \tag{6}$$

where $\bar{s}_x = \sum_i \nu_{x,i}'' s_{x,i}$. Note that the notation "s" in our model denotes survival (as opposed to a selection coefficient).

Analysis of the Model

We consider a scenario where two populations enter into secondary contact after divergence in allopatry. Divergence corresponds to variation maintained at the trait locus, with each allele most frequent in one population. Initially, allele A is most frequent in habitat 1 and similarly for allele a in habitat 2. This initial divergence between habitats can result from drift, or from local adaptation if survival depends on the trait locus. Divergence is measured as the frequency of allele A in habitat 1 (i.e., $p_{A,1} = \sum_x p_{x,A,1}$). Under the assumptions of our model, allele frequencies will be symmetric across habitats when divergence occurs at equilibrium ($p_{A,1} = 1 - p_{A,2} = p_{a,2}$). Otherwise, one allele invades both habitats. We further assume an even sex ratio in offspring, such that the allele frequencies are the same in males and females at this initial life stage. Under this assumption, following female demography is sufficient to track genotypic frequencies in both males and females at the scale of the population (see Supporting Information files). Note, however, that genotypic
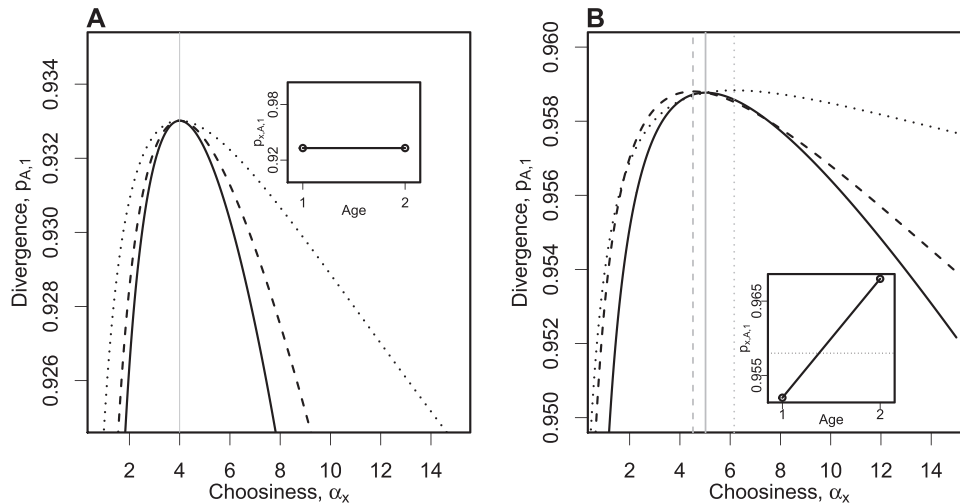
Figure 1. Divergence as a function of choosiness without viability selection (A) and with viability selection (B). The insets represent the allele frequencies at age 1 and 2 at maximum divergence. The base life cycle corresponds to $f_{1,.} = 1$, $f_{2,.} = 1$, $s_{1,.} = 0.7$, $s_{2,.} = 0$, where $f_{x,.}$ and $s_{x,.}$ denote fecundity and survival at age x for all genotypes, respectively. Migration between habitats occurs right after breeding at rate $m = 0.02$. Viability selection is modeled by assuming that the locally adapted allele provides the base survival and subtracting a factor $\Delta_S = 0.3$ for the survival rate at age 1 of individuals with the foreign allele. For both panels, solid black line: choosiness is the same at both ages, dashed black line: choosiness at age 2 is fixed at $\hat{\alpha}$, dotted black line: choosiness at age 1 is fixed at $\hat{\alpha}$; the solid, dashed, and dotted gray lines represent the vectors of age-specific choosiness maximizing divergence $\{\hat{\alpha}, \hat{\alpha}\}$, $\{\hat{\alpha}_1, \hat{\alpha}\}$, and $\{\hat{\alpha}, \hat{\alpha}_2\}$, respectively. Note that in Panel (A) all vertical lines are superposed.

frequencies can still differ between choosy females of a given age and males (see below).

As is shown below, in order to understand the forces behind the evolution of choosiness at different ages, we must first determine the effect of age-specific choosiness on divergence. Previous work showed that, following secondary contact of populations that have diverged in allopatry, phenotype matching favors the evolution of an intermediate choosiness value that maximizes population divergence at equilibrium (Servedio 2011). Phenotype matching triggers positive frequency-dependent sexual selection, whereby common males, corresponding to the most common type of choosy females, have a higher mating success than rare males. When secondary contact occurs, intermediate choosiness values maximize positive frequency-dependent selection in favor of the trait most frequent in each species (see Servedio 2011). We concentrate the investigation on secondary contact scenarios where divergence is maintained at equilibrium (but see Cotto and Servedio 2017, for a more thorough investigation of the effect of choosiness, migration, and selection, on divergence). Once we have established how age-specific choosiness affects divergence, we can then turn to how age-specific choosiness can evolve. We present our results for a life cycle with two reproducing age classes, but we obtain similar outcomes with three age-classes (see Appendix S1 and Fig. S1). The code corresponding to the analyses is provided in Supporting Information 1 and 2.

ESTABLISHED AGE-SPECIFIC CHOOSINESS
Maximizing divergence

First, we find intermediate age-specific choosiness values that maximize divergence. This can be seen using an algorithm to find the age-specific choosiness values that maximize divergence in our model with overlapping generations. To reduce the parameter space for investigation, we assume that mutations affecting choosiness at all ages reach fixation before the occurrence of mutations affecting age-specific choosiness (we later relax this assumption without affecting our conclusions; Fig. S2). We first used an algorithm to find the age-independent choosiness value that maximizes divergence, and that we denote by $\hat{\alpha}$. We then search for the choosiness value at age x, $\hat{\alpha}_x$, that maximizes divergence, assuming that choosiness at the other ages is fixed at $\hat{\alpha}$ (Algorithm 1 presented in Appendix S2).

A numerical example

Let's first consider an arbitrary life cycle with two reproducing age classes and investigate how age-specific choosiness affects divergence (Fig. 1). In this case, we find that age-specific choosiness values evolve to maximize divergence when viability selection or migration result in age-specific changes in allele frequencies.

The solid lines in Figure 1A and B (respectively, without and with viability selection) show divergence as a function of choosiness when *all* ages are equally choosy. Consistent with previous

studies (Servedio 2011), we find that in this case divergence is maximized at an intermediate choosiness value $\hat{\alpha}$.

We next consider cases where choosiness can vary with age, corresponding to the discontinuous lines in Figure 1. The insets represent the difference in allele frequency at the trait locus between ages one and two at equilibrium. In Figure 1A, migration occurs immediately after breeding (e.g., natal dispersal), and there is sexual selection only (both trait alleles provide the same survival in both habitats) such that allele frequencies are unchanged among the different cohorts of reproductive adults (Fig. 1A, inset). We find that in this case divergence peaks at a unique choosiness value $\hat{\alpha}$, which is not age specific (Fig. 1A, all vertical lines overlap).

Figure 1B shows the same life cycle as Figure 1A but with selection on survival within the reproductive period, between ages one and two. As a result of selection, the frequency of the locally adapted allele increases between both ages (Fig. 1B inset). Yet, because male age is not a criterion for mating, the trait frequency in each cohort of females differs from that of the pool of male partners, as well as from the trait frequency in the whole population (dashed horizontal line in Fig. 1B inset). In this case, we find that age-specific choosiness affects divergence (e.g. Fig. 1A dashed- and dotted- lines as compared to solid lines). Specifically, we find vectors of age-specific choosiness $\{\hat{\alpha}, \hat{\alpha}_2\}$ (where the positions in the vector match the choosiness at ages 1 and 2, respectively) and $\{\hat{\alpha}_1, \hat{\alpha}\}$, that increase divergence further than $\{\hat{\alpha}, \hat{\alpha}\}$ (compare the solid vertical gray line with the dashed and dotted ones in Fig. 1B).

We further verified that $\{\hat{\alpha}_1, \hat{\alpha}_2\}$ maximizes divergence. In other words, compared with the case where choosiness is not age specific and maximizes divergence, both decrease in choosiness at age 1 and an increase in choosiness at age 2 leads to a slight increase in divergence (Fig. 1B).

Lastly, in Figure 1A and B, migration occurs right after breeding such that it does not affect the allele frequencies in the reproductive ages. When migration occurs during the reproductive period, it also generates a difference in allele frequency between reproductive cohorts. The trait frequency in each cohort of choosy females can thus also be different from that of the male population. In that case, we similarly find vectors $\{\hat{\alpha}_1, \hat{\alpha}_2\} \neq \{\hat{\alpha}, \hat{\alpha}\}$ (see Fig. S6). In the following, we focus on cases where migration occurs after breeding such that viability selection is the only source of variation in allele frequency along a cohort lifetime.

Sensitivity analysis

To investigate whether the above observation holds for a wide spectrum of parameters, we draw random life cycles with two age-classes, migration rates, and selection strengths, and investigated the effect of age-specific choosiness on divergence. The life

cycles are drawn such that they are viable (asymptotic growth rate $\lambda \geq 1$, calculated as the dominant eigenvalue of the Leslie matrix corresponding to each drawn life cycle). Migration is assumed to occur after breeding, with a rate drawn from a uniform distribution $U(0, 0.1)$. The strength of selection $\Delta_s = |s_{1,A} - s_{1,a}|$ (the sign of the difference in survival depends on the habitat) is drawn from a uniform distribution such that the survival rate at age 1 is always positive (i.e., $\Delta_s\, U(0, \max[s_{1,i}]_{i \in A,a})$). Lastly, we keep only the parameters (life cycle, migration rate, and selection strength) allowing divergence to be maintained at equilibrium under random mating to avoid special cases where divergence is maintained in a narrow window of choosiness values (see Cotto and Servedio 2017).

We find that an increase in choosiness with age is correlated with an increase in frequency of the mating trait with age, although with some variation (Fig. 2A). The increase in choosiness from age 1 to age 2 can be more than threefold for an increase in frequency of the trait between these two ages of about 0.1. When we find age-specific choosiness values that maximize divergence, then their mean (weighted by the frequency of each age class) is equal to the choosiness value that maximizes divergence when choosiness is not age specific (Fig. 2B). Therefore, at the scale of the metapopulation, age-specific choosiness does not affect reproductive isolation. Considering the range of frequencies of the divergent trait that we obtained, the maximum divergence reached when choosiness is not age specific is similar to the maximum divergence reached with age-specific choosiness (Fig. 2C). Looking closely, however, the maximum divergence obtained with age-specific choosiness is slightly larger than the maximum divergence obtained when choosiness is not age-specific (Fig. 2D), similar to the example of Figure 1B. In the following section, we examine more closely why a difference in trait frequency with age, resulting in a difference in frequency between cohorts of choosy females and males, affects the age-specific choosiness values that maximize divergence.

Mechanisms

The previous numerical results indicate that the choosiness value maximizing divergence at the population scale does not depend on whether choosiness is age specific. However, the population-level choosiness value that maximizes divergence can result from a large age-specific variation in choosiness. To understand the mechanism that drives this last observation, we focus the analysis on a cohort of females of age x in a single population.

Since male age is not a criterion for female mating preference, males are perceived as a single pool of potential partners where the allele frequency corresponds to that of the local population of reproducing individuals ($p_A^* = \bar{p}_A$). Any change in allele frequency through a cohort's lifetime results in different allele frequencies between females of a given age ($p_{x,A}$) and their male
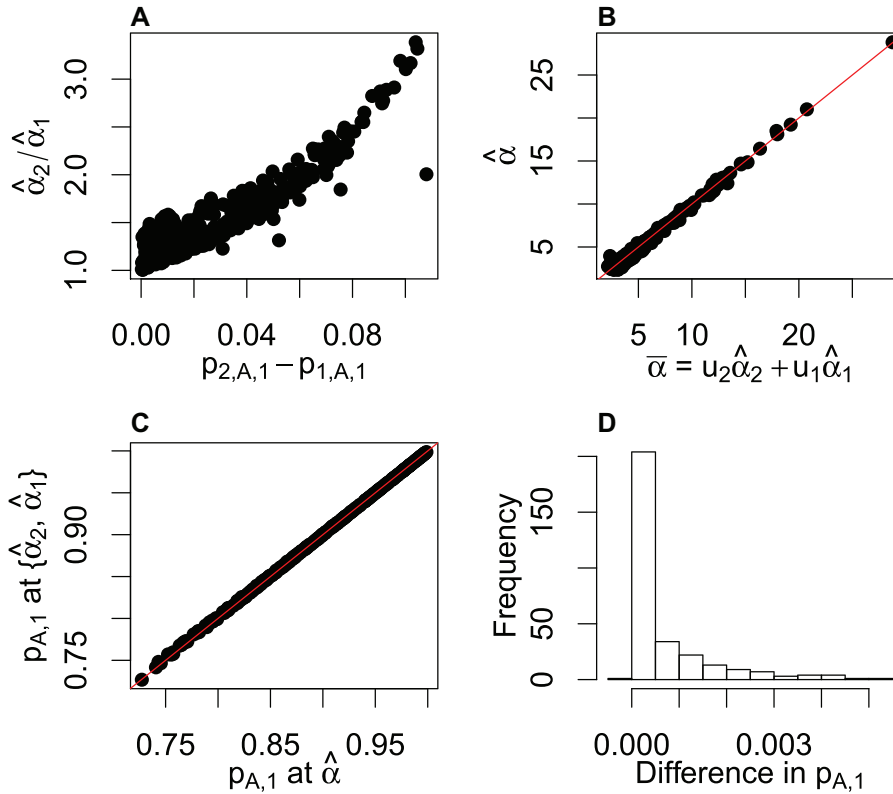
Figure 2. Link between life history, age-specific choosiness and divergence in simulated life histories. (A) Ratio of the age-specific choosiness values maximizing divergence as a function of the difference in frequency with age of the trait allele. (B) Age-independent choosiness value that maximizes divergence as a function of the mean choosiness value when choosiness is age specific. u_1 and u_2 correspond to the frequency of age 1 and 2 in the population at the stable age structure. (C) Maximum divergence with age-specific choosiness as a function of that with age-independent choosiness. (D) Distribution of the difference in divergence obtained when choosiness is age specific minus that when choosiness is independent of age. The method used to draw the life cycles, migration rate, and selection strength is described in the main text.

partners (p_A^*). Notice that such a difference in frequency between males and females can result from many causes, including sex-specific selection. Our analysis below is relevant regardless of the cause of the difference in frequency between males and females. We use δ to denote the difference in frequency of the A allele between females with age x and males, such that $p_{x,A} = p_A^* + \delta$. Substituting this expression in equation 4, we obtain

$$p_{0,A}^x = \left[p_A^* \left(M_{A,A}^x + M_{A,a}^x/2 \right) + (1 - p_A^*) M_{a,A}^x/2 \right] + p_\delta^x \quad (7)$$

where

$$p_\delta^x = \delta \left[M_{A,A}^x + 1/2 \left(M_{A,a}^x - M_{a,A}^x \right) \right] \quad (8)$$

The term in brackets in Equation 7 corresponds to the case where the allele frequencies are the same in both sexes. This term is maximized at $\hat{\alpha}$ (see demonstration in Servedio 2011). Equation 8 gives the effect of δ on the allele frequency in offspring. Replac-

ing the $M_{i,j}^x$ by their expression as a function of p_A^* and α_x, we obtain

$$p_\delta^x = \delta \frac{(1 + \alpha_x)(1 + 2\alpha_x p_A^*(1 - p_A^*))}{2(1 + \alpha_x p_A^*)(1 + \alpha_x(1 - p_A^*))} \quad (9)$$

that is of the sign of δ and where the ratio is a monotonically increasing function of α_x. When the frequency of allele A is larger in females than in males ($\delta > 0$), increasing female choosiness always increases the frequency of allele A in offspring relative to random mating. It follows that, for A-carrying males, the cost of losing mating opportunities with a-carrying females with age x is offset by the gain in mating opportunities resulting from an increase in frequency of A-carrying females with age x. We find that, as a result, the choosiness value that maximizes the frequency of A in offspring from aged-x females (with $\delta > 0$), $\hat{\alpha}_x$, is larger than $\hat{\alpha}$ (Fig. 3). In Appendix S3, we investigate the strength of selection on the mating trait using the selection coefficient approach proposed by Barton and Turelli (1991) for two populations undergoing viability selection and connected by migration.
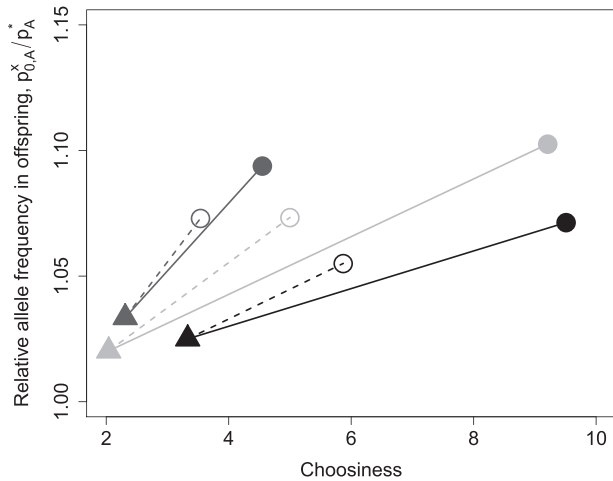
Figure 3. Effect of a difference in frequency between aged-*x* females and males on the choosiness value maximizing allele *A* frequency in offspring from aged-*x* females, $p_{0,A}^x$. We measure $p_{0,A}^x$ relative to that in males, p_A^*. The triangles represent the choosiness values that maximize allele *A* frequency in offspring when the allele frequencies are the same in both sexes ($\delta = 0$, term in brackets in Eq. 7). The circles correspond to the choosiness value that maximizes allele *A* frequency in offspring when the frequency of allele *A* is larger in females than in males ($\delta \neq 0$, adding the effect of p_δ^x). Black: $p_A^* = 0.9$, dark gray: $p_A^* = 0.75$, gray: $p_A^* = 0.6$, solid lines and symbols: $\delta = 0.06$, and dashed lines and open symbols: $\delta = 0.04$.

The analysis of selection on the mating trait confirms the above conclusion, and demonstrates that it holds when directly incorporating the effect of migration and viability selection on the allele frequency (Appendix S3, One locus analysis).

We further demonstrated that the choosiness value that maximizes the mating success of *A*-males always increases with the frequency of *A*-females (see Appendix S4). Consider n_x females with age *x*, $n_{x,A} = p_{x,A} n_x$ carrying allele *A*. We measure the mating success as the expected number of mating events X_A obtained by an *A*-carrying male with this cohort of females. We find that X_A follows approximately a normal distribution (Appendix S4) with mean

$$E[X_A] = n_{x,A} R_{x,AA} + n_{x,a} R_{x,aA}, \qquad (10)$$

where $R_{x,AA} = \frac{(1+\alpha_x)p_{x,A}}{1+\alpha_x p_{x,A}}$ and $R_{x,aA} = \frac{p_{x,A}}{1+\alpha_x p_{x,a}}$ are the mating probabilities of an *A*-carrying female of age *x* and an *a*-carrying female of age *x* with an *A*-carrying male, respectively. Equation 10 shows again the trade-off between mating with *A*-carrying females exclusively and getting some mating opportunities with *a*-carrying females. The only positive root of the derivative of equation 10 with respect to α_x is presented in Figure 4. The female choosiness value that maximizes X_A increases with $p_{x,A}$. At a low choosiness value, the gain in mating success
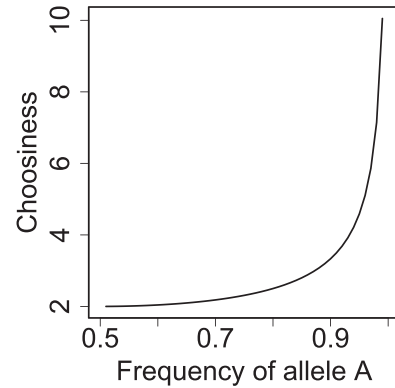

Figure 4. Choosiness value of *A*-carrying females that maximizes the mating success of *A*-carrying males as a function of the frequency of *A*-carrying females. It corresponds to the only positive root of the derivative of equation 10 with respect to α_x. (See also fig. 5B in Servedio 2011 for a related in result in a population without age structure).

of *A*-carrying males with *A*-carrying females increases faster with female choosiness than the loss of mating success with *a*-carrying females (preferring *a*-carrying males). At strong choosiness, however, the loss of mating success with *a*-carrying females increases faster with choosiness than the gain in mating success with *A*-carrying females. The choosiness value where the tipping point between these two dynamics occurs increases with the frequency of *A*-carrying females. In other words, the rarer the *a*-carrying females are, the more choosy they need to be for rare males to benefit from their preference. This effect is reversed when the frequency of allele *A* decreases in females.

EVOLVING AGE-SPECIFIC CHOOSINESS

Now that we understand the mechanisms by which age-specific choosiness affects divergence, we are well positioned to ask: Will age-specific choosiness evolve? To address this question, we numerically investigate whether age-specific choosiness strategies are evolutionarily stable and consider the case where allele *M* and *m* at the choosiness locus can code for different age-specific choosiness values. The population is first at migration-selection equilibrium at the trait locus for the ancestral age-specific choosiness, which is encoded by allele *M*. This equilibrium arises from secondary contact between initially divergent populations. A modifier *m*, coding for a new age-specific choosiness value, is introduced at linkage equilibrium with the trait locus and in the same frequency in both habitats ($p_m = 0.1$). We build pairwise invasibility plots (PIPs) where the state of the population is recorded after 20,000 time units. The PIPs illustrate the change in frequency of the mutant allele *m* depending on the choosiness that it encodes and on the choosiness encoded by the resident allele
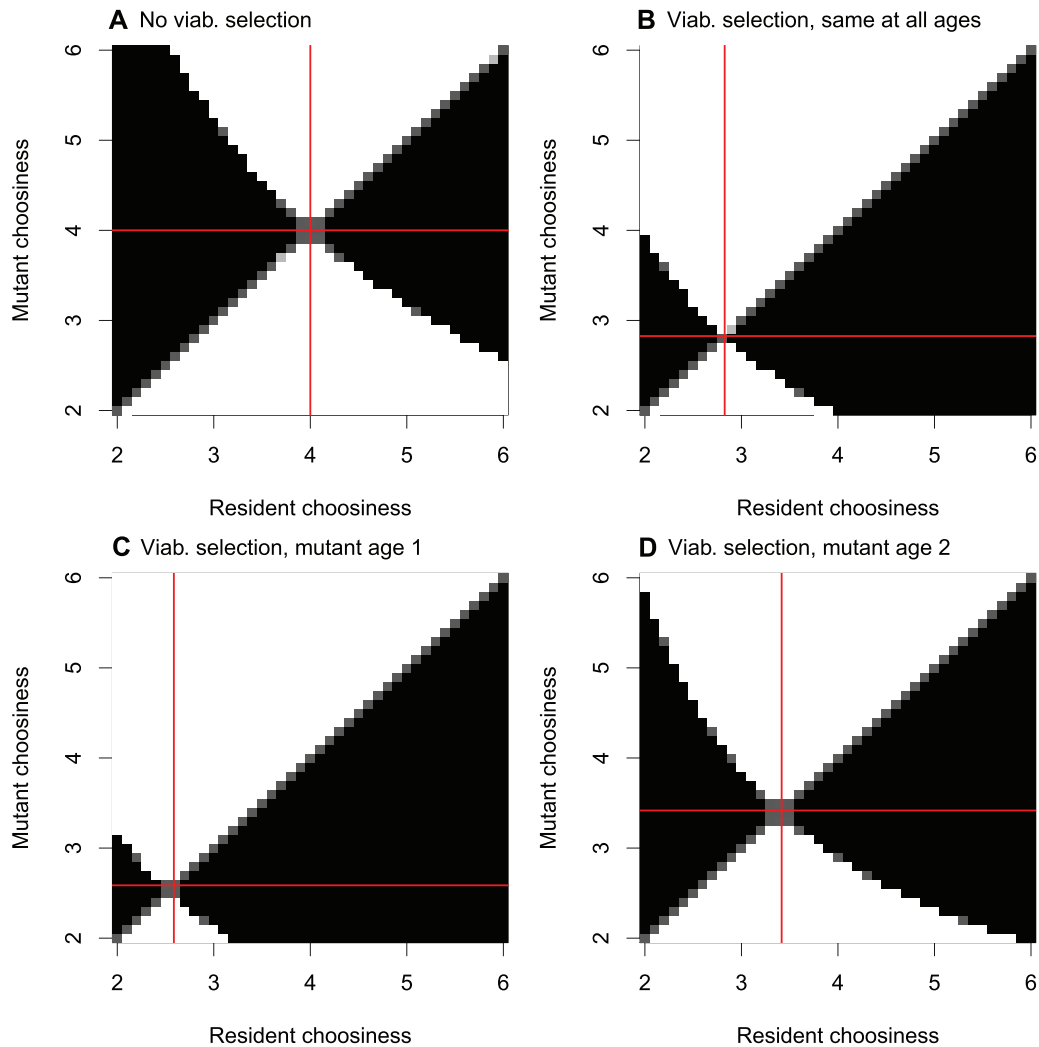
Figure 5. Pairwise invasibility plots (PIPs) for different scenarios of selection and age effects of mutations at the choosiness locus. Black: the mutant will ultimately invade ($p_m > 0.101$), white: the mutant will ultimately disappear ($p_m < 0.099$), and gray: frequencies change very slowly ($0.099 < p_m < 0.101$). The PIPs represented with a continuous gray-scale are in Figure S7. Red lines: Choosiness that maximizes divergence. (A) Sexual selection only ; life cycle and parameters as in Figure 1A. (B) Sexual and viability selection, the mutant affects choosiness at all ages (the ESS is $\hat{\alpha}$). (C) The mutant affects choosiness at age 1 only while choosiness at age 2 is fixed at $\hat{\alpha}$. (D) The mutant affects choosiness at age 2 only while choosiness at age 1 is fixed at $\hat{\alpha}$. For panels (B-D), life cycle and parameters are as in Figure 1B.

M. The PIPs therefore inform us of the evolutionary properties of the different strategies (Geritz et al. 1998). For the purpose of illustration, the PIPs are divided into three zones corresponding to the fixation (black), loss (white), and stability (gray) of the mutant (Fig. 5). We verified that intermediate allele frequencies at the modifier locus (i.e., no fixation) at the end of the simulations correspond to ongoing (slow) dynamics and not to a stable polymorphism. We expect that invasion or loss of the modifier will eventually occur.

In all cases, we find that choosiness evolves to maximize divergence. When the modifier of choosiness affects both age classes similarly, choosiness evolves to the value that maximizes divergence $\hat{\alpha}$ when choosiness is not age specific (Fig. 5A and B).

But when the modifier of choosiness has an effect specific to age, the choosiness values that evolve departs from $\hat{\alpha}$ to reach the age-specific choosiness value that maximizes divergence $\hat{\alpha}_i$. The PIPs indicate that the age-specific choosiness values maximizing divergence (the $\hat{\alpha}_i$'s) are evolutionary stable (hereafter ES) (Fig. 5C and D). The fitness surface is very flat around the ES strategies, however, resulting in slow evolution at the modifier locus (Fig. S7).

We studied the numerical results from the PIPs with further analysis. First, we verified that the age-specific ES choosi-

ness strategy evolves from a random mating population. We implemented an algorithm where modifiers coding for choosiness either at age 1 or 2 are recursively tested for invasion, from a population mating randomly (Algorithm 2 in Appendix S2). The age-specific choosiness strategies evolve toward the age-specific choosiness values maximizing divergence $\{\hat{\alpha}_1, \hat{\alpha}_2\}$, confirming the results from the PIPs (Fig. 5, and see Fig. S2). Then, we verified that the ES age-specific choosiness strategy corresponds to a selective optimum. For this aim, we expanded the expression of the selection coefficients Barton and Turelli (1991) to add the locus modifier of choosiness (see Appendix S3). We find that the selective advantage of males carrying the locally adapted trait, including sexual and viability selection, is maximized when the age-specific choosiness values of females are that maximizing divergence (Fig. S5). Lastly, we directly tested whether the age-specific choosiness values maximizing divergence $\{\hat{\alpha}_1, \hat{\alpha}_2\}$ were evolutionarily stable. We introduced modifiers coding for alternative, but close, choosiness values in a population with resident strategy $\{\hat{\alpha}_1, \hat{\alpha}_2\}$. For all parameters tested, modifiers coding for age-specific choosiness values different from those maximizing divergence decreased in frequency (Fig. S8). The above analyses together confirm the ES property of the strategy $\{\hat{\alpha}_1, \hat{\alpha}_2\}$. We conclude that speciation by sexual selection can result in the evolution of age-specific choosiness. Such evolution can result in a strong variation in choosiness with age. In our simple two-age-class model, females of age 2 can indeed evolve to be as much as three times more choosy than females of age 1 (Fig. 2A).

Discussion

We investigated the evolution of age-specific choosiness by sexual selection in the context of diverging populations. We find that an increase in female choosiness with age is evolutionarily stable when there is local selection on age-specific survival and when male age is not a mating cue. Our numerical analysis suggests that the increase in choosiness with age can be as high as two- or threefold (Fig. 2A). This prediction is consistent with observations that, in birds, heterospecific pairs are observed in the first reproductive ages but not in old individuals (Alatalo et al. 1990), or that conspecific recognition increases from metamorphosis in frogs (Baugh and Ryan 2010). Our prediction contrasts with previous (but rare) empirical studies not in a speciation context, which observed a declined in choosiness with age (e.g., Moore and Moore 2001). Speciation might thus result in different patterns of age-specific choosiness than those proposed to evolve as a life history strategy (but see Gilman et al. 2018).

The ultimate cause of this result is that selection on survival increases the frequency of the locally adapted trait with age in females. Because male age itself is not used as a mating cue, the frequency of the locally adapted trait in old females tends to be larger than that of their male partners, and the converse is true in young females. We show that the choosiness value that maximizes sexual selection in favor of the locally adapted males increases with the frequency of the locally adapted trait in females, selecting for an increase of choosiness with age. Such an increase in frequency of the locally adapted traits with age has been documented in systems where ecological divergence promotes the evolution of reproductive isolation (e.g., Nosil et al. 2003). More generally, our model suggests that choosiness should vary with age whenever the frequency of the mating trait varies with age and age is not a mating cue. Further empirical investigations are required to link age-specific changes in locally-adapted allele frequency to age-specific choosiness patterns in systems undergoing speciation.

While we find strong differences in choosiness with age at evolutionary equilibrium, this does not affect divergence at the population scale. We show that when choosiness depends on age, it only slightly increases divergence relative to the case where choosiness is not age specific. The larger frequency of the locally adapted trait in old females than in males is counterbalanced by the low frequency of the old age classes, which reduces the overall effect of age-specific choosiness on divergence. This slight increase in divergence nevertheless explains the evolution of age-specific choosiness. Similarly, we find that the population mean choosiness is equal to the value that maximizes divergence in a model without age structure (e.g., Servedio 2011).

Overall, our approach links the study of the variation in mate choice with age to the theory of speciation. On the one hand, our results provide an alternative interpretation to the observation of variation in choosiness with age, by showing that speciation can generate such variation. On the other hand, we show that allowing choosiness to vary with age has little effect on the process of speciation itself. For simplicity, most models of speciation do not include overlapping generations. We have demonstrated that this general approach is justified, at least under the assumptions of our model (shared by many models of speciation with gene flow).

Even though scarce, previous theory on the evolution of age-specific choosiness is based on the assumption that physiological capacity for mating decreases with age (Sozou and Seymour 2003, and see Introduction). The ubiquity of age-specific trade-offs for reproduction has, however, been questioned in wild populations (Hassall et al. 2015). Moreover, age-specific selection on choosiness may not be able to optimize choosiness at all ages, especially in young and old reproducers (Cotto and Day 2021). The predictions from our model do not depend on the variation of the physiological state with age (which can affect age-specific patterns of fecundities or survival rates). Instead, the mechanism underlying the variation in choosiness with age in our model depends on the difference in "relative" gene frequencies within and between cohorts triggered by selection or mi-

gration. Therefore, our model proposes a new mechanism that shapes age-specific choosiness, complementing previous theory based on optimization of costs and benefits of choosiness. Our predictions are particularly relevant in systems undergoing speciation, where hybrids have low or zero fitness. Selection on age-specific choosiness induced by the evolution of stable incomplete reproductive isolation is relatively weak. But we demonstrated that age-specific variation of choosiness with age is an evolutionary equilibrium, which could be reached over the long timescale of speciation.

The increase with age of the frequency of the locally adapted alleles is similar to the selective disappearance of low quality individuals in natural populations (Vaupel et al. 1979). With selective disappearance, if individuals of low quality die younger, older age classes will be composed of a nonrandom subset of individuals with high quality. Selective disappearance has been proposed to select for a preference of females for old males, as an old age would then be an indicator of male quality (Kokko and Lindström 1996, Kokko 1998). The evolution of direct preference for male age has been criticized, especially because deleterious mutations might accumulate in the male germ line (e.g., Beck et al. 2002). In our model, viability selection occurs similarly in both males and females, such that selective disappearance also occurs in males. The frequency of the locally favored allele therefore also increases with male age. Local females thus mate more often with old males, which tend to be better locally adapted, than what is expected from their frequency. This generates a by-product correlation between mating success and age in males (see a review in Bonduriansky et al. 2008). This correlation however does not result from a direct preference toward old males. Direct preference for old males can rather evolve as a result of an increase in the investment in advertisement with age (Proulx et al. 2002).

Finally our analysis demonstrates that, in general, a difference in allele frequency between females and males can affect the evolution of choosiness (see section "Mechanisms"). Sex-specific selection is another common source of variation in allele frequency between males and females, that could in turn affect the evolution of choosiness. We predict that female choosiness should increase with age as a result of the frequency of the locally adapted allele being higher in old females than in the male population (since male age is not a mating cue in our model). It follows that if selection on the ecological trait is stronger in females than in males, the ESS choosiness would vary more with age than in the case where selection is similar in both sexes. This would be the case, for example, if the ecological trait depends on genes on a sex chromosome in male heterogametic taxa, or if, as recent studies suggest is the case for many genes, expression depends on sex (e.g., Cheng and Kirkpatrick 2016). Sexually antagonistic selection on the mating trait can also lead to large differences in its distribution between males and females, with potential effect on the evolution of age-specific choosiness. We conclude that the mechanisms of age-specific sexual selection that we describe, originating from a differential in allele frequency between males and choosy females, have broad implications for the evolution of choosiness and deserve further investigation.

AUTHOR CONTRIBUTIONS

ACKNOWLEDGMENTS

LITERATURE CITED

Alatalo, R. V., D. Eriksson, L. Gustafsson, and A. Lundberg. 1990. Hybridization between pied and collared flycatchers—sexual selection and speciation theory. J. Evol. Biol. 3:375–389.

Barton, N., and M. Turelli. 1991. Natural and sexual selection on many loci. Genet. 127:229–255.

Baugh, A. T., and M. J. Ryan. 2010. The development of sexual behavior in tungara frogs (physalaemus pustulosus). J. Comp. Psychol. 124:66.

Beck, C. W., B. Shapiro, S. Choksi, and D. E. Promislow. 2002. A genetic algorithm approach to study the evolution of female preference based on male age. Evol. Ecol. Res. 4:275–292.

Bonduriansky, R., A. Maklakov, F. Zajitschek, and R. Brooks. 2008. Sexual selection, sexual conflict and the evolution of ageing and life span. Funct. Ecol. 22:443–453.

Bouwhuis, S., B. Sheldon, S. Verhulst, and A. Charmantier. 2009. Great tits growing old: selective disappearance and the partitioning of senescence to stages within the breeding cycle. Proc. Roy. Soc. B: Biol. Sci. 276:2769–2777.

Cheng, C., and M. Kirkpatrick. 2016. Sex-specific selection and sex-biased gene expression in humans and flies. PLoS Genet. 12:e1006170.

Cotto, O., and T. Day. 2021. The evolution of age-specific choosiness when mating. J. Evol. Biol. 34:477–485.

Cotto, O., and M. R. Servedio. 2017. The roles of sexual and viability selection in the evolution of incomplete reproductive isolation: from allopatry to sympatry. Am. Nat. 190:680–693.

Etienne, L., F. Rousset, B. Godelle, and A. Courtiol. 2014. How choosy should i be? the relative searching time predicts evolution of choosiness under direct sexual selection. Proc. Roy. Soc. B: Biol. Sci. 281: 20140190.

Gavrilets, S. 2004. Fitness landscapes and the origin of species (MPB-41), vol. 41. Princeton Univ. Press, Princeton, NJ.

Geritz, S. A., G. Mesze, J. A. Metz, et al. 1998. Evolutionarily singular strategies and the adaptive growth and branching of the evolutionary tree. Evol. Ecol. 12:35–57.

Gilman, R. T., K. Fowler-Finn, and E. A. Hebets. 2018. A probable case of incipient speciation in schizocosa wolf spiders driven by allochrony, habitat use, and female mate choice. Amer. Nat. 192:332–346.

A Gray, D. 1999. Intrinsic factors affecting female choice in house crickets: time cost, female age, nutritional condition, body size, and size-relative reproductive investment. J. Insect Behav. 12:691–700.

Hassall, C., T. N. Sherratt, P. C. Watts, and D. J. Thompson. 2015. Live fast, die old: no evidence of reproductive senescence or costs of mating in a damselfly (odonata: Zygoptera). J. Anim. Ecol. 84: 1542–1554.

M Henshaw, J. 2018. Finding the one: optimal choosiness under sequential mate choice. J. Evol. Biol. 31:1193–1203.

C Janetos, A. 1980. Strategies of female mate choice: a theoretical analysis. Behav. Ecol. Sociobiol. 7:107–112.

Janicke, T., L. Marie-Orleach, T. G. Aubier, C. Perrier, and E. H. Morrow. 2019. Assortative mating in animals and its role for speciation. Amer. Nat. 194:865–875.

Jennions, M. D., and M. Petrie. 1997. Variation in mate choice and mating preferences: a review of causes and consequences. Biol. Rev. 72:283–327.

Jiang, Y., D. I. Bolnick, and M. Kirkpatrick. 2013. Assortative mating in animals. Am. Nat. 181:E125–E138.

Jiggins, C. D., R. E. Naisbit, R. L. Coe, and J. Mallet. 2001. Reproductive isolation caused by colour pattern mimicry. Nature 411:302–305.

Jouventin, P., B. Lequette, and F. S. Dobson. 1999. Age-related mate choice in the wandering albatross. Anim. Behav. 57:1099–1106.

Kodric-Brown, A., and P. F. Nicoletto. 2001. Age and experience affect female choice in the guppy (poecilia reticulata). Am. Nat. 157: 316–323.

Kokko, H. 1998. Good genes, old age and life-history trade-offs. Evol. Ecol. 12:739–750.

Kokko, H., and J. Lindström. 1996. Evolution of female preference for old mates. Proc. Roy. Soc. Lond. Ser. B: Biol. Sci. 263: 1533–1538.

Kopp, M., M. R. Servedio, T. C. Mendelson, R. J. Safran, R. L. Rodríguez, M. E. Hauber, E. C. Scordato, L. B. Symes, C. N. Balakrishnan, D. M. Zonana, et al. 2018. Mechanisms of assortative mating in speciation with gene flow: connecting theory and empirical research. Am. Nat. 191:1–20.

Kronforst, M. R., L. G. Young, D. D. Kapan, C. McNeely, R. J. O'Neill, and L. E. Gilbert. 2006. Linkage of butterfly mate preference and wing color preference cue at the genomic location of wingless. Proc. Nat. Acad. Sci. U.S.A. 103:6575–6580.

Moore, P. J., and A. J. Moore. 2001. Reproductive aging and mating: the ticking of the biological clock in female cockroaches. Proc. Nat. Acad. Sci. U.S.A. 98:9171–9176.

Moran, P. 1959. The theory of some genetical effects of population subdivision. Aust. J. Biol. Sci. 12:109–116.

Nosil, P., B. Crespi, and C. Sandoval. 2003. Reproductive isolation driven by the combined effects of ecological adaptation and reinforcement. Proc. Roy. Soc. London. Ser. B: Biol. Sci. 270:1911–1918.

Priklopil, T., E. Kisdi, and M. Gyllenberg. 2015. Evolutionarily stable mating decisions for sequentially searching females and the stability of reproductive isolation by assortative mating. Evol. 69:1015–1026.

Proulx, S. R., T. Day, and L. Rowe. 2002. Older males signal more reliably. Proc. Roy. Soc. Lond. Ser. B: Biol. Sci. 269:2291–2299.

Ronald, K. L., E. Fernández-Juricic, and J. R. Lucas. 2012. Taking the sensory approach: how individual differences in sensory perception can influence mate choice. Anim. Behav. 84:1283–1294.

Rosenthal, G. G. 2017. Mate choice: the evolution of sexual decision making from microbes to humans. Princeton Univ. Press, Princeton, NJ.

R Servedio, M. 2011. Limits to the evolution of assortative mating by female choice under restricted gene flow. Proc. R. Soc. B: Biol. Sci. 278:179–187.

Servedio, M. R., and J. W. Boughman. 2017. The role of sexual selection in local adaptation and speciation. Annu. Rev. Ecol. Evol. Systematics. 48:85–109.

Servedio, M. R., and R. Bürger. 2020. The effectiveness of pseudomagic traits in promoting divergence and enhancing local adaptation. Evol.

Servedio, M. R., and J. Hermisson. 2020. The evolution of partial reproductive isolation as an adaptive optimum. Evolution 74:4–14.

Servedio, M. R., G. S. Van Doorn, M. Kopp, A. M. Frame, and P. Nosil. 2011. Magic traits in speciation: 'magic' but not rare? Trends Ecol. Evol. 26:389–397.

Sozou, P. D., and R. M. Seymour. 2003. Augmented discounting: interaction between ageing and time–preference behaviour. Proc. Roy. Soc. London. Ser. B: Biol. Sci. 270(1519): 1047–1053.

Summers, K., R. Symula, M. Clough, and T. Cronin. 1999. Visual mate choice in poison frogs. Proc. Roy. Soc. Lond. Ser. B: Biol. Sci. 266:2141–2145.

Vaupel, J. W., K. G. Manton, and E. Stallard. 1979. The impact of heterogeneity in individual frailty on the dynamics of mortality. Demography 16:439–454.

Associate Editor: Dr. Tim Connallon
Handling Editor: Prof. Tracey Chapman

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1: Divergence as function of choosiness (A) and choosiness as a function of age (B) for the life cycle with 3 age classes

Figure S2: Choosiness that maximizes divergence (solid lines) at age 1 (black) and at age 2 (red) and age-specific choosiness predicted from algorithm 2 (dashed lines)

Figure S3: Choosiness that maximizes selection on the A in males (a*T in eq. S.3) as a function of the difference in frequency between males and females (for different frequencies in males p*A)

Figure S4: Choosiness that maximizes selection on the A in males (a*T in eq.S.3) as a function of the strength of viability selection wA

Figure S5: Selection coefficient on the mating trait in males as a function of the difference between the choosiness encoded by the modifier and the choosiness maximizing divergence

Figure S6: Divergence as a function of choosiness when reproductive adults (here of age 1) disperse between habitats and with sexual selection only (no viability selection)

Figure S7: PIPs with the actual frequencies (in grey scale) corresponding to Figure 4

Figure S8: Frequency of the modifier after 20000 time units when the resident allele codes for the age-specific choosiness values that maximizes divergence $\{\hat{\alpha}_1, \hat{\alpha}_2\}$ for different scenarios of migration and selection.